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A PROFESSIONAL CORPORATION

ENVIRONMENTAL LAW

July 14, 2025

Chair Laura Capps
Santa Barbara County Board of Supervisors
105 E. Anapamu Street, Fourth Floor
Santa Barbara, California 93101

RE: Item A-42: Flood Control District Annual Maintenance Plan

Chair Capps and Honorable Members of the Board of Supervisors:

This office represents the Committees for Land, Air, Water, and Species, Friends of Mission Canyon, and the Santa Barbara Audubon Society. Although these groups have signed a separate group letter, these groups feel, as many others, that sensitive ecological resources have suffered from past Flood Control District maintenance activities that must not continue as proposed without substantial expansion of environmental impact analysis and enhanced avoidance and mitigation measures. It is our belief and contention that the 2001 PEIR and the subsequent environmental review of individual projects – many of which purport to utilize CEQA exemptions and addenda with no public review process - have been and are inadequate to avoid adverse impacts to resources that can and should be protected. Although a renewed public consultation process is necessary, in the mean time, the Flood Control District (FCD) must not be allowed to proceed with continuation of past routine “maintenance” activities without additional environmental analysis. The Board may not make the CEQA or Plan Approval findings as they are not supported by substantial evidence. The Findings fail to address the Plan and FCD’s program’s consistency with Local Coastal, General and Community Plan policies that are cornerstones of local planning and required by law.

It is surprising that such a substantial and controversial Project is proposed for consideration on the Board’s Administrative Calendar. The item should be continued and referred to a future Departmental Agenda hearing.

Summary of Comments

We object to the proposed approval of the Flood Control District Annual Maintenance Plan (Project) and the attempt to circumvent CEQA review of the significant adverse environmental impacts of its implementation. Reliance on the 2001 PEIR, CEQA exemptions and perfunctory addenda for the 2025-2026 Annual Maintenance Plan are improper and legally flawed. The Plan has expanded well beyond the projects, baseline conditions, and impacts that were considered in the 2001 PEIR. Conditions in the creeks and their riparian zones have changed considerably in the 25 years since conditions were assessed for the 2001 PEIR. The cumulative effects of development in every watershed have increased impervious surfaces, exacerbated the flashiness

of flows that affect native riparian and aquatic species, and encroached into riparian buffers and habitat areas. Many new species of plants and animals have been added to state and federal endangered and threatened species lists, and Critical Habitat designations added (such as for southern Steelhead in 2005) and the impacts on these species have not previously been considered, avoided, or mitigated. Climate and land use change is having many effects on local creek and riparian ecosystems, including prolonged and severe droughts, warming water temperatures, wildfires, more intense rains and subsequent debris flows, increased erosion rates, and species movement in response to changing conditions. When the effects of the Project are superimposed on all these changing conditions, substantially more significant environmental impacts occur than were considered previously.

The 2025-26 CEQA addenda are inadequate based on substantial changes to “the circumstances under which the project is undertaken which will require major revisions of the previous EIR ... due to the involvement of new significant environmental effects” or “new information of substantial importance” shows that “the project will have one or more significant effects not discussed in the previous EIR” or that “Significant effects previously examined will be substantially more severe than shown in the previous EIR” rendering a subsequent EIR necessary under Guidelines § 15162 (1) and/or (2).

CEQA disallows the use of a Categorical Exemption when there is a reasonable possibility that the activity will have significant impacts due to unusual circumstances, such as location (in Environmentally Sensitive Habitat Areas) or from location in a particularly sensitive habitat. CEQA Guidelines § 15300.2(c). The particularly sensitive nature of biologically-significant habitat in many creeks, creek beds and riparian areas is well established in the County’s Community, Local Coastal and General Plans. In many of these habitat areas, FCD activities have caused initial damage, the habitat began to recover over the course of 1-2 years, then the habitat was damaged again. These repeated and serial impacts in Environmentally Sensitive Habitat Areas and other sensitive habitats have caused significant cumulative impacts. Guideline § 15300.2(b). Other physical actions in these habitats, such as driving all-terrain vehicles through State-owned Ecological Reserves without permission and in violation of applicable law and in wetlands and identified County mitigation areas by Mosquito and Vector Management District staff, under contract to the Flood Control District and other agencies and landowners, have caused cumulative impacts to these habitats. Impacts include crushed sensitive plants, destruction of ground nesting birds and their habitat, compacted soils, and the mortality or displacement of aquatic invertebrates and various endangered and sensitive species, such as *Oncorhynchus mykiss* (steelhead and rainbow trout), *Eucyclogobius newberryi* (tidewater goby), and countless shorebirds and other native animals. The Caltrans’ Rt. 217 and Highway 101 San Jose Creek Bridge projects have had separate impacts on the same creeks and areas that FCD activity has affected and must be considered when FCD considers the cumulative impacts of proposed routine operations.

This is déjà vu all over again. In response to massive ecological damage from aggressive Flood Control District activities in the 1980s, the Flood Control District prepared a programmatic EIR

in 1991 that recognized many of the significant impacts from these activities, and shifted the Department's approach, to some degree, in adopting a dual role of both managing flood flows while actively managing the sensitive environmental and ecological resources that exist in the County's creeks and riparian corridors. Since that time and more recently, the awareness and elevation of creek health as a concomitant Departmental and County priority have been lost. As a result, Santa Barbara County's creeks and riparian areas, and the environmentally sensitive habitat present in those areas, have suffered past and present negative impacts, and under the Project will have future significant adverse environmental impacts. Regrettably, Flood Control District activities do not affirmatively seek to avoid and mitigate all significant impacts to these habitat areas but, instead, cause adverse ecological harm through aggressive and serial cutting and, in some cases maceration, of wetland woodlands and buffer areas, the application of herbicides harmful to aquatic animals, including invertebrates, dredging and grading creek bottoms and banks, increasing and altering sediment patterns and deposition locations, and other forms of habitat destruction, all of which in any other development review context would be considered to be impacts on extremely important natural habitat and protected Environmentally Sensitive Habitat Areas.

By this letter we incorporate by reference the 1991 Santa Barbara County Flood Control District PEIR, all listings of state and federal endangered species, all Critical Habitat designations for listed animals that rely on creeks included in the 2025-26 Project, and all native plants and animals that occasionally or regularly occupy or rely on creek and riparian habitat in Santa Barbara County. We also incorporate by reference a series of studies and reports identified as Exhibits and in the attached bibliography. Although the project description and impact analysis are opaque (from a 25 year old programmatic study, a number of vague and non-specific CEQA exemptions, cursory and incomplete CEQA addenda, and the conclusory and incomplete individual project descriptions in the proposed Annual Plan), the attached and referenced documents are substantial evidence supporting a fair argument of the unusual circumstances and significant impacts associated with and caused by the proposed Project. As such, neither the Project nor any individual projects may proceed without more complete project descriptions and project-specific CEQA compliance.

Public process

There has not been an open and adequate public review or engagement process for the Project. The few public workshops required pre-registration, excluding attempted drop-in participation, and the few public comments that were offered were not all well-received by an often defensive agency staff, chilling further participation. Further, one of the groups represented here (Audubon) met with Flood Control staff in an attempt to correct this process in advance to address concerns for FCD activities at Atascadero Creek, but instead were largely rebuffed in a contentious response. See Exhibit E, photos of FCD impacts to Atascadero Creek. The 1991 PEIR and Annual Maintenance Planning and programming process included a public review panel that has been abandoned, but should be reactivated.

Although the PEIR states that the FCD's processes associated with exemptions and addenda include specific follow-up project reports, none of those documents are available on the FCD website. It is impossible to determine how the projects approved by the current or previous Annual Plans proceeded, what type of environmental review process was undertaken, and the effectiveness of the purported mitigation measures (other than the few projects that required project-specific MNDs). This lack of transparency, combined with various instances of ecological harm around Project sites, reduce public confidence in the integrity of agency practices. As noted in the Environmental Defense Center's 2022 report (attached as Exhibit F) that evaluated the effectiveness of FCD's revegetation projects and found that 1) FCD does not measure success based on CDFW's measurable performance standards, 2) CDFW does not monitor revegetation sites in person and relies on FCD's qualitative evaluation of effectiveness, and 3) FCD's revegetation sites often do not meet the measurable performance standards and this may be evidence that mitigation is inadequate. Willows and other vegetation were recently removed from a restoration site on San Jose Creek that had been widened and its concrete bottom replaced with a channel bed of articulated concrete blocks, asymmetrical shape, and weirs to enable steelhead passage. This caused the build up of sediment and growth of riparian woodland vegetation. This vegetation was mowed (saplings 10-15 feet tall, 4" diameter at ground level) circa Dec-Jan 2024-25, without apparent CEQA analysis or permit authorization. The CEQA exemption was only for spot spraying of up to 500 square feet with herbicide in a concrete channel. The existence of the restoration project, sediment accumulation, and growth of willow woodland is "new information of substantial importance, which was not known and could not have been known with the exercise of due diligence at the time the previous EIR was certified" which shows "significant effects previously examined will be substantially more severe than shown in the previous EIR." The exemption is based on the existence of a concrete channel that does not accumulate sediment, is not habitat for steelhead, and does not support riparian vegetation.

Extensive repeated destruction of coastal shrubland and riparian vegetation, as well as grading along the edge of Atascadero Creek between the Parker Trust and SoCalGas parcels adjacent to Goleta Slough, some potentially owing to purported "emergency" actions and others from routine practices repeated over a number of years, has substantially impacted and impaired habitat and degraded water quality from excessive sedimentation, herbicides, and unknown agents causing discoloration of waters in disturbed areas. See Exhibit E. The cumulative effect of the Project, its individual projects, and other projects undertaken by the FCD and other agencies in the same area and impacting the same or connected linear natural resources, has been significant. It appears that great ecological damage is occurring in south coast watersheds and streams possessing and supporting important wildlife.

It is clear that Flood Control District activities have far exceeded activities authorized by previous Annual Maintenance Plans and their environmental review documentation (compare, for example, the project descriptions in the PEIR and exemptions with the FCD website and evidence of activity impacts). Serial CEQA and permitting exemptions, including inappropriate prophylactic "emergency" actions, have worked to evade necessary environmental review and

coastal permitting processes. Although an Annual Maintenance Plan can offer efficiencies and even benefits by describing comprehensive mitigation programs, County projects have been piecemeal, cumulative impacts from other projects in the vicinity of exempted projects (such as Caltrans' grading and construction in Environmentally Sensitive Habitat Areas adjacent to San Jose and Atascadero Creeks for the Rt. 217 bridge replacement project) have been ignored, and both environmental review and coastal permitting has been shortcut.

The Annual Maintenance Planning Program is in need of reform, increased transparency, and a commitment to not only remove sediment and manage vegetation in creeks but to also, at an equal level, protect and enhance the habitat and natural resources present in project areas.

PEIR, Categorical Exemption and Addenda Inadequacies

The County may not tier from the 2001 PEIR or rely on either Categorical Exemptions or Addenda because there is a reasonable probability that implementation of the Plan and the various authorized and otherwise related individual projects will cause significant direct and cumulative adverse environmental impacts to a uniquely sensitive environment and other unusual circumstances. Guidelines § 15300.2 (a-c). The 2001 PEIR is incompetent to serve as an environmental review document for the Plan and projects, and subsequent environmental review is required because the Plan and subsequent projects entail substantial changes from what was studied in the 2001 PEIR, there have been substantial changes in circumstances under which the projects will be undertaken that require major revisions to the 2001 PEIR, and new information of substantial importance to the Plan and projects that was not known at the time of the 2001 PEIR certification. Public Resource Code § 21166, Guidelines § 15162. The existence of any one of these conditions precludes reliance on a CEQA addendum. Guidelines § 15164. The Board must direct FCD to commence preparation of a new PEIR for the Annual Maintenance Plan, and ensure that all FCD activities in watersheds subject to the Plan are identified and considered, all potentially significant impacts are studied, current environmental conditions are established, and a robust program for the pro-active management of these sensitive areas be developed for both managing flood conveyances AND protecting and enhancing habitat values.

1. The Project Description and Analysis Ignores Current Evidence on the Carcinogenicity and Adverse Ecological Effects From Use of Herbicides.

a. Ecological Harm

Although herbicides are proposed for use in many watersheds, including those containing salmonids (to which glyphosates even at low concentrations can be toxic) the identity of the type, concentration and quantities of each application are omitted. As noted in the scientific literature, there is new information of substantial importance that was not known at the time of the 2001 PEIR that the exposure of many fish and invertebrates to herbicides containing glyphosates causes significant physiological effects, which ultimately harms these populations and their

viability. (see Exhibits A - D). Despite the numerous documented impacts of glyphosate on human and environmental health, the FCD does not regularly (or possibly ever) measure the concentrations of herbicides in affected waters, even though studies confirm that low concentrations can have adverse physiological impacts on non-target species. Cumulative effects from Mosquito and Vector Management District applications of Methoprene in the same waters have not been considered. Both glyphosate-based herbicides and Methoprene have been demonstrated to adversely affect aquatic invertebrate populations that are one of the “primary constituent elements essential for conservation” of local steelhead populations. 70 C.F.R. 52488, 52537, see also 52521 (9/2/2005). “Methoprene is classified as highly toxic to the planktonic crustacean *Daphnia magna*. It has adverse effects on freshwater amphipods, *Gammarus* sp. (Breud et al. 1977), lobster (Walker et al. 2005), blue crab, *Callinectes sapidus* (Horst and Walker 1999), fiddler crab (Stueckle et al. 2008), shrimp (Brown et al. 1998; Wirth et al. 2001; Ghekiere et al. 2007), a mayfly species, *Callibaetis pacificus*, non-biting midges (Chironomidae), and a dytiscid beetle, *Laccophilus* sp. (Norland and Mulla 1975).” See Exhibit H.

b. Human Health Harm

New information establishes that applications of glyphosate-based herbicides poses human health risks, with young and unborn children at particular risk. Since much of the applications authorized by the Project are near or upstream from residential areas, in waters that are not signed to avoid direct contact, there is potential harm during and after application to both staff and others that may be affected.

The manufacturer of Round-Up, Rodeo, Aquamaster and other glyphosate-based herbicides have contended these products were benign and could be liberally applied in a multitude of settings, evidence developed and disclosed since the 2001 PEIR has established the toxicity and carcinogenicity of these materials. Glyphosate was added to California’s Proposition 65 list on July 7, 2017. This listing was based on the determination by the International Agency for Research on Cancer (IARC) in 2015 that glyphosate is “probably carcinogenic to humans” (Group 2A). California’s Office of Environmental Health Hazard Assessment (OEHHA) used this classification under the "Labor Code" mechanism of Prop 65. The manufacturer of Roundup, whose products are proposed to be authorized for use by the Board’s approval of the Project, has paid nearly \$11,000,000,000 (eleven billion dollars) to settle only one class action over the sale of this product. Approximately 177,000 other lawsuits against Monsanto (now Bayer) remain pending. The County’s knowing use of this chemical places it in line for exposure to the legal consequences of its use.

Commenters question the County’s compliance with Prop 65 in alerting nearby residents, biologists, recreationalists, employees and contractors of exposure to known carcinogens. See Exhibit C, Toxic Effects of Glyphosate on the Nervous System: A Systematic Review (Abstract: “exposure to this pesticide during the early stages of life can seriously affect normal cell development by deregulating some of the signaling pathways involved in this process, leading to alterations in differentiation, neuronal growth, and myelination. Glyphosate also seems to exert a

significant toxic effect on neurotransmission and to induce oxidative stress, neuroinflammation and mitochondrial dysfunction, processes that lead to neuronal death due to autophagy, necrosis, or apoptosis, as well as the appearance of behavioral and motor disorders. The doses of glyphosate that produce these neurotoxic effects vary widely but are lower than the limits set by regulatory agencies.”)

The PEIR is inadequate as an informational document and the CEQA exemptions and addenda inapplicable if the County does not identify the quantity, location and specific, known toxic herbicide(s) that are applied as a common treatment in many watersheds. The FCD also does not undertake any follow-up studies of the fate and correlative environmental impacts of herbicide use, such as water testing and systematic assessments of herbicide effects on rare, sensitive and endangered species. Application of large quantities of carcinogenic materials in public places likely affects members of the public and members of our local ecosystems.

2. Project description inadequacies – physical activities

The FCD website states the following:

Flood Control Maintenance

The Flood Control District's aggressive channel maintenance program is directed at preserving and maximizing the flood carrying capacity of existing creeks, channels and rivers. Channels are cleared of obstructive vegetation and deposited sediments in order to allow flood waters to flow unhindered. In addition, the maintenance program operates and maintains a series of debris basins, retardation basins, and groundwater recharge basins. Routine maintenance of these facilities is essential to preserving their operating effectiveness.

Flood control maintenance is accomplished through three basic methods: heavy equipment work, hand clearing, and herbicide application. Throughout the County, natural and man-made flood control facilities in the County are subject to damage and loss of capacity through sedimentation, vegetation growth, bank erosion, and other obstructions by debris. The District uses heavy equipment to remove sediment, clear obstructive vegetation, and correct erosion problems. In many streams on the South Coast, obstructive vegetation is removed by hand crews using chain saws and other hand tools. Throughout the County, herbicides are used to inhibit the growth of obstructive vegetation and to control weed growth on a variety of sites.

The PEIR makes no reference to aggressive tactics, and heavy equipment work is downplayed. Page 101 of the PEIR describes Maintenance Practices, and makes no mention of use of heavy equipment or sediment removal. It is well known that debris and retention dams trap fine sediment, starving local beaches of sand; that they act as barriers to steelhead migration and the movement of other stream organisms; that they degrade stream continuity and convert stream reaches from free-flowing streams to a stagnant pond or mud flat; and that any habitat created by

FCD dams is regularly destroyed by maintenance activities. Stream channelization, including concrete channels, degrade stream habitat quality and reduce the habitat and biological diversity of streams.

3. Biological Survey Work Cited in PEIR is Obsolete

As noted herein, FCD has prioritized activities to maintain channel capacity and paid little attention to resource protection. In various individual projects listed in the PEIR, biological surveys are over decades old and patently inadequate to characterize the current status of populations of rare, threatened and endangered species that are present. In the absence of a requirement that the FCD develop and maintain a publicly-accessible current and accurate survey of the biological resources present in each Project area, it is impossible to determine if resources are being avoided or impacts mitigated.

The CEQA analysis fails to address past FCD restoration failures, and relies on an improbable mitigation bank with very limited justification. While the creation of new habitat for mitigation banking is warranted, restoration is a County goal elsewhere (see General and Community Plans) and should not generate credit authorizing the destruction of existing habitat.

4. Reliance on Ancillary Permitting is Illusory

The PEIR attempts to rely on ancillary permitting by outside agencies, such as the US Army Corps of Engineers, Regional Water Quality Control Board, US Fish and Wildlife Service, and California Department of Fish and Wildlife to meet regulatory requirements for its activities. In fact, FCD has relied on general permits with long duration and avoided detailed analyses of the impacts of most specific projects. Many of those permits have or will shortly expire, and cannot be relied on for the term of the Project. Further, severe weakening of many federal environmental programs and the decimation of federal resource and regulatory agencies constrains the County's reliance on the efficacy of these laws and agencies to provide a balance to FCD's aggressive work in highly sensitive areas.

5. The Impacts of Serial Emergency Exemptions in Areas of Project Work Are Significant and Must be Considered

The Flood Control District has sought and relied excessively on emergency exemptions from permits for activities that have substantial significant impacts on the same resources affected by routine annual "maintenance". As a consequence, the County needs to consider the cumulative impacts of routine maintenance activities against a background of extreme stream and riparian alterations carried out by FCD under emergency permits and exceptions along with projects in the same watersheds proceeding under individual environmental review and permitting, such as Atascadero Creek. **6. Character and Evidence of Impacts**

Serial annual destruction of habitat in our creeks by FCD has caused adverse effects on the County's creeks and riparian areas.

Below is a description of key impacts with reference to scientific sources and agency reports, incorporated herein by reference:

1. Loss of Riparian Vegetation and Habitat Structure

Impact: Mechanical vegetation removal and bank grading degrade riparian habitat by reducing canopy cover, eliminating native plant species, and disrupting wildlife corridors.

Sources:

- Kondolf, G.M. et al. (2006). *Process-based ecological river restoration: Visualizing three-dimensional connectivity and dynamic vectors to recover lost linkages*. **Ecology and Society**, 11(2): 5.
 - Notes that vegetation removal impairs habitat complexity, thermal regulation, and bank stability. Ironically, the removal of riparian vegetation can cause bank failure during floods, exacerbating flooding impacts.
- California Riparian Habitat Joint Venture (2004). *Riparian Habitat Conservation*.
 - Emphasizes that clearing riparian vegetation reduces biodiversity and contributes to streambank erosion.

2. Stream Channel Incision and Sediment Transport Disruption

Impact: Channel dredging and straightening can cause incision (lowering of the streambed), disconnecting the stream from its floodplain, lowering groundwater tables, and reducing wetland and riparian function.

Sources:

- Kondolf, G.M. (1997). *Hungry Water: Effects of Dams and Gravel Mining on River Channels*. **Environmental Management**, 21(4): 533–551.
 - Highlights the role of sediment removal in degrading channel morphology and habitat.
- Stillwater Sciences (2007). *Riparian and Aquatic Habitat Assessment of the Santa Clara River Watershed*.
 - Discusses how repeated excavation alters sediment regimes and leads to entrenched channels.

3. Reduced Aquatic Habitat Quality and Biodiversity

Impact: Simplified channels and frequent disturbances diminish habitat for aquatic species (e.g., salmonids, amphibians), reduce invertebrate populations, and impair water quality through increased turbidity and temperature.

Sources:

- NMFS (2012). *Southern California Steelhead Recovery Plan*.
 - Identifies flood control structures and maintenance as key threats to steelhead habitat quality.

- Holmes, R.W. et al. (2008). *Sources and Pathways of Sediment and Nutrients in the Ventura River Watershed*.
 - Connects sediment removal to declines in aquatic macroinvertebrate communities and fish populations.

4. Disruption of Natural Hydrologic Processes

Impact: Levees, culverts, and channel modifications disrupt the timing, frequency, and magnitude of natural flows, which are critical for sustaining the ecological functions of riparian systems.

Sources:

- Mount, J. (1995). *California Rivers and Streams: The Conflict Between Fluvial Process and Land Use*. University of California Press.
 - Documents how flood control interferes with sediment deposition, channel migration, and seasonal inundation regimes.
- USGS & California Department of Fish and Wildlife (various).
 - Numerous studies emphasize the ecological importance of flow variability for maintaining species diversity and geomorphic function.

5. Cumulative and Long-Term Impacts

Impact: Chronic maintenance activities prevent natural recovery and adaptation of riparian ecosystems, leading to long-term ecological degradation and reduced resilience to climate-driven hydrologic changes.

Sources:

- Opperman, J.J. et al. (2005). *Influence of Floodplains on Flood Risk and Hydrologic Connectivity in a Changing Climate*. **Ecological Applications**, 15(5): 1499–1510.
 - Argues for floodplain reconnection as a more sustainable alternative to traditional flood control.
- California Natural Resources Agency (2020). *Safeguarding California: Climate Adaptation Strategy*.
 - Recommends ecologically sensitive flood management to protect biodiversity and ecosystem services.

The Board’s Findings Are Not Supported by Evidence and Are Incomplete

The cursory findings included in staff’s materials are directly contradicted by incontrovertible evidence and fail to provide the analytical route for approving the Project and finding CEQA compliance. The Staff Report and analysis fail to examine the requirements of the County’s Local Coastal, General and Community Plans. Every “action, program or project” is subject to the General Plan authority, and must be “in agreement or harmony” with applicable policies. *Friends of Lagoon Valley v. City of Vacaville* (2007) 154 Cal.App.4th 807, 817; see also OPR General Plan Guidelines.

The County's Conservation Element's goal is "to ensure that Santa Barbara County's ecosystems will remain in 50 or 100 years pretty much as they are today." P. 163. Policies from the Land Use Element, Open Space Element, Safety Element govern the Plan, but are not specifically addressed. Local Coastal Plan policies identify coastal creeks as Environmentally Sensitive Habitat Areas and apply substantive protection requirements, in furtherance of Public Resource Code § 30231, which mandates protection and restoration of the "biological productivity and quality of . . . streams [and] wetlands." Numerous creek- and wetland-specific natural resources protection policies are present in each of the various Community Plans (Eastern Goleta Valley Community Plan, Gaviota Coast Plan, Orcutt Community Plan, Montecito Community Plan, Summerland Community Plan, Mission Canyon Community Plan). For example, the Eastern Goleta Valley Community Plan Policy ECO-EGV-3.1 requires preservation and enhancement of creeks, streams and waterways, fish passage, riparian vegetation and wildlife corridors. Policy ECO-EGV-3.3 establishes that "typical wildlife corridors are provided by drainage courses and similar undeveloped areas." Not only is the 2001 PEIR silent on evaluating impacts to wildlife migration, but the 2001 PEIR was certified 15 years before the EGVCP was adopted. To the extent the 2001 PEIR made cursory findings or General Plan consistency based on conditions 25 years ago, that PEIR predated many applicable policies and fails to consider current conditions, including climate-changed drought and flood patterns and the cumulative effect these conditions have upon local wildlife and habitat quality.

For all the reasons stated in this letter, public comment, referenced materials and the on-the-ground conditions, the Board lacks substantial evidence to support the proposed, inadequate findings.

Conclusion

For all the reasons listed above, Commenters object to any reliance on the outdated 2001 PEIR, and separately object to each and every CEQA exemption and addendum referenced, relied upon, or otherwise in support of any project approved by the proposed action.

Commenters respectfully request that the Board of Supervisors continue this matter to a full Departmental hearing, with sufficient notice for interested members of the public to freely express their concerns about this project and FCD practices. The multitude of CEQA exemptions and addenda relied on to support the Project and subsequent individual projects are not supported by the law and expose the County to legal risks. We implore the Board to direct FCD staff to engage productively with representatives of commenting non-governmental organizations to develop interim measures for the 2025-26 clearing program to proceed while commencing the preparation of a new Programmatic Environmental Impact Report to replace the 25 year old document that the FCD currently relies on. The 2001 PEIR is outdated based on substantial new information and conditions that did not exist in 2000 when it was prepared, and is thus inadequate for current use. The FCD cannot tier from the 2001 PEIR or utilize addenda that rely on it due to the major changes in the project descriptions, baseline conditions, and new

information. Proposed CEQA exemptions are defective due to impacts from unusual circumstances and the extreme sensitivity of the affected environment, and reliance on any of the cited CEQA shortcuts subject the Plan and all related projects to legal challenge. A new Flood Control District PEIR is required to replace the 25-year old 2001 PEIR in its entirety. The new PEIR should be prepared by an independent environmental consulting firm using current conditions in order to adequately consider the FCD's future actions and activities.

Findings for approval are not supported by substantial evidence in light of the evidence presented herein and in public comment of these changed conditions, new information, and changed project description.

We request that your Board decline to approve this matter on the Administrative calendar and defer action until a full public hearing with adequate public notification is scheduled and undertaken.

Respectfully Submitted,

LAW OFFICE OF MARC CHYTILO, APC



By: Marc Chytilo

Exhibits and Bibliography (incorporated by reference)

A: Lissandra Gluszczak, Denise dos Santos Miron, Bibiana Silveira Moraes, Róli Rodrigues Simões, Maria Rosa Chitolina Schetinger, Vera Maria Morsch, Vânia Lucia Loro, *Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen)*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, Volume 146, Issue 4, November 2007, Pages 519-524.
<https://www.sciencedirect.com/science/article/abs/pii/S1532045607001470>

B: Gonzalo Luis Pérez, National Scientific and Technical Research Council, María Solange Vera, Leandro Andrés Miranda, *Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems*, In book: Herbicides and Environment, Chapter: Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems, (pp.343-368)
https://www.researchgate.net/publication/255897901_Effects_of_Herbicide_Glyphosate_and_Glyphosate-Based_Formulations_on_Aquatic_Ecosystems

C: Carmen Costas-Ferreira 1, Rafael Durán 1, Lilian R F Faro, *Toxic Effects of Glyphosate on the Nervous System: A Systematic Review*, Int J Mol Sci. 2022 Apr 21;23(9):4605. doi: [10.3390/ijms23094605](https://doi.org/10.3390/ijms23094605)

D: Van Bruggen A.H.C. a b, He M.M. a c, Shin K. a b, Mai V. a, Jeong K.C. a, Finckh M.R. d, Morris J.G. Jr., *Environmental and health effects of the herbicide glyphosate*, Science of The Total Environment, Volumes 616–617, March 2018, Pages 255-268

E: Photos of effects of FCD activities along Atascadero Creek, 2023-2025 (credit: Marc Chytilo) (attached)

F: Blackwelder, Natalie, Trautwein, Brian, *San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank – Review and Recommendations*, Environmental Defense Center, October 24, 2022

G: P.S. Evalen, E.N. Barnhardt, J. Ryu, Z.R. Stahlschmidt, *Toxicity of glyphosate to animals: A meta-analytical approach*, Environmental Pollution 347 (2024) 123669

H: Peterson, Robert K.D., Rolston, Marni G., *Larval Mosquito management and risk to aquatic ecosystems: A comparative approach*, Transgenic Res (2022) 31:489-504.

Bibliography of Reports and Studies documenting adverse environmental impacts from clearing and flood control activities in creeks, each of which is incorporated by reference into this letter.

1. Kondolf, G.M. (1997). "Hungry Water: Effects of Dams and Gravel Mining on River Channels." *Environmental Management*, 21(4), 533–551.

This foundational article explores how sediment removal and flow regulation, both common in flood control, lead to channel incision, habitat simplification, and disconnection of rivers from floodplains. Kondolf emphasizes geomorphic and ecological consequences, many of which directly result from routine flood control maintenance.

2. Kondolf, G.M., Boulton, A.J., O’Daniel, S., Poole, G.C., Rahel, F.J., Stanley, E.H., et al. (2006). "Process-based ecological river restoration: Visualizing three-dimensional connectivity and dynamic vectors to recover lost linkages." *Ecology and Society*, 11(2): 5.

This article outlines how river restoration must consider connectivity disrupted by flood control. It explains the ecological importance of lateral and longitudinal connectivity, which is often reduced or eliminated by levees, channelization, and vegetation removal.

3. California Riparian Habitat Joint Venture (2004). Riparian Habitat Conservation.

This comprehensive guidance document discusses the ecological functions of riparian systems and the threats posed by routine maintenance, including vegetation clearing and bank grading. It emphasizes the need for integrated habitat conservation planning in flood management.

4. National Marine Fisheries Service (NMFS). (2012). Southern California Steelhead Recovery Plan.

This recovery plan identifies flood control infrastructure and maintenance practices as major barriers to the recovery of endangered Southern California steelhead. It includes a scientific rationale for maintaining riparian vegetation and restoring hydrologic connectivity.

5. Stillwater Sciences. (2007). Riparian and Aquatic Habitat Assessment of the Santa Clara River Watershed.

This technical report evaluates the ecological health of a major coastal watershed and attributes many of the impacts to flood control practices such as sediment removal, channelization, and vegetation management. It provides data on physical habitat degradation and species decline.

6. Mount, J. (1995). California Rivers and Streams: The Conflict Between Fluvial Process and Land Use. University of California Press.

Mount provides a detailed explanation of natural river processes and how human interventions—especially flood control—have disrupted them. He offers scientific and practical insight into how these activities degrade riparian and aquatic ecosystems over time.

7. Holmes, R.W., Mazor, R.D., Gillett, D.J., Brown, L.R., & May, J.T. (2008). "Benthic macroinvertebrate assemblages and habitat in a regulated California river: Correspondence of ecological patterns with land use and water management." *River Research and Applications*, 24(6), 756–774.

This study documents the sensitivity of aquatic invertebrate communities to habitat alteration from flood control and urbanization. It reinforces the importance of complex, vegetated, and dynamic channels to sustain biodiversity.

8. Opperman, J.J., Luster, R.A., McKenney, B.A., Roberts, M., & Meadows, A.W. (2005). "Floodplains: A natural system to reduce flood risk." *Frontiers in Ecology and the Environment*, 3(10), 524–531.

This article presents floodplains as ecological assets that can mitigate flood risks naturally. It critiques traditional flood control for reducing the ecological and hydrologic benefits of floodplain connectivity.

9. California Natural Resources Agency. (2020). Safeguarding California: Climate Adaptation Strategy.

This strategic document encourages a shift toward ecologically sensitive flood management that supports both climate resilience and ecosystem function. It highlights how conventional flood control undermines these goals, particularly in coastal areas.



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Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*)

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Abstract

Silver catfish (*Rhamdia quelen*; Teleostei) were exposed to commercial formulation Roundup®, a glyphosate herbicide: 0 (control), 0.2 or 0.4mg/L for 96h. Fish exposed to glyphosate showed an increase in hepatic glycogen, but a reduction in muscle glycogen at both concentrations tested. Glucose decreased in liver and increased in muscle of fish at both herbicide concentrations. Glyphosate exposure increased lactate levels in liver and white muscle at both concentrations. Protein levels increased in liver and decreased in white muscle while levels of ammonia in both tissues increased in fish at both glyphosate concentrations. Specific AChE activity was reduced in brain after treatments, no changes were observed in muscle tissue. Catalase activity in liver did not change during of exposure. Fish exposed to glyphosate demonstrated increased TBARS production in muscle tissue at both concentrations tested. For both glyphosate concentrations tested brain showed a reduction of TBARS after 96h of exposure. The

present results showed that in 96h, glyphosate changed AChE activity, metabolic parameters and TBARS production. The parameters measured can be used as herbicide toxicity indicators considering environmentally relevant concentration.

Introduction

The biological response of an aquatic organism to pollutants frequently induces changes at cellular and biochemical levels, leading to changes in the structure and function of the cells, tissues, physiology and behavior of the organism (Parvez and Raisuddin, 2005).

According to Jiraungkoorskul et al. (2002), the formulation of Roundup® is isopropylamine (IPA) salt of glyphosate 480g/L, water and polyethoxylated tallow amine surfactant, POEA. The surfactant POEA is a non-ionic substance used in herbicide formulations to increase the efficacy of active ingredients and promotes penetration of herbicide into plant cuticles (Brausch and Smith, 2007). The glyphosate formulations frequently use surfactant POEA that is more acutely toxic than the glyphosate itself (Giesy et al., 2000). This herbicide is non-selective and has been widely used in recent years. Glyphosate formulation rapidly dissipates from surface waters and soil microflora biodegrade it into AMPA and CO₂. Thus, the active ingredient persists in the water environment for only a short time (Giesy et al., 2000, Jiraungkoorskul et al., 2002). Commercial formulations of glyphosate including Rodeo®, Roundup® and Aquamaster® have been investigated for their potential to produce adverse effects in non-target organisms like fish (Gardner and Grue, 1996, Williams et al., 2000). The glyphosate concentration used in rice and soybean cultures in Southern Brazil ranges from 0.36 to 2.16mg/L. The half-life of glyphosate in the soil is around 30 to 90days and its water solubility is 157µg/L (Rodrigues and Almeida, 1998).

Teleostean fish may be good indicators of contamination by pollutants such as herbicides because their biochemical responses are similar to those found in mammals (Sancho et al., 2000, Begum, 2004). The measurement of acetylcholinesterase (AChE) activity present in the cholinergic synapses and motor end plates has been used by different authors to monitor carbamate and organophosphate effects in insects and vertebrates, such as fish (Chuiko, 2000, Fernández-Vega et al., 2002, Filho et al., 2004). However, brain AChE activity has been modified by pesticides of other classes, such as organochlorine endosulfan (Dutta and Arends, 2003) and isooxazolidinone clomazone (Miron et al., 2005).

Many environmental pollutants may induce the formation of reactive oxygen species

(ROS) (Ahmad et al., 2000, Sevgiler et al., 2004). Due to their high reactivity, these species may cause damage to lipids, proteins, carbohydrates, or nucleic acids (Fraga et al., 1996, Parvez and Raisuddin, 2005). Pollutant-induced lipid peroxidation (LPO), as in the case of herbicides, has been observed in several fish species (Schlenk et al., 1997, Sevgiler et al., 2004). Variations in the activities of antioxidant enzymes have been proposed as indicators of pollutant mediated oxidative stress (Ahmad et al., 2000, Li et al., 2003). The silver catfish (*Rhamdia quelen*) was chosen because it is a native freshwater species of economical importance in Southern Brazil. The aim of this study was to investigate the effects of short-term formulation of glyphosate exposure on metabolic and enzymatic parameters in *R. quelen* as possible indicators of herbicide toxicity.

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Section snippets

Chemicals

The herbicide used in this study was commercial formulation Roundup® (48% (480 CE) acid equivalent Monsanto Company, St Louis MO, USA) and dissolved in water.

Acetylthiocholine, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), bovine serum albumin, Triton X-100, (H₂O₂), malondialdehyde (MDA), 2-thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). ...

Fish

Juvenile silver catfish (*R. quelen*, Heptapteridae, Siluriformes) of both sexes ...

Results

AChE activity in the brain of fish exposed to glyphosate (Roundup®) was lower than in

the control group (reduction of activity ranging from 25–27% in both concentrations tested). However, exposure to glyphosate herbicide did not cause alterations of AChE activity in muscle (Fig. 1). In this study, silver catfish exposed to glyphosate exhibited an increased in liver glycogen, lactate, protein and ammonia levels, but decreased of glucose levels when compared to control values (Table 1). In ...

Discussion

The results of our study showed that silver catfish exposed to glyphosate significantly decreased brain AChE activity at both concentrations tested (maximum inhibition 27% in the 0.2 mg/L). However, this exposure did not cause alterations of AChE activity in muscle. It is known that AChE sensitivity is variable among species with being the brain and muscle the most frequent tissues to study acetylcholine hydrolysis (Sancho et al., 2000). Our results concerning brain AChE activity are in ...

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References (41)

Ahmad I. *et al.*

[Raisuddin, S. Induction of hepatic antioxidants in freshwater catfish \(*Channa punctatus* Bloch\) is a biomarker of paper mill effluent exposure](#)

Biochim. Biophys. Acta (2000)

Begum G.

[Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* \(Linn\) and recovery response](#)

Aquat. Toxicol. (2004)

Begum G. *et al.*

[Effect of acute exposure of the organophosphate insecticide rogor on some biochemical aspects of *Clarias batrachus* \(Linnaeus\)](#)

Environ. Res. (1999)

Bradford M.M.

A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding

Anl. Biochem. (1976)

ChuikoG.M.

Comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several freshwater fish: specific activities and in vitro inhibition by DDVP, an organophosphorus pesticide

Comp. Biochem. Physiol. C (2000)

CrestaniM. *et al.*

Effects of clomazone hebicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*

Ecotoxicol. Environ. Saf. (2006)

CrestaniM. *et al.*

Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern

Chemosphere (2007)

DavidM. *et al.*

Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles

Chemosphere (2004)

DuttaH.M. *et al.*

Effects of endosulfan on brain acetylcholinesterase activity in juvenile bluegill sunfish

Environ. Res. (2003)

EllmanG.L. *et al.*

A new and rapid colorimetric determination of acetylcholinesterase activity

Biochem. Pharmacol. (1961)



View more references

Cited by (226)

Potential toxic effects of glyphosate and its commercial formulations below regulatory limits

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Environmentally induced oxidative stress in aquatic animals

2011, Aquatic Toxicology

[Show abstract](#) 

Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity

2010, Chemosphere

[Show abstract](#) 

Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*

2010, Chemosphere

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Contaminant-induced oxidative stress in fish: a mechanistic approach

2016, Fish Physiology and Biochemistry

Impact of glyphosate and glyphosate-based herbicides on the freshwater environment

2014, Journal of Applied Toxicology



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Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems

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1. Introduction

Public awareness of worldwide increase herbicides use and their adverse effects on ecosystems has been growing over the past decades. Herbicides may reach water bodies via agricultural runoff and leaching processes, as well as by direct applications to control noxious aquatic weeds. Once in the aquatic ecosystems, herbicides may reduce environmental quality and influence essential ecosystem functioning by reducing species diversity and community structures, modifying food chains, changing patterns of energy flow and nutrient cycling and changing the stability and resilience of ecosystems. The aim of this chapter is to provide a general notion of the current knowledge concerning the direct and indirect effects of glyphosate and commercial formulations of glyphosate on aquatic ecosystems. Glyphosate based products are the leading post-emergent, systemic and non-selective herbicides for the control of annual and perennial weeds in the world. Here, we present a revision of their toxicity to non-target species of algae, aquatic plants, protozoa, crustaceans, molluscs, fish and amphibians. In addition, we describe the importance of each group of organisms in the functioning and health of aquatic ecosystems. With this information, a conceptual framework can be developed contributing to enhance our attention and concern about human impacts on ecosystems.

2. The scenario where glyphosate appeared on stage

The transition from biologically based to intensive-chemical based agricultural production systems advanced in North America and Europe soon after World War II. This change was supported by growing availability of inorganic fertilizers and organically synthesized pesticides. Afterwards, this type of agriculture has been adopted by other major crop production areas throughout the world during the 1960s and 1970s. The intensive cropping systems are characterized as large-scale production enterprises that utilize high inputs of chemical fertilizers and pesticides. Little emphasis is given to managing soil organic matter through use of traditional crop rotations, cover crops, or organic soil amendments that are central to maintaining the biological activity and allowing the long-term preservation of

agroecological systems in biologically based cropping systems (Yamada et al., 2009). One of the most significant inputs necessary for successful intensive crop production are herbicides for management of the variety of weed infestations especially encountered in row cropping systems. This technology was rapidly adopted because most weeds could be controlled when matched with selective herbicides, which were compatible with the crop, and was considered more cost-effective than cultural methods of weed management. In this scenario the herbicide glyphosate appeared on stage.

Glyphosate under the trade name Roundup® was introduced in the market by Monsanto Company during the 1970s. It was initially registered as a broad-spectrum, non selective, systemic herbicide for certain non-crop and plantation crop uses (fallow fields, orchards, vineyards and timber plantations) and for the control of annual and perennial weeds before the emergence of agronomic crops (Folmar et al., 1979; Woodburn, 2000). The development of minimum and no-tillage cultivation systems (zero-till) for row-cropping systems greatly expanded the use of herbicides, such as glyphosate, as it became standard practice to apply herbicides to growing weeds in fields prior to planting. This “burndown” application eliminated the need for traditional tillage (such as plow tillage) and allowed farmers to plant crop seeds directly into soil beneath a mulch of dead plant residues. The no-till practice was rapidly adopted around the world and really booming in some countries of South America like Brazil, Argentina, Paraguay and Uruguay (Altieri & Pengue, 2006). The reasons for the rapid growth of this practice are manifold, but the most important aspects are mainly economical (less work for field preparation, few expenses on fuel and machinery and higher profits). Important ecological aspects have been also pointed out. Non-till practice improves soil quality avoiding organic matter lost (Bayer et al., 2006) and water evaporation, despite of an increment in the use of herbicides. In addition, this cultivation system protects soil from erosion. For example in the southern of Brazil, no-till practice was adopted to reduce extensive soil erosion resulting from intensive row-cropping (Bolliger et al., 2006).

However, glyphosate became the most widely used herbicide worldwide with the introduction of genetically modified (GM) glyphosate-resistant (GR) crops (Woodburn, 2000). Monsanto’s glyphosate-tolerant Roundup Ready (RR) soybean was the first GR crops to be commercialized (Dill et al., 2008). In 1996, RR soybean was commercially available for the first time in the USA. These crops greatly improved conventional farmers' ability to control weeds, since glyphosate could be applied before seeding and sprayed several times during growth without damage the crop. Nowadays, glyphosate has established itself as the leading herbicide for the control of annual, perennial weeds and volunteer crops in a wide range of different situations (Woodburn, 2000). The arrival of GR soybean was followed by GR cotton, maize, canola, alfalfa and sugarbeet (Dill et al., 2008). These transgenic solutions (GR seeds + glyphosate) lead a sharp increase of worldwide areas under GR crops with concomitant increase of glyphosate use. The worldwide GR hectares planted during 1998 to 2008, increased from about 15 millions to more than 130 millions (Dill et al., 2008; James, 2008). Under these circumstances, only in USA, glyphosate usage increased from 3 10⁶ kg of a.i. (active ingredient) in 1987 to more than 54 10⁶ kg of a.i. in 2007 (Fig. 1). The two other countries with large areas under GR crops are Argentina and Brazil, however data set concerning glyphosate use in these countries are scarce.

The reported substantial increase in the global use of glyphosate has been also related with other items like herbicide price cuts and aggressive marketing, as well as the increased reliance on herbicides for weed control (Pengue, 2005). The latter issue is represented in the occurrence of weed population shifts toward less sensitive species and the evolution of

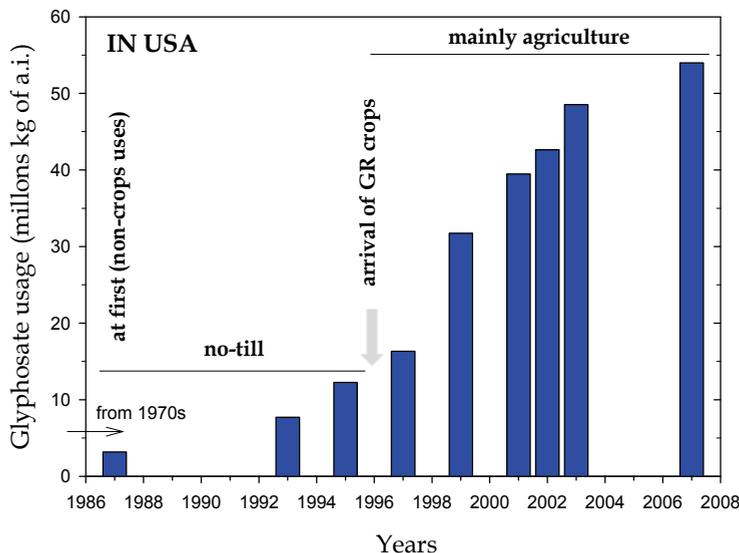


Fig. 1. Evolution of glyphosate usage in USA. Sources: USEPA, USDA.

herbicide-resistant weed populations. Glyphosate has been used worldwide since 1974 and, despite its widespread and long-term use, no case of evolved resistance to glyphosate under field conditions had been identified by 1993 (Holt et al., 1993). However, in 1996 the first case of weed resistance to glyphosate was documented in two accessions of the rigid ryegrass *Lolium rigidum*, from an orchard in Australia (Powles et al., 1998; Pratley et al., 1999). Since then, an increasing number of cases of glyphosate resistant biotypes have been reported. Currently, 14 GR weeds have been documented worldwide (Van Gessel, 2001; Pérez & Kogan, 2002; Powles, 2008; Binimelis et al., 2009; among others). Consequently, the average of glyphosate application per Ha showed a marked global increase associated with the appearance of a growing number of tolerant or resistant weeds. Bonny (2008) pointed out that the amount of glyphosate spread over the total US soybean land raised from less than 0.1 Kg/Ha in 1990 to more than 1.4 kg/Ha in 2006. Higher application rates (up to 5.6 a.i. kg/Ha) have been reported by Giesy et al. (2000). Regarding the use of other herbicides, at first the rapid growth in the use of glyphosate was accompanied by a decrease in the consumption of other former herbicides. However, for example in Argentina the consumption of the herbicides atrazine and 2,4-D, have risen again during the growing seasons of (2005-2006) (Binimelis et al., 2009). These observations coincide with Bonny (2008) who concluded that the total amount of herbicides applied per Ha in USA decreased initially between 1996 and 2001, but tended to rise afterwards.

3. Glyphosate (the molecule)

3.1 Chemistry

The chemical (technical-grade) name of glyphosate is N-(phosphonomethyl) glycine (IUPAC), an acid that belongs to chemical group of Phosphonoglycine or more generic: Organophosphonate herbicides (Fig. 2). Its main degradation product is the metabolite aminomethyl phosphonic acid (AMPA).

Glyphosate is an aminophosphonic analogue of the natural amino acid glycine and the name is a contraction of *glycine*, *phos-*, and *-ate*. The molecule has several dissociable hydrogens, especially the first hydrogen of the phosphate group. Technical-grade glyphosate has relatively low solubility in water (1.2 % at 25 ° C), and is insoluble in other solvents. Strong intermolecular hydrogen bonds stabilize the crystal lattice, causing the low water solubility. Various salts of glyphosate have much higher solubility, and do not lose any of the herbicidal properties of the parent compound (Franz, 1985). Glyphosate is commonly formulated in its form of isopropylamine salt (IPA salt), though other related chemical form are also commercialized. Glyphosate concentration is commonly expressed as mg a.i./L or mg a.e./L, where: a.e. (acid equivalents). Glyphosate is an unusual herbicide, in that essentially no structurally related compounds show any herbicidal activity (Hollander & Amrhein, 1980; Franz, 1985), with the exception of glyphosine, which has reduced herbicidal effects but shows some interesting plant growth regulatory effects, such as enhancing ripening of sugar cane (Franz, 1985). The herbicidal properties of glyphosate were reported in 1971 (Baird et al., 1971). The compound was found during a study of the herbicidal effects of tertiary aminomethylphosphonic acids derived from various primary and secondary amines (Moedritzer & Irani, 1966). Only two of the compounds produced showed some herbicidal activity, but both had very low unit activities. Attempts to find other tertiary aminomethylphosphonic acids with improved herbicidal activity failed. As a last resort, it was suggested that degradation of the two compounds might give rise to a common, active metabolite (contrary to the general trend that metabolism reduces toxicity). Glyphosate was among the possible metabolites of the two compounds, and was found to have extremely high herbicidal activity (Franz, 1985).

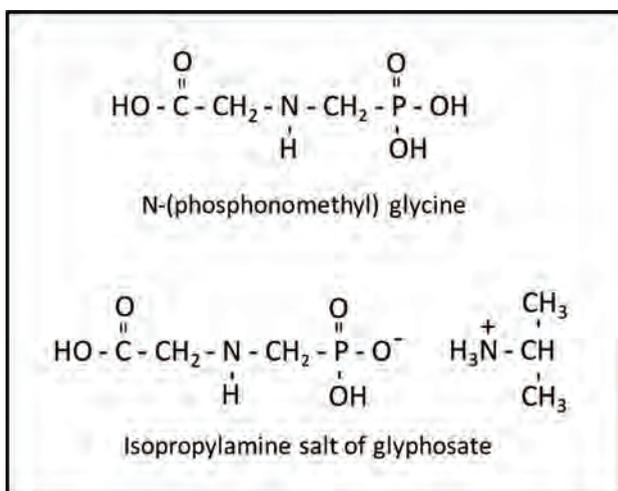


Fig. 2. Glyphosate molecule (as an acid and IPA salt).

3.2 Mode of action and biochemistry

Glyphosate is a systemic herbicide that is phloem mobile and is readily translocated throughout the plant. From the leaf surface, glyphosate molecules are absorbed into the plant cells where they are symplastically translocated to the meristems of growing plants

(Laerke, 1995). Unlike many contact herbicides, phytotoxic symptoms of glyphosate injury often develop slowly. Visible effects on most annual weeds occur within two to four days and may not occur for 7 days or more on most perennial weeds. Visual symptoms are a gradual wilting and yellowing of the plant which advances to complete browning and finally with the total deterioration and death of the plant.

Although glyphosate may ultimately perturb a variety of biochemical processes including protein synthesis, nucleic acid synthesis, photosynthesis and respiration, the primary mode of action of glyphosate was localized to the shikimate pathway of aromatic amino acid biosynthesis, a pathway that links primary and secondary metabolism. Its mode of action is the competitive inhibition of the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, a chloroplast-localized enzyme in the shikimate pathway (Steinrücken & Amrhein, 1980; Bode et al., 1984a; Rubin et al., 1984). This inhibition prevents the production of chorismate, which is the last common precursor in the biosynthesis of numerous aromatic compounds in bacteria, fungi and plants. Essential aromatic amino acids are used by plants in protein synthesis and to produce many secondary plant products (e.g. growth promoters, growth inhibitors, lignin precursors, flavonoids, tannins, and other phenolic compounds). The major end products are the amino acids phenylalanine, tyrosine, and tryptophan.

Evidence for the involvement of this pathway in glyphosate toxicity has come from a variety of studies in a wide range of microorganisms, plant cell cultures and plants (e.g. Jaworsky, 1972; Haderlie et al., 1977; Gresshoff, 1979; Duke et al., 1980; Hollander & Amrhein, 1980; among others). Glyphosate caused a massive accumulation of shikimate in treated cells and tissues (Amrhein et al., 1980; Berlin & Witte, 1981; Bode et al., 1984; Rubin et al., 1984). These studies narrowed the possible site of action to three enzymes, involved in the conversion of shikimate to chorismate. The enzyme that was finally implicated was EPSP synthase; its inhibition by glyphosate is competitive with phosphoenolpyruvate (PEP), and non-competitive with respect to shikimate-3-phosphate with a single glyphosate binding site on the enzyme.

3.3 Commercial formulations of glyphosate

When the toxicity of herbicides is discussed, the focus is mostly on the active compound (in this case glyphosate acid or glyphosate salts). However, herbicides are formulated to increase their efficacy against target plants. Commercial glyphosate based herbicides contain other components, which are called inert ingredients. These inert ingredients are mainly surfactants, solvents and antifoam compounds. Shortly, surfactant refers to chemicals that have pronounced surface activity in aqueous solutions that can decrease surface tension and perturb membrane permeability or transport function of membranes including permeability to glyphosate (Riechers et al., 1994). Antifoam compounds are chemical additives that reduce and hinder the formation of foam in industrial process liquids. Numerous contributions have demonstrated that inert ingredients in glyphosate formulations have several folds higher toxicity on non-target organisms than glyphosate alone (e.g. Folmar et al. 1979; Wan et al. 1989; Cedergreen & Streibig, 2005; among others). Therefore, glyphosate formulations are chemical mixtures and must be considered as mixtures in toxicity assessments. In this context, studies regarding specific toxicity or generalization about toxicology of inert ingredients (e.g. surfactants) must be conducted on glyphosate, inert ingredient and commercial formulation separately. The lack of such data will render any predictions about the effects of the formulations on glyphosate highly uncertain (Diamond & Durkin, 1997).

Glyphosate concentration, as well as nature and concentration of inert additives, depend on commercial formulations; though available information about inert ingredients in glyphosate products is commonly not listed on the label. The commercial formulation of glyphosate, Roundup®, is a most popular brand name for glyphosate herbicides. Roundup® contains IPA salt of glyphosate (35-50 %) plus inert ingredients. Roundup® concentration is commonly expressed as mg a.i./L or mg a.e./L of glyphosate, while also as mg of Roundup® (whole product) per L. We considered 1 mg a.i./L equal to 0.75 mg a.e./L (Relyea, 2006).

The surfactant in Roundup®, as well as in some other glyphosate based products, is the highly toxic polyethoxylated tallowamine compound (Smith & Oehme, 1992; Giesy et al., 2000). This material is referred to in the literature as MON0818, or polyoxyethyleneamine (POEA), present at about 15% in Roundup®. Presumably POEA is a derivative of tallow, a complex mixture of fat from the fatty tissue of cattle or sheep. Other trade names of glyphosate based herbicides include Aquamaster®, Filedmaster®, Touchdown®, Glyphos®, Duramax®, Durango®, Glyphomax®, Fosato®, Ron-do®, Vision®, Rodeo®, Sulfosato®, etc.

4. Glyphosate in aquatic environments

4.1 Offsite movement and direct applications

The use of herbicides and other chemical agents in agriculture may result in accidental introduction into waters. When it is applied as post emergence spray, herbicides may enter aquatic systems through accidental offsite movement in herbicide spray drift, or through transport in leaching and surface run-off.

Particularly, under field conditions, glyphosate is usually assumed to be rapidly and tightly adsorbed to soil. Consequently, glyphosate is unlikely to leach into ground waters or runoff significantly into surface waters following application. In several laboratories (Rueppel et al., 1977; Crisanto et al., 1994; among others) and some field studies (Roy et al., 1989), the immobility of glyphosate in soils has been demonstrated. In contrast, other field studies showed detectable concentrations of glyphosate in flume and streams after applications. Even though it was only restricted to a relatively brief window of time (about 1 day post application), due to the fast dissipation kinetics of glyphosate in the field. In natural waters, glyphosate dissipate rapidly (half lives < 4 days) being removed from water due to adsorption to suspended particulates followed by subsequent sedimentation and or biodegradation. However, longer half lives were reported in hard waters, where glyphosate residues could be measured after 11 days post application (Pérez et al., 2007).

Edwards et al. (1980) reported important glyphosate concentrations in runoff from natural rainfall following early springtime treatments in no-tillage agriculture soils. The highest concentration of glyphosate in runoff waters (5.2 mg/L) was found in runoff occurring 1 day after treatment at the highest rate (8.6 Kg/Ha of Roundup®) (Edwards et al., 1980). The maximum amount of glyphosate transport by runoff was 1.85% of the amount applied, most of which occurred during a single storm on the day after application. In addition, Feng et al. (1990) found in a treated watershed, a dramatic increase of glyphosate concentrations (about 1.1 and 1.5 mg/L) in two oversprayed streams in response to first rainfall event 27 h post application. Authors attributed their observations to several source of input mobilization of residues in ephemeral stream channels feeding the tributary; wash off of unabsorbed residues from overhanging vegetation, surface runoff and subsurface flow. Regarding POEA residues, based on adsorption and degradation data, leaching and runoff potential is

expected to be small. POEA strongly adsorb to soil (Giesy et al., 2000), although little information about POEA offsite movement is nowadays available.

Offsite movement of glyphosate is also possible through spray drift (e.g. Payne et al., 1990; Payne, 1992). Although the spray drift of pesticides is not compound specific, this is relevant when non-target effects of glyphosate based herbicides are considered, and several studies have specifically addressed the issue. Some studies reported that the spray deposition decreased to around 10 % of the application rate in the first 30 m and less to 5 % at a distance of 200 m (Payne et al., 1990; Riley et al., 1991). Other studies suggested that drift rates would be greater. For instance, residues have been measured 400 m downwind from applications sites (Yates et al., 1978; Payne & Thompson, 1992).

Considering offsite movement of glyphosate from treated soils through drift and run-off, Giesy et al. (2000) estimated an acute scenario considering worst-case exposure conditions. The estimate was based on two assumptions, (a) that runoff (2%) from 10 Ha field treated at the maximum single use rate of Roundup® entered to 1 Ha pond (2 m deep) and (b) that 10% of maximum single application rate per hectare entered the pond through drift, assuming aerial application. Based on these assumptions, maximum concentrations of Roundup® in natural water would range from 0.27 to 0.41 mg/L (Giesy et al., 2000). However, clearly higher concentrations in surface waters could be expected if assumptions are changed. For instance, some authors have reported that glyphosate can be readily desorbed from soil and has the potential to be extensively mobile in the soil environment. Adsorption of glyphosate to soil particulates is determined by chemical and physical characteristic of soils, which in turn affect the potential for off-target movement of the herbicide through water runoff or subsurface flow. Interestingly, given that glyphosate is bound to soil through its phosphonic acid moiety, the addition of inorganic phosphorus could potentially release glyphosate from soil particles through competition for sorption sites (Franz et al. 1997; Pechlaner, 2002). Piccolo et al. (1994) reported in an experimental study with some European soils that desorption varied from around 15 to 80% of the absorbed herbicide according to the soil characteristic. These observations, as well as supposing higher rates of terrestrial uses and higher spray drift due to weather conditions, could elicit elevated off-target movements of glyphosate formulations in to water ecosystems. Particularly, these impacts will be more important in ponds, ephemeral streams and ditchbank areas of irrigation canals due to their low water volume, and higher perimeter and area /volume proportions.

On the other hand, some glyphosate based herbicides (e.g. Rodeo® and AquaMaster®) were specially formulated to be used as aquatic herbicides, and have been employed extensively to control noxious aquatic weeds and algal blooms (Seddon, 1981; Diamond & Durkin, 1997; Siemering et al., 2008). For this purpose, glyphosate based herbicides are directly applied in aquatic ecosystems and their residues can be expected to be higher than that resulting from agricultural and other non aquatic uses. Furthermore, glyphosate can move considerable distances in canal or stream waters affecting undesired places (Duke, 1988). Fifty-eight percent of applied glyphosate was detected at distances 8 and 14.4 Km downstream from sites of introduction (Comes et al., 1976). Regardless herbicide sources, it is very important to set up the amount of glyphosate that have been measured in the field. Unluckily, there are few relevant field data on the concentration of glyphosate in aquatic habitats. The highest concentrations that have been observed in nature were: 1.24 mg a.e./L (Newton et al., 1994); 1.54 mg a.e./L (Couture et al., 1995); 2.8 mg a.e./L (Legris & Couture, 1989) and 5.2 mg a.e. /L (Edwards et al., 1980).

4.2 Toxicity of glyphosate based herbicides in aquatic environments

4.2.1 Toxicity assessment

In this chapter, we extensively reviewed published contributions about glyphosate, glyphosate formulations and surfactants effects on non-target aquatic organisms. Different parameters (lethal and sublethal effects) were evaluated in reviewed studies to characterize the hazard of chemicals (e.g. mortality, growth, biomass, ^{14}C uptake, weight, density, length, pigments, mobility, reproduction, metabolism, etc). Results were expressed as LC (concentration lethal to 10% and 50% of test organisms), EC (effective concentration causing specified effects in 10% and 50% of test organisms) and IC (inhibition concentration to specified effects in 10% and 50% of test organisms). In addition, when available, values of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) were pointed out.

When dose-response curves were not available or were not calculated due to experimental design (e.g. field studies carried out in micro- and mesocosms), contributions were described as concentrations of treatments and obtained outcomes (generally in % of control values). A complete resume of published outcomes in acute and chronic tests and field experiments were listed in tables (from Table 1 to Table 6). Concentrations were preferentially expressed as were originally reported in each reviewed contribution.

In order to relate the aquatic toxicity of the herbicide to realistic exposure levels, the expected environmental concentration (EEC) was taken as a reference value. We considered a EEC of 2.6 mg a.i./L, (following Relyea, 2005). Similar values were estimated by other authors: 1.87 mg a.i./L (Chen et al., 2004) and 3.73 mg a.i./L (Perkins et al., 2000); though higher values were also evaluated (e.g. 10.13 mg a.i./L; Mann and Bidwell, 1999).

4.2.2 Effects on non-target aquatic plants and algae

Herbicides are mainly designed to kill unwanted terrestrial plants. Consequently the most sensitive group of aquatic non-target organisms is expected to be aquatic plants and algae. Aquatic plants and algae play a pivotal role for the function of aquatic ecosystems (Scheffer et al., 1993). Aquatic plants aid in stabilizing the sediment both in lakes and running waters, and their presence affects sedimentation rates, flow velocity, nutrient uptake and recirculation. In addition, they provide refuges for insects, crustaceans and fish, and act as substrates for surface-living microorganisms, snails and other epiphyte grazers. Microalgae (phytoplankton and periphyton communities) provide the basis for a range of food-webs in the aquatic environment and are therefore fundamental to the functioning of aquatic ecosystems (Wetzel, 2001).

Single species test in algae and aquatic plants treated with glyphosate alone (i.e. technical grade acid or salts of glyphosate), showed a wide range of EC and IC values; indicating different sensibilities. Microalgae presented EC_{50} values for glyphosate treatments ranging from 0.68 mg a.e./L in the diatom *Skeletonema costatum* (Malcolm Pirnie, 1987) to around 600 mg a.e./L in the green algae *Chlorella pyrenoidosa* (Maule & Wright, 1984) (Table 1). It is important to clarify that these values indicate the concentration that elicited the 50 % of reduction in the evaluated parameter. Some works showed that 10 % of reduction (EC_{10}) could be reached between 3 to 16 folds lower concentrations than EC_{50} . For instance, 10% growth inhibition in the green algae *Scenedesmus subpicatus* was observed in treatments with 1.6 mg/L of glyphosate acid (Vedrell et al., 2009). In addition, Christy et al. (1981) reported a 10% growth inhibition in *Chlorella sorokiniana* at the concentration of 2 mg a.e./L. Regarding macrophytes, generally lower values of EC and IC were reported, indicating a higher

sensibility. For example, IC_{50} and EC_{50} values ranged from 0.22 mg a.i./L for *Myriophyllum aquaticum* (Turgut & Fomin, 2002) to 46.9 mg/L for *Lemma minor* (Cedergreen & Streibig, 2005) (Table 1).

The relative toxicity of glyphosate itself vs. commercial formulations and surfactants only can be evaluated in studies specially designed to this purpose. In general, commercial formulations (e.g. Ron-do® and Roundup®) were more toxic than glyphosate alone. For example, Tsui & Chu (2003) observed a 7 folds higher toxicity of Roundup® than the IPA salt of glyphosate in the green algae *Selenastrum capricornutum* (Table 1). Alike results were reported for *Selenastrum capricornutum* and the macrophyte *Lemma minor*, showing 4 folds higher toxicity of Roundup® than glyphosate (Cedergreen & Streibig, 2005). Lower differences in toxicity were registered by other authors (e.g. Sáenz et al., 1997; Sobrero et al., 2007), reporting between 1.2 to 1.8 folds higher toxicity of commercial formulations than active ingredient (Table 1). POEA itself contributed to Roundup® toxicity with values ranged from about 45% for *Skeletonema costatum* to 85% for *Selenastrum capricornutum* (Tsui & Chu, 2003).

Numerous studies have been published about pesticide toxicity assessment on microalgae, using single species tests. However, Bérard et al. (1999) demonstrated that single-species tests may fail to predict indirect or system responses to toxicants, such as changes in population competition or succession. According to these authors, studies focusing on the whole natural community provide more reliable predictions about herbicide safety in aquatic environments. In studies assessing communities, significant direct and indirect effects of commercial glyphosate formulations have been reported. For example, Schaffer & Sebetich (2004) reported an increment of 161% in net primary production for phytoplankton community treated with 0.13 mg a.i./L of Rodeo® (commercial formulation without POEA). In contrast, Goldsborough & Brown (1988), registered a 50% of reduction in periphyton primary production at values varying from 35.4 to 69.7 mg a.i./L of Roundup® (Table 1). However, in this contribution, 4 of the 6 treated ponds showed a reduction in the mean values of primary production with much lower concentrations (a dosage of 0.89 mg a.i./L). In microcosms experiments with natural marine microbial community, significant effects in species number and relative abundance of phytoplankton were observed at 10 µg a.i./L of Roundup® (Stachowski-Haberkorn et al., 2008). Comparable results were obtained by Pesce et al. (2009), reporting changes in riverine algal communities exposed to about 10 µg/L of glyphosate alone, in a microcosms experiment. In addition, mesocosms studies showed remarkable results with a single pulse application of Roundup® at concentrations of 6 and 8 mg a.i./L (Pérez et al., 2007; Vera et al., 2010). Even if these two contributions assessed herbicide effects in worst case scenarios, glyphosate concentration at the end of the experiments (11 and 14 days respectively), were around 2 mg a.i./L and effects were still clearly observed. At day 11, significant differences were observed in chemical and biological variables (Pérez et al., 2007). For example, we observed changes in phytoplankton assemblage fractions, with a reduction of micro and nanophytoplankton densities (2.5 folds) and a concomitant increase of picocyanobacteria (PICY) densities (40 folds) accompanied by an increase of primary production. These results can be expected by either direct effect of herbicide (differences in sensibility among species) or indirect effects duo to interspecific competition. In addition, Vera et al. (2010) found that Roundup® produced a clear delay in periphytic colonization in treated mesocosms. The periphytic mass variables: dry weight (DW), ash-free dry weight (AFDW) and chlorophyll a, were always higher in control mesocosms. Despite the mortality of algae, (mainly diatoms), cyanobacteria was favoured in treated mesocosms. We also observed that Roundup® produced a long term shift in the typology of mesocosms, "clear" turning to "turbid" state due to an eutrophication process.

AQUATIC ALGAE & PLANTS	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
- Phytoplankton and Periphyton					
<i>Chlorococcumhynnosporum</i>	Gly. (acid)	SST (7 d.)	Growth	96h EC ₅₀ = 68.0	Maule & Wright, 1984
<i>Chlorella pyrenoidosa</i>				96h EC ₅₀ = 590.0	
<i>Skeletonema costatum</i>	Gly. (acid)	SST (7 d.)	Biomass	EC ₅₀ = 0.64 ; NOEC = 0.28	Malcolm Pirnie, 1987
(Periphyton community)	Roundup®	LTNC (4 h.)	NPP	4h EC ₅₀ = (between 35.4 to 69.7)	Goldsborough & Brown, 1988
<i>Scenedesmus acutus</i>	Gly. (IPA salt)	SST (4d.)	Density	96h EC ₅₀ = 10.2 ; NOEC = 2.0	Sáenz et al., 1997
	Ron-do®			96h EC ₅₀ = 9 ; NOEC = 3.2	
<i>Ankistrodesmus sp</i>	Rodeo®	SST (10 d.)	Density	96h EC ₅₀ = 74.0	Gardner et al., 1997
(Phytoplankton community)	Rodeo®	LTNC (6 h.)	NPP	0.13 mg/L elicited NPP increment	Schaffer & Sebetich, 2004
<i>Selenastrum capricornutum</i>	Gly. (IPA salt)	SST (4 d.)	Growth	96h EC ₅₀ = 41.0	Tsui & Chu, 2003
	Roundup®			96h EC ₅₀ = 5.81	
	POEA			96h EC ₅₀ = 3.91	
<i>Skeletonema costatum</i>	Gly. (IPA salt)	SST (4 d.)	Growth	96h EC ₅₀ = 5.89	Tsui & Chu, 2003
	Roundup®			96h EC ₅₀ = 1.85	
	POEA			96h EC ₅₀ = 3.35	
<i>Selenastrum capricornutum</i>	Gly. (n.c.)	SST (2 d.)	Growth	48h EC ₁₀ = 95.5 ; 48h EC ₅₀ = 270.0	Cedergreen & Streibig, 2005
	Roundup®			48h EC ₁₀ = 13.6 ; 48h EC ₅₀ = 64.7	
(Phytoplankton & periphyton community)	Roundup®	MES (11 d.)	Density #	6 mg/L elicited changes in community structure	Pérez et al., 2007
(Microbial community)	Roundup®	MIS (7 d.)	Community structure #	10 µg/L elicited a reduction in species number.	Stachowski-Haberhorn et al., 2008
(Microbial community)	Gly. (n.c.)	MIS (14 d.)	Community structure #	~ 10 µg/L elicited changes in algal community structure.	Pesce et al., 2009
<i>Chlorella saccharophila</i>	Gly. (acid)	SST (3 d.)	Growth	72h EC ₁₀ = 3.0 ; 72h EC ₅₀ = 46.6	Vedrell et al., 2009
<i>Scenedesmus subspicatus</i>				72h EC ₁₀ = 1.6 ; 72h EC ₅₀ = 26.0	
(Periphyton community)	Roundup®	MES (42 d.)	Density #	8 mg/L elicited changes in community structure	Vera et al., 2010
- Macrophytes					
<i>Myriophyllum sibiricum</i>	Gly. (acid)	SST (14d.)	Root length #	14d IC ₁₀ = 0.59 ; 14d IC ₅₀ = 0.84	Roshon et al., 1999
	Roundup®			14d IC ₅₀ = 1.22	
<i>Myriophyllum aquaticum</i>	Gly. (n.c.)	SST (14d.)	Growth & chl <i>a</i> #	14d EC ₅₀ = 0.22 (for growth)	Turgut & Fomin, 2002
				14d EC ₅₀ = 0.22 (for chl <i>a</i>)	
<i>Lemma minor</i>	Gly. (n.c.)	SST (7 d.)	Growth	7d EC ₁₀ = 3.8 ; 7d EC ₅₀ = 46.9	Cedergreen & Streibig, 2005
	Roundup®			7d EC ₁₀ = 3.5 ; 7d EC ₅₀ = 11.2	
<i>Myriophyllum spicatum</i>	Roundup®	SST (21 d.)	Weight & length #	21d IC ₅₀ = 1.0 (for weight) 21d IC ₅₀ = 2.8 (for length)	Sánchez et al., 2007
<i>Lemma gibba</i>	Gly. (acid)	SST (10 d.)	Growth #	10d IC ₁₀ = 4.6 ; 10d IC ₅₀ = 20.5	Sobrero et al., 2007
	Roundup®			10d IC ₁₀ = 2.5 ; 10d IC ₅₀ = 11.6	

Abbreviations and acronyms: gly. (glyphosate); n.c. (not clarified); n.a (not available); d. (days); SST (single species laboratory tests); LTNC (laboratory tests with natural communities), MES (mesocosms studies); (MIS) microcosms studies; SSFE (single species field experiments); chl *a* (chlorophyll *a*); NPP (net primary production). Notes: (##) Effects concentrations were expressed as mg/L of formulation, mg a.i./L or mg a.e./L, see bibliographic references to clarify. (#) Several parameters were assessed in these contributions; remarkable examples of the reported outcomes were listed in tables.

Table 1. Effects of glyphosate, different commercial formulations of glyphosate and POEA on algae and aquatic plants

4.2.3 Effects on non-target aquatic bacteria and protozoa

The majority of the available pesticide data regarding aquatic microorganisms is for algae. Far fewer pesticide studies exist for aquatic bacteria and protozoa. Aquatic bacteria and protozoa (e.g. amoeboids, flagellates, ciliates and sporozoans) have key roles in the functioning of aquatics environments. Shortly aquatic bacteria occupy an important position

in the aquatic food web since they are major actors in the decomposition of dead material, and thereby in the recycling of nutrients and carbon. They are extremely important in “Lake metabolism”, being involved in mineralization processes and in the chemical transformation of elements between reduced and oxidized forms. Protozoans are ecologically important as key links in food chains. Ubiquitous in aquatic environments, protozoans prey upon algae, bacteria, and other organisms and are themselves consumed by animals such as microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is very important (in the traditional food web and in the microbial loop). On the other hand, some protozoa are important as parasites and symbionts of multicellular animals.

Concentration effects of glyphosate itself on bacteria and protozoa varied widely and seem to indicate low sensibility (Table 2). For instance, EC₅₀ values obtained in treatments with glyphosate ranging from 18.2 mg/L for the bacteria *Vibrio fischeri* (Bonnet et al., 2007) to 386.0 mg a.e./L for the ciliate *Tetrahymena pyriformis* (Tsui & Chu, 2003) (Table 2). However, lower concentrations have been reported to produce observable effects (Everett & Dickerson, 2003). These authors registered a LOEC value of 5 mg/L for the parasite ciliate *Ichthyophthirius multifiliis* treated with glyphosate acid.

Roundup® showed higher toxicity than glyphosate for bacteria and protozoa in the revised bibliography. Tsui & Chu (2003) reported 6 folds higher sensibility of *Vibrio fischeri* to

MICROORGANISMS	ASSESSED CHEMICAL	TYPE OF STUDY	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCE
-Bacteria and Protozoa					
<i>Ichthyophthirius multifiliis</i>	Gly. (acid)	SST (5 h)	Mortality	NOEC = 2.5 , LOEC = 5.1	Everett & Dickerson, 2003
	Roundup®			NOEC = n.a., LOEC = 0.07	
<i>Tetrahymena thermophila</i>	Gly. (acid)	SST (24 h)	Mortality	NOEC = 10.1 , LOEC = n.a.	Everett & Dickerson, 2003
	Roundup®			NOEC = n. a., LOEC = 0.31	
<i>Brachionus calyciflorus</i>	Gly. (n.c.)	SST (24 h)	Growth #	24h EC ₅₀ = 28.0	Xi & Feng, 2004
	Gly. (IPA salt)			5min EC ₅₀ = 162.0	
<i>Vibrio fischeri</i>	Roundup®	SST (5 min.)	Growth	5min EC ₅₀ = 24.9	Tsui & Chu, 2003
	POEA			5min EC ₅₀ = 10.2	
	Gly. (IPA salt)			40h EC ₅₀ = 386.0	
<i>Tetrahymena pyriformis</i>	Roundup®	SST (40 h.)	Growth	40h EC ₅₀ = 29.5	Tsui & Chu, 2003
	POEA			40h EC ₅₀ = 4.96	
	Gly. (IPA salt)			48h EC ₅₀ = 64.1	
<i>Euplotes vannus</i>	Roundup®	SST (48 h.)	Growth	48h EC ₅₀ = 23.5	Tsui & Chu, 2003
	POEA			48h EC ₅₀ = 5.0	
	Gly. (IPA salt)			48h EC ₅₀ = 64.1	
<i>Euglena gracilis</i> *	Roundup®	SST (7 d.)	Velocity #	NOEC = 0.05 , LOEC = 0.1	Pettersson & Ekelund, 2006
	Avans®			NOEC = 0.05 , LOEC = 0.1	
<i>Vibrio fischeri</i>	Gly. (acid)	SST (15 min.)	Bioluminescence	15min EC ₅₀ = 18.2	Bonnet et al., 2007
	AMPA			15min EC ₅₀ = 53.4	
<i>Tetrahymena pyriformis</i>	Gly. (acid)	SST (45 min.)	Enzyme activities #	45min EC ₅₀ = 87.9	Bonnet et al., 2007
	AMPA			45min EC ₅₀ = 166.5	

Abbreviations and acronyms: see Table 1. Notes: (*) *Euglena gracilis* is a mixotrophic green flagellated; although in this resume was grouped with protozoa; see Table 1 for other notes.

Table 2. Effects of glyphosate, different commercial formulations of glyphosate, AMPA and POEA on bacteria and protozoa

Roundup® than to glyphosate acid. In addition, these authors observed 2.7 and 13 folds higher sensibility of the ciliates *Euplotes vannus* and *Tetrahymena pyriformis* to Roundup®, respectively. In addition, the ciliate parasite *Ichthyophthirius multifiliis* showed an accentuated response, being several times more sensible to Roundup® (Everett & Dickerson, 2003) (Table 3). Values of EC₅₀ obtained in Roundup® treatments ranged from 23.5 to 29.5 mg a.e./L (Tsui & Chu, 2003); though lower values produced observable effects. For example, Everett & Dickerson (2003) registered LOEC values of 0.07 and 0.31 mg a.e./L for two ciliates. Besides, values of 0.1 mg a.i./L of Roundup® and Avans® (other glyphosate commercial formulation) elicited reduction of at least 50% in the swimming velocity of *Euglena gracilis* (Pettersson & Ekelund, 2006).

AQUATIC INVERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
-Crustaceans (copepods, cladocerans and amphipods)					
<i>Daphnia magna</i>	Roundup®	SST (48h)	Immobilization	48h EC ₅₀ = 3.0	Folmar et al., 1979
<i>Gammarus pseudolimnaeus</i>	Roundup®	SST (48h)	Mortality	48h LC ₅₀ = 62.0	Folmar et al., 1979
<i>Daphnia pulex</i>	Roundup®	SST (96 h.)	Immobilization	96h EC ₅₀ = 8.5	Servizi et al., 1987
	POEA			96h EC ₅₀ = 2.0	
<i>Daphnia magna</i>	Ron-Do®	SST (48h)	Immobilization	48h EC ₅₀ = 61.7	Alberdi et al., 1996
<i>Daphnia spinulata</i>	Ron-Do®	SST (48h)	Immobilization	48h EC ₅₀ = 66.2	Alberdi et al., 1996
<i>Simocephalus vetulus</i>	Vision®	SST (8 d)	Survival and reproduction #	0.75 mg/L elicited survival and reproduction reduction	Chen et al., 2004
	Rodeo®			48h LC ₅₀ = 415.0	
<i>Ceriodaphnia dubia</i>	Roundup bioactive®	SST (48 h.)	Mortality#	48h LC ₅₀ = 81.5	Tsui & Chu, 2004
	Roundup®			48h LC ₅₀ = 5.7	
	Rodeo®			48h LC ₅₀ = 225.0	
<i>Hyalella azteca</i>	Roundup bioactive®	SST (48 h.)	Mortality#	48h LC ₅₀ = 120.0	Tsui & Chu, 2004
	Roundup®			48h LC ₅₀ = 1.5	
	Gly. (IPA salt)			48h LC ₅₀ = 415.0	
<i>Ceriodaphnia dubia</i>	Roundup®	SST (48 h.)	Mortality	48h LC ₅₀ = 5.4	Tsui & Chu, 2003
	POEA			48h LC ₅₀ = 1.2	
	Gly. (IPA salt)			48h LC ₅₀ = 49.3	
<i>Acartia tonsa</i>	Roundup®	SST (48 h.)	Mortality	48h LC ₅₀ = 1.77	Tsui & Chu, 2003
	POEA			48h LC ₅₀ = 0.57	
	POEA (5:1)			48h LC ₅₀ = 0.18	
<i>Daphnia magna</i>	POEA (10:1)	SST (48 h.)	Mortality	48h LC ₅₀ = 0.097	Brausch et al., 2007
	POEA (15:1)			48h LC ₅₀ = 0.85	
-Molluscs (snails and mussels)					
<i>Pseudosuccinea columella</i>	Gly. (n. c.)	SST (12 d.)	Growth and hatching #	1 mg/L elicited growth increment and 10 mg/L inhibited hatching.	Tate et al., 1997
<i>Pseudosuccinea columella</i>	Gly. (n. c.)	SST (12 d.)	Metabolism	0.1 mg/L elicited an increment in free amino acids.	Tate et al., 2000
<i>Utterbackia imbecillis</i>	Roundup®	SST (24 h.)	Mortality #	48h LC ₅₀ = 18.3	Connors & Black, 2004
-Others (insects and worms)					
<i>Chironomus plumosus</i>	Gly. (acid)	SST (48h)	Immobilization	48h EC ₅₀ = 55.0	Folmar et al., 1979
	Roundup®			48h EC ₅₀ = 18.0	
	POEA			48h EC ₅₀ = 13.0	
<i>Lumbriculus variegatus</i>	Gly. (acid)	SST (2-4 d.)	Bioaccumulation & enzyme action	0.05 mg/L elicited increase in sGST activity.	Contardo-Jara et al., 2009
	Roundup Ultra®			0.05 mg/L elicited an increase in sGST activity.	

Abbreviations, acronyms and notes: see Table 1

Table 3. Effects of glyphosate, different commercial formulations of glyphosate and POEA on aquatic invertebrates

Surfactant POEA itself resulted more toxic to bacteria and ciliates (between 2.4 to 5.8 folds) than Roundup® (Tsui & Chu, 2003); though the degradation product of glyphosate (AMPA) resulted less toxic than glyphosate acid (Bonnet et al., 2007).

4.2.4 Effects on non-target aquatic invertebrates

Invertebrates comprise a large group of aquatic species with a wide variety in shape and size, and evolved to utilize different habitats and resources (e.g., insects, worms, snails, hydroids, crustacean, etc.). They can be practically divided in micro and macro groups. Micro-invertebrates (zooplankton) are keystone species (e.g. as food for predators and top-down controller of phytoplankton, periphyton and detritus) (Montenegro Rayo, 2004). The micro-invertebrates (rotifers and copepods) are usually more abundant in number of individuals, but their smaller size limit their impact to size discrimination of phytoplankton rather than a reduction of total algal biomass (Scheffer, 1998). Large cladocerans (e.g., *Daphnia spp.*) can feed efficiently on a wide range of particle types and sizes. Moreover, cladocerans are also important as a major food source for many fish species and predatory invertebrates. Some crustacean species (e.g., shrimps, crabs and crayfish) are important as food resource for men. Insects are foraging on zooplankton, periphytic algae, and detritus and themselves prey for fish and waterfowl. In addition, aquatic molluscs (i.e. snails and mussels) have also a significant importance in aquatic environments and in human life (e.g. parasite vectors; invasive species; top-down controller of phytoplankton, periphyton and detritus; source of food to higher trophic levels, as well as human food resources) (Brönmark & Hansson, 2005).

Aquatic invertebrates seem to have low sensibility to glyphosate itself (Table 3). Values of LC₅₀ obtained in treatments with glyphosate ranged from 49.3 mg a.e./L for the marine copepod *Acartia tonsa* to 415 mg a.e./L for the cladoceran *Ceriodaphnia dubia* (Tsui & Chu, 2003). However, Tate et al. (1997 and 2000), reported remarkable results in the snail *Pseudosuccinea columella* (an intermediate snail host of *Fasciola hepatica*) treated with lower glyphosate concentrations (0.1, 1 and 10 mg/L). Concentrations of 1 mg/L elicited an increment in the growth in the third-generation of snails, as well as 0.1 and 10 mg/L elicited an inhibition of eggs hatching, abnormalities and polyembryony (Tate et al., 1997). Same authors observed significant differences in the metabolism of *P. columella*. Glyphosate concentrations of 0.1 mg/L induced about 2 folds increment in five free amino acids (Tate et al., 2000). In addition, Contardo-Jara et al. (2009) reported significant increment in the enzymes activities (sGST and SOD) of worm *Lumbriculus variegatus* at 0.05 and 0.1 mg a.i./L of glyphosate.

On the other hand, invertebrates showed higher sensibility to commercial formulations. For instance, Roundup® showed between 3 folds to 76 folds higher toxicity than glyphosate itself. Values of LC₅₀ obtained in Roundup® treatments ranged from 1.5 mg a.e./L for the amphipod *Hyalella azteca* (Tsui & Chu, 2004) to 62.0 mg a.i./L for other amphipod *Gammarus pseudolimnaeu* (Folmar et al., 1979) (Table 3). In addition, 0.7 mg a.e./L of Vision® (a commercial formulation containing POEA) elicited a 100 % of mortality and more than 50 % reduction in total neonates per female in the cladoceran *Simocephalus vetulus* at values of pH = 7.5 (Chen et al., 2004).

Sublethal effects were observed at much lower concentration of Roundup (1.1 µg/L) in the Clam, *Ruditapes decussates*, showing histological alterations (Abdel-Nabi, et al., 2007; El-Shenawy, et al., 2009).

The surfactant POEA was several times more toxic for invertebrates than glyphosate itself and Roundup®. For example, Folmar et al. (1979) reported almost 1.4 folds higher toxicity of POEA relative to Roundup® in the midge larvae *Chironomous plumosus*; contributing with

about 66 % of the Roundup® toxicity. Higher values were reported by Servizi et al. (1987) and Tsui & Chu (2003), being POEA 3 and 5 folds more toxic than Roundup®, respectively. POEA can contribute with more than the 90% of Roundup® toxicity (Tsui & Chu, 2003) On the other hand, commercial formulations without POEA (e.g. Ron-Do®, Rodeo® and Roundup bioactive®) showed lower toxicity (Alberdi et al., 1996; Tsui & Chu, 2004) than other formulations (Table 3).

4.2.5 Effects on non-target aquatic vertebrates

Fishes are well appreciated in human societies in many ways (e.g., economical, recreational, ecological). In many countries, commercial fishing has a large economic importance as national food supply and as an export product. Fishes are one of the most demanded pets and there are a lot of people who enjoy sport fishing. This vertebrate group has an amazing diversity in morphology, size and color, which reflects their life history adaptations (e.g., feeding behavior reproduction and habitat selection). It is well known that fish populations have both direct and indirect effects on ecosystem function and structure in general (e.g., nutrient dynamics and cycling, zooplanktonic community composition), and especially in freshwater ecosystems where they are top consumers on lower trophic levels (e.g., piscivore, planktivore, benthivore fish) (Scheffer, 1998; Montenegro Rayo, 2004; Brönmark & Hansson, 2005).

The major groups of amphibians found in lakes and ponds are frogs, toads and salamanders. Some species live their whole life in freshwater whereas other species are completely terrestrial and depended on water for their reproduction. Most tadpoles have a feeding apparatus that allows them to trap bacteria, phytoplankton, and other small particles suspended in the water. Many species also graze on periphytic algae and some species even have mouth parts adapted for a predatory feeding mode. Salamanders start to feed on zooplankton but as they grow they include larger invertebrates in their diet and some species even prey on tadpoles. Tadpoles, frogs, toads and salamanders are important source of food for fish and birds.

Aquatic fish and amphibians appear to have low sensibility to glyphosate itself (Table 4 and 5). Values of LC₅₀ in glyphosate treatments ranged from 130 mg a.i./L for the channel catfish *Ictalurus punctatus* (Folmar et al., 1979) to 620 mg a.i./L for the carp *Cyprinus carpio* (Neškovic et al., 1996) (Table 4). In amphibians, values of LC₅₀ obtained with glyphosate (IPA salt) treatments, varied from 340 to 460 mg a.e./L in four tadpoles of Australian frogs (Table 5). However, lower values were obtained in treatments with glyphosate as an acid, reporting values of LC₅₀ from 82 to 121 mg a.e./L (Mann & Bidwell, 1999). Similar toxicity was registered for Roundup® Biactive, a commercial formulation without surfactant POEA (Mann & Bidwell, 1999).

Wide differences were observed in the toxicity of glyphosate itself and commercial formulations. In fish, values of LC₅₀ obtained with Roundup® treatments ranged from 2.3 mg a.i./L for the fathead minnows *Pimephales promelas* (Folmar et al., 1979) to 14.5 mg /L for *Ictalurus punctatus* (Abdelghani et al., 1997). Treatments with Vision® showed middle LC₅₀ (10.42 mg a.i./L) for the rainbow trout *Oncorhynchus mykiss* (Morgan & Kiceniuk 1992). In addition, values of 4 and 12 mg a.i./L of Eskoba III Max® (a commercial formulation with unknown surfactant) elicited the 25 % and 20% of mortality in juveniles of Pejerrey *Odontesthes bonariensis* and adults of Tosquero *Jenynsia lineata* respectively; though not lethal effects were observed in glyphosate treatments (Pérez & Miranda unpublished) (Fig. 3). However, much lower concentrations of Roundup® have shown to cause effects in the biometry, metabolism and enzyme activities of fish. For instance, Gluszcak et al. (2007) reported a significant decrease in AChE activity (enzyme presents in cholinergic synapses and motor end plates) and

TBARS levels (a measure of oxidative stress) in the brain of silver catfish *Rhamdia quelen* exposed to 0.2 and 0.4 mg/L. Besides, significant reduction in the biometry of Piava *Leporinus obtusidens* was observed in treatments with 1 mg/L, eliciting a reduction of length (15%) and weight (50%) (Salbego et al., 2010). Other recent study, indicated molecular responses for the flounder *Platichthys flesus* treated with low doses of herbicide cocktail (< 10 µg/L of glyphosate) during a long-term contamination (62 days) (Evrard et al., 2010).

VERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCE
<i>Fish</i>					
<i>Oncorhynchus mykiss</i>	Gly. (acid)	SST (96h)	Mortality	96h LC ₅₀ =140.0	Folmar et al., 1979
	Roundup®			96h LC ₅₀ =8.3	
	POEA			96h LC ₅₀ =2.0	
<i>Pimphales promelas</i>	Gly. (acid)	SST (96h)	Mortality	96h LC ₅₀ =97.0	Folmar et al., 1979
	Roundup®			96h LC ₅₀ =2.3	
	POEA			96h LC ₅₀ =1.0	
<i>Ictalurus punctatus</i>	Gly. (acid)	SST (96h)	Mortality	96h LC ₅₀ =130.0	Folmar et al., 1979
	Roundup®			96h LC ₅₀ =13.0	
	POEA			96h LC ₅₀ =13.0	
<i>Oncorhynchus mykiss</i>	Roundup®	SSFE (96h)	Mortality	96h LC ₅₀ =52.0	Hildebrand et al., 1982
<i>Oncorhynchus mykiss</i>	Roundup®	SST (96h)	Mortality	96h LC ₅₀ =8.5	Servizi et al., 1987
	POEA			96h LC ₅₀ =3.2	
<i>Oncorhynchus nerka</i>	Roundup®	SST (96h)	Mortality	96h LC ₅₀ =8.1	Servizi et al., 1987
	POEA			96h LC ₅₀ =2.6	
<i>Oncorhynchus mykiss</i>	Vision®	SST (96h)	Mortality	96h LC ₅₀ =10.2	Morgan & Kiceniuk, 1992.
<i>Cyprinus carpio</i>	Gly. (acid)	SST (96h)	Mortality #	96h LC ₅₀ =620.0	Neškovic et al., 1996
<i>Ictalurus punctatus</i>	Roundup®	SST (96h)	Mortality	96h LC ₅₀ =14.5	Abdelghani et al., 1997
<i>Lepomis macrochirus</i>	Roundup®	SST (96h)	Mortality	96h LC ₅₀ =13.0	Abdelghani et al., 1997
<i>Rhamdia quelen</i>	Roundup®	SST (96h)	Metabolism & enzyme activity#	0.2 mg/L elicited a decrease in AChE and TBARS.	Glusczak et al., 2007
<i>Prochilodus lineatus</i>	Roundup®	SST (96h)	Mortality & physiology #	96h LC ₅₀ =13.7	Carmo Langiano & Martinez, 2008
<i>Leporinus obtusidens</i>	Roundup®	SST (90 d.)	Biometry & enzyme activity#	1 mg/L elicited a decrease in length and weight and AChE	Salbego et al., 2010
<i>Platichthys flesus</i>	Roundup® + AMPA	SST (62 d.)	Molecular and physiology	0.16 µg/L elicited liver injury	Evrard et al., 2010
<i>Odontesthes bonariensis</i>	Gly. (IPA salt)	SST (96h.)	Mortality	Not observed lethal effect	Pérez & Miranda (unpublished)
	Eskoba III Max®			4 mg/l elicited the 25% of mortality	
<i>Jenynsia lineata</i>	Gly. (IPA salt)	SST (96h.)	Mortality	Not observed lethal effect	Pérez & Miranda (unpublished)
	Eskoba III Max®			12 mg/l elicited the 20% of mortality	

Abbreviations, acronyms and notes: see Table 1

Table 4. Effects of glyphosate, different commercial formulations of glyphosate and POEA on fish.

Comparable outcomes with treatments of Roundup® and Glyphos® (other commercial formulation containing POEA) were reported for amphibians (Table 5). In laboratory tests, values of LC₅₀ varied from 2.6 mg/L of Glyphos® for tadpoles of the hylid *Scinax nasicus* (Lajmanovich et al., 2003) to 11.6 mg a.e./L of Roundup® for tadpoles of *Litoria moorei* (Mann & Bidwell, 1999). Middle concentrations (8 mg a.i./L) caused 100% of mortality in tadpoles of the toad *Rhinella arenarum* (Pérez & Miranda unpublished) exposed to Eskoba III Max® (Fig. 6), though LC values of 3.2 mg a.i./L were reported for this toad exposed to Roundup Ultra-Max (Lajmanovich et al., 2010). However, lower concentrations have shown significant effects in

mortality and growth. For instance, Chen et al. (2004) reported 100 % of mortality in tadpoles of *Rana pipiens* treated with 0.75 mg a.e./L of Vision® at pH of 7.5. Cauble & Wagner (2005) observed 50% of mortality for tadpoles of *Rana cascadae* treated with 1.94 mg a.i./L of Roundup® and an earlier metamorphosis time with 1 mg a.i. /L (Table 5). In addition, 2 mg a.i./L significantly reduce the survival and growth in three of five tadpoles exposed to Roundup® (Relyea, 2004). The same author reported in a mesocosms experiment a 100% of mortality for the *Rana sylvatica* and *Hyla versicolor* tadpoles and around 98 % of mortality for *Rana pipiens* and *Bufo americanus* tadpoles due to a direct herbicide effect (Relyea, 2005). Tadpoles seem to be more sensible to commercial formulations than juveniles and adults.

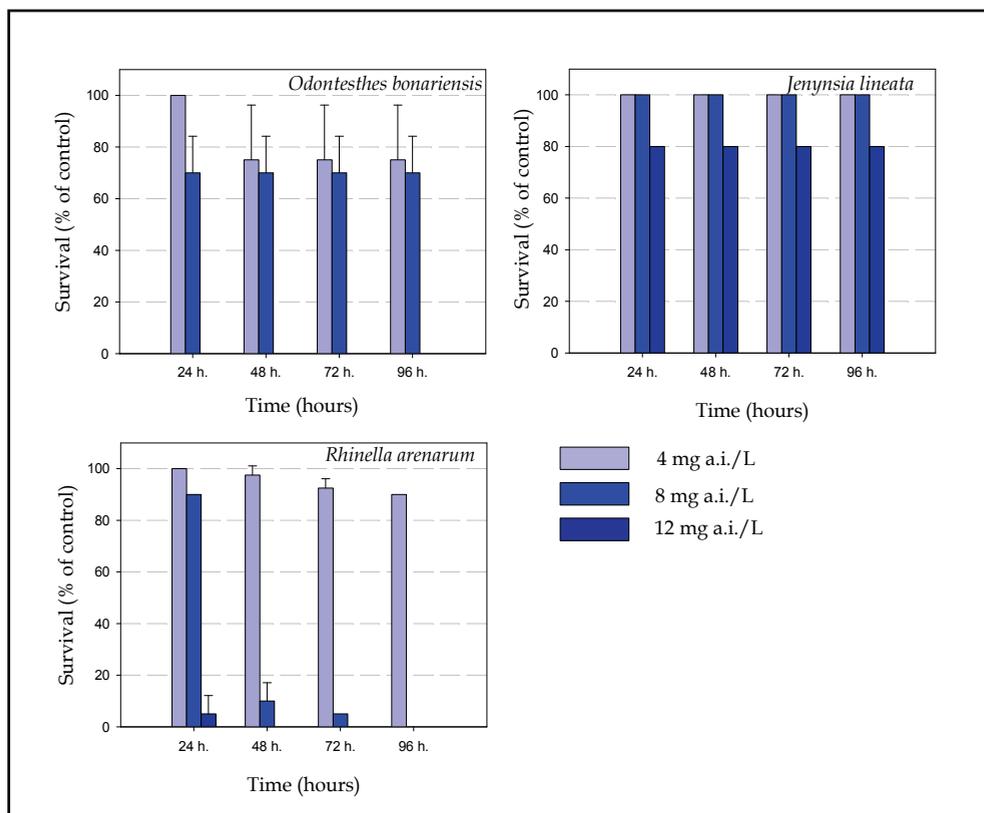


Fig. 3. Acute lethal effects of Eskoba III Max® on two fish species (*Odontesthes bonariensis* and *Jenynsia lineata*) and on tadpoles from *Rhinella arenarum*

POEA itself resulted more toxic than Roundup®, being this surfactant the more noxious component of several commercial formulations. Different authors concluded that the high mortality in fish and amphibian are actually due mainly to POEA surfactant and not to glyphosate itself (Folmar et al., 1979; Servizi et al., 1987; Perkins et al., 2000). In the fish *Pimephales promelas*, the relative contribution of glyphosate acid to the toxicity of Roundup® was about 30% (Folmar et al., 1979), while glyphosate (as IPA salt) was not toxic for 5 species of Australian frogs, and therefore without any contribution to Roundup® toxicity

(Mann & Bidwell, 1999). In fish, values of LC₅₀ obtained in POEA treatments varied from 1 to 13 mg a.i./L (Folmar et al., 1979; Servizi et al., 1987), being these values up to 4 fold more toxic than Roundup®. Besides, Perkins et al. (2000), found LC₅₀ values of 6.8 mg a.e./L for POEA treatments in African *Xenopus laevis* tadpoles, showing 1.8 fold higher toxicity than commercial formulation.

VERTEBRATES	ASSESSED CHEMICAL	STUDY TYPE	ASSESSED PARAMETER	EFFECTS CONCENTRATION (mg/L) ##	REFERENCES
-Amphibians (frogs and toads)					
<i>Lymnodynastes dorsalis</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC ₅₀ = > 400	Mann & Bidwell, 1999
	Roundup®			48h LC ₅₀ = 3.0	
	Roundup® Biactive			48h LC ₅₀ = > 400	
	Touchdown®			48h LC ₅₀ = 12.0	
<i>Heleioporus eyrei</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC ₅₀ => 373	Mann & Bidwell, 1999
	Roundup®			48h LC ₅₀ = 6.3	
	Roundup® Biactive			48h LC ₅₀ => 427	
	Touchdown®			48h LC ₅₀ = 16.1	
<i>Crinia insignifera</i>	Gly. (IPA salt)	SST (48 h.)	Morality	48h LC ₅₀ = > 466	Mann & Bidwell, 1999
	Roundup®			48h LC ₅₀ = 3.6	
	Roundup® Biactive			48h LC ₅₀ = > 494	
	Touchdown®			48h LC ₅₀ = 9.0	
<i>Xenopus laevis</i>	Rodeo®	SST (96 h.)	Morality	96h LC ₅₀ = 5407	Perkins et al. 2000
	Roundup®			96h LC ₅₀ = 9.4	
	POEA			96h LC ₅₀ = 2.7	
<i>Scinax nasicus</i>	Glyphos®	SST (96 h.)	Mortality #	96h LC ₅₀ = 2.6	Lajmanovich et al., 2003
<i>Rana pipiens</i>	Vision®	SST (8 d.)	Mortality #	0.75 mg/L elicited 100 % mortality	Chen et al., 2004
<i>Rana pipiens</i>				Not observed significant effects	
<i>Rana clamitans</i>				2 mg/L reduce survival & growth	Relyea, 2004
<i>Rana catesbeiana</i>	Roundup®	SST (16 d.)	Mortality & growth	2 mg/L reduce survival & growth	
<i>Bufo americanus</i>				2 mg/L reduce growth	
<i>Hyla versicolor</i>				Not observed significant effects	
<i>Rana sylvatica</i>				3.8 mg/L elicited 100% mortality	Relyea, 2005
<i>Rana pipiens</i>				3.8 mg/L elicited 98% mortality	
<i>Bufo americanus</i>	Roundup®	MES (15 d.)	Mortality & biomass #	3.8 mg/L elicited 98% mortality	
<i>Hyla versicolor</i>				3.8 mg/L elicited 100% mortality	
<i>Pseudacris crucifer</i>				Not observed significant effects	
<i>Rana cascadae</i>	Roundup®	SST (42 d.)	Mortality & metamorphosis#	1.94 mg/L elicited mortality and earlier metamorphosis times	Cauble & Wagner, 2005
<i>Rhinella arenarum</i>	Gly. (IPA salt)	SST (96h.)	Morality	Not observed significant effects	Pérez & Miranda (unpublished)
	Eskoba III Max®			8 mg/L elicited 100% mortality	

Abbreviations, acronyms and notes: see Table 1

Table 5. Effects of glyphosate, different commercial formulations of glyphosate and POEA on frogs and toads.

5. Conclusions

- Reviewing the available information on toxicity of glyphosate and its formulations on different groups of aquatic organisms, we have concluded that they are hazardous to the

aquatic environment. Several contributions reviewed here reported significant effects of the herbicide at concentrations lower than EEC (2.6 mg a.i./L). Herbicide could be very noxious in standing waters like ponds, or in irrigation canals and impounded waters, where EEC can be reached. In these scenarios, toxicity could be exacerbated by other stressors and water characteristics (e.g. high temperature and pH, low O₂ concentration, presence of clay colloids, water hardness and other chemicals). Besides, toxicity also has showed to depend on organism life stage.

- Overall, ecotoxicological sub-lethal endpoints based on behavioral traits (e.g., predator avoidance, feeding, and locomotion) and other endpoints (e.g. growth, reproduction and metabolism) seem to be more sensitive indicators of effects (i.e. reporting lower effective concentrations) and give more insights into patterns of toxicity than survivorship tests (i.e. lethality). In addition, in doses dependent effects studies, commonly results are expressed as LC₅₀ or EC₅₀. However, it is not possible to predict, for instance, if the 10 % of mortality or reduction in growth (i.e at lower herbicide concentration) do not have significant effects on population and eventually in the community. On the other hand, studies focused in natural or assembled communities (e.g. microcosm and mesocosms experiments) have provided interesting and significant outcomes regarding direct and indirect herbicide effects that could not be reached in single species laboratory tests. Although these laboratory tests are an essential protocol to rapidly identify the direct impacts of pesticides on organisms, they prevent an assessment of effects on organisms embedded in their natural ecological contexts.

- Glyphosate itself (as acid or salt) is generally considered to be slightly or moderately toxic to aquatic organisms (i.e., LC₅₀ or EC₅₀ between >1 to < 100 mg/L). However, some algae and aquatic plants showed higher sensibility, being glyphosate very toxic (EC₅₀ between >0.1 to < 1 mg/L). Aquatic plants seem to be more sensitive to glyphosate than microalgae. The high toxicity of glyphosate in algae and aquatic plants is related with the mode of action of this compound (an herbicide) that interferes with plant metabolisms. On the other hand, much lower glyphosate toxicity was observed for other aquatic organisms (i.e. bacteria, protozoa, invertebrates, fish and amphibians). However, snails and worms seem to be exceptions; showing significant effects in growth, reproduction and metabolism at concentrations of < 1 mg/L of glyphosate.

- Commercial formulations and specially those containing the surfactant POEA, showed higher toxicity than the active ingredient itself for all the aquatic organisms studied. Roundup® showed to be up to 7 folds more noxious than glyphosate in algae and aquatic plants, up to 13 folds in protozoa, up to 42 folds in fish, up 70 folds in crustaceans, and up to 130 folds in frogs and toads. Algae and aquatic plants, showed significant effects with concentrations < 3 mg a.i./L. however, lower values were registered in studies of periphyton and micro plankton communities. Roundup® concentration of 10 µg a.i./L elicited changes in marine microbial community structure and 0.13 mg a.i./L of Rodeo® caused an increment in periphyton primary production. In addition, significant effects at concentrations relevant to environmental toxicity thresholds were also observed for other groups of aquatic organism. In protozoa, *Euglena gracilis* showed high sensibility, with 0.1 mg a.i. /L of Roundup® and Avans® eliciting significant sublethal effects. Different species of crustaceans showed lethal effects with values lower than 3 mg a.i./L of Roundup®. In Frogs and toads, relevant concentrations of glyphosate based products (< 2 mg a.i./L) elicited lethal and sublethal effects. Fish seems to be less sensitive to commercial

formulations, though some contributions showed significant sublethal effects in metabolism and enzyme activity at concentrations (< 2.5 mg a.i./L) of Roundup®

- The high toxicity observed for several commercial formulation of glyphosate was generally related with the content of POEA. In protozoa and invertebrates, POEA contributed with more than the 80% of Roundup® toxicity. Crustaceans showed values of EC_{50} and LC_{50} that ranged from 0.097 to 2 mg/L of POEA. In fish, glyphosate only contributed to the toxicity of Roundup® with around 30%, and values of EC_{50} ranged from 1 to 13 mg/L of POEA. In frogs and toads POEA seems to be the most toxic compound in commercial formulations. Glyphosate alone (as IPA salt) was not toxic for 5 species of Australian frogs ($LC_{50} > 343$ mg a.e./L) and therefore contributed little to Roundup® toxicity. In contrast, POEA could show lower contribution in algae and aquatic plants (as from about 46%).
- Stated the hazard of glyphosate and commercial formulations of glyphosate on aquatic environments and ecological implication of the effects reviewed here, we stressed the paucity of contributions studying the effects of glyphosate on several potential endangered aquatic species (e.g. hydroids, sponges, worms, flatworms, insects, and urodela species). We also emphasize the necessity of studies in natural communities or in assembled communities in order to evaluate direct and indirect effects upon different trophic levels.
- Finally we consider that glyphosate and commercial formulations of glyphosate could have particularly significant disruptive effects to waterbodies like ponds. Ponds have been widely recognized as very important freshwater habitats. These relative small and shallow still aquatic environments are very rich in genetic and taxonomic biodiversity; they are important refuges for amphibians and also for a bewildering variety of plants and animals, including many scarce and endangered species. In addition ponds are important places for insects hatching, fish larvae and juveniles refuges and net sites for wetland birds.

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7. References

- Abdel-Nabi, I.M.; El-Shenawy, N.S.; Taha I.A. & Moawad, T.I. (2007). Oxidative stress biomarkers and bioconcentration of Reldan and Roundup by the edible clam *Ruditapes decussates*. *Acta Zool. Sinica*, 53: 910-920
- Abdelghani A. A., P. B. Tchounwou, A. C. Anderson, H. Sujono, L. R. Heyer, & Monkiedje, A. (1997). Toxicity evaluation of single and chemical mixtures of Roundup, Garlon-3A, 2,4-D, and Syndets surfactant to channel catfish (*Ictalurus punctatus*), bluegill sunfish (*Lepomis microchirus*), and crawfish (*Procambarus spp.*). *Environ. Toxicol. Water. Qual.* 12, 237-243
- Alberdi, J. L.; Sáenz, M. E. Di Marzio, W. D. & Tortorelli, M. C. (1996). Comparative acute toxicity of two herbicides, Paraquat and Glyphosate, to *Daphnia magna* y *D. spinulata*. *Bull. Environ. Contam. Toxicol.*, 57, 229-235
- Amrhein N., Deus B., Gehrke P. and Steinrucken H.C. (1980). The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation in vivo and in vitro. *Plant Physiol.* 66, 830-834

- Altieri, M.A. & Pengue, W. (2006). GM soybean: Latin America's new colonizer. *Seedling*, January issue
- Baird, D. D.; Upchurch, R. P.; Homesley, W. B. & Franz, J. E. (1971). Toxicity of glyphosate. *Proc. North Cent. Weed Control Conf.*, 26, 64
- Bayer, C.; Lovato, T.; Dieckow, J.; Zanatta, J. A. & Mielniczuk, J. (2006). A method for estimating coefficients of soil organic matter dynamics based on long-term experiments. *Soil and Tillage Research*, 91, 217-226
- Bérard, A., Leboulanger, C., Pelte, T. (1999). Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine in natural phytoplankton populations and in pure culture: influence of season and temperature. *Arch. Environ. Contam. Toxicol.*, 37, 472-479
- Berlin, J. & Witte, L. (1981). Effects of glyphosate on shikimic acid accumulation in tobacco cell cultures with low and high yields of cinnamoyl putrescines. *Z. Naturforsch Sect. C.*, 36, 210-214
- Binimelis, R.; Pengue, W. & Monterroso, I. (2009). "Transgenic treadmill": Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina. *Geoforum*, 40, 623-633
- Bode, R.; Melo, C. & Birnbaum, D. (1984). Mode of action of glyphosate in *Candida maltosa*. *Arch. Microbiol.*, 140, 83-85
- Bolliger, A.; Magrid, J.; Amado, T. J. C.; Neto, F. S.; Ribeiro, M. F. S.; Calegari, A.; Ralisch, R. & Neergaard, A. (2006). Taking stock of the Brazilian "zero-till revolution": a review of landmark research and farmers' practice. *Adv. Agron.*, 91, 47-110
- Bonnet, J. L.; Bonnemoy, F.; Dusser, M. & Bohatier, J. (2007). Assessment of the potential toxicity of herbicides and their degradation products to non target cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate *Tetrahymena pyriformis*. *Environ. Toxicol.*, 22, 78-91
- Bonny, S. (2009). Genetically Modified Glyphosate-Tolerant Soybean in the USA: Adoption Factors, Impacts and Prospects - A Review. *Agron. Sustain. Dev.*, 28, 257-272
- Brausch, J.; Beall, B. & Smith, P. (2007). Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.*, 78, 510-514
- Brönmark, C. & Hansson, L.-A. (2005). *The Biology of Lakes and Ponds*, Oxford University Press, New York.
- Cauble, K. & Wagner, R. S. (2005). Sublethal effects of the herbicide glyphosate on amphibian metamorphosis and development. *Bull. Environ. Contam. Toxicol.*, 75, 429-435
- Cedergreen, N. & Streibig, J. C. (2005). The toxicity of herbicides to nontarget aquatic plants and algae: Assessment of predictive factors and hazard. *Pest Manag. Sci.*, 61, 1152-1160
- Chen, C. Y.; Hathaway, K. M. & Folt, C. L. (2004). Multiple stress effects of Vision® herbicide, pH, and food on zooplankton and larval amphibian species from forest wetlands. *Environ. Toxicol. Chem.*, 23, 823-831
- Christy, S.L.; Karlander, E.P. & Parochetti, J.V. (1981). Effects of glyphosate on the growth rate of *Chlorella*. *Weed. Sci.*, 29, 5-7
- Comes, R. D.; Bruns, V. F. & Kelly, A. D. (1976). Residues and persistence of glyphosate in irrigation water. *Weed Sci.*, 24, 47-

- Conners D. E. & Black, M. C. (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch. Environ. Contam. Toxicol.*, 46, 362-371
- Contardo-Jara, V.; Klingelmann, E. & Wiegand, C. (2009). Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environ. Pollut.*, 157, 57-63
- Couture, G.; Legris, J. & Langevin, R. (1995). Evaluation des impacts du glyphosate utilise dans le milieu forestier. Ministere des Ressources Naturelles, Direction de Penviron- ment forestier, Service du suivi environnemental, Charles- bourg, Quebec, Canada.
- Crisanto T.; Sanchez-Martín, M. J. & Sanchez-Camazano, M. (1994). Mobility of pesticides in soil. Influence of soil properties and pesticide structure. *Toxicol. Environ. Chem.*, 45, 97-104
- Diamond. G. & Durkin, P. (1997). Effects of Surfactants on the Toxicity of Glyphosate, with Specific Reference to Rodeo. Prepared under USDA FS Contract No. 53-3187-5- 12. Final Report.
- Dill, G. M.; Cajacob, C. A. & Padgette, S. R. (2008). Glyphosate-resistant crops: adoption, use and future considerations. *Pest Manag. Sci.*, 64, 326-331
- do Carmo Langiano, V. & Martinez, C. B. R., (2008). Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comp. Biochem. Physiol.*, 147, 222 - 231
- Duke, S. O.; Hoagland, R. E. & Elmore, C. D. (1980). Effects of glyphosate on metabolism of phenolic compounds. V. L-a-aminoxy-B-phenylpropionic acid and glyphosate effects on phenylalanine ammonia-lyase in soybean seedlings. *Plant Physiol.*, 65, 17-21
- Duke, S. O. (1988). Glyphosate. In: *Herbicides, Chemistry, degradation and mode of action*, Kearney, P.C. & Kaufman, D.D. (Ed.), 1-70 pp, Marcel Dekker, New York
- Edwards, W. M.; Triplett, G. B. & Kramer, R. M. (1980). A watershed study of glyphosate transport in runoff. *J. Environ. Qual.*, 9, 661-665
- Everett, K. D. E. & Dickerson, H. W. (2003). *Ichthyophthirius multifiliis* and *Tetrahymena thermophila* tolerate glyphosate but not a commercial herbicidal formulation. *Bull. Environ. Contam. Toxicol.*, 70, 731-734
- El-Shenawy, N.S.; Moawad, T.I.S.; Mohallal, M.E.; Abdel-Nabi, I.M. & Taha, I.A. (2009). Histopathologic biomarker response of clam *Ruditapes decussates* to Reldan and Roundup: A laboratory study. *Ocean Sci. J.*, 44: 27-34.
- Evrard, E.; Marchand, J.; Theron, M.; Pichavant-Rafini, K.; Durand, G.; Quiniou, L. & Laroche, J. (2010). Impacts of mixtures of herbicides on molecular and physiological responses of the European flounder *Platichthys flesus*. *Comparative Biochemistry and Physiology Part C*, 152, 321-331
- Feng, J. C.; Thompson, D. G. & Reynolds, P. E. (1990). Fate of Glyphosate in a Canadian Forest Watershed. 1. Aquatic Residues and Off Target Deposit Assessment. *J. Agric. Food Chem.*, 38, 1110-1118
- Folmar, L. C.; Sanders, J. O. & Julin A. M. (1979). Toxicity of the Herbicide Glyphosate and Several of its Formulations to Fish and Aquatic Invertebrates. *Arch. Environ. Contain. Toxicol.*, 8, 269-278

- Franz, J. E. (1985). Discovery, development and chemistry of glyphosate. In: *The Herbicide Glyphosate*, Grossbard, E. & Atkinson, D. (Eds.), 3-17. Butterworth & Co, London, UK
- Franz, J. E.; Mao, M. K. & Sikorski, J. A. (1997). *Glyphosate: A Unique Global Herbicide*. ACS Monograph 189. American Chemical Society, 65-97, Washington, DC
- Gardner, S. C.; Grue, C. E.; Grassley, J. M.; Lenz, L. A.; Lindenauer, J. M. & Seeley, M. E. (1997). Single species algal (*Ankistrodesmus*) toxicity tests with Rodeo® and Garlon® 3A. *Bull. Environ. Contam. Toxicol.*, 59, 492-499
- Giesy, J.P.; Dobson, S. & Soloman, K. R. (2000). Ecotoxicological risk assessment for Roundup® herbicide. *Rev. Environ. Contam. Toxicol.*, 167, 35-120
- Goldsborough, L. G. & Brown, D. J. (1988). Effect of Glyphosate (Roundup® Formulation) on Periphytic Algal Photosynthesis. *Bull. Environ. Contam. Toxicol.*, 41, 253- 260
- Gluszczak, L.; Miron, D. S.; Moraes, B. S.; Simões, R. R.; Schetinger, M. R. C.; Morsch, V. M. & Loro, V. L. (2007). Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). *Comp. Biochem. Physiol. C.*, 146, 519-524
- Gresshoff, P. M. (1979). Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine. *Aust. J. Plant. Physiol.*, 6, 177-185
- Haderlie, L. C.; Widholm, J. & Slife, F. W. (1977). Effect of glyphosate on carrot and tobacco cells. *Plant Physiol.* 60, 40-3.
- Hildebrand, L. D.; Sullivan, D. S. & Sullivan, T. P. (1982). Experimental studies of rainbow trout populations exposed to field applications of Roundup herbicide. *Arch. Environ. Contam. Toxicol.*, 11, 93-98
- Hollander. H. & Amrhein, N. (1980). The site of action of the inhibition of the shikimate pathway by glyphosate. I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Fagopyrum esculentum* Moench). *Plant Physiol.*, 66, 823-829
- Holt J.; Powles, S. & Holtum, J. (1993). Mechanisms and agronomic aspects of herbicide resistance. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44, 203 - 229
- James, C. (2008). Global status of commercialized biotech/GM crops: 2008. The first thirteen years, 1996 to 2008. ISAAA Brief No. 39. International Service for the Acquisition of Agri-biotech Associations, Ithaca, New York, USA
- Jaworski, E. G. (1972). Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. *J. Agric. Food Chem.*, 20, 1195-1198
- Laerke, P. E. (1995). Foliar absorption of some glyphosate formulation and their efficacy on plants. *Pesticide Science*, 44, 107-116
- Lajmanovich, R. C.; Sandoval, M. T. & Peltzer, P. M. (2003). Induction of mortality and Malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. *Bull. Environ. Contam. Toxicol.*, 70, 612-618.
- Lajmanovich, R. C.; Attademo, A.M.; Peltzer, P.M.; Junges C. & Cabagna, M. (2010). Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitions. *Arch. Environ. Contam. Toxicol.* doi:10.1007/s00244-010-9578-2
- Legris, J. & Couture, G. (1989). Residus de glyphosate dans l'eau et les sediments suite a des pulverisations terrestres en milieu forestier en 1986. Publication #3322.

- Gouvernement du Quebec, Ministere de l'Energie et des Ressources, Direction de la Conservation, Charlesbourg, Quebec, Canada
- Malcolm, P., Inc. (1987) The toxicity of glyphosate technical to *Skeletonema costatum*. White Plains, New York, (Unpublished report submitted by Monsanto Ltd)
- Mann, R. M. & Bidwell, J. R. (1999). The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. *Arch. Environ. Contam. Toxicol.*, 26, 193-199
- Maule, A. & Wright, S. J. L. (1984). Herbicide effects on the population growth of some green algae and cyanobacteria. *J. Appl. Bacteriol.*, 57, 369-376
- Moedritzer, K. & Irani, R. R. (1966). The direct synthesis of aminomethyl phosphonic acid. *J. Org. Chem.*, 31, 1603-1607
- Montenegro Rayo, K. (2004). *Ecotoxicological effects of DDT and glyphosate on aquatic organisms: a case study*, Lunds Universitet, Lund
- Morgan, M. J. & Kiceniuk, J. W. (1992). Response of rainbow trout to a two month exposure to Vision®, a glyphosate herbicide. *Bull. Environ. Contam. Toxicol.*, 48, 772-780
- Neškovic, N. K.; Poleksic, V.; Elezovic, I.; Karan, V. & Budimir, M. (1996). Biochemical and histopathological effects of glyphosate on carp (*Cyprinus carpio*). *Bull. Environ. Contam. Toxicol.*, 56, 295-302
- Newton, M.; Horner, L. H.; Cowell, J. E.; White, D. E. & Cole E. C. (1994). Dissipation of glyphosate and aminomethylphosphonic acid in North American forests. *Journal of Agriculture and Food Chemistry*, 42, 1795-1802.
- Payne N. J.; Feng J.C. & Reynolds, P. E. (1990). Off-target deposits and buffer zones required around water for aerial glyphosate applications. *Pestic. Science*, 30, 183-198
- Payne, N. J. 1992. Off-target glyphosate from aerial silvicultural applications and buffer zones required around sensitive areas. *Pestic. Science*, 34, 1-8.
- Payne, N. J. & Thompson, D. G. (1992). Off-target glyphosate deposits from Aerial silvicultural applications under various meteorological conditions. *Pestic. Science*, 34, 53-59
- Pechlaner, R. (2002). Glyphosate in herbicides: an overlooked threat to microbial bottom-up processes in freshwater systems. *Verhand. Intern. Verien. Limnol.*, 28, 1831 - 1835.
- Pengue, W. (2005). "Transgenic crops in Argentina: the ecological and social debt" *Bulletin of Science, Technology and Society*, 25, 314-322
- Perez, A. & Kogan, M. (2003). Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. *Weed Res.*, 43, 12-19
- Pérez, G. L.; Torremorell, A.; Mugni, H.; Rodríguez, P.; Vera, M. S.; Do Nascimento, M.; Allende, L.; Bustingorry, J.; Escaray, R.; Ferraro, M.; Izaguirre, I.; Pizarro, H.; Bonetto, C.; Morris, D. P. & Zagarese, H. (2007). Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecol. Appl.*, 17, 2310-2322
- Perkins, P. J.; Boermans, H. J. & Stephenson, G. R. (2000). Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay-Xenopus. *Environ. Toxicol. Chem.*, 19:940-945.
- Pesce, S.; Batisson, I.; Bardot, C.; Fajon, C.; Portelli, C.; Montuelle, B. & Bohatier, J. (2009). Response of spring and summer riverine microbial communities following glyphosate exposure. *Ecotoxicol. Environ. Saf.*, 72, 1905-1912
- Pettersson, M. & Ekelund, N. G. (2006). Effects of the Herbicides Roundup and Avans on *Euglena gracilis*. *Arch. Environ. Contam. Toxicol.*, 50, 175-181

- Piccolo, A.; Celano, G. & Pietramellara, G. (1992). Adsorption of the herbicide glyphosate on a metal-humic acid complex. *Sci. Total Environ.*, 123/124, 77-82
- Powles, S. B.; Lorraine-Colwill, D. F., Dellow, J. J. & Preston, C. (1998) Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci.*, 46, 604-607
- Powles, S. B. (2008). Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest. Manag. Sci.*, 64, 360-365
- Pratley, J.; Urwin, N.; Stanton, R.; Baines, P.; Broster, J.; Cullis, K.; Schafer, D.; Bohn, J. & Krueger, R. (1999). Resistance to glyphosate in *Lolium rigidum*. I. Bioevaluation. *Weed Sci.*, 47, 405-411.
- Relyea, R. A. (2004). Growth and survival of five amphibian species exposed to combinations of pesticides. *Environ. Toxicol. Chem.*, 23, 1737-1742
- Relyea, R.A. (2005). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.*, 15, 618-627
- Relyea, R. A. (2006). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities: response. *Ecol. Appl.*, 16, 2027-2034.
- Riechers, D. E.; Wax, L. M.; Liebl, R. A. & Bullock, D. G. (1995). Surfactant effects on glyphosate efficacy. *Weed Technol.*, 9, 281 - 285
- Riley, C. M.; Wisener, C. J.; Sexsmith, W. A. (1991). Estimating off-target spray deposition on the ground following the aerial application of glyphosate for conifer release in New Brunswick. *J. Environ. Sci. Health B.*, 26, 185-208
- Roshon, R. D.; McCann, J. H.; Thompson, D. G. & Stephenson, G. R. (1999). Effects of seven forestry management herbicides on *Myriophyllum sibiricum*, as compared with other nontarget aquatic organisms. *Can. J. For. Res.*, 29, 1158-1169
- Roy, D. N.; Konar, S. K.; Banerjee, S.; Charles, D. A.; Thompson, D. G. & Prasad, R. (1989). Persistence, movement and degradation of glyphosate in selected Canadian boreal forest soils. *J. Agric. Food Chem.*, 37, 437-440
- Rubin, J. L.; Gaines, C. G. & Jensen, R. A. (1984). Glyphosate inhibition of 5-enolpyruvylshikimate 3-phosphate synthase from suspension-cultured cells of *Nicotiana glauca*. *Plant Physiol.*, 75, 839-845.
- Rueppel, M. L.; Brightwell, B. B.; Schaefer J. & Marvel J. T. (1977). Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.*, 25, 517-528
- Sáenz, M. E.; Di Marzio, W. D.; Alberdi, J. L. & del Carmen Tortorelli, M. (1997). Effects of technical grade and a commercial formulation of glyphosate on algal population growth. *Bull. Environ. Contam. Toxicol.*, 59, 638-344
- Salbego, J.; Pretto, A.; Gioda, C.; de Menezes, C., Lazzari, R., Radünz Neto, J., Baldisserotto, B. & Loro, V. (2010). Herbicide Formulation with Glyphosate Affects Growth, Acetylcholinesterase Activity, and Metabolic and Hematological Parameters in *Piava (Leporinus obtusidens)*. *Arch. Environ. Contam. Toxicol.*, 58, 740-745
- Sánchez, D.; Graça, M. & Canhoto, J. (2007). Testing the Use of the Water Milfoil (*Myriophyllum spicatum* L.) in Laboratory Toxicity Assays. *Bull. Environ. Contam. Toxicol.*, 78, 421-426
- Schaffer, J. D. & Sebetich, M. J. (2004). Effects of aquatic herbicides on primary productivity of phytoplankton in the laboratory. *Bull. Environ. Contam. Toxicol.*, 72:1032-1037
- Scheffer, M.; Hosper, S. H.; Meijer, M. L.; Moss B. & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology and Evolution*, 8, 275-279
- Scheffer, M. (1998). *Ecology of Shallow Lakes*, Chapman & Hall, London, UK.

- Seddon, J. C. (1981). The control of aquatic weeds with the isopropylamine salt of N-phosphonomethyl glycine. *Proceedings Aquatic Weeds and their Control, Assoc. Appl. Biol. Natl. Veg. Res. Stn., Wellesbourne, Warwick, England*, 141-148
- Servizi, J. A.; Gordon, R. W. & Martens, D. W. (1987). Acute toxicity of Garlon 4 and Roundup herbicides to salmon, *Daphnia*, and trout. *Bull. Environ. Contam. Toxicol.*, 39, 15-22
- Siemering, G.; Hayworth, J. & Greenfield, B. (2008). Assessment of potential herbicide impacts to California aquatic ecosystems. *Arch. Environ. Contam. Toxicol.*, 55, 415-431
- Smith, E. A. & Oehme, F. W. (1992). The biological activity of glyphosate to plants and animals: a literature review. *Vet. Hum. Toxicol.*, 34, 531-543
- Sobrero, M.C., Rimoldi, F., Ronco, A.E., 2007. Effects of the glyphosate active ingredient and a formulation on *Lemma gibba* L. at different exposure levels and assessment endpoints. *Bull. Environ. Contam. Toxicol.*, 79, 537-543
- Stachowski-Haberkorn, S.; Becker, B.; Marie, D.; Haberkorn, H.; Coroller, L. & de la Broise, D. (2008). Impact of Roundup on the marine microbial community, as shown by an in situ microcosm experiment. *Aquat. Toxicol.*, 89, 232-241
- Steinrücken, H. C. & Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. *Biochem. Biophys. Res. Commun.*, 94, 1207-1212
- Tate, T. M.; Spurlock, J. O. & Christian, F. A. (1997). Effect of glyphosate on the development of *Pseudosuccinea columella* snails. *Arch. Environ. Contam. Toxicol.*, 33, 286-289
- Tate, T.; Jackson, R. & Christian, F. (2000). Effects of glyphosate and dalapon on total free amino acid profiles of *Pseudosuccinea columella* Snails. *Bull. Environ. Contam. Toxicol.*, 64, 258-262
- Turgut, C. & Fomin, A. (2002). Sensitivity of the rooted macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC50. *Bull. Environ. Contam. Toxicol.*, 69, 601-608
- Tsui, M. T. K. & Chu, L. M. (2003). Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere*, 52, 1189- 1197
- Tsui, M. T. K., & Chu, L. M. (2004). Comparative toxicity of glyphosate-based herbicides: Aqueous and sediment porewater exposures. *Arch. Environ. Contam. Toxicol.*, 46, 316-323
- USEPA (United States Environmental Protection Agency). Pesticide Industry Sales and Usage 1994-2001. Marked estimates. <http://www.epa.gov/opp00001/pestsales/>
- USDA (United States Department of Agriculture). Agricultural Chemical Usage - Field Crops. National Agricultural Statistics Service. <http://usda.mannlib.cornell.edu>
- Vendrell, E.; Ferraz, D. G.; Sabater, C. & Carrasco, J. M. (2009). Effect of glyphosate on growth of four freshwater species of phytoplankton: a microplate bioassay. *Bull. Environ. Contam. Toxicol.*, 82, 538-42
- Vera, M. S.; Lagomarsino, L.; Sylvester, M.; Pérez, G. L.; Rodriguez, P., Mugni, H.; Sinistro, R.; Ferraro, M.; Bonetto, C.; Zagarese, H. & Pizarro, H. (2010). New evidences of Roundup® (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology*, 19, 710-719

- Wan, M. T.; Watts, R. G. & Moul, D. J. (1989). Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. *Bull. Environ. Contam. Toxicol.*, 43, 378-385
- Wax, L. M.; Leibl, R. M. & Bush, D. R. (1994). Surfactant-increased glyphosate uptake into plant membrane vesicles isolated from common lambsquarters leaves. *Plant Physiol.*, 105, 1419-25
- Van Gessel, M.J. (2001). Confirming glyphosate-resistant horseweed (*Conyza canadensis*) in Delaware. *Weed Sci.*, 49, 703-712
- Wetzel, R. (2001). *Limnology, Lake and River Ecosystems*, Academic Press. San Diego, California.
- Woodburn, A. T. (2000). Glyphosate: production, pricing and use worldwide. *Pest Manage. Sci.*, 56, 309-312
- Xi, Y. L. & Feng, L. K. (2004). Effects of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calyciflorus* Pallas. *Bull. Environ. Contam. Toxicol.*, 73, 644-651
- Yamada, T.; Kremer, R. J.; de Camargo e Castro, P. R. & Wood, B. W. (2009). Glyphosate interactions with physiology, nutrition, and diseases of plants: Threat to agricultural sustainability? *Europ. J. Agronomy*, 31, 111-113
- Yates, W. E., Akesson, N. B. & Bayer, D. E. (1978). Drift of glyphosate sprays applied with aerial and ground equipment. *Weed Science*, 26, 597-604



Herbicides and Environment

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Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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Review

Toxic Effects of Glyphosate on the Nervous System: A Systematic Review

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Abstract: Glyphosate, a non-selective systemic biocide with broad-spectrum activity, is the most widely used herbicide in the world. It can persist in the environment for days or months, and its intensive and large-scale use can constitute a major environmental and health problem. In this systematic review, we investigate the current state of our knowledge related to the effects of this pesticide on the nervous system of various animal species and humans. The information provided indicates that exposure to glyphosate or its commercial formulations induces several neurotoxic effects. It has been shown that exposure to this pesticide during the early stages of life can seriously affect normal cell development by deregulating some of the signaling pathways involved in this process, leading to alterations in differentiation, neuronal growth, and myelination. Glyphosate also seems to exert a significant toxic effect on neurotransmission and to induce oxidative stress, neuroinflammation and mitochondrial dysfunction, processes that lead to neuronal death due to autophagy, necrosis, or apoptosis, as well as the appearance of behavioral and motor disorders. The doses of glyphosate that produce these neurotoxic effects vary widely but are lower than the limits set by regulatory agencies. Although there are important discrepancies between the analyzed findings, it is unequivocal that exposure to glyphosate produces important alterations in the structure and function of the nervous system of humans, rodents, fish, and invertebrates.



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Keywords: glyphosate; glyphosate-based herbicides (GBH); neurotoxic effects; human; rodent; fish

1. Introduction

Glyphosate, N-(phosphonomethyl) glycine is the most widely used herbicide in the world [1]. It is a non-selective systemic biocide with broad-spectrum activity and was introduced in 1974 for the control of weeds in agricultural production fields [2]. The widespread use of glyphosate in agriculture and forestry has contributed to the development of numerous commercial formulations containing this compound. Herbicide formulations containing this active ingredient represent approximately 60% of the global market for non-selective herbicides [3].

In recent years, the use of glyphosate has spread worldwide due to the development of glyphosate-resistant crops. The main driver of the enormous success of this technology has been the economic benefits obtained in the agricultural sector after the introduction of genetically modified crops [4]. This has contributed to the positioning of glyphosate-based herbicides (GBH) as leaders in the global pesticide market. Thus, around 600.000 to 750.000 tons of glyphosate are used each year, and it is estimated that its use will increase reaching between 740.000 and 920.000 tons by 2025 [5].

The mechanism of action of glyphosate is associated with its ability to block the shikimic acid pathway, which is involved in the synthesis of aromatic amino acids in plants, fungi, and some microorganisms [6]. Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is the penultimate step in the shikimate pathway [7]. This inhibition leads to a reduction in the synthesis of the aromatic amino acids tyrosine, phenylalanine, and tryptophan, as well as a decrease in protein synthesis [8] (Figure 1). Therefore,

blocking this metabolic pathway eventually causes the death of the target organism within a few days [9].

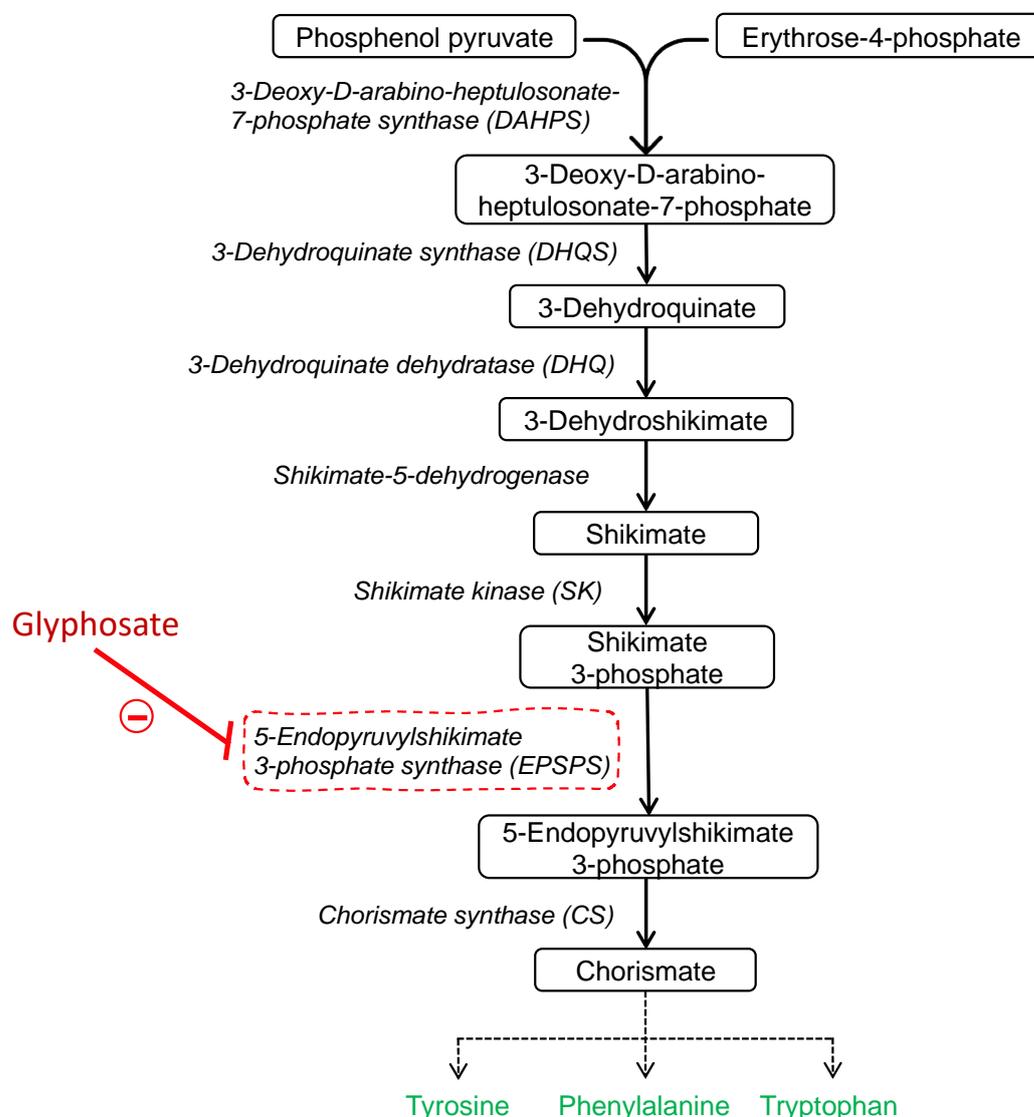


Figure 1. Glyphosate inhibits the enzymatic activity of the 5-endopyruvylshikimate 3-phosphate synthase (EPSPS) in the shikimate pathway, preventing the synthesis of the aromatic amino acids tyrosine, phenylalanine, and tryptophan.

The absence of the shikimate pathway in animals has led to the conclusion that GBH does not pose a health risk to animals and humans [10]. Moreover, many investigations on glyphosate toxicity in animals have suggested the low toxicity of this compound, the adverse effects of which have only been observed after exposure to relatively high doses [8,11,12]. These data led to the classification of glyphosate in the least toxic category (category IV, practically non-toxic and non-irritating) by the United States Environmental Protection Agency (EPA) [13,14].

In general, in the different environmental compartments, glyphosate is mainly degraded by microorganisms, so that its persistence is considered to be low to moderate, although it is considerably variable. On the one hand, although glyphosate is assumed to be readily degraded in soil, its biodegradation is influenced by numerous factors, including physico-chemical, biological properties and soil composition [15]. Thus, the half-life of glyphosate in soil can range from 1 to 280 days, while that of aminomethylphosphonic acid (AMPA), its main metabolite, ranges from 23 to 958 days [16–18]. In soil, glyphosate

can bind strongly to its constituent particles and remain biologically inactive, or it can reach groundwater, due to its high water solubility [19]. However, repeated applications of glyphosate have been shown to result in a gradual difficulty for its biodegradation in the soil, which could increase the risk of groundwater contamination [20,21]. In water, the permanence of glyphosate is also widely variable and depends on factors such as light and temperature, being more persistent and toxic under conditions of darkness and higher water temperatures [22]. In general, the half-life of glyphosate in water varies from a few days to 91 days [23,24], although it has been found to remain for up to 315 days in marine waters [25]. On the other hand, while the persistence of glyphosate in vegetation may be only days, several studies have detected its presence in many foods and crops even a year after application [26,27].

Therefore, although the concentrations of glyphosate residues that persist over time are relatively low, it is possible that due to extensive use on a large scale they may accumulate and become a risk to animal and human health, as they are chronically exposed to residues in the water and food they consume [23,28,29]. This has been confirmed by the detection of glyphosate in the organs and urine of a high proportion of farm animals and farmers [30–33]. In addition, residues were also found in the urine of 60–80% of the general population in the United States at medium and maximum concentrations of 2–3 and 233 µg/L, respectively. In Europe, residues were also detected in the urine of 44% of the population, although their average and maximum concentrations were lower: <1 and 5 µg/L, respectively [9,28].

Recently, data on glyphosate contamination in the environment suggest that acute toxicity may not be as relevant as toxicity from chronic exposure to lower concentrations of this compound. Therefore, the number of publications demonstrating the chronic toxicity of glyphosate in animals and humans has increased considerably. This has led to increased concern about the potential harmful side effects that chronic exposure to glyphosate could have on animal and human health. Glyphosate has recently received increasing attention from the scientific community and from national and international regulatory agencies. Based on research on the chronic side effects of glyphosate, in 2015, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) reclassified glyphosate as probably carcinogenic to humans. Nevertheless, IARC's conclusion has not been confirmed by European Union assessment or the recent joint assessment by the Food and Agriculture Organization of the United Nations (FAO)/OMS [34]. This highlights the existence of significant disagreement between regulatory agencies, as well as the need to reach consensus and update safety standards for glyphosate in order to protect public health and the environment.

Numerous commercial formulations of glyphosate contain several adjuvants, which improve the penetration of the active ingredient into the target plants and increase their efficacy [35,36]. It has been postulated that the activity of GBH is not exclusively due to the active ingredient but could be due to the intrinsic toxicity of the adjuvants or the possible synergy between glyphosate and the other ingredients of the formulation [37]. In fact, polyethoxylated tallow amine, the predominant surfactant in several commercial formulations, has been found to increase glyphosate-induced toxicity by facilitating its penetration through plasma membranes [38–42]. Therefore, it is important for research to evaluate the toxicity of both commercial formulations and pure glyphosate [43].

Taken together, above information shows that the intensive and widescale use of glyphosate can constitute a major environmental and health problem. In recent years, there has been an increase in the number of scientific publications dealing with the possible side effects of glyphosate and its commercial formulations. Nevertheless, a full review of research articles on the effects of glyphosate on the nervous system is still needed.

Therefore, in the present study, we carry out a systematic review of the scientific literature available on the effects and mechanisms of action of glyphosate and its commercial formulations on the nervous system of various animal species and humans. The main objective of this review is to understand the risks arising from increasing exposure to glyphosate residues in the environment and in food.

2. Methodology

The present review was carried out with the aim of unifying the results of the most recent studies on the effects of glyphosate and its commercial formulations on animal health. For this purpose, a systemic review was performed following the guidelines established by the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [44]. Searches were carried out in the specialized databases PubMed, Scopus, and Web of Science in February 2022, with a time restriction limited to studies published in the last ten years.

The following terms referring to glyphosate and its commercial formulations were used to select the scientific articles to be included in this review: “glyphosate”, “Roundup”, and “GBH”. Each of the three terms mentioned above was entered into the databases according to the following search strategies: “((glyphosate OR Roundup OR GBH) AND (nervous system))”; “((glyphosate OR Roundup OR GBH) AND (neurotoxicity))”; “((glyphosate OR Roundup OR GBH) AND (nervous system) AND (effects))”; “((glyphosate OR Roundup OR GBH) AND (nervous system) AND (toxicity))”.

Exclusion and Inclusion Criteria

The articles included in this review met the following inclusion criteria: (1) original studies in article format; (2) in English or Spanish; and (3) studying the effects of the pesticide on the nervous system. Articles were excluded according to the following exclusion criteria: (1) theoretical articles or reviews; (2) studies in which glyphosate was administered in combination with other pesticides; (3) studies evaluating the effects of pesticides other than glyphosate; (4) case studies; and (5) studies that used glyphosate doses above the no-observed-adverse-effect levels (NOAEL) established by regulatory agencies.

As a result of the searches carried out in the three databases, 922 articles published in the last ten years were identified. The articles were then exported to Refworks to eliminate duplicates. In accordance with the inclusion and exclusion criteria, 163 titles and abstracts were screened to verify whether they met the previously mentioned criteria. After this procedure, 112 articles were excluded for the reasons summarized in Figure 2. Ultimately, 51 articles were included in the present systematic review.

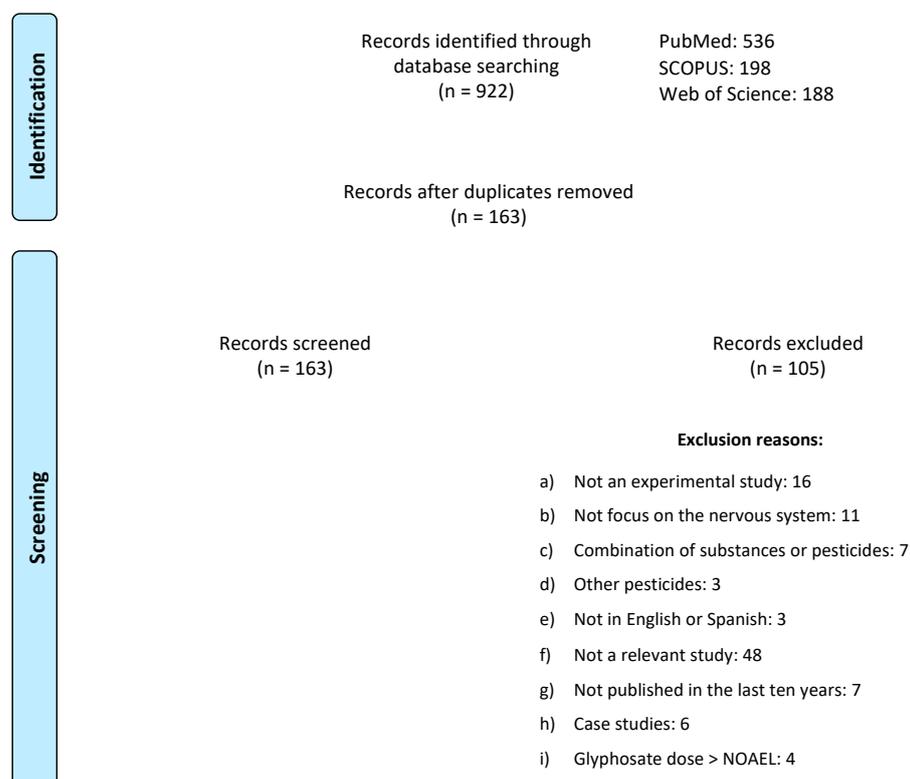


Figure 2. Cont.

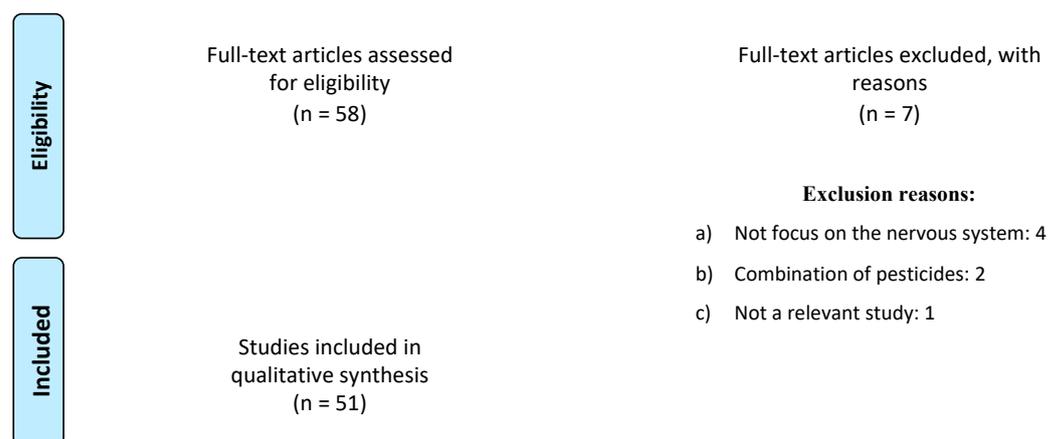


Figure 2. Flow diagram of the systematic search process.

3. Results

3.1. Effects of Glyphosate in Humans

A series of studies show that glyphosate and its commercial formulations can produce detrimental effects on the human nervous system. These investigations have shown that glyphosate can cross and affect the blood–brain barrier (BBB) and cause various types of short-term or long-term disturbances in the human nervous system (Table 1).

Table 1. Studies on the effects of glyphosate and/or its commercial formulations in humans.

Type of Study	Toxic Agent	Exposure Mode/Objectives	Results	Reference
Transversal study	GBH	Occupational exposure	- Positive association between GBH exposure and visual memory impairment	[45]
Prospective cohort study	GBH	Not specified	- ↑ in S100B protein levels in patients with neurological complications - S100B protein was a predictor of neurological complications in GLY-poisoned patients	[46]
Population-based case-control study	GBH	Occupational exposure	- Prenatal and infant exposure increases the risk of autism spectrum disorder - Exposure during childhood appears to increase the risk of developing more severely impaired phenotypes with comorbid intellectual disability	[47]
Cohort study	GBH	Occupational exposure	- GBHs were associated with lower toxicity to farmers' health compared to other non-GBHs	[48]
Cohort study	GBH	Occupational exposure	- No relationship was found between GBH use and peripheral nerve conduction abnormalities in farmers	[49]
In vitro SH-SY5Y cell line	GLY alone or mixed with other formulants: 5.33 to 3.200 µg/mL for 24 h	Investigate whether GBH toxicity is related to formulants	- Inhibition of cell proliferation when GLY was administered with other formulants but not when it was administered alone	[50]
In vitro IMR90-c4 iPSCs line	GLY, AMPA: 0.1 to 1000 µM for 24 or 48 h	Investigate the effect of GLY on the BBB in vitro and compare it with that of AMPA and glycine	- GLY and its metabolite altered the integrity of the BBB - GLY can be released through the BBB - High doses of GLY and AMPA altered glucose uptake by microvascular endothelial cells in the brain and metabolic activity of neurons	[51]
In vitro SH-SY5Y cell line	GLY, AMPA: 0.1 to 20 mM for 48 h	Investigate the effects of GLY and AMPA on oxidative stress, neurodevelopment, and cell death.	- ↓ cell viability and increased leakage of LDH - ↑ production of MDA, NO, and ROS - ↑ caspase-3/7 activity - GLY ↑ the levels of mRNA-Wnt3a, -Wnt5a, and -Wnt7a - GLY positively regulated IL-6 and TNF-α genes - GLY ↑ the expression of CAMK2A and CAMK2B mRNA - GLY and AMPA downregulated the expression of the <i>TUBB3</i> and <i>GAP43</i> genes - GLY and AMPA altered the gene expression of cell death pathways	[52]

Abbreviations: GBH, glyphosate-based herbicide; ↑, increase; S100B, S100 calcium-binding protein B; GLY, glyphosate; iPSCs, induced pluripotent stem cells; AMPA, aminomethylphosphonic acid; BBB, blood–brain barrier; ↓, decrease; LDH, lactate dehydrogenase; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; CAMK2, Ca²⁺/calmodulin-dependent protein kinase 2.

3.1.1. Descriptive and Analytical Studies

Although most of studies in humans mainly describe the consequences of glyphosate poisoning after suicide attempts, it appears that occupational or chronic exposure to this pesticide (via inhalation and dermal routes) may also cause neurotoxic effects. In a study by Fuhrmann et al. [45], the authors describe that glyphosate exposure has been associated with the development of visual memory impairment in Ugandan smallholder farmers. However, other studies have not found an association between occupational exposure to glyphosate and increased risk of health problems, such as nerve conduction abnormalities [48,49]. These results have led some authors to postulate that glyphosate is less toxic to farmers' health than other pesticides. Therefore, future research is needed to follow up and compare the potential toxic effects that agricultural use of different pesticides may exert on human health.

Another consideration about the effects of glyphosate is the fact that its effects do not appear immediately after exposure but one or two days later. In this sense, Lee et al. [46] found that S100 calcium-binding protein B (S100B) could be an important predictor of neurological complications in patients poisoned with glyphosate because its levels were increased in the group that was exposed to this substance and that presented neurological alterations. S100B levels peaked on the second day after exposure, indicating that glyphosate can reach the brain parenchyma and cause maximum brain damage after some time [46].

3.1.2. In Vitro Studies with Human Line Cells

The ability of glyphosate to cross the BBB was also reported in an in vitro study by Martínez and Al-Ahmad [51]. In this study, the authors observed that both glyphosate and its metabolite AMPA can increase BBB permeability, possibly by interfering with the proteins that mediate the hermetic junctions between the endothelial cells that comprise the BBB. This study also showed that glucose uptake by brain endothelial cells increased after exposure to high doses of glyphosate [51]. Glucose is the main source of energy for the brain, and its entry into the central nervous system (CNS) is mediated by microvascular endothelial cells. Therefore, increased availability of glucose after exposure to glyphosate could alter the metabolic activity of neurons, as seen in a study by Martínez and Al-Ahmad [51]. This investigation also documented a decrease in cellular metabolism that did not appear to be related to neurotoxicity, as neurite density and formation were not affected by treatment with glyphosate.

Exposure to glyphosate appears to affect neuronal development in the human CNS, altering the expression of molecules involved in the growth and maturation of neurons. GBH administration has been shown to alter the proliferation of cells in culture, although this effect did not occur when glyphosate was administered alone [50]. In addition, exposure to glyphosate and its metabolite induced a negative regulation in the expression of the *TUBB3* and *GAP43* genes, which are responsible for the synthesis of neuronal cytoskeleton proteins and axonal growth cones, respectively [52].

Likewise, exposure to glyphosate induced an increase in the levels of mRNA-Wnt3a, -Wnt5a, and -Wnt7a, which are also related to the regulation of neuronal development [52]. Given that the components of the Wnt signaling pathways are expressed in a strictly controlled manner during development, their deregulation by glyphosate could promote neuronal morphological defects and cause changes in the correct neuronal development [53,54].

In line with this, there is evidence of the participation of Wnt signaling in various neurocognitive developmental disorders, such as autism [55,56]. Thus, the deregulation of this pathway by glyphosate in human cells in vitro could be related to a higher incidence of developmental and autism spectrum disorders in children whose mothers were exposed to pesticides [57–59], including glyphosate, during pregnancy [47].

Martínez and colleagues [52] also showed that glyphosate increased the mRNA expression of the two isoforms of calcium-calmodulin-dependent protein kinase 2 (CAMK2A and CAMK2B). The product of these genes appears to have a dual role, as, although they

are related to neuronal development and survival, they also contribute to regulation of neuronal death in response to a variety of insults [60,61]. Thus, the increased expression of this glyphosate-induced mRNA could be related to neuronal apoptosis triggered in response to oxidative stress. However, according to a study by Martínez et al. [52], AMPA decreased the expression levels of this mRNA, which led the authors to suggest that glyphosate metabolism could partially prevent neuronal death.

Another effect observed in a study by Martínez et al. [52] is an increase in oxidative stress, evidenced as an increase in the production of reactive oxygen species (ROS) and nitric oxide (NO), as well as lipid peroxidation (LPO). In addition, glyphosate and its metabolite also potentiated an inflammatory response by upregulating the expression of the proinflammatory cytokine interleukin 6 (IL-6) genes and tumor necrosis factor-alpha (TNF- α).

As mentioned previously, injuries caused by glyphosate, such as neuroinflammation or oxidative stress, can cause neuronal death. It was shown that both glyphosate and AMPA reduced the viability of human cells and increased the leakage of lactate dehydrogenase (LDH) [52]. LDH is involved in energy production, is found in almost every organ in the body (including the brain), and, when an organ or tissue is damaged, is released into the blood. Thus, glyphosate-induced LDH increases could be indicative of damage caused by the herbicide in the CNS. Likewise, there was also an upregulation in the gene expression of the different pathways of cell death (apoptosis, autophagy, and necrosis), including caspases 3 and 7, involved in the execution of apoptosis [52]. These results corroborate the possible involvement of mechanisms of apoptosis, autophagy, and necrosis in glyphosate-induced cell death in humans.

3.2. Effects of Glyphosate in Rodents

Several studies show that exposure to glyphosate or GBH produces many toxic effects on both the CNS and peripheral nervous system (PNS) of rodents. The main effects observed include changes in the development of the nervous system and in the neurotransmission systems, as well as oxidative stress and neuroinflammation, processes that lead to neuronal death and the appearance of behavioral changes (Tables 2 and 3). Most of these studies show the neurotoxic effects of glyphosate administered at early ages during the intrauterine period and lactation, although chronic or acute exposure in adulthood also causes important alterations in the function and structure of the nervous system.

Table 2. In vivo effects of glyphosate and/or its commercial formulations in rodents.

Species	Dose and Exposition	Time Exposition	Objectives	Results	Reference
Swiss mice	Roundup [®] : 250 or 500 mg/kg/day orally	Subchronic exposition: 6 weeks Chronic exposition: 12 weeks	Assess the effects of acute or repeated GBH exposure on the developing brain of young and adult mice	Chronic/subchronic exposure: - \downarrow locomotion - \uparrow anxiety, depressive behavior - \downarrow 5-HT immunoreactivity Chronic exposure: \downarrow TH immunoreactivity	[62]
Swiss mice	Roundup [®] : 250 or 500 mg/kg/day orally	Subchronic exposition: 6 weeks Chronic exposition: 12 weeks	Evaluate the effects of GBH on learning and memory functions, AChE activity, and oxidation/antioxidation homeostasis	Chronic/subchronic exposure: - Impaired recognition and retention memory - \downarrow AChE activity - \downarrow SOD and peroxidase activity Chronic exposure caused impairment in working memory	[63]
Swiss mice	Roundup [®] : 250 or 500 mg/kg/day orally	From GD0 to PND21	Evaluate the behavioral (PND5-PND25) and biochemical (PND60) effects of gestational and lactational exposure to GBH on offspring	- Delayed sensorimotor development from PND5 to PND25 - \downarrow locomotion, anxiety, \downarrow sociability, cognitive impairment - \downarrow number of TH ⁺ cells - \downarrow AChE activity - Activation of microglia and astrocytes. \uparrow TNF- α expression and \downarrow BDNF. \uparrow TrkB levels	[64]

Table 2. Cont.

Species	Dose and Exposition	Time Exposition	Objectives	Results	Reference
CF-1 mice	Glifloglex [®] : 50 mg/kg/day intranasally	Three days a week for four weeks	Assess the neurobehavioral effects of repeated intranasal administration of a GBH	<ul style="list-style-type: none"> - ↓ locomotion - ↑ anxiety - Impaired recognition memory 	[65]
Wistar rats	Roundup [®] : 70 mg/kg/day orally	Chronic exposition: from GD5 to PND15. Acute exposition: 30 min in vitro	Determine the neurotoxic effects of GBH on the hippocampal function of immature rats after chronic exposure (pregnancy and lactation) and after acute in vitro exposition.	<p>Acute in vitro exposition:</p> <ul style="list-style-type: none"> - ↑ glutamate release, activation of NMDAR and L-VDCC, and ↑ Ca²⁺ influx - CaMKII and ERK activation - LPO, ↓ GGT and G6PD activity, ↓ GSH content - ↓ cellular viability <p>Acute and chronic exposure:</p> <ul style="list-style-type: none"> - Impaired glutamate metabolism in astrocytes: ↓ reuptake and metabolism, inhibition of glutamine synthetase - ↑ uptake of Ca²⁺ - ↑ accumulation of C-MeAIB 	[37]
Wistar rats	Roundup [®] : 1% in drinking water (0.38% GLY)	Subchronic exposition: from GD5 to PND21. Chronic exposition: from GD5 to PND60.	Investigate the effects of subchronic exposure to GBH on neurochemical and behavioral parameters in immature and adult offspring	<ul style="list-style-type: none"> - ↓ uptake and ↑ glutamate release - Activation of NMDARs and influx of Ca²⁺ - GLY can bind to the glutamate and glycine cavities of NMDAR - ↓ AChE activity in offspring - Depressive behavior - ↓ levels of GSH (acute exposure) - ↓ GST after acute exposure, ↑ after chronic exposure - ↓ SOD and G6PD activity - ERK1/2 overactivation - ↓ NF-κB activation - ↓ levels of S100B protein during development, which later increased in adult offspring 	[3]
Wistar rats	Roundup [®] : 70 mg/kg/day orally	Subchronic exposition: from GD5 to PND15.	Investigate possible biochemical and cell-persistent effects in the brain of adult rats following perinatal exposure to GBH	<ul style="list-style-type: none"> - Changes in the peptide expression in the SN - ↓ expression of peptides from the dynorphin family - ↓ dynorphin immunoreactivity in the SN and hippocampus - ↑ number of nestin-positive hippocampus cells 	[66]
Wistar rats	GLY: 24 or 35 mg/kg intraperitoneally	Dams received injections every 48 h from GD8 to GD20, totaling seven injections over two weeks	Evaluate the neurobehavioral effects of GLY in neonate rats after gestational exposure	<ul style="list-style-type: none"> - Delay in the development of neonatal reflexes in offspring - ↓ locomotion - Learning and memory deficits - Negative regulation of the Wnt5a/CaMKII pathway 	[67]
Sprague-Dawley rats	GLY, Roundup [®] : 5 mg/kg/day orally	From GD10 to PND22	Compare the potential effects of a low dose of GLY and GBH on maternal behavior and maternal neuroplasticity, focusing on the hippocampus and cingulate gyrus	<ul style="list-style-type: none"> - Initial reduction in maternal licking behavior, followed by a subsequent increase - Impairment of neurogenesis and plasticity in the mother's hippocampus - Alteration in synaptophysin expression 	[68]
CF-1 mice	Glifloglex [®] : 50 mg/kg/day intranasally	Four weeks (three injections per week)	Elucidate the mechanisms by which the intranasal administration of a GBH exerts its neuropathological effects	<ul style="list-style-type: none"> - ↓ total thiol content and CAT activity - ↓ expression of ChAT and α7 nAChRs and AChE activity - ↑ number of astrocytes - ↓ GPT and GOT transaminase activity 	[69]
Sprague-Dawley rats	GLY: 50, 100, or 150 mg/kg intraperitoneally	Two weeks (three injections per week)	Assess the integrity of the nigrostriatal and mesolimbic dopaminergic systems and their relationship with spontaneous locomotor activity after repeated or acute exposure to GLY	<ul style="list-style-type: none"> - ↓ locomotion in the short term - ↓ specific binding of an antagonist to dopamine D1 receptors in the short term - ↓ levels of extracellular dopamine in the short term 	[70]

Table 2. Cont.

Species	Dose and Exposition	Time Exposition	Objectives	Results	Reference
ICR mice	Roundup®: 50 mg/kg/day orally	From GD14 to PND7	Assess the miRNA expression patterns in the PFC of mouse offspring after exposure to GBH during pregnancy and lactation	- Dysregulation of 53 miRNAs involved in brain development and in the pathogenesis of non-destructive diseases	[71]
Balb/c mice	Roundup®: 25, 50 or 100 mg/kg orally	Acute exposure	Investigate the behavioral effects induced by acute exposure to a GBH in increasing doses	- ↓ exploratory capacity of females - ↑ immobility time	[72]
Wistar rats	GBH: 2.5, 5, 10, 20 or 40 mM	Single dose	Assess the inhibitory potency of a GBH on AChE activity in rat tissues	- GBH is a weak inhibitor of AChE activity	[73]
Wistar rats	GLY: 35 or 70 mg/kg subcutaneous injection	From PND7 to PND27	Evaluate the effects of glyphosate on hippocampal synapses and cognitive functioning	- Impairment of spatial memory and recognition - ↓ expression of CAMKII - ↓ expression of synaptic proteins	[74]
Wistar rats	GLY: 35, 75, 150 or 800 mg/kg/day orally	Six days	Determine the effects of GLY on the levels of DA, NE, and 5-HT and their metabolites, as well as the turnover in striatum, hippocampus, PFC, hypothalamus, and midbrain.	- Significant dose- and region-dependent decreases in 5-HT, DA, and NE contents - ↑ turnover of 5-HIAA/5-HT in the striatum and DOPAC+HVA/DA in the PFC and hippocampus - ↓ turnover of NE/MHPG in the PFC and hypothalamus	[13]

Abbreviations: GBH, glyphosate-based herbicide; ↓, decrease; ↑, increase; 5-HT, serotonin; TH, tyrosine hydroxylase; AChE, acetylcholinesterase; SOD, superoxide dismutase; GD, gestational day; PND, postnatal day; TNF- α , tumor necrosis factor alpha; BDNF, brain-derived neurotrophic factor; TrkB, tyrosine-related kinase receptor B; NMDAR: N-methyl-D-aspartate receptor; L-VDCC, voltage-dependent calcium channels; CaMKII, Ca²⁺/calmodulin-dependent protein kinase II; ERK, extracellular signal-regulated kinases; LPO, lipid peroxidation; GGT, gamma-glutamyl transferase; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; C-MeAIB, C-methylaminoisobutyric acid; GLY, glyphosate; GST, glutathione S-transferase; NF-kB, nuclear factor-kB; S100B, S100 calcium-binding protein B; SN, substantia nigra; CAT, catalase; ChAT, choline acetyltransferase; nAChRs, nicotinic acetylcholine receptors; GPT, glutamate-pyruvate transaminase; GOT, glutamate-oxaloacetate transaminase; PFC, prefrontal cortex; DA, dopamine; NE, noradrenaline; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MHPG, methoxy-4-hydroxyphenylglycol.

Table 3. In vitro effects of glyphosate and/or its commercial formulations in rodents.

Cellular Line	Dose and Time of Exposure	Objectives	Results	Reference
PC12 cells	GLY: 0, 5, 10, 20, or 40 mM for 12, 24, 48, or 72 h	Investigate the neurotoxicity of GLY in differentiated rat PC12 cells and explore the role of apoptosis and autophagy pathways in toxicity	- ↓ cell viability - Activation of autophagic and apoptotic cell death pathways	[75]
Hippocampal pyramidal cells	GLY: 0.5 or 1 mg/mL for five or ten days	Examine the effects of glyphosate on synapse formation and maturation in the hippocampus	- ↓ dendritic complexity and synaptic column formation and maturation - ↓ synapse formation in hippocampal neurons	[74]
NSC	GLY: 0.1, 700, 7000, or 36,000 μ g/L for 24 h	Understand the effects of two maximum permissible concentrations of GLY on the basic processes of neurogenesis in NSCs of the postnatal mouse subventricular zone.	- ↓ cell viability and induction of cytotoxicity - ↓ cell migration and differentiation - ↓ expression of neuronal and astrocytic genes - ↓ expression of the <i>CYP1A1</i> gene - ↑ expression of the <i>SOD1</i> gene - ↑ Ca ²⁺ signaling	[76]
Astrogloma (C6)	GLY: concentrations from 0 to 160 mM for 24 h	Determine the activity of enzymes related to energy metabolism, as well as parameters of oxidative stress, mitochondrial mass, nuclear area, and autophagy in astrocytes treated with GBH	- ↓ cell viability - ↓ in the activity of the enzymes of the mitochondrial respiratory chain - ↓ CK activity - ↓ mitochondrial mass - ↑ non-protein thiol levels - ↑ autophagic protein levels	[77]

Table 3. Cont.

Cellular Line	Dose and Time of Exposure	Objectives	Results	Reference
Embryonic DRG and pure Schwann cells	GLY, Roundup [®] : 0.0005% and 0.005% for ten days (DRG) or 72 h (Schwann cells)	Investigate the effects of pure GLY and GBH in murine embryonic DRG cultures	- GBH had a demyelinating effect, but this effect was not observed after treatment with GLY - GBH ↑ expression of TNF-α in DRG and in Schwann cells - GBH ↑ NO release in Schwann cells	[78]
Embryonic DRG and pure Schwann cells	GLY, Roundup [®] : doses not specified for ten days (DRG) or 72 h (Schwann cells)	Study and compare the effects of pure GLY and GBH in murine embryonic DRG explant cultures	- GBH had a concentration-dependent demyelinating effect - GBH ↑ TNF-α expression and NO release in Schwann cells	[79]

Abbreviations: PC-12, pheochromocytoma; GLY, glyphosate; ↓, decrease; NSC, neural stem cells; ↑, increase; SOD, superoxide dismutase; GBH, glyphosate-based herbicide; CK, creatine kinase; DRG, dorsal root ganglia; TNF-α, tumor necrosis factor alpha; NO, nitric oxide.

3.2.1. Development of Nervous System

Data from *in vitro* and *ex vivo* studies show that exposure to glyphosate in the early stages of neuronal development induces dysregulation of various signaling pathways and cascades, leading to delayed neuronal differentiation, growth, migration, and myelination processes in both the CNS and PNS during this period [76,78,79]. Coullery et al. [67] showed that glyphosate downregulates the Wnt5a/CaMKII signaling pathway in embryonic hippocampal neurons, a cascade that controls neuronal circuit formation and integration. Similarly, Luna et al. [74] found that glyphosate administered in the early postnatal stage reduced the expression of CAMKII in the hippocampus. Because of this deregulation, glyphosate would alter the process of neuronal differentiation and the subsequent formation of synaptic connections between hippocampal neurons [80]. This was verified by the team of Luna et al. [74], who observed that glyphosate administration was associated with a decrease in dendritic complexity and synapse formation in hippocampal neurons. However, Cattani et al. [37] observed a different effect, as GBH exposure activated the CaMKII pathway. These authors related CaMKII activation to the increase in glutamate-induced Ca²⁺ influx in the hippocampus of GBH-treated animals. The difference between studies may be related to the time of exposure, as Coullery et al. [67] and Luna et al. [74] applied a subchronic treatment with glyphosate, whereas Cattani and colleagues [37] applied a single exposure to GBH *in vitro*.

It was also observed that GBH downregulated the expression of brain-derived neurotrophic factor (BDNF) in the prefrontal cortex (PFC) and hippocampus [64]. BDNF plays an essential role in the processes of neurogenesis, growth, survival, and neuronal plasticity. In the brain, the BDNF binds to tyrosine-related kinase receptor B (TrkB) and activates an intracellular signaling cascade to promote synaptic survival and plasticity [81,82]. In this regard, GBH exposure upregulated TrkB expression in the PFC, which could reflect a compensatory mechanism to balance the reduction in BDNF levels [64].

Exposure to GBH also modified the expression of S100B. *In vitro* exposure to GBH induced a downregulation of this protein during development, but its levels increased in adult offspring after chronic exposure [3]. The S100B protein is mainly found in the glial cells (predominantly astrocytes) and exerts trophic or toxic effects, depending on its concentration [83]. At nanomolar concentrations, S100B acts as a neurotrophic factor that promotes neuronal growth and survival in the developing or injured nervous system. Conversely, at micromolar levels, S100B is thought to stimulate the expression of inflammatory cytokines that cause cell death. Thus, increased levels of this protein are considered a marker of nervous system damage. Down-regulation of S100B levels in the early stages of development could reflect one of the mechanisms by which glyphosate alters proper neuronal development, whereas upregulation of this protein during adulthood could be indicative of damage to the CNS induced by glyphosate.

Nuclear factor kappa B (NF-κB) expression is another parameter modified after GBH exposure [3]. NF-κB proteins represent a family of transcription factors that are expressed in both neuronal and non-neuronal cells. These proteins are involved in a wide variety of

functions, including differentiation, cell survival, synaptic plasticity, or adult neurogenesis, among many others. Exposure to glyphosate during the periods of pregnancy and lactation induced a decrease in NF- κ B activation in the hippocampus of immature rats, indicating that early exposure to this compound could affect neurogenesis and therefore the correct development of CNS [3].

Likewise, Ji et al. [71] demonstrated that GBH exposure modified the expression of numerous microRNAs that are implicated in brain development and in the pathogenesis of various diseases. MicroRNAs are small, non-coding RNAs that are involved in gene silencing and thus can shape the landscape of post-transcriptional gene expression [84]. They are also involved in numerous biological processes, such as early neurogenesis, circuit development, or synaptic plasticity, and their dysregulation is linked to the onset of numerous diseases. In an in vitro study, Masood et al. [76] observed the ability of glyphosate to modify gene expression, as its administration negatively regulated the expression of the cytoprotective *CYP1A1* gene. These results reflect the fact that altered gene expression patterns could be another mechanism underlying glyphosate-induced neurotoxicity.

GBH-induced neurotoxicity in the hippocampus has also been linked to the recruitment of signal transduction pathways, leading to the activation of kinase cascades, including extracellular signal-regulated kinase (ERK) [3,37]. The ERK cascade is involved in the regulation of a wide variety of cellular functions, such as proliferation, differentiation, neuronal survival, and synaptic plasticity [85,86]. However, previous studies have shown that ERK activation can also mediate cell death [87,88]. Specifically, Jiang et al. [89] and Satoh et al. [90] found that activation of this pathway was involved in glutamate-induced neuronal apoptosis. Given that in a study by Cattani et al. [37], it was shown that GBH, in addition to ERK overactivation, also increased glutamate levels, the authors linked the activation of this pathway to increases in glutamate levels and subsequent neuronal death by apoptosis.

The glutamate excitotoxicity and calcium overload observed by Cattani et al. [37] may be related to the decrease in the expression of proteins of the dynorphin family observed later by the same authors [66]. This is because dynorphins are opioid receptor agonists [91], but they can also have other effects, such as the modulation of N-methyl-D-aspartate glutamatergic receptors (NMDAR) in the hippocampus and voltage-gated calcium channels (VDCCs) [92–94], and a decrease in their levels can trigger the previously observed effects on neurotransmission. Furthermore, dynorphins act as protectors of dopaminergic neurons and reduce the progression of Parkinson's disease [95]. Thus, the decrease in dynorphin levels observed by Cattani et al. [66] in the substantia nigra could also be related to increased vulnerability of dopaminergic neurons to environmental pollutants, such as glyphosate.

The previously mentioned alterations could be implicated in the delay in sensorimotor development observed after GBH exposure in a study by Ait-Bali et al. [64]. Specifically, GBH exposure caused a delay in the development of innate reflexes and a deficit in the motor development of the offspring.

However, glyphosate not only appears to affect neural development in immature offspring but could also alter neurogenesis during adulthood. Various brain regions in adult females undergo remodeling during the peripartum period to adapt their behavior to the needs of the offspring [96,97]. One such region is the hippocampus, which has received much attention due to its persistent ability to generate new neurons even in adulthood [98]. It has been shown that glyphosate alone or in formulation affected aspects of neurogenesis in the maternal hippocampus during the postnatal period [68].

3.2.2. Effects on Neurotransmission

Neurotransmission is another fundamental process in the functioning of the nervous system on which glyphosate seems to exert a toxic effect, the glutamatergic system being one of the most affected after exposure to the pesticide. Glutamate is the most abundant excitatory neurotransmitter in the nervous system and is involved in various cognitive functions, such as learning and memory. Under physiological conditions, glutamate is

maintained mainly in the intracellular medium, and only a small fraction exists outside the cells, as an increase in the extracellular levels of this neurotransmitter and its interaction with pre- or postsynaptic receptors can induce neuronal death by excitotoxicity [99–101].

Studies by Ait-Bali et al. [64] and Cattani et al. [3,37] show the effects of early GBH exposure on glutamatergic neurotransmission in immature or adult offspring. Acute or chronic treatment with GBH increased *in vitro* glutamate release, decreased its reuptake by astrocytes, and increased Ca^{2+} influx into hippocampal terminals. Furthermore, GBH increased the expression of NMDAR in both the hippocampus and the PFC [64] and activated these receptors and L-type voltage-dependent calcium channels (L-VDCC) in the hippocampus [3,37]. On the other hand, it was also demonstrated that GBH may alter glutamate metabolism by decreasing the activity of glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) [69]. Furthermore, an increase in glutamine transport into neurons was observed after chronic GBH exposure [37].

On the other hand, both early and late exposure to glyphosate seems to also affect cholinergic neurotransmission. Acetylcholine is a neurotransmitter that plays a central role in learning, attention, and synaptic plasticity [102,103]. In a study by Gallegos et al. [69], it was shown that GBH exposure reduced the number of cholinergic neurons in the medial septum, which was evidenced by a decrease in the expression of choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine. GBH also reduced levels of the $\alpha 7$ -type nicotinic acetylcholine receptor in the hippocampus. These data suggest that exposure to glyphosate alters the functioning of the septo-hippocampal cholinergic pathway, which is involved in the processing of memory [104–106].

Several of the studies reviewed here show that glyphosate or GBH was able to induce a slight decrease in acetylcholinesterase (AChE) activity in different brain areas [3,63,64,69,73]. The effect of glyphosate on AChE activity differs considerably depending on treatment and brain area analyzed, but in all cases, it is observed that, unlike other organophosphate pesticides, glyphosate is a weak inhibitor of AChE. Therefore, concentrations much higher than ambient levels of glyphosate that would be required to produce an effective inhibition of the activity of this enzyme in the brain [73].

Another neurotransmitter system affected by glyphosate exposure is the dopaminergic system. Dopamine is a biogenic amine involved in the modulation of locomotor activity, affectivity, and neuroendocrine communication in the CNS, and its alteration can lead to the development of neurodegenerative disorders, such as Parkinson's disease [106]. Studies by Ait-Bali et al. [62,64], Hernández-Plata et al. [70], and Martínez et al. [13] show the effects of glyphosate on mesocorticolimbic and nigrostriatal dopaminergic neurotransmission. Ait-Bali et al. [62,64] have shown that early or adult exposure to GBH caused a decrease in the number of dopaminergic neurons, observed as a decrease in immunoreactivity for the enzyme tyrosine hydroxylase (TH) in the substantia nigra pars compacta and ventral tegmental area.

Furthermore, in studies by Hernández-Plata et al. [70] and Martínez et al. [13], the authors documented that systemic administration of glyphosate in adult rats significantly decreased total dopamine content, especially in the striatum, PFC, and hippocampus, in addition to increasing dopamine turnover in the PFC and hippocampus. Likewise, Hernández-Plata et al. [70] demonstrated that glyphosate decreased extracellular levels of dopamine and its metabolites in the striatum, as well as the specific binding of dopamine to its D1-type receptor in the nucleus accumbens. Thus, glyphosate could affect mechanisms that regulate the density or affinity of dopaminergic D1 receptors. However, these alterations in dopaminergic neurotransmission appear to occur when a critical concentration of glyphosate is present in the system but disappear over time. Taken together, the results of these studies seem to indicate that glyphosate exposure produces short-term effects on dopaminergic neurotransmission in various brain regions.

Other alterations in neurotransmitter processes documented after exposure to glyphosate or GBH include a decrease in the number of serotonergic neurons and in the contents of noradrenaline and serotonin in various brain areas [13,62]. Both serotonin and noradrenaline

play a fundamental role in the modulation of mood and emotions, so alterations in these systems induced by glyphosate could favor the onset of depression or anxiety, according to observations in some studies analyzed herein [3,62,64,65].

Another effect observed on neurotransmitter processes is the alteration of synaptophysin expression after perinatal exposure to glyphosate or GBH. Specifically, an increase in synaptophysin expression was observed in the dentate gyrus and CA3 regions of the hippocampus, while its expression was downregulated in the cingulate gyrus [68]. Synaptophysin is a membrane protein present on the surface of synaptic vesicles that is expressed in most CNS neurons [107]. Glyphosate-induced changes in the expression of this protein could indicate its ability to alter neurotransmitter release and metabolism in various brain regions.

3.2.3. Effects on Behavior

Exposure to glyphosate also induces important changes in rodent behavior, possibly because of alterations in neurotransmission. Early or late exposure to glyphosate or GBH has been shown to cause a decrease in locomotion, which could be associated with the changes in the dopaminergic system discussed previously [62,64,65,67,70]. Furthermore, an increase in anxiety levels and depression-like behavior of the animals was also observed [3,62,64,65]. In general, alterations in mood could arise because of the decrease in serotonin and noradrenaline levels caused by glyphosate. Likewise, cognitive functioning also seems to be affected by the action of this compound. In this regard, it was shown that exposure to GBH or glyphosate caused an impairment in learning and memory processes, which could be due to alterations in the functioning of the cholinergic system [63,65,67,74].

However, early exposure to glyphosate not only affects offspring behavior but also maternal behavior. Glyphosate and GBH treatments altered maternal licking behavior toward pups [68]. The changes induced by glyphosate in maternal behavior could favor the subsequent development of behavioral alterations in the offspring, such as the decreased social activity in adult mice observed in a study by Ait-Bali et al. [64].

3.2.4. Induction of Oxidative Stress and Inflammation

Many of the most widely used pesticides worldwide exert their neurotoxic effects through oxidative stress mechanisms. Oxidative stress occurs when there is an imbalance between the production of ROS and the antioxidant capacity of the system responsible for detoxifying these reactive products. Consequently, oxidative damage of essential biomolecules, such as proteins, lipids, and DNA, occurs. The nervous system is particularly vulnerable to oxidative damage, mainly due to its low level of antioxidant activity, high oxygen requirement, and high lipid composition [108,109].

Glyphosate has been shown to induce oxidative stress immediately after administration, evidenced by an increase in LPO, which is induced by free radical action [37]. Oxidative stress induced by glyphosate exposure was also evidenced by alterations in protein and non-protein thiol concentrations [69,77]. Thiols are powerful antioxidants with the capacity to protect the organism from oxidative attack, and changes in their levels are used as indicators of the antioxidant status of organisms [110,111].

The enzymatic antioxidant defense system of living cells constitutes an adaptive mechanism in which the activities of the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) stand out [108,112]. Studies have shown that glyphosate can alter the activity of these enzyme systems in the nervous system of intoxicated animals. Decreases in SOD, CAT, and peroxidase activity [3,63,69], as well as increases in GPx activity and SOD expression [76] have been observed. In this regard, it is important to note that any change in the expression or activity of antioxidant enzymes, whether increased or decreased, is indicative of oxidative stress [113].

The oxidative damage induced by glyphosate was also confirmed by the inhibition of the enzymatic activity of gamma-glutamyl transferase (GGT) and glucose-6-phosphate dehydrogenase (G6PD), which are involved in the synthesis and reduction of glutathione

(GSH), respectively [3,37]. GSH is one of the most efficient intrinsic antioxidants in the brain, and its decreased availability markedly promotes the production of free radicals that enhance oxidative damage [114,115]. The ability of GBH to decrease GSH content after acute exposure has been documented, although the change was not sustained over time [3,37]. This inhibitory effect was also observed for glutathione-S-transferases (GSTs), which are involved in catalyzing the conjugation of GSH to various hydrophobic and electrophilic substrates, with the aim of protecting cells from oxidative attack [116]. Therefore, it is possible that after the initial depletion of GSH, the organism undergoes various adaptive modifications to restore the levels of this antioxidant to normal concentrations in order to mitigate the neurotoxic consequences of exposition to glyphosate.

On the other hand, the main source of ROS inside cells is the mitochondria, organelles whose function is also severely altered under oxidative stress conditions. In this regard, Da Silva et al. [77] showed that *in vitro* exposure to GBH in astroglomas inhibited the activity of mitochondrial respiratory chain enzymes and creatine kinase (CK), an enzyme related to energy metabolism in nervous tissue. The effect of glyphosate on mitochondrial functioning and cell survival was previously demonstrated in a study by Astiz et al. [117]. These authors demonstrated that glyphosate alone or in combination with other pesticides induced loss of mitochondrial membrane potential and reduced concentrations of cardiolipin, a phospholipid involved in the electron transport chain that is highly vulnerable to oxidative stress due to its richness in fatty acids, leading to increased LPO and neuronal death in the substantia nigra. Taken together, these results suggest that glyphosate and GBH can severely alter the functioning of mitochondria and consequently cause their elimination by mitophagy, as evidenced by the loss of mitochondrial mass observed by Da Silva et al. [77]. It would be interesting for future research to clarify whether mitochondrial dysfunction is a cause or a consequence of oxidative stress caused by glyphosate.

Neuroinflammation appears to be another process contributing to neurotoxicity induced by glyphosate. The inflammatory reaction plays a healthy role in helping the immune system cope with certain pathological conditions, but when this reaction becomes unbalanced or prolonged over time, it can damage the CNS [118]. During the inflammatory process, activation of microglia and astrocytes occurs, which release a variety of molecular signals, such as TNF- α , that contribute to the inflammatory state of the CNS [119,120]. Furthermore, during the process, activated glial cells expressing the enzyme nitric oxide synthase (NOS) can generate excessive amounts of NO, a molecule that leads to the formation of reactive nitrogen species that promote oxidative damage in the brain [121].

In this regard, the data analyzed in the present review show that exposure to glyphosate or GBH produces some proinflammatory effects measured as increases in the number and activation of microglia and astrocytes and increased expression of TNF- α in the CNS of mice [64,69], as well as increased concentrations of NO in murine PNS [78,79].

3.2.5. Induction of Apoptosis and Autophagy

All of the neurotoxicity mechanisms triggered by glyphosate discussed previously could ultimately lead to neuronal death. Several studies have shown that exposure to glyphosate at any life stage reduces cell viability [37,75–77]. Specifically, it has been shown that both apoptosis and autophagy processes could be involved in the glyphosate-induced decrease in neuronal viability and neuronal death in rodents.

Apoptosis is a form of programmed cell death in which cells induce their own death through different pathways when subjected to certain types of stimuli. When the mitochondrial apoptotic pathway is activated, the Bax protein, which promotes neuronal death, inserts into the mitochondrial membrane and enables the release of cytochrome C into the cytoplasm, which ultimately leads to cell death [122]. In line with this, it has been observed that glyphosate treatment led to an increase in Bax protein expression while reducing levels of the anti-apoptotic protein Bcl-2 [75]. Bcl-2 promotes cell survival and inhibits the action of apoptotic proteins [123–125]. These data show the pro-apoptotic action of glyphosate in rat PC12 cells.

On the other hand, autophagy constitutes a mechanism for the turnover or destruction of dysfunctional or unnecessary cytoplasmic components within cells [126]. In a study by Gui et al. [75], it was observed that glyphosate treatment increased the concentration of the lipidated form of microtubule-associated protein 1A/1B light chain 3 (LC3-II), which is used as a marker for autophagosomes. The authors hypothesize that it is possible that glyphosate-induced autophagy is related to the need to remove cellular components damaged by this compound, such as mitochondria. Furthermore, these results were corroborated by the glyphosate-induced increase in the expression of the gene encoding Beclin-1, a protein that acts as a potential regulator of both [75].

3.3. Effects of Glyphosate in Fish

The zebrafish (*Danio rerio*) is a small freshwater teleost fish classically used as an experimental model for biomedical research and aquaculture breeding. After the mouse, the zebrafish has become one of the most widely used animal models for the study of various types of alterations in the nervous system, such as neurodegenerative and motor diseases, as well as for the study of neurotoxicity of environmental pollutants [127–129]. The main advantages of zebrafish, besides the high fecundity rate, rapid development, and low cost, are the organization of its nervous system, which is very similar to that of the human nervous system, and the similarity between the neurotransmitter systems [130,131].

Concerning the effects of glyphosate on fish, many of the studies included in this review describe the toxic effects of glyphosate or GBH on the nervous system of zebrafish, although the effects on other fish species have also been analyzed. Consistent with observations in for rodents, the analyzed studies show that glyphosate mainly affects nervous system development, neurotransmission, behavior, and energy metabolism, as well as producing oxidative stress and inflammation. The effects of glyphosate on fish are described in Table 4.

Table 4. Effects of glyphosate and/or its commercial formulations in fish.

Species	Dose and Time Exposure	Objectives	Results	Reference
<i>Cnesterodon decemmaculatus</i> (Ten-spotted livebearer)	GLY: 1 or 10 mg/L for 96 h	Assess the effect of seasonal variability on AChE activity in fish exposed to chlorpyrifos and GLY	<ul style="list-style-type: none"> - ↓ AChE activity dose-dependently (all seasons) - Fish were more susceptible to GLY in autumn - No inhibitory effect on AChE was observed when in vitro tests were performed at a wide range of GLY concentrations 	[132]
<i>Colossoma macropomum</i> (Blackfin pacu)	Roundup®: 10 or 15 mg/L for 96 h	Investigate the effects of GBH on gill morphology and function, hematological parameters, biotransformation enzymes, the antioxidant system in the gills and liver, as well as on both neurological and erythrocytic DNA damage	<ul style="list-style-type: none"> - ↓ AChE activity 	[133]
<i>Danio rerio</i> (Zebrafish)	Roundup®, GLY: 0.01, 0.065, or 0.5 mg/L for 96 h	Evaluate the effects of GLY and GBH on morphological and behavioral parameters in larvae and adult zebrafish	GLY and GBH caused: <ul style="list-style-type: none"> - Alteration of locomotion and aversive behavior in larvae - ↓ locomotion and aggressive behavior in adults - GBH caused memory impairment in adults 	[134]
<i>Danio rerio</i> (Zebrafish)	GLY: 5, 10, or 50 µg/mL for 96 h	Identify a possible mechanism of toxicity for GLY related to changes in microtubule stability, which could alter the distribution and dynamics of cytoskeletal components	<ul style="list-style-type: none"> - GLY (50 µg/mL) ↓ levels of acetylated α-tubulin - GLY (10 and 50 µg/mL) ↓ percentage of polymeric tubulin There was no impairment of the stability of the actin filaments or the expression patterns of α-tubulin	[135]
Pintado da Amazônia	Roundup®: 0.37, 0.75, 2.25, 4.5, 7.5, 11.25, 15, 22.5, or 30 mg/L for 24, 48, 72, or 96 h	Evaluate the lethal concentration of the GLY and the oxidative stress parameters in tests with sublethal concentrations	<ul style="list-style-type: none"> - ↑ content of carbonyl protein - Brain LPO levels remained unchanged - ↑ brain AChE activity 	[136]

Table 4. Cont.

Species	Dose and Time Exposure	Objectives	Results	Reference
<i>Danio rerio</i> (Zebrafish)	GLY: 0.3 or 3 µg/L for 2 weeks	Analyze the neurotoxicity of GLY in adult zebrafish after exposure through water to environmentally relevant concentrations	<ul style="list-style-type: none"> - Deterioration of exploratory and social behaviors, with increased anxiety - ↑ levels of 5-HT in the anterior brain - ↑ DA, DOPAC, and HVA in the anterior brain; ↑ DOPAC/DA and HVA/DA turnover - Downregulation of the expression of genes involved in the dopaminergic system: <i>th1</i>, <i>th2</i>, <i>comb</i>, and <i>scl6a3</i> - ↑ LPO and alteration of brain antioxidant status: ↑CAT and SOD activity and ↓ GSH 	[130]
<i>Danio rerio</i> (Zebrafish)	GLY: 0.05 to 10.000 µg/L for a period of 1.5 to 120 h after fertilization	Explore the effects of the use of different concentrations of GLY on anatomy and behavior of fish	<p>High concentrations of GLY (≥1000 µg/L) caused:</p> <ul style="list-style-type: none"> - Electrophysiological changes in the midbrain and ↓ locomotion <p>High and low concentrations of GLY were associated with:</p> <ul style="list-style-type: none"> - Morphological signs of microglia activation - Dysregulation of genetic pathways involved in neuronal physiology, synaptic transmission, and inflammation - Absence of neurovascular structural malformations 	[137]
<i>Hypomesus transpacificus</i> (Delta smelt)	Roundup®: 0.064, 0.64, 6.4, 64, or 640 mg/L for 6 h	Compare the sublethal toxicity of four herbicides (penoxsulam, imazamox, fluridone, and GBH).	<ul style="list-style-type: none"> - GBH did not cause alterations in AChE activity 	[138]
<i>Cyprinus carpio</i> (European carp)	GLY: 0.02, 0.05, 0.07, or 0.1 mg/L for 24, 48, 72, or 96 h	Analyze the effect of GLY and atrazine on the hematological and biochemical parameters of blood and on behavioral aspects	<ul style="list-style-type: none"> - GLY caused behavioral disturbances 	[139]
<i>Danio rerio</i> (Zebrafish)	Roundup®: 2, 5, or 8.5 µg/mL for 72 h	Investigate the lethal and sublethal developmental effects, neurotoxic potential, and oxidative stress responses after GBH exposure	<p>High concentrations of GBH caused:</p> <ul style="list-style-type: none"> - Developmental toxicity - Malformations <p>Low concentrations of GBH:</p> <ul style="list-style-type: none"> - Did not have teratogenic effects and did not alter oxidative stress, neurotransmission, or the regulation of energy metabolism - Did not cause histopathological changes in the brain 	[140]
<i>Danio rerio</i> (Zebrafish)	Roundup®: 1, 2, or 5 µg/mL for 72 h	Assess GBH effects at environmentally relevant concentrations through a set of behavioral patterns	<ul style="list-style-type: none"> - Did not alter exploratory or social behavior but did induce changes in avoidance behavior <p>Cortisol levels were not altered</p>	[141]
<i>Carassius auratus</i> (Goldenfish)	Nongtेशi®: 0.22, 0.44, or 0.88 mmol/L for 96 h	Investigate the toxic effects of GBH exposure using a metabolomic approach supplemented with histological inspection and hematological evaluation	<ul style="list-style-type: none"> - Destruction of the microscopic structure of the brain - High concentrations of GBH caused behavioral disturbances - Alteration of the balance of neurotransmitters: ↓ glutamate and GABA levels, ↑ glutamine levels - ↓ levels of the glial marker myoinositol and NAA - ↓ levels of brain creatine/phosphocreatine 	[142]
<i>Carassius auratus</i>	Nongtेशi®: 0.2 mmol/L for 90 days	Assess GBH toxicity after prolonged exposure	<p>Results in the brain:</p> <ul style="list-style-type: none"> - ↓ choline, phosphocholine, and betaine levels - ↑ 3-hydroxybutyrate levels - ↓ AMP levels - ↓ succinate and citrate - Positive correlations between choline and phosphocholine, choline and betaine, glycine, and sarcosine 	[143]

Table 4. Cont.

Species	Dose and Time Exposure	Objectives	Results	Reference
<i>Danio rerio</i> (Zebrafish)	GLY: 5 or 10 mg/L for 24 and 96 h	Evaluate oxidative stress parameters, as well as the activity and expression of AChE	<ul style="list-style-type: none"> - ↓ brain LPO 24 h after exposure to GLY (10 mg/L) - No alterations in the generation of ROS - No alterations in AChE activity were detected <p>AChE gene expression in the brain decreased after 24 h for both GLY concentrations and improved 96 h after exposure to 10 mg/L GLY</p>	[144]
<i>Cnesterodon decemmaculatus</i>	GLY: 1, 17.5, or 35 mg/L for 96 h	Assess the toxic effect of acute exposure to sublethal GLY concentrations on AChE activity in different parts of the body	<ul style="list-style-type: none"> - ↓ AChE activity in the anterior and middle sections of the body but not in the posterior section 	[145]
<i>Odontesthes bonariensis</i> (Argentinian silverside)	GBH: 1 or 10 mg/L for 15 days	Determine the basal levels of adenylates, phosphagens, and the AEC index in the brain, muscle, and liver, as well as the impact of exposure to sublethal GBH on the subcellular energy balance	<ul style="list-style-type: none"> - There was no alteration of cerebral AEC 	[146]
<i>Danio rerio</i> (Zebrafish)	GBH, GLY: 0.065, 1, 10, 160, 1.6×10^3 , 4×10^3 , or 8×10^3 mg/L for 3 h (in vitro) or for 7 days (in vivo)	Investigate the neurotoxic effects of GBH by focusing on acute toxicity, activity, and transcription levels of mitochondrial respiratory chain complexes, mitochondrial membrane potential, reactive species formation, and behavioral repertoire	<p>In vivo exposure to GBH (7 days) caused:</p> <ul style="list-style-type: none"> - Modulation of gene expression related to mitochondrial complexes - Increased ROS production - Mitochondrial hyperpolarization in brain cells - Behavioral disturbances <p>Low concentrations of GBH (0.065, 1 mg/L) caused:</p> <ul style="list-style-type: none"> - Inhibition of NADH dehydrogenase and cytochrome C 	[147]
<i>Danio rerio</i> (Zebrafish)	Roundup [®] , GLY: 50 µg/mL for 24 h	Investigate the neurotoxic effects of GBH and GLY exposure on the developing brain	<p>Both GBH and GLY caused:</p> <ul style="list-style-type: none"> - Loss of delineated cerebral ventricles and reductions in the cephalic and ocular regions <p>↓ gene expression in the forebrain, midbrain, and eye, but no changes were detected in the rhombencephalon</p>	[148]
<i>Jenynsia multidentate</i> (Onesided livebearer)	Roundup [®] (Original, Transorb or WG): 0.5 mg/L for 96 h	Evaluate and compare the effects of three GBH formulations on behavior patterns	<p>Roundup WG[®] was the most harmful formulation and negatively affected:</p> <ul style="list-style-type: none"> - Social interaction - Space exploration and swimming performance - Long-term memory consolidation <p>Roundup Transorb[®] had more severe effects on sexual behavior</p>	[149]
<i>Rhamdia quelen</i> (Silver catfish)	GLY: 6.5 mg/L for 12, 24, 48, or 72 h	Investigate the effects of GLY on the antioxidant system, as well as the neurotoxic effects on eggs and larvae	<ul style="list-style-type: none"> - ↑ AChE activity at 12 and 48 h of treatment, ↓ between 48 and 72 h - There were no changes in CAT or GST activity - ↑ activity of the GR at 12 and 24 h - ↑ LPO at 48 h of treatment 	[150]
<i>Danio rerio</i> (Zebrafish)	GLY: 0.01, 0.1, 0.5, 1, 5, 10, 100, 200, 400, or 600 mg/L from 3 hpf until 96 hpf	Assess the developmental, morphological, and genetic effects of GLY in zebrafish embryos	<ul style="list-style-type: none"> - Concentrations of GLY higher than 100 mg/L caused delay and alterations in development, as well as embryonic death - Damage to axons of primary caudal motor neurons in embryos and increased locomotion in larvae 	[151]

Abbreviations: GLY, glyphosate; ↓, decrease; AChE: acetylcholinesterase; GBH, glyphosate-based herbicide; ↑, increase; LPO, lipid peroxidation; 5-HT, serotonin; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; GABA, gamma-aminobutyric acid; NAA, N-acetyl-L-aspartate; AMP, adenosine monophosphate; ROS, reactive oxygen species; AEC, adenylate energy charge; NADH, nicotinamide adenine dinucleotide dehydrogenase; GST, glutathione S-transferase; GR, glutathione reductase; hpf, hours post fertilization.

3.3.1. Development of Nervous System

Early exposure to glyphosate or GBH causes dysregulation of genetic pathways directly involved in neuronal physiology and synaptic transmission in zebrafish, especially affecting the development of the forebrain, midbrain, and eye structure [137,148]. Dysregulation of these pathways can lead to the appearance of different alterations and malformations in the brain, such as those observed in the studies of Lanzarin et al. [140], Roy et al. [148], and Zhang et al. [151].

Likewise, it has also been shown that treatment with high doses of glyphosate during development can alter the dynamics and structure of the components of the cell cytoskeleton [135]. Specifically, exposure to glyphosate reduced the levels of acetylated α -tubulin in the polymeric fraction of zebrafish embryos, suggesting a decrease in microtubule stability.

3.3.2. Effects on Behavior

The use of the zebrafish model to evaluate behavioral alterations provides a series of very relevant information for the investigation of the effects of environmental toxicants on the nervous system. Half of the studies reviewed herein reported glyphosate-induced behavioral alterations in these animals. In a study by Zhang et al. [151], an increase in locomotor activity of larvae during the day was observed, which, according to the authors, could be due to damage in the axons of primary motor neurons caused by glyphosate. In contrast, in the investigations of Forner-Piquer et al. [137] and Bridi et al. [134], early exposure to glyphosate or GBH caused a decrease in locomotion in both larvae and adults. It is possible that this apparent contrast is due to the difference in doses and exposure times used in each study. An increase in locomotion was documented by Zhang et al. [151] when exposing animals to the highest doses used (100–600 mg/L), whereas Forner-Piquer et al. [137] and Bridi et al. [134] used doses in the 0.05 to 10 mg/L range.

Another change observed after treatment with glyphosate and GBH was a decrease in aggressive behavior [134], which could be related to alterations in serotonergic neurotransmission [152]. Likewise, in a study by Faria et al. [130], glyphosate exposure caused impairment of exploratory and social behavior consistent with increased anxiety. However, the team of Lanzarin et al. [141] did not find such an association between GBH treatment and alterations in exploratory behavior and anxiety, the latter measured by alterations in hormone cortisol. Exposure to glyphosate also induced avoidance in the presence of aversive stimulus [134,141]. These results are likely related to the decreased exploratory ability observed in fish after glyphosate exposure. Finally, the occurrence of memory impairment in zebrafish after exposure to GBH was also documented, which could be related to an incorrect functioning of the cholinergic system in these animals [134].

3.3.3. Effects on Neurotransmission

Several of the studies included herein investigated glyphosate-induced changes in AChE activity in various species, and the data reflect a heterogeneity of effects. Sobjak et al. [150] observed that exposure of eggs and larvae of silver catfish (*Rhamdia quelen*) to glyphosate caused an unexpected increase in AChE activity at 48 h after exposure, followed by a decline at 96 h. These results suggest that exposure to glyphosate during early developmental stages may cause an early induction of AChE activity and a subsequent difficulty in maintaining elevated AChE activity at later times.

On the other hand, regarding the effects of glyphosate on AChE in young and adult fish, several studies support its inhibitory potential against the activity of this enzyme [132,133,145]. This enzymatic inhibition would affect the anterior and middle regions of the fish body and seems to depend on the concentration and time of year, being more potent in autumn [132,145]. In contrast, in a study by Teixeira et al. [136], an increase in brain AChE activity was observed, whereas Jin et al. [138] and Lopes et al. [144] did not detect any alteration in its activity level. The considerable variability of these results is probably due to the wide diversity of species and doses of glyphosate used in each of the investigations.

Another of the changes observed is an alteration of the balance between glutamine, glutamate, and the amino acid gamma-aminobutyric acid (GABA), which are fundamental components of brain metabolism and function. Glutamine is produced from glutamate reuptake by astrocytes, and although it does not fulfill a neurotransmitter function, it is the main precursor for glutamate and GABA synthesis [153]. In this regard, Li et al. [142] observed that GBH exposure reduced both GABA and glutamate, resulting in an elevation in brain glutamine levels and the subsequent appearance of various behavioral alterations.

Furthermore, Faria et al. [130] documented an increase in the content of dopamine and its acidic metabolites (3,4-dihydroxyphenylacetic acid, DOPAC, and homovanillic acid, HVA) and in dopamine turnover in the forebrain. However, no variations were found in the levels of 3-methoxytyramine, an extracellular metabolite of dopamine. Therefore, because DOPAC is an intracellular metabolite and HVA reflects both intra- and extraneuronal metabolism, the changes observed after glyphosate exposure suggest that it could increase the intraneuronal metabolism of dopamine but without inducing increased release of the neurotransmitter. Glyphosate also downregulated the expression of several genes involved in dopamine synthesis, degradation, and transport [130]. In this same study, the authors also reported that glyphosate exposure increased serotonin levels in the forebrain, which could influence the mood and behavior of the animals.

The alterations in neurotransmission could explain the behavioral alterations observed by Sánchez et al. [149] in one-sided livebearer (*Jenysia multidentate*). In this study, exposure to 0.5 mg/L GBH for 96 h was associated with a decrease in spatial exploration and swimming performance, as well as impaired long-term memory consolidation. Furthermore, these authors also observed a negative effect of GBH on social interaction and sexual behavior of fish.

3.3.4. Induction of Oxidative Stress and Inflammation

As previously discussed, many of the most widely used pesticides exert their neurotoxicity by altering the balance between ROS production and the ability of the organism's antioxidant system to neutralize them. In this regard, Faria et al. [130], Pereira et al. [147], and Sobjak et al. [150] have documented the ability of glyphosate and GBH to increase ROS concentrations in the nervous system of fish, as well as the LPO caused by them. The exception is the work of Lopes et al. [144], wherein a decrease in LPO levels was observed in zebrafish after exposure to glyphosate. Although the team of Teixeira et al. [136] found no changes in LPO levels after treatment of pintado da Amazônia fish with sublethal doses of GBH, they did observe an increase in carbonyl protein content. These data suggest the existence of an alteration in normal protein metabolism that can be used as a marker of protein oxidative damage.

Mitochondria are cellular organelles that contribute considerably to ROS production and thereby to oxidative stress. In a study by Pereira et al. [147], in vivo exposure to GBH induced mitochondrial dysfunction in brain cells after 7 days of treatment, evidenced by a decrease in the activity of the nicotinamide adenine dinucleotide (NADH) dehydrogenase and cytochrome C, enzymes of mitochondrial complexes I and IV, respectively. These alterations were also associated with changes in the transcription of genes encoding electron transfer proteins in the mitochondrial respiratory chain and a subsequent hyperpolarization of the mitochondrial membrane potential. In this regard, when the mitochondrial membrane potential is high, the mitochondrial respiratory chain becomes a producer of ROS, which poses a serious risk to cell integrity [154].

GBH exposure also caused a decrease in the levels of choline; its derivative, phosphocholine; and its metabolite, betaine, in the brain. Choline constitutes an essential component of different phospholipids of lipid bilayers and is necessary for the maintenance of structural integrity of cell membranes [155]. Therefore, because cell membrane phospholipids are the main target of oxidative attack, the decrease in levels of choline and its derivatives after glyphosate exposure could be indicative of an accelerated use of these components to repair cell membranes impaired by free radicals.

Another marker of oxidative stress induced by exposure to neurotoxic agents is the alteration in the activity of enzymes responsible for scavenging free radicals and restoring the normal antioxidant status of nerve tissue. It has been documented that exposure to glyphosate induced an increase in the activity of the antioxidant enzymes CAT, SOD, and glutathione reductase (GR), with a concomitant decrease in GSH reserves, which could reflect an attempt by the cells to neutralize the excessive levels of ROS caused by glyphosate [130]. However, Sobjak et al. [150] found no changes in CAT and GST activity in the brain of silver catfish after exposure to glyphosate.

Glyphosate exposure also appears to induce an inflammatory reaction in the nervous system of zebrafish. This was demonstrated by a study by Forner-Piquer et al. [137], who administered concentrations of 0.05 and 10.000 µg/L glyphosate and detected the presence of amoeboid cells, suggesting microglial activation and therefore activation of the inflammatory process. In addition, transcriptomic analysis revealed dysregulation of genetic pathways involved in inflammation. In contrast, Li et al. [142] demonstrated that exposure of goldfish (*Carassius auratus*) to 0.22, 0.44, or 0.88 mmol/L of GBH induced a decrease in the levels of myoinositol, a compound that is mainly present in glial cells and is therefore considered a glial marker [156].

3.3.5. Effects on Energy Metabolism

The effect of glyphosate on brain energy metabolism is an aspect not addressed in studies in other species analyzed so far, although the available evidence supports the potential of pesticides to interfere with energy production [157]. Glucose is the main source of energy in the brain, and its catabolism via glycolysis results in pyruvate, which is subsequently transferred to mitochondria to produce adenosine triphosphate (ATP) via the tricarboxylic acid (TCA) cycle and the mitochondrial respiratory chain [158]. GBH exposure was shown to deregulate glucose metabolism in the goldfish brain, which was evidenced by decreased succinate and citrate concentrations [143]. Because both are intermediates of the TCA cycle, the reduction in their levels suggests that glyphosate may block the TCA cycle and thereby generate an insufficient amount of ATP.

To survive this energy crisis, the body can use additional resources of energy production. For example, creatine and phosphocreatine are important energy metabolites because phosphocreatine hydrolysis donates a phosphate group to adenosine diphosphate (ADP) to synthesize ATP [159]. In this regard, a decrease in creatine and phosphocreatine levels was observed in the brain of fish dosed with GBH, suggesting the activation of the phosphocreatine reaction with ADP in order to supply the need of ATP [142]. Likewise, Li et al. [143] observed increased levels of ketone 3-hydroxybutyrate in the brain tissue of GBH-treated goldfish. In the brain, ketones are the main alternative fuel to glucose, so they could be used to meet the insufficient energy supply caused by glyphosate exposure [160].

When glucose is unable to maintain brain homeostasis, other substrates, such as acetate, can also be used as metabolic precursors [161]. In line with this, Li et al. [142] found that GBH exposure induced a considerable reduction in brain levels of the amino acid N-acetyl-L-aspartate (NAA), the second most abundant amino acid in the CNS, which can be degraded to aspartate for energy production [162].

On the other hand, adenosine monophosphate (AMP)-activated kinase is the main sensor and regulator of cellular energy homeostasis. Under low-energy conditions, this kinase is activated and inhibits ATP-using (anabolic) processes while stimulating ATP-producing (catabolic) processes [163]. Therefore, the reduction in AMP levels in brain tissues of fish exposed to GBH described by Li et al. [143] may affect the AMP-activated kinase pathway and thus alter downstream metabolic processes. In contrast to these results, in a study by Menéndez-Helman et al. [146], GBH treatment did not produce alterations in adenylate energy charge (AEC), a measure reflecting the amount of energy available from the adenylate pool (ATP, ADP, and AMP).

Overall, the changes at the molecular level induced by glyphosate discussed previously can lead to the manifestation of important alterations both in structure and in brain function.

This is clearly reflected in the destruction of the microscopic structure of the brain or in the appearance of abnormal and variable brain activity in the midbrain of fish exposed to glyphosate alone or in formulation [137,141]. Nevertheless, when considering these studies, the heterogeneity of the obtained results is evident, which, as mentioned above, may be due to the use of different species, as well as to treatment with doses and exposure periods that differ considerably. This makes it difficult to draw rigorous conclusions and demonstrates the need for more research in this area.

3.4. Effects of Glyphosate on Invertebrates

The number of recent studies on the effects of glyphosate on the nervous system of invertebrates is limited. Two studies were identified with the worm *Caenorhabditis elegans* describing that GBH also produces neurotoxicity in this species, which was evidenced by altered neuronal development, mitochondrial damage, oxidative stress, and in behavioral patterns.

On the one hand, it has been shown that GBH treatment affected cell development in worms, which was evidenced by a decrease in the number and size of dopaminergic neurons from the fourth larval stage onwards [164]. These authors also observed that GBH caused a marked increase in superoxide levels during the fourth larval stage [164] and hydrogen peroxide in treated worms [165]. As has been observed in rodents, GBH exposure also affects mitochondrial function in these animals. In addition, Burchfield et al. [165] showed that glyphosate induced inhibition of mitochondrial complex II, and consequently, a decrease in ATP levels occurred.

4. Discussion

4.1. Overview of the Main Mechanisms of Action of Glyphosate on the Nervous System

A wide variety of studies show the correlation between pesticide exposure and the development of various types of diseases. Organophosphate exposure has been reported to be associated with various human conditions, such as mood disorders, attention deficit hyperactivity disorder, cancer, kidney damage, and autism, among others [166–169]. Furthermore, it has been postulated that pesticides may be the main environmental factor associated with the etiology of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease [59,170].

Regarding glyphosate, some previous studies have reported DNA damage in human and rodent cells after exposure to this compound and GBH [171–173]. The teratogenic action of GBH in vertebrates was also described [174]. Likewise, human clinical reports on the effects of intoxication with glyphosate formulations have described harmful effects on the nervous system, including parkinsonism [175].

The data analyzed in the present review show that exposure to glyphosate or GBH generates various types of neurotoxic effects in all the species studied (Figure 3). The study of glyphosate effects in humans is based on the observation of clinical signs and symptoms in people accidentally or intentionally exposed to the pesticide or from in vitro studies with human cell lines. Although these in vitro studies are quite heterogeneous, in general, the results corroborate those described for the other species studied. Some of the findings of these investigations corroborate the ability of glyphosate to cross the human BBB and produce neurotoxic effects in the CNS. This ability of glyphosate to cross both the placental barrier and the BBB in humans was also observed in other previous studies that detected this compound in the brain and cerebrospinal fluid of individuals who had been exposed to GBH [176–178].

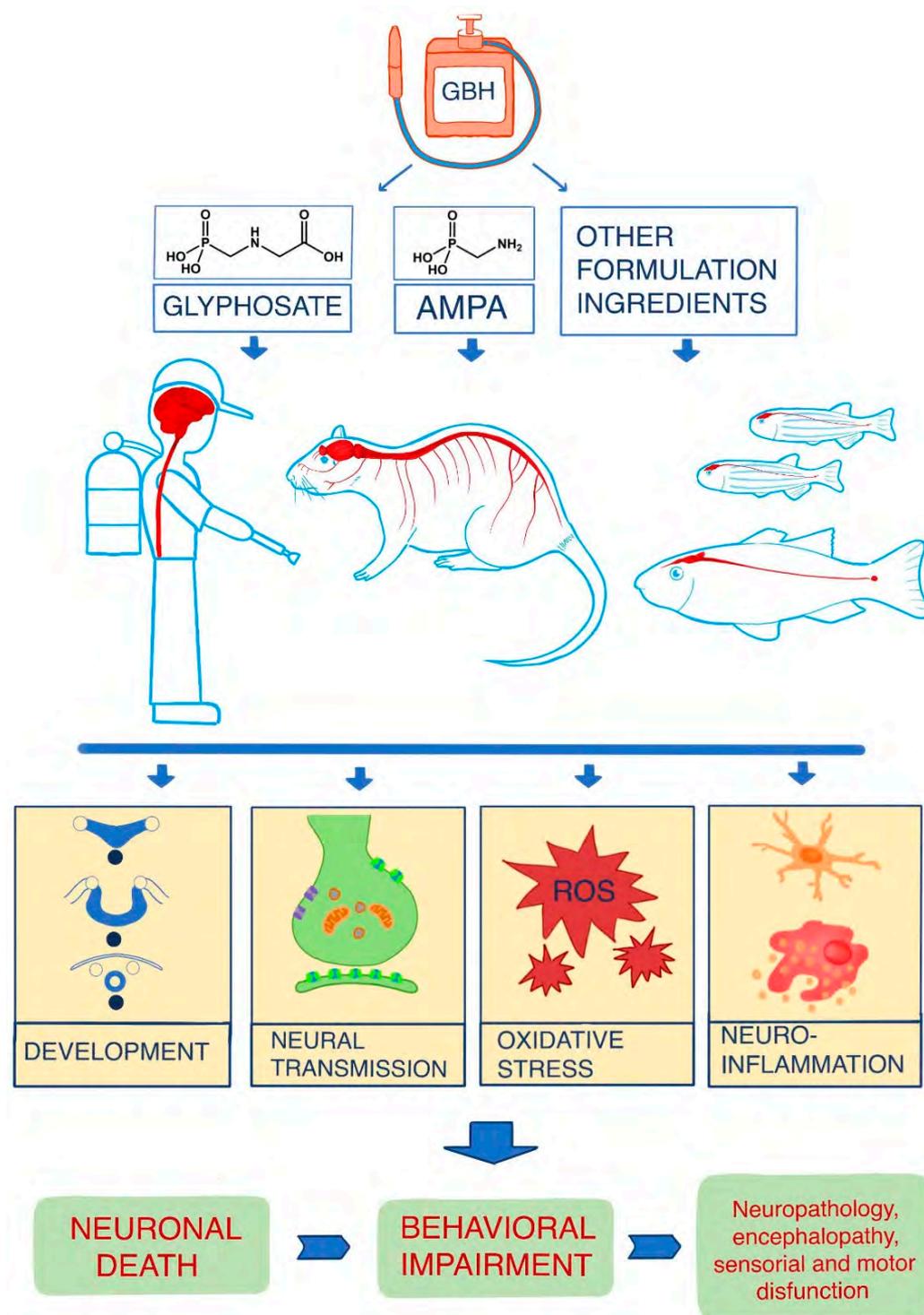


Figure 3. Exposition to glyphosate; its main metabolite, AMPA (aminomethylphosphonic acid); or its commercial formulations induces neurotoxic effects in all studied species. The main modes of action include changes in the development of the nervous system and in the neurotransmission systems, oxidative stress, neuroinflammation, processes that lead to neuronal death, and the appearance of behavioral changes. Changes in the structure and function of neurons lead to the development of neuropathology, encephalopathy, and sensory and motor dysfunctions.

Nevertheless, it appears that in adult humans, glyphosate does not produce toxic effects immediately after exposure but takes one or two days to do so. It is possible that this delay is due, at least in part, to the fact that the pesticide takes time to alter the integrity

of the BBB, cross it, and subsequently distribute itself in the CNS. Clinical observations also show that the adverse effects caused by glyphosate disappear as the concentration of the compound in the body decreases, although there are certain sequelae that seem to be maintained over time, such as memory disturbances.

Figure 4 summarizes the main mechanisms by which glyphosate produces its toxic effects on the nervous system. However, the existence of important discrepancies between the results obtained in studies carried out with humans, rodents, and fish must be considered. The data analyzed suggest that glyphosate induces the entry of Na^+ and Ca^{2+} from the extracellular medium through different mechanisms. Glyphosate causes an overstimulation of NMDAR, which leads to an influx of these ions and membrane depolarization. On the other hand, the inhibition of AChE by glyphosate produces an increase in acetylcholine levels and the stimulation of its receptors, including the $\alpha 7$ and $\alpha 4\beta 2$ receptors. The opening of these channels also induces an Na^+ and Ca^{2+} influx. Furthermore, although $\alpha 4\beta 2$ receptors are rapidly desensitized, $\alpha 7$ receptors can remain open for longer in the presence of agonists, further increasing Ca^{2+} influx [179]. It is possible that the sustained activity of these receptors induced by chronic exposure to glyphosate eventually leads to a decrease in their expression. Taken together, depolarization resulting from activation of glutamatergic and cholinergic receptors can cause VDCCs to open, allowing more Ca^{2+} to enter the cell. Some studies have shown that because of excess glutamate and acetylcholine, their metabotropic receptors are also activated [37,180,181]. The mGluR1 and mGluR5 receptors and mAChRs 1, 3, and 5 act on the same signaling pathway by activating associated G proteins, which in turn activate phospholipase C and the production of inositol triphosphate (IP3). The resulting IP3 diffuses through the cytoplasm and binds to specific receptors in the endoplasmic reticulum (ER), causing the release of Ca^{2+} to the cytosol [182].

Similarly, exposure of human cells to glyphosate has been shown to induce an increase in the expression of Wnt-5a mRNA, although this increase was not observed in rodents. Wnt-5a can interact with the Frizzled receptor, the activation of which also leads to an increase in IP3 levels and the consequential release of Ca^{2+} to the cytoplasm [183]. Likewise, Ca^{2+} ions inside the cell can bind to ryanodine receptors, Ca^{2+} channels present in the ER, the opening of which allows for the exit of this cation into the cytoplasm [182]. Ca^{2+} is an intracellular messenger involved in multiple signaling pathways, the concentrations of which are strictly controlled. Thus, this increase causes numerous changes in the cell. One such change is its binding to calmodulin, which leads to the activation of neuronal nitric oxide synthase (nNOS), which contributes to oxidative stress through the release of large amounts of NO [184].

As has been amply demonstrated, glyphosate produces a marked increase in intracellular ROS concentrations, which induce the activation of a series of mechanisms to neutralize oxidative stress conditions. Among these mechanisms is the activity of endogenous antioxidants, both enzymatic (SOD, CAT, GPx, peroxidase (PRX), GST, GGT, and G6PD) and non-enzymatic (GSH and thiols). However, these mechanisms appear to be insufficient to counteract the large amount of free radicals resulting from exposure to the pesticide. ROS act on the membranes of organelles, proteins, and DNA, causing their oxidation. In this situation, the cell can adopt various strategies to repair its components, such as increasing the activity of alkaline phosphatase to repair its DNA.

On the other hand, glyphosate has been shown to induce neuroinflammation characterized by the activation of both microglia and astrocytes. Because of their activation, these cells release large amounts of inflammatory cytokines, such as $\text{TNF-}\alpha$ and IL-6. In addition, exposure to glyphosate could also promote the release of the S100B protein by astrocytes, reaching its maximum levels one day after intoxication. At micromolar concentrations, the S100B protein can act on the receptor for advanced glycation end products (RAGE) at the neuronal membrane, favoring the overproduction of ROS [185]. Furthermore, this protein also binds to RAGEs present in the glial membrane, where it amplifies the inflammatory response [186]. In line with this, both the S100B protein and some inflammatory cytokines can

trigger inducible nitric oxide synthase (iNOS) activation in astrocytes [187]. Consequently, these glial cells produce large amounts of NO, which further enhances oxidative stress.

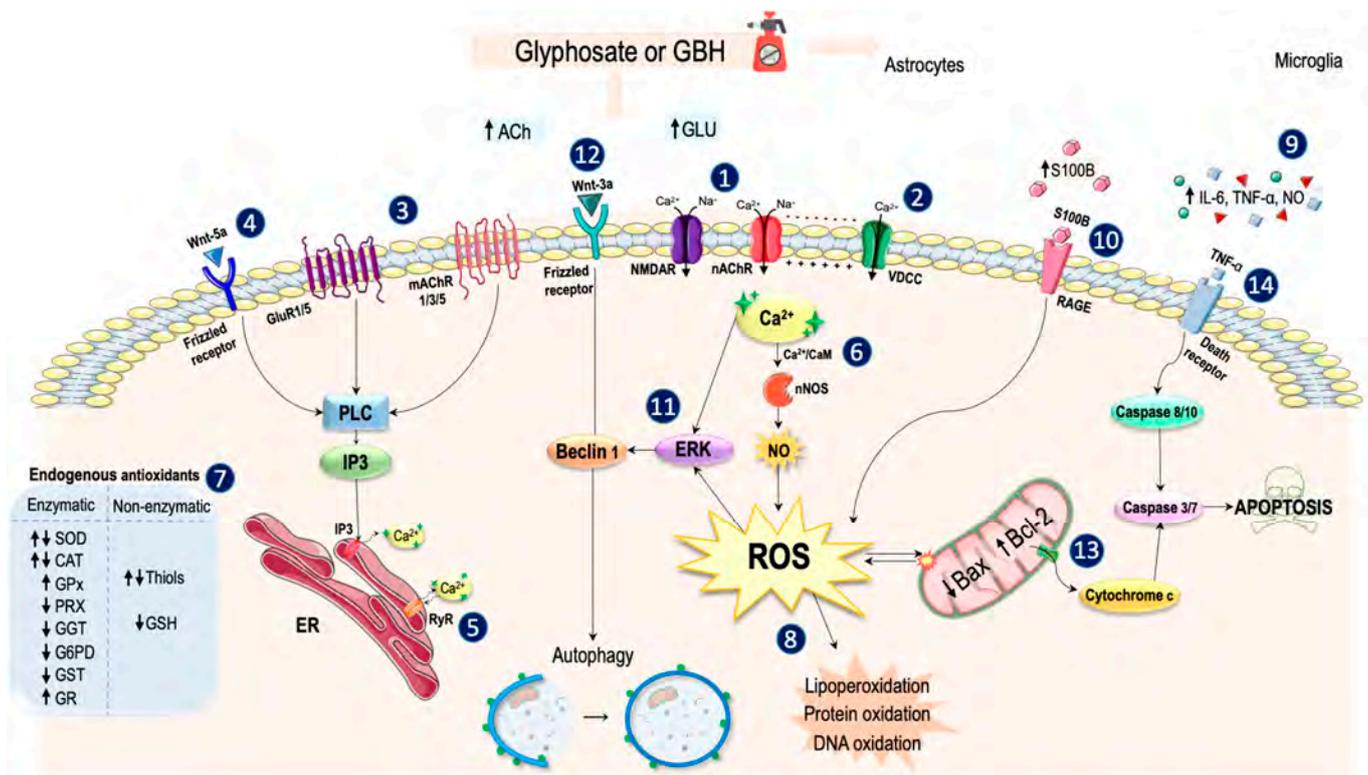


Figure 4. Possible mechanism of action of glyphosate or GBH in the nervous system. The presence of glyphosate induces several changes, including (1) opening of nAChRs and NMDA receptors, as well as entry of Na⁺ and Ca²⁺ into the cell due to increased levels of ACh and GLU and/or the direct binding of glyphosate to the cavities of NMDAR; (2) opening of the VDCCs by cellular depolarization and entry of Ca²⁺; (3) activation of the metabotropic GLU and ACh receptors, which stimulate the PLC to generate IP₃, which causes the release of Ca²⁺ from inside the ER; (4) increase in the levels of Wnt-5a, which binds to Frizzled receptors and triggers the generation of IP₃, with the consequential release of Ca²⁺ from the interior of the ER; (5) Ca²⁺ binding to ryanodine receptors and Ca²⁺ release from inside the ER; (6) binding of Ca²⁺ to calmodulin and activation of nNOS, which releases NO; (7) modification of the activity and/or concentrations of endogenous antioxidants; (8) excessive levels of ROS, leading to oxidation of lipids, proteins, and DNA; (9) activation of glial cells, which release inflammatory cytokines and NO; (10) release of S100B protein, which binds to neuronal RAGEs and increases ROS overproduction; (11) activation of ERK due to excessive levels of Ca²⁺ and ROS, which activates Beclin 1 and induces autophagy; (12) increased levels of Wnt-3a, which binds to Frizzled receptors and induces autophagy; (13) mitochondrial dysfunction, leading to activation of the intrinsic apoptosis pathway; and (14) binding of the ligand TNF-α to the death receptor, activating the extrinsic apoptosis pathway. Parts of the figure were created using templates from Servier Medical Art, which are licensed under a Creative Commons Attribution 3.0 Unported License (<http://smart.servier.com/> accessed on 17 February 2022). Abbreviations: GBH: glyphosate-based herbicide; GLU: glutamate; nAChR: nicotinic acetylcholine receptor; NMDAR: N-methyl-D-aspartate receptor; VDCC: voltage-dependent calcium channel; PLC: phospholipase C; IP₃: inositol trisphosphate; ER: endoplasmic reticulum; CaM: calmodulin; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; PRX: peroxidase; GGT: gamma-glutamyl transferase; G6PD: glucose-6-phosphate dehydrogenase; GST: glutathione S-transferase; GR: glutathione reductase; GSH: glutathione; ROS: reactive oxygen species; IL-6: interleukin-6; TNF-α: tumor necrosis factor alpha; S100B: S100 calcium-binding protein B; RAGE: receptor for advanced glycation end products; ERK: extracellular signal-regulated kinase; ↑, increase; ↓, decrease.

To eliminate the organelles damaged by the action of ROS and obtain energy, the cells can induce the process of autophagy. Thus, the increase in Ca^{2+} and ROS concentrations caused by glyphosate has been related to increased activation of ERK, which can activate the Beclin 1 protein and induce autophagy [188]. In addition, this natural recycling process can also be induced by other mechanisms observed in studies with glyphosate, such as the binding of the Wnt-3a ligand to the Frizzled receptor or through the deficient production of ATP in mitochondria [189].

Although autophagy favors cell survival in stressful situations or under nutrient deprivation, excessive induction of this mechanism can be destructive to the cell and lead to apoptosis [190]. The conditions of oxidative stress induced by glyphosate can trigger the intrinsic or mitochondrial apoptotic pathway because the ability of these organelles to produce energy and maintain Ca^{2+} homeostasis is severely affected. Therefore, the increase in the intracellular Ca^{2+} also leads to a marked increase in its levels in the mitochondria. Consequently, activation of the Bax protein occurs. This activated protein forms pores in the mitochondrial membrane and allows for the release of cytochrome C, which activates the caspases responsible for orchestrating apoptosis [191].

Exposure to glyphosate can also activate the extrinsic apoptotic pathway. As previously mentioned, glial activation by the pesticide causes the release of inflammatory cytokines, such as $\text{TNF-}\alpha$, which can act as a ligand for the death receptors at the membrane. As a result of the activation of these receptors, the activation of caspase 8 occurs, which clears other caspases and finally causes neuronal death by apoptosis [192].

These findings also suggest that when exposure occurs early in life, the pesticide can induce serious disturbances in the development of the nervous system, possibly by deregulating some of the signaling pathways involved in this process. On the other hand, a common aspect that can be drawn from *in vivo* and *in vitro* studies with rodents and humans is that both in early age and in adulthood, the hippocampus is especially vulnerable to the action of glyphosate. As a result of this damage, exposure to different concentrations of glyphosate has usually been associated with severe memory impairment that is sometimes irreversible.

4.2. Relationship between Glyphosate Doses and Neurotoxic Effects in Rodents and Humans

The findings reported in this review coincide in pointing out the neurotoxic potential of glyphosate in the different animal species studied. Although many of the studies used doses higher than those commonly found in the environment, the main objective of these studies was the evaluation of the neurotoxic mechanisms of action of the pesticide on the nervous system. Therefore, to assess the real toxic risk that exposure to glyphosate may pose in animals, it is necessary to consider environmental concentrations of the pesticide.

The first point to consider is that there are several discrepancies with respect to the values established as NOAEL. Several previous investigations have established a NOAEL for glyphosate at considerably high values, such as 1000 mg/kg/day, which established for maternal and developmental toxicity in rats [14]. However, these guideline values were established considering carcinogenicity and toxicity in organs other than the brain. Therefore, a considerable number of regulatory toxicity studies found no evidence that high doses of glyphosate caused neurotoxic effects in rodents. This has led to some subsequent studies to establish a NOAEL for neurotoxicity in rats ranging from 2000 to 20,000 mg/kg/day of glyphosate [193]. Finally, more recent research established a more moderate NOAEL value of 617 mg/kg/day [34].

Most of studies of rodents analyzed in this review used doses of glyphosate or GBH that did not exceed the current NOAEL and ranged from 5 mg/kg [68] to 800 mg/kg [13]. These doses are not representative of human environmental exposures, which are in the range of $\mu\text{g}/\text{kg}/\text{day}$. However, the data provided by these studies suggest that even oral treatment with the lowest used dose of glyphosate or GBH (5 mg/kg) was associated with alterations in behavior and neuroplasticity in rats [68]. Likewise, the dose of glyphosate most used in the studies with rodents was 50 mg/kg, which is also much lower than the

established NOAEL, and it was associated with important alterations in the structure and functioning of the CNS. Therefore, although it appears that more conservative guideline values for the neurotoxicity of glyphosate and GBH have been adopted in recent years, the data discussed herein suggest that these values may still be too high. The results analyzed in this review suggest that the ability of glyphosate, alone or in formulation, to produce neurotoxic effects in rodents has been underestimated.

The current evidence analyzed in this review raises concern and indicates the need for more research, mainly in rodents. This is because rodent studies provide essential information for the establishment of reference values for humans. In line with this, the reference dose of glyphosate established by the EPA (USA) is 1.75 mg/kg/day, whereas the JMPR/WHO is more restrictive and limits the maximum dose to 1 mg/kg/day [194]. The EPA has estimated that the exposure of the general population to glyphosate through food and water is 0.088 mg/kg/day (range 0.058–0.230 mg/kg/day) [195], some studies have indicated that glyphosate is habitually found in urine in concentrations corresponding to a daily intake of approximately 0.1–0.3 µg/kg/day [28]. These data indicate that the general population is exposed to glyphosate levels lower than the reference dose and the acceptable daily intakes proposed by several regulatory agencies, not representing an immediate risk to health. However, under certain circumstances, the established reference values can be clearly exceeded, as in the case of some accidental or intentional exposures. Thus, the most serious neurotoxic effects reported have been observed after suicide attempts, with exposures to doses of glyphosate much higher than those normally found in the environment.

Studies on occupational toxicity in rural populations are of particular relevance. In such studies, glyphosate concentrations commonly used in agricultural practice or those found in residential environments close to growing areas were considered. These studies reveal a possible relationship between exposure to glyphosate during prenatal and childhood and an increased risk of developing diseases such as autism spectrum disorder [47]. Likewise, peripheral neuropathy and memory impairment have also been reported in farmers occupationally exposed to the pesticide [45,196]. These data suggest that pesticide concentrations found in agricultural settings could represent a health risk to children and adults.

4.3. Exposure Levels and Neurotoxic Effects in Fish

When GBH is applied to crops, it can remain in the soil until it is degraded by microorganisms or mobilized by wind, rain, or irrigation. These factors increase infiltration and surface runoff, favoring the arrival of glyphosate in aquatic environments [146]. Varied concentrations of glyphosate have been reported in aquatic environments, such as concentrations of 0.1–1.48 mg/L detected in waters of Argentina and Brazil, or the maximum concentration of 0.65 mg/L detected in waters in Europe [149,197]. During the glyphosate use season, its concentration reaches 10 mg/L in Taihu Lake (China) [48].

Therefore, Roundup® concentrations below 10 mg/L can be considered environmentally realistic considering that with current application rates, a bare water body can have a maximum glyphosate concentration of 3.7 mg/L, which corresponds to 9 mg/L of Roundup® [198]. Almost all the fish studies analyzed in this review used doses that ranged from 0.064 to 10 mg/L of GBH or glyphosate. Exposure to these environmentally relevant doses of glyphosate was associated with a wide range of neurotoxic effects, including alterations in the development of the nervous system and the appearance of alterations in behavior. In addition, although, given the physicochemical characteristics of glyphosate, it is not expected that it can bioaccumulate in the tissues of animals, the available evidence supports that this compound can accumulate in the tissues of some aquatic species, which could result in an increase in its neurotoxicity [199–202].

Although elevated concentrations of glyphosate have been detected in some aquatic environments, some countries have adopted more restrictive limits. Thus, the maximum level of glyphosate accepted in the United States is 0.7 mg/L, whereas in Europe, the maxi-

imum accepted for a standard environmental quality is 0.028 mg/L [43,203]. In line with this, Bridi et al. [134] showed that exposure of zebrafish to glyphosate or GBH (0.01–0.5 mg/L) caused alterations in locomotion, aversive and aggressive behavior, as well as memory impairment. In a study by Khan et al. [139], treatment with 0.02–0.1 mg/L of glyphosate also altered the behavior in European carp (*Cyprinus carpio*). Similarly, Faria et al. [130] found that the exposure of zebrafish to concentrations of 0.3 or 3 µg/L of glyphosate for two weeks caused a deterioration in exploratory and social behavior, as well as an increase in anxiety. Likewise, the team of Sánchez et al. [149] observed that treatment of one-sided livebearer (*Jenynsia multidentata*) with 0.5 mg/L GBH reduced social interaction, spatial exploration, swimming performance, and long-term memory consolidation and even caused severe alterations in sexual behavior.

These results suggest that exposure to glyphosate or GBH, although in doses that are within legally accepted limits, can result in neurotoxicity in fish and alter behaviors essential for the survival of these species. Ultimately, this could have important ecological consequences, especially in agricultural areas where agrochemicals are applied regularly. Therefore, these results suggest the need to adopt more conservative contamination limits that consider the neurotoxicity induced by glyphosate.

5. Limitations

This systematic review is not free of limitations, which should be considered when generalizing the results. First, most of the studies analyzed used commercial formulations of glyphosate, which are mixtures of glyphosate (active ingredient) with other adjuvants. Thus, it is not possible to determine precisely which component(s) of the formulation was (or were) responsible for the observed neurotoxic effects. In addition, the results of various studies support the greater toxicity of commercial glyphosate formulations compared to glyphosate administered alone [147,204–206]. For this reason, the results analyzed herein cannot be attributed exclusively to glyphosate, as they could have been caused by other components of the formulation or even by possible synergy between these components and glyphosate [65,207–209].

On the other hand, most research has studied the effects of higher doses of glyphosate than the concentrations to which the general population is routinely exposed. Thus, it is possible that exposure to environmental concentrations of glyphosate does not result in the wide range of neurotoxic effects documented here. Furthermore, when analyzing many studies, a great heterogeneity in the results obtained is observed; the various discrepancies identified when comparing the studies could be due to differences in their experimental conditions, such as the route of exposure, the compound used (glyphosate, AMPA, or GBH), the total duration of treatment, and the time at which the evaluations were performed since the end of the treatment.

6. Conclusions

The information summarized in the present review indicates that exposure to glyphosate, AMPA, or GBH could induce several toxic effects on the nervous system of all species studied. Exposure to glyphosate during the early stages of life can severely affect normal cell development by deregulating some of the signaling pathways involved in this process, leading to alterations in differentiation, neuronal growth, migration, and myelination. Glyphosate also seems to exert a significant toxic effect on neurotransmission, with the glutamatergic system being one of the most affected systems. Glyphosate was found to increase glutamate release and decreased its reuptake, in addition to activating NMDAR and L-VDCC, thus increasing the influx of Ca²⁺ into neurons. Likewise, the results analyzed herein reflect the capacity of glyphosate to induce oxidative stress, neuroinflammation, and mitochondrial dysfunction, processes that lead to neuronal death by autophagia, necrosis, or apoptosis, as well as the appearance of behavioral and motor disorders. Although there are important discrepancies between the findings analyzed in this review, it is unequivocal that exposure to glyphosate, alone or in commercial formulations, can produce important

alterations in the structure and function of the nervous system of humans, rodents, fish, and invertebrate animals.

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References

- Dayan, F.E.; Barker, A.; Takano, H.; Bough, R.; Ortiz, M.; Duke, S.O. Herbicide mechanism of action and resistance. In *Comprehensive Biotechnology*, 3rd ed.; Moo-Young, M., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; Volume 4, p. 4826.
- Duke, S.O. The history and current status of glyphosate. *Pest Manag. Sci.* **2018**, *74*, 1027–1034. [[CrossRef](#)]
- Cattani, D.; Cesconetto, P.A.; Tavares, M.K.; Parisotto, E.B.; Oliveira, P.V.; Rieg, C.E.H.; Leite, M.C.; Prediger, R.; Wendt, N.; Razzera, G.; et al. Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: Implication of glutamate excitotoxicity and oxidative stress. *Toxicology* **2017**, *387*, 67–80. [[CrossRef](#)]
- Klümper, W.; Qaim, M. A Meta-Analysis of the Impacts of Genetically Modified Crops. *PLoS ONE* **2014**, *9*, e111629. [[CrossRef](#)]
- Maggi, F.; la Cecilia, D.; Tang, F.H.; McBratney, A. The global environmental hazard of glyphosate use. *Sci. Total Environ.* **2020**, *717*, 137167. [[CrossRef](#)] [[PubMed](#)]
- Saunders, L.E.; Pezeshki, R. Glyphosate in Runoff Waters and in the Root-Zone: A Review. *Toxics* **2015**, *3*, 462–480. [[CrossRef](#)] [[PubMed](#)]
- Funke, T.; Han, H.; Healy-Fried, M.L.; Fischer, M.; Schönbrunn, E. Molecular basis for the herbicide resistance of Roundup Ready crops. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13010–13015. [[CrossRef](#)] [[PubMed](#)]
- Meftaul, I.M.; Venkateswarlu, K.; Dharmarajan, R.; Annamalai, P.; Asaduzzaman; Parven, A.; Megharaj, M. Controversies over human health and ecological impacts of glyphosate: Is it to be banned in modern agriculture? *Environ. Pollut.* **2020**, *263*, 114372. [[CrossRef](#)]
- Van Bruggen, A.H.C.; He, M.M.; Shin, K.; Mai, V.; Jeong, K.C.; Finckh, M.R.; Morris, J.G., Jr. Environmental and health effects of the herbicide glyphosate. *Sci. Total Environ.* **2018**, *616–617*, 255–268. [[CrossRef](#)]
- Cox, C. Glyphosate (roundup). *J. Pestic. Reform.* **1998**, *18*, 3–17.
- Kier, L.D.; Kirkland, D.J. Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit. Rev. Toxicol.* **2013**, *43*, 283–315. [[CrossRef](#)]
- Williams, G.M.; Aardema, M.; Acquavella, J.; Berry, S.C.; Brusick, D.; Burns, M.M.; De Camargo, J.L.V.; Garabrant, D.; Greim, H.A.; Kier, L.D.; et al. A review of the carcinogenic potential of glyphosate by four independent expert panels and comparison to the IARC assessment. *Crit. Rev. Toxicol.* **2016**, *46*, 3–20. [[CrossRef](#)] [[PubMed](#)]
- Martínez, M.-A.; Ares, I.; Rodríguez, J.-L.; Martínez, M.; Martínez-Larrañaga, M.-R.; Anadón, A. Neurotransmitter changes in rat brain regions following glyphosate exposure. *Environ. Res.* **2018**, *161*, 212–219. [[CrossRef](#)] [[PubMed](#)]
- Williams, G.M.; Kroes, R.; Munro, I.C. Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans. *Regul. Toxicol. Pharmacol.* **2000**, *31*, 117–165. [[CrossRef](#)] [[PubMed](#)]
- Kanissery, R.; Gairhe, B.; Kadyampakeni, D.; Batuman, O.; Alferez, F. Glyphosate: Its Environmental Persistence and Impact on Crop Health and Nutrition. *Plants* **2019**, *8*, 499. [[CrossRef](#)]
- Bento, C.P.M.; Yang, X.; Gort, G.; Xue, S.; van Dam, R.; Zomer, P.; Mol, H.G.J.; Ritsema, C.J.; Geissen, V. Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. *Sci. Total Environ.* **2016**, *572*, 301–311. [[CrossRef](#)] [[PubMed](#)]
- Bergström, L.; Börjesson, E.; Stenström, J. Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil. *J. Environ. Qual.* **2011**, *40*, 98–108. [[CrossRef](#)]
- Laitinen, P.; Siimes, K.; Eronen, L.; Rämö, S.; Welling, L.; Oinonen, S.; Mattsoff, L.; Ruohonen-Lehto, M. Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils. *Pest Manag. Sci.* **2006**, *62*, 473–491. [[CrossRef](#)]
- Tzanetou, E.; Karasali, H. Glyphosate Residues in Soil and Air: An Integrated Review. In *Pests, Weeds and Diseases in Agricultural Crop and Animal Husbandry Production*; IntechOpen: London, UK, 2020. [[CrossRef](#)]

20. De Andrea, M.M.; Peres, T.B.; Luchini, L.C.; Bazarin, S.; Papini, S.; Matallo, M.B.; Savoy, V.L.T. Influence of repeated applications of glyphosate on its persistence and soil bioactivity. *Pesqui. Agropecuária Bras.* **2003**, *38*, 1329–1335. [[CrossRef](#)]
21. Carretta, L.; Cardinali, A.; Onofri, A.; Masin, R.; Zanin, G. Dynamics of Glyphosate and Aminomethylphosphonic Acid in Soil Under Conventional and Conservation Tillage. *Int. J. Environ. Res.* **2021**, *15*, 1037–1055. [[CrossRef](#)]
22. Leoci, R.; Ruberti, M. Glyphosate in Agriculture: Environmental Persistence and Effects on Animals. A Review. *J. Agric. Environ. Int. Dev.* **2020**, *114*, 99–122. [[CrossRef](#)]
23. Battaglin, W.A.; Meyer, M.T.; Kuivila, K.M.; Dietze, J.E. Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation. *JAWRA J. Am. Water Resour. Assoc.* **2014**, *50*, 275–290. [[CrossRef](#)]
24. Berman, M.C.; Marino, D.; Quiroga, M.V.; Zagarese, H. Occurrence and levels of glyphosate and AMPA in shallow lakes from the Pampean and Patagonian regions of Argentina. *Chemosphere* **2018**, *200*, 513–522. [[CrossRef](#)] [[PubMed](#)]
25. Mercurio, P.; Flores, F.; Mueller, J.F.; Carter, S.; Negri, A.P. Glyphosate persistence in seawater. *Mar. Pollut. Bull.* **2014**, *85*, 385–390. [[CrossRef](#)] [[PubMed](#)]
26. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005. *EFSA J.* **2018**, *16*. [[CrossRef](#)]
27. Edge, C.; Brown, M.; Hartz, S.; Thompson, D.; Ritter, L.; Ramadoss, M. The Persistence of Glyphosate in Vegetation One Year after Application. *Forests* **2021**, *12*, 601. [[CrossRef](#)]
28. Niemann, L.; Sieke, C.; Pfeil, R.; Solecki, R. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. *J. Verbr. Lebensm.* **2015**, *10*, 3–12. [[CrossRef](#)]
29. Reddy, K.N.; Rimando, A.M.; Duke, S.O.; Nandula, V.K. Aminomethylphosphonic Acid Accumulation in Plant Species Treated with Glyphosate. *J. Agric. Food Chem.* **2008**, *56*, 2125–2130. [[CrossRef](#)]
30. Acquavella, J.F.; Alexander, B.H.; Mandel, J.S.; Gustin, C.; Baker, B.; Chapman, P.; Bleeke, M. Glyphosate biomonitoring for farmers and their families: Results from the Farm Family Exposure Study. *Environ. Health Perspect.* **2004**, *112*, 321–326. [[CrossRef](#)]
31. Conrad, A.; Schröter-Kermani, C.; Hoppe, H.-W.; Rütger, M.; Pieper, S.; Kolossa-Gehring, M. Glyphosate in German adults—Time trend (2001 to 2015) of human exposure to a widely used herbicide. *Int. J. Hyg. Environ. Health* **2017**, *220*, 8–16. [[CrossRef](#)]
32. Krüger, M.; Schledorn, P.; Schrödl, W.; Hoppe, H.W.; Lutz, W.; Shehata, A.A. Detection of Glyphosate Residues in Animals and Humans. *J. Environ. Anal. Toxicol.* **2014**, *4*, 1000210. [[CrossRef](#)]
33. Krüger, M.; Schrödl, W.; Pedersen, I. Detection of Glyphosate in Malformed Piglets. *J. Environ. Anal. Toxicol.* **2014**, *4*, 1000230. [[CrossRef](#)]
34. Tarazona, J.V.; Court-Marques, D.; Tiramani, M.; Reich, H.; Pfeil, R.; Istace, F.; Crivellente, F. Glyphosate toxicity and carcinogenicity: A review of the scientific basis of the European Union assessment and its differences with IARC. *Arch. Toxicol.* **2017**, *91*, 2723–2743. [[CrossRef](#)] [[PubMed](#)]
35. Currie, Z.; Prosser, R.S.; Rodriguez-Gil, J.L.; Mahon, K.; Poirier, D.; Solomon, K.R. Toxicity of Cúspide 480SL[®] spray mixture formulation of glyphosate to aquatic organisms. *Environ. Toxicol. Chem.* **2015**, *34*, 1178–1184. [[CrossRef](#)] [[PubMed](#)]
36. Li, J.; Smeda, R.J.; Sellers, B.A.; Johnson, W.G. Influence of formulation and glyphosate salt on absorption and translocation in three annual weeds. *Weed Sci.* **2005**, *53*, 153–159. [[CrossRef](#)]
37. Cattani, D.; Cavalli, V.L.D.L.O.; Rieg, C.E.H.; Domingues, J.T.; Dal-Cim, T.; Tasca, C.I.; Silva, F.R.M.B.; Zamoner, A. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. *Toxicology* **2014**, *320*, 34–45. [[CrossRef](#)]
38. Bringolf, R.B.; Cope, W.G.; Mosher, S.; Barnhart, M.C.; Shea, D. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of lamprolaima siliquoidea (unionidae). *Environ. Toxicol. Chem.* **2007**, *26*, 2094–2100. [[CrossRef](#)]
39. Rodrigues, L.D.B.; de Oliveira, R.; Abe, F.R.; Brito, L.B.; Moura, D.S.; Valadares, M.C.; Grisolia, C.K.; de Oliveira, D.P.; de Oliveira, G.A.R. Ecotoxicological assessment of glyphosate-based herbicides: Effects on different organisms. *Environ. Toxicol. Chem.* **2016**, *36*, 1755–1763. [[CrossRef](#)]
40. Mesnage, R.; Bernay, B.; Séralini, G.-E. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology* **2013**, *313*, 122–128. [[CrossRef](#)]
41. Nobels, I.; Spanoghe, P.; Haesaert, G.; Robbens, J.; Blust, R. Toxicity Ranking and Toxic Mode of Action Evaluation of Commonly Used Agricultural Adjuvants on the Basis of Bacterial Gene Expression Profiles. *PLoS ONE* **2011**, *6*, e24139. [[CrossRef](#)]
42. Prosser, R.S.; Rodriguez-Gil, J.L.; Solomon, K.R.; Sibley, P.K.; Poirier, D.G. Effects of the herbicide surfactant MON 0818 on oviposition and viability of eggs of the ramshorn snail (*Planorbella pilsbryi*). *Environ. Toxicol. Chem.* **2016**, *36*, 522–531. [[CrossRef](#)]
43. Bai, S.H.; Ogbourne, S. Glyphosate: Environmental contamination, toxicity and potential risks to human health via food contamination. *Environ. Sci. Pollut. Res.* **2016**, *23*, 18988–19001. [[CrossRef](#)] [[PubMed](#)]
44. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. [[CrossRef](#)] [[PubMed](#)]
45. Fuhrmann, S.; Farnham, A.; Staudacher, P.; Atuhaire, A.; Manfioletti, T.; Niwagaba, C.B.; Namirembe, S.; Mugweri, J.; Winkler, M.S.; Portengen, L.; et al. Exposure to multiple pesticides and neurobehavioral outcomes among smallholder farmers in Uganda. *Environ. Int.* **2021**, *152*, 106477. [[CrossRef](#)]
46. Lee, J.-W.; Choi, Y.-J.; Park, S.; Gil, H.-W.; Song, H.-Y.; Hong, S.-Y. Serum S100 protein could predict altered consciousness in glyphosate or glufosinate poisoning patients. *Clin. Toxicol.* **2017**, *55*, 357–359. [[CrossRef](#)] [[PubMed](#)]

47. Von Ehrenstein, O.S.; Ling, C.; Cui, X.; Cockburn, M.; Park, A.S.; Yu, F.; Wu, J.; Ritz, B. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study. *BMJ* **2019**, *364*, 1962. [[CrossRef](#)]
48. Zhang, C.; Hu, R.; Huang, J.; Huang, X.; Shi, G.; Li, Y.; Yin, Y.; Chen, Z. Health effect of agricultural pesticide use in China: Implications for the development of GM crops. *Sci. Rep.* **2016**, *6*, 34918. [[CrossRef](#)]
49. Zhang, C.; Sun, Y.; Hu, R.; Huang, J.; Huang, X.; Li, Y.; Yin, Y.; Chen, Z. A comparison of the effects of agricultural pesticide uses on peripheral nerve conduction in China. *Sci. Rep.* **2018**, *8*, 9621. [[CrossRef](#)]
50. Hao, Y.; Zhang, Y.; Ni, H.; Gao, J.; Yang, Y.; Xu, W.; Tao, L. Evaluation of the cytotoxic effects of glyphosate herbicides in human liver, lung, and nerve. *J. Environ. Sci. Health Part B* **2019**, *54*, 737–744. [[CrossRef](#)]
51. Martinez, A.; Al-Ahmad, A.J. Effects of glyphosate and aminomethylphosphonic acid on an isogenic model of the human blood-brain barrier. *Toxicol. Lett.* **2018**, *304*, 39–49. [[CrossRef](#)]
52. Martínez, M.-A.; Rodríguez-Gutierrez, J.-L.; Torres, B.L.; Martínez, M.; Martínez-Larrañaga, M.-R.; Maximiliano, J.-E.; Anadón, A.; Ares, I. Use of human neuroblastoma SH-SY5Y cells to evaluate glyphosate-induced effects on oxidative stress, neuronal development and cell death signaling pathways. *Environ. Int.* **2019**, *135*, 105414. [[CrossRef](#)]
53. Luo, J.; Chen, J.; Deng, Z.-L.; Luo, X.; Song, W.-X.; Sharff, K.A.; Tang, N.; Haydon, R.C.; Luu, H.H.; He, T.-C. Wnt signaling and human diseases: What are the therapeutic implications? *Lab. Invest.* **2007**, *87*, 97–103. [[CrossRef](#)]
54. Okerlund, N.D.; Cheyette, B.N.R. Synaptic Wnt signaling—a contributor to major psychiatric disorders? *J. Neurodev. Disord.* **2011**, *3*, 162–174. [[CrossRef](#)] [[PubMed](#)]
55. Kwan, V.; Unda, B.K.; Singh, K.K. Wnt signaling networks in autism spectrum disorder and intellectual disability. *J. Neurodev. Disord.* **2016**, *8*, 45. [[CrossRef](#)] [[PubMed](#)]
56. Zhang, Y.; Yuan, X.-S.; Wang, Z.; Li, R. The canonical Wnt signaling pathway in autism. *CNS Neurol. Disord.—Drug Targets* **2014**, *13*, 765–770. [[CrossRef](#)] [[PubMed](#)]
57. Good, P. Evidence the U.S. autism epidemic initiated by acetaminophen (Tylenol) is aggravated by oral antibiotic amoxicillin/clavulanate (Augmentin) and now exponentially by herbicide glyphosate (Roundup). *Clin. Nutr. ESPEN* **2018**, *23*, 171–183. [[CrossRef](#)] [[PubMed](#)]
58. Pu, Y.; Yang, J.; Chang, L.; Qu, Y.; Wang, S.; Zhang, K.; Xiong, Z.; Zhang, J.; Tan, Y.; Wang, X.; et al. Maternal glyphosate exposure causes autism-like behaviors in offspring through increased expression of soluble epoxide hydrolase. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 11753–11759. [[CrossRef](#)]
59. Swanson, N.L.; Leu, A.; Abrahamson, J.; Wallet, B. Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. *JOS* **2014**, *9*, 6–37.
60. Coultrap, S.J.; Vest, R.S.; Ashpole, N.M.; Hudmon, A.; Bayer, K.U. CaMKII in cerebral ischemia. *Acta Pharmacol. Sin.* **2011**, *32*, 861–872. [[CrossRef](#)]
61. Zhang, X.; Connelly, J.; Levitan, E.S.; Sun, D.; Wang, J.Q. Calcium/Calmodulin-Dependent Protein Kinase II in Cerebrovascular Diseases. *Transl. Stroke Res.* **2021**, *12*, 513–529. [[CrossRef](#)]
62. Bali, Y.A.; Ba-Mhamed, S.; Bennis, M. Behavioral and Immunohistochemical Study of the Effects of Subchronic and Chronic Exposure to Glyphosate in Mice. *Front. Behav. Neurosci.* **2017**, *11*, 146. [[CrossRef](#)]
63. Bali, Y.A.; Kaikai, N.-E.; Ba-M'Hamed, S.; Bennis, M. Learning and memory impairments associated to acetylcholinesterase inhibition and oxidative stress following glyphosate based-herbicide exposure in mice. *Toxicology* **2019**, *415*, 18–25. [[CrossRef](#)] [[PubMed](#)]
64. Ait-Bali, Y.; Ba-M'Hamed, S.; Gambarotta, G.; Sassoè-Pognetto, M.; Giustetto, M.; Bennis, M. Pre- and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments in adult mice: Evidence of cortical ad hippocampal dysfunction. *Arch. Toxicol.* **2020**, *94*, 1703–1723. [[CrossRef](#)] [[PubMed](#)]
65. Baier, C.J.; Gallegos, C.E.; Raisman-Vozari, R.; Minetti, A. Behavioral impairments following repeated intranasal glyphosate-based herbicide administration in mice. *Neurotoxicology Teratol.* **2017**, *64*, 63–72. [[CrossRef](#)] [[PubMed](#)]
66. Cattani, D.; Struyf, N.; Steffensen, V.; Bergquist, J.; Zamoner, A.; Brittebo, E.; Andersson, M. Perinatal exposure to a glyphosate-based herbicide causes dysregulation of dynorphins and an increase of neural precursor cells in the brain of adult male rats. *Toxicology* **2021**, *461*, 152922. [[CrossRef](#)]
67. Coullery, R.; Pacchioni, A.M.; Rosso, S.B. Exposure to glyphosate during pregnancy induces neurobehavioral alterations and downregulation of Wnt5a-CaMKII pathway. *Reprod. Toxicol.* **2020**, *96*, 390–398. [[CrossRef](#)]
68. Dechartres, J.; Pawluski, J.L.; Gueguen, M.; Jablaoui, A.; Maguin, E.; Rhimi, M.; Charlier, T.D. Glyphosate and glyphosate-based herbicide exposure during the peripartum period affects maternal brain plasticity, maternal behaviour and microbiome. *J. Neuroendocr.* **2019**, *31*, e12731. [[CrossRef](#)]
69. Gallegos, C.E.; Bartos, M.; Gumilar, F.; Raisman-Vozari, R.; Minetti, A.; Baier, C.J. Intranasal glyphosate-based herbicide administration alters the redox balance and the cholinergic system in the mouse brain. *NeuroToxicology* **2020**, *77*, 205–215. [[CrossRef](#)]
70. Hernández-Plata, I.; Giordano, M.; Díaz-Muñoz, M.; Rodríguez, V.M. The herbicide glyphosate causes behavioral changes and alterations in dopaminergic markers in male Sprague-Dawley rat. *NeuroToxicology* **2015**, *46*, 79–91. [[CrossRef](#)]
71. Ji, H.; Xu, L.; Wang, Z.; Fan, X.; Wu, L. Differential microRNA expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation. *Exp. Ther. Med.* **2017**, *15*, 2457–2467. [[CrossRef](#)]

72. Joaquim, A.; Spinosa, H.; Macrini, D.J.; Rodrigues, P.A.; Ricci, E.L.; Artioli, T.S.; Moreira, N.; Suffredini, I.B.; Bernardi, M.M. Behavioral effects of acute glyphosate exposure in male and female Balb/c mice. *Braz. J. Vet. Res. Anim. Sci.* **2012**, *49*, 367–376. [[CrossRef](#)]
73. Larsen, K.E.; Lifschitz, A.L.; Lanasse, C.E.; Virkel, G.L. The herbicide glyphosate is a weak inhibitor of acetylcholinesterase in rats. *Environ. Toxicol. Pharmacol.* **2016**, *45*, 41–44. [[CrossRef](#)] [[PubMed](#)]
74. Luna, S.; Neila, L.P.; Vena, R.; Borgatello, C.; Rosso, S.B. Glyphosate exposure induces synaptic impairment in hippocampal neurons and cognitive deficits in developing rats. *Arch. Toxicol.* **2021**, *95*, 2137–2150. [[CrossRef](#)] [[PubMed](#)]
75. Gui, Y.-X.; Fan, X.-N.; Wang, H.-M.; Wang, G.; Chen, S.-D. Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicol. Teratol.* **2012**, *34*, 344–349. [[CrossRef](#)] [[PubMed](#)]
76. Masood, M.I.; Naseem, M.; Warda, S.A.; Tapia-Laliena, M.; Rehman, H.U.; Nasim, M.J.; Schäfer, K.H. Environment permissible concentrations of glyphosate in drinking water can influence the fate of neural stem cells from the subventricular zone of the postnatal mouse. *Environ. Pollut.* **2020**, *270*, 116179. [[CrossRef](#)] [[PubMed](#)]
77. Da Silva, K.N.; Cappellaro, L.G.; Ueda, C.N.; Rodrigues, L.; Remor, A.P.; Martins, R.D.P.; Latini, A.; Glaser, V. Glyphosate-based herbicide impairs energy metabolism and increases autophagy in C6 astrogloma cell line. *J. Toxicol. Environ. Health Part A* **2020**, *83*, 153–167. [[CrossRef](#)] [[PubMed](#)]
78. Szepanowski, F.; Szepanowski, L.-P.; Mausberg, A.K.; Albrecht, P.; Kleinschnitz, C.; Kieseier, B.C.; Stettner, M. Differential impact of pure glyphosate and glyphosate-based herbicide in a model of peripheral nervous system myelination. *Acta Neuropathol.* **2018**, *136*, 979–982. [[CrossRef](#)]
79. Szepanowski, F.; Kleinschnitz, C.; Stettner, M. Glyphosate-based herbicide: A risk factor for demyelinating conditions of the peripheral nervous system? *Neural Regen. Res.* **2019**, *14*, 2079–2080. [[CrossRef](#)]
80. Arredondo, S.B.; Guerrero, F.G.; Herrera-Soto, A.; Jensen-Flores, J.; Bustamante, D.B.; Oñate-Ponce, A.; Henny, P.; Varas-Godoy, M.; Inestrosa, N.C.; Varela-Nallar, L. Wnt5a promotes differentiation and development of adult-born neurons in the hippocampus by noncanonical Wnt signaling. *Stem Cells* **2019**, *38*, 422–436. [[CrossRef](#)]
81. Habtemariam, S. The brain-derived neurotrophic factor in neuronal plasticity and neuroregeneration: New pharmacological concepts for old and new drugs. *Neural Regen. Res.* **2018**, *13*, 983–984. [[CrossRef](#)]
82. Murray, P.S.; Holmes, P.V. An Overview of Brain-Derived Neurotrophic Factor and Implications for Excitotoxic Vulnerability in the Hippocampus. *Int. J. Pept.* **2011**, *2011*, 1–12. [[CrossRef](#)]
83. Rothermundt, M.; Peters, M.; Prehn, J.; Arolt, V. S100B in brain damage and neurodegeneration. *Microsc. Res. Tech.* **2003**, *60*, 614–632. [[CrossRef](#)] [[PubMed](#)]
84. Pomper, N.; Liu, Y.; Hoyer, M.L.; Dougherty, J.D.; Miller, T.M. CNS microRNA profiles: A database for cell type enriched microRNA expression across the mouse central nervous system. *Sci. Rep.* **2020**, *10*, 4921. [[CrossRef](#)] [[PubMed](#)]
85. Cruz, C.D.; Cruz, F. The ERK 1 and 2 Pathway in the Nervous System: From Basic Aspects to Possible Clinical Applications in Pain and Visceral Dysfunction. *Curr. Neuropharmacol.* **2007**, *5*, 244–252. [[CrossRef](#)] [[PubMed](#)]
86. Sun, J.; Nan, G. The extracellular signal-regulated kinase 1/2 pathway in neurological diseases: A potential therapeutic target (Review). *Int. J. Mol. Med.* **2017**, *39*, 1338–1346. [[CrossRef](#)] [[PubMed](#)]
87. Subramaniam, S.; Unsicker, K. ERK and cell death: ERK1/2 in neuronal death. *FEBS J.* **2009**, *277*, 22–29. [[CrossRef](#)]
88. Zhao, Y.; Luo, P.; Guo, Q.; Li, S.; Zhang, L.; Zhao, M.; Xu, H.; Yang, Y.; Poon, W.; Fei, Z. Interactions between SIRT1 and MAPK/ERK regulate neuronal apoptosis induced by traumatic brain injury in vitro and in vivo. *Exp. Neurol.* **2012**, *237*, 489–498. [[CrossRef](#)]
89. Jiang, Q.; Gu, Z.; Zhang, G.; Jing, G. Diphosphorylation and involvement of extracellular signal-regulated kinases (ERK1/2) in glutamate-induced apoptotic-like death in cultured rat cortical neurons. *Brain Res.* **2000**, *857*, 71–77. [[CrossRef](#)]
90. Satoh, T.; Nakatsuka, D.; Watanabe, Y.; Nagata, I.; Kikuchi, H.; Namura, S. Neuroprotection by MAPK/ERK kinase inhibition with U0126 against oxidative stress in a mouse neuronal cell line and rat primary cultured cortical neurons. *Neurosci. Lett.* **2000**, *288*, 163–166. [[CrossRef](#)]
91. Schwarzer, C. 30 years of dynorphins—New insights on their functions in neuropsychiatric diseases. *Pharmacol. Ther.* **2009**, *123*, 353–370. [[CrossRef](#)]
92. Albillos, A.; Carbone, E.; Gandia, L.; Garcia, A.G.; Polio, A. Opioid Inhibition of Ca²⁺ Channel Subtypes in Bovine Chromaffin Cells: Selectivity of Action and Voltage-dependence. *Eur. J. Neurosci.* **1996**, *8*, 1561–1570. [[CrossRef](#)]
93. Rittase, W.B.; Dong, Y.; Barksdale, D.; Galdzicki, Z.; Bausch, S.B. Dynorphin up-regulation in the dentate granule cell mossy fiber pathway following chronic inhibition of GluN2B-containing NMDAR is associated with increased CREB (Ser 133) phosphorylation, but is independent of BDNF/TrkB signaling pathways. *Mol. Cell. Neurosci.* **2014**, *60*, 63–71. [[CrossRef](#)] [[PubMed](#)]
94. Wagner, J.J.; Terman, G.W.; Chavkin, C. Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in the hippocampus. *Nat.* **1993**, *363*, 451–454. [[CrossRef](#)] [[PubMed](#)]
95. Sgroi, S.; Tonini, R. Opioidergic Modulation of Striatal Circuits, Implications in Parkinson’s Disease and Levodopa Induced Dyskinesia. *Front. Neurol.* **2018**, *9*, 524. [[CrossRef](#)] [[PubMed](#)]
96. Bridges, R.S. Long-term alterations in neural and endocrine processes induced by motherhood in mammals. *Horm. Behav.* **2015**, *77*, 193–203. [[CrossRef](#)] [[PubMed](#)]
97. Kohl, J.; Autry, A.E.; Dulac, C. The neurobiology of parenting: A neural circuit perspective. *BioEssays* **2016**, *39*, e201600159-11. [[CrossRef](#)]

98. Pawluski, J.L.; Lambert, K.G.; Kinsley, C.H. Neuroplasticity in the maternal hippocampus: Relation to cognition and effects of repeated stress. *Horm. Behav.* **2016**, *77*, 86–97. [[CrossRef](#)]
99. Lau, A.; Tymianski, M. Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Arch.-Eur. J. Physiol.* **2010**, *460*, 525–542. [[CrossRef](#)]
100. Moussawi, K.; Riegel, A.; Nair, S.; Kalivas, P.W. Extracellular Glutamate: Functional Compartments Operate in Different Concentration Ranges. *Front. Syst. Neurosci.* **2011**, *5*, 94. [[CrossRef](#)]
101. Simões, A.P.; Silva, C.S.G.; Marques, J.; Pochmann, D.; Porciúncula, L.O.; Ferreira, S.; Osés, J.P.; Beleza, R.O.; Real, J.I.; Köfalvi, A.; et al. Glutamate-induced and NMDA receptor-mediated neurodegeneration entails P2Y1 receptor activation. *Cell Death Dis.* **2018**, *9*, 297. [[CrossRef](#)]
102. Maurer, S.V.; Williams, C.L. The Cholinergic System Modulates Memory and Hippocampal Plasticity via Its Interactions with Non-Neuronal Cells. *Front. Immunol.* **2017**, *8*, 1489. [[CrossRef](#)]
103. Picciotto, M.R.; Higley, M.J.; Mineur, Y.S. Acetylcholine as a Neuromodulator: Cholinergic Signaling Shapes Nervous System Function and Behavior. *Neuron* **2012**, *76*, 116–129. [[CrossRef](#)] [[PubMed](#)]
104. Jaffard, R.; Galey, D.; Micheau, J.; Durkin, T. The cholinergic septo-hippocampal pathway, learning and memory. In *Brain Plasticity, Learning, and Memory*; Will, B.E., Schmitt, P., Dalrymple-Alford, J.C., Eds.; Springer: Boston, MA, USA, 1985; pp. 167–181.
105. Nicoll, R.A. The septo-hippocampal projection: A model cholinergic pathway. *Trends Neurosci.* **1985**, *8*, 533–536. [[CrossRef](#)]
106. Bahena-Trujillo, R.; Flores, G.; Arias-Montaña, J.A. Dopamina: Síntesis, liberación y receptores en el Sistema Nervioso Central. *Rev. Biomed.* **2000**, *11*, 39–60. [[CrossRef](#)]
107. Gudi, V.; Gai, L.; Herder, V.; Tejedor, L.S.; Kipp, M.; Amor, S.; Sühs, K.-W.; Hansmann, F.; Beineke, A.; Baumgärtner, W.; et al. Synaptophysin Is a Reliable Marker for Axonal Damage. *J. Neuropathol. Exp. Neurol.* **2017**, *76*, 109–125. [[CrossRef](#)]
108. Salim, S. Oxidative Stress and the Central Nervous System. *J. Pharmacol. Exp. Ther.* **2017**, *360*, 201–205. [[CrossRef](#)]
109. Wang, X.; Michaelis, E.K. Selective neuronal vulnerability to oxidative stress in the brain. *Front. Aging Neurosci.* **2010**, *2*, 12. [[CrossRef](#)]
110. Baba, S.P.; Bhatnagar, A. Role of thiols in oxidative stress. *Curr. Opin. Toxicol.* **2018**, *7*, 133–139. [[CrossRef](#)]
111. Harris, C.; Hansen, J.M. Oxidative stress, thiols, and redox profiles. In *Developmental Toxicology*; Harris, C., Hansen, J., Eds.; Humana Press: Totowa, NJ, USA, 2012; pp. 325–346.
112. Grant, R.S.; Guest, J.A. Effects of dietary derived antioxidants on the central nervous system. *Int. J. Nutr. Pharmacol. Neurol. Dis.* **2012**, *2*, 185. [[CrossRef](#)]
113. Peng, L. Mice Brain Tissue Injury Induced by Diisononyl Phthalate Exposure and the Protective Application of Vitamin, E.J. *Biochem. Mol. Toxicol.* **2015**, *29*, 311–320. [[CrossRef](#)]
114. Kwon, D.H.; Cha, H.-J.; Lee, H.; Hong, S.-H.; Park, C.; Park, S.-H.; Kim, G.-Y.; Kim, S.; Kim, H.-S.; Hwang, H.-J.; et al. Protective Effect of Glutathione against Oxidative Stress-induced Cytotoxicity in RAW 264.7 Macrophages through Activating the Nuclear Factor Erythroid 2-Related Factor-2/Heme Oxygenase-1 Pathway. *Antioxidants* **2019**, *8*, 82. [[CrossRef](#)]
115. Tan, B.L.; Norhaizan, M.E.; Liew, W.-P.-P.; Rahman, H.S. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Front. Pharmacol.* **2018**, *9*, 1162. [[CrossRef](#)]
116. Kumar, S.; Trivedi, P.K. Glutathione S-Transferases: Role in Combating Abiotic Stresses Including Arsenic Detoxification in Plants. *Front. Plant Sci.* **2018**, *9*, 751. [[CrossRef](#)] [[PubMed](#)]
117. Astiz, M.; de Alaniz, M.J.; Marra, C.A. Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 2025–2032. [[CrossRef](#)] [[PubMed](#)]
118. Yang, Q.; Zhou, J. Neuroinflammation in the central nervous system: Symphony of glial cells. *Glia* **2018**, *67*, 1017–1035. [[CrossRef](#)]
119. Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **2018**, *9*, 7204–7218. [[CrossRef](#)] [[PubMed](#)]
120. Glass, C.K.; Saijo, K.; Winner, B.; Marchetto, M.C.; Gage, F.H. Mechanisms Underlying Inflammation in Neurodegeneration. *Cell* **2010**, *140*, 918–934. [[CrossRef](#)]
121. Yuste, J.E.; Tarragon, E.; Campuzano, C.M.; Cros, E.T. Implications of glial nitric oxide in neurodegenerative diseases. *Front. Cell. Neurosci.* **2015**, *9*, 322. [[CrossRef](#)] [[PubMed](#)]
122. Saraste, A.; Pulkki, K. Morphologic and biochemical hallmarks of apoptosis. *Cardiovasc. Res.* **2000**, *45*, 528–537. [[CrossRef](#)]
123. Badadani, M. Autophagy Mechanism, Regulation, Functions, and Disorders. *ISRN Cell Biol.* **2012**, *2012*, 927064. [[CrossRef](#)]
124. Edlich, F. BCL-2 proteins and apoptosis: Recent insights and unknowns. *Biochem. Biophys. Res. Commun.* **2018**, *500*, 26–34. [[CrossRef](#)]
125. Mizushima, N. Autophagy: Process and function. *Genes Dev.* **2007**, *21*, 2861–2873. [[CrossRef](#)] [[PubMed](#)]
126. Stavoe, A.; Holzbaur, E.L.F. Autophagy in Neurons. *Annu. Rev. Cell Dev. Biol.* **2019**, *35*, 477–500. [[CrossRef](#)] [[PubMed](#)]
127. Babin, P.J.; Goizet, C.; Raldúa, D. Zebrafish models of human motor neuron diseases: Advantages and limitations. *Prog. Neurobiol.* **2014**, *118*, 36–58. [[CrossRef](#)] [[PubMed](#)]
128. Fitzgerald, J.A.; Könemann, S.; Krümpelmann, L.; Županič, A.; Berg, C.V. Approaches to Test the Neurotoxicity of Environmental Contaminants in the Zebrafish Model: From Behavior to Molecular Mechanisms. *Environ. Toxicol. Chem.* **2020**, *40*, 989–1006. [[CrossRef](#)]
129. Flinn, L.; Bretaud, S.; Lo, C.; Ingham, P.W.; Bandmann, O. Zebrafish as a new animal model for movement disorders. *J. Neurochem.* **2008**, *106*, 1991–1997. [[CrossRef](#)]

130. Faria, M.; Bedrossiantz, J.; Ramírez, J.R.R.; Mayol, M.; García, G.H.; Bellot, M.; Prats, E.; Garcia-Reyero, N.; Gómez-Canela, C.; Gómez-Oliván, L.M.; et al. Glyphosate targets fish monoaminergic systems leading to oxidative stress and anxiety. *Environ. Int.* **2020**, *146*, 106253. [[CrossRef](#)] [[PubMed](#)]
131. Horzmann, K.A.; Freeman, J.L. Zebrafish Get Connected: Investigating Neurotransmission Targets and Alterations in Chemical Toxicity. *Toxics* **2016**, *4*, 19. [[CrossRef](#)]
132. Bernal-Rey, D.L.; Cantera, C.G.; Afonso, M.D.S.; Menéndez-Helman, R.J. Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate. *Ecotoxicol. Environ. Saf.* **2019**, *187*, 109673. [[CrossRef](#)]
133. Braz-Mota, S.; Sadauskas-Henrique, H.; Duarte, R.; Val, A.; Almeida-Val, V.M. Roundup® exposure promotes gills and liver impairments, DNA damage and inhibition of brain cholinergic activity in the Amazon teleost fish *Colossoma macropomum*. *Chemosphere* **2015**, *135*, 53–60. [[CrossRef](#)]
134. Bridi, D.; Altenhofen, S.; Gonzalez, J.B.; Reolon, G.K.; Bonan, C.D. Glyphosate and Roundup® alter morphology and behavior in zebrafish. *Toxicology* **2017**, *392*, 32–39. [[CrossRef](#)]
135. Díaz-Martín, R.D.; Valencia-Hernández, J.D.; Betancourt-Lozano, M.; Yáñez-Rivera, B. Changes in microtubule stability in zebrafish (*Danio rerio*) embryos after glyphosate exposure. *Heliyon* **2021**, *7*, e06027. [[CrossRef](#)] [[PubMed](#)]
136. Teixeira, J.M.D.S.; Lima, V.D.S.; De Moura, F.R.; Marisco, P.D.C.; Sinhorin, A.P.; Sinhorin, V.D.G. Acute toxicity and effects of Roundup Original® on pintado da Amazônia. *Environ. Sci. Pollut. Res.* **2018**, *25*, 25383–25389. [[CrossRef](#)] [[PubMed](#)]
137. Forner-Piquer, I.; Faucherre, A.; Byram, J.; Blaquiere, M.; de Bock, F.; Gamet-Payraastre, L.; Ellero-Simatós, S.; Audinat, E.; Jopling, C.; Marchi, N. Differential impact of dose-range glyphosate on locomotor behavior, neuronal activity, glio-cerebrovascular structures, and transcript regulations in zebrafish larvae. *Chemosphere* **2020**, *267*, 128986. [[CrossRef](#)] [[PubMed](#)]
138. Jin, J.; Kurobe, T.; Ramírez-Duarte, W.F.; Bolotaolo, M.B.; Lam, C.H.; Pandey, P.K.; Hung, T.-C.; Stillway, M.E.; Zweig, L.; Caudill, J.; et al. Sub-lethal effects of herbicides penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (*Hypomesus transpacificus*). *Aquat. Toxicol.* **2018**, *197*, 79–88. [[CrossRef](#)] [[PubMed](#)]
139. Khan, A.K.; Shah, N.; Gul, A.; Sahar, N.U.; Ismail, A.; Muhammad, M.; Aziz, F.; Farooq, M.; Adnan, M.; Rizwan, M. Comparative Study of Toxicological Impinge of Glyphosate and Atrazine (Herbicide) on Stress Biomarkers; Blood Biochemical and Hematological Parameters of the Freshwater Common Carp (*Cyprinus carpio*). *Pol. J. Environ. Stud.* **2016**, *25*, 1995–2001. [[CrossRef](#)]
140. Lanzarin, G.A.B.; Félix, L.M.; Santos, D.; Venâncio, C.A.S.; Monteiro, S.M. Dose-dependent effects of a glyphosate commercial formulation—Roundup® UltraMax—on the early zebrafish embryogenesis. *Chemosphere* **2019**, *223*, 514–522. [[CrossRef](#)] [[PubMed](#)]
141. Lanzarin, G.A.; Venâncio, C.; Monteiro, S.M.; Félix, L.M. Behavioural toxicity of environmental relevant concentrations of a glyphosate commercial formulation—RoundUp® UltraMax—During zebrafish embryogenesis. *Chemosphere* **2020**, *253*, 126636. [[CrossRef](#)]
142. Li, M.-H.; Xu, H.-D.; Liu, Y.; Chen, T.; Jiang, L.; Fu, Y.-H.; Wang, J.-S. Multi-tissue metabolic responses of goldfish (*Carassius auratus*) exposed to glyphosate-based herbicide. *Toxicol. Res.* **2016**, *5*, 1039–1052. [[CrossRef](#)]
143. Li, M.; Ruan, L.-Y.; Zhou, J.-W.; Fu, Y.-H.; Jiang, L.; Zhao, H.; Wang, J.-S. Metabolic profiling of goldfish (*Carassius auratus*) after long-term glyphosate-based herbicide exposure. *Aquat. Toxicol.* **2017**, *188*, 159–169. [[CrossRef](#)]
144. Lopes, F.M.; Caldas, S.S.; Primel, E.G.; da Rosa, C.E. Glyphosate Adversely Affects *Danio rerio* Males: Acetylcholinesterase Modulation and Oxidative Stress. *Zebrafish* **2017**, *14*, 97–105. [[CrossRef](#)]
145. Menéndez-Helman, R.J.; Ferreyroa, G.V.; Afonso, M.D.S.; Salibián, A. Glyphosate as an Acetylcholinesterase Inhibitor in *Cnesterodon decemmaculatus*. *Bull. Environ. Contam. Toxicol.* **2011**, *88*, 6–9. [[CrossRef](#)] [[PubMed](#)]
146. Menéndez-Helman, R.J.; Miranda, L.A.; Afonso, M.D.S.; Salibián, A. Subcellular energy balance of *Odontesthes bonariensis* exposed to a glyphosate-based herbicide. *Ecotoxicol. Environ. Saf.* **2015**, *114*, 157–163. [[CrossRef](#)] [[PubMed](#)]
147. Pereira, A.G.; Jaramillo, M.L.; Remor, A.P.; Latini, A.; Davico, C.E.; da Silva, M.L.; Müller, Y.M.; Ammar, D.; Nazari, E.M. Low-concentration exposure to glyphosate-based herbicide modulates the complexes of the mitochondrial respiratory chain and induces mitochondrial hyperpolarization in the *Danio rerio* brain. *Chemosphere* **2018**, *209*, 353–362. [[CrossRef](#)] [[PubMed](#)]
148. Roy, N.M.; Carneiro, B.; Ochs, J. Glyphosate induces neurotoxicity in zebrafish. *Environ. Toxicol. Pharmacol.* **2016**, *42*, 45–54. [[CrossRef](#)]
149. Sánchez, J.A.A.; Barros, D.M.; Bistoni, M.D.L.A.; Ballesteros, M.L.; Roggio, M.A.; Martins, C.D.G.M. Glyphosate-based herbicides affect behavioural patterns of the livebearer *Jenynsia multidentata*. *Environ. Sci. Pollut. Res.* **2021**, *28*, 29958–29970. [[CrossRef](#)]
150. Sobjak, T.M.; Romão, S.; Nascimento, C.Z.D.; dos Santos, A.F.P.; Vogel, L.; Guimarães, A.T.B. Assessment of the oxidative and neurotoxic effects of glyphosate pesticide on the larvae of *Rhamdia quelen* fish. *Chemosphere* **2017**, *182*, 267–275. [[CrossRef](#)]
151. Zhang, S.; Xu, J.; Kuang, X.; Li, S.; Li, X.; Chen, D.; Zhao, X.; Feng, X. Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (*Danio rerio*). *Chemosphere* **2017**, *181*, 270–280. [[CrossRef](#)]
152. Carhart-Harris, R.L.; Nutt, D.J. Serotonin and brain function: A tale of two receptors. *J. Psychopharmacol.* **2017**, *31*, 1091–1120. [[CrossRef](#)]
153. Strużyńska, L.; Sulkowski, G. Relationships between glutamine, glutamate, and GABA in nerve endings under Pb-toxicity conditions. *J. Inorg. Biochem.* **2004**, *98*, 951–958. [[CrossRef](#)]
154. Zorova, L.D.; Popkov, V.A.; Plotnikov, E.Y.; Silachev, D.N.; Pevzner, I.B.; Jankauskas, S.S.; Babenko, V.A.; Zorov, S.D.; Balakireva, A.V.; Juhaszova, M.; et al. Mitochondrial membrane potential. *Anal. Biochem.* **2018**, *552*, 50–59. [[CrossRef](#)]

155. Tayebati, S.; Marucci, G.; Santinelli, C.; Buccioni, M.; Amenta, F. Choline-Containing Phospholipids: Structure-Activity Relationships Versus Therapeutic Applications. *Curr. Med. Chem.* **2015**, *22*, 4328–4340. [[CrossRef](#)] [[PubMed](#)]
156. Zahr, N.M.; Mayer, D.; Rohlfing, T.; Sullivan, E.V.; Pfefferbaum, A. Imaging Neuroinflammation? A Perspective from MR Spectroscopy. *Brain Pathol.* **2014**, *24*, 654–664. [[CrossRef](#)] [[PubMed](#)]
157. He, B.; Ni, Y.; Jin, Y.; Fu, Z. Pesticides-induced energy metabolic disorders. *Sci. Total Environ.* **2020**, *729*, 139033. [[CrossRef](#)] [[PubMed](#)]
158. Diemel, G.A. Brain Glucose Metabolism: Integration of Energetics with Function. *Physiol. Rev.* **2019**, *99*, 949–1045. [[CrossRef](#)]
159. Holtzman, D. Brain Creatine Kinases and Phosphocreatine: An Update. *Dev. Neurosci.* **1996**, *18*, 522–523. [[CrossRef](#)]
160. Cunnane, S.C.; Courchesne-Loyer, A.; Vandenberghe, C.; St-Pierre, V.; Fortier, M.; Hennebelle, M.; Croteau, E.; Bocti, C.; Fulop, T.; Castellano, C.-A. Can Ketones Help Rescue Brain Fuel Supply in Later Life? Implications for Cognitive Health during Aging and the Treatment of Alzheimer's Disease. *Front. Mol. Neurosci.* **2016**, *9*, 53. [[CrossRef](#)]
161. Tovar-Franco, J.A.; Ortiz, M.J.S.; Morales, L.; Espindola, C. Regulación de la utilización del aspartato por los astrocitos durante la prelactancia. *Univ. Sci.* **2009**, *14*, 151–163. [[CrossRef](#)]
162. Moffett, J.R.; Arun, P.; Ariyannur, P.S.; Namboodiri, A.M. N-Acetylaspartate reductions in brain injury: Impact on post-injury neuroenergetics, lipid synthesis, and protein acetylation. *Front. Neuroenergetics* **2013**, *5*, 11. [[CrossRef](#)]
163. Domise, M.; Sauvé, F.; Didier, S.; Caillerez, R.; Bégard, S.; Carrier, S.; Colin, M.; Marinangeli, C.; Buée, L.; Vingtdeux, V. Neuronal AMP-activated protein kinase hyper-activation induces synaptic loss by an autophagy-mediated process. *Cell Death Dis.* **2019**, *10*, 221. [[CrossRef](#)]
164. McVey, K.A.; Snapp, I.B.; Johnson, M.B.; Negga, R.; Pressley, A.S.; Fitsanakis, V.A. Exposure of *C. elegans* eggs to a glyphosate-containing herbicide leads to abnormal neuronal morphology. *Neurotoxicol. Teratol.* **2016**, *55*, 23–31. [[CrossRef](#)]
165. Burchfield, S.L.; Bailey, D.C.; Todt, C.E.; Denney, R.D.; Negga, R.; Fitsanakis, V.A. Acute exposure to a glyphosate-containing herbicide formulation inhibits Complex II and increases hydrogen peroxide in the model organism *Caenorhabditis elegans*. *Environ. Toxicol. Pharmacol.* **2018**, *66*, 36–42. [[CrossRef](#)] [[PubMed](#)]
166. Fortes, C.; Mastroeni, S.; Segatto, M.M.; Hohmann, C.; Miligi, L.; Bakos, L.; Bonamigo, R. Occupational Exposure to Pesticides With Occupational Sun Exposure Increases the Risk for Cutaneous Melanoma. *J. Occup. Environ. Med.* **2016**, *58*, 370–375. [[CrossRef](#)] [[PubMed](#)]
167. Jayasumana, C.; Gunatilake, S.; Senanayake, P. Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka? *Int. J. Environ. Res. Public Health* **2014**, *11*, 2125–2147. [[CrossRef](#)] [[PubMed](#)]
168. Kamel, F.; Hoppin, J.A. Association of Pesticide Exposure with Neurologic Dysfunction and Disease. *Environ. Health Perspect.* **2004**, *112*, 950–958. [[CrossRef](#)] [[PubMed](#)]
169. Lee, W.J.; Alavanja, M.C.; Hoppin, J.; Rusiecki, J.A.; Kamel, F.; Blair, A.; Sandler, D.P. Mortality among Pesticide Applicators Exposed to Chlorpyrifos in the Agricultural Health Study. *Environ. Health Perspect.* **2007**, *115*, 528–534. [[CrossRef](#)]
170. Le Couteur, D.; McLean, A.; Taylor, M.; Woodham, B.; Board, P. Pesticides and Parkinson's disease. *Biomed. Pharmacother.* **1999**, *53*, 122–130. [[CrossRef](#)]
171. Gasnier, C.; Dumont, C.; Benachour, N.; Clair, E.; Chagnon, M.-C.; Séralini, G.-E. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* **2009**, *262*, 184–191. [[CrossRef](#)] [[PubMed](#)]
172. Koller, V.J.; Fürhacker, M.; Nersesyan, A.; Mišík, M.; Eisenbauer, M.; Knasmueller, S. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch. Toxicol.* **2012**, *86*, 805–813. [[CrossRef](#)]
173. Prasad, S.; Srivastava, S.; Singh, M.; Shukla, Y. Clastogenic Effects of Glyphosate in Bone Marrow Cells of Swiss Albino Mice. *J. Toxicol.* **2009**, *2009*, 308985. [[CrossRef](#)]
174. Lajmanovich, R.C.; Sandoval, M.T.; Peltzer, P.M. Induction of Mortality and Malformation in *Scinax nasicus* Tadpoles Exposed to Glyphosate Formulations. *Bull. Environ. Contam. Toxicol.* **2003**, *70*, 612–618. [[CrossRef](#)]
175. Barbosa, E.R.; da Costa, M.D.L.; Bacheschi, L.A.; Scaff, M.; Leite, C.C. Parkinsonism after glycine-derivate exposure. *Mov. Disord.* **2001**, *16*, 565–568. [[CrossRef](#)] [[PubMed](#)]
176. Menkes, D.; Temple, W.; Edwards, I. Intentional Self-Poisoning with Glyphosate-Containing Herbicides. *Hum. Exp. Toxicol.* **1991**, *10*, 103–107. [[CrossRef](#)] [[PubMed](#)]
177. Mose, T.; Kjaerstad, M.B.; Mathiesen, L.; Nielsen, J.B.; Edelfors, S.; Knudsen, L.E. Placental Passage of Benzoic acid, Caffeine, and Glyphosate in an Ex Vivo Human Perfusion System. *J. Toxicol. Environ. Health Part A* **2008**, *71*, 984–991. [[CrossRef](#)] [[PubMed](#)]
178. Poulsen, M.S.; Rytting, E.; Mose, T.; Knudsen, L.E. Modeling placental transport: Correlation of in vitro BeWo cell permeability and ex vivo human placental perfusion. *Toxicol. Vitro.* **2009**, *23*, 1380–1386. [[CrossRef](#)]
179. Woollorton, J.; Pidoplichko, V.I.; Broide, R.S.; Dani, J.A. Differential Desensitization and Distribution of Nicotinic Acetylcholine Receptor Subtypes in Midbrain Dopamine Areas. *J. Neurosci.* **2003**, *23*, 3176–3185. [[CrossRef](#)]
180. Balali-Mood, M.; Saber, H. Recent Advances in the Treatment of Organophosphorous Poisonings. *Iran. J. Med. Sci.* **2012**, *37*, 74–91.
181. Scheffel, C.; Niessen, K.V.; Rappenglück, S.; Wanner, K.; Thiermann, H.; Worek, F.; Seeger, T. Counteracting desensitization of human $\alpha 7$ -nicotinic acetylcholine receptors with bispyridinium compounds as an approach against organophosphorus poisoning. *Toxicol. Lett.* **2018**, *293*, 149–156. [[CrossRef](#)]
182. Wojda, U.; Salinska, E.; Kuznicki, J. Calcium ions in neuronal degeneration. *IUBMB Life* **2008**, *60*, 575–590. [[CrossRef](#)]
183. De, A. Wnt/Ca²⁺ signaling pathway: A brief overview. *Acta Biochim. Biophys. Sin.* **2011**, *43*, 745–756. [[CrossRef](#)]

184. McMurry, J.L.; Chrestensen, C.A.; Scott, I.M.; Lee, E.W.; Rahn, A.M.; Johansen, A.M.; Forsberg, B.J.; Harris, K.D.; Salerno, J.C. Rate, affinity and calcium dependence of nitric oxide synthase isoform binding to the primary physiological regulator calmodulin. *FEBS J.* **2011**, *278*, 4943–4954. [CrossRef]
185. Donato, R.; Sorci, G.; Riuuzzi, F.; Arcuri, C.; Bianchi, R.; Brozzi, F.; Tubaro, C.; Giambanco, I. S100B's double life: Intracellular regulator and extracellular signal. *Biochim. Biophys. Acta* **2009**, *1793*, 1008–1022. [CrossRef] [PubMed]
186. Bianchi, R.; Giambanco, I.; Donato, R. S100B/RAGE-dependent activation of microglia via NF- κ B and AP-1: Co-regulation of COX-2 expression by S100B, IL-1 β and TNF- α . *Neurobiol. Aging* **2010**, *31*, 665–677. [CrossRef]
187. Petrova, T.V.; Hu, J.; Van Eldik, L.J. Modulation of glial activation by astrocyte-derived protein S100B: Differential responses of astrocyte and microglial cultures. *Brain Res.* **2000**, *853*, 74–80. [CrossRef]
188. Sun, F.; Xu, X.; Wang, X.; Zhang, B. Regulation of autophagy by Ca²⁺. *Tumor Biol.* **2016**, *37*, 15467–15476. [CrossRef] [PubMed]
189. Ríos, J.A.; Godoy, J.A.; Inestrosa, N.C. Wnt3a ligand facilitates autophagy in hippocampal neurons by modulating a novel GSK-3 β -AMPK axis. *Cell Commun. Signal.* **2018**, *16*, 15. [CrossRef] [PubMed]
190. Lorzadeh, S.; Kohan, L.; Ghavami, S.; Azarpira, N. Autophagy and the Wnt signaling pathway: A focus on Wnt/ β -catenin signaling. *Biochim. Biophys. Acta* **2020**, *1868*, 118926. [CrossRef]
191. Hollville, E.; Romero, S.E.; Deshmukh, M. Apoptotic cell death regulation in neurons. *FEBS J.* **2019**, *286*, 3276–3298. [CrossRef]
192. Fricker, M.; Tolkovsky, A.M.; Borutaite, V.; Coleman, M.; Brown, G.C. Neuronal Cell Death. *Physiol. Rev. Am. Physiol. Soc.* **2018**, *98*, 813–880. [CrossRef]
193. World Health Organization. Pesticide Residues in Food-2004: Evaluations 2004: Part II-Toxicological (Vol. 178). World Health Organization. 2006. Available online: http://apps.who.int/iris/bitstream/handle/10665/43624/9241665203_eng.pdf;jsessionid=98409D614439EBC6C58392A3809839C4?sequence=1 (accessed on 30 July 2021).
194. Solomon, K.R. Glyphosate in the general population and in applicators: A critical review of studies on exposures. *Crit. Rev. Toxicol.* **2016**, *46*, 21–27. [CrossRef]
195. Paumgartten, F.J.R. To be or not to be a carcinogen; delving into the glyphosate classification controversy. *Braz. J. Pharm. Sci.* **2019**, *55*, 2175-97902019000118217. [CrossRef]
196. Kawagashira, Y.; Koike, H.; Kawabata, K.; Takahashi, M.; Ohyama, K.; Hashimoto, R.; Iijima, M.; Katsuno, M.; Sobue, G. Vasculitic Neuropathy Following Exposure to a Glyphosate-based Herbicide. *Intern. Med.* **2017**, *56*, 1431–1434. [CrossRef]
197. Ramwell, C.T.; Heather, A.I.J.; Shepherd, A.J. Herbicide loss following application to a roadside. *Pest Manag. Sci.* **2002**, *58*, 695–701. [CrossRef] [PubMed]
198. Langiano, V.D.C.; Martinez, C. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2008**, *147*, 222–231. [CrossRef] [PubMed]
199. Contardo-Jara, V.; Klingelmann, E.; Wiegand, C. Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environ. Pollut.* **2008**, *157*, 57–63. [CrossRef] [PubMed]
200. Dey, S.; Samanta, P.; Pal, S.; Mukherjee, A.K.; Kole, D.; Ghosh, A.R. Integrative assessment of biomarker responses in teleostean fishes exposed to glyphosate-based herbicide (Excel Mera 71). *Emerg. Contam.* **2016**, *2*, 191–203. [CrossRef]
201. El-Sheshtawy, S.M.; Nada, M.M.; Elhafeez, M.S.A.; Samak, D.H. Protective Effect of Supplementation with Powdered Mulberry Leaves on Glyphosate-Induced Toxicity in Catfish (*Clarias gariepinus*). *Adv. Anim. Vet.-Sci.* **2021**, *9*, 1718–1731. [CrossRef]
202. Rossi, A.S.; Fantón, N.; Michlig, M.P.; Repetti, M.R.; Cazenave, J. Fish inhabiting rice fields: Bioaccumulation, oxidative stress and neurotoxic effects after pesticides application. *Ecol. Indic.* **2020**, *113*, 106186. [CrossRef]
203. Fiorino, E.; Sehonova, P.; Plhalova, L.; Blahova, J.; Svobodova, Z.; Faggio, C. Effects of glyphosate on early life stages: Comparison between *Cyprinus carpio* and *Danio rerio*. *Environ. Sci. Pollut. Res.* **2018**, *25*, 8542–8549. [CrossRef]
204. Benachour, N.; Seralini, G.-E. Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. *Chem. Res. Toxicol.* **2009**, *22*, 97–105. [CrossRef]
205. Mesnage, R.; Ibragim, M.; Mandrioli, D.; Falcioni, L.; Tibaldi, E.; Belpoggi, F.; Brandsma, I.; Bourne, E.; Savage, E.; Mein, C.A.; et al. Comparative Toxicogenomics of Glyphosate and Roundup Herbicides by Mammalian Stem Cell-Based Genotoxicity Assays and Molecular Profiling in Sprague-Dawley Rats. *Toxicol. Sci.* **2021**, *186*, 83–101. [CrossRef]
206. Tsui, M.T.; Chu, L.M. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. *Chemosphere* **2003**, *52*, 1189–1197. [CrossRef]
207. El-Shenawy, N.S. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. *Environ. Toxicol. Pharmacol.* **2009**, *28*, 379–385. [CrossRef] [PubMed]
208. Peixoto, F. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere* **2005**, *61*, 1115–1122. [CrossRef] [PubMed]
209. Richard, S.; Moslemi, S.; Sipahutar, H.; Benachour, N.; Seralini, G.-E. Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase. *Environ. Health Perspect.* **2005**, *113*, 716–720. [CrossRef] [PubMed]



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San Jose Creek Flood Control Revegetation Sites **And Los Carneros Mitigation Bank** **Review and Recommendations**



ENVIRONMENTAL DEFENSE CENTER

October 24, 2022

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Cover Photo: Norma Revegetation Site on San Jose Creek with tip-rap bank stabilization and willow woodland habitat installed by Santa Barbara County Flood Control District.

Table Of Contents

I.	Executive Summary	4
	A. Background	4
	B. Introduction	5
	C. Findings and Recommendations	7
	D. Acknowledgements	8
II.	Purpose, Goals, and Methods	8
	A. Purpose	8
	B. Goals	8
	C. Methods	9
III.	Habitat Mitigation for Flood Control Annual Maintenance	10
	A. CDFW Performance Standards	10
	B. Mitigation Ratios	11
	C. Mitigation Locations and In-kind Mitigation	12
	D. Mitigation Banks	12
	E. Mitigation for Repeated Impacts in the Same Locations	13
IV.	San Jose Creek Revegetation Project Sites	14
	A. Vineyard Road	15
	B. Norma (Site 1)	30
	C. Paseo Cameo (Site 11)	37
	D. North Cathedral Oaks Road (Site 2)	39
	E. Cathedral Oaks Village Association #1- #4	43
	F. Old Footbridge in University Circle Open Space (Site 4)	78
	G. Upstream Seventy-five Feet From Berkeley Footbridge (Site 5)	85
	H. Berkeley Road Footbridge by Goleta Water District (GWD) Building (Site 6)	87
	I. Three-hundred Feet Downstream from Berkeley Footbridge, East Bank Repair (Site 23)	90
	J. Berkeley Road Downstream from Berkeley Footbridge, West Side of Creek (Site 13)	97
	K. Somerset Road Access Ramp	101
	L. Downstream from Somerset Road Access Ramp (Site 10)	106
	M. Downstream from HWY 101, West Bank (Site 7)	109
	N. Hansen Aggregate Site	112
	O. La Goleta Condominiums	116
	P. Armitos Road Arundo Removal	134
V.	Lake Los Carneros Mitigation Bank	140
	I. Site A	140
	II. Site D	140
	III. Sites E, F, and G	142
	IV. Sites H and I	142
	V. Site J	143
	VI. Site K	143
	VII. Site L	144
	VIII. Sites M and N	145
	IX. Site O	145
VI.	Global Recommendations	152
Appendix I:	Acronyms	159



I. Executive Summary

A. Background

The Santa Barbara County Flood Control and Water Conservation District (“SBCFCWCD”) maintains creeks throughout Santa Barbara County to reduce the threat of flooding. In 1987, creek maintenance involving clearing riparian¹ habitat on San Jose Creek led to public concern over the effects of creek maintenance on fish, wildlife, and habitat along local creeks.² The Santa Barbara County Board of Supervisors acting as the SBCFCWCD Board of Directors required the SBCFCWCD to evaluate its Annual Maintenance Program pursuant to the California Environmental Quality Act (“CEQA”). The SBCFCWCD prepared a programmatic environmental impact report (“EIR”) in 1991 to assess the environmental effects of annual creek maintenance.³ A second EIR was prepared in 2001.⁴ Projects such as the Annual Maintenance Program may only be approved if they avoid or substantially lessen significant environmental impacts whenever feasible.⁵ The 2001 EIR includes alternatives and mitigation measures termed Best Management Practices designed to lessen unavoidable environmental impacts.⁶ The SBCFCWCD also secured a California Department of Fish and Wildlife (“CDFW”) Stream Bed or Lake Alteration Agreement (“SAA”) pursuant to Fish and Game Code Section 1601. If an activity such as the Annual Maintenance Program may substantially affect fish and wildlife resources, CDFW may issue a SAA “that includes reasonable measures necessary to protect the resource.”⁷ The SAA which CDFW issued to SBCFCWCD is a multi-year permit authorizing SBCFCWCD’s Annual Maintenance Program subject to measures CDFW deems necessary to protect fish and wildlife resources in local creeks subject to CDFW jurisdiction.⁸ The most recent SAA for the Annual Maintenance Program was approved in 2015 and expires on December 31, 2025.⁹

Mitigation measures developed in the EIRs and permit conditions, including measures in the SAA, are intended to avoid, minimize, and compensate for environmental impacts, including removal of native vegetation and impacts to native habitats and species caused by creek maintenance. Numerous measures to protect water quality, riparian and aquatic habitat, and sensitive species are employed by SBCFCWCD.¹⁰ The primary method for compensating for

¹ Riparian means “relating to or living or located on the bank of a natural watercourse (such as a river) or sometimes of a lake or a tidewater.” <https://www.merriam-webster.com/dictionary/riparian>

² Keith Dalton, Santa Barbara News-Press, *Creek Bed Caper Creating Waves* (June 1987).

³ SBCFCWCD *Program Environmental Impact Report for Routine Maintenance Activities* (1991).

⁴ SBCFCWCD *Final Program Environmental Impact Report for Updated Routine Maintenance Program* (November 2001). (“SBCFCWCD (2001)”).

⁵ California Public Resources Code § 21002; CEQA Guidelines § 15092.

⁶ SBCFCWCD (2001) Chapters 5.0 and 7.0; *See also* SBCFCWCD *Annual Routine Maintenance Plan Fiscal Year 2021-2022* at i-5 (“SBCFCWCD (2022)”).

⁷ California Fish and Game Code § 1602 (a)(4)(B).

⁸ Letter from Betty J. Courtney, Environmental Program Manager, California Department of Fish and Wildlife to Seth Shank, SBCFCWCD re *Final Lake or Streambed Alteration Agreement Notification No. 1600-2015-0053-R5 Annual Routine Maintenance Program* at 4 (August 5, 2015) (“CDFW (2015)”).

⁹ CDFW (2015) at 21.

¹⁰ SBCFCWCD (2001) Chapter 5.0; *See also* CDFW (2015) at 7 - 17.



temporary and permanent impacts to habitats is creation or restoration of habitat, a process called revegetation. This report focusses on riparian habitat revegetation projects implemented by SBCFCWCD along San Jose Creek between 1992 and 2021 and at the Los Carneros Mitigation Bank between 2005 and 2020 to compensate for the effects of creek maintenance on riparian habitats.

B. Overview of the SBCFCWCD Annual Maintenance Program

The SBCFCWCD Annual Maintenance Program includes projects in Santa Barbara County creeks to protect public safety and infrastructure by lessening the potential for flooding and erosion. Maintenance projects involve removing obstructive vegetation from creek beds, reducing buildup of sediment which can constrict channel capacity, and stabilizing creek banks.¹¹ More specifically, maintenance activities include limbing of downed trees and limbs, brushing, herbicide application, desilting, channel shaping, bank stabilization, and repair and construction of check structures.¹² Work typically involves the use of hand tools including chain saws, application of herbicide to prevent regrowth of in-channel vegetation, and heavy equipment work in select areas to shore up banks, remove vegetation from creek beds, and facilitate sediment movement.¹³

The SBCFCWCD conducts springtime annual creek walks in north and south County creeks to identify locations where work is required.¹⁴ The SBCFCWCD prepares an Annual Maintenance Plan in May and June of each year.¹⁵ Annual Plans are approved by the SBCFCWCD Board of Directors in June or July.¹⁶ Projects are implemented in the Fall, typically during a narrow window “Between August 1 and December 15” after bird nesting season and before steelhead migration season.¹⁷

As part of the Annual Maintenance Plan, SBCFCWCD implements revegetation projects to mitigate the effects of vegetation and habitat removal. The current requirement to mitigate impacts comes from the 2001 SBCFCWCD Program EIR¹⁸ and regulatory permits. Regulatory permits and approvals include California Coastal Commission Coastal Development Permits or Coastal Consistency Determinations for projects in the Coastal Zone.¹⁹ Army Corps of Engineers Clean Water Act Section 404 permits are required for projects “such as bank stabilization or channel shaping” which discharge fill or dredged material into wetlands or Waters of the US.²⁰

¹¹ SBCFCWCD *Annual Routine Maintenance Plan – Fiscal Year 2021-2022* at i-2 (2021-2022) (“SBCFCWCD (2021-2022)”).

¹² CDFW (2015).

¹³ *Id.*

¹⁴ SBCFCWCD (2021-2022) at i-2 – i-3.

¹⁵ *Id.* at i-4.

¹⁶ *Id.* at i-10.

¹⁷ *Id.* at i-12.

¹⁸ SBCFCWCD (2001) at 4.4 – 4.10.

¹⁹ SBCFCWCD (2021-2022) at i-10 and i-12; *See also*: California Coastal Commission *Staff Report and Adopted Findings for Application 4-19-1158, Agenda Item F15a*, Atascadero Creek Flood Control Project, Santa Barbara County available at <https://www.coastal.ca.gov/meetings/agenda/#/2020/7> (July 10, 2020).

²⁰ SBCFCWCD (2021-2022) at i-10.



The State Water Resources Control Board issues a 401B Water Quality Certification “for projects that also require a 404 permit from the Army Corps of Engineers.”²¹

Maintenance projects which alter the streambed, bank, or riparian habitat are also regulated by the CDFW SAA.²² The SAA is a permit issued by CDFW authorizing SBCFCWCD to implement the Annual Maintenance Plans subject to permit conditions requiring protection of biological and other natural resources such as habitats, species, and water quality. It requires SBCFCWCD to implement revegetation projects to replace native habitats that are removed during maintenance activities.²³ The SAA sets forth measurable performance standards called “success criteria” to evaluate effectiveness of revegetation projects. Criteria include plant survival rates, tree growth rates, absence of woody invasive species, percent cover of herbaceous nonnative plants, and percent cover of native species.²⁴ The SAA requires SBCFCWCD to track and report on the implementation of the Annual Maintenance Plans, including revegetation projects and “their ability to meet success criteria.”²⁵ Tracking and reporting on revegetation projects is essential so that CDFW can measure and track success pursuant to the success criteria and ultimately sign-off on completed revegetation projects to ensure that the impacts of SBCFCWCD’s maintenance projects are mitigated.

The SBCFCWCD prepares Annual Maintenance and Revegetation Reports which are “designed to incorporate all the information that each agency has requested” to demonstrate implementation of maintenance projects and revegetation projects.²⁶ Annual Maintenance and Revegetation Reports are required to provide “a detailed discussion of data collection, ability to meet success criteria, monitoring activities, and tasks performed at each restoration site.”²⁷ They are intended to demonstrate that the revegetation projects are sufficiently mitigating the impacts of maintenance projects. These reports are therefore critical for determining whether revegetation projects fulfill the quantitative success criteria and comply with permits, including the SAA. These reports must therefore contain a detailed accounting and quantitative analysis of success pursuant to the criteria. As discussed below, EDC’s analysis concludes that these reports do not contain adequately detailed data collection or assessment of revegetation sites’ performance pursuant to the required success criteria.

CDFW evaluates revegetation sites to determine whether they are successful. In this case, CDFW signed off on the San Jose Creek revegetation sites as having been successful.²⁸ Yet CDFW may lack resources to inspect the numerous revegetation sites on each of the thirty-six south coast drainages, including at least nineteen revegetation sites on San Jose Creek.²⁹ Given this, CDFW must rely on the SBCFCWCD’s Annual Maintenance and Revegetation Reports to

²¹ *Id.*

²² *Id.*; *See also* CDFW (2015).

²³ *Id.* Section 3.7 at 15-16.

²⁴ *Id.* Section 3.9 at 17.

²⁵ *Id.* Section 3.5 at 15-16.

²⁶ SBCFCWCD (2020-2021) at i-13.

²⁷ CDFW (2015) at 15.

²⁸ Spencer (2022).

²⁹ CDFW (2015) at 1; *See also* Sarah Rains, CDFW phone call with Brian Trautwein (2021) (“Rains (2021)”).

gage success.³⁰ The Reports, however, do not consistently include specific information necessary to demonstrate success of revegetation projects pursuant to the SAA's success criteria, including tree growth rates, plant survival rates, presence of invasive woody species, percent cover of herbaceous invasive nonnative plants, and percent cover of native plants, as described below, and are thus insufficient to demonstrate compliance with the SAA's success criteria.³¹

C. Findings and Recommendations

This report includes an evaluation of the success of nineteen revegetation sites on San Jose Creek and fifteen revegetation polygons in the Lake Los Carneros Mitigation Bank. The Environmental Defense Center ("EDC") concludes that some revegetation sites, including the Lake Los Carneros Mitigation Bank, continue to function very well with few exotic plant species present and substantial cover by native plant species. All sites have reportedly met the applicable success criteria outlined in the CDFW SAA.³² SBCFCWCD state that, "Restoration sites are monitored by District Environmental staff for success criteria, percent cover, mortality, and tree-height. Generally, most revegetation sites achieve the success criteria within 3 to 5 years. Results and photos are submitted each year in the Post-Project Report."³³ However, EDC's analysis demonstrates that percent cover, tree height, and mortality rates are not recorded in SBCFCWCD's Annual Maintenance and Revegetation Reports and that most revegetation sites do not currently meet the SAA's specific measurable success criteria.³⁴ Some sites have become dominated by nonnative plants, including aggressive invasive species which undermine the long-term success of revegetation and raise concerns that SBCFCWCD's revegetation program may not provide long-term replacement of riparian habitat.

EDC's report sets forth site-specific recommendations to achieve compliance with the SAA's success criteria and ensure that existing revegetation sites successfully mitigate the effects of creek maintenance on riparian habitats over the long term. Programmatic recommendations are also included to ensure that SBCFCWCD's future revegetation projects fully compensate for losses in riparian habitat quantity and quality. Recommendations are intended to ensure that SBCFCWCD and permitting agencies, including CDFW, effectively monitor, track, and record short-term and long-term success pursuant to the SAA's measurable success criteria. The Annual Maintenance and Revegetation Reports must provide a more detailed and quantitative assessment of the success of restoration sites to demonstrate whether the measurable success criteria are met. In addition, CDFW should visit each revegetation site annually and only approve revegetation projects when all success criteria set forth in the SAA are met.

³⁰ SBCFCWCD *Annual Maintenance and Revegetation Reports* (2000-2001 through 2020-2021)

³¹ *Id.*

³² Maureen Spencer, Operations and Environmental Manager, SBCFCWCD, phone call with Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 25, 2022) ("Spencer (2022)").

³³ SBCFCWCD *Four-Year Status Report 2016-2019 Annual Routine Maintenance Program* at 6 (January 2020).

³⁴ Some revegetation sites were required under previous SAAs. However, EDC used the most recent SAA's success criteria to gage success of existing revegetation sites.

D. Acknowledgements

UCSB Associated Students Coastal Fund, MUFG Union Bank, and Clif Family Foundation provided generous grant funding to enable revegetation site surveys, research, meetings with SBCFCWCD staff, and preparation of this report. UCSB Associated Students Coastal Fund also provided a generous stipend to EDC's Watershed Program Intern and Coauthor, Natalie Blackwelder.

The authors of this report are extremely grateful to Maureen Spencer, SBCFCWCD Operations and Environmental Manager, for providing Annual Plans, Annual Maintenance and Revegetation Reports, Lake Los Carneros Mitigation Bank reports and information in a timely manner, taking time to speak with us to explain the SBCFCWCD's revegetation program, and accompanying us in the field. Without this exceptionally helpful assistance, EDC would not have been able to understand the details of the SBCFCWCD's revegetation program or been able to produce this detailed report.

EDC is grateful for Sarah Rains, CDFW Environmental Scientist, for providing EDC with the SAA and other documents related to SBCFCWCD's Maintenance Program and providing commentary on CDFW's process for evaluating revegetation success.

II. Purpose, Goals, and Methods

A. Purpose

This analysis evaluates the effectiveness of SBCFCWCD revegetation projects required to mitigate the effects of Annual Maintenance Plans on riparian habitat pursuant to CEQA and CDFW's SAA. EDC intends for this report to be utilized by the SBCFCWCD to improve the effectiveness of its revegetation program to ensure long-term success and prevent net loss in the extent or quality of riparian habitat along creeks in Santa Barbara County. EDC intends for this report to inform permitting agencies, including CDFW, regarding the long-term success of revegetation projects and to inform future Annual Maintenance Program permitting and tracking and monitoring of effectiveness pursuant to CDFW's success criteria.

B. Goals

In order to fulfill this purpose, the goals of this report are to:

- Identify successes and shortcomings in SBCFCWCD's existing revegetation projects.
- Identify site-specific recommendations for existing revegetation projects.
- Identify global recommendations for SBCFCWCD and for CDFW and other permitting agencies to ensure successful monitoring, effective revegetation, and tracking of success.

C. Methods

1. Process of Evaluating Success of Revegetation Projects

The following steps were undertaken to evaluate success of SBCFCWCD revegetation sites on San Jose Creek and within the Lake Los Carneros Mitigation Bank:

1. Reviewed all Annual Plans dated from 1993-94 through 2021-22.
2. Reviewed all available Maintenance and Revegetation Reports dated 1993-94 through 2020-21.
3. Created chart tracking revegetation sites and revegetation square footage at each site on San Jose Creek.
4. Created map showing each revegetation site on San Jose Creek.
5. Reviewed CDFW's SAA.
6. Researched revegetation and habitat mitigation ratios for permanent and temporary impacts.
7. Researched pros and cons of mitigation banks.
8. Met with Maureen Spencer, SBCFCWCD Operations and Environmental Manager.
9. Visited all known revegetation sites on San Jose Creek and at the Lake Los Carneros Mitigation Bank.
10. Photo-documented each site.
11. Counted or estimated and recorded the number of installed plants observed and calculated the estimated percent survival,³⁵ general condition, plant heights and/or lateral spread, percent cover of native species, presence of woody nonnative species, and extent of invasive nonnative herbaceous understory species.
12. Documented nonnative and invasive exotic species.
13. Identified recommendations to enhance the success of revegetation projects at each site, to enhance the success of future revegetation efforts, and to improve tracking and reporting of success.

This report will be submitted to the SBCFCWCD, CDFW, United States Army Corps of Engineers, National Marine Fisheries Service, the City of Goleta, and nonprofit organization involved with creek preservation, management, and restoration, agencies. EDC will follow up with such agencies to discuss the EDC's findings and recommendations.

³⁵ It was only possible to estimate percent survival when the number of plants planted was recorded by SBCFCWCD. When the number of plants planted was not recorded in SBCFCWCD documents and is therefore unknown, it was infeasible to estimate percent survival unless zero surviving plants were observed (0% survival).

2. Problems Encountered Evaluating Revegetation Project Success

- **Inadequate Mapping and Field Demarcations.** Difficulty identifying locations and boundaries due to inadequate mapping, field demarcations, and descriptions of locations.
- **Failure to Record Plant Numbers.** Difficulty tracking survival rates because number of overall plants and number of plants of each species planted at each site plants of each species not recorded.
- **Failure to Record Species Planted.** Species planted are frequently not documented in Maintenance and Revegetation Reports.

III. Habitat Mitigation for Flood Control Annual Maintenance

A. CDFW Performance Standards

SBCFCWCD must create or restore riparian habitat through a process called revegetation to compensate for temporary and permanent habitat losses and degradation caused by the Annual Maintenance Program. CDFW established “Success Criteria for Restoration Areas” to measure success of habitat and tree replacement through revegetation.³⁶ Native trees that are removed must be replaced. If those trees are three inches to six inches in diameter breast height (“DBH”), then they must be replaced at a two-to-one (2:1) ratio. If those trees are six inches or more DBH, then they must be replaced at a ten-to-one (10:1) ratio.³⁷ Successful tree replacement requires achieving three-year and five-year height-based milestones.³⁸ The success criteria for tree replacement depends on the species. Sycamore trees, for example, must be at least five feet tall after three years and nine feet tall after five years, to be considered successful.³⁹

Revegetation sites may be considered successful after they complete a five-year period of monitoring, maintenance, and reporting.⁴⁰ At the end of the five-year period, no single species planted can constitute more than sixty percent (60%) of the vegetative cover with the exception of willow trees, which can constitute eighty percent (80%) cover.⁴¹ No woody invasive species can be present.⁴² Herbaceous nonnative invasive species must be limited to less than five percent (5%) cover in revegetation sites.⁴³ Additionally, there must be no supplemental irrigation for at

³⁶ CDFW (2015) Section 3.9 at 17.

³⁷ *Id.* Section 3.2 at 15.

³⁸ *Id.* Section 3.8 at 16.

³⁹ *Id.*

⁴⁰ *Id.* Section 3.9a at 17.

⁴¹ *Id.* Section 3.9b at 17.

⁴² *Id.* Section 3.9c at 17.

⁴³ *Id.*

least 2-years.⁴⁴ When planted plants perish, replacement plants are required. Replacement plants must be monitored with the same growth and survival requirement for five years after planting.⁴⁵

Each species planted must have at least eighty percent (80%) survival for the first year and one hundred percent (100%) survival for every subsequent year and/or sites must exhibit seventy-five percent (75%) cover after three years and ninety percent (90%) cover after five years “and for the life of the project” in order for the sites to be deemed successful.⁴⁶ “In some scenarios, if it can be demonstrated that the restoration area has achieved its goals and met the success criteria, CDFW can provide concurrence that no further restoration activities are required.”⁴⁷

Lake Los Carneros Mitigation Bank success criteria are divided into 3-year and 5-year criteria.⁴⁸ When the 3-year success criteria are met in a given restored area, half of the available banking credits become available and the other half after the 5-year success criteria are met.⁴⁹

B. Mitigation Ratios

According to the CDFW, the SBCFCWCD must provide mitigation for any removal of riparian habitat on a one-to-one (1:1) basis for temporary impacts.⁵⁰ SBCFCWCD activities that result in permanent impacts require a three-to-one (3:1) mitigation ratio. The one-to-one (1:1) ratio was established in SBCFCWCD’s 2001 Program EIR and the CDFW SAA.⁵¹ The three-to-one ratio (3:1) is required by the CDFW SAA.⁵² However, if revegetation to offset temporary impacts does not take place within the next growing season, the CDFW SAA may require a higher but unspecified ratio.⁵³ At the Lake Los Carneros Mitigation Bank, riparian habitat is mitigated on a 1:1 ratio, while upland habitats must be mitigated on a .75:1 ratio.⁵⁴

Permanent impacts caused by SBCFCWCD’s maintenance program must be mitigated at three-to-one (3:1) ratio.⁵⁵ Examples of permanent impacts include “activities such as new grade stabilizers and non-vegetated bank protection,” including the use of cement or grouted rock, or other intrusions that cannot easily be removed.⁵⁶

⁴⁴ *Id.* Section 3.9d at 17.

⁴⁵ *Id.* Section 3.9g at 17.

⁴⁶ *Id.* Section 3.9f at 17.

⁴⁷ *Id.* Section 3.9h at 17

⁴⁸ *Id.* at 16.

⁴⁹ *Id.*

⁵⁰ A 1:1 mitigation ration requires replacement of every area impacted with an equal or larger area of replacement habitat.

⁵¹ Spencer (2022); *See also* SBCFCWCD (2001) at 4-7 – 4-11; *See also* CDFW (2015) at 15.

⁵² Spencer (2022); *See also* CDFW (2015) at 15. Note the 2001 Program EIR requires a two-to-one (2:1) ratio for permanent impacts but the CDFW SAA requires a three-to-one (3:1) ratio.

⁵³ CDFW (2015) Section 3.1 Mitigation for Impacts to Habitat at 15.

⁵⁴ *Id.* at 16.

⁵⁵ CDFW (2015) Section 3.1 at 15.

⁵⁶ *Id.*

C. Revegetation Locations and In-kind Mitigation

Whenever possible, mitigation efforts should take place directly where the impacts occurred.⁵⁷ If it is infeasible to mitigate onsite, then another nearby location within the same creek should be required. In the event there are no other revegetation sites available in the watershed, a nearby watershed is another less desirable offsite revegetation option. As outlined in the CDFW SAA, “Restoration is conducted with the primary intent that it will be located at the project areas or along the same drainage near the project/impact area. If these locations are not suitable, restoration is then located in an adjacent watershed.”⁵⁸ As a last result, the impact may be mitigated in another area known as a “mitigation bank.” In Santa Barbara County, the Lake Los Carneros Mitigation Bank has served as SBCFCWCD’s alternative restoration site which includes a total of 6.5 acres of revegetated habitats.

The County’s CEQA Thresholds and Guidelines Manual also directs mitigation to be located onsite or as close to the area of impact as possible whenever feasible, and that offsite mitigation is least preferred.⁵⁹ SBCFCWCD employs this same hierarchical approach favoring onsite mitigation, offsite but within the same creek, offsite in a nearby creek, or as a last resort, at the Lake Los Carneros Mitigation Bank.⁶⁰

Mitigation should also be in-kind with the replacement vegetation matched to the habitat type impacted.⁶¹ During surveys EDC noted that some SBCFCWCD revegetation sites, such as the Cathedral Oaks Village Association (“COVA”) sites described below, include upland species planted to replace riparian species. The Lake Los Carneros Mitigation Bank allows this but employs a lower ratio (.75:1) for upland vegetation to compensate for not replacing riparian species in-kind with riparian species.⁶²

D. Mitigation Banks

The SAA requires mitigation in the form of revegetation at or near the site of the impact but allows for revegetation at the Lake Los Carneros Mitigation Bank when nearby sites are not available.⁶³ “Mitigation banking is a system of credits and debits devised to ensure that ecological loss, especially loss to wetlands and streams resulting from various development works, is compensated by the preservation and restoration of wetlands, natural habitats, and

⁵⁷ CDFW (2015) Section 3.4 Revegetation Plan and Restoration Goals at 15; *See also* Santa Barbara County, *CEQA Thresholds and Guidelines Manual* at 32 – 33 available at <https://www.countyofsb.org/ceo/asset.c/479> (2008) (“Santa Barbara County (2008)”).

⁵⁸ CDFW (2015) Section 3.4 Revegetation and Restoration Goals at 15.

⁵⁹ Santa Barbara County (2008).

⁶⁰ Maureen Spencer, Operations Manager, SBCFCWCD, personal communication with Natalie Blackwelder, Watershed Program Intern, EDC, and Brian Trautwein, Environmental Analyst/ Watershed Program Coordinator, EDC (February 19, 2022).

⁶¹ Santa Barbara County (2008).

⁶² SBCFCWCD, *Los Carneros Mitigation Bank Goleta, CA Progress Report* at 1, 2, and 13 (2021).

⁶³ CDFW (2015) at 4.

streams in other areas so that there is no net loss to the environment.”⁶⁴ Mitigation banks can be an effective way to compensate for the loss of habitat because they may result in larger contiguous areas of habitat and thereby reduce the potential for edge effects, which are adverse ecological effects that occur when natural habitats have large perimeters relative to size and may decrease the efficacy of a restored area.⁶⁵ Edge effects can allow new invasive, nonnative species that are better adapted to disruptions to take root, undermining revegetation goals.⁶⁶ Mitigation banks are typically larger areas which have smaller perimeter to size ratios and therefore may reduce edge effects.

Further, mitigation banks are more economical to build and manage over the long-run.⁶⁷ For example, it is easier to install irrigation on a one-acre area than ten different irrigation systems in ten .1-acre areas.

However, in the context of ecological restoration, mitigation banks can be less desirable for several reasons. For example, they often only require a one-to-one (1:1) mitigation ratio as opposed to site-specific revegetation sites that may require higher ratios.⁶⁸ Further, mitigation banks allow revegetation to occur offsite from the direct ecological impact, which prevents those impacted areas from getting restored. As a result, mitigation banks can result in fewer and more widely dispersed albeit larger habitats separated by greater distances which may impair wildlife movement between habitats.

In preparing this report, EDC considered the mitigation ratios required in the CDFW SAA, the SAA’s ten-year mitigation reset period discussed below in I.E., information about mitigation ratios used for other temporary and permanent habitat removal projects, the SAA success criteria and requirements for tracking and reporting success, and the short-term and long-term mitigation success at the SBCFCWCD’s revegetation sites. EDC identified recommendations regarding mitigation ratios for SBCFCWCD’s revegetation program which are included at the end of this report.

E. Mitigation for Repeated Impacts in the Same Locations

SBCFCWCD is required to mitigate one time for repeated temporary impacts in the same location for a period of ten years. Thus, if creek maintenance temporarily removes habitat from the same location multiple times during a ten-year period, SBCFCWCD must only mitigate the impacts of that repeated impact once per decade. However, mitigation would again be required the first time this same area is impacted after the decade has elapsed.

⁶⁴ Virkam Jhavar, *Understanding the Basics of Mitigation Banking* available at <https://www.investopedia.com/articles/dictionary/031615/understanding-basics-mitigation-banking.asp> (January 31, 2022).

⁶⁵ DUDEK, *Mitigation Bank Advantages* (2011) (“DUDEK (2011)”).

⁶⁶ Vallejo, David, *Edge effects and habitat fragmentation* (2018).

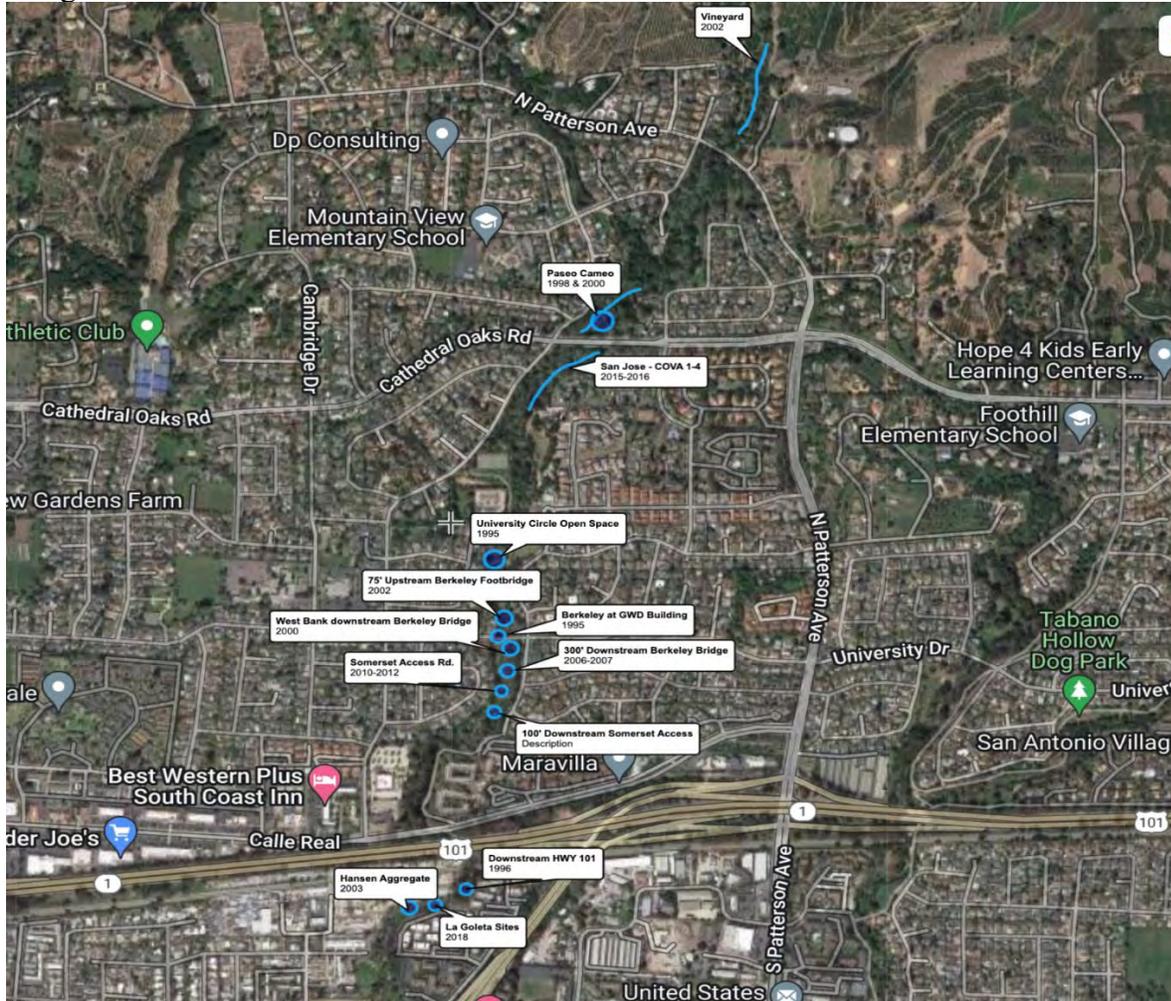
⁶⁷ DUDEK (2011).

⁶⁸ *Id.*



IV. SAN JOSE CREEK REVEGETATION SITES⁶⁹

EDC’s analysis of the success of nineteen revegetation sites on San Jose Creek is set forth below along with site-specific recommendations. The SBCFCWCD’s descriptions of the nineteen revegetation projects are found in the SBCFCWCD’s Annual Plans and Maintenance and Revegetation Reports. Information regarding the long-term success and current status of the nineteen revegetation sites is derived from observations during EDC’s surveys of creek revegetation sites.



Map of the revegetation sites along San Jose Creek assessed in this report. Scribble Maps. Blackwelder. 2022.

⁶⁹ EDC evaluated nineteen revegetation sites on San Jose Creek. The following three sites failed and/or could not be located and were not visited: Cavaletto Site (Site 3) was unsuccessful. (SBCFCWCD, *Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1, 1998)); The site one hundred feet downstream from Berkeley Footbridge (Site 6) was having limited success. (SBCFCWCD *Maintenance Work Performed During 1995/1996 Maintenance Season and Revegetation Update* (December 1, 1996)); The site upstream from Hollister Avenue behind pipe and wire revetment (Site 9) SBCFCWCD personnel were unable to provide a location or directions to this site due to the fact it predated current staff’s employment by the SBCFCWCD.

A. Vineyard Road Site

SBCFCWCD revegetated a site on the west bank and western terrace of San Jose Creek between Vineyard Road to the north and North Patterson Avenue to the south in 2002.⁷⁰ (Figure 1)

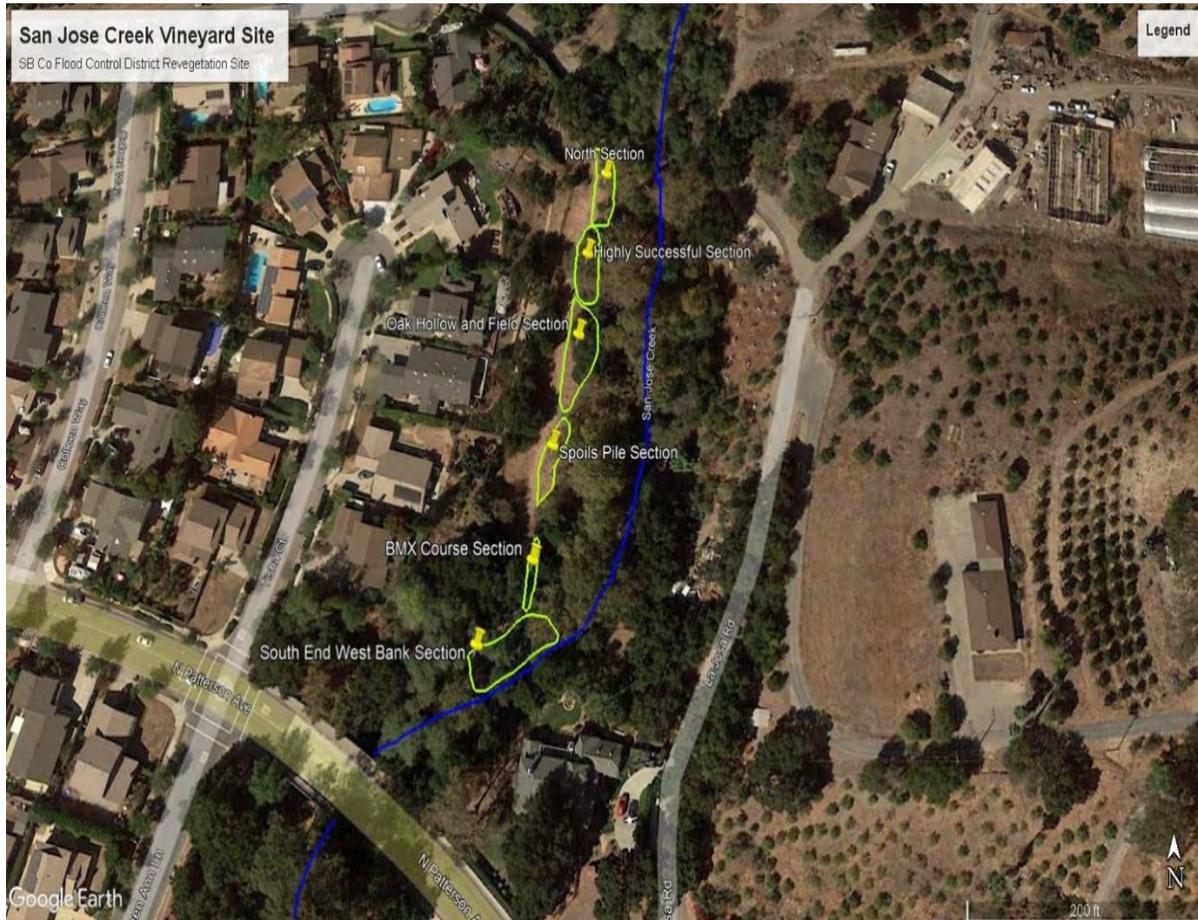


Figure 1. EDC divided the Vineyard Road revegetation site into six sections for evaluation. Google Earth. 2021.

Year Planted: 2002

Size:

- Reported: 10,000 square feet⁷¹
- Observed: 14,380 square feet⁷²

Site Visits: January 24 and 25, 2022

⁷⁰ SBCFCWCD *Maintenance and Revegetation Report 2003/2004 Maintenance Season* (2004).

⁷¹ SBCFCWCD *Annual Maintenance and Revegetation Report* (2010-2011).

⁷² EDC estimated this site at 14,380 square feet.

Plant Table

Species Planted⁷³	# Planted	# Observed⁷⁴	Avg height or spread	General Condition	Estimated % Survival
Coast live oak	7	7	2'-15'h	Fair to Good	100
Black cottonwood	10	8 - 10 ⁷⁵	3'-10'h ⁷⁶	Fair to Good	80 - 100
Western sycamore	4	0	-	-	0
Willow	89	3 - 7	5'-10'h ⁷⁷	Fair to Good	<10
Elderberry	11	2 - 3	1-10	Poor to Good	<30
Coyote brush	23	0 - 3	5'-7'h	Good	<15
Coffeeberry	26	0		-	0
Santa Barbara honeysuckle	13	2 - 4	3'-5'h	Good	<33
Wild blackberry	100	15 - 25	1'-4'h	Fair to Good	15 - 25
Mugwort	50	20 - 28	1' - 6'h .5 - 2' spread	Good	40 - 56
Toyon	13	10	1' - 15'h	Poor to Good	77
California sage	26	6 - 9	3' - 7' h 2' - 10' spread	Good	24 - 34
Gooseberry	20	4	3' - 8'h	Good	20

⁷³ SBCFCWCD Annual Maintenance and Revegetation Report (2010-2011).

⁷⁴ Number of plants observed are estimates of surviving plants installed as part of the revegetation project based on field observations on January 24 and 25, 2022. Numbers may not represent the total number of surviving plants given (1) the potential for surviving plants to have been overlooked during surveys and (2) the potential for naturally-occurring plants to have been mistaken for plants that were installed as part of the revegetation project.

⁷⁵ A range of plants observed is listed when it is unclear whether observed plants were planted or are naturally occurring.

⁷⁶ The current SAA would require black cottonwoods to be at least twelve feet tall after five years but these plants measure only three to ten feet tall after twenty years.

⁷⁷ The current SAA would require red, black, and arroyo willows to be at least fifteen to eighteen feet tall after five years but these trees measure only five to ten feet tall after twenty years.

*Estimated Percent Total Cover:*⁷⁸

- 4,662 square feet / 14,380 square feet **32.4%** (based on 14,380 square feet⁷⁹)
- 4,662 square feet / 10,000 square feet **46.6%** (based on 10,000 square feet⁸⁰)

Estimated Percent Total Cover by Section:

- North Section: 500 sq ft / 2,500 sq ft (20%)
- Highly Successful Section: 2,850 sq ft / 3,000 sq ft (95%)
- Oak Hollow Section (understory): 300 sq ft / 3,000 sq ft (10%)
- Field Section: 160 sq ft / 1,600 sq ft (10%)
- Spoil Pile Area Section: 600 sq ft / 1,200 sq ft (50%)
- BMX Area Section (understory): 0 sq ft / 600 sq ft (0%)
- South End West Bank Section: 252 sq ft / 2,480 sq ft (10%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Rockrose (west of trail, spreading east toward revegetation site and creek)
- Vinca (South End West Bank Section)
- Arundo (creek banks and west of trail)
- Shamel ash (South End West Bank Section by trail)
- Algerian or English ivy (climbing up sycamore trees)
- Castor bean (in creek, north end)
- Brazilian pepper (in creek, north end)
- Nonnative grasses (throughout; Spoil Pile Area Section, Field Section, South End West Bank Section; and areas between trail and successful revegetation locations)

Vineyard Road Site Highlights:

- The Highly Successful Section south of Vineyard Road has performed extremely well with nearly a 100% cover by native species including wild blackberry, sage species, and Santa Barbara honeysuckle.
- The large size of this site allows for establishment of continuous habitat and reduces edge effects compared to smaller restoration sites.

⁷⁸ Estimated cover is the percentage of cover provided by installed native plants over the revegetation site as a whole. For example, if half of the revegetation site is covered by planted native vegetation, the cover is fifty percent.

⁷⁹ EDC estimated the Vineyard Road Revegetation Site as 14,380 square feet based on field measurements and visual estimates of areas planted as described by SBCFCWCD in the 2003 Maintenance and Revegetation Report i.e., “essentially from the top of [west] bank out to the dirt walking/bike path seen in the picture.” EDC included the area below the top of the west bank in the south end upstream from North Patterson Avenue which EDC estimates at 2,480 square feet.

⁸⁰ SBCFCWCD estimates the Vineyard Revegetation Site as 10,000 square feet in the 2003 Maintenance and Revegetation Report.

- The perennial nature of the Creek creates hydrological conditions which support native plant establishment.
- The project revegetates a section of San Jose Creek used by resident and potentially anadromous endangered southern California steelhead and threatened California red-legged frogs.

Vineyard Road Site Recommendations:

The following actions are recommended to fulfill the current CDFW SAA revegetation success criteria:⁸¹

- Entire site: Eradicate perennial invasive and aggressive non-native plants listed above and control nonnative grasses and forbs.
- South End West Bank
 - Remove or solarize all nonnative grasses for three consecutive years and then plant 200 blackberries on 3' centers to establish native understory.
 - Plant toe of wet bank three feet above creek bed with red, black, and/or arroyo willow trees on ten-foot centers. Plant mugwort underneath willows.
 - Plant lower two-thirds of west bank with twenty-five black cottonwoods on average fifteen-foot centers. Plant five coast live oaks on top third of west bank.
- North End
 - Remove nonnative grasses and weeds prior to seed set or solarize these invasive species west and east of the trail for three consecutive years. Then plant wild blackberries on six-foot centers, five to ten gooseberries, four to six Santa Barbara honeysuckle plants, and four to six coast live oak trees between the trail and the areas of successful revegetation.
- BMX Course
 - Chip wood pile and spread as woodchips.
 - Plant sixty wild blackberries on six-foot centers. Cage plants using chicken wire to prevent vandalism for five to ten years or until blackberries reach fifty percent cover then remove cages using wire cutters to avoid damaging vines.
- Spoil Pile Area
 - Remove nonnative grasses prior to seed set for two to three consecutive years or solarize and plant spoil pile and open field south of spoil pile with blackberries on six-foot centers and fifteen to twenty coast live oak trees.

⁸¹ EDC acknowledges that the current SAA was approved in 2015 and that revegetation occurred prior to the approval of the current SAA but believes the 2015 SAA provides useful success criteria for measuring long-term effectiveness of revegetation projects.

- Oak Hollow / Field
 - Plant fifty to eighty blackberries under the large oak tree's dripline. Cage plants using chicken wire to prevent vandalism.
 - Remove all grasses and weeds from field by removing for three consecutive years prior to seed set or solarize these invasives. Plant with fifty blackberries, three to five gooseberries, and four to six Santa Barbara honeysuckle plants. Cage to prevent vandalism.
 - Remove cages after five to ten years or once blackberries reach fifty percent cover. Remove cages using wire cutters to avoid damaging vines.

Photos



Figure 2. North End of Vineyard Site. Successful establishment of blackberry understory with gooseberry in center of photo. Trautwein. January 25, 2022.



Figure 3. North end of Vineyard Site looking north from trail. Note the failure to establish native vegetation cover between the trail and the Creek. Successful establishment of oaks (presumably two of seven planted at Vineyard site). Trautwein. January 25, 2022.



Figure 4. North end of Vineyard Site looking southeast. Successful establishment of toyon and Santa Barbara honeysuckle. Trautwein. January 25, 2022.



Figure 5. Highly Successful Section of Vineyard Site with estimated 95% cover (estimated 2,850 square feet out of 3,000 square feet). Gooseberry and California sage. Trautwein. January 25, 2022.



Figure 6. Southwest edge of Highly Successful Section of Vineyard Site looking north by northeast. Large toyon in center of image. Note lack of native vegetation between toyon and trail in lower left and bottom portions of photo. Trautwein. January 25, 2022.



Figure 7. Highly Successful Section of Vineyard Site looking south across area with nearly 100% native cover of blackberries and California sage. Trautwein. January 25, 2022.



Figure 8. Looking south along trail from Highly Successful Section of Vineyard Site to Oak Hollow Section (center) and Spoil Pile Area Section (center right). Note toyon on left is the same plant shown in Figure 6. Trautwein. January 25, 2022.



Figure 9. Spoil Pile Area Section with oaks established on spoil pile. Herbaceous layer is dominated by nonnative grasses. Scattered wild blackberry and mugwort also present. Trautwein. January 25, 2022.



Figure 10. Spoil Pile Area Section (right side) looking north along trail to Field Section containing nonnative grasses and Oak Hollow Section (upper center). Trautwein. January 25, 2022.



Figure 11. Field Section containing mostly nonnative weeds between Highly Successful Section (to left of picture), Spoil Pile Section (center right), east of trail, and west of San Jose Creek bank. Trautwein. January 25, 2022.



Figure 12. Oak Hollow Section. Scattered wild blackberry in understory. Lack of wild blackberry understory may be the result of vandalism. Trautwein. January 25, 2022.



Figure 13. BMX Section. No native understory plants established likely due to BMX course established in this location and associated removal of native understory. Trautwein. January 25, 2022.



Figure 14. Pile of deadwood likely created during clearing of area to construct BMX course. Trautwein. January 25, 2022.



Figure 15. South End West Bank Section dominated by nonnative grasses. Note mugwort plants (tall thin plants center and right), and coast live oak and cottonwood saplings (orange flagging tape center left). Trautwein. January 25, 2022.



Figure 16. South End West Bank Section. Mugwort plants amidst dense nonnative grasses dominating this section of Vineyard Revegetation Site near North Patterson Avenue. Trautwein. January 25, 2022.



Figure 17. Small, four-foot tall, healthy black cottonwood sapling. South End West Bank Section. Trautwein. January 25, 2022.



Figure 18. Successful willow saplings near toe of west bank at South End West Bank Section, near North Patterson Avenue. Note extensive nonnative grass understory. Trautwein. January 25, 2022.



Figure 19. Scattered mugwort plants surrounded by dense nonnative grasses. South End West Bank Section near North Patterson Avenue. Trautwein. January 25, 2022.



Figure 20. Successful willow sapling (left side of image) established on South End West Bank Section near North Patterson. Smaller cottonwood sapling to right of willow. Note extensive nonnative grasses. Trautwein. January 25, 2022.



Figure 21. Small thirty-inch-tall black cottonwood sapling at South End West Bank Section of Vineyard Site. Note extensive nonnative grasses. Trautwein. January 25, 2022.



Figure 22. Small cottonwood sapling on South End West Bank Section of Vineyard Site. Trautwein. January 25, 2022.



Figure 23. Trail at South End West bank Section of Vineyard Site. Note Patterson Avenue Bridge in background. Nonnative oxalis and grasses dominate strip along trail's edge. Trautwein. January 25, 2022.



Figure 24. Toyon in shade of oak canopy. South End West Bank Section of Vineyard Site adjacent to trail near Patterson Avenue Bridge. Understory dominated by nonnative oxalis and vinca. Trautwein. January 25, 2022.

B. Norma Site (Site 1)

SBCFCWCD installed rip rap to repair an eroded bank and planted many willow trees to restore riparian habitat.⁸²

Year Planted: First planting 1996, second planting 1997, third planting 1998

Size: 300 feet x 20 feet = 6,000 square feet⁸³

Site Visit: February 25, 2022

⁸² SBCFCWCD *Annual Maintenance and Revegetation Report 1996/1997 Maintenance Season (1997)*.

⁸³ EDC observed a revegetated area that measured approximately two hundred feet by twenty feet, or 4,000 square feet.

Plant Table

Species	# Planted	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	Unknown	>40	20-35' tall, 4-10" DBH	Fair-Good some dead	Unknown
Coast Live Oak	Unknown	-	15' tall	Good	Unknown
Blackberry	Unknown	-	-	Good	Unknown
Wild Cucumber	Unknown	-	-	Good	Unknown

Estimated Total Percent Cover:

- Understory 75-80% native vegetation
- Overstory 95% native vegetation

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Wild Grasses
- Arundo
- Shamel Ash
- Wild Radish
- Eucalyptus

Norma Site Highlights:

- Remarkably high native willow canopy percent cover.
- Good example of revegetating bank that had been eroded by high flows.
- Apparent perennial or near perennial flows support healthy willow woodland.
- A lot of willows were present and in fairly good condition

Observation:

- Rip rap was used to stabilize the Creek bank. It appears that the willows are growing above the rip rap but not from within it. It is unclear if willow wattling was used within the rip rap.

Norma Site Recommendations:

- Remove invasive Arundo, aggressive nonnative Shamel ash, and naturalized olive tree to fulfill success criteria related to presence of woody nonnative vegetation.⁸⁴
- Diversify the understory with more mugwort, blackberries, and giant rye.
- Diversify the riparian tree overstory with more cottonwoods, bay laurels, and sycamore trees.
- Remove eucalyptus tree(s) on east of site.



Figure 25. Looking downstream San Jose creek at the Norma Site (Site 1). Successful establishing of willow woodland. Note the riprap installed on the west bank. This site was visited in late February/early March and water was still flowing. All of the surviving revegetation is located above the installed rip rap. Trautwein. March 7, 2022.

⁸⁴ CDFW (2015) at 17.



Figure 26. Looking down the west bank of San Jose Creek at the Norma Site. Note the understory is primarily nonnative grasses, and poison oak, however in the lower foreground are young black cottonwoods and a coast live oak sapling about 3' tall. These appear to be from natural recruitment. Blackwelder. March 7, 2022.



Figure 27. West bank of San Jose Creek at Norma Site above the installed rip rap. The understory is primarily nonnative grasses. Coast live oak sapling about four feet tall in the right third of the photo. There is nonnative, invasive Arundo in the background along the upper mid-right edge. Blackwelder. March 7, 2022.



Figure 28. Nonnative grasses and oxalis on west bank San Jose Creek at the of Norma Site. There are also oaks and willows in the background near the top of the image. Blackwelder. March 7, 2022.

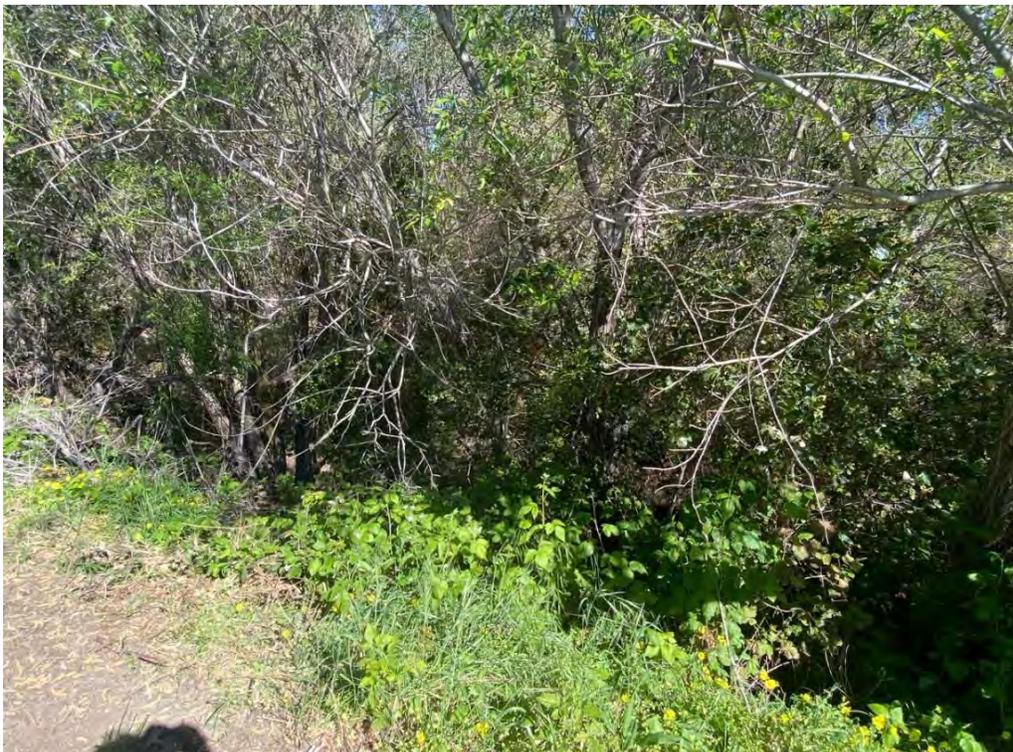


Figure 29. Along the trail west of the Norma Site on San Jose Creek. Understory is primarily nonnative grasses and poison oak. The upper half of this image shows the thick cover of willow trees planted on the west bank, with coast live oak saplings appearing through natural recruitment. Blackwelder. March 7, 2022.



Figure 30. Naturalized olive tree (dark waxy lanceolate leaves), poison oak and native wild cucumber. Ground covered includes nonnative oxalis. Blackwelder. March 7, 2022.



Figure 31. Looking north along the trail at the Norma Revegetation Site on the west bank of San Jose Creek. The trail runs parallel to the Creek. Note the dense willow woodland along the right side of the trail. These trees contribute to a healthy native riparian habitat. Ideally there would be more plant species diversity in the overstory and groundcover along this section of the Creek. Blackwelder. March 7,

C. Paseo Cameo (Site 11)

The Paseo Cameo Site located within a County Open Space on Paseo Cameo Road experienced significant erosion after a heavy storm in 1997 which eroded the east bank, parkland, and part of Paseo Cameo.⁸⁵ SBCFCWCD repaired the tall east bank of San Jose Creek which had eroded in 1998, installed rip rap at the base of the bank, and planted willow trees to restore habitat and stabilize the bank.⁸⁶

Year Planted: April 1998 and Spring 2000

Size: 150 feet x 40 feet = 6,000 square feet⁸⁷

Site Visit: March 7, 2022

Plant Table

Species	# Planted	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	100	25	< 45' h	Fair to Good. 6 dead	25%

Estimated Percent Cover: 95-100%

Other native plants present:

- Coast live oak
- Wild blackberry

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Nonnative grasses including wild oats and brome

Paseo Cameo Site Highlights:

- In 2022, twenty-two to twenty-four years after planting, the site boasts nearly 100% cover of native willow trees installed by SBCDCWCD, and approximately ten coast live oak saplings and several bay laurel saplings which have been added through natural recruitment. While percent survival is below the success criterion in the 2015 CDFW SAA⁸⁸ the 100% cover indicates the site has been successful in establishing native riparian trees.

⁸⁵ Neighbor on Paseo Cameo, personal communication with Natalie Blackwelder, EDC Watershed Program Intern, and Brian Trautwein, Environmental Analyst/Watershed Program Coordinator, EDC (February 22, 2022).

⁸⁶ SBCFCWCD *Annual Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1998).

⁸⁷ SBCFCWCD *Annual Maintenance and Revegetation Report* (2000).

⁸⁸ CDFW (2015) at 17.



Paseo Cameo Site Recommendations:

- Introduce more riparian tree and shrub diversity (i.e., cottonwoods, sycamores, coast live oaks, toyon, gooseberry, and coffeeberry).
- Remove nasturtium and nonnative grasses and plant the understory with greater diversity of native groundcovers (i.e., mugwort, wild blackberry, and giant wild rye grass).
- Relocate the wood rail fence twenty feet out from the top of the bank and plant coast live oak trees and native oak woodland understory species such as mugwort, hummingbird sage, and giant wild rye grass in this area.

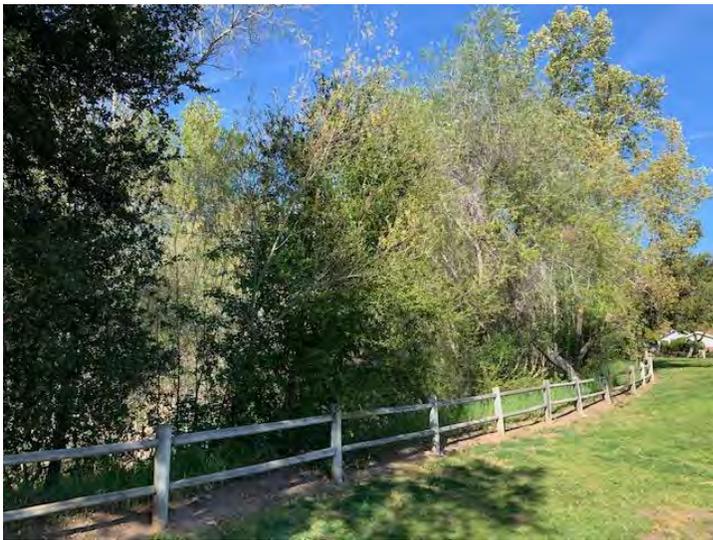


Figure 32. Paseo Cameo Site. Wood rail fence installed after 1998 storm eroded bank approximately twenty-five feet into street. One hundred willows were planted on the east creek bank inside the fence. Site experiencing natural recruitment of coast live oak and bay laurel saplings. Trautwein. March 7, 2022.



Figure 33. Paseo Cameo. Willow trees as tall as forty feet comprise the riparian canopy but the understory is mostly devoid of vegetation and contains nonnative species. Trautwein. March 7, 2022.



Figure 34. Paseo Cameo site with wood rail fence in foreground. Note the amputated branches of the willow tree above the fence. Most trees along this stretch seem to be in fair to good condition. Trautwein. March 7, 2022.

D. North of Cathedral Oaks (Site 2)⁸⁹

SBCFCWCD stabilized the east bank in 1995 and planted willow and sycamore trees and wild blackberry between 1996 and 2000 north of the Cathedral Oaks Bridge.⁹⁰ In 1987 maintenance work resulted at this location impacted riparian habitat and a steelhead spawning pool leading to public complaints which led to preparation of the first Program EIR in 1990, approval of a CDFW SAA, and significant changes in SBCFCWCD’s standard maintenance practices.

Year Planted: First planting Spring 1996; second planting April 1998 (willow and sycamore trees); third planting Spring 2000 (blackberries).

Size: 300 feet x 10 feet = 3,000 square feet

Site Visits: March 7 and March 22, 2022

⁸⁹ SBCFCWCD describes this site as being located on the outside bank (east bank) upstream from the Cathedral Oaks Road Bridge.

⁹⁰ SBCFCWCD *Annual Maintenance and Revegetation Report 2000/20001 Maintenance Season* (2001).

Plant Table

Species	# Planted⁹¹	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	Unknown	11 ⁹²	15' – 30' h	Fair	Unknown
Sycamore	“a couple”	8 ⁹³	20' - 45' h	Good	High
Blackberry	Unknown	Four stands	10' - 40' s	Fair to Good ⁹⁴	Unknown

Estimated Total Percent Cover:

- The willows, blackberries, and naturally occurring bay and oak saplings located above the pipe and wire revetment create an estimated 70% - 80% cover.
- The sycamores and blackberries near the northern terminus of the access ramp form a nearly 100% cover.⁹⁵

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Shamel ash trees
- Brazilian pepper tree
- Vinca
- Cape ivy
- Nasturtium
- Oxalis

North of Cathedral Oaks Site Highlights:

- The Site boasts a high percent cover on the east bank with blackberries and sycamores⁹⁶ where the access ramp terminates on the Creek bank.
- The size of the sycamores as tall as forty-five feet demonstrates excellent growth and success at the access ramp’s terminus.⁹⁷

⁹¹ SBCFCWCD *Maintenance and Revegetation Report, 1997-1998 Maintenance Season* (December 1998).

⁹² Given the planting date of 1998, it is unclear how many of the willow trees were planted and how many may be naturally occurring. Of the eleven observed, six were located above the revetment and believed to be planted. The remaining five are located below the revetment and are likely natural occurring because we do not believe the SBCFCWCD planted trees in the streambed.

⁹³ The sycamores ranged from 4” to 12” DBH and 20’ to 45’ tall. Given that only a “couple” were planted, it is likely that several are naturally occurring., including two that appear to be growing below the pipe and wire revetment.

⁹⁴ The health and size of the blackberry stands increased going from downstream near the Cathedral Oaks Bridge to upstream adjacent to the access ramp.

⁹⁵ The sycamores and blackberries near the access ramp may be naturally occurring.

⁹⁶ *Id.*

⁹⁷ *Id.*



- Natural recruitment of four California bay laurel, and several coast live oak and black cottonwood saplings above and east of the pipe and wire revetment indicates that this riparian habitat revegetation site is developing into a more robust, diverse, and mature plant community.

North of Cathedral Oaks Site Recommendations:

- Eradicate cape ivy in area north of Cathedral Oaks and along access road.
- Remove *Arundo* patch on east bank near Cathedral Oaks Road.
- Eradicate patches of *Arundo* on west bank near access ramp.
- Eradicate periwinkle, oxalis, and nasturtium on east Creek bank and along the access road and ramp. Control nonnative grasses.
- Plant cottonwood trees on top of rock revetment on west bank near access ramp.⁹⁸
- Plant willow poles within rock revetment on west bank near access ramp.
- Extend plantings east from narrow strip of plantings atop east bank near Cathedral Oaks Bridge to widen riparian habitat and enhance Creek buffer.

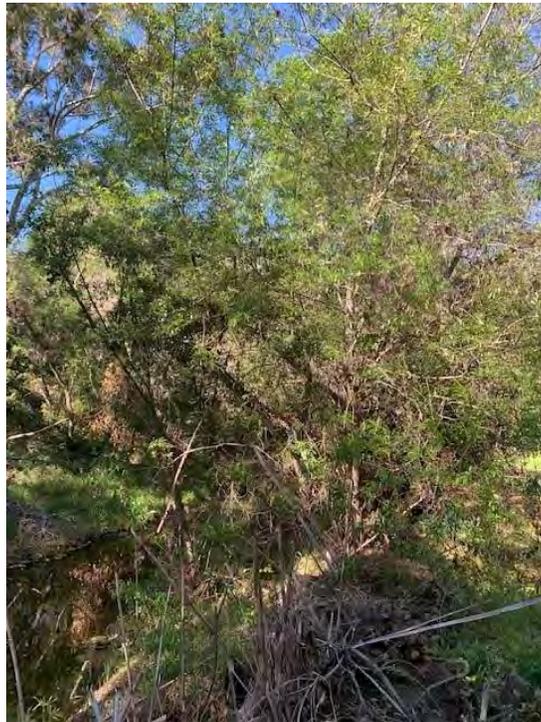
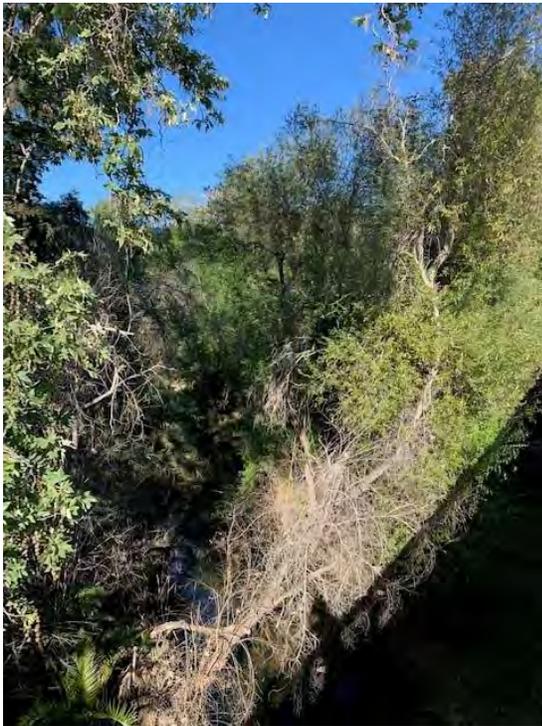


Figure 35. San Jose Creek looking upstream (north by northeast) from Cathedral Oaks Bridge at willows planted on east (right) bank.

Figure 36. Willow on east bank and dead *Arundo donax* stalks in foreground likely remaining after SBCFCWCD eradication efforts. Trautwein. March 22, 2022.

⁹⁸ While the site is limited to the east bank, and west bank near the access ramp was reconstructed using rock rip rap and lacks riparian tree cover.



Figure 37. Willows on east bank of San Jose Creek and Arundo recovering between trees.

Figure 38. Arundo not fully eradicated is surviving around base of willow tree. Trautwein. March 22, 2022.



Figure 39. Nasturtium in riparian understory below willow tree on east bank upstream from Cathedral Oaks.

Figure 40. Willows along east bank next to SBCFCWCD access road. Trautwein. March 22, 2022.



Figure 41. Willow trees on east bank of San Jose Creek at North of Cathedral Oaks Site.

Figure 42. Willow trees with bay laurel sapling recruitment and wild blackberry. Trautwein. March 22, 2022.

E. Cathedral Oaks Village Association Sites

SBCFCWCD obtained approval from COVA in 2015-2018 to revegetate four sites on COVA property along San Jose Creek near Cathedral Oaks Road and Kellogg Avenue.⁹⁹ (Figure 43) These four sites are evaluated below. COVA was required by CDFW to revegetate riparian habitat to mitigate the impacts of unauthorized clearing of riparian vegetation adjacent to Cathedral Oaks Village. However, EDC did not identify any revegetation sites attributed to COVA to mitigate the impacts of its clearing of riparian habitat. Instead, EDC observed SBCFCWCD’s four revegetation sites planted as mitigation for the Annual Maintenance Program’s impacts to San Jose Creek. Thus, it appears that COVA did not implement revegetation projects as required by CDFW, or COVA has improperly double counted SBCFCWCD’s revegetation projects as mitigation for COVA’s clearing of riparian habitat. In either case, COVA has yet to implement revegetation for the extensive riparian habitat COVA removed along the east side of San Jose Creek

COVA #1

This site is located on a steep north-facing upland slope south of Cathedral Oaks Road below Cathedral Oaks Village. SBCFCWCD obtained COVA’s approval to revegetate COVA Sites #1¹⁰⁰ and #2, provided all labor and materials, including irrigation lines and was credited with mitigation for both Sites.¹⁰¹ (Figure 43) COVA supplied irrigation water. However, COVA Sites #1 and #2 were required by CDFW to serve as mitigation for COVA’s clearing of riparian

⁹⁹ SBCFCWCD *Maintenance and Revegetation Report, 2020-2021 Maintenance Season* (July 2021); Personal Communication, Andrew Raaf, Environmental Team Leader, SBCFCWD to Brian Trautwein, Senior Analyst/Watershed Program Director, EDC (August 25, 2022) (“Raaf (2022)”).

¹⁰⁰ COVA Site #1 does not appear to have been successfully revegetated as described herein.

¹⁰¹ SBCFCWCD *Maintenance and Revegetation Report 2020-2021 Maintenance Season* at 49-50 (July 2021); Raaf (2022).

habitat within San Jose Creek.¹⁰² Given that these sites were revegetated by SBCFCWCD, COVA has yet to implement revegetation required by CDFW to mitigate the effects of COVA’s riparian habitat clearing along San Jose Creek.¹⁰³

Year Planted: 2015-2018

Size: 1,900 square feet¹⁰⁴

Site Visits: January 23, 2022, January 27, 2022, and January 30, 2022

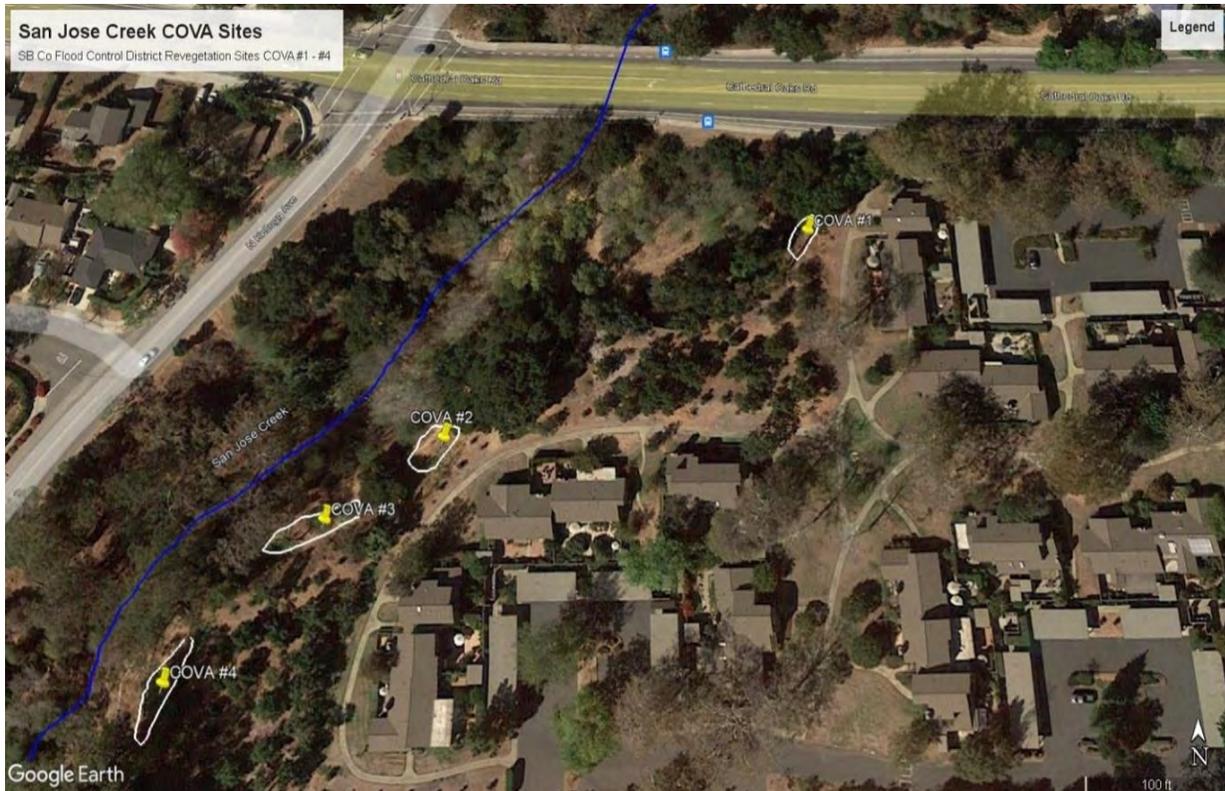


Figure 43. The four COVA revegetation sites east of San Jose Creek south of Cathedral Oaks Road. Google Earth. 2021.

Plant Table

Species Planted	# Planted¹⁰⁵	# Observed	Avg Height or Spread	General Condition	Estimated % Survival¹⁰⁶
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¹⁰² Email from Natasha Lohmus, retired CDFW, to Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 28, 2022) (“Lohmus (2022)”); *See also* CDFW Streamed Alteration Agreement 1600-2012-0132-R5 (2012).

¹⁰³ Lohmus (2022).

¹⁰⁴ SBCFCWCD *Annual Maintenance and Revegetation Report* (2020-2021).

¹⁰⁵ “Approximately 400 plants were planted at the two sites” (COVA 1 and COVA 2), SBCFCWCD *Annual Maintenance and Revegetation Report* (2015-2016).

¹⁰⁶ Only twenty-nine surviving plants were observed at COVA Sites #1 and #2 combined, less than 10% survival.

Willows	Unknown	0	-	-	0
Elderberry	Unknown	4 ¹⁰⁷	2' – 8' h	Poor to Good	Unknown
Blackberry	Unknown	0	-	-	Unknown
Wildrye	Unknown	1	10 sq ft s	Fair	Unknown
Deergrass	Unknown	0	-	-	0
Toyon	Unknown	0	-	-	0

Estimated Percent Total Cover:

- Fifty percent (50%) cover including mugwort which is not on the planting list.¹⁰⁸
- Estimated percent total cover is less than ten percent (10%) excluding mugwort.

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Kikuyu grass
- Shamel ash
- Oxalis
- Yucca tree
- Cape ivy

COVA Site #1 Highlights:

- Cooperation with landowners is beneficial because it greatly increases opportunities for onsite mitigation.
- Location of COVA Site #1 adjacent to UCC’s San Jose Creek Restoration Site has the potential to magnify environmental enhancement and benefits for birds and wildlife.

COVA Site #1 Recommendations:

- Control invasive plant species through weeding and/or solarization then seed with mugwort from locally collected source.
- Remove ash and yucca trees.
- Replant wild blackberries at five-foot centers.
- Plant three coast live oak trees and five elderberries.

¹⁰⁷ Two of these four are large healthy elderberry plants but are located outside of COVA #1 and may be naturally occurring or may have been planted by UCC or Audubon Society as part of the County-approved San Jose Creek Restoration Project.

¹⁰⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2015-2016).

- Increase plant diversity by planting additional shrub and understory species such as toyon, coffeeberry, hummingbird sage, gooseberry, and wild rose propagated from plant materials collected along San Jose Creek near COVA.

Figure 44. COVA Site #1. This site was one of the sites COVA was supposed to plant as mitigation for COVA's clearing of riparian habitat, but it was planted by SBCFCWCD as part of its Annual Maintenance Program, therefore COVA must still implement revegetation to mitigate the effects of COVA's riparian vegetation removal. This site is dominated by nonnative weeds, including oxalis, kikuyu grass, dandelion, and cape ivy which are visible using binoculars from the Cathedral Oaks Road Right of Way. (Figure 44b) Yucca trees and a Shamel ash are also present. Four hundred native deergrass, willow trees, elderberry, toyon blackberry, and wildrye plants were reportedly installed by SBCFCWCD at COVA Site #1 and #2 in 2016, however only one giant wild ryegrass, and one to two elderberries including one which has been knocked down by a fallen limb are present at COVA Site #1. Two other elderberries are located outside COVA Site #1 and it is unclear if they were planted by SBCFCWCD or as part of UCC's San Jose Creek Restoration Project or are naturally-occurring. COVA Site #1 is largely dominated (over 50% cover) by invasive nonnative herbaceous vegetation including oxalis and kikuyu grass. Mugwort is also common but is not one of the species planted. CDFW SAA success criteria require <5% cover by herbaceous invasive plants but this criterion is not being met. Irrigation lines are still present but appear to be in disuse. Trautwein. January 30, 2022. Figure 44b. Photographer location. On-X.

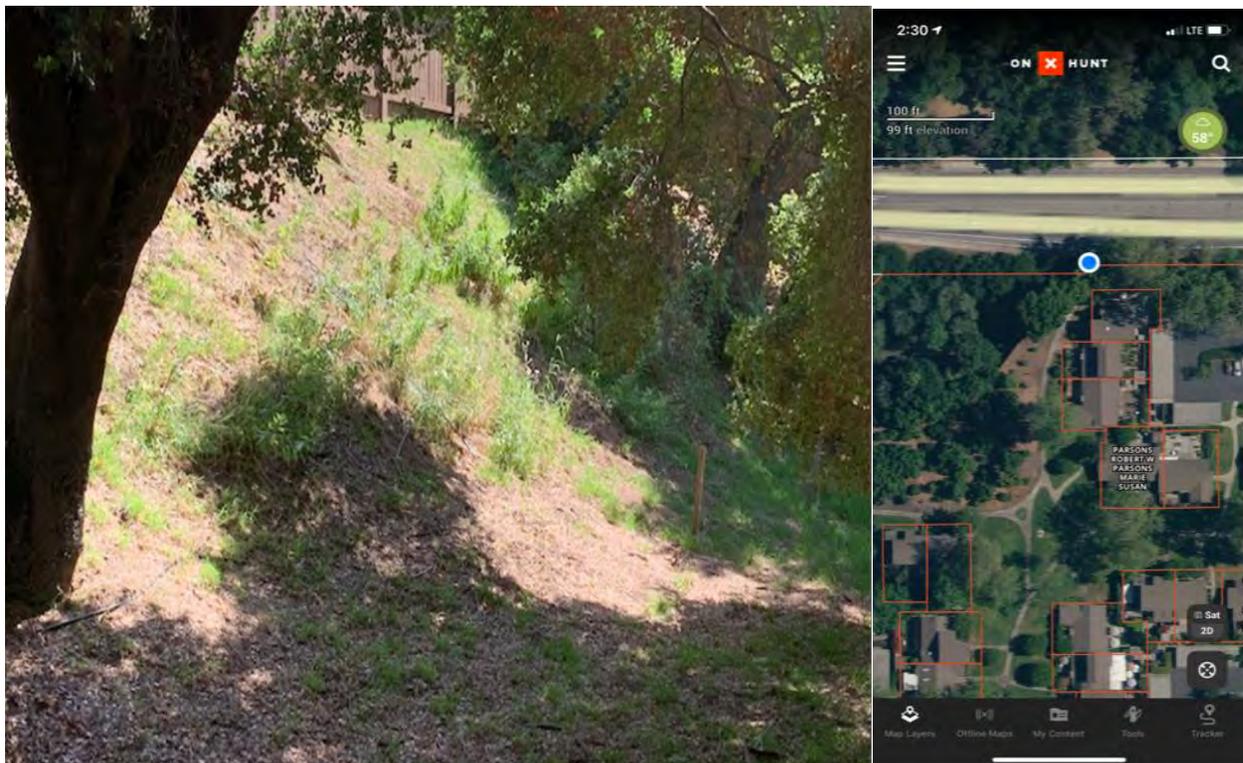




Figure 45. COVA Site #1 is located on the slope behind and under trees in the background of this image. The center of this image is a portion of UCC's San Jose Creek Restoration Project Site #1 which was cleared incrementally by COVA between 2007 and 2018. Note one of two healthy elderberries naturally occurring or planted near COVA Site #1 (upper right quadrant right of image). Trautwein. January 2022.

COVA #2

SBCFCWCD obtained COVA's approval to plant this northwest-facing slope and provided all the materials (other than irrigation water) and labor.¹⁰⁹ The slope is in an upland setting and was planted primarily with upland species rather than riparian species.

Area: 2,700 square feet¹¹⁰

Year Planted: 2015-2016¹¹¹

Site Visits: January 23, 2022, January 27, and January 30, 2022

¹⁰⁹ Raaf (2022).

¹¹⁰ SBCFCWCD *Maintenance and Revegetation Report* for 2020-2021 Season at 49 (July 2021).

¹¹¹ *Id.* at 49 – 52.

Plant Table

Species Planted¹¹²	# Planted¹¹³	# Observed	Avg Height or Spread	General Conditions	Estimated % Survival¹¹⁴
Willow	Unknown	1	10' h	Poor	Unknown
Deergrass	Unknown	1	3' s	Fair	Unknown
Giant wildrye	Unknown	9	6' s	Fair to Good	Unknown
Mulefat	Unknown	4	5' - 10' h	Fair to Good	Unknown
Toyon	Unknown	3	7' h	Fair	Unknown
Coyote brush ¹¹⁵	Unknown	1	5' h	Fair to Good	Unknown
Coast live oak	Unknown	1	15'	Good	Unknown
California sage	Unknown	3	3' h, 3' – 5' s	Fair to Good	Unknown
White sage	Unknown Unknown	1	2' h X 2' s	Fair	Unknown

Estimated Percent Cover: Eighty Percent (80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Oxalis

COVA Site #2 Highlights:

- Upland species such as sages and coast live oak are performing well.
- Restoration of upland habitat adjacent to riparian habitat increases plant and habitat diversity and provides transitional areas for wildlife species.

¹¹² At least one species is not the local native variety which raises the concern that none of the species planted in COVA Site #2 are local genotypes.

¹¹³ The number of plants installed at COVA Site #2 is not provided in the Maintenance and Revegetation Reports however approximately 400 native plants were installed at COVA Sites #1 and #2 combined. SBCFCWCD 2017-2018 Maintenance and Revegetation Report at 55.

¹¹⁴ Only twenty-nine surviving plants were observed at COVA Sites #1 and #2 combined, less than 10% survival.

¹¹⁵ The coyote brush installed at COVA Site #2 does not appear to be the local native variety of coyote brush and should be replaced by appropriate native species of local genotype. Planting non-local genotypes may result in hybridization and impacts to the local native gene pool and is inconsistent with proper restoration protocol which requires use of local genotypes.

COVA Site #2 Recommendations:

- Remove nonnative species and replace them with native oak woodland and coastal sage species.
- Plant riparian vegetation in the cleared area west of COVA Site #2 to restore dense willow riparian woodlands removed by COVA between 2013 and 2015. (See below.)
Note: COVA Site #3 was planted by SBCFCWCD and credited as mitigation for the Annual Maintenance Program.¹¹⁶ COVA should be required to restore riparian vegetation cleared by COVA adjacent to COVA Site #3 to mitigate the impacts of COVA's riparian habitat clearing.



Figure 46. COVA Site #2 showing California sage, mulefat, and toyon surviving with portion of site in lower left quadrant covered by invasive herbaceous species. Trautwein. January 23, 2022.

¹¹⁶ Raaf (2022).



Figure 47. COVA Site #2 with giant wild ryegrass (center left), deergrass (lower left), coast live oak sapling (upper center-right), and mule fat (left side). Note area in foreground was cleared of riparian vegetation and consists of one hundred percent nonnative species. Trautwein. January 30, 2022.

COVA Site #3

SBCFCWCD planted an upland slope on COVA property with approval from COVA.¹¹⁷ Adjacent riparian habitat was cleared by COVA around the same time; however, EDC’s surveys did not identify revegetation areas planted by COVA.

Area: 2,300 square feet¹¹⁸

Year Planted: 2015-2018¹¹⁹

Site Visits: January 23, 2022, January 27, 2022, and January 30, 2022

Plant Table

Species Planted	# Planted ¹²⁰	Number Observed	Avg. Height or Spread	General Condition	Estimated % Survival ¹²¹
Black sage	Unknown	10	3’ – 6’ s	Fair to Good	Unknown
Nightshade	Unknown	1	4’ h	Fair	Unknown
Willow	Unknown	2	5’ – 10’ h	Poor	Unknown
Ceanothus ¹²²	Unknown	2	6’ h	Fair	Unknown

¹¹⁷ SBCFCWCD (2021) at 49; Raaf (2022).; *See also* Aaron Stein, COVA Homeowners’ Association personal communication with Brian Trautwein, Senior Analyst/Watershed Program Director (September 9, 2022).

¹¹⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2017-2018) at 56.

¹¹⁹ *Id.*

¹²⁰ The number of plants planted was not provided in Maintenance and Revegetation Reports.

¹²¹ It is impossible to estimate percent survival because the number of plants planted was not provided.

¹²² Ceanothus installed at this site is not the local native variety of greenbark or spiny ceanothus (*Ceanothus megacarpa* or *Ceanothus spinosa*) and should be replaced with native plants of local genotypes consistent with habitat restoration protocols.

Coyote brush ¹²³	Unknown	8	5' h	Fair to Good	Unknown
California sage	Unknown	3-5	3' – 4' h	Fair to Good	Unknown
Nightshade	Unknown	1	4' h	Fair	Unknown
Coast live oak tree	Unknown	1	10' h	Good	Unknown

Estimated Percent Cover:

- 70% including non-local or nonnative coyote brush and ceanothus
- 50% excluding these two species.

Nonnative and Aggressive Plant Species Present in and near Revegetation Site:

- Kikuyu grass
- Nasturtium
- Nonnative grasses
- *Eucalyptus citriodora*

*Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:*¹²⁴

- Jacaranda (observed spreading in other portions of San Jose Creek, including La Goleta Pollinator Garden (See Section II.O. below)).
- Palm tree
- Lemon-scented gum (eucalyptus) tree

COVA Site #3 Highlights:

- Upland coastal sage scrub species and coast live oak trees are well established and healthy.

COVA Site #3 Recommendations:

- Remove all Kikuyu grass by rhizomes for three consecutive years or by solarization.
- Remove medium-sized multi-trunked *eucalyptus citriodora* tree west of Site. This tree was retained when native willows and riparian habitat was removed during 2013-2015 in violation of CDFW SAA No. 1600-2012-0132-R5.

¹²³ Coyote brush (*Baccharis pilularis*) installed at this site is not a local variety and should be replaced with native plants of local genotypes.

¹²⁴ These species were retained while high quality riparian habitat was cleared.

- Remove nonnative or non-local ceanothus and coyote brush and other native species determined to be non-local genotypes.
- Plant local genotype oak woodland species to ensure 80-100% cover within five years pursuant to CDFW SAA success criteria.
- Maintain weeds to ensure < 5% cover by invasive herbaceous species pursuant to CDFW SAA success criteria.
- COVA must plant cleared area west of COVA Site #3 to restore dense willow riparian woodlands removed by COVA between 2013 and 2015 in apparent violation of CDFW SAA No. 1600-2012-0132-R5. (See below.) *Note: SBCFCWCD paid for the plants and labor to install and maintain COVA Site #3 as mitigation for the Annual Maintenance Program. COVA will need to revegetate an area(s) such as the cleared area west of COVA Site #3 as mitigation for COVA's clearing of sensitive riparian habitat in this area.*



Figure 48. COVA Site #3. Large palm tree retained on edge of site when riparian habitat was cleared. Planted species on slope are primarily non-riparian upland species including California sage, black sage, coyote brush and ceanothus. A large area of dense riparian habitat had been removed (lower half of image). Trautwein. January 30, 2022.



Figure 49. COVA Site #3. Nonnative jacaranda tree retained within revegetation site is shading native upland species such as California shade which prefer full sunlight. COVA requested SBCFCWCD to retain nonnative trees in revegetation areas. Understory is comprised of aggressive nonnative herbaceous species including nasturtium. Weedy area in foreground is where riparian habitat was cleared. Trautwein. January 30, 2022.



Figure 50. COVA Site #3. Note healthy coast live oak sapling in upper middle portion of image. Weedy area in lower portion of image is where riparian habitat was cleared. Trautwein. January 30, 2022.



Figure 51. COVA Site #3 (upper right quadrant of image). Lower half of image is where COVA cleared dense willow-riparian woodland with nearly impenetrable understory of blackberries. Area is now comprised of invasive nonnative grasses. Note multi-trunked *Eucalyptus citriodora* tree behind tri-trunked sycamore (left of middle, upper portion of image). Trautwein. January 30, 2022



Figure 52. COVA Site #3. Understory in southern portion of site comprised of nasturtium and other aggressive nonnative herbaceous species such as exotic grasses. Trautwein. January 30, 2022.



Figure 53. COVA Site #3. Site is upland plant community which is not in-kind mitigation for loss of riparian vegetation. Trautwein. January 30, 2022.

COVA Site #4

This is the fourth site planted by SBCFCWCD with approval by the landowner, COVA.¹²⁵ It is adjacent to the area of riparian habitat that COVA cleared including a large field and approximately one hundred linear feet of the east bank of San Jose Creek. (Figures 54 – 58)

Area: 3,000 square feet¹²⁶

Year Planted: 2019¹²⁷

Site Visits: January 27, 2022 and January 30, 2022

Plant Table

Species Planted	# Planted¹²⁸	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Elderberry	Unknown	10	7' – 12'h	Good	Unknown
Blackberry	Unknown	1,400 sq ft	1,400 sq ft	Good	Unknown
Coyote brush	Unknown	2	8'h	Good	Unknown

¹²⁵ Raaf (2022).

¹²⁶ SBCFCWCD *Annual Maintenance and Revegetation Report at 50* (2020-2021).

¹²⁷ *Id.*

¹²⁸ Two-hundred and fifty plants were installed at COVA Site #4 but there is no information on how many plants of each species were planted. SBCFCWCD *Annual Maintenance and Revegetation Report at 50* (2020-2021).

Mulefat	Unknown	1 patch	10' X 7's	Good	Unknown
Willow	Unknown	1	5' – 10'	Poor	Unknown

Estimated Percent Cover: 75 – 80%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Yucca tree
- Nasturtium
- Oxalis
- Italian thistle
- Mallow
- Shamel ash and castorbean along San Jose Creek adjoining COVA Site #4

COVA Site #4 Highlights:

- The elderberries and blackberries planted at this site are performing very well.

COVA Site #4 Recommendations:

- Remove or control oxalis, nasturtium, Italian thistle, mallow, plumbago, and yucca tree.
- Remove Shamel ash on east Creek bank located adjacent to site.
- Remove castor bean (*Ricinus communis*) from Creek area.
- Replace nonnative plants with native coast live oak woodland species from local genotypes to ensure >80% cover within five years pursuant to CDFW SAA success criteria.
- Remove and control weeds to ensure < 5% invasive herbaceous ground cover pursuant to CDFW SAA success criteria.
- Remove exotic vegetation including nonnative grasses and replant adjacent cleared riparian habitat (now a grassy field ~1,200 square feet) and eastern bank of San Jose Creek (~100 linear feet) with native riparian species including arroyo willow (*Salix lasiolepis*), black cottonwood (*Populus trichocarpas*), and wild blackberry (*Rubus ursinus*) as mitigation for COVA's clearing of this area between 2013 and 2015. (See above.)

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 57 of 159



Figure 54. COVA Site #4 (upper left quadrant of image). Note large area (approximately 90' by 30') of once dense willow riparian woodland cleared by COVA circa 2015 now covered by 100% invasive nonnative grasses. See Figure 66. Trautwein. January 30, 2022.



Figure 55. COVA Site #4 (Upper left quadrant of image) Note large, cleared area approximately 90' by 30' covered by exotic grasses. This area was a dense riparian woodland prior to clearing by COVA in 2015. This revegetation site supports ten healthy elderberry plants and an established stand of blackberries mixed with native wild cucumber and nonnative nasturtium at the south end of the Site. COVA Site #4 has achieved an estimated cover of 75-80% of native species. Trautwein. January 30, 2022.



Figure 56. COVA Site #4 with healthy elderberries at north end of site. Note exotic invasive herbaceous species on slope below elderberries. Italian thistle, nasturtium, mallow, and oxalis dominate site's understory. The area in the foreground / lower portion of image was cleared of high-quality riparian habitat. Trautwein. January 30, 2022.



Figure 57. COVA Site #4. A large yucca was retained within this revegetation site detracting from its success. COVA cleared riparian habitat in lower right half of this image. Trautwein. January 30, 2022.



Figure 58. COVA Site #4. South end of Site supports large stand of wild blackberry mixed with native wild cucumber and aggressive, exotic nasturtium. Note the grassy area in the lower portion of Figure 58 is where COVA cleared extensive riparian habitat. Trautwein. January 30, 2022.

1. COVA Cleared Substantial Areas of Riparian Habitat and Was Required by CDFW to Undertake Revegetation to Mitigate the Impacts, However SBCFCWCD Improperly Claimed COVA's Revegetation Sites as Mitigation for SBCFCWCD's Annual Maintenance Program.

During 2007 through 2018, COVA removed extensive areas of high-quality, dense willow riparian woodland with a thick understory of native riparian plants dominated by wild blackberry adjacent to COVA Site #1 as shown below. (Figures 59-66; *See also* Figures 45, 48-51, and 54-58) The clearings took place on COVA and Santa Barbara County properties, including within Santa Barbara Urban Creeks Council's ("UCC") County-approved San Jose Creek Restoration Site #1 south of Cathedral Oaks Road and east of San Jose Creek.¹²⁹

EDC reported the clearing of riparian habitat to CDFW as a potential Fish and Game Code violation circa 2010. (Figure 60) CDFW issued SAA No. 1600-2012-0132-R5. COVA agreed to plant native vegetation in two locations on an embankment (COVA Sites #1 and #2) to mitigate COVA's removal of riparian habitat near COVA Site #1.¹³⁰ Instead, SBCFCWCD undertook revegetation at COVA Sites #1 and #2 with COVA's approval to mitigate the impacts of the Annual Maintenance Program.¹³¹ Therefore COVA never undertook revegetation required to mitigate the impacts of COVA's clearing of riparian habitat pursuant to CDFW SAA No. 1600-2012-0132-R5.

Between 2013 and 2018, COVA cleared additional high-quality riparian habitat adjacent to COVA Sites #2 - #4, including an estimated one hundred linear feet of Creek bank and constructed an access route for trucks. (Figures 64 – 66; *See also* Figures 54 – 58, 72 – 74, and 76 - 83) EDC reported these clearings to CDFW in 2022.¹³²

- a. *COVA Sites #1 and COVA #2 Were Planted to Mitigate the Impacts of SBCFCWCD's Annual Maintenance Program Therefore COVA Must be Required to Revegetate Additional Areas to Mitigate the Impacts of COVA's Extensive Clearing of Riparian Habitat.*

The San Jose Creek riparian habitat cleared by COVA immediately south of Cathedral Oaks Road exhibited one hundred percent cover of riparian trees (willows, black cottonwoods,

¹²⁹ The UCC's San Jose Creek Restoration Project is a voluntary restoration project approved by the County, including Santa Barbara County Fire Chief Jim Thomas, Santa Barbara County Public Works Department and SBCFCWCD Director Phil Demery, Santa Barbara County Parks Department Director Jennifer Briggs, Santa Barbara County Counsel Steven Shane Stark, and Santa Barbara Risk Manager Charles Mitchell.¹²⁹ The Project received support from numerous other agencies including CDFW. The Project was funded with a State Department of Water Resources Urban Streams Restoration Program Grant of \$41,000 issued to SBCFCWCD on behalf of UCC and SBCFCWCD.

¹³⁰ Lohmus (2022).

¹³¹ *Id.*; *See also* Raaf (2022).

¹³² Email from Brian Trautwein, Senior Analyst/Watershed Program Director, to Warden Joseph Gonzales, CDFW (April 22, 2022).

and western sycamores) and understory plants (wild blackberry, etc.) in 2006. (Figure 59) However, much of this native riparian vegetation was sequentially removed between 2007 and 2018 by COVA which was issued SAA No. 1600-2012-0132-R5 in 2012 after COVA's clearing project was reported to CDFW. COVA never implemented revegetation required by that SAA.¹³³ (Figures 59 - 63)

As discussed above, SBCFCWCD obtained COVA's approval to revegetate COVA Sites #1¹³⁴ and #2 and claimed mitigation credit for both Sites.¹³⁵ (Figure 43) However, COVA was required by CDFW to mitigate for COVA's clearing of riparian habitat within San Jose Creek.¹³⁶ Therefore, COVA must be required to revegetate other areas to mitigate the impacts of COVA's riparian vegetation clearing program.



Figure 59. UCC San Jose Creek Restoration Project Site #1 (green polygon in middle and right side of image) covered with native riparian vegetation installed by UCC and established through natural recolonization processes. Google Earth. 2006.

¹³³ COVA claimed a portion of the UCC's San Jose Creek Restoration Project was on COVA property. Clearing riparian habitat on private property requires a Streambed Alteration Agreement; See also CDFW SAA No. 1600-2012-0132-R5 at 4 requiring 5:1 replacement of willow trees and "90% cover after 5 years for the life of the project."

¹³⁴ COVA Site #1 does not appear to have been successfully revegetated as described herein.

¹³⁵ SBCFCWCD *Maintenance and Revegetation Report 2020-2021 Maintenance Season* at 49-50 (July 2021); See also Raaf (2022)

¹³⁶ Lohmus (2022).



Figure 60. Riparian habitat in UCC's Site #1 cleared by COVA (red polygons) in 2007-2010. Google Earth. 2010.

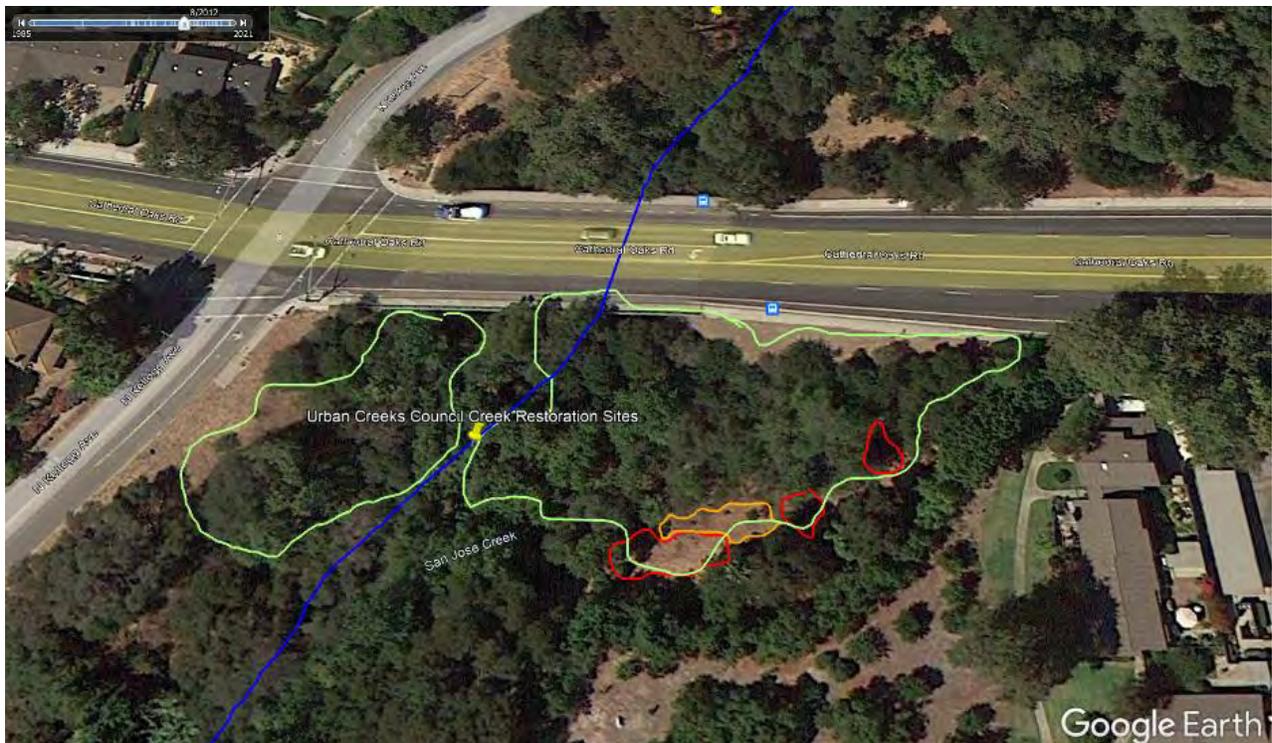


Figure 61. Additionally riparian habitat cleared by COVA (orange polygon) in 2010-2012. Google Earth. 2012.



Figure 62. More riparian habitat cleared by COVA (yellow polygons) in 2013-2014. Google Earth, 2014.



Figure 63. Further clearing of riparian habitat by COVA (purple polygon) in 2018. Google Earth, 2018.

- b. *SBCFCWCD Credits COVA's Upland Revegetation Sites #3 and #4 as Mitigation for the Annual Maintenance Plan Therefore COVA cannot also Claim Sites #3 and #4 as Mitigation for COVA's Removal of Riparian Habitat.*

COVA cleared additional dense riparian habitat along the east side of San Jose Creek between 2013 and 2015 adjacent to COVA Sites #2, #3, and #4, including approximately one hundred linear feet of riparian woodland from the east bank of San Jose Creek. (Figures 64 – 66, 71 – 74, and 76 - 83) Based on aerial photos, EDC estimates the riparian clearings measure a minimum of 8,100 square feet, plus additional areas of oak riparian understory vegetation not visible in aerial photos. EDC's surveys did not detect any revegetation sites planted by COVA as mitigation for its riparian habitat clearing program pursuant to CDFW SAA No. 1600-2012-0132-R5.¹³⁷ COVA should be required to provide onsite replacement of the riparian habitat removed south of Cathedral Oaks Road extending to the southwest corner of COVA's property west of COVA Site #4 and adjoining County property which was also cleared.

SBCFCWCD planted native upland vegetation at COVA sites #3 - #4 in 2017-2019.¹³⁸ SBCFCWCD credits COVA Sites #3 and #4 as mitigation for its Annual Maintenance Program. Given that COVA Sites #3 - #4 are claimed by SBCFCWCD as mitigation, there has been no mitigation for COVA's riparian habitat clearings as required by CDFW.¹³⁹ COVA Sites #3 - #4 cannot be considered mitigation for both COVA's and SBCFCWCD's riparian habitat removal projects.

COVA should be required to replant COVA Site #1, complete restoration of COVA Sites #2 - #4, and restore riparian habitat within all of the cleared locations to mitigate the impacts caused by COVA's clearing of riparian habitat. EDC recommends that riparian habitat removed by COVA be replaced at a minimum 3:1 ratio onsite. If necessary, offsite within nearby portions of San Jose Creek should be restored to ensure a minimum 3:1 replacement.

¹³⁷ CDFW directed COVA to restore riparian habitat to mitigate the impacts of COVA's clearing near Cathedral Oaks Road. Lohmus (2022); *See also* CDFW SAA No. 1600-2012-0132-R5.

¹³⁸ Raaf (2022); *See also* SBCFCWCD Maintenance and Revegetation Report 2020/2021 Maintenance Season at 49-50. See COVA Site #3 and COVA Site #4 Plant Tables below which demonstrate that these sites are upland plantings containing native and nonnative chaparral and coastal sage scrub species, including nonnative ceanothus, coyote brush, white sage, black sage, and California sage. Several willows planted on these upland slopes are struggling to survive despite irrigation.

¹³⁹ Lohmus (2022); *See also* CDFW SAA No. 1600-2012-0132-R5.

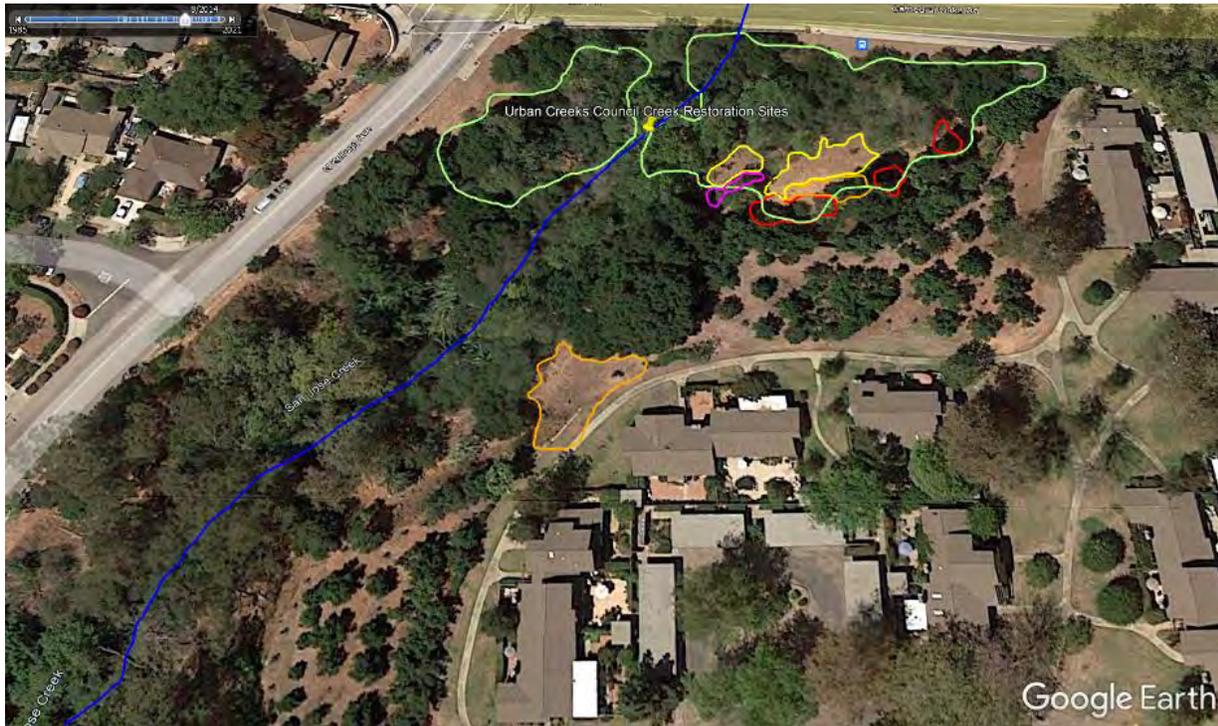


Figure 64. COVA cleared additional natural riparian habitat in 2014 (lower orange polygon in center of image). Google Earth. August 2014.



Figure 65. Prior to COVA clearing of riparian habitat in 2014. Note lower orange polygon in center of image contained dense riparian vegetation including willow trees in December 2013, which were removed in 2014 as shown in Figure 64 above. Google Earth. December 2013.

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations
Page 66 of 159



Figure 66. Riparian tree and understory vegetation appears to have been removed from additional areas east of the Creek adjacent to COVA between 2013 and 2015 (yellow polygons in center to lower left section of image). As shown in Figure 67 below, substantial portions of the two lower yellow polygons are located on Santa Barbara County property. Google Earth. 2015.



Figure 67. Parcel Map showing a portion of the cleared habitat was located on County property. On-X. January 29, 2022.



Figure 68. The western portion of Urban Creeks Council's San Jose Creek Restoration Project Site #1 located on County property was cleared by COVA. CDFW directed COVA to undertake revegetation to mitigate the impacts of the unpermitted removal of riparian habitat but this revegetation never occurred. Trautwein. January 2022.



Figure 69. Southern portion of Urban Creeks Council's County-approved San Jose Creek Restoration Site #1 was cleared by COVA. CDFW subsequently required COVA to revegetate COVA Sites #1 and #2 as mitigation for the unpermitted habitat removal, but these sites were revegetated by SBCFCWCD as part of its Annual Maintenance Plan. Trautwein. January 30, 2022.



Figure 70. COVA also cleared the native understory from the entirety of Urban Creeks Council's San Jose Creek Restoration Project Site #1. Note the black cottonwood and arroyo willow trees in Urban Creek's Council's County-approved San Jose Creek Restoration Project Site #1 are underlain by a 100% nonnative understory. The understory was 100% native cover consisting primarily of wild blackberries prior to COVA's clearing of the Site. Trautwein. January 30, 2022.



Figure 71. COVA cleared oak riparian woodland understory on City property south of the Cathedral Oaks Road Bridge over San Jose Creek. Trautwein. January 30, 2022.



Figure 72. Oak riparian woodland understory was cleared and oak trees were pruned along east side of San Jose Creek west of COVA between 2009 and 2015 providing vehicle access along the Creek bank. Trautwein. January 23, 2022.



Figure 73. Cleared riparian habitat now provides vehicle access along Creek bank near COVA Sites #2 and #3. Trautwein. January 23, 2022.



Figure 74. COVA Site #2 with cleared riparian habitat in foreground. Wild blackberry (right side) and California sage (center) present. COVA required SBCFCWD to retain nonnative trees in the revegetation sites. Large yucca tree (upper left) was retained in revegetation site. Trautwein. January 23, 2022.



Figure 75. COVA Site #2 showing California sage on left, small white sage in center, and mulefat on right. Understory is dominated by invasive nonnative oxalis. Trautwein. January 23, 2022.



Figure 76. COVA Site #2 with California sage. COVA directed SBCFCWCD to retain nonnative trees in the revegetation sites. Note palm tree and jacaranda tree were inappropriately retained in and adjacent to restoration site. Trautwein. January 23, 2022.



Figure 77. COVA cleared a dense willow riparian habitat including mature willow trees and native understory plants creating a large field of nonnative grasses and forbs approximately ninety feet by thirty feet immediately west of COVA Site #4. Trautwein. January 30, 2022.

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 73 of 159



Figure 78. COVA cleared riparian woodland from approximately one hundred linear feet of the east bank of San Jose Creek on City property west of COVA Site #4. Looking downstream, south by southwest. Trautwein. January 30, 2022.

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 74 of 159



Figure 79. COVA cleared riparian woodland from approximately one hundred linear feet of the east bank of San Jose Creek on City property west of COVA Site #4. Looking upstream, north by northeast. Trautwein. January 30, 2022.



Figure 80. COVA cleared riparian habitat near COVA Site #4. Looking north by northeast to south by southeast. San Jose Creek is located on left side of image. Trautwein. January 30, 2022.



Figure 81. COVA's large riparian habitat removal site (grassy area) west of COVA #4 (upper center portion of image). Trautwein. January 30, 2022.



Figure 82. Dense, high-quality riparian habitat was cleared from County property near COVA Site #4. COVA Site #4 is visible in upper right portion of image. Trautwein. January 23, 2022.



Figure 83. COVA Site #4 with native plants covering 75% of embankment. Note grassy area is where COVA cleared riparian habitat. Trautwein. January 23, 2022.

c. *Recommendations to Mitigate the Impacts of COVA's Riparian Habitat Clearing Projects.*

- CDFW should require COVA to replant all areas COVA cleared of riparian habitat and achieve one hundred percent (100%) cover of native overstory and understory species to replace habitat removed between 2007 and 2018.¹⁴⁰ (Figures 59 – 66)
- CDFW should require COVA to revegetate additional riparian areas near COVA Sites #1 - #4 to mitigate the temporal impacts of removing high-quality riparian habitat at minimum three-to-one (3:1) ratio. Revegetation should achieve ninety percent (90%) total cover within five years and exhibit less than five percent (5%) cover by nonnative herbaceous vegetation as required by CDFW SAA No. 1600-2012-0132-R5.
- CDFW should determine whether SAA No. 1600-2012-0132-R5 authorized all of COVA's riparian habitat removal projects. If not, CDFW should issue a Notice of Violation of the California Fish and Game Code for COVA's removal of high-quality riparian habitat.
- CDFW should require COVA to remove nonnative and non-local varieties of native vegetation in the cleared areas.
- CDFW should require COVA to allow SBCFCWCD to remove nonnative and non-local native plants from Sites #1 - #4, including:
 - Nonnative varieties of native species such as ceanothus and coyote brush (*Baccharis pilularis*) & replant COVA Sites #3 and #4 with local genotype native riparian and upland;¹⁴¹
 - Palm, eucalyptus, yucca, and jacaranda trees; and
 - Invasive and aggressive weedy herbaceous species including oxalis, nasturtium, Italian thistle, mallow, and nonnative grasses, including kikuyu grass present in all four COVA Sites and all areas cleared by COVA.
- Mitigation requirements for COVA and SBCFCWCD should be clarified to ensure each entity's mitigation requirement is fulfilled.
- CDFW should require COVA to (1) preserve native vegetation along the creek and revegetation areas, and (2) place all riparian and upland revegetation and restoration sites in a permanent conservation easement or deed restriction to prevent future removal of this sensitive habitat.

¹⁴⁰ COVA continues to maintain the cleared areas preventing regrowth of native riparian vegetation.

¹⁴¹ See e.g., City of Goleta (2020) CWMP Strategy 11.1.2.

F. Old Footbridge in University Circle Park (Site 4)

SBCFCWCD installed rip rap bank protection on the west bank following storms in 1995 which caused bank erosion.¹⁴² Willows were planted within the rip rap as wattling.

Year Planted: 1995

Size:

- Reported: 50 feet x 10 feet = 500 square feet as measured by SBCFCWCD in 1995.¹⁴³
- Observed: 64 feet x 8 feet = 512 ft² as measured by EDC in 2022.

Site Visit: February 25, 2022

Plant Table: n/a¹⁴⁴

Estimated Percent Cover:

- 50-75% native tree canopy
- 50-80% cover by nonnative understory species

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Cape ivy (Figures 84 and 86)
- Kikuyu grass (Figure 86)
- Oxalis (Figure 86)

Old Footbridge Site Highlights:

- This site was described as a “textbook site to view successful willow wattling” in 1998.¹⁴⁵ However, twenty-seven years after installation, only two willows remain. They are in fair condition with multiple trunks. Each is approximately 30’ tall and about 6-8” DBH.
- One black cottonwood is present in fair to good condition. It is approximately thirty-five feet tall with an eight-inch DBH.¹⁴⁶
- The rip rap remains in good condition however, concrete with rebar and asphalt blocks were mixed in with boulders. (Figure 88)

¹⁴² SBCFCWCD *Annual Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1998) (“SBCFCWCD (1998)”).

¹⁴³ SBCFCWCD (1998)

¹⁴⁴ Willow wattling was installed but the number of willows installed was not provided. No other species were planted at this location.

¹⁴⁵ SBCFCWCD (1998).

¹⁴⁶ This tree appears to be naturally occurring.

Old Footbridge Site Recommendations:

- Remove and replace nonnative understory, including Cape ivy, oxalis, and Kikuyu grass with natives, including mugwort, wild blackberry, giant wild ryegrass.
- Remove Shamel ash trees and Arundo donax on east bank opposite rip rap.
- Do not use concrete or asphalt in rip rap. Use naturally occurring rocks.
- Plant willow and black cottonwood trees around the rip rap.

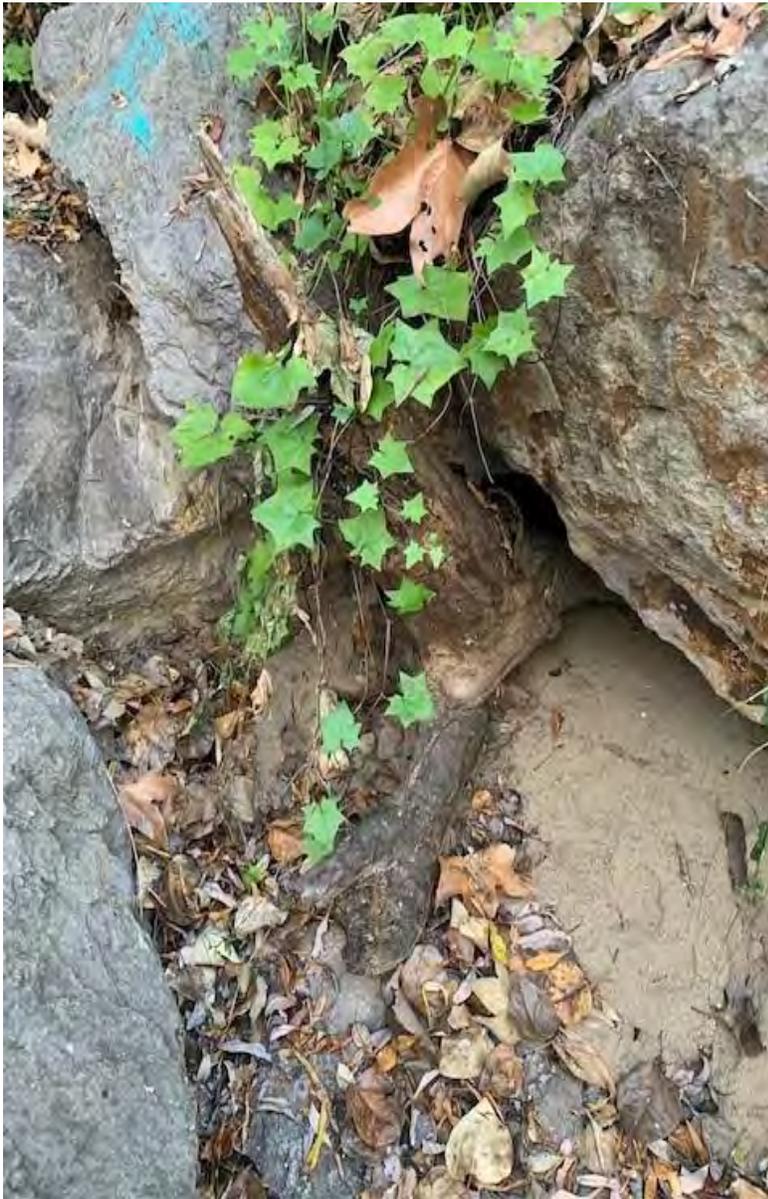


Figure 84. Nonnative, invasive Cape Ivy growing over installed rip rap. Trautwein. February 25, 2022.



Figure 85. University Circle Open Space. Many large willows, sycamores and cottonwoods grow along San Jose Creek. Trautwein near the Old Footbridge Site. February 25, 2022.



Figure 86. Nonnative, invasive grasses, oxalis, and cape ivy growing over rip rap that was installed as bank stabilization. Trautwein. February 25, 2022.



Figure 87. Looking up San Jose Creek at the Old Footbridge Site near University Circle Open Space. Rip rap was installed and is now overgrown with nonnative grasses, oxalis, and invasive Cape ivy. Woody tree in the foreground is one of two surviving willows believed to be installed as wattling in the rip rap in 1995. Trautwein. February 25, 2022.



Figure 88. Rip rap includes and asphalt and concrete with rebar used as bank stabilization. Potential for concrete to alter pH of Creek water. Rebar also poses a potential safety hazard. Trautwein. February 25, 2022.



Figure 89. Rip rap covered with native poison oak and nonnative oxalis, kikuyu grass, and cape ivy. One of two remaining willow trees (left side of image) and one naturally-occurring black cottonwood tree is present on upper bank (center of image). Trautwein. February 25, 2022.



Figure 90. Rip rap with one of two surviving willow trees installed in 1995. February 25, 2022.



Figure 91. Black cottonwood on creek bank growing above rip rap at Old Footbridge Site. This is believed to be a naturally-occurring tree because only willows were planted in the rip rap. Trautwein. February 25, 2022.

G. Seventy-five Feet Upstream of Berkeley Footbridge (Site 5)

SBCFCWCD installed rip rap with willow wattling in this location where high flows caused bank erosion at the outside of a sharp bend in the southern end of the University Circle Open Space.¹⁴⁷ SBCFCWCD installed the willow wattling 1995.

Year Planted: 1995¹⁴⁸

Area: 25 feet x 10 feet = 250 square feet

Date of Site Visit: February 25, 2022

Plant Table: n/a¹⁴⁹

Estimated Percent Cover:

- 50% - 60%

Native Species Present:

- Willows
- Cottonwood
- Creek clematis

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Periwinkle
- Nasturtium
- Cape ivy
- Algerian ivy

Seventy-five Feet Upstream of Berkeley Bridge Site Highlights:

- Rip rap installed by Flood Control District is creating roughness and helping establish a pool at this location.

Seventy-five Feet Upstream of Berkeley Bridge Site Recommendations:

- Remove residential shed located on public property. (Figure 92)
- Plant native trees and understory around the rip rap to achieve a higher percent cover at this site.

¹⁴⁷ SBCFCWCD (1998).

¹⁴⁸ *Id.*

¹⁴⁹ Willow wattling was planted underneath the rip rap but the number of willow branches planted was not disclosed and no other plants were installed.



Figure 92. Site known as Seventy-five Feet Upstream from the Berkeley Footbridge. Installed rip rap is supporting native black cottonwood tree. Bank downstream from rip rap lacks native riparian vegetation. Trautwein. February 2022.



Figure 93. View of rip rap, looking upstream of San Jose Creek. Willows growing out of the rocks and bank. Trautwein. February 2022.

H. Berkeley Footbridge East of Goleta Water District Well Building (Site 6)¹⁵⁰

SBCFCWCD planted this small patch of native plants next to the Berkely Footbridge in 1995.¹⁵¹

Year Planted: 1995¹⁵²

Area:

- Reported: 180 feet x 15 feet = 2,700 square feet as reported by SBCFCWCD.¹⁵³
- Observed: 800 square feet (elderberries and willow trees) plus 200 square feet (sycamore trees) = 1,000 square feet observed by EDC in 2022.

Date of Site Visit: January 27, 2022

Description: This site contains several willow and sycamore trees, elderberries, and wild rose west of the west bank of San Jose Creek south of the Berkely Footbridge. The sycamore trees were planted along the Creek’s west bank east of the footpath.

Plant Table

Species Planted	# Planted	# Observed	Avg. Height	General Condition	Estimated % Survival
Sycamore	Unknown	2	20’ - 25’h	Good	Unknown
Willow	Unknown	1	15’h	Fair to Good	Unknown
Elderberry	Unknown	4	15’’h	Fair to Good	Unknown
Wild rose	Unknown	2	3’h	Fair	Unknown
Coyote Brush	Unknown ¹⁵⁴	2	6’h	Fair	Unknown

Estimated Total Percent Cover:

- Overstory: Greater than eighty percent (80%) native species within the eight hundred (800) square foot revegetation area.
- Understory: One hundred percent (100%) nonnative invasive herbaceous species.

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses.

Berkeley Road Footbridge Site Highlights:

¹⁵⁰ Uncertain if this is part of Site 6; the site numbering system from report to report is inconsistent and unclear.

¹⁵¹ SBCFCWCD (1998).

¹⁵² Uncertain if this is part of Site 6; the site numbering system from report to report is inconsistent and unclear.

¹⁵³ SBCFCWCD (1998).

¹⁵⁴ Uncertain if planted, might be from natural recruitment.



- The two sycamore trees are growing strong and large, indicating great success in their establishment.

Berkeley Footbridge Site Recommendations:

- Manually remove all nonnative understory plants under willows, sycamores, wild rose, and elderberries and within 20 feet of driplines.
- Plant twenty-five wild blackberry plants on six-foot centers.
- Control weeds until blackberries establish a minimum ninety-five percent cover.



Figure 94. Berkeley Footbridge Site East of Goleta Water District Well Building. Willows, elderberries, and wild rose present by understory is 100% exotic invasive herbaceous plants. Trautwein. January 23, 2022.



Figure 95. One of two healthy sycamore trees SBCFCWCD planted at the Berkeley Footbridge Site. Trautwein. January 23, 2022.

I. Three Hundred Feet Downstream from Berkeley Road Footbridge East Bank Repair (Site 23)

East bank repair: installation of rock rip rap and willow brush layering; east and west bank revegetation.¹⁵⁵

Years Planted: 2006-2007

Area:

- East bank: 80 feet x 15 feet = 1,200 square feet
- West bank: 12 feet x 6 feet = 72 square feet

Date of Site Visits: January 22, 2022, January 27, 2022, and February 18, 2022

Plant Table

Species Planted	# Planted	# Observed	Avg Height or Spread	General Condition	% Survival
East bank	-----	-----	-----	-----	-----
Mugwort (<i>Artemesia</i>)	25	0	-	-	0
Sunflower (<i>Venegasia</i>) ¹⁵⁶	10	1	-	-	10
Elderberry (<i>Sambucus</i>)	2	1	8' h	Good	50
Sycamore (<i>Platanus racemosa</i>)	1	0	-	-	0
Blackberry (<i>Rubus ursinus</i>)	15	2	2x2' s and 10x4' s	Fair	13.3
Lemonade berry (<i>Rhus integrifolia</i>)	3	0	-	-	0
Brush layering with Willow branches	n/a	3	5-15' h	Fair	-
West Bank	-----	-----	-----	-----	-----
Willow (<i>Salix</i>)	10	7 (3 dead ¹⁵⁷)	10' to 20' h	Fair to Good	70
Wild blackberry (<i>Rubus ursinus</i>)	8	0	-	-	0
Clematis	10	0	-	-	0

¹⁵⁵ SBCFCWCD *Maintenance and Revegetation Report 2006/2007 Maintenance Season* (2007).

¹⁵⁶ Might have seen one canyon sunflower but were unable to conclusively identify species.

¹⁵⁷ Not sure if these were planted by SBCFCWCD or if they are natural recruits that died.

Estimated Total Percent Cover:

- East Bank: Understory 10-40%
Overstory 50%
- West Bank: Understory 10%
Overstory 60-70%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Shamel ash
- Nonnative grasses
- Nasturtium

Three Hundred Feet South of Berkeley Road East Bank Repair Site Highlights:

- SBCFCWCD retained a pool at this site below the rock rip rap.
- One large willow appears to have grown from the brush layering within the rock rip rap.

Three Hundred Feet South of Berkeley Road East Bank Repair Site Recommendations:

- Eradicate nonnative invasive species from east bank above rock rip rap
- Plant additional willow and/or cottonwood poles into the rip rap.
- Eradicate the Shamel ash tree growing in the rip rap.



Figure 96. Bank repair. East bank of San Jose Creek approximately three hundred feet south of Berkeley Footbridge. Note invasive Shamel ash (green and yellow compound leaves) established at bank repair site. East bank above rip rap bank armoring supports nonnative invasive species. One single large willow appears to remain (behind ash tree) in upper right quadrant of image. Trautwein. January 27, 2022.



Figure 97. Site 23, approximately 300' downstream of Berkeley Footbridge on the east bank of San Jose Creek. Note: the white PVC pipe coming out of the foliage was installed to irrigate the layered willows that were placed between rip rap layers. Trautwein. March 7, 2022.



Figure 98. Riprap located on the east bank of Site 23. Young willows are growing out of the rocks are a result of willow brush layering at the time of mitigation. Understory is about 50% nonnative grasses. Trautwein. March 7, 2022.



Figure 99. Rip rap and exposed willow layering. Note the upshoots of new willow growth. Trautwein. March 7, 2022.



Figure 100. Site 23, about three hundred feet downstream of the Berkeley Footbridge, on the west bank. The understory is primarily nonnative grasses and nasturtium. The overstory is primarily willow, sycamore, and black cottonwood. Trautwein. March 7, 2022.



Figure 101. West bank of San Jose Creek at Site 23. Large sycamore in the background from natural recruitment. Understory is primarily nonnative grasses. Willow saplings present on lower bank of Creek. Trautwein. March 7, 2022.



Figure 102. Young willow growing out of a poison oak. Note the nonnative grasses in the foreground. Trautwein. March 7, 2022.



Figure 103. Site 23, approximately three hundred feet downstream of Berkeley Footbridge on the west bank. Note the nonnative, naturalized bottlebrush bush that is growing in the center of the photo. There are also nonnative grasses and nasturtium in the foreground, and willow and oak trees behind the bottlebrush. Trautwein. March 7, 2022.

J. Berkeley Road Downstream from Berkeley Footbridge, West Side of Creek near Access Road (Site 13)

SBCFCWCD installed rip rap, rebuilt the bank, and planted this site with willows, cottonwoods, sycamores, and elderberries in 2000 after storms eroded the Creek’s west bank and adjacent flood control access road which serves as a neighborhood trail.¹⁵⁸ The only plants to survive are located adjacent to the bank top.

Year Planted: 2000¹⁵⁹

Area: 1,307 square feet¹⁶⁰

Dates of Site Visits: January 22, 2022, January 27, 2022, and February 18, 2022

Plant Table

Species	# Planted ¹⁶¹	# Observed ¹⁶²	Avg Height or Spread	General Condition	% Survival
Willow	5	1-2	10’ - 20’ h	Fair	20 – 40%
Cottonwood	2	2	10’ h	Fair	100%
Elderberry	2	2	15’ – 20’ h	Fair	100%
California wild rose	5	0	-	-	0%
Sycamore	1	1	20’ h	Fair	100%
Wild blackberry	20	0	-	-	0%

Estimated Cover:

- Fifty Percent (50%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Nonnative grasses

Site Highlights:

- The elderberries on the bank-top have grown exceptionally large and likely produce numerous berries to nurture birds and wildlife.

¹⁵⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2000) (“SBCFCWCD (2020)”).

¹⁵⁹ *Id.*

¹⁶⁰ *Id.* stating site is .03 acres in size.

¹⁶¹ *Id.*

¹⁶² The only remaining plants are located adjacent to the west creek bank. All plants installed beyond 10 feet from the bank to the access road are gone with only invasive herbaceous plants growing there now.



Three Hundred Feet Downstream from Berkeley Road West Side of Creek by Access Road Site Recommendations:

- Remove all herbaceous plants between access road and creek bank.
- Replant area with blackberries, elderberries, coast live oaks, and coast live oak and riparian understory species to ensure >80% cover within 5 years pursuant to CDFW performance standards. Cage plants to prevent vandalism.
- Control weeds to ensure <5% cover pursuant to CDFW performance criteria.
- Confirm coast live oak planted as mitigation for Somerset Road oak pruning.



Figure 104. Site 13.
Sycamore tree on west
bank of San Jose Creek
approximately 300 feet
south of Berkeley
Footbridge. Trautwein.
January 27, 2022.



Figure 105. Site 13. Large elderberry on west bank of San Jose Creek approximately three hundred feet downstream from Berkeley Footbridge. Trautwein. January 27, 2022.



Figure 106. Site 13. Looking south. Approximately three hundred feet south of Berkeley Footbridge on west bank. Note lack of native plants and 100% cover by invasive exotic herbaceous plants except for the immediate bank top. Note sole oak tree in center of image planted in SBCFCWCD's Site 13. It is EDC's understanding that this oak tree was required as mitigation for oak pruning by a Somerset Avenue resident which EDC reported to the City of Goleta. If so, this oak tree cannot be double-counted as mitigation for SBCFCWCD. Trautwein. January 27, 2022.



Figure 107. Site 13 looking north toward Berkeley Road. Note sole oak tree believed to be planted as a result of City of Goleta enforcement regarding neighbor oak pruning reported by EDC northeast of Somerset Avenue Accessway. Note absence of native understory plants. Trautwein. January 27, 2022.



Figure 108. Site #13 in July 2000. Looking north towards Berkeley Road (same perspective as Figure 107 above). Note plants extended west (left) from top of west bank (right side of image) to almost access road/trail. As shown in Figure 107 above, only plants planted immediately atop the Creek bank survived. The remainder of the planted area is dominated by non-native invasive herbaceous plants including oxalis and exotic grasses. Image from SBCFCWCD Annual Maintenance and Revegetation Report. 2000.

K. Somerset Road Access Ramp

SBCFCWCD decommissioned an access ramp on the west bank, stabilized the west bank using rock rip rap, and revegetated it with willows, mulefat, giant wild ryegrass, elderberry cuttings, and other species.¹⁶³

Year Planted: 2010-2012

Area: 1,000 square feet

Date of Site Visits: January 23, 2022 and January 27, 2022.

Plant Table

Species	# Planted ¹⁶⁴	# Observed	Avg Height or Spread	General Condition	% Survival
Willows	Unknown	1	~12' h ¹⁶⁵	Fair	Unknown
Oak Trees	Unknown	0	-	-	0
Blackberry	Unknown	0	-	-	0
Mulefat	Unknown	1	-	-	Unknown
Coyote brush	Unknown	0	-	-	0
Ryegrass	Unknown	7-8	60-100 sq ft total	Fair to Good	Unknown
Elderberry	Unknown	4	5'-8' h	Fair	Unknown

Estimated Percent Total Cover:

- Fifty to seventy percent (50-70%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Nonnative grasses

¹⁶³ SBCFCWCD *Annual Maintenance and Revegetation Report 2011/2012 Maintenance Season* (2012).

¹⁶⁴ The number planted was not found in any of the Annual Plans or Maintenance and Revegetation Reports, so it is impossible to calculate percent survival.

¹⁶⁵ These plantings were reported to have reached over 14' tall in 2015 in the 2015-2016 Annual Maintenance and Revegetation Report at 82 however EDC did not find photographs in subsequent Maintenance and Revegetation Reports showing plants. The 2015-16 Report shows one photograph from 2015 but the plants appear to be approximately five feet tall with the exception of one taller willow or mulefat plant. Currently, the tallest plant is the lone willow which is 12 feet tall and does not appear to have ever exceeded 12 feet in height.



Figure 109. Somerset Access Ramp Site showing giant wild ryegrass (lower portion of image), mulefat (center of image), lone willow (left side of image), and herbaceous nonnative understory vegetation. Trautwein. January 27, 2022.

Somerset Access Ramp Site Highlights:

- The giant wildrye grass is well established in this location.
- The District successfully established elderberries from cuttings demonstrating a potentially more efficient way to propagate elderberries than growing plants from seeds.

Somerset Access Ramp Site Recommendations:

- Remove oxalis and nonnative grasses for two consecutive years.
- Remove Shamel ash sapling at base of rock rip rap bank repair site
- Plant two coast live oak trees.
- Replant twenty wild blackberry and 10 giant wild ryegrass plants at 5-ft centers after oxalis and nonnative grasses are eradicated.
- Plant one hundred mugwort plants on one-foot centers.
- Water and weed until established, percent cover of invasive herbaceous plants is <5%, and percent cover of native plants increases to at least 80% pursuant to CDFW SAA success criteria.



Figure 110. Looking downstream San Jose Creek at the Somerset Access Ramp Site. The west bank hosts a large sycamore, some oaks, nonnative grasses, elderberry, wild giant rye grass, and mulefat. Trautwein. February 2022.



Figure 111. West bank of San Jose Creek at Somerset Access Ramp Site. Riprap in the foreground along the bottom edge of the image with oxalis and nonnative grasses, such as kikuyu. Large sycamore in the background center. SBCFCWCD installed willows, mulefat, several elderberries, and giant wild ryegrass at this Site. Trautwein. February 2022.



Figure 112. Nonnative Shamel ash sapling in the center foreground growing out of rock riprap along with nonnative grasses. Trautwein. February 2022.

L. One-hundred Feet Downstream from Former Somerset Access Ramp, West Bank Repair (Site 10)¹⁶⁶

SBCFCWCD reconstructed the eroding west bank, replaced a corroded metal stormwater culvert which was contributing to erosion with a plastic culvert, replaced failing pipe and wire revetment with rock rip rap (retaining one or two pipe revetment poles to support a large sycamore tree), installed willow wattling in the rip rap, and then planted the bank approximately two hundred feet downstream from the decommissioned Somerset Drive access ramp. Currently, the west bank which is located along a sharp curve upstream from the Elks Club is experiencing significant erosion which is threatening the back yard of a home located on Somerset Drive. Large patches of *Arundo* are present and native vegetation is lacking on the west bank.

Years Planted: 1996, 1997, and 1998

Area: 50 feet x 10 feet = 500 square feet

Date of Site Visits: January 27 and February 4, 2022.

Plant Table: n/a¹⁶⁷

Natives Observed:

- Willow
- Elderberry
- Wild Cucumber

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- *Arundo donax* (two large patches)

Downstream from Somerset Access Ramp Site Highlights:

- Most of the rip rap is intact.

Downstream from Somerset Access Ramp Recommendations:

- Conduct an engineering feasibility analysis. If feasible, engineer and install a live willow crib wall in base of the eroding bank. Use rip rap with willow and/or cottonwood wattling at the foot of slope in front of the crib wall if engineering analysis determines a crib wall alone would be insufficient to protect and stabilize the bank.
- Remove *Arundo* and other nonnative species and revegetate bank with sycamores, willows, blackberry, mugwort, and giant wild ryegrass.

¹⁶⁶ Uncertain whether this is the correct site number. Site numbering is very confusing and inconsistent throughout the Flood Control Maintenance and Revegetation Reports.

¹⁶⁷ No plant table is included because EDC was not able to find a list of the species other than the willow wattling or number of plants planted.



Figure 113. About one hundred to two hundred feet downstream the site of the decommissioned Somerset Drive Access Ramp on the west bank. Note the chain-link fence at the top of this slope delineates a backyard. This slope has eroded severely at the upstream end of SBCFCWCD's 1996 bank repair project. Consideration should be given to engineering and installing a live willow crib wall. Trautwein. February 2022.



Figure 114. Looking down stream of San Jose Creek at the erosion site show above (Figure 113). A large sycamore stretches over the Creek bed providing shade. Nonnative Arundo is abundant on either side of the sycamore. Kikuyu grass is present in the bottom left corner. Trautwein. February 2022.



Figure 115. Eroded west bank of San Jose Creek one to two hundred feet downstream of the site of the decommissioned Somerset Drive Access Ramp. Displaced rip rap in the bottom right corner. Young willows grow out of the remaining riprap in the center of the photo. Trautwein. February 2022.

M. Downstream from Highway 101 West Bank (Site 7)

This Site included a large patch of castor bean which SBCFCWCD eradicated before planting willow trees in 1996 to try to screen the sand and gravel operation to the west of the Site.¹⁶⁸ The Site contains several remaining willows trees but the understory is almost entirely nonnative species. Records of the numbers of plants installed were not kept. The location near Highway 101 places this Site near a well-known homeless encampment.

Date Planted: 1996¹⁶⁹

Area: 100 feet by 15 feet = 1,500 square feet¹⁷⁰

Site Visit: February 18, 2022

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	% Survival
Willows	Unknown	6	~6-8" DBH	Good	Unknown
Mugwort	Unknown	Few	-	-	0
Blackberry	Unknown	Few	-	-	0

Estimated Percent Total Cover:

- Canopy: Sixty percent (60%)
- Groundcover: Less than ten percent (10%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Oxalis
- Palm Tree
- Eucalyptus
- Castor
- Cape Ivy
- Nonnative Grasses
- Tree Tobacco

Site-specific Recommendations:

¹⁶⁸ SBCFCWCD *Maintenance and Revegetation Report, 2017-2018 Maintenance Season* (December 1998).

¹⁶⁹ SBCFCWCD *Maintenance and Revegetation Report* (2000).

¹⁷⁰ *Id.*



- Remove the nonnative plants listed above and increase the groundcover with native understory species, including wild blackberry, hummingbird sage, poison oak, and mugwort.
- Plant native shrubs including gooseberry, toyon, coffeeberry, lemonade berry, and wild rose.
- Plant native trees including black cottonwood and sycamore to increase the canopy cover.



Figure 116. Nonnative understory of primarily nasturtium. Nonnative palm tree in background and eucalyptus canopy (upper portion of photo). Trautwein. February 18, 2022.



Figure 117. San Jose Creek Highway 101 Revegetation Site on west bank looking east across Creek. Several remaining willows from the 1996 revegetation project. Trautwein. February 18, 2022.



Figure 118. View from west bank looking northeast showing several remaining willows from 1996 revegetation project. Ground cover is nonnative grasses and nasturtium. Trautwein. February 18, 2022.

N. Hansen Aggregate Site

A portion of this Site was planted in 2003 when SBCFCWCD regraded the west bank where it had experienced erosion.¹⁷¹ Other sections of the bank were planted along a two-hundred-and-fifty-foot reach.¹⁷² Coconut fiber was laid down on the bank.¹⁷³ An adjacent property had added a large amount of impervious surface and not properly addressed runoff threatening to exacerbate erosion.¹⁷⁴ The operation eventually punched a hole in the bank to allow water to drain into the creek so it would not pond within the sand and gravel facility.¹⁷⁵ Subsequently, the sand and gravel operation installed a storm drain after working with regulatory agencies.¹⁷⁶ Currently, the west bank adjacent to the aggregate operation supports several large sycamore trees. There is a concrete block wall adjacent to the top of the bank installed in approximately 2000-2005. It is unclear if this block wall received permits from the City of Goleta or CDFW. Encampments have left significant amounts of trash on the west bank and Creek bed. Several willow and cottonwoods occur below the sycamore canopy and two spreading patches of wild rose are present on the bank. However, the vast majority of the bank is covered by nonnative groundcover plants.

Year Planted: 2003¹⁷⁷

Area: 2,500 square feet¹⁷⁸

Date of Site Visit: February 18, 2022

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	30	14	Variable	Fair to Good	<50%
Cottonwood	5	2	>15' h	Good	40%
Wildrose	10	At least 2 patches	Patches ranged from 8' by 25' and 8' by 20'	Good	at least 20%
Sycamore	2	0	-	-	0%

Estimated Percent Cover:

- Groundcover: Ten percent (10%)
- Canopy: Forty to sixty percent (40%-60%)

¹⁷¹ SBCFCWCD *Maintenance and Revegetation Report* (December 2003).

¹⁷² *Id.*

¹⁷³ *Id.*

¹⁷⁴ *Id.*

¹⁷⁵ *Id.*

¹⁷⁶ *Id.*

¹⁷⁷ *Id.*

¹⁷⁸ *Id.*



Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- *Arundo donax*
- Oxalis
- Nonnative Grasses
- Shamel ash tree

Hanson Aggregate Site Highlights:

- The south-facing bank adjacent to the aggregate facility makes it an ideal site for revegetation which once complete will help shield the creek from the noise and lights associated with the facility.

Hanson Aggregate Site Recommendations:

- Remove the nonnative species present and replace with native understory, including blackberry, native shrubs, including wild rose, toyon, and lemonade berry, as well as native trees, such as willows, sycamores, California bay laurels and/or cottonwoods.
- Report the block wall to the City and CDFW to ascertain permit status.



Figure 119. This is the West bank of the Hansen Aggregate site, located just downstream from the train tracks and along Hansen Aggregate company grounds. This site hosts a robust homeless population which collectively produces a lot of trash and subsequent creek pollution. Trautwein. February 18, 2022.



Figure 120. West bank of San Jose Creek at the Hansen Aggregate site. Slope is covered in nonnative grasses and fallen sycamore leaves from the large sycamore in the upper right corner of the photo. One of several remaining willow trees is shown in the upper left corner of the photo. Trautwein. February 18, 2022.



Figure 121. Several remaining willow trees are present at the Hanson Aggregate Site on the west bank of San Jose Creek beneath large sycamores. The bank is covered by nonnative plants such as oxalis and kikuyu grass. Note the concrete block wall at the top of the bank. Trautwein. February 18, 2022.



Figure 122. Looking downstream along the west bank of San Jose Creek at the Hansen Aggregate Site. One of remaining willow trees from 2003 revegetation is present in the foreground and several large sycamores in background. Willows. Trautwein. February 18, 2022.



Figure 123. Looking downstream along San Jose Creek at the Hansen Aggregate site. There are wild roses surviving from the 2022 revegetation project and these are spreading underneath the canopy of sycamores and willows. Trautwein. February 18, 2022.

O. La Goleta Condominiums Site¹⁷⁹

The La Goleta Revegetation Site is located on the south/south-east bank of San Jose Creek behind the La Goleta Condominium complex located at Armitos Avenue and Dearborn Place. Approximately 600 plants were installed at this Site in 2018 through a cooperative effort between SBCFCWCD and the La Goleta Homeowners Association (“HOA”).¹⁸⁰ EDC divided the Site into six sections for the purpose of evaluating success of revegetation efforts. Several of the subsections are performing well. The HOA planted a pollinator garden, including nonnative species, which should not be credited toward SBCFCWCD’s revegetation program, as discussed below.

¹⁷⁹ EDC segmented the La Goleta Site into six sections (not including the pollinator garden) for the purpose of tallying plants and estimating percent cover.

¹⁸⁰ SBCFCWCD *Maintenance and Revegetation Report 2020/2021 Maintenance Season* (July 2021) at 58.

Year Planted: 2018¹⁸¹

Size: 6,500 square feet¹⁸²

Dates of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.



Figure 124. La Goleta Condominiums Revegetation Site and La Goleta Homeowners Association Pollinator Garden. Google Earth. 2021.

i. La Goleta Condominiums Site Section #1

Section 1 is 450 square feet and is located at the west end of the La Goleta Revegetation Site northwest of the La Goleta Pollinator Garden discussed below and is directly behind 5514 Armitos Avenue.

Year Planted: 2018

Size: 15 feet x 30 feet = 450 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

¹⁸¹ *Id.*

¹⁸² *Id.*

Plant Table

Species	# Planted¹⁸³	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	5	~8.5' h	Good	Unknown
Sycamore	Unknown	1	15' h	Good	Unknown
Black Sage	Unknown	3	3' – 4' h, 5' – 6' s	Good	Unknown
Mulefat	Unknown	1	3 - 4' h	Good	Unknown
Coyote brush	Unknown	1	4' h	Good	Unknown



Figure 125. La Goleta Site Section #1. Healthy black sage (lower third of image) and sycamore sapling middle center. Trautwein. January 31, 2022.

¹⁸³ The SBCFCWCD Maintenance and Revegetation Report for Maintenance Season 2020-2021 states at 58 that “~600 native plants” were installed in an area “over 6500 square feet” at the La Goleta Condo Site but does not provide the number of plants planted by species. Given the relatively recent planting date, EDC was generally able to discern planted plants from naturally occurring plants to populate the Plant table for the La Goleta Condo Site.

Estimated Percent Total Cover:

- Seventy-five to eighty percent (75 - 80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)
- Sow thistle
- Shamel ash tree
- Yucca
- Mallow
- Evening primrose
- Jacaranda tree
- Carolina cherry¹⁸⁴

La Goleta Condo Site Section #1 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy, especially the black sage. (Figure 125)
- The location of Section 1 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo. Site #1 Recommendations:

- Do not count the pollinator garden toward SBCFCWCD mitigation requirements.
- Replace nonnative, invasive plants and horticultural variants of local native plants in the pollinator garden with local native varieties.
- Remove the large jacaranda, yucca and Shamel ash trees which shade the site and pose a threat of spreading in the riparian habitat. (The jacaranda tree is spreading and EDC considers Shamel ash invasive due to the extensive spread in Goleta's creeks.)
- Increase percent cover of native riparian vegetation by about 10% or more.

ii. La Goleta Condominiums Site Section #2

Section 2 is located on the south/south-east bank of San Jose Creek behind the La Goleta Condominium complex. This Section begins at the east end of Section 1 behind the pollinator garden and continues northeast along the creek bank.

Year Planted: 2018

¹⁸⁴ EDC used the Seek app for preliminary identify of this plant as Carolina cherry. However, the plant appeared similar to coffeeberry, a native shrub found in riparian areas, and may be reclassified upon further evaluation. The plant was growing under the riparian canopy and measured 11-12 feet tall. It was in good condition.

Size: 10 feet x 70 feet = 700 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Coast Live Oak	Unknown	1	10-15' h	Fair	Unknown
Black sage	Unknown	4	3' h	Good	Unknown
Purple sage	Unknown	1	5' h	Good	Unknown
Coyote brush	Unknown	4	6' h	Good	Unknown
Lemonade berry	Unknown	1	7' h	Good	Unknown
Mugwort	Unknown	3	3' h	Good	Unknown
Seacliff wild buckwheat	Unknown	3	2' h	Fair	Unknown

Estimated Cover: 75% - 85%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (e.g., kikuyu grass)
- Jacaranda tree

La Goleta Condo Site Section 2 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section 2 provides a good buffer between the condominiums, parking lot, and Creek

La Goleta Condo Site Section 2 Recommendations:

- Remove nonnative plants including jacaranda.
- Add understory species to increase understory percent cover.

iii. La Goleta Condominiums Site Section #3

Section #3 begins at the east end of Section #2 and continues northeast along the Creek's east bank.

Year Planted: 2018

Size: 100 feet x 20 feet = 2,000 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	15	6' h	Fair	Unknown
Black sage	Unknown	5	2'- 4' h	Fair to Good ¹⁸⁵	Unknown
Mugwort	Unknown	2	2' h	Good	Unknown
Lemonade berry	Unknown	4	3' h	Fair	Unknown
Holly leaf cherry	Unknown	2	5' h	Good	Unknown
Purple sage	Unknown	4	4' h	Poor to Good ¹⁸⁶	Unknown
Wild blackberry	Unknown	2	5' s	Good	Unknown
Coast live oak	Unknown	10	6-18' h	Poor to Fair to Good ¹⁸⁷	Unknown
Coyote brush	Unknown	8	5' h	Fair to Good	Unknown
Seacliff wild buckwheat	Unknown	1	2' h	Poor	Unknown

¹⁸⁵ Sage planted under nonnative jacaranda trees received too little sunlight and some plants were in poor condition.

¹⁸⁶ Sage planted under nonnative jacaranda trees received too little sunlight and some plants were in poor condition.

¹⁸⁷ Oaks were either planted to close together or represent natural recruitment.

Estimated Percent Total Cover:

- Seventy-five to eighty-five percent (75-80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Jacaranda (3)
- *Eucalyptus citriodora*
- Pines (2)

La Goleta Condo Site Section #3 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section 3 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo Site Section #3 Recommendations:

- Remove the jacarandas, eucalyptus, and pine trees.
- Control exotic invasive species such as nonnative grasses.
- Plant additional riparian plants to increase percent cover of native species.

iv. La Goleta Condominiums Site Section #4

Section 4 begins at the east end of Section 3 and continues northeast along the east bank of San Jose Creek.

Year Planted: 2018

Size: 85 feet x 15 feet = 1,275 square feet

Dates of Site Visits: January 24, 2022 and January 31, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	5	1' h	Poor to Dead ¹⁸⁸	0
Sycamore	Unknown	2	3' h	Fair ¹⁸⁹	Unknown
Black sage	Unknown	1	5' h	Fair	Unknown
Mulefat	Unknown	1	6' h	Fair	Unknown
Deadly nightshade	Unknown	1	1' h	Good	Unknown
Holly leaf cherry ¹⁹⁰	Unknown	1	5' h	Fair	Unknown
Purple sage	Unknown	1	3' h	Fair	Unknown
Wild blackberry	Unknown	2	5' s	Poor to Fair	Unknown
Coast live oak	Unknown	2	8' h	Fair to Good ¹⁹¹	Unknown
Coyote brush	Unknown	3	4' h	Fair	Unknown

Estimated Cover:

- Thirty-five to forty-five percent (35-40%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., Kikuyu Grass)
- Lemon-scented gum (*Eucalyptus citriodora*) (3)
- *Arundo donax* (appears to have been cut back)
- English/Algerian Ivy

¹⁸⁸ The planting location appears to be too dry to support willows without substantial irrigation.

¹⁸⁹ Sycamores planted under mature eucalyptus received too little sun.

¹⁹⁰ The Seek app identified this as holly leaf cherry however it appeared to be a Catalina island cherry. A definitive identification should be made and only holly leaf cherries retained.

¹⁹¹ Some oaks were either planted too close together or represent natural recruitment at the site.



La Goleta Condo Site Section #4 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #4 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo Site Section #4 Recommendations:

- A willow snag was cut out – this should have remained as habitat for resident species.
- Remove English/Algerian ivy to protect sycamore and other native species in the area. (Figure 127)
- Remove Eucalyptus – they are shading out native plants.
- Remove *Arundo donax*.
- Increase percent cover of natives to 80% - 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Ensure future planting of oaks, willows, and sycamores are planted with ample room to grow (not too close to each other).
- Install drip irrigation or plant willows and sycamores closer to the Creek in more mesic conditions.

v. La Goleta Condominiums Site Section #5

La Goleta Condominium Site Section #5 begins at the east end of Section #4 and continues northeast along the east Creek bank.

Year Planted: 2018

Size: 85 feet x 17 feet = 1.445 square feet

Dates of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	10	6-10' h	Fair ¹⁹²	Unknown
Sycamore	Unknown	4	4-20' h	Fair	Unknown
Black sage	Unknown	4	5' h	Good	Unknown
Lemonade berry	Unknown	6	4' h	Fair to Good	Unknown
Mugwort	Unknown	2	5' s	Good	Unknown
Black cottonwood	Unknown	2	10' h	Fair	Unknown
Purple sage	Unknown	1	4' h	Good	Unknown
Wild blackberry	Unknown	3	4'-10' s	Good	Unknown
Coast live oak	Unknown	1	22' h	Good	Unknown
Coyote brush ¹⁹³	Unknown	10	4' h	Poor	Unknown
Seacliff wild buckwheat	Unknown	2	6' s	Good	Unknown

Estimated Percent Total Cover:

- Seventy-five percent (75%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)

La Goleta Condominium Site Section #5 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #5 provides a good buffer between the condominiums, parking lot, and Creek.

¹⁹² Willows planted too far from the creek received too little water and were in poor to fair condition.

¹⁹³ Numerous coyote brush throughout the site were severely pruned adversely affecting their general condition and contribution to the restored habitat. (Figure 128)



La Goleta Condominium Site Section #5 Recommendations:

- Do not prune or trim the coyote brush – these plants have been unnecessarily cut back. (Figure 128)
- Remove the nonnative kikuyu grass.
- Increase percent cover of natives to 80% or 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Future planting of willows should be placed closer to the creek bed where water access is more available.

vi. La Goleta Condominiums Site Section #6

Section #6 of the La Goleta Condominium Site begins at the east end of Section #5 and continues northeast along the Creek bank for one hundred feet.

Year Planted: 2018

Size: 100 feet x 5 – 10 feet = 500 – 1,000 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table:

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	4	3-12' h	Dead to Fair *2 dead, 1 good	Unknown
Sycamore	Unknown	1	4' h	Fair	Unknown
Purple sage	Unknown	1	-	Dead	Unknown
Seacliff wild buckwheat	Unknown	3	3'-10' s	Good	Unknown
Mugwort	Unknown	3	2'-8' s	Fair	Unknown
Lemonade berry	Unknown	4	6'-9' h	Poor	Unknown
Wild blackberry	Unknown	2	15' s	Good	Unknown
Coyote brush	Unknown	3	6' h	Good	Unknown

Estimated Percent Total Cover:

- Seventy-five percent ($\approx 75\%$)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)
- Oxalis
- Myoporum (within and adjacent to Site #6)
- Bird of paradise
- Castor bean (Figure 126)

La Goleta Condominium Site Section #6 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #6 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condominium Site Section #6 Recommendations:

- Remove the nonnative and invasive species listed above.
- Increase percent cover of natives to 80% - 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Plant more willows, closer to the creek bed, and if water levels are low, install drip irrigation.

La Goleta Site Overall Plant Table

Species	# Planted	# Observed	Estimated % Survival
Willow	Unknown	39	Unknown
Sycamore	Unknown	8	Unknown
Black cottonwood	Unknown	2	Unknown
Coast live oak	Unknown	14	Unknown
Lemonade berry	Unknown	15	Unknown
Holly leaf cherry	Unknown	3	Unknown
Black sage	Unknown	17	Unknown
Purple sage	Unknown	8	Unknown
Mulefat	Unknown	2	Unknown
Coyote brush	Unknown	29	Unknown
Seacliff wild buckwheat	Unknown	9	Unknown
Nightshade	Unknown	1	Unknown
Mugwort ¹⁹⁴	Unknown	10	Unknown
Wild blackberry ¹⁹⁵	Unknown	9	Unknown
Total	~600	166	26.7%

¹⁹⁴ Number observed includes individual plants believed to be planted, however mugwort plants are actively spreading indicating successful establishment.

¹⁹⁵ Number observed includes individual plants believed to be planted, however wild blackberry plants are actively spreading indicating successful establishment.





Figure 126. Castor bean at La Goleta Revegetation was flagged and retained. Trautwein. January 2022.



Figure 127. Algerian ivy at the La Goleta Site Section #4 is smothering this native tree. Trautwein. January 2022.



Figure 128. Coyote brush, purple sage, black sage, lemonade berry, and sycamore at La Goleta Site Section 5. Coyote brush was pruned heavily. Trautwein. February 2022.

vii. La Goleta Condominium Pollinator Garden

Kitson Nursery planted a pollinator garden for the HOA east of Section #1. SBCFCWCD references a “pollinator” garden of “native” plants which “will supplement the riparian plantings.”¹⁹⁶ This garden is location directly behind 5514 Armitos Avenue in the La Goleta Condominium complex. This garden appears to have been installed using wildflower seed packets. It contains non-local, horticultural varieties of native species which could escape into the riparian habitat or hybridize with local varieties of these species. There are several nonnative invasive species populating the area. The pollinator garden contains non-riparian species. The pollinator garden was not counted towards SBCFCWCD’s mitigation requirements. However, inclusion in the Annual Maintenance and Monitoring Reports may create the appearance that it was counted as mitigation by SBCFCWCD.

Date Planted: 2018 or later

Size: 82 feet x 18 feet = 1,476 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

*Horticultural varieties of native species present:*¹⁹⁷

- California poppy
- Elegant clarkia
- Lupine
- Matilija poppy
- Coyote brush
- Goldenrod
- Vervain
- Encelia¹⁹⁸

Invasive and Naturalized Nonnative Plants Present in and Near Revegetation Site:

- Mallow
- Kikuyu grass
- Sow thistle
- Jacaranda (seedling)

La Goleta Condominium Pollinator Garden Recommendations:

- The HOA should replace plants with local varieties of native flowering plants to prevent spread of non-local varieties and non-native plants in riparian corridor.
- Clarify in future reports that the pollinator garden does not count towards SBCFCWCD mitigation credits.

¹⁹⁶ SBCFCWCD Maintenance and Revegetation Report (2020-2021) at 58.

¹⁹⁷ Some of these species are not native to Goleta watersheds e.g., Matilija poppy.

¹⁹⁸ Two Encelia shrubs appeared to have been planted as container plants rather than from seed packets.



Figure 129. The La Goleta Condominium Pollinator Garden. The seed mix planted here contains California natives and non-local horticultural varieties of local native species, such as the elegant clarkia above. It is not habitat restoration and not riparian habitat. It was not counted toward SBCFCWCD's mitigation requirements. Trautwein. January 31, 2022.

P. Armitos Road Arundo Removal (Site 8)

SBCFCWCD removed *Arundo donax* from this site, installed rock rip rap, and planted native vegetation in the early to mid-1990s.¹⁹⁹ Vandalism occurred requiring replanting several times. This was one of SBCFCWCD’s first Arundo removal sites. After first replanting in 1993, storms, vandalism and unsuccessful planting required the Flood Control District to replant several more times in 1995, 1997 and lastly in 1998. The 1997-1998 Maintenance and Revegetation Report says that the Creek bank was destroyed and had to be “rebuilt.”²⁰⁰ We assumed this to mean that riprap was installed at the Armitos Road Site. The Report is vague in its location description so we cannot be one hundred percent confident that we analyzed the exact Site. However, we assume the Site we analyzed is the correct location given the description of being adjacent to the La Goleta Condominiums and Armitos Road and the presence of rip rap.

Years Planted: 1991, 1995, 1997, and 1998

Size:

- Reported: 219 feet x 20 feet = 4,380 square feet²⁰¹
- Observed: 171 feet x 20 feet = 3,420 square feet recorded during field survey

Date of Site Visit: February 28, 2022.

Estimated Percent Total Cover:

- Understory ~50%
- Canopy ~ 90%

Plant Table

Species ²⁰²	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	Above riprap: 7 In riprap: 3	20-30’ h	Good	Unknown
Sycamore	Unknown	2	35-45’ h	Good	Unknown
Cottonwood	Unknown	6	45-50’ h	Good	Unknown
Coast Live Oak	Unknown	1	25’ h	Good	Unknown
Mulefat	Unknown	-	6’ h	Good	Unknown
Blackberry	Unknown	1	-	Good	Unknown
Wild Cucumber	Unknown	-	-	Good	Unknown

¹⁹⁹ SBCFCWCD *Maintenance and Revegetation Report 1995/1996 Maintenance Season* (December 1, 1996).

²⁰⁰ SBCFCWCD *Maintenance and Revegetation Report, 1997-1998 Maintenance Season* (December 1998) (“SBCFCWCD (1998)”).

²⁰¹ SBCFCWCD (1998).

²⁰² The species included in the Armitos Road Site Plant Table are based on species observed at the Site because we found no list of species planted in the Maintenance and Revegetation Report for the 1997-1998 Maintenance Season.



Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Shamel ash (2) – 12” DBH
- Eucalyptus (2) – 45-50’ tall
- Nonnative grasses
- Oxalis
- Umbrella plant
- Castor bean
- Bottlebrush
- Yucca tree

Armitos Road (Site 8) Highlights:

- Overall, the site looks particularly good and healthy. The trees are well-established and the understory includes dense patches of native plants, including poison oak and wild blackberry.

Armitos Road (Site 8) Recommendations:

- Remove nonnative species from understory and replace with native mug wort, blackberry, and giant rye grass.
- Remove Shamel ash, castor bean, bottlebrush, and Eucalyptus trees. Replace with native woody species like cottonwood, sycamore, and willow and oak trees farther away from the Creek.



Figure 130. Four native tree species including black cottonwood, sycamore, willow, and coast live oak are present at the Armitos Road Site. Blackwelder. February 28, 2022.



Figure 131. Looking upstream at the Armitos Site. Several young willow and cottonwood trees populating the east bank of San Jose Creek. Blackwelder. February 28, 2022.



Figure 132. Rip rap at the Armitos Road Site. Horizontal willow with several new up shoots. In the back left corner is a nonnative, invasive Shamel ash tree. Middle left is a nonnative eucalyptus tree. Upper right corner is a nonnative Yucca tree. The vines growing over the riprap are native wild cucumber. Blackwelder. February 28, 2022.



Figure 133. Native wild cucumber within native willow branches. Two large nonnative, invasive eucalyptus trees in the background. Blackwelder. February 28, 2022.



Figure 134. Looking upstream at the Armitos Site on San Jose Creek. The Creek bed is clear of obstructive vegetation although a small cottonwood sucker is sprouting up in the creek bed. Blackwelder. February 28, 2022.

V. LAKE LOS CARNEROS MITIGATION BANK

EDC also reviewed the success of the Lake Los Carneros Mitigation Bank. As discussed above on page 13, there are pros and cons with mitigation banks. The Lake Los Carneros Mitigation Bank, although an offsite mitigation location, nonetheless appears to be successful by creating a large continuous habitat with value for birds and wildlife.

Site A:

Site A appears to be well-established although drought-stressed. It contains a mix of upland and riparian species. The 2020 Annual Report found that upland areas were performing well with riparian species becoming established.²⁰³ Without sufficient rainfall or supplemental watering, this site may transition from containing riparian species such as willows to upland species dominated by coyote brush and/or exotic species.

Highlight:

- Two fifteen-foot-high bay laurel trees have become established in Site A and are flowering despite cessation of irrigation and despite the climate change-induced drought.

Recommendations:

- Continue to monitor Site A. If it currently considered all or part riparian habitat and it transitions into upland habitat due to the ongoing drought, consider tracking it at a .75:1 credit for mitigation.²⁰⁴
- Control nonnative weeds in the understory of riparian trees and around and between upland shrubs.
- Plant additional native species in open area between shrubs near the western side of Lake Los Carneros Dam.

Site D

Site D is one of the moister polygons and the largest polygon in the bank. It currently supports willows, sycamores, coast live oaks, cottonwoods, and at least one small bay sapling and an understory consisting almost entirely of wild blackberry in the southern portion of the Site. The cottonwoods approach 10" DBH and appear to be spreading by root suckers. The willows and sycamores appear healthy although some may be drought-stressed. Some willows have succumbed to the drought in drier portions of Site D. (Figure 135) Overall the southern portion of Site D is in good condition while the northern portion is in fair to good condition.

²⁰³ SBCFCWCD, *Los Carneros Mitigation Bank Progress Report* at 2 (Summer 2021).

²⁰⁴ *Id.*; See also CDFW SAA at 16.



Figure 135. Some willows have not survived the climate change-induced drought in Site D, but overall, the Site is in fair to good condition despite the climate change drought. Trautwein. February 7, 2022.

Highlights:

- Eradication of pampas grass in the southwest portion of Site D was remarkably successful and no pampas grass plants were observed during surveys.
- This site contains healthy and well-established riparian woodland vegetation, including canopy trees and understory, especially in the moister southern portion of Site D.
- Natural recruitment of coast live oaks is occurring.
- An exceptionally large greenback ceanothus shrub at least twenty feet high is present and likely predated revegetation efforts by several decades.

Recommendations:

- Continue to monitor Site D annually or every other year using aerial photographs and ground surveys.
- Document and contain invasions of nonnative plants.

- Leave trees that perish as snags.
- If riparian vegetation transitions to upland vegetation, ensure nonnative plants do not invade, consider planting upland species in such areas to facilitate the transition, and apply the .75:1 credit ratio for upland areas.
- The understory consists of almost entirely blackberry, although mugwort is also present. The understory should be diversified through planting additional understory species including wild giant ryegrass, gooseberry, hummingbird sage, snowberry, and mugwort.
- Remove Shamel ash trees located in this Site close to Calle Real. This species is known to outcompete willows in riparian areas and could significantly displace the riparian vegetation installed in Site D. Monitor for invasion by Shamel ash trees.

Sites E, F, and G

The three southeastern sites contain cottonwood, sycamore, willows, and bay laurel trees and these trees generally appear to be in good condition. The northern part of Site E contains open areas lacking tree cover. EDC's surveys viewed these Sites from a nearby trail so we did not get close enough to examine the understory.

Highlights:

- Site contains diversity of habitats and tree species.
- Location near grasslands to northeast may support white-tailed kite and raptor nesting, roosts, or perches.

Recommendations:

- Continue to monitor Sites E, F, and G annually or every other year.
- If riparian vegetation transitions to upland vegetation due to drought, retain snags, plant upland species, control nonnative weeds, and apply the .75:1 credit ratio for upland areas.

Sites H and I

Sites H and I are located closest to Calle Real which appears to pond water or slow the flow of groundwater south due to soil compaction associated with the road, resulting in moist conditions conducive to riparian plant establishment. These sites appear to be in good condition. They support willows, sycamores, and oak trees, including natural recruitment of oak trees. The understory is primarily native vegetation including blackberries, wild giant ryegrass, and snowberries.

Highlights:

- Good moisture retention benefits riparian species.
- Presence of snowberries.
- Natural oak recruitment.



Recommendations:

- Continue to monitor Sites H and I annually or every other year using aerial photos and ground surveys.
- Search for pampas grass and other nonnatives and remove within one year of detection.
- If riparian vegetation transitions to upland vegetation, plant upland species to facilitate transition, control nonnative weeds which might colonize during the transition, and apply the .75:1 credit ratio for upland areas.
- Eradicate castorbean located along Calle Real.

Site J

Site J contains large cottonwood trees approaching forty feet tall and eight-inch DBH. It contains at least one large gooseberry, as well as mugwort and naturally occurring oak saplings.

Highlights:

- The large cottonwoods are phenomenally successful.

Recommendations:

- Continue to monitor Site J annually or every other year using a combination of aerial photographs and field surveys.
- If riparian vegetation transitions to upland vegetation, plant upland species, control nonnative weeds they may colonize during transition, and apply the .75:1 credit ratio for upland areas.
- Monitor and control invasive species to prevent significant infestations.
- Plant native plants around the perimeter of the woodland where native plants are absent.

Site K

Site K is located toward the southern end of the Lake Los Carneros Mitigation Bank. It is a moist area suitable for riparian species. Willows and wild blackberries installed by SBCFCWCD thrive here. Rushes were noted in or near Site K. (Figure 136)



Figure 136. Rushes in or near Site K. Trautwein. February 7, 2022.

Highlights:

- Moist area supports healthy riparian vegetation installed by SBCFCWCD.

Recommendations:

- Continue to monitor Site K annually or every other year.

Site L

This site contains coast live oak trees, cottonwood trees, wild giant ryegrass, and coyote brush. This site appears to be transitional between riparian and upland. Given the drought, this site could transition to upland and become dominated by coyote brush and/or nonnative weeds such as mustard.

Recommendations:

- Continue to monitor Site L annually or every other year using aerial photos and field surveys.
- If riparian vegetation transitions to upland vegetation, plant upland species, control nonnatives that may colonize the area, and apply the .75:1 credit ratio for upland areas.
- Eradicate Cape ivy in this section and monitor for reinfestations.
- Eradicate exotic Cape smilax denoted by pink flags underneath large coast live oak tree on west wide of Site along main trail.²⁰⁵ (Figures 137 a and b)

²⁰⁵ The Seek App identified this species as Cape smilax.



Figure 137 a. and b. Nonnative Cape smilax under oak in Site L. Trautwein. February 7, 2022.

Sites M and N

These Sites are on the far west side of the bank. They support dense willow thickets with wild blackberry understory. They are well established but in fair condition given the drought.

Highlights:

- Wood rat nests were observed in this area indicating wildlife use in restored areas.

Recommendations:

- Continue to monitor Sites M and N annually or every other year using both aerial photographs and field surveys.
- If willows succumb to the drought, retain snags.
- If riparian vegetation transitions to upland vegetation, monitor to ensure native upland species colonize the area, plant upland species if necessary to maintain native plant cover and diversity native upland plant community, control nonnative weeds which may colonize the area, and apply the .75:1 credit ratio for upland areas.

Site O

Site O located south of the Dam is one of the largest sites in the Lake Los Carneros Mitigation Bank and also the most upland sites. It contains upland species, including sumac, blackberry, oak, , lemonade berry coyote brush, buckwheat (Figure 138), Encelia, Santa Barbara honeysuckle, coffeeberry, purple sage, and California sage, as well as riparian species including

sycamore. (Figure 139) Providing upland plant communities enhances the functionality of the adjoining riparian woodlands by allowing for use by a greater number of wildlife species.²⁰⁶ Overall plant health ranges from poor to good with riparian species closest to the Dam (northern portion of Site O) generally in the poor to fair range with some dead willows. (Figure 140) Some sycamores in this area are in good condition.

The western portion is almost solid coyote brush, which may not meet the CDFW success criteria related to percent cover by diverse native species.²⁰⁷

Aerial photos from as recently as 2019 showed good percent cover of natives e.g., 75-80%, however, it appears that since watering ceased during the current megadrought, some native plants have not fared well. The percent cover still appears to be in the range of 70-80% but lower in the northern portion of Site O. (Figures 141 and 145) Nonnative herbaceous plant communities are present in the northern portion of Site O and dominate the understory. Overall, Site O may be the least successful polygon in the Lake Los Carneros Mitigation Bank.

Highlights:

- Large number of upland species established.
- Presence of Santa Barbara honeysuckle, a California Rare Native Plant species.

Recommendations:

- Continue to monitor annually or every other year using aerial photos and field surveys to ensure riparian sites remain riparian and do not transition to native or nonnative upland plant communities because such a transition would signal long-term failure of mitigation.
- If riparian vegetation transitions to upland vegetation, monitor to ensure nonnative plants do not colonize the area, control nonnative species which may colonize the area, plant native upland plants to ensure species diversity, apply the .75:1 credit ratio for upland areas.
- Control invasive herbaceous understory species especially in northern portion of Site O.
- Replant upland species in open northern portions of Site O dominated by nonnative species.
- Increase upland plant diversity in western portion of Site O currently dominated by coyote brush.

²⁰⁶ SBCFCWCD, *Los Carneros Mitigation Bank Progress Report* at 2 (Summer 2021).

²⁰⁷ CDFW SAA Success Criterion 3.9(b) at 17.



Figure 138. Buckwheat in northern portion of Site O. Trautwein. February 7, 2022.



Figure 139. Upland plant community in northern portion of Site O including coyote brush, lemonade berry, and buckwheat, with nonnative mustard growing between native plants. Note dam in background. Trautwein. February 7, 2022.



Figure 140. Some willows have died due to apparent lack of water in the northern portion of Site O, including this one which grew around an irrigation line. Trautwein. February 7, 2022.



Figure 141. Northern portion of Site O closest to the dam contains open areas dominated by nonnative plants. Trautwein. February 7, 2022.

Lake Los Carneros Mitigation Bank Overall Highlights:

- Eradication of large number of large pampas grass and German ivy plants with no young pampas grasses or German ivy apparent.
- Large contiguous area of habitat created.
- Success of riparian trees and understory species, especially in southern polygons (Sites K, J, I, H, and D).
- Co-location of upland and riparian areas to increase habitat diversity and benefit birds and wildlife.

Lake Los Carneros Mitigation Bank Overall Recommendations:

- Continue to monitor annually or every other year using aerial photos and field surveys to ensure riparian sites remain riparian and do not transition to native or nonnative upland plant communities because such a transition would signal long-term failure of mitigation.
- If riparian vegetation transitions to upland vegetation, apply the .75:1 credit ratio for upland areas.
- Control invasive species including Shamel ash and Cape ivy.
- Monitor for other invasive species and control to prevent significant infestations, e.g., castorbean, pampas grass, and mustard.
- Retain Monterey cypress snags.
- Remove all irrigation lines once sites are established. Reinstall only if needed to prevent loss of riparian vegetation due to the megadrought. (Figure 142)



Figure 142. Irrigation lines are broken in some areas and should be removed to prevent plastic pollution. Trautwein. February 7, 2022.



Figure 143. Los Carneros Mitigation Banks site prior to removal of pampas grass. Google Earth. November 10, 2003.



Figure 144. Lake Los Carneros Mitigation Bank Polygon Map. Safter pampas grass removal and before maturation of plantings. SBCFCWCD. Circa 2009.

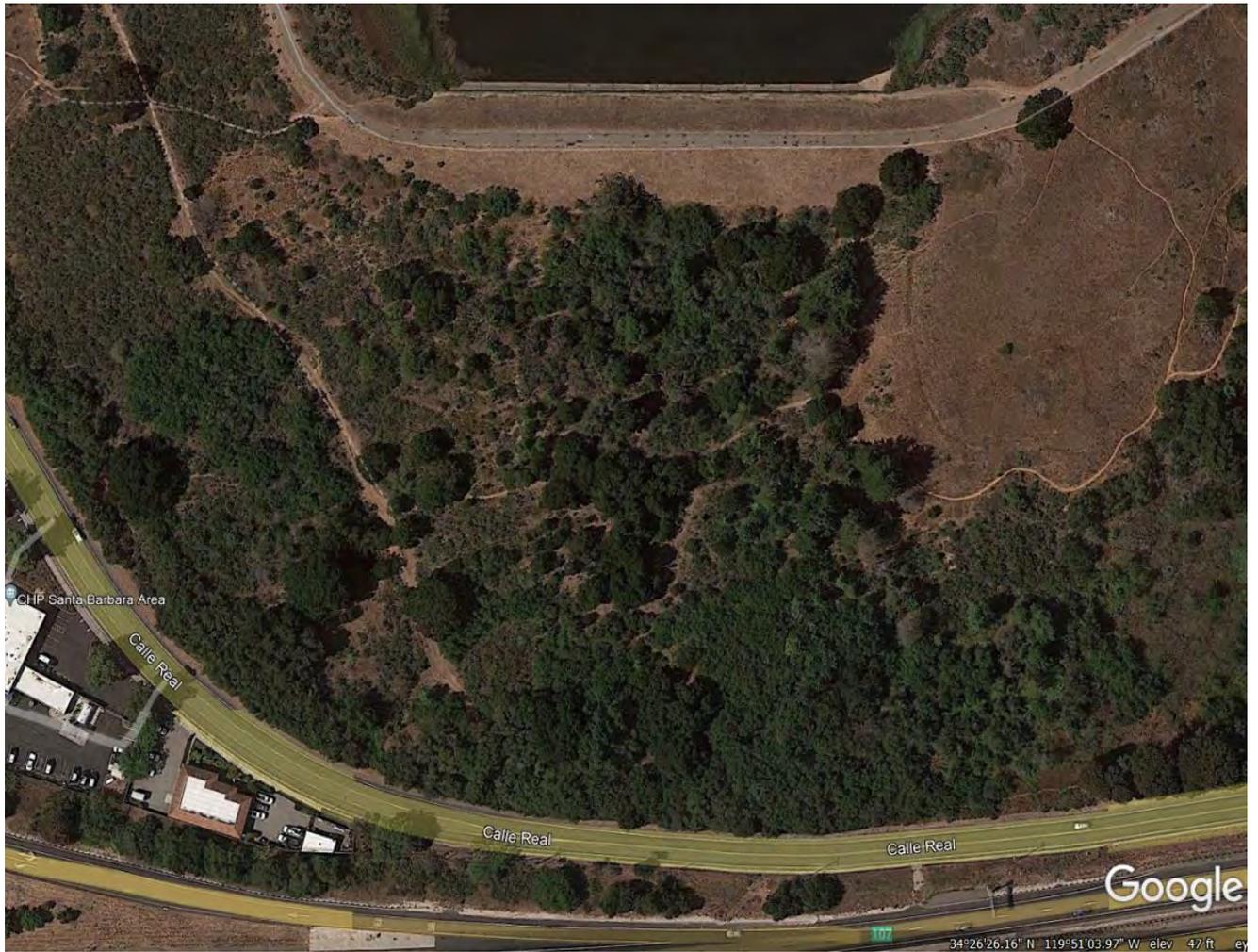


Figure 145. Los Carneros Mitigation Bank after maturation of plantings. Google Earth. August 2019.

VI. GLOBAL RECOMMENDATIONS FOR SBCFCWCD REVEGETATION

The following revegetation recommendations for SBCFCWCD and permitting agencies such as CDFW are designed to (1) ensure impacts to the quality and quantity of riparian vegetation are mitigated over the long-term through successful establishment of permanent riparian habitat by revegetation projects, (2) improve the success of riparian revegetation projects, and (3) enable more effective and quantitative monitoring and tracking of revegetation sites by providing measurable data relevant to CDFW's SAA success criteria, such as percent cover, plant survival rates, plant growth rates, and presence and percent cover of invasive species.²⁰⁸

- A. Mitigation Ratio. Discussion:** Observations indicate that 2015 SAA success criteria are not always met in the short term and long term and that compensatory mitigation is inadequate to offset or substantially lessen impacts.²⁰⁹ Specifically, observations documented herein demonstrate that a number of revegetation projects do not meet success criteria related percent cover, percent cover of herbaceous invasive understory, tree growth rates, and presence of woody nonnative vegetation over the short and long term. Success criteria also indicate that ratios should be greater than 1:1 for impacts which are not mitigated by implementing revegetation within a year.²¹⁰ Observations and review of SBCFCWCD Maintenance and Revegetation Reports indicate that mitigation for temporary impacts is not always implemented with a year, and/or is not successful the first year and requires replanting. Despite this, the SBCFCWCD appears to only credit mitigation for temporary impacts at 1:1. Furthermore, revegetation sites typically contain lower species diversity compared to adjacent natural habitats.

Recommendation: Given the failure of some revegetation sites to meet and maintain success with respect to the measurable criteria, the mitigation ratio for temporary impacts must be increased to ensure adequate mitigation. The ratio should also be increased because despite the requirement of Criterion 3.1 SBCFCWCD does not undertake mitigation at a ratio greater than 1:1 for revegetation projects occurring after the first growing season following impacts. Therefore, EDC recommends that CDFW and other agencies amend the SAA and other permits to increase mitigation ratio for temporary impacts (a) from 1:1 to 2:1 to account for failure to achieve and maintain success pursuant to the criteria, and (b) from 1:1 to 3:1 for revegetation projects successfully initiated later than the first growing season after the impact occurs.

- B. One-time Mitigation of Temporary Impacts Within Ten Years. Discussion:** Instream riparian habitat that is cleared typically recovers and creates important habitat within five years if not re-cleared. Currently the trigger for mitigating

²⁰⁸ CDFW SAA at 17.

²⁰⁹ Success Criterion 3.9(f) notably involves attainment "for the life of the project." CDFW (2015) at 17.

²¹⁰ Criterion 3.1 requires, "a 1:1 acreage replacement ration *if habitat is replaced the following growing season.*" (*Emphasis added.*) CDFW (2015) Criterion 3.1 at 15.

temporary impacts is ten years. However, when riparian habitat in the same location is re-cleared after a five-year period, new temporary vegetation and habitat impacts result, triggering the need for new mitigation in the form of revegetation.

Recommendation: Amend the SAA and other permits to require revegetation each time a temporary impact occurs in the same location after five years (i.e., reduce the permit trigger from ten years to five years).²¹¹ Keep the permit term at ten years unless it must match the permit trigger, in which case reduce the permit term to five years to match the mitigation trigger.

- C. In-kind Mitigation.** Discussion: Revegetation sites such as COVA are upland plant communities (e.g., Ceanothus, California sage, coyote brush, white sage, black sage, etc.) which do not mitigate loss of riparian habitat. Recommendation: Amend the SAA and other permits to not count upland revegetation sites as mitigation for loss of riparian species or to count upland revegetation sites at lower credit ratio, i.e., 50%.²¹²
- D. In-watershed Mitigation and Use of Lake Los Carneros Mitigation Bank.** Discussion: SBCFCWCD appropriately attempts to mitigate within the site of disturbance or within the same watershed whenever possible, but there is no decision tree or process for determining when onsite or in-watershed mitigation is infeasible. Recommendation: Amend the SAA and other permits to include a decision tree or enforceable process to ensure revegetation occurs in the watershed where impact occurs to the maximum extent possible.²¹³ Permitting agencies should develop an objective, quantitative method for determining when the Lake Los Carneros Mitigation Bank must be used. For example, a decision tree which includes mapping and assessing the feasibility of available onsite public and private revegetation locations, including landowner cooperation, could be used to determine when onsite or in-watershed revegetation is infeasible thereby triggering the use of the Lake Los Carneros Mitigation Bank.
- E. Increase Species Diversity.** Discussion: Revegetation projects often include a low species diversity compared to the adjacent native riparian habitats. Recommendation: Amend the SAA and other permits to increase diversity of species planted at revegetation sites.
- F. Credit Eradication of Invasive Nonnative Plant Species as Mitigation.** Discussion: Invasive plant species are spreading in San Jose Creek and other

²¹¹ CDFW (2015) Criterion 3.2 at 15.

²¹² The Lake Los Carneros Mitigation Bank only credits upland vegetation at a .75:1 ratio.

²¹³ Santa Barbara County (2008); *See e.g.*, 2020-2021 Maintenance and Revegetation Report at 37 stating that SBCFCWCD “may draw from the surplus restoration for the other watersheds in the Goleta Slough system.”

riparian habitats which are maintained by SBCFCWCD.²¹⁴ As discussed above, revegetation projects do not always successfully mitigate the impacts of the SBCFCWCD Annual Maintenance Program over the long term. Therefore, there should be a requirement for SBCFCWCD to eradicate or control specific invasive species, including but not limited to Shamel ash, castorbean, tamarix, and *Arundo donax*, to prevent their spread in areas subject to maintenance in a given year's Annual Plan. We acknowledge SBCFCWCD's statement that it is not funded specifically to restore habitat. However, successful restoration is required by resource agency permits and CEQA. Effective removal of invasive species would create additional areas for onsite revegetation and would help ensure successful mitigation to fulfill permit requirements.

Recommendation F1: Regulatory agencies should require SBCFCWCD to remove specified invasive species, including seedlings, saplings, and plants from the riparian corridor to the maximum extent feasible within areas subject to maintenance in each Annual Maintenance Plan to mitigate the effects of the Annual Maintenance Program.

In the absence of a requirement to remove invasive species as part of the Annual Maintenance Plan, there should be an effective incentive for SBCFCWCD to remove these species.

Recommendation F2: Regulatory agencies should credit effective eradication of exotic invasive species to SBCFCWCD's mitigation program when SBCFCWCD (1) documents removal of such species from sections of creek subject to maintenance, (2) successfully revegetates areas over one hundred square feet where invasive species are effectively removed, and (3) continues to remove and document removal of invasive species seedlings, saplings, and plants for five years following initial eradication. If invasive species are successfully eradicated over five years in a given area and the area is successfully revegetated pursuant to success criteria, the areas should count as 100% mitigation credit (i.e., one hundred square feet of eradication and revegetation should count as one hundred square feet of mitigation). Areas where over one hundred square feet of invasive plants are successfully eradicated must be revegetated. If areas where invasive species are successfully eradicated are less than one hundred square feet and not revegetated, the areas of eradication should count as 50% mitigation. For Recommendation F2 to be effective at improving the success of mitigation, long-term monitoring by SBCFCWCD and regulatory agencies must demonstrate that the targeted invasives are being effectively minimized and replaced with native species.

G. Increase Weed Control Efforts and Ensure Removal of Nonnative Trees in Revegetation Sites. Discussion: EDC observed significant levels of nonnative invasive woody vegetation and nonnative invasive herbaceous plants often over

²¹⁴ EDC, *Goleta Watersheds and Wildland Urban Interfaces: Enhancing Fire Safety and Riparian Forest Health at 178-187* (November 2021)

50-90% at revegetation sites even though Success Criterion 3.9(c) states, “no woody invasive species shall be present, and herbaceous invasive species shall not exceed five percent.”²¹⁵ SBCFCWCD stated that when it obtains approval for revegetation projects on private land, such as at COVA, landowners sometimes request them to preserve nonnative trees within the revegetation area, and SBCFCWCD has no option but to comply if it wants to plant on private land. EDC observed nonnative trees, including eucalyptus, yucca, and jacaranda retained in several revegetation sites located on private property, such as the COVA and La Goleta sites. This is problematic because these tree species shade revegetation sites and were observed spreading near the riparian habitat revegetation sites.

The SAA requires that invasive herbaceous species cover no more than five percent of revegetation sites. SBCFCWCD stated that invasive herbaceous species like oxalis are widespread in local watersheds and it would take significant resources to try to control them within revegetation sites, so SBCFCWCD tolerates these species and does not expend the resources necessary to remove these species to try to meet the success criterion for invasive herbaceous species.²¹⁶

Permitting agencies generally do not hold SBCFCWCD to the quantitative success criteria.²¹⁷ According to SBCFCWCD, it is better to track density and presence of multilayered canopies than percent survival when monitoring success of revegetation sites.²¹⁸ Part of the reason for this is because it is hard to count the number of surviving plants for species which grow by rhizome such as California rose and wild blackberry. SBCFCWCD would prefer to have Success Criterion 3(g) (percent survival) removed from the permits but the permitting agencies prefer to retain the criteria.²¹⁹

Recommendation G1: Retain all success criteria in the SAA.

Recommendation G2: Require increased weed control efforts at revegetation sites to fulfill CDFW Success Criterion 3.9(g) to achieve zero percent presence of invasive woody species and less than five percent cover of invasive herbaceous vegetation at revegetation sites as required under Success Criterion 3.9(g).

Recommendation G3: Permitting agencies should require SBCFCWCD to collaborate with landowners to ensure that nonnative and especially invasive woody species are removed from SBCFCWCD revegetation sites to the maximum extent possible before mitigation is accepted as successful. Retaining invasive or nonnative woody plants in revegetation sites should be prohibited to the maximum extent possible and accepted only as a last result when necessary to

²¹⁵ CDFW (2015) at 17.

²¹⁶ Raaf (2022).

²¹⁷ *Id.*

²¹⁸ *Id.*

²¹⁹ *Id.*

obtain landowner approval to conduct revegetation. The landowner’s requirement to retain nonnative plants must be demonstrated in writing to the permitting agencies in such situations, and SBCFCWCD must be required to prevent the spread of such plants

Recommendation G4: Track success pursuant to established success criteria in CDFW SAA and also track density and presence of multilayered canopies (i.e., groundcover, shrub layer, and tree canopy) as an additional measure of success.

Recommendation G5: Track success of plants which spread by rhizomes e.g., California rose and wild blackberry by percent cover rather than percent survival.

H. Improve Tracking and Reporting in Annual Maintenance and Revegetation

Reports: Discussion: The following recommendations are intended to ensure that the success of revegetation projects can be tracked pursuant to the CDFW SAA, which requires that, “each chapter includes a detailed discussion of data collection, ability to meet success criteria, monitoring activities... species used, and so forth.” The Maintenance and Revegetation Reports do not contain an assessment of specific success criteria used in the SAA, including percent survival,²²⁰ percent cover,²²¹ tree heights,²²² percent cover of invasive herbaceous vegetation,²²³ or presence of woody invasive nonnative species.²²⁴ Annual Maintenance and Revegetation Reports do not consistently set forth the species planted or the number of plants installed.²²⁵ SBCFCWCD assesses success qualitatively.²²⁶ SBCFCWCD can do a better job of counting the number of plants planted and surviving.²²⁷

Recommendations: The following recommendations are necessary for SBCFCWCD and CDFW and other permitting agencies to accurately measure success pursuant to permits including the CDFW SAA’s Success Criteria.

1. Map revegetation site boundaries using GPS coordinates at site boundaries to document precise locations. Include maps in each year’s Maintenance and Revegetation Report.
2. Demarcate boundaries of revegetation sites in the field using durable markers and replace markers if removed.
3. Consistently name or number each revegetation site.
4. Number all figures in Maintenance and Revegetation Reports.

²²⁰ The CDFW SAA requires “80% survival, by species, the first year and 100% survival thereafter and/or shall retain 75% cover after 3 years and 90% cover after 5 years for the life of the project.” Success Criterion 3.9(f) (Emphasis added.)

²²¹ *Id.*

²²² CDFW SAA Section 3.8 Criteria for Tree Replacement at 16 and Success Criterion 3.9(e) at 17.

²²³ CDFW SAA Success Criteria 3.9(c) at 17.

²²⁴ *Id.*

²²⁵ SBCFCWCD, *Maintenance and Revegetation Reports* (1993-1994 – 2020-21); *See also* Annual Plans (1993-94 – 2021-22).

²²⁶ Maureen Spencer, Operations Manager, SBCFCWCD, personal communication with Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 2022.)

²²⁷ Raaf (2022).

5. Add photos of each site from set reference point locations in annual Maintenance and Revegetation Reports.
6. Document all species planted at each site.
7. Document number of plants of each species planted at each site for tracking pursuant to SAA Success Criterion 3.9(f).
8. Document and track survival rates annually (i.e., number and percent of installed plants surviving) pursuant to SAA Success Criterion 3.9(f).
9. Annually track year-to-year plant height and growth pursuant to SAA Section 3.8 Criterion for Tree Replacement.
10. Annually track percent cover of native species pursuant to SAA Success Criterion 3.9(f).
11. Annually track percent cover of herbaceous nonnative species present pursuant to SAA Success Criterion 3.9(c).
12. Annually track presence of woody invasive species pursuant to SAA Success Criterion 3.9(c).
13. Annually track general health and condition of plants installed.

I. SBCFCWCD Should Encourage Restoration Projects by Nonprofits.

Discussion: SBCFCWCD has an informal policy to prohibit nonprofit groups from conducting creek revegetation projects on County owned land or easements in order to preserve these areas for SBCFCWCD future revegetation projects.²²⁸

This precludes enhancement of the region's riparian habitat and habitat for listed species such as steelhead. There are many locations where nonprofits could enhance riparian vegetation if this policy were lifted. Moreover, such areas are not being used by the SBCFCWCD for revegetation.

Recommendation: Encourage and support nonprofit efforts to conduct voluntary habitat restoration projects along County-owned properties and easements. Consider allowing SBCFCWCD to credit a percentage of this restoration as mitigation, for example, if SBCFCWCD provides half the plants, labor, and maintenance then 50% of the revegetation area could be credited as mitigation.

J. Exclude Voluntary Community Restoration Projects. Discussion: The potential exists for SBCFCWCD to claim mitigation credit for a nonprofit group's creek revegetation projects when done in tandem with SBCFCWCD.

Recommendation: SBCFCWCD and permitting agencies should ensure that SBCFCWCD does not count voluntary restoration projects undertaken by nonprofit groups, Homeowners Associations, and community groups as mitigation for SBCFCWCD maintenance projects.²²⁹

²²⁸ Personal communication, Tom Fayram, former Deputy Director, SBCFCWCD (circa 1997).

²²⁹ Urban Creeks Council's joint project with SBCFCWCD at Las Vegas Creek counted as 50% mitigation for SBCFCWCD maintenance activities. Joint revegetation projects such as this must be tracked carefully to ensure the correct acreage is attributed to SBCFCWCD's revegetation program.

- K. No Double-counting Mitigation Projects.** Discussion: SBCFCWCD has revegetated areas where a private entity (COVA) was required to undertake revegetation to mitigate the impacts of COVA's clearing of riparian habitat. Recommendation: SBCFCWCD and permitting agencies must develop a method to ensure that revegetation projects are not double-counted as mitigation for both SBCFCWCD maintenance projects and private projects.²³⁰
- L. Annual Maintenance and Revegetation Reports Should Clearly Exclude Community Landscaping Projects.** Discussion: SBCFCWCD must clarify that SBCFCWCD does not take mitigation credit for community landscaping projects such as the La Goleta Condominiums Pollinator Garden (which includes nonnative and non-local plant species) as mitigation for SBCFCWCD's maintenance program. (See pages 131 - 132 above.) Recommendation: SBCFCWCD and permitting agencies should ensure that non-restoration community landscaping projects such as pollinator gardens are not credited as mitigation.²³¹
- M. Require Local Genotype Plants.** Discussion: In at least one instance, horticultural, non-local varieties of native plants were apparently used in a revegetation project credited as SBCFCWCD mitigation. Restoration practitioners and agencies generally require local genotype plants to protect the genetic integrity of local native plant populations.²³²
- Recommendation: CDFW and other permitting agencies permits should require plants installed to be from local genotypes grown from seeds or cuttings collected along the creek where the impact occurs whenever feasible, or within proximity (e.g., the Goleta Watershed) to protect the genetic integrity of local native plant populations.
- N. Increase the Monitoring and Maintenance Period to Ten Years.** Discussion: The current program's use of a five-year maintenance and monitoring period has resulted in unsuccessful revegetation projects as documented in this report. Revegetation projects may initially succeed when watered and weeded but often become overrun with exotics and thwarted by mortality (note percent covers and survival rates in plant tables above). The problem is likely to worsen given climate change-induced droughts.

²³⁰ See e.g., discussion of COVA mitigation projects being counted as SBCFCWCD mitigation projects.

²³¹ The pollinator garden planted by Kitson Nursery with the La Goleta Condominium residents includes a mixture of native and non-native flowering plants which appears to be a commercial seed mix rather than local native species.

²³² See e.g., City of Goleta, *Creek and Watershed Management Plan Strategy* 11.1 and Action 11.1.2 at 258 – 259 (November 2020); See also Belnap, Jayne, *Genetic Integrity: Why Do We Care? An Overview of the Issues* https://www.fs.fed.us/rm/pubs/int_gtr315/5_belnap.pdf (December 26, 2021); Ken Owens, Executive Director, Channel Islands Restoration, personal communication to Watershed Alliance of South Coast Organizations stating that most sycamore seedlings found in local creeks are hybrids with London plane trees, and to ensure local sycamore population's genetic integrity is maintained, propagation must occur using cuttings from heritage trees which predate importation of London plane trees to this region. (April 14, 2022).

Recommendation: The SAA and other permits should require maintenance and monitoring for a minimum of ten years and longer if needed to achieve the measurable success criteria in the SAA and other permits and to increase long-term success and effectively mitigate impacts.

- O. CDFW Inspections of Revegetation Sites.** Discussion: CDFW does not inspect all of the many revegetation sites and instead relies on SBCFCWCD’s Maintenance and Revegetation Reports.²³³

Recommendation: CDFW and all permitting agencies should visit each revegetation site and measure success pursuant to the measurable success criteria before signing off on revegetation sites as successful.

- P. Plant a More Upland Plant Palette for Climate Change Resiliency.**

Discussion: EDC has observed many local creeks that used to flow year-round have become intermittent due to the climate change-induced drought. Projections indicate our region will have a warmer climate with less frequent winter storms, a diminished water supply, and increasing reliance on groundwater, SBCFCWCD should consider planting oak riparian woodland plant communities where willow woodland and other riparian communities once thrived.

Appendix I

ACRONYMS

CDFW	California Department of Fish and Wildlife
CEQA	California Environmental Quality Act
COVA	Cathedral Oaks Village Association
DBH	Diameter at Breast Height
EDC	Environmental Defense Center
EIR	Environmental Impact Report
HOA	Homeowners Association
SAA	Streambed Alteration Agreement
SBCFCWCD	Santa Barbara County Flood Control and Water Conservation District
ROW	Right of Way
UCC	Urban Creeks Council

²³³ Rains (2021).

Exhibit E

Photos and evidence of habitat impacts from Flood Control District activities in and near Atascadero Creek, 2023 to present

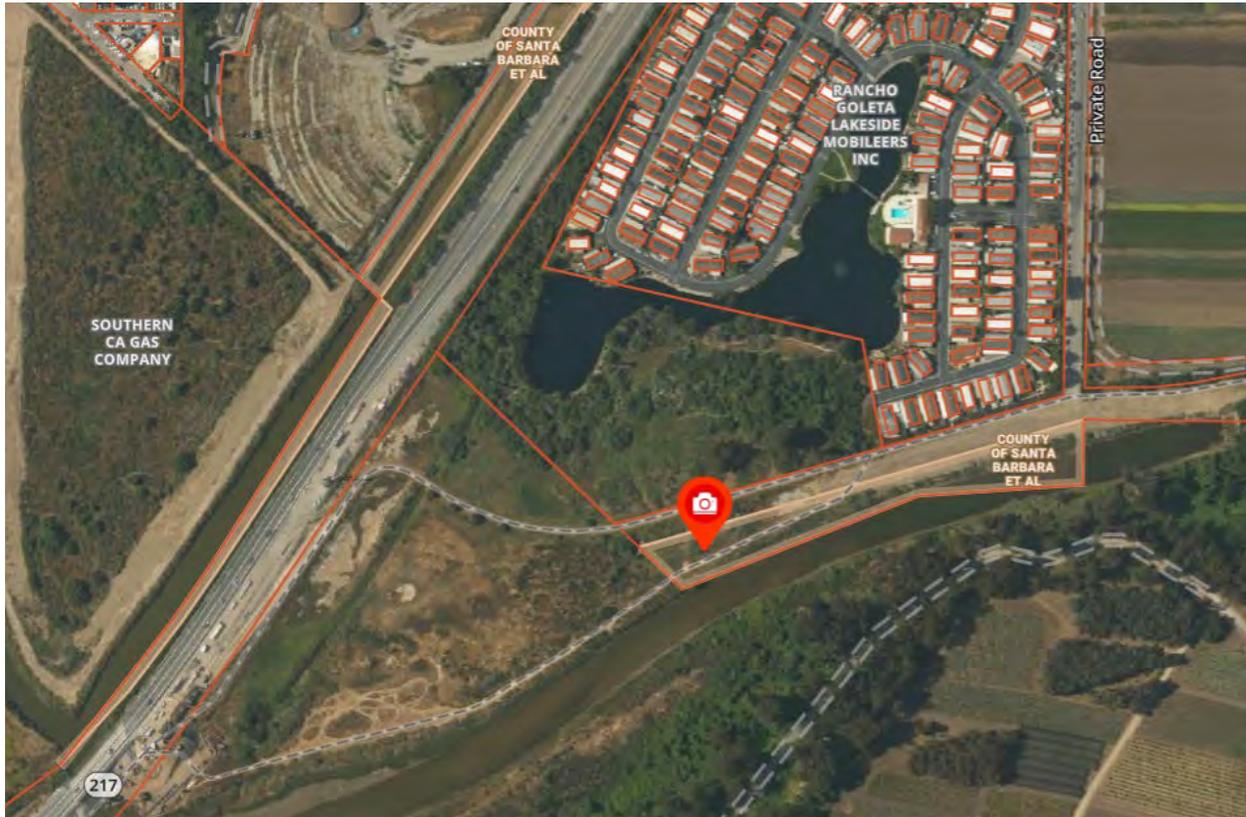




Exhibit E to LOMC Comments to FCD AMP





Exhibit E to LOMC Comments to FCD AMP



Exhibit E to LOMC Comments to FCD AMP



Exhibit E to LOMC Comments to FCD AMP





Exhibit E to LOMC Comments to FCD AMP



Sickly red water coloration calls for water quality testing following In-creek heavy equipment



environmental
DEFENSE CENTER

San Jose Creek Flood Control Revegetation Sites **And Los Carneros Mitigation Bank** **Review and Recommendations**



ENVIRONMENTAL DEFENSE CENTER

October 24, 2022

Natalie Blackwelder, EDC Watershed Program Intern
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Cover Photo: Norma Revegetation Site on San Jose Creek with tip-rap bank stabilization and willow woodland habitat installed by Santa Barbara County Flood Control District.

Table Of Contents

I.	Executive Summary	4
A.	Background	4
B.	Introduction	5
C.	Findings and Recommendations	7
D.	Acknowledgements	8
II.	Purpose, Goals, and Methods	8
A.	Purpose	8
B.	Goals	8
C.	Methods	9
III.	Habitat Mitigation for Flood Control Annual Maintenance	10
A.	CDFW Performance Standards	10
B.	Mitigation Ratios	11
C.	Mitigation Locations and In-kind Mitigation	12
D.	Mitigation Banks	12
E.	Mitigation for Repeated Impacts in the Same Locations	13
IV.	San Jose Creek Revegetation Project Sites	14
A.	Vineyard Road	15
B.	Norma (Site 1)	30
C.	Paseo Cameo (Site 11)	37
D.	North Cathedral Oaks Road (Site 2)	39
E.	Cathedral Oaks Village Association #1- #4	43
F.	Old Footbridge in University Circle Open Space (Site 4)	78
G.	Upstream Seventy-five Feet From Berkeley Footbridge (Site 5)	85
H.	Berkeley Road Footbridge by Goleta Water District (GWD) Building (Site 6)	87
I.	Three-hundred Feet Downstream from Berkeley Footbridge, East Bank Repair (Site 23)	90
J.	Berkeley Road Downstream from Berkeley Footbridge, West Side of Creek (Site 13)	97
K.	Somerset Road Access Ramp	101
L.	Downstream from Somerset Road Access Ramp (Site 10)	106
M.	Downstream from HWY 101, West Bank (Site 7)	109
N.	Hansen Aggregate Site	112
O.	La Goleta Condominiums	116
P.	Armitos Road Arundo Removal	134
V.	Lake Los Carneros Mitigation Bank	140
I.	Site A	140
II.	Site D	140
III.	Sites E, F, and G	142
IV.	Sites H and I	142
V.	Site J	143
VI.	Site K	143
VII.	Site L	144
VIII.	Sites M and N	145
IX.	Site O	145
VI.	Global Recommendations	152
Appendix I:	Acronyms	159



I. Executive Summary

A. Background

The Santa Barbara County Flood Control and Water Conservation District (“SBCFCWCD”) maintains creeks throughout Santa Barbara County to reduce the threat of flooding. In 1987, creek maintenance involving clearing riparian¹ habitat on San Jose Creek led to public concern over the effects of creek maintenance on fish, wildlife, and habitat along local creeks.² The Santa Barbara County Board of Supervisors acting as the SBCFCWCD Board of Directors required the SBCFCWCD to evaluate its Annual Maintenance Program pursuant to the California Environmental Quality Act (“CEQA”). The SBCFCWCD prepared a programmatic environmental impact report (“EIR”) in 1991 to assess the environmental effects of annual creek maintenance.³ A second EIR was prepared in 2001.⁴ Projects such as the Annual Maintenance Program may only be approved if they avoid or substantially lessen significant environmental impacts whenever feasible.⁵ The 2001 EIR includes alternatives and mitigation measures termed Best Management Practices designed to lessen unavoidable environmental impacts.⁶ The SBCFCWCD also secured a California Department of Fish and Wildlife (“CDFW”) Stream Bed or Lake Alteration Agreement (“SAA”) pursuant to Fish and Game Code Section 1601. If an activity such as the Annual Maintenance Program may substantially affect fish and wildlife resources, CDFW may issue a SAA “that includes reasonable measures necessary to protect the resource.”⁷ The SAA which CDFW issued to SBCFCWCD is a multi-year permit authorizing SBCFCWCD’s Annual Maintenance Program subject to measures CDFW deems necessary to protect fish and wildlife resources in local creeks subject to CDFW jurisdiction.⁸ The most recent SAA for the Annual Maintenance Program was approved in 2015 and expires on December 31, 2025.⁹

Mitigation measures developed in the EIRs and permit conditions, including measures in the SAA, are intended to avoid, minimize, and compensate for environmental impacts, including removal of native vegetation and impacts to native habitats and species caused by creek maintenance. Numerous measures to protect water quality, riparian and aquatic habitat, and sensitive species are employed by SBCFCWCD.¹⁰ The primary method for compensating for

¹ Riparian means “relating to or living or located on the bank of a natural watercourse (such as a river) or sometimes of a lake or a tidewater.” <https://www.merriam-webster.com/dictionary/riparian>

² Keith Dalton, Santa Barbara News-Press, *Creek Bed Caper Creating Waves* (June 1987).

³ SBCFCWCD *Program Environmental Impact Report for Routine Maintenance Activities* (1991).

⁴ SBCFCWCD *Final Program Environmental Impact Report for Updated Routine Maintenance Program* (November 2001). (“SBCFCWCD (2001)”).

⁵ California Public Resources Code § 21002; CEQA Guidelines § 15092.

⁶ SBCFCWCD (2001) Chapters 5.0 and 7.0; *See also* SBCFCWCD *Annual Routine Maintenance Plan Fiscal Year 2021-2022* at i-5 (“SBCFCWCD (2022)”).

⁷ California Fish and Game Code § 1602 (a)(4)(B).

⁸ Letter from Betty J. Courtney, Environmental Program Manager, California Department of Fish and Wildlife to Seth Shank, SBCFCWCD re *Final Lake or Streambed Alteration Agreement Notification No. 1600-2015-0053-R5 Annual Routine Maintenance Program* at 4 (August 5, 2015) (“CDFW (2015)”).

⁹ CDFW (2015) at 21.

¹⁰ SBCFCWCD (2001) Chapter 5.0; *See also* CDFW (2015) at 7 - 17.



temporary and permanent impacts to habitats is creation or restoration of habitat, a process called revegetation. This report focusses on riparian habitat revegetation projects implemented by SBCFCWCD along San Jose Creek between 1992 and 2021 and at the Los Carneros Mitigation Bank between 2005 and 2020 to compensate for the effects of creek maintenance on riparian habitats.

B. Overview of the SBCFCWCD Annual Maintenance Program

The SBCFCWCD Annual Maintenance Program includes projects in Santa Barbara County creeks to protect public safety and infrastructure by lessening the potential for flooding and erosion. Maintenance projects involve removing obstructive vegetation from creek beds, reducing buildup of sediment which can constrict channel capacity, and stabilizing creek banks.¹¹ More specifically, maintenance activities include limbing of downed trees and limbs, brushing, herbicide application, desilting, channel shaping, bank stabilization, and repair and construction of check structures.¹² Work typically involves the use of hand tools including chain saws, application of herbicide to prevent regrowth of in-channel vegetation, and heavy equipment work in select areas to shore up banks, remove vegetation from creek beds, and facilitate sediment movement.¹³

The SBCFCWCD conducts springtime annual creek walks in north and south County creeks to identify locations where work is required.¹⁴ The SBCFCWCD prepares an Annual Maintenance Plan in May and June of each year.¹⁵ Annual Plans are approved by the SBCFCWCD Board of Directors in June or July.¹⁶ Projects are implemented in the Fall, typically during a narrow window “Between August 1 and December 15” after bird nesting season and before steelhead migration season.¹⁷

As part of the Annual Maintenance Plan, SBCFCWCD implements revegetation projects to mitigate the effects of vegetation and habitat removal. The current requirement to mitigate impacts comes from the 2001 SBCFCWCD Program EIR¹⁸ and regulatory permits. Regulatory permits and approvals include California Coastal Commission Coastal Development Permits or Coastal Consistency Determinations for projects in the Coastal Zone.¹⁹ Army Corps of Engineers Clean Water Act Section 404 permits are required for projects “such as bank stabilization or channel shaping” which discharge fill or dredged material into wetlands or Waters of the US.²⁰

¹¹ SBCFCWCD *Annual Routine Maintenance Plan – Fiscal Year 2021-2022* at i-2 (2021-2022) (“SBCFCWCD (2021-2022)”).

¹² CDFW (2015).

¹³ *Id.*

¹⁴ SBCFCWCD (2021-2022) at i-2 – i-3.

¹⁵ *Id.* at i-4.

¹⁶ *Id.* at i-10.

¹⁷ *Id.* at i-12.

¹⁸ SBCFCWCD (2001) at 4.4 – 4.10.

¹⁹ SBCFCWCD (2021-2022) at i-10 and i-12; *See also*: California Coastal Commission *Staff Report and Adopted Findings for Application 4-19-1158, Agenda Item F15a*, Atascadero Creek Flood Control Project, Santa Barbara County available at <https://www.coastal.ca.gov/meetings/agenda/#/2020/7> (July 10, 2020).

²⁰ SBCFCWCD (2021-2022) at i-10.



The State Water Resources Control Board issues a 401B Water Quality Certification “for projects that also require a 404 permit from the Army Corps of Engineers.”²¹

Maintenance projects which alter the streambed, bank, or riparian habitat are also regulated by the CDFW SAA.²² The SAA is a permit issued by CDFW authorizing SBCFCWCD to implement the Annual Maintenance Plans subject to permit conditions requiring protection of biological and other natural resources such as habitats, species, and water quality. It requires SBCFCWCD to implement revegetation projects to replace native habitats that are removed during maintenance activities.²³ The SAA sets forth measurable performance standards called “success criteria” to evaluate effectiveness of revegetation projects. Criteria include plant survival rates, tree growth rates, absence of woody invasive species, percent cover of herbaceous nonnative plants, and percent cover of native species.²⁴ The SAA requires SBCFCWCD to track and report on the implementation of the Annual Maintenance Plans, including revegetation projects and “their ability to meet success criteria.”²⁵ Tracking and reporting on revegetation projects is essential so that CDFW can measure and track success pursuant to the success criteria and ultimately sign-off on completed revegetation projects to ensure that the impacts of SBCFCWCD’s maintenance projects are mitigated.

The SBCFCWCD prepares Annual Maintenance and Revegetation Reports which are “designed to incorporate all the information that each agency has requested” to demonstrate implementation of maintenance projects and revegetation projects.²⁶ Annual Maintenance and Revegetation Reports are required to provide “a detailed discussion of data collection, ability to meet success criteria, monitoring activities, and tasks performed at each restoration site.”²⁷ They are intended to demonstrate that the revegetation projects are sufficiently mitigating the impacts of maintenance projects. These reports are therefore critical for determining whether revegetation projects fulfill the quantitative success criteria and comply with permits, including the SAA. These reports must therefore contain a detailed accounting and quantitative analysis of success pursuant to the criteria. As discussed below, EDC’s analysis concludes that these reports do not contain adequately detailed data collection or assessment of revegetation sites’ performance pursuant to the required success criteria.

CDFW evaluates revegetation sites to determine whether they are successful. In this case, CDFW signed off on the San Jose Creek revegetation sites as having been successful.²⁸ Yet CDFW may lack resources to inspect the numerous revegetation sites on each of the thirty-six south coast drainages, including at least nineteen revegetation sites on San Jose Creek.²⁹ Given this, CDFW must rely on the SBCFCWCD’s Annual Maintenance and Revegetation Reports to

²¹ *Id.*

²² *Id.*; *See also* CDFW (2015).

²³ *Id.* Section 3.7 at 15-16.

²⁴ *Id.* Section 3.9 at 17.

²⁵ *Id.* Section 3.5 at 15-16.

²⁶ SBCFCWCD (2020-2021) at i-13.

²⁷ CDFW (2015) at 15.

²⁸ Spencer (2022).

²⁹ CDFW (2015) at 1; *See also* Sarah Rains, CDFW phone call with Brian Trautwein (2021) (“Rains (2021)”).

gage success.³⁰ The Reports, however, do not consistently include specific information necessary to demonstrate success of revegetation projects pursuant to the SAA's success criteria, including tree growth rates, plant survival rates, presence of invasive woody species, percent cover of herbaceous invasive nonnative plants, and percent cover of native plants, as described below, and are thus insufficient to demonstrate compliance with the SAA's success criteria.³¹

C. Findings and Recommendations

This report includes an evaluation of the success of nineteen revegetation sites on San Jose Creek and fifteen revegetation polygons in the Lake Los Carneros Mitigation Bank. The Environmental Defense Center ("EDC") concludes that some revegetation sites, including the Lake Los Carneros Mitigation Bank, continue to function very well with few exotic plant species present and substantial cover by native plant species. All sites have reportedly met the applicable success criteria outlined in the CDFW SAA.³² SBCFCWCD state that, "Restoration sites are monitored by District Environmental staff for success criteria, percent cover, mortality, and tree-height. Generally, most revegetation sites achieve the success criteria within 3 to 5 years. Results and photos are submitted each year in the Post-Project Report."³³ However, EDC's analysis demonstrates that percent cover, tree height, and mortality rates are not recorded in SBCFCWCD's Annual Maintenance and Revegetation Reports and that most revegetation sites do not currently meet the SAA's specific measurable success criteria.³⁴ Some sites have become dominated by nonnative plants, including aggressive invasive species which undermine the long-term success of revegetation and raise concerns that SBCFCWCD's revegetation program may not provide long-term replacement of riparian habitat.

EDC's report sets forth site-specific recommendations to achieve compliance with the SAA's success criteria and ensure that existing revegetation sites successfully mitigate the effects of creek maintenance on riparian habitats over the long term. Programmatic recommendations are also included to ensure that SBCFCWCD's future revegetation projects fully compensate for losses in riparian habitat quantity and quality. Recommendations are intended to ensure that SBCFCWCD and permitting agencies, including CDFW, effectively monitor, track, and record short-term and long-term success pursuant to the SAA's measurable success criteria. The Annual Maintenance and Revegetation Reports must provide a more detailed and quantitative assessment of the success of restoration sites to demonstrate whether the measurable success criteria are met. In addition, CDFW should visit each revegetation site annually and only approve revegetation projects when all success criteria set forth in the SAA are met.

³⁰ SBCFCWCD *Annual Maintenance and Revegetation Reports* (2000-2001 through 2020-2021)

³¹ *Id.*

³² Maureen Spencer, Operations and Environmental Manager, SBCFCWCD, phone call with Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 25, 2022) ("Spencer (2022)").

³³ SBCFCWCD *Four-Year Status Report 2016-2019 Annual Routine Maintenance Program* at 6 (January 2020).

³⁴ Some revegetation sites were required under previous SAAs. However, EDC used the most recent SAA's success criteria to gage success of existing revegetation sites.

D. Acknowledgements

UCSB Associated Students Coastal Fund, MUFG Union Bank, and Clif Family Foundation provided generous grant funding to enable revegetation site surveys, research, meetings with SBCFCWCD staff, and preparation of this report. UCSB Associated Students Coastal Fund also provided a generous stipend to EDC's Watershed Program Intern and Coauthor, Natalie Blackwelder.

The authors of this report are extremely grateful to Maureen Spencer, SBCFCWCD Operations and Environmental Manager, for providing Annual Plans, Annual Maintenance and Revegetation Reports, Lake Los Carneros Mitigation Bank reports and information in a timely manner, taking time to speak with us to explain the SBCFCWCD's revegetation program, and accompanying us in the field. Without this exceptionally helpful assistance, EDC would not have been able to understand the details of the SBCFCWCD's revegetation program or been able to produce this detailed report.

EDC is grateful for Sarah Rains, CDFW Environmental Scientist, for providing EDC with the SAA and other documents related to SBCFCWCD's Maintenance Program and providing commentary on CDFW's process for evaluating revegetation success.

II. Purpose, Goals, and Methods

A. Purpose

This analysis evaluates the effectiveness of SBCFCWCD revegetation projects required to mitigate the effects of Annual Maintenance Plans on riparian habitat pursuant to CEQA and CDFW's SAA. EDC intends for this report to be utilized by the SBCFCWCD to improve the effectiveness of its revegetation program to ensure long-term success and prevent net loss in the extent or quality of riparian habitat along creeks in Santa Barbara County. EDC intends for this report to inform permitting agencies, including CDFW, regarding the long-term success of revegetation projects and to inform future Annual Maintenance Program permitting and tracking and monitoring of effectiveness pursuant to CDFW's success criteria.

B. Goals

In order to fulfill this purpose, the goals of this report are to:

- Identify successes and shortcomings in SBCFCWCD's existing revegetation projects.
- Identify site-specific recommendations for existing revegetation projects.
- Identify global recommendations for SBCFCWCD and for CDFW and other permitting agencies to ensure successful monitoring, effective revegetation, and tracking of success.

C. Methods

1. Process of Evaluating Success of Revegetation Projects

The following steps were undertaken to evaluate success of SBCFCWCD revegetation sites on San Jose Creek and within the Lake Los Carneros Mitigation Bank:

1. Reviewed all Annual Plans dated from 1993-94 through 2021-22.
2. Reviewed all available Maintenance and Revegetation Reports dated 1993-94 through 2020-21.
3. Created chart tracking revegetation sites and revegetation square footage at each site on San Jose Creek.
4. Created map showing each revegetation site on San Jose Creek.
5. Reviewed CDFW's SAA.
6. Researched revegetation and habitat mitigation ratios for permanent and temporary impacts.
7. Researched pros and cons of mitigation banks.
8. Met with Maureen Spencer, SBCFCWCD Operations and Environmental Manager.
9. Visited all known revegetation sites on San Jose Creek and at the Lake Los Carneros Mitigation Bank.
10. Photo-documented each site.
11. Counted or estimated and recorded the number of installed plants observed and calculated the estimated percent survival,³⁵ general condition, plant heights and/or lateral spread, percent cover of native species, presence of woody nonnative species, and extent of invasive nonnative herbaceous understory species.
12. Documented nonnative and invasive exotic species.
13. Identified recommendations to enhance the success of revegetation projects at each site, to enhance the success of future revegetation efforts, and to improve tracking and reporting of success.

This report will be submitted to the SBCFCWCD, CDFW, United States Army Corps of Engineers, National Marine Fisheries Service, the City of Goleta, and nonprofit organization involved with creek preservation, management, and restoration, agencies. EDC will follow up with such agencies to discuss the EDC's findings and recommendations.

³⁵ It was only possible to estimate percent survival when the number of plants planted was recorded by SBCFCWCD. When the number of plants planted was not recorded in SBCFCWCD documents and is therefore unknown, it was infeasible to estimate percent survival unless zero surviving plants were observed (0% survival).

2. Problems Encountered Evaluating Revegetation Project Success

- **Inadequate Mapping and Field Demarcations.** Difficulty identifying locations and boundaries due to inadequate mapping, field demarcations, and descriptions of locations.
- **Failure to Record Plant Numbers.** Difficulty tracking survival rates because number of overall plants and number of plants of each species planted at each site plants of each species not recorded.
- **Failure to Record Species Planted.** Species planted are frequently not documented in Maintenance and Revegetation Reports.

III. Habitat Mitigation for Flood Control Annual Maintenance

A. **CDFW Performance Standards**

SBCFCWCD must create or restore riparian habitat through a process called revegetation to compensate for temporary and permanent habitat losses and degradation caused by the Annual Maintenance Program. CDFW established “Success Criteria for Restoration Areas” to measure success of habitat and tree replacement through revegetation.³⁶ Native trees that are removed must be replaced. If those trees are three inches to six inches in diameter breast height (“DBH”), then they must be replaced at a two-to-one (2:1) ratio. If those trees are six inches or more DBH, then they must be replaced at a ten-to-one (10:1) ratio.³⁷ Successful tree replacement requires achieving three-year and five-year height-based milestones.³⁸ The success criteria for tree replacement depends on the species. Sycamore trees, for example, must be at least five feet tall after three years and nine feet tall after five years, to be considered successful.³⁹

Revegetation sites may be considered successful after they complete a five-year period of monitoring, maintenance, and reporting.⁴⁰ At the end of the five-year period, no single species planted can constitute more than sixty percent (60%) of the vegetative cover with the exception of willow trees, which can constitute eighty percent (80%) cover.⁴¹ No woody invasive species can be present.⁴² Herbaceous nonnative invasive species must be limited to less than five percent (5%) cover in revegetation sites.⁴³ Additionally, there must be no supplemental irrigation for at

³⁶ CDFW (2015) Section 3.9 at 17.

³⁷ *Id.* Section 3.2 at 15.

³⁸ *Id.* Section 3.8 at 16.

³⁹ *Id.*

⁴⁰ *Id.* Section 3.9a at 17.

⁴¹ *Id.* Section 3.9b at 17.

⁴² *Id.* Section 3.9c at 17.

⁴³ *Id.*

least 2-years.⁴⁴ When planted plants perish, replacement plants are required. Replacement plants must be monitored with the same growth and survival requirement for five years after planting.⁴⁵

Each species planted must have at least eighty percent (80%) survival for the first year and one hundred percent (100%) survival for every subsequent year and/or sites must exhibit seventy-five percent (75%) cover after three years and ninety percent (90%) cover after five years “and for the life of the project” in order for the sites to be deemed successful.⁴⁶ “In some scenarios, if it can be demonstrated that the restoration area has achieved its goals and met the success criteria, CDFW can provide concurrence that no further restoration activities are required.”⁴⁷

Lake Los Carneros Mitigation Bank success criteria are divided into 3-year and 5-year criteria.⁴⁸ When the 3-year success criteria are met in a given restored area, half of the available banking credits become available and the other half after the 5-year success criteria are met.⁴⁹

B. Mitigation Ratios

According to the CDFW, the SBCFCWCD must provide mitigation for any removal of riparian habitat on a one-to-one (1:1) basis for temporary impacts.⁵⁰ SBCFCWCD activities that result in permanent impacts require a three-to-one (3:1) mitigation ratio. The one-to-one (1:1) ratio was established in SBCFCWCD’s 2001 Program EIR and the CDFW SAA.⁵¹ The three-to-one ratio (3:1) is required by the CDFW SAA.⁵² However, if revegetation to offset temporary impacts does not take place within the next growing season, the CDFW SAA may require a higher but unspecified ratio.⁵³ At the Lake Los Carneros Mitigation Bank, riparian habitat is mitigated on a 1:1 ratio, while upland habitats must be mitigated on a .75:1 ratio.⁵⁴

Permanent impacts caused by SBCFCWCD’s maintenance program must be mitigated at three-to-one (3:1) ratio.⁵⁵ Examples of permanent impacts include “activities such as new grade stabilizers and non-vegetated bank protection,” including the use of cement or grouted rock, or other intrusions that cannot easily be removed.⁵⁶

⁴⁴ *Id.* Section 3.9d at 17.

⁴⁵ *Id.* Section 3.9g at 17.

⁴⁶ *Id.* Section 3.9f at 17.

⁴⁷ *Id.* Section 3.9h at 17

⁴⁸ *Id.* at 16.

⁴⁹ *Id.*

⁵⁰ A 1:1 mitigation ration requires replacement of every area impacted with an equal or larger area of replacement habitat.

⁵¹ Spencer (2022); *See also* SBCFCWCD (2001) at 4-7 – 4-11; *See also* CDFW (2015) at 15.

⁵² Spencer (2022); *See also* CDFW (2015) at 15. Note the 2001 Program EIR requires a two-to-one (2:1) ratio for permanent impacts but the CDFW SAA requires a three-to-one (3:1) ratio.

⁵³ CDFW (2015) Section 3.1 Mitigation for Impacts to Habitat at 15.

⁵⁴ *Id.* at 16.

⁵⁵ CDFW (2015) Section 3.1 at 15.

⁵⁶ *Id.*

C. Revegetation Locations and In-kind Mitigation

Whenever possible, mitigation efforts should take place directly where the impacts occurred.⁵⁷ If it is infeasible to mitigate onsite, then another nearby location within the same creek should be required. In the event there are no other revegetation sites available in the watershed, a nearby watershed is another less desirable offsite revegetation option. As outlined in the CDFW SAA, “Restoration is conducted with the primary intent that it will be located at the project areas or along the same drainage near the project/impact area. If these locations are not suitable, restoration is then located in an adjacent watershed.”⁵⁸ As a last result, the impact may be mitigated in another area known as a “mitigation bank.” In Santa Barbara County, the Lake Los Carneros Mitigation Bank has served as SBCFCWCD’s alternative restoration site which includes a total of 6.5 acres of revegetated habitats.

The County’s CEQA Thresholds and Guidelines Manual also directs mitigation to be located onsite or as close to the area of impact as possible whenever feasible, and that offsite mitigation is least preferred.⁵⁹ SBCFCWCD employs this same hierarchical approach favoring onsite mitigation, offsite but within the same creek, offsite in a nearby creek, or as a last resort, at the Lake Los Carneros Mitigation Bank.⁶⁰

Mitigation should also be in-kind with the replacement vegetation matched to the habitat type impacted.⁶¹ During surveys EDC noted that some SBCFCWCD revegetation sites, such as the Cathedral Oaks Village Association (“COVA”) sites described below, include upland species planted to replace riparian species. The Lake Los Carneros Mitigation Bank allows this but employs a lower ratio (.75:1) for upland vegetation to compensate for not replacing riparian species in-kind with riparian species.⁶²

D. Mitigation Banks

The SAA requires mitigation in the form of revegetation at or near the site of the impact but allows for revegetation at the Lake Los Carneros Mitigation Bank when nearby sites are not available.⁶³ “Mitigation banking is a system of credits and debits devised to ensure that ecological loss, especially loss to wetlands and streams resulting from various development works, is compensated by the preservation and restoration of wetlands, natural habitats, and

⁵⁷ CDFW (2015) Section 3.4 Revegetation Plan and Restoration Goals at 15; *See also* Santa Barbara County, *CEQA Thresholds and Guidelines Manual* at 32 – 33 available at <https://www.countyofsb.org/ceo/asset.c/479> (2008) (“Santa Barbara County (2008)”).

⁵⁸ CDFW (2015) Section 3.4 Revegetation and Restoration Goals at 15.

⁵⁹ Santa Barbara County (2008).

⁶⁰ Maureen Spencer, Operations Manager, SBCFCWCD, personal communication with Natalie Blackwelder, Watershed Program Intern, EDC, and Brian Trautwein, Environmental Analyst/ Watershed Program Coordinator, EDC (February 19, 2022).

⁶¹ Santa Barbara County (2008).

⁶² SBCFCWCD, *Los Carneros Mitigation Bank Goleta, CA Progress Report* at 1, 2, and 13 (2021).

⁶³ CDFW (2015) at 4.

streams in other areas so that there is no net loss to the environment.”⁶⁴ Mitigation banks can be an effective way to compensate for the loss of habitat because they may result in larger contiguous areas of habitat and thereby reduce the potential for edge effects, which are adverse ecological effects that occur when natural habitats have large perimeters relative to size and may decrease the efficacy of a restored area.⁶⁵ Edge effects can allow new invasive, nonnative species that are better adapted to disruptions to take root, undermining revegetation goals.⁶⁶ Mitigation banks are typically larger areas which have smaller perimeter to size ratios and therefore may reduce edge effects.

Further, mitigation banks are more economical to build and manage over the long-run.⁶⁷ For example, it is easier to install irrigation on a one-acre area than ten different irrigation systems in ten .1-acre areas.

However, in the context of ecological restoration, mitigation banks can be less desirable for several reasons. For example, they often only require a one-to-one (1:1) mitigation ratio as opposed to site-specific revegetation sites that may require higher ratios.⁶⁸ Further, mitigation banks allow revegetation to occur offsite from the direct ecological impact, which prevents those impacted areas from getting restored. As a result, mitigation banks can result in fewer and more widely dispersed albeit larger habitats separated by greater distances which may impair wildlife movement between habitats.

In preparing this report, EDC considered the mitigation ratios required in the CDFW SAA, the SAA’s ten-year mitigation reset period discussed below in I.E., information about mitigation ratios used for other temporary and permanent habitat removal projects, the SAA success criteria and requirements for tracking and reporting success, and the short-term and long-term mitigation success at the SBCFCWCD’s revegetation sites. EDC identified recommendations regarding mitigation ratios for SBCFCWCD’s revegetation program which are included at the end of this report.

E. Mitigation for Repeated Impacts in the Same Locations

SBCFCWCD is required to mitigate one time for repeated temporary impacts in the same location for a period of ten years. Thus, if creek maintenance temporarily removes habitat from the same location multiple times during a ten-year period, SBCFCWCD must only mitigate the impacts of that repeated impact once per decade. However, mitigation would again be required the first time this same area is impacted after the decade has elapsed.

⁶⁴ Virkam Jhavar, *Understanding the Basics of Mitigation Banking* available at <https://www.investopedia.com/articles/dictionary/031615/understanding-basics-mitigation-banking.asp> (January 31, 2022).

⁶⁵ DUDEK, *Mitigation Bank Advantages* (2011) (“DUDEK (2011)”).

⁶⁶ Vallejo, David, *Edge effects and habitat fragmentation* (2018).

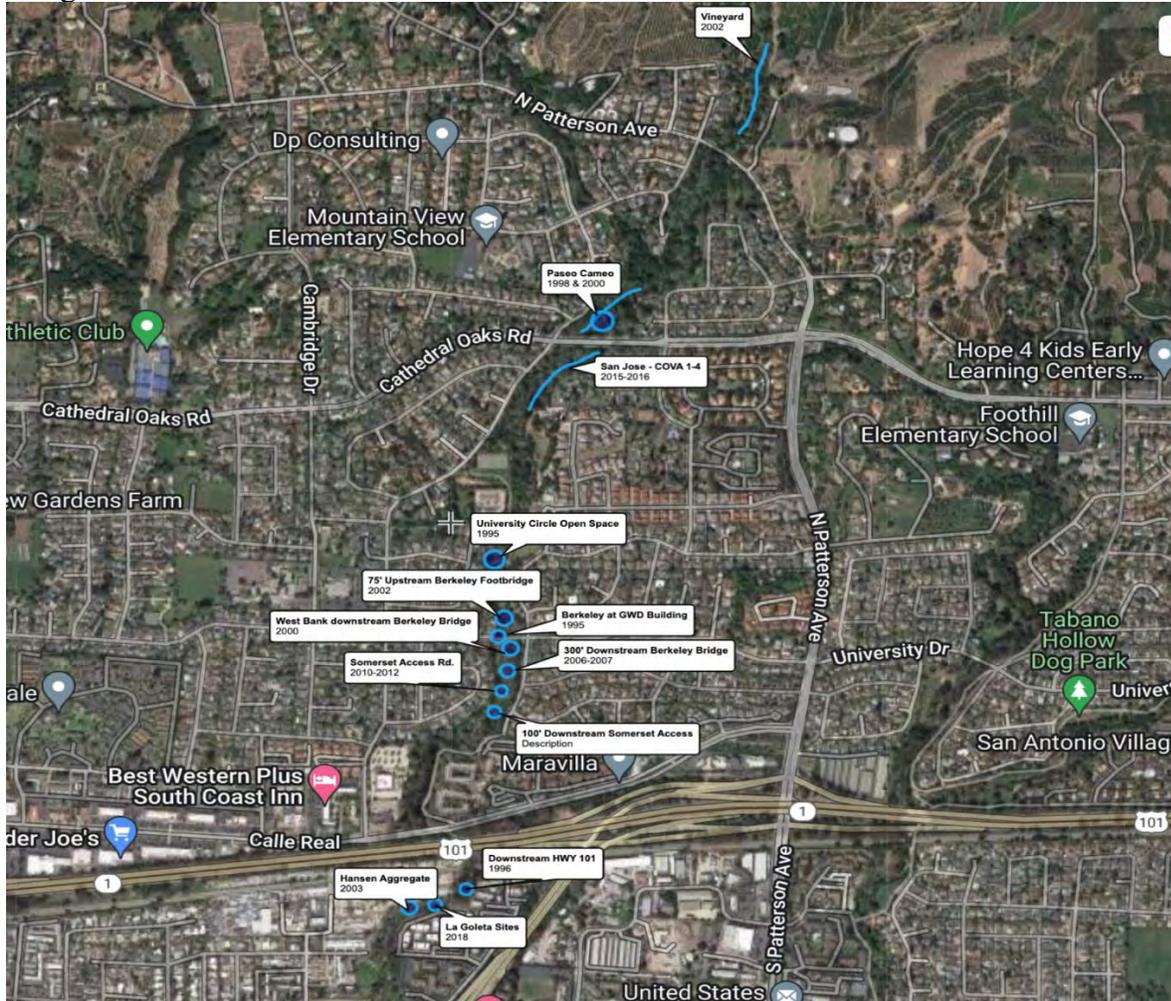
⁶⁷ DUDEK (2011).

⁶⁸ *Id.*



IV. SAN JOSE CREEK REVEGETATION SITES⁶⁹

EDC’s analysis of the success of nineteen revegetation sites on San Jose Creek is set forth below along with site-specific recommendations. The SBCFCWCD’s descriptions of the nineteen revegetation projects are found in the SBCFCWCD’s Annual Plans and Maintenance and Revegetation Reports. Information regarding the long-term success and current status of the nineteen revegetation sites is derived from observations during EDC’s surveys of creek revegetation sites.



Map of the revegetation sites along San Jose Creek assessed in this report. Scribble Maps. Blackwelder. 2022.

⁶⁹ EDC evaluated nineteen revegetation sites on San Jose Creek. The following three sites failed and/or could not be located and were not visited: Cavaletto Site (Site 3) was unsuccessful. (SBCFCWCD, *Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1, 1998)); The site one hundred feet downstream from Berkeley Footbridge (Site 6) was having limited success. (SBCFCWCD *Maintenance Work Performed During 1995/1996 Maintenance Season and Revegetation Update* (December 1, 1996)); The site upstream from Hollister Avenue behind pipe and wire revetment (Site 9) SBCFCWCD personnel were unable to provide a location or directions to this site due to the fact it predated current staff’s employment by the SBCFCWCD.

A. Vineyard Road Site

SBCFCWCD revegetated a site on the west bank and western terrace of San Jose Creek between Vineyard Road to the north and North Patterson Avenue to the south in 2002.⁷⁰ (Figure 1)

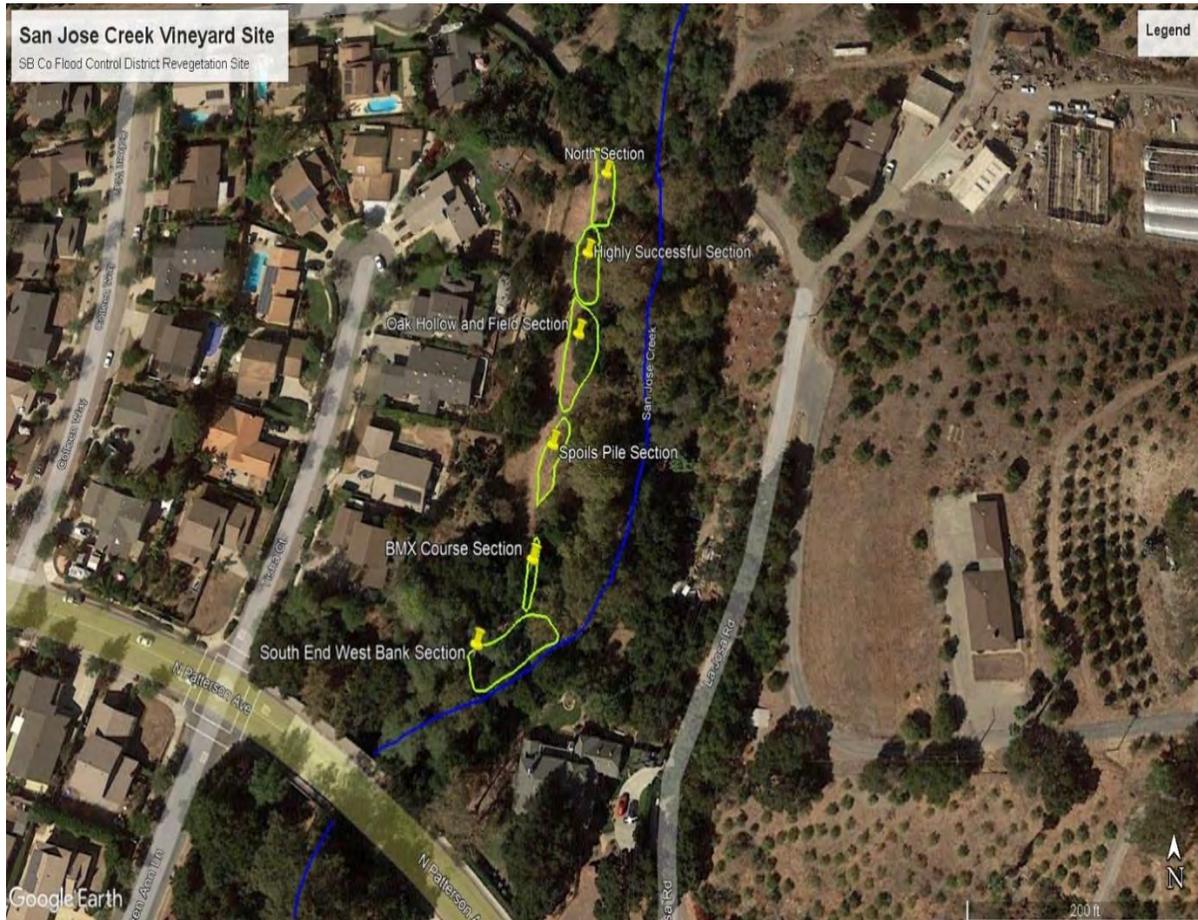


Figure 1. EDC divided the Vineyard Road revegetation site into six sections for evaluation. Google Earth. 2021.

Year Planted: 2002

Size:

- Reported: 10,000 square feet⁷¹
- Observed: 14,380 square feet⁷²

Site Visits: January 24 and 25, 2022

⁷⁰ SBCFCWCD *Maintenance and Revegetation Report 2003/2004 Maintenance Season* (2004).

⁷¹ SBCFCWCD *Annual Maintenance and Revegetation Report* (2010-2011).

⁷² EDC estimated this site at 14,380 square feet.

Plant Table

Species Planted⁷³	# Planted	# Observed⁷⁴	Avg height or spread	General Condition	Estimated % Survival
Coast live oak	7	7	2'-15'h	Fair to Good	100
Black cottonwood	10	8 - 10 ⁷⁵	3'-10'h ⁷⁶	Fair to Good	80 - 100
Western sycamore	4	0	-	-	0
Willow	89	3 - 7	5'-10'h ⁷⁷	Fair to Good	<10
Elderberry	11	2 - 3	1-10	Poor to Good	<30
Coyote brush	23	0 - 3	5'-7'h	Good	<15
Coffeeberry	26	0		-	0
Santa Barbara honeysuckle	13	2 - 4	3'-5'h	Good	<33
Wild blackberry	100	15 - 25	1'-4'h	Fair to Good	15 - 25
Mugwort	50	20 - 28	1' - 6'h .5 - 2' spread	Good	40 - 56
Toyon	13	10	1' - 15'h	Poor to Good	77
California sage	26	6 - 9	3' - 7' h 2' - 10' spread	Good	24 - 34
Gooseberry	20	4	3' - 8'h	Good	20

⁷³ SBCFCWCD Annual Maintenance and Revegetation Report (2010-2011).

⁷⁴ Number of plants observed are estimates of surviving plants installed as part of the revegetation project based on field observations on January 24 and 25, 2022. Numbers may not represent the total number of surviving plants given (1) the potential for surviving plants to have been overlooked during surveys and (2) the potential for naturally-occurring plants to have been mistaken for plants that were installed as part of the revegetation project.

⁷⁵ A range of plants observed is listed when it is unclear whether observed plants were planted or are naturally occurring.

⁷⁶ The current SAA would require black cottonwoods to be at least twelve feet tall after five years but these plants measure only three to ten feet tall after twenty years.

⁷⁷ The current SAA would require red, black, and arroyo willows to be at least fifteen to eighteen feet tall after five years but these trees measure only five to ten feet tall after twenty years.

*Estimated Percent Total Cover:*⁷⁸

- 4,662 square feet / 14,380 square feet **32.4%** (based on 14,380 square feet⁷⁹)
- 4,662 square feet / 10,000 square feet **46.6%** (based on 10,000 square feet⁸⁰)

Estimated Percent Total Cover by Section:

- North Section: 500 sq ft / 2,500 sq ft (20%)
- Highly Successful Section: 2,850 sq ft / 3,000 sq ft (95%)
- Oak Hollow Section (understory): 300 sq ft / 3,000 sq ft (10%)
- Field Section: 160 sq ft / 1,600 sq ft (10%)
- Spoil Pile Area Section: 600 sq ft / 1,200 sq ft (50%)
- BMX Area Section (understory): 0 sq ft / 600 sq ft (0%)
- South End West Bank Section: 252 sq ft / 2,480 sq ft (10%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Rockrose (west of trail, spreading east toward revegetation site and creek)
- Vinca (South End West Bank Section)
- Arundo (creek banks and west of trail)
- Shamel ash (South End West Bank Section by trail)
- Algerian or English ivy (climbing up sycamore trees)
- Castor bean (in creek, north end)
- Brazilian pepper (in creek, north end)
- Nonnative grasses (throughout; Spoil Pile Area Section, Field Section, South End West Bank Section; and areas between trail and successful revegetation locations)

Vineyard Road Site Highlights:

- The Highly Successful Section south of Vineyard Road has performed extremely well with nearly a 100% cover by native species including wild blackberry, sage species, and Santa Barbara honeysuckle.
- The large size of this site allows for establishment of continuous habitat and reduces edge effects compared to smaller restoration sites.

⁷⁸ Estimated cover is the percentage of cover provided by installed native plants over the revegetation site as a whole. For example, if half of the revegetation site is covered by planted native vegetation, the cover is fifty percent.

⁷⁹ EDC estimated the Vineyard Road Revegetation Site as 14,380 square feet based on field measurements and visual estimates of areas planted as described by SBCFCWCD in the 2003 Maintenance and Revegetation Report i.e., “essentially from the top of [west] bank out to the dirt walking/bike path seen in the picture.” EDC included the area below the top of the west bank in the south end upstream from North Patterson Avenue which EDC estimates at 2,480 square feet.

⁸⁰ SBCFCWCD estimates the Vineyard Revegetation Site as 10,000 square feet in the 2003 Maintenance and Revegetation Report.

- The perennial nature of the Creek creates hydrological conditions which support native plant establishment.
- The project revegetates a section of San Jose Creek used by resident and potentially anadromous endangered southern California steelhead and threatened California red-legged frogs.

Vineyard Road Site Recommendations:

The following actions are recommended to fulfill the current CDFW SAA revegetation success criteria:⁸¹

- Entire site: Eradicate perennial invasive and aggressive non-native plants listed above and control nonnative grasses and forbs.
- South End West Bank
 - Remove or solarize all nonnative grasses for three consecutive years and then plant 200 blackberries on 3' centers to establish native understory.
 - Plant toe of wet bank three feet above creek bed with red, black, and/or arroyo willow trees on ten-foot centers. Plant mugwort underneath willows.
 - Plant lower two-thirds of west bank with twenty-five black cottonwoods on average fifteen-foot centers. Plant five coast live oaks on top third of west bank.
- North End
 - Remove nonnative grasses and weeds prior to seed set or solarize these invasive species west and east of the trail for three consecutive years. Then plant wild blackberries on six-foot centers, five to ten gooseberries, four to six Santa Barbara honeysuckle plants, and four to six coast live oak trees between the trail and the areas of successful revegetation.
- BMX Course
 - Chip wood pile and spread as woodchips.
 - Plant sixty wild blackberries on six-foot centers. Cage plants using chicken wire to prevent vandalism for five to ten years or until blackberries reach fifty percent cover then remove cages using wire cutters to avoid damaging vines.
- Spoil Pile Area
 - Remove nonnative grasses prior to seed set for two to three consecutive years or solarize and plant spoil pile and open field south of spoil pile with blackberries on six-foot centers and fifteen to twenty coast live oak trees.

⁸¹ EDC acknowledges that the current SAA was approved in 2015 and that revegetation occurred prior to the approval of the current SAA but believes the 2015 SAA provides useful success criteria for measuring long-term effectiveness of revegetation projects.

- Oak Hollow / Field
 - Plant fifty to eighty blackberries under the large oak tree's dripline. Cage plants using chicken wire to prevent vandalism.
 - Remove all grasses and weeds from field by removing for three consecutive years prior to seed set or solarize these invasives. Plant with fifty blackberries, three to five gooseberries, and four to six Santa Barbara honeysuckle plants. Cage to prevent vandalism.
 - Remove cages after five to ten years or once blackberries reach fifty percent cover. Remove cages using wire cutters to avoid damaging vines.

Photos



Figure 2. North End of Vineyard Site. Successful establishment of blackberry understory with gooseberry in center of photo. Trautwein. January 25, 2022.



Figure 3. North end of Vineyard Site looking north from trail. Note the failure to establish native vegetation cover between the trail and the Creek. Successful establishment of oaks (presumably two of seven planted at Vineyard site). Trautwein. January 25, 2022.



Figure 4. North end of Vineyard Site looking southeast. Successful establishment of toyon and Santa Barbara honeysuckle. Trautwein. January 25, 2022.



Figure 5. Highly Successful Section of Vineyard Site with estimated 95% cover (estimated 2,850 square feet out of 3,000 square feet). Gooseberry and California sage. Trautwein. January 25, 2022.



Figure 6. Southwest edge of Highly Successful Section of Vineyard Site looking north by northeast. Large toyon in center of image. Note lack of native vegetation between toyon and trail in lower left and bottom portions of photo. Trautwein. January 25, 2022.



Figure 7. Highly Successful Section of Vineyard Site looking south across area with nearly 100% native cover of blackberries and California sage. Trautwein. January 25, 2022.



Figure 8. Looking south along trail from Highly Successful Section of Vineyard Site to Oak Hollow Section (center) and Spoil Pile Area Section (center right). Note toyon on left is the same plant shown in Figure 6. Trautwein. January 25, 2022.



Figure 9. Spoil Pile Area Section with oaks established on spoil pile. Herbaceous layer is dominated by nonnative grasses. Scattered wild blackberry and mugwort also present. Trautwein. January 25, 2022.



Figure 10. Spoil Pile Area Section (right side) looking north along trail to Field Section containing nonnative grasses and Oak Hollow Section (upper center). Trautwein. January 25, 2022.



Figure 11. Field Section containing mostly nonnative weeds between Highly Successful Section (to left of picture), Spoil Pile Section (center right), east of trail, and west of San Jose Creek bank. Trautwein. January 25, 2022.



Figure 12. Oak Hollow Section. Scattered wild blackberry in understory. Lack of wild blackberry understory may be the result of vandalism. Trautwein. January 25, 2022.



Figure 13. BMX Section. No native understory plants established likely due to BMX course established in this location and associated removal of native understory. Trautwein. January 25, 2022.



Figure 14. Pile of deadwood likely created during clearing of area to construct BMX course. Trautwein. January 25, 2022.



Figure 15. South End West Bank Section dominated by nonnative grasses. Note mugwort plants (tall thin plants center and right), and coast live oak and cottonwood saplings (orange flagging tape center left). Trautwein. January 25, 2022.



Figure 16. South End West Bank Section. Mugwort plants amidst dense nonnative grasses dominating this section of Vineyard Revegetation Site near North Patterson Avenue. Trautwein. January 25, 2022.



Figure 17. Small, four-foot tall, healthy black cottonwood sapling. South End West Bank Section. Trautwein. January 25, 2022.



Figure 18. Successful willow saplings near toe of west bank at South End West Bank Section, near North Patterson Avenue. Note extensive nonnative grass understory. Trautwein. January 25, 2022.



Figure 19. Scattered mugwort plants surrounded by dense nonnative grasses. South End West Bank Section near North Patterson Avenue. Trautwein. January 25, 2022.



Figure 20. Successful willow sapling (left side of image) established on South End West Bank Section near North Patterson. Smaller cottonwood sapling to right of willow. Note extensive nonnative grasses. Trautwein. January 25, 2022.



Figure 21. Small thirty-inch-tall black cottonwood sapling at South End West Bank Section of Vineyard Site. Note extensive nonnative grasses. Trautwein. January 25, 2022.



Figure 22. Small cottonwood sapling on South End West Bank Section of Vineyard Site. Trautwein. January 25, 2022.



Figure 23. Trail at South End West bank Section of Vineyard Site. Note Patterson Avenue Bridge in background. Nonnative oxalis and grasses dominate strip along trail's edge. Trautwein. January 25, 2022.

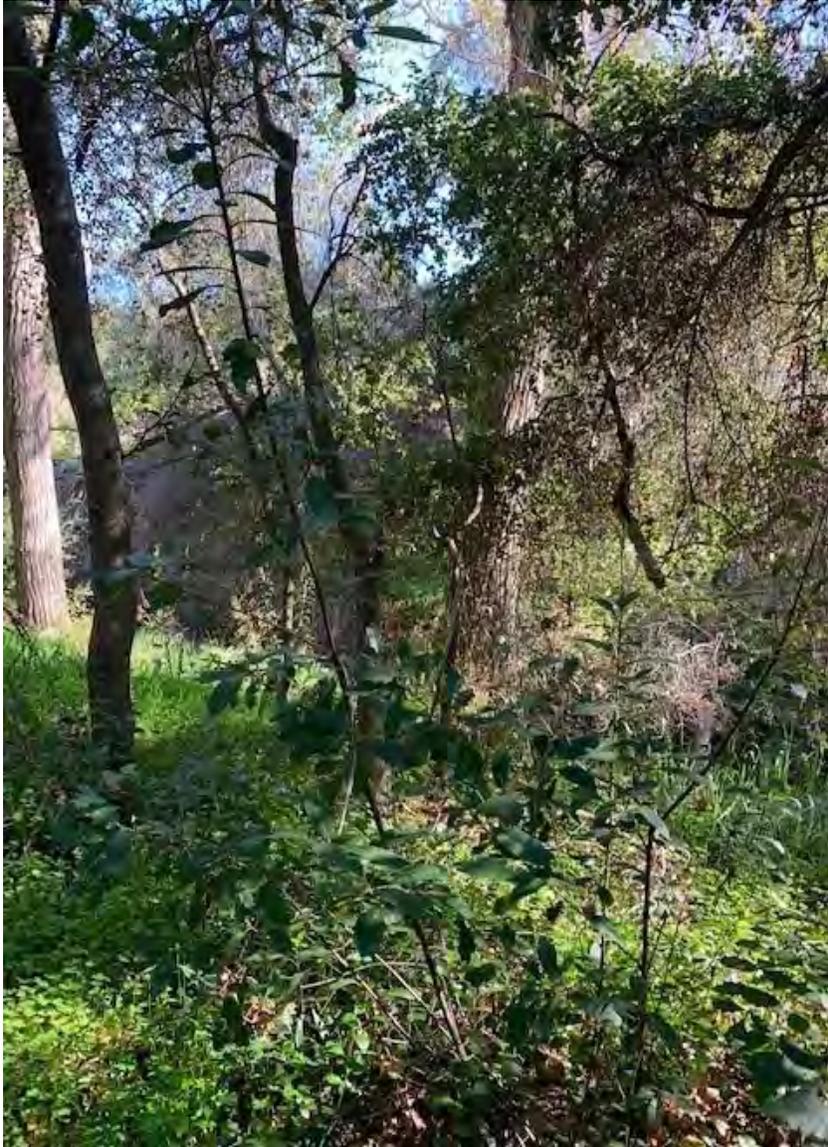


Figure 24. Toyon in shade of oak canopy. South End West Bank Section of Vineyard Site adjacent to trail near Patterson Avenue Bridge. Understory dominated by nonnative oxalis and vinca. Trautwein. January 25, 2022.

B. Norma Site (Site 1)

SBCFCWCD installed rip rap to repair an eroded bank and planted many willow trees to restore riparian habitat.⁸²

Year Planted: First planting 1996, second planting 1997, third planting 1998

Size: 300 feet x 20 feet = 6,000 square feet⁸³

Site Visit: February 25, 2022

⁸² SBCFCWCD *Annual Maintenance and Revegetation Report 1996/1997 Maintenance Season (1997)*.

⁸³ EDC observed a revegetated area that measured approximately two hundred feet by twenty feet, or 4,000 square feet.

Plant Table

Species	# Planted	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	Unknown	>40	20-35' tall, 4-10" DBH	Fair-Good some dead	Unknown
Coast Live Oak	Unknown	-	15' tall	Good	Unknown
Blackberry	Unknown	-	-	Good	Unknown
Wild Cucumber	Unknown	-	-	Good	Unknown

Estimated Total Percent Cover:

- Understory 75-80% native vegetation
- Overstory 95% native vegetation

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Wild Grasses
- Arundo
- Shamel Ash
- Wild Radish
- Eucalyptus

Norma Site Highlights:

- Remarkably high native willow canopy percent cover.
- Good example of revegetating bank that had been eroded by high flows.
- Apparent perennial or near perennial flows support healthy willow woodland.
- A lot of willows were present and in fairly good condition

Observation:

- Rip rap was used to stabilize the Creek bank. It appears that the willows are growing above the rip rap but not from within it. It is unclear if willow wattling was used within the rip rap.

Norma Site Recommendations:

- Remove invasive Arundo, aggressive nonnative Shamel ash, and naturalized olive tree to fulfill success criteria related to presence of woody nonnative vegetation.⁸⁴
- Diversify the understory with more mugwort, blackberries, and giant rye.
- Diversify the riparian tree overstory with more cottonwoods, bay laurels, and sycamore trees.
- Remove eucalyptus tree(s) on east of site.



Figure 25. Looking downstream San Jose creek at the Norma Site (Site 1). Successful establishing of willow woodland. Note the riprap installed on the west bank. This site was visited in late February/early March and water was still flowing. All of the surviving revegetation is located above the installed rip rap. Trautwein. March 7, 2022.

⁸⁴ CDFW (2015) at 17.



Figure 26. Looking down the west bank of San Jose Creek at the Norma Site. Note the understory is primarily nonnative grasses, and poison oak, however in the lower foreground are young black cottonwoods and a coast live oak sapling about 3' tall. These appear to be from natural recruitment. Blackwelder. March 7, 2022.



Figure 27. West bank of San Jose Creek at Norma Site above the installed rip rap. The understory is primarily nonnative grasses. Coast live oak sapling about four feet tall in the right third of the photo. There is nonnative, invasive Arundo in the background along the upper mid-right edge. Blackwelder. March 7, 2022.



Figure 28. Nonnative grasses and oxalis on west bank San Jose Creek at the of Norma Site. There are also oaks and willows in the background near the top of the image. Blackwelder. March 7, 2022.

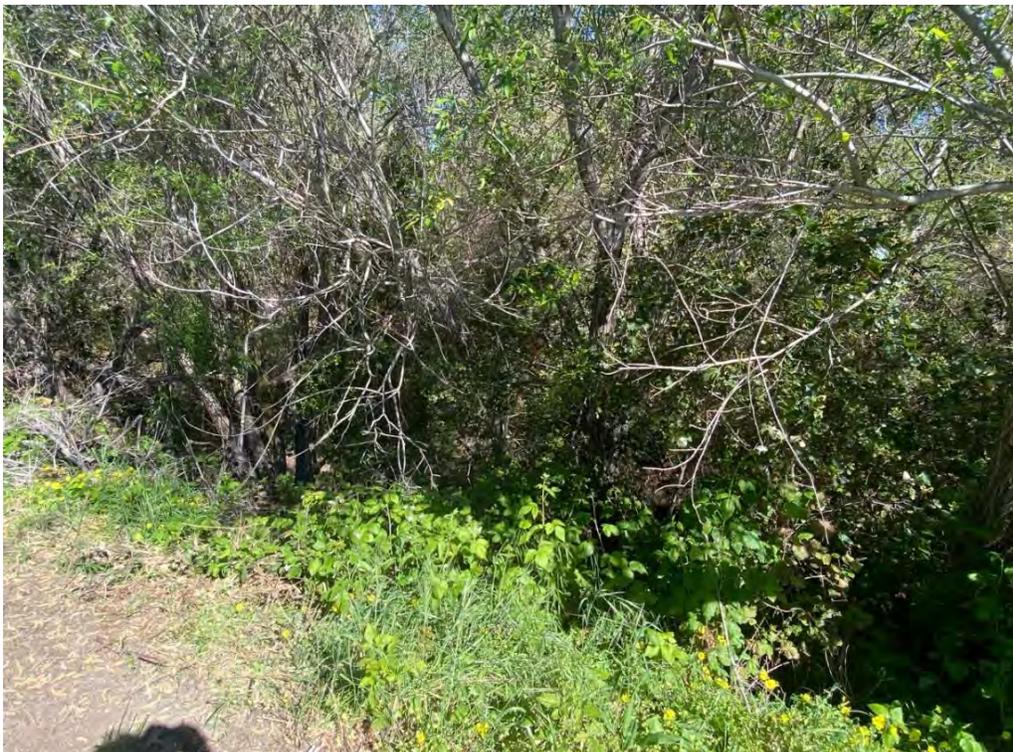


Figure 29. Along the trail west of the Norma Site on San Jose Creek. Understory is primarily nonnative grasses and poison oak. The upper half of this image shows the thick cover of willow trees planted on the west bank, with coast live oak saplings appearing through natural recruitment. Blackwelder. March 7, 2022.



Figure 30. Naturalized olive tree (dark waxy lanceolate leaves), poison oak and native wild cucumber. Ground covered includes nonnative oxalis. Blackwelder. March 7, 2022.

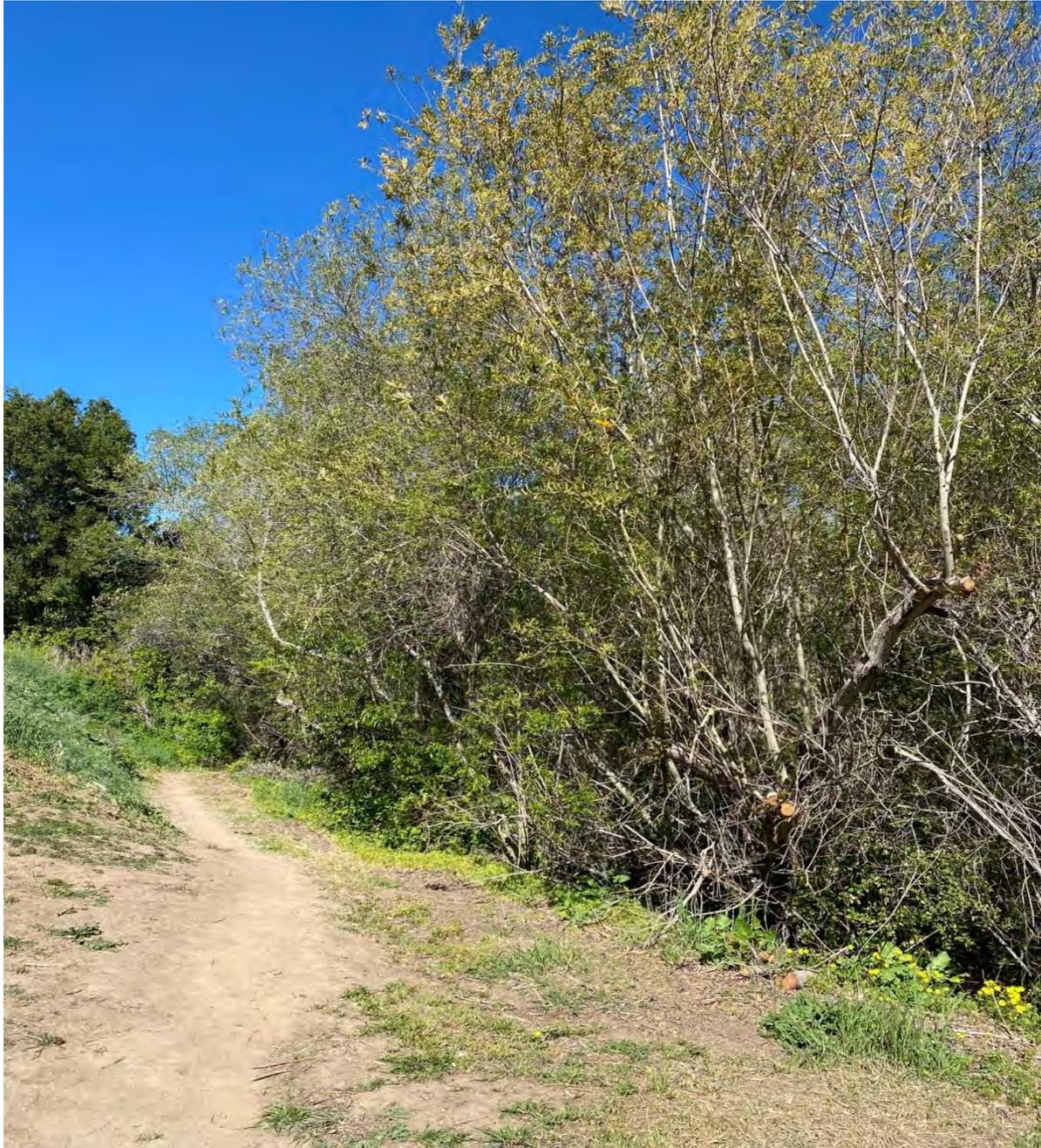


Figure 31. Looking north along the trail at the Norma Revegetation Site on the west bank of San Jose Creek. The trail runs parallel to the Creek. Note the dense willow woodland along the right side of the trail. These trees contribute to a healthy native riparian habitat. Ideally there would be more plant species diversity in the overstory and groundcover along this section of the Creek. Blackwelder. March 7,

C. Paseo Cameo (Site 11)

The Paseo Cameo Site located within a County Open Space on Paseo Cameo Road experienced significant erosion after a heavy storm in 1997 which eroded the east bank, parkland, and part of Paseo Cameo.⁸⁵ SBCFCWCD repaired the tall east bank of San Jose Creek which had eroded in 1998, installed rip rap at the base of the bank, and planted willow trees to restore habitat and stabilize the bank.⁸⁶

Year Planted: April 1998 and Spring 2000

Size: 150 feet x 40 feet = 6,000 square feet⁸⁷

Site Visit: March 7, 2022

Plant Table

Species	# Planted	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	100	25	< 45' h	Fair to Good. 6 dead	25%

Estimated Percent Cover: 95-100%

Other native plants present:

- Coast live oak
- Wild blackberry

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Nonnative grasses including wild oats and brome

Paseo Cameo Site Highlights:

- In 2022, twenty-two to twenty-four years after planting, the site boasts nearly 100% cover of native willow trees installed by SBCDCWCD, and approximately ten coast live oak saplings and several bay laurel saplings which have been added through natural recruitment. While percent survival is below the success criterion in the 2015 CDFW SAA⁸⁸ the 100% cover indicates the site has been successful in establishing native riparian trees.

⁸⁵ Neighbor on Paseo Cameo, personal communication with Natalie Blackwelder, EDC Watershed Program Intern, and Brian Trautwein, Environmental Analyst/Watershed Program Coordinator, EDC (February 22, 2022).

⁸⁶ SBCFCWCD *Annual Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1998).

⁸⁷ SBCFCWCD *Annual Maintenance and Revegetation Report* (2000).

⁸⁸ CDFW (2015) at 17.



Paseo Cameo Site Recommendations:

- Introduce more riparian tree and shrub diversity (i.e., cottonwoods, sycamores, coast live oaks, toyon, gooseberry, and coffeeberry).
- Remove nasturtium and nonnative grasses and plant the understory with greater diversity of native groundcovers (i.e., mugwort, wild blackberry, and giant wild rye grass).
- Relocate the wood rail fence twenty feet out from the top of the bank and plant coast live oak trees and native oak woodland understory species such as mugwort, hummingbird sage, and giant wild rye grass in this area.

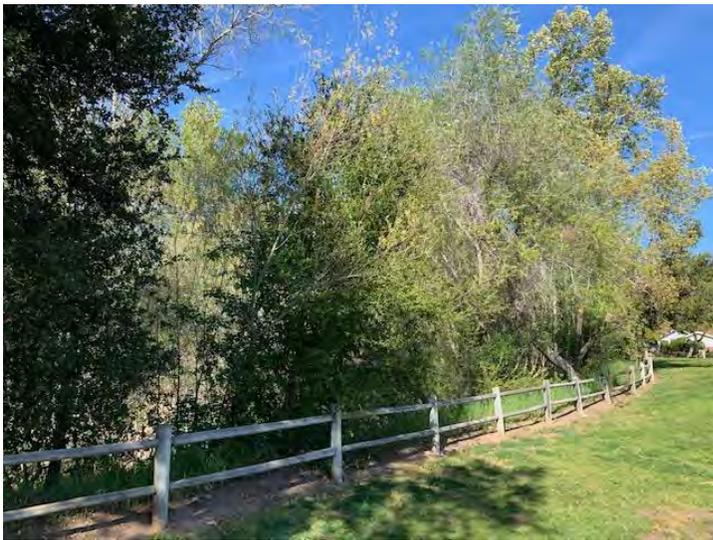


Figure 32. Paseo Cameo Site. Wood rail fence installed after 1998 storm eroded bank approximately twenty-five feet into street. One hundred willows were planted on the east creek bank inside the fence. Site experiencing natural recruitment of coast live oak and bay laurel saplings. Trautwein. March 7, 2022.



Figure 33. Paseo Cameo. Willow trees as tall as forty feet comprise the riparian canopy but the understory is mostly devoid of vegetation and contains nonnative species. Trautwein. March 7, 2022.



Figure 34. Paseo Cameo site with wood rail fence in foreground. Note the amputated branches of the willow tree above the fence. Most trees along this stretch seem to be in fair to good condition. Trautwein. March 7, 2022.

D. North of Cathedral Oaks (Site 2)⁸⁹

SBCFCWCD stabilized the east bank in 1995 and planted willow and sycamore trees and wild blackberry between 1996 and 2000 north of the Cathedral Oaks Bridge.⁹⁰ In 1987 maintenance work resulted at this location impacted riparian habitat and a steelhead spawning pool leading to public complaints which led to preparation of the first Program EIR in 1990, approval of a CDFW SAA, and significant changes in SBCFCWCD’s standard maintenance practices.

Year Planted: First planting Spring 1996; second planting April 1998 (willow and sycamore trees); third planting Spring 2000 (blackberries).

Size: 300 feet x 10 feet = 3,000 square feet

Site Visits: March 7 and March 22, 2022

⁸⁹ SBCFCWCD describes this site as being located on the outside bank (east bank) upstream from the Cathedral Oaks Road Bridge.

⁹⁰ SBCFCWCD *Annual Maintenance and Revegetation Report 2000/20001 Maintenance Season* (2001).

Plant Table

Species	# Planted⁹¹	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Willow	Unknown	11 ⁹²	15' – 30' h	Fair	Unknown
Sycamore	“a couple”	8 ⁹³	20' - 45' h	Good	High
Blackberry	Unknown	Four stands	10' - 40' s	Fair to Good ⁹⁴	Unknown

Estimated Total Percent Cover:

- The willows, blackberries, and naturally occurring bay and oak saplings located above the pipe and wire revetment create an estimated 70% - 80% cover.
- The sycamores and blackberries near the northern terminus of the access ramp form a nearly 100% cover.⁹⁵

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Shamel ash trees
- Brazilian pepper tree
- Vinca
- Cape ivy
- Nasturtium
- Oxalis

North of Cathedral Oaks Site Highlights:

- The Site boasts a high percent cover on the east bank with blackberries and sycamores⁹⁶ where the access ramp terminates on the Creek bank.
- The size of the sycamores as tall as forty-five feet demonstrates excellent growth and success at the access ramp’s terminus.⁹⁷

⁹¹ SBCFCWCD *Maintenance and Revegetation Report, 1997-1998 Maintenance Season* (December 1998).

⁹² Given the planting date of 1998, it is unclear how many of the willow trees were planted and how many may be naturally occurring. Of the eleven observed, six were located above the revetment and believed to be planted. The remaining five are located below the revetment and are likely natural occurring because we do not believe the SBCFCWCD planted trees in the streambed.

⁹³ The sycamores ranged from 4” to 12” DBH and 20’ to 45’ tall. Given that only a “couple” were planted, it is likely that several are naturally occurring., including two that appear to be growing below the pipe and wire revetment.

⁹⁴ The health and size of the blackberry stands increased going from downstream near the Cathedral Oaks Bridge to upstream adjacent to the access ramp.

⁹⁵ The sycamores and blackberries near the access ramp may be naturally occurring.

⁹⁶ *Id.*

⁹⁷ *Id.*



- Natural recruitment of four California bay laurel, and several coast live oak and black cottonwood saplings above and east of the pipe and wire revetment indicates that this riparian habitat revegetation site is developing into a more robust, diverse, and mature plant community.

North of Cathedral Oaks Site Recommendations:

- Eradicate cape ivy in area north of Cathedral Oaks and along access road.
- Remove *Arundo* patch on east bank near Cathedral Oaks Road.
- Eradicate patches of *Arundo* on west bank near access ramp.
- Eradicate periwinkle, oxalis, and nasturtium on east Creek bank and along the access road and ramp. Control nonnative grasses.
- Plant cottonwood trees on top of rock revetment on west bank near access ramp.⁹⁸
- Plant willow poles within rock revetment on west bank near access ramp.
- Extend plantings east from narrow strip of plantings atop east bank near Cathedral Oaks Bridge to widen riparian habitat and enhance Creek buffer.

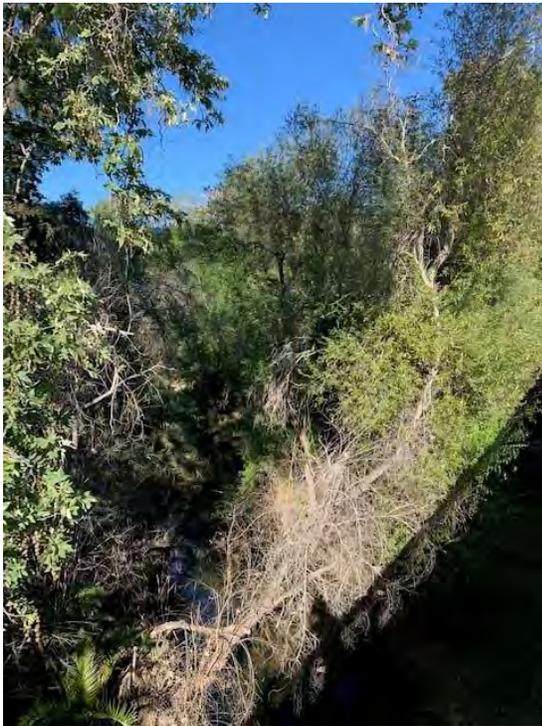


Figure 35. San Jose Creek looking upstream (north by northeast) from Cathedral Oaks Bridge at willows planted on east (right) bank.

Figure 36. Willow on east bank and dead *Arundo donax* stalks in foreground likely remaining after SBCFCWCD eradication efforts. Trautwein. March 22, 2022.

⁹⁸ While the site is limited to the east bank, and west bank near the access ramp was reconstructed using rock rip rap and lacks riparian tree cover.



Figure 37. Willows on east bank of San Jose Creek and Arundo recovering between trees.

Figure 38. Arundo not fully eradicated is surviving around base of willow tree. Trautwein. March 22, 2022.



Figure 39. Nasturtium in riparian understory below willow tree on east bank upstream from Cathedral Oaks.

Figure 40. Willows along east bank next to SBCFCWCD access road. Trautwein. March 22, 2022.



Figure 41. Willow trees on east bank of San Jose Creek at North of Cathedral Oaks Site.

Figure 42. Willow trees with bay laurel sapling recruitment and wild blackberry. Trautwein. March 22, 2022.

E. Cathedral Oaks Village Association Sites

SBCFCWCD obtained approval from COVA in 2015-2018 to revegetate four sites on COVA property along San Jose Creek near Cathedral Oaks Road and Kellogg Avenue.⁹⁹ (Figure 43) These four sites are evaluated below. COVA was required by CDFW to revegetate riparian habitat to mitigate the impacts of unauthorized clearing of riparian vegetation adjacent to Cathedral Oaks Village. However, EDC did not identify any revegetation sites attributed to COVA to mitigate the impacts of its clearing of riparian habitat. Instead, EDC observed SBCFCWCD’s four revegetation sites planted as mitigation for the Annual Maintenance Program’s impacts to San Jose Creek. Thus, it appears that COVA did not implement revegetation projects as required by CDFW, or COVA has improperly double counted SBCFCWCD’s revegetation projects as mitigation for COVA’s clearing of riparian habitat. In either case, COVA has yet to implement revegetation for the extensive riparian habitat COVA removed along the east side of San Jose Creek

COVA #1

This site is located on a steep north-facing upland slope south of Cathedral Oaks Road below Cathedral Oaks Village. SBCFCWCD obtained COVA’s approval to revegetate COVA Sites #1¹⁰⁰ and #2, provided all labor and materials, including irrigation lines and was credited with mitigation for both Sites.¹⁰¹ (Figure 43) COVA supplied irrigation water. However, COVA Sites #1 and #2 were required by CDFW to serve as mitigation for COVA’s clearing of riparian

⁹⁹ SBCFCWCD *Maintenance and Revegetation Report, 2020-2021 Maintenance Season* (July 2021); Personal Communication, Andrew Raaf, Environmental Team Leader, SBCFCWD to Brian Trautwein, Senior Analyst/Watershed Program Director, EDC (August 25, 2022) (“Raaf (2022)”).

¹⁰⁰ COVA Site #1 does not appear to have been successfully revegetated as described herein.

¹⁰¹ SBCFCWCD *Maintenance and Revegetation Report 2020-2021 Maintenance Season* at 49-50 (July 2021); Raaf (2022).

habitat within San Jose Creek.¹⁰² Given that these sites were revegetated by SBCFCWCD, COVA has yet to implement revegetation required by CDFW to mitigate the effects of COVA’s riparian habitat clearing along San Jose Creek.¹⁰³

Year Planted: 2015-2018

Size: 1,900 square feet¹⁰⁴

Site Visits: January 23, 2022, January 27, 2022, and January 30, 2022



Figure 43. The four COVA revegetation sites east of San Jose Creek south of Cathedral Oaks Road. Google Earth. 2021.

Plant Table

Species Planted	# Planted¹⁰⁵	# Observed	Avg Height or Spread	General Condition	Estimated % Survival¹⁰⁶
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¹⁰² Email from Natasha Lohmus, retired CDFW, to Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 28, 2022) (“Lohmus (2022)”); *See also* CDFW Streamed Alteration Agreement 1600-2012-0132-R5 (2012).

¹⁰³ Lohmus (2022).

¹⁰⁴ SBCFCWCD *Annual Maintenance and Revegetation Report* (2020-2021).

¹⁰⁵ “Approximately 400 plants were planted at the two sites” (COVA 1 and COVA 2), SBCFCWCD *Annual Maintenance and Revegetation Report* (2015-2016).

¹⁰⁶ Only twenty-nine surviving plants were observed at COVA Sites #1 and #2 combined, less than 10% survival.

Willows	Unknown	0	-	-	0
Elderberry	Unknown	4 ¹⁰⁷	2' – 8' h	Poor to Good	Unknown
Blackberry	Unknown	0	-	-	Unknown
Wildrye	Unknown	1	10 sq ft s	Fair	Unknown
Deergrass	Unknown	0	-	-	0
Toyon	Unknown	0	-	-	0

Estimated Percent Total Cover:

- Fifty percent (50%) cover including mugwort which is not on the planting list.¹⁰⁸
- Estimated percent total cover is less than ten percent (10%) excluding mugwort.

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Kikuyu grass
- Shamel ash
- Oxalis
- Yucca tree
- Cape ivy

COVA Site #1 Highlights:

- Cooperation with landowners is beneficial because it greatly increases opportunities for onsite mitigation.
- Location of COVA Site #1 adjacent to UCC’s San Jose Creek Restoration Site has the potential to magnify environmental enhancement and benefits for birds and wildlife.

COVA Site #1 Recommendations:

- Control invasive plant species through weeding and/or solarization then seed with mugwort from locally collected source.
- Remove ash and yucca trees.
- Replant wild blackberries at five-foot centers.
- Plant three coast live oak trees and five elderberries.

¹⁰⁷ Two of these four are large healthy elderberry plants but are located outside of COVA #1 and may be naturally occurring or may have been planted by UCC or Audubon Society as part of the County-approved San Jose Creek Restoration Project.

¹⁰⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2015-2016).

- Increase plant diversity by planting additional shrub and understory species such as toyon, coffeeberry, hummingbird sage, gooseberry, and wild rose propagated from plant materials collected along San Jose Creek near COVA.

Figure 44. COVA Site #1. This site was one of the sites COVA was supposed to plant as mitigation for COVA's clearing of riparian habitat, but it was planted by SBCFCWCD as part of its Annual Maintenance Program, therefore COVA must still implement revegetation to mitigate the effects of COVA's riparian vegetation removal. This site is dominated by nonnative weeds, including oxalis, kikuyu grass, dandelion, and cape ivy which are visible using binoculars from the Cathedral Oaks Road Right of Way. (Figure 44b) Yucca trees and a Shamel ash are also present. Four hundred native deergrass, willow trees, elderberry, toyon blackberry, and wildrye plants were reportedly installed by SBCFCWCD at COVA Site #1 and #2 in 2016, however only one giant wild ryegrass, and one to two elderberries including one which has been knocked down by a fallen limb are present at COVA Site #1. Two other elderberries are located outside COVA Site #1 and it is unclear if they were planted by SBCFCWCD or as part of UCC's San Jose Creek Restoration Project or are naturally-occurring. COVA Site #1 is largely dominated (over 50% cover) by invasive nonnative herbaceous vegetation including oxalis and kikuyu grass. Mugwort is also common but is not one of the species planted. CDFW SAA success criteria require <5% cover by herbaceous invasive plants but this criterion is not being met. Irrigation lines are still present but appear to be in disuse. Trautwein. January 30, 2022. Figure 44b. Photographer location. On-X.

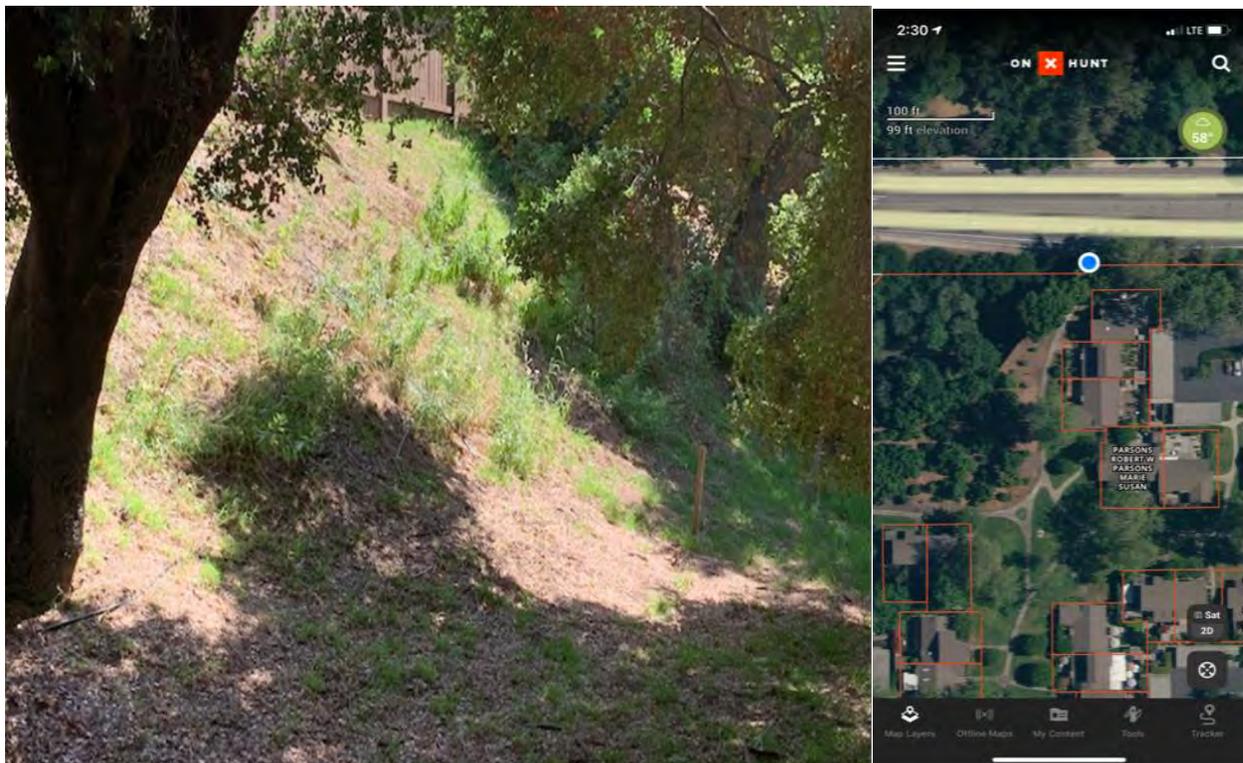




Figure 45. COVA Site #1 is located on the slope behind and under trees in the background of this image. The center of this image is a portion of UCC's San Jose Creek Restoration Project Site #1 which was cleared incrementally by COVA between 2007 and 2018. Note one of two healthy elderberries naturally occurring or planted near COVA Site #1 (upper right quadrant right of image). Trautwein. January 2022.

COVA #2

SBCFCWCD obtained COVA's approval to plant this northwest-facing slope and provided all the materials (other than irrigation water) and labor.¹⁰⁹ The slope is in an upland setting and was planted primarily with upland species rather than riparian species.

Area: 2,700 square feet¹¹⁰

Year Planted: 2015-2016¹¹¹

Site Visits: January 23, 2022, January 27, and January 30, 2022

¹⁰⁹ Raaf (2022).

¹¹⁰ SBCFCWCD *Maintenance and Revegetation Report* for 2020-2021 Season at 49 (July 2021).

¹¹¹ *Id.* at 49 – 52.

Plant Table

Species Planted¹¹²	# Planted¹¹³	# Observed	Avg Height or Spread	General Conditions	Estimated % Survival¹¹⁴
Willow	Unknown	1	10' h	Poor	Unknown
Deergrass	Unknown	1	3' s	Fair	Unknown
Giant wildrye	Unknown	9	6' s	Fair to Good	Unknown
Mulefat	Unknown	4	5' - 10' h	Fair to Good	Unknown
Toyon	Unknown	3	7' h	Fair	Unknown
Coyote brush ¹¹⁵	Unknown	1	5' h	Fair to Good	Unknown
Coast live oak	Unknown	1	15'	Good	Unknown
California sage	Unknown	3	3' h, 3' – 5' s	Fair to Good	Unknown
White sage	Unknown Unknown	1	2' h X 2' s	Fair	Unknown

Estimated Percent Cover: Eighty Percent (80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Oxalis

COVA Site #2 Highlights:

- Upland species such as sages and coast live oak are performing well.
- Restoration of upland habitat adjacent to riparian habitat increases plant and habitat diversity and provides transitional areas for wildlife species.

¹¹² At least one species is not the local native variety which raises the concern that none of the species planted in COVA Site #2 are local genotypes.

¹¹³ The number of plants installed at COVA Site #2 is not provided in the Maintenance and Revegetation Reports however approximately 400 native plants were installed at COVA Sites #1 and #2 combined. SBCFCWCD 2017-2018 Maintenance and Revegetation Report at 55.

¹¹⁴ Only twenty-nine surviving plants were observed at COVA Sites #1 and #2 combined, less than 10% survival.

¹¹⁵ The coyote brush installed at COVA Site #2 does not appear to be the local native variety of coyote brush and should be replaced by appropriate native species of local genotype. Planting non-local genotypes may result in hybridization and impacts to the local native gene pool and is inconsistent with proper restoration protocol which requires use of local genotypes.



COVA Site #2 Recommendations:

- Remove nonnative species and replace them with native oak woodland and coastal sage species.
- Plant riparian vegetation in the cleared area west of COVA Site #2 to restore dense willow riparian woodlands removed by COVA between 2013 and 2015. (See below.)
Note: COVA Site #3 was planted by SBCFCWCD and credited as mitigation for the Annual Maintenance Program.¹¹⁶ COVA should be required to restore riparian vegetation cleared by COVA adjacent to COVA Site #3 to mitigate the impacts of COVA's riparian habitat clearing.



Figure 46. COVA Site #2 showing California sage, mulefat, and toyon surviving with portion of site in lower left quadrant covered by invasive herbaceous species. Trautwein. January 23, 2022.

¹¹⁶ Raaf (2022).



Figure 47. COVA Site #2 with giant wild ryegrass (center left), deergrass (lower left), coast live oak sapling (upper center-right), and mule fat (left side). Note area in foreground was cleared of riparian vegetation and consists of one hundred percent nonnative species. Trautwein. January 30, 2022.

COVA Site #3

SBCFCWCD planted an upland slope on COVA property with approval from COVA.¹¹⁷ Adjacent riparian habitat was cleared by COVA around the same time; however, EDC’s surveys did not identify revegetation areas planted by COVA.

Area: 2,300 square feet¹¹⁸

Year Planted: 2015-2018¹¹⁹

Site Visits: January 23, 2022, January 27, 2022, and January 30, 2022

Plant Table

Species Planted	# Planted ¹²⁰	Number Observed	Avg. Height or Spread	General Condition	Estimated % Survival ¹²¹
Black sage	Unknown	10	3’ – 6’ s	Fair to Good	Unknown
Nightshade	Unknown	1	4’ h	Fair	Unknown
Willow	Unknown	2	5’ – 10’ h	Poor	Unknown
Ceanothus ¹²²	Unknown	2	6’ h	Fair	Unknown

¹¹⁷ SBCFCWCD (2021) at 49; Raaf (2022).; *See also* Aaron Stein, COVA Homeowners’ Association personal communication with Brian Trautwein, Senior Analyst/Watershed Program Director (September 9, 2022).

¹¹⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2017-2018) at 56.

¹¹⁹ *Id.*

¹²⁰ The number of plants planted was not provided in Maintenance and Revegetation Reports.

¹²¹ It is impossible to estimate percent survival because the number of plants planted was not provided.

¹²² Ceanothus installed at this site is not the local native variety of greenbark or spiny ceanothus (*Ceanothus megacarpa* or *Ceanothus spinosa*) and should be replaced with native plants of local genotypes consistent with habitat restoration protocols.

Coyote brush ¹²³	Unknown	8	5' h	Fair to Good	Unknown
California sage	Unknown	3-5	3' – 4' h	Fair to Good	Unknown
Nightshade	Unknown	1	4' h	Fair	Unknown
Coast live oak tree	Unknown	1	10' h	Good	Unknown

Estimated Percent Cover:

- 70% including non-local or nonnative coyote brush and ceanothus
- 50% excluding these two species.

Nonnative and Aggressive Plant Species Present in and near Revegetation Site:

- Kikuyu grass
- Nasturtium
- Nonnative grasses
- *Eucalyptus citriodora*

*Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:*¹²⁴

- Jacaranda (observed spreading in other portions of San Jose Creek, including La Goleta Pollinator Garden (See Section II.O. below)).
- Palm tree
- Lemon-scented gum (eucalyptus) tree

COVA Site #3 Highlights:

- Upland coastal sage scrub species and coast live oak trees are well established and healthy.

COVA Site #3 Recommendations:

- Remove all Kikuyu grass by rhizomes for three consecutive years or by solarization.
- Remove medium-sized multi-trunked *eucalyptus citriodora* tree west of Site. This tree was retained when native willows and riparian habitat was removed during 2013-2015 in violation of CDFW SAA No. 1600-2012-0132-R5.

¹²³ Coyote brush (*Baccharis pilularis*) installed at this site is not a local variety and should be replaced with native plants of local genotypes.

¹²⁴ These species were retained while high quality riparian habitat was cleared.

- Remove nonnative or non-local ceanothus and coyote brush and other native species determined to be non-local genotypes.
- Plant local genotype oak woodland species to ensure 80-100% cover within five years pursuant to CDFW SAA success criteria.
- Maintain weeds to ensure < 5% cover by invasive herbaceous species pursuant to CDFW SAA success criteria.
- COVA must plant cleared area west of COVA Site #3 to restore dense willow riparian woodlands removed by COVA between 2013 and 2015 in apparent violation of CDFW SAA No. 1600-2012-0132-R5. (See below.) *Note: SBCFCWCD paid for the plants and labor to install and maintain COVA Site #3 as mitigation for the Annual Maintenance Program. COVA will need to revegetate an area(s) such as the cleared area west of COVA Site #3 as mitigation for COVA's clearing of sensitive riparian habitat in this area.*



Figure 48. COVA Site #3. Large palm tree retained on edge of site when riparian habitat was cleared. Planted species on slope are primarily non-riparian upland species including California sage, black sage, coyote brush and ceanothus. A large area of dense riparian habitat had been removed (lower half of image). Trautwein. January 30, 2022.



Figure 49. COVA Site #3. Nonnative jacaranda tree retained within revegetation site is shading native upland species such as California shade which prefer full sunlight. COVA requested SBCFCWCD to retain nonnative trees in revegetation areas. Understory is comprised of aggressive nonnative herbaceous species including nasturtium. Weedy area in foreground is where riparian habitat was cleared. Trautwein. January 30, 2022.



Figure 50. COVA Site #3. Note healthy coast live oak sapling in upper middle portion of image. Weedy area in lower portion of image is where riparian habitat was cleared. Trautwein. January 30, 2022.



Figure 51. COVA Site #3 (upper right quadrant of image). Lower half of image is where COVA cleared dense willow-riparian woodland with nearly impenetrable understory of blackberries. Area is now comprised of invasive nonnative grasses. Note multi-trunked *Eucalyptus citriodora* tree behind tri-trunked sycamore (left of middle, upper portion of image). Trautwein. January 30, 2022



Figure 52. COVA Site #3. Understory in southern portion of site comprised of nasturtium and other aggressive nonnative herbaceous species such as exotic grasses. Trautwein. January 30, 2022.



Figure 53. COVA Site #3. Site is upland plant community which is not in-kind mitigation for loss of riparian vegetation. Trautwein. January 30, 2022.

COVA Site #4

This is the fourth site planted by SBCFCWCD with approval by the landowner, COVA.¹²⁵ It is adjacent to the area of riparian habitat that COVA cleared including a large field and approximately one hundred linear feet of the east bank of San Jose Creek. (Figures 54 – 58)

Area: 3,000 square feet¹²⁶

Year Planted: 2019¹²⁷

Site Visits: January 27, 2022 and January 30, 2022

Plant Table

Species Planted	# Planted¹²⁸	# Observed	Avg. Height or Spread	General Condition	Estimated % Survival
Elderberry	Unknown	10	7' – 12'h	Good	Unknown
Blackberry	Unknown	1,400 sq ft	1,400 sq ft	Good	Unknown
Coyote brush	Unknown	2	8'h	Good	Unknown

¹²⁵ Raaf (2022).

¹²⁶ SBCFCWCD *Annual Maintenance and Revegetation Report at 50* (2020-2021).

¹²⁷ *Id.*

¹²⁸ Two-hundred and fifty plants were installed at COVA Site #4 but there is no information on how many plants of each species were planted. SBCFCWCD *Annual Maintenance and Revegetation Report at 50* (2020-2021).

Mulefat	Unknown	1 patch	10' X 7's	Good	Unknown
Willow	Unknown	1	5' – 10'	Poor	Unknown

Estimated Percent Cover: 75 – 80%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Yucca tree
- Nasturtium
- Oxalis
- Italian thistle
- Mallow
- Shamel ash and castorbean along San Jose Creek adjoining COVA Site #4

COVA Site #4 Highlights:

- The elderberries and blackberries planted at this site are performing very well.

COVA Site #4 Recommendations:

- Remove or control oxalis, nasturtium, Italian thistle, mallow, plumbago, and yucca tree.
- Remove Shamel ash on east Creek bank located adjacent to site.
- Remove castor bean (*Ricinus communis*) from Creek area.
- Replace nonnative plants with native coast live oak woodland species from local genotypes to ensure >80% cover within five years pursuant to CDFW SAA success criteria.
- Remove and control weeds to ensure < 5% invasive herbaceous ground cover pursuant to CDFW SAA success criteria.
- Remove exotic vegetation including nonnative grasses and replant adjacent cleared riparian habitat (now a grassy field ~1,200 square feet) and eastern bank of San Jose Creek (~100 linear feet) with native riparian species including arroyo willow (*Salix lasiolepis*), black cottonwood (*Populus trichocarpas*), and wild blackberry (*Rubus ursinus*) as mitigation for COVA's clearing of this area between 2013 and 2015. (See above.)

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 57 of 159



Figure 54. COVA Site #4 (upper left quadrant of image). Note large area (approximately 90' by 30') of once dense willow riparian woodland cleared by COVA circa 2015 now covered by 100% invasive nonnative grasses. See Figure 66. Trautwein. January 30, 2022.



Figure 55. COVA Site #4 (Upper left quadrant of image) Note large, cleared area approximately 90' by 30' covered by exotic grasses. This area was a dense riparian woodland prior to clearing by COVA in 2015. This revegetation site supports ten healthy elderberry plants and an established stand of blackberries mixed with native wild cucumber and nonnative nasturtium at the south end of the Site. COVA Site #4 has achieved an estimated cover of 75-80% of native species. Trautwein. January 30, 2022.



Figure 56. COVA Site #4 with healthy elderberries at north end of site. Note exotic invasive herbaceous species on slope below elderberries. Italian thistle, nasturtium, mallow, and oxalis dominate site's understory. The area in the foreground / lower portion of image was cleared of high-quality riparian habitat. Trautwein. January 30, 2022.



Figure 57. COVA Site #4. A large yucca was retained within this revegetation site detracting from its success. COVA cleared riparian habitat in lower right half of this image. Trautwein. January 30, 2022.



Figure 58. COVA Site #4. South end of Site supports large stand of wild blackberry mixed with native wild cucumber and aggressive, exotic nasturtium. Note the grassy area in the lower portion of Figure 58 is where COVA cleared extensive riparian habitat. Trautwein. January 30, 2022.

1. COVA Cleared Substantial Areas of Riparian Habitat and Was Required by CDFW to Undertake Revegetation to Mitigate the Impacts, However SBCFCWCD Improperly Claimed COVA's Revegetation Sites as Mitigation for SBCFCWCD's Annual Maintenance Program.

During 2007 through 2018, COVA removed extensive areas of high-quality, dense willow riparian woodland with a thick understory of native riparian plants dominated by wild blackberry adjacent to COVA Site #1 as shown below. (Figures 59-66; *See also* Figures 45, 48-51, and 54-58) The clearings took place on COVA and Santa Barbara County properties, including within Santa Barbara Urban Creeks Council's ("UCC") County-approved San Jose Creek Restoration Site #1 south of Cathedral Oaks Road and east of San Jose Creek.¹²⁹

EDC reported the clearing of riparian habitat to CDFW as a potential Fish and Game Code violation circa 2010. (Figure 60) CDFW issued SAA No. 1600-2012-0132-R5. COVA agreed to plant native vegetation in two locations on an embankment (COVA Sites #1 and #2) to mitigate COVA's removal of riparian habitat near COVA Site #1.¹³⁰ Instead, SBCFCWCD undertook revegetation at COVA Sites #1 and #2 with COVA's approval to mitigate the impacts of the Annual Maintenance Program.¹³¹ Therefore COVA never undertook revegetation required to mitigate the impacts of COVA's clearing of riparian habitat pursuant to CDFW SAA No. 1600-2012-0132-R5.

Between 2013 and 2018, COVA cleared additional high-quality riparian habitat adjacent to COVA Sites #2 - #4, including an estimated one hundred linear feet of Creek bank and constructed an access route for trucks. (Figures 64 – 66; *See also* Figures 54 – 58, 72 – 74, and 76 - 83) EDC reported these clearings to CDFW in 2022.¹³²

- a. *COVA Sites #1 and COVA #2 Were Planted to Mitigate the Impacts of SBCFCWCD's Annual Maintenance Program Therefore COVA Must be Required to Revegetate Additional Areas to Mitigate the Impacts of COVA's Extensive Clearing of Riparian Habitat.*

The San Jose Creek riparian habitat cleared by COVA immediately south of Cathedral Oaks Road exhibited one hundred percent cover of riparian trees (willows, black cottonwoods,

¹²⁹ The UCC's San Jose Creek Restoration Project is a voluntary restoration project approved by the County, including Santa Barbara County Fire Chief Jim Thomas, Santa Barbara County Public Works Department and SBCFCWCD Director Phil Demery, Santa Barbara County Parks Department Director Jennifer Briggs, Santa Barbara County Counsel Steven Shane Stark, and Santa Barbara Risk Manager Charles Mitchell.¹²⁹ The Project received support from numerous other agencies including CDFW. The Project was funded with a State Department of Water Resources Urban Streams Restoration Program Grant of \$41,000 issued to SBCFCWCD on behalf of UCC and SBCFCWCD.

¹³⁰ Lohmus (2022).

¹³¹ *Id.*; *See also* Raaf (2022).

¹³² Email from Brian Trautwein, Senior Analyst/Watershed Program Director, to Warden Joseph Gonzales, CDFW (April 22, 2022).

and western sycamores) and understory plants (wild blackberry, etc.) in 2006. (Figure 59) However, much of this native riparian vegetation was sequentially removed between 2007 and 2018 by COVA which was issued SAA No. 1600-2012-0132-R5 in 2012 after COVA's clearing project was reported to CDFW. COVA never implemented revegetation required by that SAA.¹³³ (Figures 59 - 63)

As discussed above, SBCFCWCD obtained COVA's approval to revegetate COVA Sites #1¹³⁴ and #2 and claimed mitigation credit for both Sites.¹³⁵ (Figure 43) However, COVA was required by CDFW to mitigate for COVA's clearing of riparian habitat within San Jose Creek.¹³⁶ Therefore, COVA must be required to revegetate other areas to mitigate the impacts of COVA's riparian vegetation clearing program.



Figure 59. UCC San Jose Creek Restoration Project Site #1 (green polygon in middle and right side of image) covered with native riparian vegetation installed by UCC and established through natural recolonization processes. Google Earth. 2006.

¹³³ COVA claimed a portion of the UCC's San Jose Creek Restoration Project was on COVA property. Clearing riparian habitat on private property requires a Streambed Alteration Agreement; See also CDFW SAA No. 1600-2012-0132-R5 at 4 requiring 5:1 replacement of willow trees and "90% cover after 5 years for the life of the project."

¹³⁴ COVA Site #1 does not appear to have been successfully revegetated as described herein.

¹³⁵ SBCFCWCD *Maintenance and Revegetation Report 2020-2021 Maintenance Season* at 49-50 (July 2021); See also Raaf (2022)

¹³⁶ Lohmus (2022).



Figure 60. Riparian habitat in UCC's Site #1 cleared by COVA (red polygons) in 2007-2010. Google Earth. 2010.

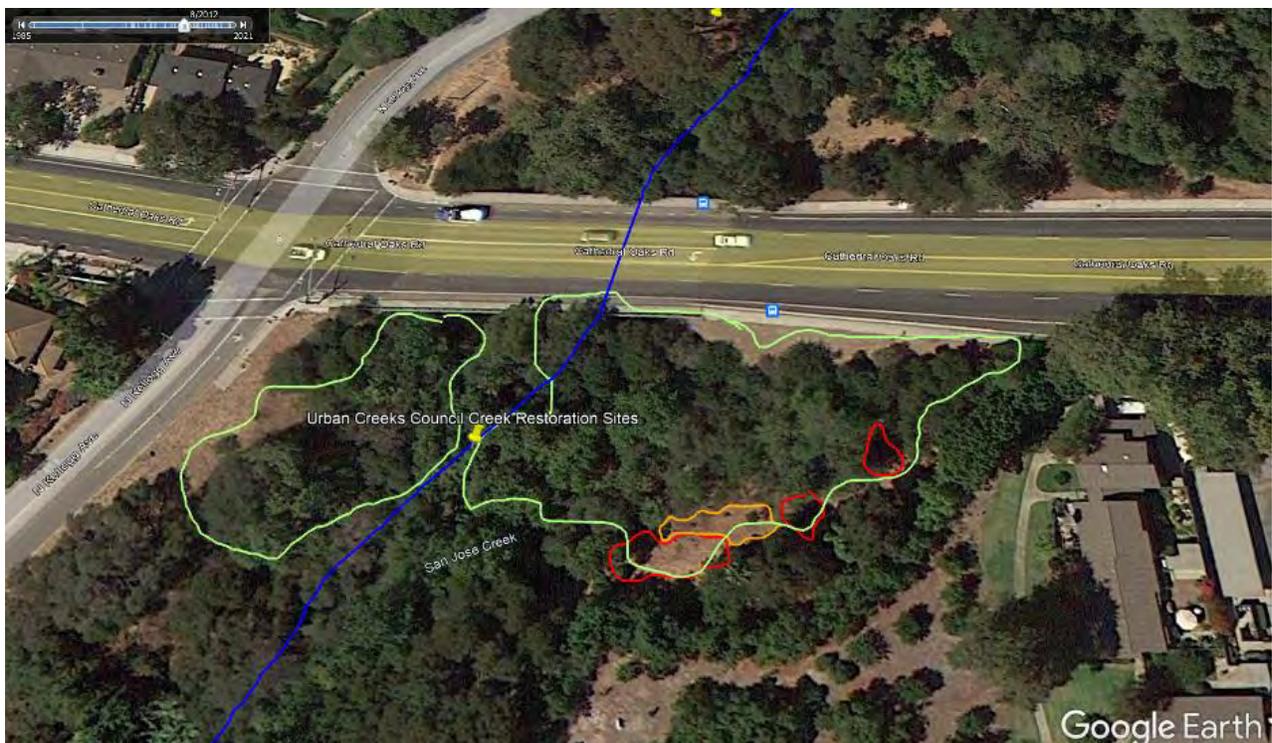


Figure 61. Additionally riparian habitat cleared by COVA (orange polygon) in 2010-2012. Google Earth. 2012.



Figure 62. More riparian habitat cleared by COVA (yellow polygons) in 2013-2014. Google Earth. 2014.

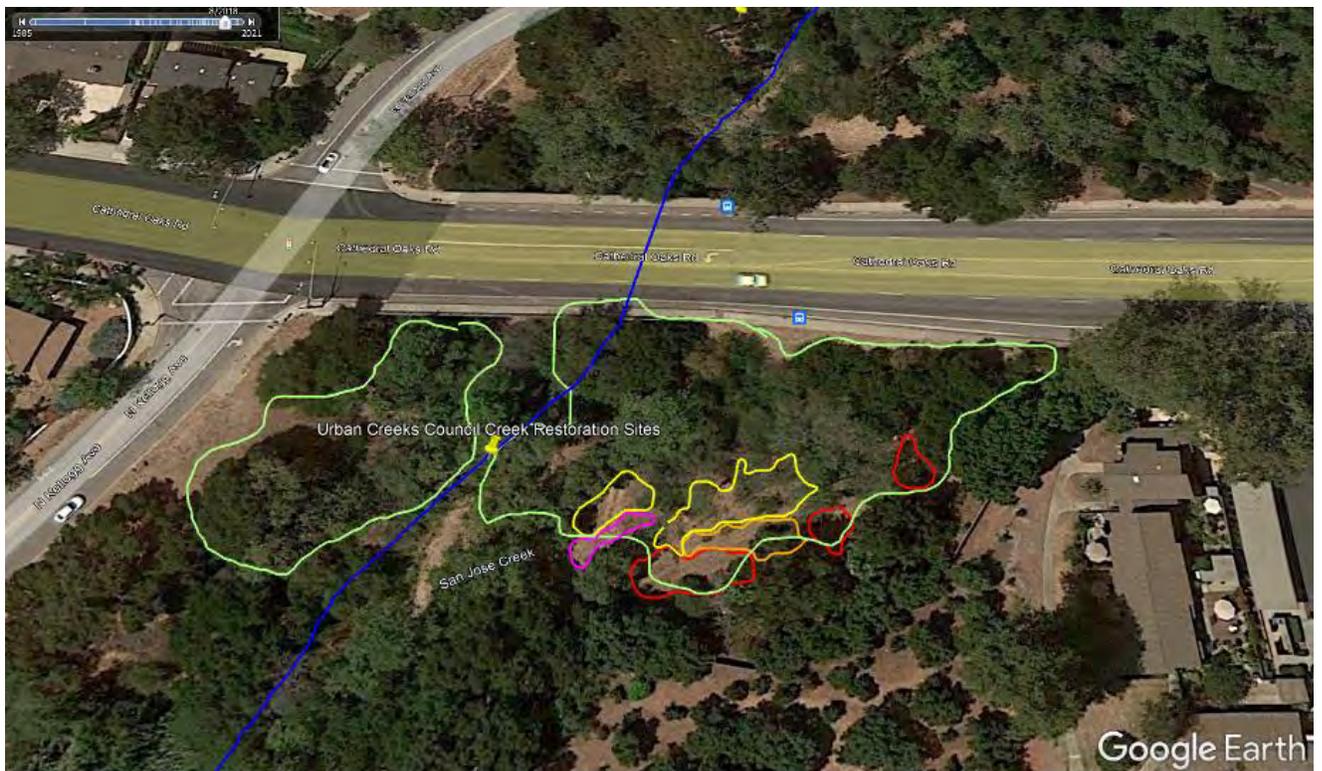


Figure 63. Further clearing of riparian habitat by COVA (purple polygon) in 2018. Google Earth. 2018.

- b. *SBCFCWCD Credits COVA's Upland Revegetation Sites #3 and #4 as Mitigation for the Annual Maintenance Plan Therefore COVA cannot also Claim Sites #3 and #4 as Mitigation for COVA's Removal of Riparian Habitat.*

COVA cleared additional dense riparian habitat along the east side of San Jose Creek between 2013 and 2015 adjacent to COVA Sites #2, #3, and #4, including approximately one hundred linear feet of riparian woodland from the east bank of San Jose Creek. (Figures 64 – 66, 71 – 74, and 76 - 83) Based on aerial photos, EDC estimates the riparian clearings measure a minimum of 8,100 square feet, plus additional areas of oak riparian understory vegetation not visible in aerial photos. EDC's surveys did not detect any revegetation sites planted by COVA as mitigation for its riparian habitat clearing program pursuant to CDFW SAA No. 1600-2012-0132-R5.¹³⁷ COVA should be required to provide onsite replacement of the riparian habitat removed south of Cathedral Oaks Road extending to the southwest corner of COVA's property west of COVA Site #4 and adjoining County property which was also cleared.

SBCFCWCD planted native upland vegetation at COVA sites #3 - #4 in 2017-2019.¹³⁸ SBCFCWCD credits COVA Sites #3 and #4 as mitigation for its Annual Maintenance Program. Given that COVA Sites #3 - #4 are claimed by SBCFCWCD as mitigation, there has been no mitigation for COVA's riparian habitat clearings as required by CDFW.¹³⁹ COVA Sites #3 - #4 cannot be considered mitigation for both COVA's and SBCFCWCD's riparian habitat removal projects.

COVA should be required to replant COVA Site #1, complete restoration of COVA Sites #2 - #4, and restore riparian habitat within all of the cleared locations to mitigate the impacts caused by COVA's clearing of riparian habitat. EDC recommends that riparian habitat removed by COVA be replaced at a minimum 3:1 ratio onsite. If necessary, offsite within nearby portions of San Jose Creek should be restored to ensure a minimum 3:1 replacement.

¹³⁷ CDFW directed COVA to restore riparian habitat to mitigate the impacts of COVA's clearing near Cathedral Oaks Road. Lohmus (2022); *See also* CDFW SAA No. 1600-2012-0132-R5.

¹³⁸ Raaf (2022); *See also* SBCFCWCD Maintenance and Revegetation Report 2020/2021 Maintenance Season at 49-50. See COVA Site #3 and COVA Site #4 Plant Tables below which demonstrate that these sites are upland plantings containing native and nonnative chaparral and coastal sage scrub species, including nonnative ceanothus, coyote brush, white sage, black sage, and California sage. Several willows planted on these upland slopes are struggling to survive despite irrigation.

¹³⁹ Lohmus (2022); *See also* CDFW SAA No. 1600-2012-0132-R5.

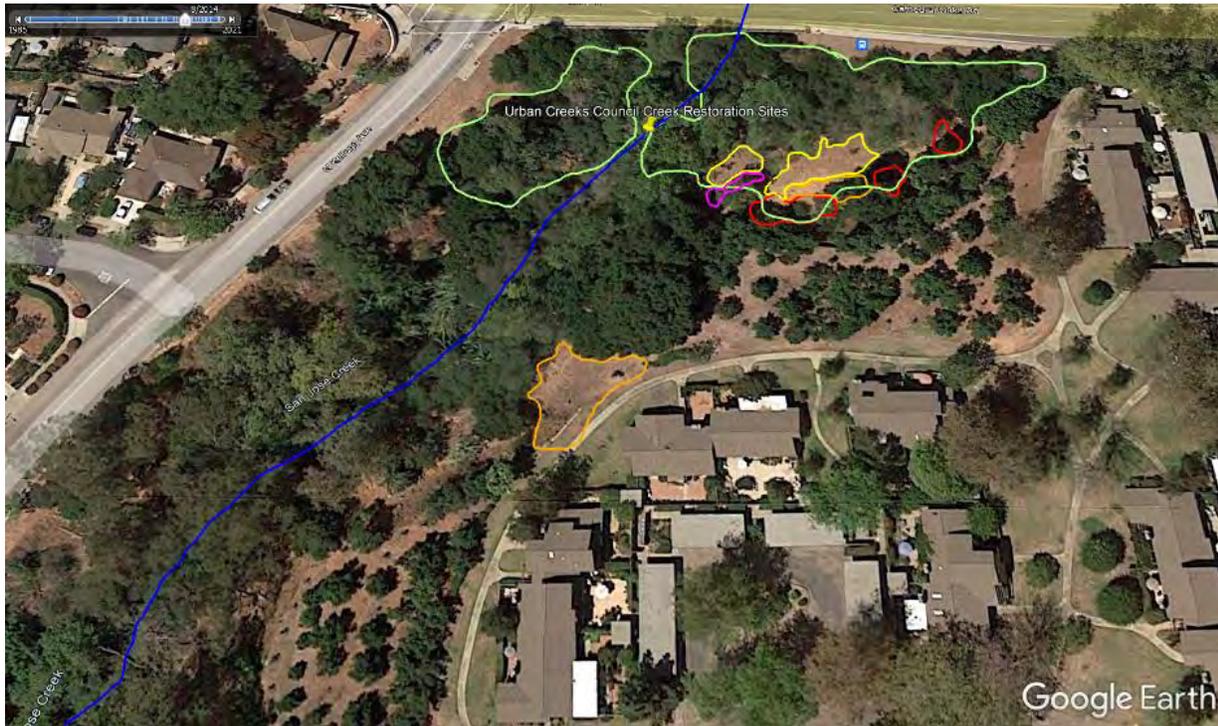


Figure 64. COVA cleared additional natural riparian habitat in 2014 (lower orange polygon in center of image). Google Earth. August 2014.



Figure 65. Prior to COVA clearing of riparian habitat in 2014. Note lower orange polygon in center of image contained dense riparian vegetation including willow trees in December 2013, which were removed in 2014 as shown in Figure 64 above. Google Earth. December 2013.

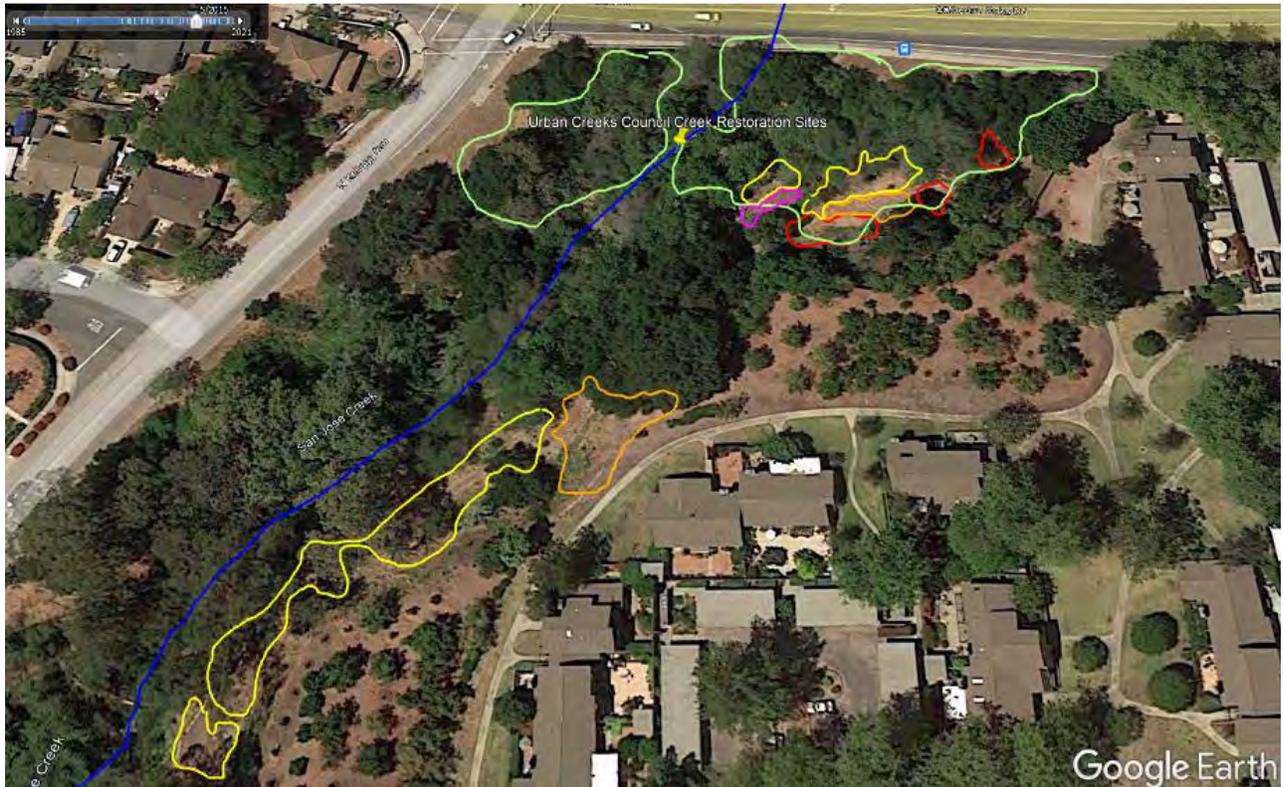


Figure 66. Riparian tree and understory vegetation appears to have been removed from additional areas east of the Creek adjacent to COVA between 2013 and 2015 (yellow polygons in center to lower left section of image). As shown in Figure 67 below, substantial portions of the two lower yellow polygons are located on Santa Barbara County property. Google Earth. 2015.



Figure 67. Parcel Map showing a portion of the cleared habitat was located on County property. On-X. January 29, 2022.



Figure 68. The western portion of Urban Creeks Council's San Jose Creek Restoration Project Site #1 located on County property was cleared by COVA. CDFW directed COVA to undertake revegetation to mitigate the impacts of the unpermitted removal of riparian habitat but this revegetation never occurred. Trautwein. January 2022.



Figure 69. Southern portion of Urban Creeks Council's County-approved San Jose Creek Restoration Site #1 was cleared by COVA. CDFW subsequently required COVA to revegetate COVA Sites #1 and #2 as mitigation for the unpermitted habitat removal, but these sites were revegetated by SBCFCWCD as part of its Annual Maintenance Plan. Trautwein. January 30, 2022.



Figure 70. COVA also cleared the native understory from the entirety of Urban Creeks Council's San Jose Creek Restoration Project Site #1. Note the black cottonwood and arroyo willow trees in Urban Creek's Council's County-approved San Jose Creek Restoration Project Site #1 are underlain by a 100% nonnative understory. The understory was 100% native cover consisting primarily of wild blackberries prior to COVA's clearing of the Site. Trautwein. January 30, 2022.



Figure 71. COVA cleared oak riparian woodland understory on City property south of the Cathedral Oaks Road Bridge over San Jose Creek. Trautwein. January 30, 2022.



Figure 72. Oak riparian woodland understory was cleared and oak trees were pruned along east side of San Jose Creek west of COVA between 2009 and 2015 providing vehicle access along the Creek bank. Trautwein. January 23, 2022.



Figure 73. Cleared riparian habitat now provides vehicle access along Creek bank near COVA Sites #2 and #3. Trautwein. January 23, 2022.



Figure 74. COVA Site #2 with cleared riparian habitat in foreground. Wild blackberry (right side) and California sage (center) present. COVA required SBCFCWD to retain nonnative trees in the revegetation sites. Large yucca tree (upper left) was retained in revegetation site. Trautwein. January 23, 2022.



Figure 75. COVA Site #2 showing California sage on left, small white sage in center, and mulefat on right. Understory is dominated by invasive nonnative oxalis. Trautwein. January 23, 2022.



Figure 76. COVA Site #2 with California sage. COVA directed SBCFCWCD to retain nonnative trees in the revegetation sites. Note palm tree and jacaranda tree were inappropriately retained in and adjacent to restoration site. Trautwein. January 23, 2022.



Figure 77. COVA cleared a dense willow riparian habitat including mature willow trees and native understory plants creating a large field of nonnative grasses and forbs approximately ninety feet by thirty feet immediately west of COVA Site #4. Trautwein. January 30, 2022.

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 73 of 159



Figure 78. COVA cleared riparian woodland from approximately one hundred linear feet of the east bank of San Jose Creek on City property west of COVA Site #4. Looking downstream, south by southwest. Trautwein. January 30, 2022.

October 24, 2022

San Jose Creek Flood Control Revegetation Sites and Los Carneros Mitigation Bank Review and Recommendations

Page 74 of 159



Figure 79. COVA cleared riparian woodland from approximately one hundred linear feet of the east bank of San Jose Creek on City property west of COVA Site #4. Looking upstream, north by northeast. Trautwein. January 30, 2022.



Figure 80. COVA cleared riparian habitat near COVA Site #4. Looking north by northeast to south by southeast. San Jose Creek is located on left side of image. Trautwein. January 30, 2022.



Figure 81. COVA's large riparian habitat removal site (grassy area) west of COVA #4 (upper center portion of image). Trautwein. January 30, 2022.



Figure 82. Dense, high-quality riparian habitat was cleared from County property near COVA Site #4. COVA Site #4 is visible in upper right portion of image. Trautwein. January 23, 2022.



Figure 83. COVA Site #4 with native plants covering 75% of embankment. Note grassy area is where COVA cleared riparian habitat. Trautwein. January 23, 2022.

c. *Recommendations to Mitigate the Impacts of COVA's Riparian Habitat Clearing Projects.*

- CDFW should require COVA to replant all areas COVA cleared of riparian habitat and achieve one hundred percent (100%) cover of native overstory and understory species to replace habitat removed between 2007 and 2018.¹⁴⁰ (Figures 59 – 66)
- CDFW should require COVA to revegetate additional riparian areas near COVA Sites #1 - #4 to mitigate the temporal impacts of removing high-quality riparian habitat at minimum three-to-one (3:1) ratio. Revegetation should achieve ninety percent (90%) total cover within five years and exhibit less than five percent (5%) cover by nonnative herbaceous vegetation as required by CDFW SAA No. 1600-2012-0132-R5.
- CDFW should determine whether SAA No. 1600-2012-0132-R5 authorized all of COVA's riparian habitat removal projects. If not, CDFW should issue a Notice of Violation of the California Fish and Game Code for COVA's removal of high-quality riparian habitat.
- CDFW should require COVA to remove nonnative and non-local varieties of native vegetation in the cleared areas.
- CDFW should require COVA to allow SBCFCWCD to remove nonnative and non-local native plants from Sites #1 - #4, including:
 - Nonnative varieties of native species such as ceanothus and coyote brush (*Baccharis pilularis*) & replant COVA Sites #3 and #4 with local genotype native riparian and upland;¹⁴¹
 - Palm, eucalyptus, yucca, and jacaranda trees; and
 - Invasive and aggressive weedy herbaceous species including oxalis, nasturtium, Italian thistle, mallow, and nonnative grasses, including kikuyu grass present in all four COVA Sites and all areas cleared by COVA.
- Mitigation requirements for COVA and SBCFCWCD should be clarified to ensure each entity's mitigation requirement is fulfilled.
- CDFW should require COVA to (1) preserve native vegetation along the creek and revegetation areas, and (2) place all riparian and upland revegetation and restoration sites in a permanent conservation easement or deed restriction to prevent future removal of this sensitive habitat.

¹⁴⁰ COVA continues to maintain the cleared areas preventing regrowth of native riparian vegetation.

¹⁴¹ See e.g., City of Goleta (2020) CWMP Strategy 11.1.2.

F. Old Footbridge in University Circle Park (Site 4)

SBCFCWCD installed rip rap bank protection on the west bank following storms in 1995 which caused bank erosion.¹⁴² Willows were planted within the rip rap as wattling.

Year Planted: 1995

Size:

- Reported: 50 feet x 10 feet = 500 square feet as measured by SBCFCWCD in 1995.¹⁴³
- Observed: 64 feet x 8 feet = 512 ft² as measured by EDC in 2022.

Site Visit: February 25, 2022

Plant Table: n/a¹⁴⁴

Estimated Percent Cover:

- 50-75% native tree canopy
- 50-80% cover by nonnative understory species

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Cape ivy (Figures 84 and 86)
- Kikuyu grass (Figure 86)
- Oxalis (Figure 86)

Old Footbridge Site Highlights:

- This site was described as a “textbook site to view successful willow wattling” in 1998.¹⁴⁵ However, twenty-seven years after installation, only two willows remain. They are in fair condition with multiple trunks. Each is approximately 30’ tall and about 6-8” DBH.
- One black cottonwood is present in fair to good condition. It is approximately thirty-five feet tall with an eight-inch DBH.¹⁴⁶
- The rip rap remains in good condition however, concrete with rebar and asphalt blocks were mixed in with boulders. (Figure 88)

¹⁴² SBCFCWCD *Annual Maintenance and Revegetation Report 1997/1998 Maintenance Season* (December 1998) (“SBCFCWCD (1998)”).

¹⁴³ SBCFCWCD (1998)

¹⁴⁴ Willow wattling was installed but the number of willows installed was not provided. No other species were planted at this location.

¹⁴⁵ SBCFCWCD (1998).

¹⁴⁶ This tree appears to be naturally occurring.

Old Footbridge Site Recommendations:

- Remove and replace nonnative understory, including Cape ivy, oxalis, and Kikuyu grass with natives, including mugwort, wild blackberry, giant wild ryegrass.
- Remove Shamel ash trees and Arundo donax on east bank opposite rip rap.
- Do not use concrete or asphalt in rip rap. Use naturally occurring rocks.
- Plant willow and black cottonwood trees around the rip rap.

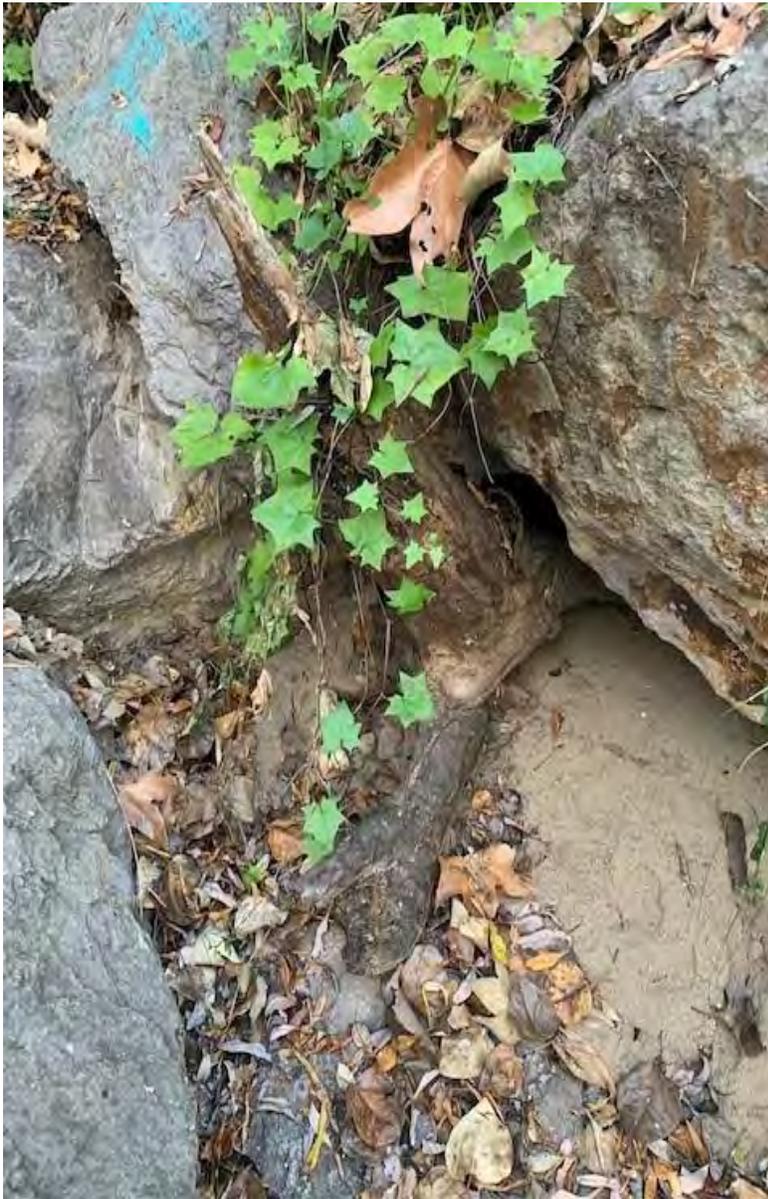


Figure 84. Nonnative, invasive Cape Ivy growing over installed rip rap. Trautwein. February 25, 2022.



Figure 85. University Circle Open Space. Many large willows, sycamores and cottonwoods grow along San Jose Creek. Trautwein near the Old Footbridge Site. February 25, 2022.



Figure 86. Nonnative, invasive grasses, oxalis, and cape ivy growing over rip rap that was installed as bank stabilization. Trautwein. February 25, 2022.



Figure 87. Looking up San Jose Creek at the Old Footbridge Site near University Circle Open Space. Rip rap was installed and is now overgrown with nonnative grasses, oxalis, and invasive Cape ivy. Woody tree in the foreground is one of two surviving willows believed to be installed as wattling in the rip rap in 1995. Trautwein. February 25, 2022.



Figure 88. Rip rap includes and asphalt and concrete with rebar used as bank stabilization. Potential for concrete to alter pH of Creek water. Rebar also poses a potential safety hazard. Trautwein. February 25, 2022.



Figure 89. Rip rap covered with native poison oak and nonnative oxalis, kikuyu grass, and cape ivy. One of two remaining willow trees (left side of image) and one naturally-occurring black cottonwood tree is present on upper bank (center of image). Trautwein. February 25, 2022.



Figure 90. Rip rap with one of two surviving willow trees installed in 1995. February 25, 2022.



Figure 91. Black cottonwood on creek bank growing above rip rap at Old Footbridge Site. This is believed to be a naturally-occurring tree because only willows were planted in the rip rap. Trautwein. February 25, 2022.

G. Seventy-five Feet Upstream of Berkeley Footbridge (Site 5)

SBCFCWCD installed rip rap with willow wattling in this location where high flows caused bank erosion at the outside of a sharp bend in the southern end of the University Circle Open Space.¹⁴⁷ SBCFCWCD installed the willow wattling 1995.

Year Planted: 1995¹⁴⁸

Area: 25 feet x 10 feet = 250 square feet

Date of Site Visit: February 25, 2022

Plant Table: n/a¹⁴⁹

Estimated Percent Cover:

- 50% - 60%

Native Species Present:

- Willows
- Cottonwood
- Creek clematis

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Periwinkle
- Nasturtium
- Cape ivy
- Algerian ivy

Seventy-five Feet Upstream of Berkeley Bridge Site Highlights:

- Rip rap installed by Flood Control District is creating roughness and helping establish a pool at this location.

Seventy-five Feet Upstream of Berkeley Bridge Site Recommendations:

- Remove residential shed located on public property. (Figure 92)
- Plant native trees and understory around the rip rap to achieve a higher percent cover at this site.

¹⁴⁷ SBCFCWCD (1998).

¹⁴⁸ *Id.*

¹⁴⁹ Willow wattling was planted underneath the rip rap but the number of willow branches planted was not disclosed and no other plants were installed.



Figure 92. Site known as Seventy-five Feet Upstream from the Berkeley Footbridge. Installed rip rap is supporting native black cottonwood tree. Bank downstream from rip rap lacks native riparian vegetation. Trautwein. February 2022.



Figure 93. View of rip rap, looking upstream of San Jose Creek. Willows growing out of the rocks and bank. Trautwein. February 2022.

H. Berkeley Footbridge East of Goleta Water District Well Building (Site 6)¹⁵⁰

SBCFCWCD planted this small patch of native plants next to the Berkely Footbridge in 1995.¹⁵¹

Year Planted: 1995¹⁵²

Area:

- Reported: 180 feet x 15 feet = 2,700 square feet as reported by SBCFCWCD.¹⁵³
- Observed: 800 square feet (elderberries and willow trees) plus 200 square feet (sycamore trees) = 1,000 square feet observed by EDC in 2022.

Date of Site Visit: January 27, 2022

Description: This site contains several willow and sycamore trees, elderberries, and wild rose west of the west bank of San Jose Creek south of the Berkely Footbridge. The sycamore trees were planted along the Creek’s west bank east of the footpath.

Plant Table

Species Planted	# Planted	# Observed	Avg. Height	General Condition	Estimated % Survival
Sycamore	Unknown	2	20’ - 25’h	Good	Unknown
Willow	Unknown	1	15’h	Fair to Good	Unknown
Elderberry	Unknown	4	15’’h	Fair to Good	Unknown
Wild rose	Unknown	2	3’h	Fair	Unknown
Coyote Brush	Unknown ¹⁵⁴	2	6’h	Fair	Unknown

Estimated Total Percent Cover:

- Overstory: Greater than eighty percent (80%) native species within the eight hundred (800) square foot revegetation area.
- Understory: One hundred percent (100%) nonnative invasive herbaceous species.

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses.

Berkeley Road Footbridge Site Highlights:

¹⁵⁰ Uncertain if this is part of Site 6; the site numbering system from report to report is inconsistent and unclear.

¹⁵¹ SBCFCWCD (1998).

¹⁵² Uncertain if this is part of Site 6; the site numbering system from report to report is inconsistent and unclear.

¹⁵³ SBCFCWCD (1998).

¹⁵⁴ Uncertain if planted, might be from natural recruitment.



- The two sycamore trees are growing strong and large, indicating great success in their establishment.

Berkeley Footbridge Site Recommendations:

- Manually remove all nonnative understory plants under willows, sycamores, wild rose, and elderberries and within 20 feet of driplines.
- Plant twenty-five wild blackberry plants on six-foot centers.
- Control weeds until blackberries establish a minimum ninety-five percent cover.



Figure 94. Berkeley Footbridge Site East of Goleta Water District Well Building. Willows, elderberries, and wild rose present by understory is 100% exotic invasive herbaceous plants. Trautwein. January 23, 2022.



Figure 95. One of two healthy sycamore trees SBCFCWCD planted at the Berkeley Footbridge Site. Trautwein. January 23, 2022.

I. Three Hundred Feet Downstream from Berkeley Road Footbridge East Bank Repair (Site 23)

East bank repair: installation of rock rip rap and willow brush layering; east and west bank revegetation.¹⁵⁵

Years Planted: 2006-2007

Area:

- East bank: 80 feet x 15 feet = 1,200 square feet
- West bank: 12 feet x 6 feet = 72 square feet

Date of Site Visits: January 22, 2022, January 27, 2022, and February 18, 2022

Plant Table

Species Planted	# Planted	# Observed	Avg Height or Spread	General Condition	% Survival
East bank	-----	-----	-----	-----	-----
Mugwort (<i>Artemesia</i>)	25	0	-	-	0
Sunflower (<i>Venegasia</i>) ¹⁵⁶	10	1	-	-	10
Elderberry (<i>Sambucus</i>)	2	1	8' h	Good	50
Sycamore (<i>Platanus racemosa</i>)	1	0	-	-	0
Blackberry (<i>Rubus ursinus</i>)	15	2	2x2' s and 10x4' s	Fair	13.3
Lemonade berry (<i>Rhus integrifolia</i>)	3	0	-	-	0
Brush layering with Willow branches	n/a	3	5-15' h	Fair	-
West Bank	-----	-----	-----	-----	-----
Willow (<i>Salix</i>)	10	7 (3 dead ¹⁵⁷)	10' to 20' h	Fair to Good	70
Wild blackberry (<i>Rubus ursinus</i>)	8	0	-	-	0
Clematis	10	0	-	-	0

¹⁵⁵ SBCFCWCD Maintenance and Revegetation Report 2006/2007 Maintenance Season (2007).

¹⁵⁶ Might have seen one canyon sunflower but were unable to conclusively identify species.

¹⁵⁷ Not sure if these were planted by SBCFCWCD or if they are natural recruits that died.

Estimated Total Percent Cover:

- East Bank: Understory 10-40%
Overstory 50%
- West Bank: Understory 10%
Overstory 60-70%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Shamel ash
- Nonnative grasses
- Nasturtium

Three Hundred Feet South of Berkeley Road East Bank Repair Site Highlights:

- SBCFCWCD retained a pool at this site below the rock rip rap.
- One large willow appears to have grown from the brush layering within the rock rip rap.

Three Hundred Feet South of Berkeley Road East Bank Repair Site Recommendations:

- Eradicate nonnative invasive species from east bank above rock rip rap
- Plant additional willow and/or cottonwood poles into the rip rap.
- Eradicate the Shamel ash tree growing in the rip rap.



Figure 96. Bank repair. East bank of San Jose Creek approximately three hundred feet south of Berkeley Footbridge. Note invasive Shamel ash (green and yellow compound leaves) established at bank repair site. East bank above rip rap bank armoring supports nonnative invasive species. One single large willow appears to remain (behind ash tree) in upper right quadrant of image. Trautwein. January 27, 2022.



Figure 97. Site 23, approximately 300' downstream of Berkeley Footbridge on the east bank of San Jose Creek. Note: the white PVC pipe coming out of the foliage was installed to irrigate the layered willows that were placed between rip rap layers. Trautwein. March 7, 2022.



Figure 98. Riprap located on the east bank of Site 23. Young willows are growing out of the rocks are a result of willow brush layering at the time of mitigation. Understory is about 50% nonnative grasses. Trautwein. March 7, 2022.



Figure 99. Rip rap and exposed willow layering. Note the upshoots of new willow growth. Trautwein. March 7, 2022.



Figure 100. Site 23, about three hundred feet downstream of the Berkeley Footbridge, on the west bank. The understory is primarily nonnative grasses and nasturtium. The overstory is primarily willow, sycamore, and black cottonwood. Trautwein. March 7, 2022.



Figure 101. West bank of San Jose Creek at Site 23. Large sycamore in the background from natural recruitment. Understory is primarily nonnative grasses. Willow saplings present on lower bank of Creek. Trautwein. March 7, 2022.



Figure 102. Young willow growing out of a poison oak. Note the nonnative grasses in the foreground. Trautwein. March 7, 2022.



Figure 103. Site 23, approximately three hundred feet downstream of Berkeley Footbridge on the west bank. Note the nonnative, naturalized bottlebrush bush that is growing in the center of the photo. There are also nonnative grasses and nasturtium in the foreground, and willow and oak trees behind the bottlebrush. Trautwein. March 7, 2022.

J. Berkeley Road Downstream from Berkeley Footbridge, West Side of Creek near Access Road (Site 13)

SBCFCWCD installed rip rap, rebuilt the bank, and planted this site with willows, cottonwoods, sycamores, and elderberries in 2000 after storms eroded the Creek’s west bank and adjacent flood control access road which serves as a neighborhood trail.¹⁵⁸ The only plants to survive are located adjacent to the bank top.

Year Planted: 2000¹⁵⁹

Area: 1,307 square feet¹⁶⁰

Dates of Site Visits: January 22, 2022, January 27, 2022, and February 18, 2022

Plant Table

Species	# Planted¹⁶¹	# Observed¹⁶²	Avg Height or Spread	General Condition	% Survival
Willow	5	1-2	10’ - 20’ h	Fair	20 – 40%
Cottonwood	2	2	10’ h	Fair	100%
Elderberry	2	2	15’ – 20’ h	Fair	100%
California wild rose	5	0	-	-	0%
Sycamore	1	1	20’ h	Fair	100%
Wild blackberry	20	0	-	-	0%

Estimated Cover:

- Fifty Percent (50%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Nonnative grasses

Site Highlights:

- The elderberries on the bank-top have grown exceptionally large and likely produce numerous berries to nurture birds and wildlife.

¹⁵⁸ SBCFCWCD *Annual Maintenance and Revegetation Report* (2000) (“SBCFCWCD (2020)”).

¹⁵⁹ *Id.*

¹⁶⁰ *Id.* stating site is .03 acres in size.

¹⁶¹ *Id.*

¹⁶² The only remaining plants are located adjacent to the west creek bank. All plants installed beyond 10 feet from the bank to the access road are gone with only invasive herbaceous plants growing there now.



Three Hundred Feet Downstream from Berkeley Road West Side of Creek by Access Road Site Recommendations:

- Remove all herbaceous plants between access road and creek bank.
- Replant area with blackberries, elderberries, coast live oaks, and coast live oak and riparian understory species to ensure >80% cover within 5 years pursuant to CDFW performance standards. Cage plants to prevent vandalism.
- Control weeds to ensure <5% cover pursuant to CDFW performance criteria.
- Confirm coast live oak planted as mitigation for Somerset Road oak pruning.



Figure 104. Site 13.
Sycamore tree on west
bank of San Jose Creek
approximately 300 feet
south of Berkeley
Footbridge. Trautwein.
January 27, 2022.



Figure 105. Site 13. Large elderberry on west bank of San Jose Creek approximately three hundred feet downstream from Berkeley Footbridge. Trautwein. January 27, 2022.



Figure 106. Site 13. Looking south. Approximately three hundred feet south of Berkeley Footbridge on west bank. Note lack of native plants and 100% cover by invasive exotic herbaceous plants except for the immediate bank top. Note sole oak tree in center of image planted in SBCFCWCD's Site 13. It is EDC's understanding that this oak tree was required as mitigation for oak pruning by a Somerset Avenue resident which EDC reported to the City of Goleta. If so, this oak tree cannot be double-counted as mitigation for SBCFCWCD. Trautwein. January 27, 2022.



Figure 107. Site 13 looking north toward Berkeley Road. Note sole oak tree believed to be planted as a result of City of Goleta enforcement regarding neighbor oak pruning reported by EDC northeast of Somerset Avenue Accessway. Note absence of native understory plants. Trautwein. January 27, 2022.



Figure 108. Site #13 in July 2000. Looking north towards Berkeley Road (same perspective as Figure 107 above). Note plants extended west (left) from top of west bank (right side of image) to almost access road/trail. As shown in Figure 107 above, only plants planted immediately atop the Creek bank survived. The remainder of the planted area is dominated by non-native invasive herbaceous plants including oxalis and exotic grasses. Image from SBCFCWCD Annual Maintenance and Revegetation Report. 2000.

K. Somerset Road Access Ramp

SBCFCWCD decommissioned an access ramp on the west bank, stabilized the west bank using rock rip rap, and revegetated it with willows, mulefat, giant wild ryegrass, elderberry cuttings, and other species.¹⁶³

Year Planted: 2010-2012

Area: 1,000 square feet

Date of Site Visits: January 23, 2022 and January 27, 2022.

Plant Table

Species	# Planted ¹⁶⁴	# Observed	Avg Height or Spread	General Condition	% Survival
Willows	Unknown	1	~12' h ¹⁶⁵	Fair	Unknown
Oak Trees	Unknown	0	-	-	0
Blackberry	Unknown	0	-	-	0
Mulefat	Unknown	1	-	-	Unknown
Coyote brush	Unknown	0	-	-	0
Ryegrass	Unknown	7-8	60-100 sq ft total	Fair to Good	Unknown
Elderberry	Unknown	4	5'-8' h	Fair	Unknown

Estimated Percent Total Cover:

- Fifty to seventy percent (50-70%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Oxalis
- Nonnative grasses

¹⁶³ SBCFCWCD *Annual Maintenance and Revegetation Report 2011/2012 Maintenance Season* (2012).

¹⁶⁴ The number planted was not found in any of the Annual Plans or Maintenance and Revegetation Reports, so it is impossible to calculate percent survival.

¹⁶⁵ These plantings were reported to have reached over 14' tall in 2015 in the 2015-2016 Annual Maintenance and Revegetation Report at 82 however EDC did not find photographs in subsequent Maintenance and Revegetation Reports showing plants. The 2015-16 Report shows one photograph from 2015 but the plants appear to be approximately five feet tall with the exception of one taller willow or mulefat plant. Currently, the tallest plant is the lone willow which is 12 feet tall and does not appear to have ever exceeded 12 feet in height.





Figure 109. Somerset Access Ramp Site showing giant wild ryegrass (lower portion of image), mulefat (center of image), lone willow (left side of image), and herbaceous nonnative understory vegetation. Trautwein. January 27, 2022.

Somerset Access Ramp Site Highlights:

- The giant wildrye grass is well established in this location.
- The District successfully established elderberries from cuttings demonstrating a potentially more efficient way to propagate elderberries than growing plants from seeds.

Somerset Access Ramp Site Recommendations:

- Remove oxalis and nonnative grasses for two consecutive years.
- Remove Shamel ash sapling at base of rock rip rap bank repair site
- Plant two coast live oak trees.
- Replant twenty wild blackberry and 10 giant wild ryegrass plants at 5-ft centers after oxalis and nonnative grasses are eradicated.
- Plant one hundred mugwort plants on one-foot centers.
- Water and weed until established, percent cover of invasive herbaceous plants is <5%, and percent cover of native plants increases to at least 80% pursuant to CDFW SAA success criteria.



Figure 110. Looking downstream San Jose Creek at the Somerset Access Ramp Site. The west bank hosts a large sycamore, some oaks, nonnative grasses, elderberry, wild giant rye grass, and mulefat. Trautwein. February 2022.



Figure 111. West bank of San Jose Creek at Somerset Access Ramp Site. Riprap in the foreground along the bottom edge of the image with oxalis and nonnative grasses, such as kikuyu. Large sycamore in the background center. SBCFCWCD installed willows, mulefat, several elderberries, and giant wild ryegrass at this Site. Trautwein. February 2022.



Figure 112. Nonnative Shamal ash sapling in the center foreground growing out of rock riprap along with nonnative grasses. Trautwein. February 2022.

L. One-hundred Feet Downstream from Former Somerset Access Ramp, West Bank Repair (Site 10)¹⁶⁶

SBCFCWCD reconstructed the eroding west bank, replaced a corroded metal stormwater culvert which was contributing to erosion with a plastic culvert, replaced failing pipe and wire revetment with rock rip rap (retaining one or two pipe revetment poles to support a large sycamore tree), installed willow wattling in the rip rap, and then planted the bank approximately two hundred feet downstream from the decommissioned Somerset Drive access ramp. Currently, the west bank which is located along a sharp curve upstream from the Elks Club is experiencing significant erosion which is threatening the back yard of a home located on Somerset Drive. Large patches of *Arundo* are present and native vegetation is lacking on the west bank.

Years Planted: 1996, 1997, and 1998

Area: 50 feet x 10 feet = 500 square feet

Date of Site Visits: January 27 and February 4, 2022.

Plant Table: n/a¹⁶⁷

Natives Observed:

- Willow
- Elderberry
- Wild Cucumber

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- *Arundo donax* (two large patches)

Downstream from Somerset Access Ramp Site Highlights:

- Most of the rip rap is intact.

Downstream from Somerset Access Ramp Recommendations:

- Conduct an engineering feasibility analysis. If feasible, engineer and install a live willow crib wall in base of the eroding bank. Use rip rap with willow and/or cottonwood wattling at the foot of slope in front of the crib wall if engineering analysis determines a crib wall alone would be insufficient to protect and stabilize the bank.
- Remove *Arundo* and other nonnative species and revegetate bank with sycamores, willows, blackberry, mugwort, and giant wild ryegrass.

¹⁶⁶ Uncertain whether this is the correct site number. Site numbering is very confusing and inconsistent throughout the Flood Control Maintenance and Revegetation Reports.

¹⁶⁷ No plant table is included because EDC was not able to find a list of the species other than the willow wattling or number of plants planted.



Figure 113. About one hundred to two hundred feet downstream the site of the decommissioned Somerset Drive Access Ramp on the west bank. Note the chain-link fence at the top of this slope delineates a backyard. This slope has eroded severely at the upstream end of SBCFCWCD's 1996 bank repair project. Consideration should be given to engineering and installing a live willow crib wall. Trautwein. February 2022.



Figure 114. Looking down stream of San Jose Creek at the erosion site show above (Figure 113). A large sycamore stretches over the Creek bed providing shade. Nonnative Arundo is abundant on either side of the sycamore. Kikuyu grass is present in the bottom left corner. Trautwein. February 2022.



Figure 115. Eroded west bank of San Jose Creek one to two hundred feet downstream of the site of the decommissioned Somerset Drive Access Ramp. Displaced rip rap in the bottom right corner. Young willows grow out of the remaining riprap in the center of the photo. Trautwein. February 2022.

M. Downstream from Highway 101 West Bank (Site 7)

This Site included a large patch of castor bean which SBCFCWCD eradicated before planting willow trees in 1996 to try to screen the sand and gravel operation to the west of the Site.¹⁶⁸ The Site contains several remaining willows trees but the understory is almost entirely nonnative species. Records of the numbers of plants installed were not kept. The location near Highway 101 places this Site near a well-known homeless encampment.

Date Planted: 1996¹⁶⁹

Area: 100 feet by 15 feet = 1,500 square feet¹⁷⁰

Site Visit: February 18, 2022

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	% Survival
Willows	Unknown	6	~6-8" DBH	Good	Unknown
Mugwort	Unknown	Few	-	-	0
Blackberry	Unknown	Few	-	-	0

Estimated Percent Total Cover:

- Canopy: Sixty percent (60%)
- Groundcover: Less than ten percent (10%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nasturtium
- Oxalis
- Palm Tree
- Eucalyptus
- Castor
- Cape Ivy
- Nonnative Grasses
- Tree Tobacco

Site-specific Recommendations:

¹⁶⁸ SBCFCWCD *Maintenance and Revegetation Report, 2017-2018 Maintenance Season* (December 1998).

¹⁶⁹ SBCFCWCD *Maintenance and Revegetation Report* (2000).

¹⁷⁰ *Id.*



- Remove the nonnative plants listed above and increase the groundcover with native understory species, including wild blackberry, hummingbird sage, poison oak, and mugwort.
- Plant native shrubs including gooseberry, toyon, coffeeberry, lemonade berry, and wild rose.
- Plant native trees including black cottonwood and sycamore to increase the canopy cover.



Figure 116. Nonnative understory of primarily nasturtium. Nonnative palm tree in background and eucalyptus canopy (upper portion of photo). Trautwein. February 18, 2022.



Figure 117. San Jose Creek Highway 101 Revegetation Site on west bank looking east across Creek. Several remaining willows from the 1996 revegetation project. Trautwein. February 18, 2022.



Figure 118. View from west bank looking northeast showing several remaining willows from 1996 revegetation project. Ground cover is nonnative grasses and nasturtium. Trautwein. February 18, 2022.

N. Hansen Aggregate Site

A portion of this Site was planted in 2003 when SBCFCWCD regraded the west bank where it had experienced erosion.¹⁷¹ Other sections of the bank were planted along a two-hundred-and-fifty-foot reach.¹⁷² Coconut fiber was laid down on the bank.¹⁷³ An adjacent property had added a large amount of impervious surface and not properly addressed runoff threatening to exacerbate erosion.¹⁷⁴ The operation eventually punched a hole in the bank to allow water to drain into the creek so it would not pond within the sand and gravel facility.¹⁷⁵ Subsequently, the sand and gravel operation installed a storm drain after working with regulatory agencies.¹⁷⁶ Currently, the west bank adjacent to the aggregate operation supports several large sycamore trees. There is a concrete block wall adjacent to the top of the bank installed in approximately 2000-2005. It is unclear if this block wall received permits from the City of Goleta or CDFW. Encampments have left significant amounts of trash on the west bank and Creek bed. Several willow and cottonwoods occur below the sycamore canopy and two spreading patches of wild rose are present on the bank. However, the vast majority of the bank is covered by nonnative groundcover plants.

Year Planted: 2003¹⁷⁷

Area: 2,500 square feet¹⁷⁸

Date of Site Visit: February 18, 2022

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	30	14	Variable	Fair to Good	<50%
Cottonwood	5	2	>15' h	Good	40%
Wildrose	10	At least 2 patches	Patches ranged from 8' by 25' and 8' by 20'	Good	at least 20%
Sycamore	2	0	-	-	0%

Estimated Percent Cover:

- Groundcover: Ten percent (10%)
- Canopy: Forty to sixty percent (40%-60%)

¹⁷¹ SBCFCWCD *Maintenance and Revegetation Report* (December 2003).

¹⁷² *Id.*

¹⁷³ *Id.*

¹⁷⁴ *Id.*

¹⁷⁵ *Id.*

¹⁷⁶ *Id.*

¹⁷⁷ *Id.*

¹⁷⁸ *Id.*



Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- *Arundo donax*
- Oxalis
- Nonnative Grasses
- Shamel ash tree

Hanson Aggregate Site Highlights:

- The south-facing bank adjacent to the aggregate facility makes it an ideal site for revegetation which once complete will help shield the creek from the noise and lights associated with the facility.

Hanson Aggregate Site Recommendations:

- Remove the nonnative species present and replace with native understory, including blackberry, native shrubs, including wild rose, toyon, and lemonade berry, as well as native trees, such as willows, sycamores, California bay laurels and/or cottonwoods.
- Report the block wall to the City and CDFW to ascertain permit status.



Figure 119. This is the West bank of the Hansen Aggregate site, located just downstream from the train tracks and along Hansen Aggregate company grounds. This site hosts a robust homeless population which collectively produces a lot of trash and subsequent creek pollution. Trautwein. February 18, 2022.



Figure 120. West bank of San Jose Creek at the Hansen Aggregate site. Slope is covered in nonnative grasses and fallen sycamore leaves from the large sycamore in the upper right corner of the photo. One of several remaining willow trees is shown in the upper left corner of the photo. Trautwein. February 18, 2022.



Figure 121. Several remaining willow trees are present at the Hanson Aggregate Site on the west bank of San Jose Creek beneath large sycamores. The bank is covered by nonnative plants such as oxalis and kikuyu grass. Note the concrete block wall at the top of the bank. Trautwein. February 18, 2022.



Figure 122. Looking downstream along the west bank of San Jose Creek at the Hansen Aggregate Site. One of remaining willow trees from 2003 revegetation is present in the foreground and several large sycamores in background. Willows. Trautwein. February 18, 2022.



Figure 123. Looking downstream along San Jose Creek at the Hansen Aggregate site. There are wild roses surviving from the 2022 revegetation project and these are spreading underneath the canopy of sycamores and willows. Trautwein. February 18, 2022.

O. La Goleta Condominiums Site¹⁷⁹

The La Goleta Revegetation Site is located on the south/south-east bank of San Jose Creek behind the La Goleta Condominium complex located at Armitos Avenue and Dearborn Place. Approximately 600 plants were installed at this Site in 2018 through a cooperative effort between SBCFCWCD and the La Goleta Homeowners Association (“HOA”).¹⁸⁰ EDC divided the Site into six sections for the purpose of evaluating success of revegetation efforts. Several of the subsections are performing well. The HOA planted a pollinator garden, including nonnative species, which should not be credited toward SBCFCWCD’s revegetation program, as discussed below.

¹⁷⁹ EDC segmented the La Goleta Site into six sections (not including the pollinator garden) for the purpose of tallying plants and estimating percent cover.

¹⁸⁰ SBCFCWCD *Maintenance and Revegetation Report 2020/2021 Maintenance Season* (July 2021) at 58.

Year Planted: 2018¹⁸¹

Size: 6,500 square feet¹⁸²

Dates of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.



Figure 124. La Goleta Condominiums Revegetation Site and La Goleta Homeowners Association Pollinator Garden. Google Earth. 2021.

i. La Goleta Condominiums Site Section #1

Section 1 is 450 square feet and is located at the west end of the La Goleta Revegetation Site northwest of the La Goleta Pollinator Garden discussed below and is directly behind 5514 Armitos Avenue.

Year Planted: 2018

Size: 15 feet x 30 feet = 450 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

¹⁸¹ *Id.*

¹⁸² *Id.*

Plant Table

Species	# Planted¹⁸³	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	5	~8.5' h	Good	Unknown
Sycamore	Unknown	1	15' h	Good	Unknown
Black Sage	Unknown	3	3' – 4' h, 5' – 6' s	Good	Unknown
Mulefat	Unknown	1	3 - 4' h	Good	Unknown
Coyote brush	Unknown	1	4' h	Good	Unknown



Figure 125. La Goleta Site Section #1. Healthy black sage (lower third of image) and sycamore sapling middle center. Trautwein. January 31, 2022.

¹⁸³ The SBCFCWCD Maintenance and Revegetation Report for Maintenance Season 2020-2021 states at 58 that “~600 native plants” were installed in an area “over 6500 square feet” at the La Goleta Condo Site but does not provide the number of plants planted by species. Given the relatively recent planting date, EDC was generally able to discern planted plants from naturally occurring plants to populate the Plant table for the La Goleta Condo Site.

Estimated Percent Total Cover:

- Seventy-five to eighty percent (75 - 80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)
- Sow thistle
- Shamel ash tree
- Yucca
- Mallow
- Evening primrose
- Jacaranda tree
- Carolina cherry¹⁸⁴

La Goleta Condo Site Section #1 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy, especially the black sage. (Figure 125)
- The location of Section 1 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo. Site #1 Recommendations:

- Do not count the pollinator garden toward SBCFCWCD mitigation requirements.
- Replace nonnative, invasive plants and horticultural variants of local native plants in the pollinator garden with local native varieties.
- Remove the large jacaranda, yucca and Shamel ash trees which shade the site and pose a threat of spreading in the riparian habitat. (The jacaranda tree is spreading and EDC considers Shamel ash invasive due to the extensive spread in Goleta's creeks.)
- Increase percent cover of native riparian vegetation by about 10% or more.

ii. La Goleta Condominiums Site Section #2

Section 2 is located on the south/south-east bank of San Jose Creek behind the La Goleta Condominium complex. This Section begins at the east end of Section 1 behind the pollinator garden and continues northeast along the creek bank.

Year Planted: 2018

¹⁸⁴ EDC used the Seek app for preliminary identify of this plant as Carolina cherry. However, the plant appeared similar to coffeeberry, a native shrub found in riparian areas, and may be reclassified upon further evaluation. The plant was growing under the riparian canopy and measured 11-12 feet tall. It was in good condition.

Size: 10 feet x 70 feet = 700 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Coast Live Oak	Unknown	1	10-15' h	Fair	Unknown
Black sage	Unknown	4	3' h	Good	Unknown
Purple sage	Unknown	1	5' h	Good	Unknown
Coyote brush	Unknown	4	6' h	Good	Unknown
Lemonade berry	Unknown	1	7' h	Good	Unknown
Mugwort	Unknown	3	3' h	Good	Unknown
Seacliff wild buckwheat	Unknown	3	2' h	Fair	Unknown

Estimated Cover: 75% - 85%

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (e.g., kikuyu grass)
- Jacaranda tree

La Goleta Condo Site Section 2 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section 2 provides a good buffer between the condominiums, parking lot, and Creek

La Goleta Condo Site Section 2 Recommendations:

- Remove nonnative plants including jacaranda.
- Add understory species to increase understory percent cover.

iii. La Goleta Condominiums Site Section #3

Section #3 begins at the east end of Section #2 and continues northeast along the Creek's east bank.

Year Planted: 2018

Size: 100 feet x 20 feet = 2,000 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	15	6' h	Fair	Unknown
Black sage	Unknown	5	2'- 4' h	Fair to Good ¹⁸⁵	Unknown
Mugwort	Unknown	2	2' h	Good	Unknown
Lemonade berry	Unknown	4	3' h	Fair	Unknown
Holly leaf cherry	Unknown	2	5' h	Good	Unknown
Purple sage	Unknown	4	4' h	Poor to Good ¹⁸⁶	Unknown
Wild blackberry	Unknown	2	5' s	Good	Unknown
Coast live oak	Unknown	10	6-18' h	Poor to Fair to Good ¹⁸⁷	Unknown
Coyote brush	Unknown	8	5' h	Fair to Good	Unknown
Seacliff wild buckwheat	Unknown	1	2' h	Poor	Unknown

¹⁸⁵ Sage planted under nonnative jacaranda trees received too little sunlight and some plants were in poor condition.

¹⁸⁶ Sage planted under nonnative jacaranda trees received too little sunlight and some plants were in poor condition.

¹⁸⁷ Oaks were either planted to close together or represent natural recruitment.

Estimated Percent Total Cover:

- Seventy-five to eighty-five percent (75-80%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Jacaranda (3)
- *Eucalyptus citriodora*
- Pines (2)

La Goleta Condo Site Section #3 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section 3 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo Site Section #3 Recommendations:

- Remove the jacarandas, eucalyptus, and pine trees.
- Control exotic invasive species such as nonnative grasses.
- Plant additional riparian plants to increase percent cover of native species.

iv. La Goleta Condominiums Site Section #4

Section 4 begins at the east end of Section 3 and continues northeast along the east bank of San Jose Creek.

Year Planted: 2018

Size: 85 feet x 15 feet = 1,275 square feet

Dates of Site Visits: January 24, 2022 and January 31, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	5	1' h	Poor to Dead ¹⁸⁸	0
Sycamore	Unknown	2	3' h	Fair ¹⁸⁹	Unknown
Black sage	Unknown	1	5' h	Fair	Unknown
Mulefat	Unknown	1	6' h	Fair	Unknown
Deadly nightshade	Unknown	1	1' h	Good	Unknown
Holly leaf cherry ¹⁹⁰	Unknown	1	5' h	Fair	Unknown
Purple sage	Unknown	1	3' h	Fair	Unknown
Wild blackberry	Unknown	2	5' s	Poor to Fair	Unknown
Coast live oak	Unknown	2	8' h	Fair to Good ¹⁹¹	Unknown
Coyote brush	Unknown	3	4' h	Fair	Unknown

Estimated Cover:

- Thirty-five to forty-five percent (35-40%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., Kikuyu Grass)
- Lemon-scented gum (*Eucalyptus citriodora*) (3)
- *Arundo donax* (appears to have been cut back)
- English/Algerian Ivy

¹⁸⁸ The planting location appears to be too dry to support willows without substantial irrigation.

¹⁸⁹ Sycamores planted under mature eucalyptus received too little sun.

¹⁹⁰ The Seek app identified this as holly leaf cherry however it appeared to be a Catalina island cherry. A definitive identification should be made and only holly leaf cherries retained.

¹⁹¹ Some oaks were either planted too close together or represent natural recruitment at the site.



La Goleta Condo Site Section #4 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #4 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condo Site Section #4 Recommendations:

- A willow snag was cut out – this should have remained as habitat for resident species.
- Remove English/Algerian ivy to protect sycamore and other native species in the area. (Figure 127)
- Remove Eucalyptus – they are shading out native plants.
- Remove *Arundo donax*.
- Increase percent cover of natives to 80% - 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Ensure future planting of oaks, willows, and sycamores are planted with ample room to grow (not too close to each other).
- Install drip irrigation or plant willows and sycamores closer to the Creek in more mesic conditions.

v. La Goleta Condominiums Site Section #5

La Goleta Condominium Site Section #5 begins at the east end of Section #4 and continues northeast along the east Creek bank.

Year Planted: 2018

Size: 85 feet x 17 feet = 1.445 square feet

Dates of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	10	6-10' h	Fair ¹⁹²	Unknown
Sycamore	Unknown	4	4-20' h	Fair	Unknown
Black sage	Unknown	4	5' h	Good	Unknown
Lemonade berry	Unknown	6	4' h	Fair to Good	Unknown
Mugwort	Unknown	2	5' s	Good	Unknown
Black cottonwood	Unknown	2	10' h	Fair	Unknown
Purple sage	Unknown	1	4' h	Good	Unknown
Wild blackberry	Unknown	3	4'-10' s	Good	Unknown
Coast live oak	Unknown	1	22' h	Good	Unknown
Coyote brush ¹⁹³	Unknown	10	4' h	Poor	Unknown
Seacliff wild buckwheat	Unknown	2	6' s	Good	Unknown

Estimated Percent Total Cover:

- Seventy-five percent (75%)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)

La Goleta Condominium Site Section #5 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #5 provides a good buffer between the condominiums, parking lot, and Creek.

¹⁹² Willows planted too far from the creek received too little water and were in poor to fair condition.

¹⁹³ Numerous coyote brush throughout the site were severely pruned adversely affecting their general condition and contribution to the restored habitat. (Figure 128)



La Goleta Condominium Site Section #5 Recommendations:

- Do not prune or trim the coyote brush – these plants have been unnecessarily cut back. (Figure 128)
- Remove the nonnative kikuyu grass.
- Increase percent cover of natives to 80% or 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Future planting of willows should be placed closer to the creek bed where water access is more available.

vi. La Goleta Condominiums Site Section #6

Section #6 of the La Goleta Condominium Site begins at the east end of Section #5 and continues northeast along the Creek bank for one hundred feet.

Year Planted: 2018

Size: 100 feet x 5 – 10 feet = 500 – 1,000 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

Plant Table:

Species	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	4	3-12' h	Dead to Fair *2 dead, 1 good	Unknown
Sycamore	Unknown	1	4' h	Fair	Unknown
Purple sage	Unknown	1	-	Dead	Unknown
Seacliff wild buckwheat	Unknown	3	3'-10' s	Good	Unknown
Mugwort	Unknown	3	2'-8' s	Fair	Unknown
Lemonade berry	Unknown	4	6'-9' h	Poor	Unknown
Wild blackberry	Unknown	2	15' s	Good	Unknown
Coyote brush	Unknown	3	6' h	Good	Unknown

Estimated Percent Total Cover:

- Seventy-five percent ($\approx 75\%$)

Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Nonnative grasses (i.e., kikuyu grass)
- Oxalis
- Myoporum (within and adjacent to Site #6)
- Bird of paradise
- Castor bean (Figure 126)

La Goleta Condominium Site Section #6 Highlights:

- This site includes greater plant species diversity than many of the other revegetation sites evaluated.
- The plants are generally healthy.
- The location of Section #6 provides a good buffer between the condominiums, parking lot, and Creek.

La Goleta Condominium Site Section #6 Recommendations:

- Remove the nonnative and invasive species listed above.
- Increase percent cover of natives to 80% - 90% to ensure CDFW cover-based performance criterion is met or exceeded.
- Plant more willows, closer to the creek bed, and if water levels are low, install drip irrigation.

La Goleta Site Overall Plant Table

Species	# Planted	# Observed	Estimated % Survival
Willow	Unknown	39	Unknown
Sycamore	Unknown	8	Unknown
Black cottonwood	Unknown	2	Unknown
Coast live oak	Unknown	14	Unknown
Lemonade berry	Unknown	15	Unknown
Holly leaf cherry	Unknown	3	Unknown
Black sage	Unknown	17	Unknown
Purple sage	Unknown	8	Unknown
Mulefat	Unknown	2	Unknown
Coyote brush	Unknown	29	Unknown
Seacliff wild buckwheat	Unknown	9	Unknown
Nightshade	Unknown	1	Unknown
Mugwort ¹⁹⁴	Unknown	10	Unknown
Wild blackberry ¹⁹⁵	Unknown	9	Unknown
Total	~600	166	26.7%

¹⁹⁴ Number observed includes individual plants believed to be planted, however mugwort plants are actively spreading indicating successful establishment.

¹⁹⁵ Number observed includes individual plants believed to be planted, however wild blackberry plants are actively spreading indicating successful establishment.





Figure 126. Castor bean at La Goleta Revegetation was flagged and retained. Trautwein. January 2022.



Figure 127. Algerian ivy at the La Goleta Site Section #4 is smothering this native tree. Trautwein. January 2022.



Figure 128. Coyote brush, purple sage, black sage, lemonade berry, and sycamore at La Goleta Site Section 5. Coyote brush was pruned heavily. Trautwein. February 2022.

vii. La Goleta Condominium Pollinator Garden

Kitson Nursery planted a pollinator garden for the HOA east of Section #1. SBCFCWCD references a “pollinator” garden of “native” plants which “will supplement the riparian plantings.”¹⁹⁶ This garden is location directly behind 5514 Armitos Avenue in the La Goleta Condominium complex. This garden appears to have been installed using wildflower seed packets. It contains non-local, horticultural varieties of native species which could escape into the riparian habitat or hybridize with local varieties of these species. There are several nonnative invasive species populating the area. The pollinator garden contains non-riparian species. The pollinator garden was not counted towards SBCFCWCD’s mitigation requirements. However, inclusion in the Annual Maintenance and Monitoring Reports may create the appearance that it was counted as mitigation by SBCFCWCD.

Date Planted: 2018 or later

Size: 82 feet x 18 feet = 1,476 square feet

Date of Site Visits: January 24, 2022, January 31, 2022, and February 18, 2022.

*Horticultural varieties of native species present:*¹⁹⁷

- California poppy
- Elegant clarkia
- Lupine
- Matilija poppy
- Coyote brush
- Goldenrod
- Vervain
- Encelia¹⁹⁸

Invasive and Naturalized Nonnative Plants Present in and Near Revegetation Site:

- Mallow
- Kikuyu grass
- Sow thistle
- Jacaranda (seedling)

La Goleta Condominium Pollinator Garden Recommendations:

- The HOA should replace plants with local varieties of native flowering plants to prevent spread of non-local varieties and non-native plants in riparian corridor.
- Clarify in future reports that the pollinator garden does not count towards SBCFCWCD mitigation credits.

¹⁹⁶ SBCFCWCD Maintenance and Revegetation Report (2020-2021) at 58.

¹⁹⁷ Some of these species are not native to Goleta watersheds e.g., Matilija poppy.

¹⁹⁸ Two Encelia shrubs appeared to have been planted as container plants rather than from seed packets.



Figure 129. The La Goleta Condominium Pollinator Garden. The seed mix planted here contains California natives and non-local horticultural varieties of local native species, such as the elegant clarkia above. It is not habitat restoration and not riparian habitat. It was not counted toward SBCFCWCD's mitigation requirements. Trautwein. January 31, 2022.

P. Armitos Road Arundo Removal (Site 8)

SBCFCWCD removed *Arundo donax* from this site, installed rock rip rap, and planted native vegetation in the early to mid-1990s.¹⁹⁹ Vandalism occurred requiring replanting several times. This was one of SBCFCWCD’s first Arundo removal sites. After first replanting in 1993, storms, vandalism and unsuccessful planting required the Flood Control District to replant several more times in 1995, 1997 and lastly in 1998. The 1997-1998 Maintenance and Revegetation Report says that the Creek bank was destroyed and had to be “rebuilt.”²⁰⁰ We assumed this to mean that riprap was installed at the Armitos Road Site. The Report is vague in its location description so we cannot be one hundred percent confident that we analyzed the exact Site. However, we assume the Site we analyzed is the correct location given the description of being adjacent to the La Goleta Condominiums and Armitos Road and the presence of rip rap.

Years Planted: 1991, 1995, 1997, and 1998

Size:

- Reported: 219 feet x 20 feet = 4,380 square feet²⁰¹
- Observed: 171 feet x 20 feet = 3,420 square feet recorded during field survey

Date of Site Visit: February 28, 2022.

Estimated Percent Total Cover:

- Understory ~50%
- Canopy ~ 90%

Plant Table

Species ²⁰²	# Planted	# Observed	Avg Height or Spread	General Condition	Estimated % Survival
Willows	Unknown	Above riprap: 7 In riprap: 3	20-30’ h	Good	Unknown
Sycamore	Unknown	2	35-45’ h	Good	Unknown
Cottonwood	Unknown	6	45-50’ h	Good	Unknown
Coast Live Oak	Unknown	1	25’ h	Good	Unknown
Mulefat	Unknown	-	6’ h	Good	Unknown
Blackberry	Unknown	1	-	Good	Unknown
Wild Cucumber	Unknown	-	-	Good	Unknown

¹⁹⁹ SBCFCWCD *Maintenance and Revegetation Report 1995/1996 Maintenance Season* (December 1, 1996).

²⁰⁰ SBCFCWCD *Maintenance and Revegetation Report, 1997-1998 Maintenance Season* (December 1998) (“SBCFCWCD (1998)”).

²⁰¹ SBCFCWCD (1998).

²⁰² The species included in the Armitos Road Site Plant Table are based on species observed at the Site because we found no list of species planted in the Maintenance and Revegetation Report for the 1997-1998 Maintenance Season.



Invasive and Aggressive Nonnative Plants Present in and Near Revegetation Site:

- Shamel ash (2) – 12” DBH
- Eucalyptus (2) – 45-50’ tall
- Nonnative grasses
- Oxalis
- Umbrella plant
- Castor bean
- Bottlebrush
- Yucca tree

Armitos Road (Site 8) Highlights:

- Overall, the site looks particularly good and healthy. The trees are well-established and the understory includes dense patches of native plants, including poison oak and wild blackberry.

Armitos Road (Site 8) Recommendations:

- Remove nonnative species from understory and replace with native mug wort, blackberry, and giant rye grass.
- Remove Shamel ash, castor bean, bottlebrush, and Eucalyptus trees. Replace with native woody species like cottonwood, sycamore, and willow and oak trees farther away from the Creek.



Figure 130. Four native tree species including black cottonwood, sycamore, willow, and coast live oak are present at the Armitos Road Site. Blackwelder. February 28, 2022.



Figure 131. Looking upstream at the Armitos Site. Several young willow and cottonwood trees populating the east bank of San Jose Creek. Blackwelder. February 28, 2022.



Figure 132. Rip rap at the Armitos Road Site. Horizontal willow with several new up shoots. In the back left corner is a nonnative, invasive Shamel ash tree. Middle left is a nonnative eucalyptus tree. Upper right corner is a nonnative Yucca tree. The vines growing over the riprap are native wild cucumber. Blackwelder. February 28, 2022.



Figure 133. Native wild cucumber within native willow branches. Two large nonnative, invasive eucalyptus trees in the background. Blackwelder. February 28, 2022.



Figure 134. Looking upstream at the Armitos Site on San Jose Creek. The Creek bed is clear of obstructive vegetation although a small cottonwood sucker is sprouting up in the creek bed. Blackwelder. February 28, 2022.

V. LAKE LOS CARNEROS MITIGATION BANK

EDC also reviewed the success of the Lake Los Carneros Mitigation Bank. As discussed above on page 13, there are pros and cons with mitigation banks. The Lake Los Carneros Mitigation Bank, although an offsite mitigation location, nonetheless appears to be successful by creating a large continuous habitat with value for birds and wildlife.

Site A:

Site A appears to be well-established although drought-stressed. It contains a mix of upland and riparian species. The 2020 Annual Report found that upland areas were performing well with riparian species becoming established.²⁰³ Without sufficient rainfall or supplemental watering, this site may transition from containing riparian species such as willows to upland species dominated by coyote brush and/or exotic species.

Highlight:

- Two fifteen-foot-high bay laurel trees have become established in Site A and are flowering despite cessation of irrigation and despite the climate change-induced drought.

Recommendations:

- Continue to monitor Site A. If it currently considered all or part riparian habitat and it transitions into upland habitat due to the ongoing drought, consider tracking it at a .75:1 credit for mitigation.²⁰⁴
- Control nonnative weeds in the understory of riparian trees and around and between upland shrubs.
- Plant additional native species in open area between shrubs near the western side of Lake Los Carneros Dam.

Site D

Site D is one of the moister polygons and the largest polygon in the bank. It currently supports willows, sycamores, coast live oaks, cottonwoods, and at least one small bay sapling and an understory consisting almost entirely of wild blackberry in the southern portion of the Site. The cottonwoods approach 10" DBH and appear to be spreading by root suckers. The willows and sycamores appear healthy although some may be drought-stressed. Some willows have succumbed to the drought in drier portions of Site D. (Figure 135) Overall the southern portion of Site D is in good condition while the northern portion is in fair to good condition.

²⁰³ SBCFCWCD, *Los Carneros Mitigation Bank Progress Report* at 2 (Summer 2021).

²⁰⁴ *Id.*; See also CDFW SAA at 16.



Figure 135. Some willows have not survived the climate change-induced drought in Site D, but overall, the Site is in fair to good condition despite the climate change drought. Trautwein. February 7, 2022.

Highlights:

- Eradication of pampas grass in the southwest portion of Site D was remarkably successful and no pampas grass plants were observed during surveys.
- This site contains healthy and well-established riparian woodland vegetation, including canopy trees and understory, especially in the moister southern portion of Site D.
- Natural recruitment of coast live oaks is occurring.
- An exceptionally large greenback ceanothus shrub at least twenty feet high is present and likely predated revegetation efforts by several decades.

Recommendations:

- Continue to monitor Site D annually or every other year using aerial photographs and ground surveys.
- Document and contain invasions of nonnative plants.

- Leave trees that perish as snags.
- If riparian vegetation transitions to upland vegetation, ensure nonnative plants do not invade, consider planting upland species in such areas to facilitate the transition, and apply the .75:1 credit ratio for upland areas.
- The understory consists of almost entirely blackberry, although mugwort is also present. The understory should be diversified through planting additional understory species including wild giant ryegrass, gooseberry, hummingbird sage, snowberry, and mugwort.
- Remove Shamel ash trees located in this Site close to Calle Real. This species is known to outcompete willows in riparian areas and could significantly displace the riparian vegetation installed in Site D. Monitor for invasion by Shamel ash trees.

Sites E, F, and G

The three southeastern sites contain cottonwood, sycamore, willows, and bay laurel trees and these trees generally appear to be in good condition. The northern part of Site E contains open areas lacking tree cover. EDC's surveys viewed these Sites from a nearby trail so we did not get close enough to examine the understory.

Highlights:

- Site contains diversity of habitats and tree species.
- Location near grasslands to northeast may support white-tailed kite and raptor nesting, roosts, or perches.

Recommendations:

- Continue to monitor Sites E, F, and G annually or every other year.
- If riparian vegetation transitions to upland vegetation due to drought, retain snags, plant upland species, control nonnative weeds, and apply the .75:1 credit ratio for upland areas.

Sites H and I

Sites H and I are located closest to Calle Real which appears to pond water or slow the flow of groundwater south due to soil compaction associated with the road, resulting in moist conditions conducive to riparian plant establishment. These sites appear to be in good condition. They support willows, sycamores, and oak trees, including natural recruitment of oak trees. The understory is primarily native vegetation including blackberries, wild giant ryegrass, and snowberries.

Highlights:

- Good moisture retention benefits riparian species.
- Presence of snowberries.
- Natural oak recruitment.

Recommendations:

- Continue to monitor Sites H and I annually or every other year using aerial photos and ground surveys.
- Search for pampas grass and other nonnatives and remove within one year of detection.
- If riparian vegetation transitions to upland vegetation, plant upland species to facilitate transition, control nonnative weeds which might colonize during the transition, and apply the .75:1 credit ratio for upland areas.
- Eradicate castorbean located along Calle Real.

Site J

Site J contains large cottonwood trees approaching forty feet tall and eight-inch DBH. It contains at least one large gooseberry, as well as mugwort and naturally occurring oak saplings.

Highlights:

- The large cottonwoods are phenomenally successful.

Recommendations:

- Continue to monitor Site J annually or every other year using a combination of aerial photographs and field surveys.
- If riparian vegetation transitions to upland vegetation, plant upland species, control nonnative weeds they may colonize during transition, and apply the .75:1 credit ratio for upland areas.
- Monitor and control invasive species to prevent significant infestations.
- Plant native plants around the perimeter of the woodland where native plants are absent.

Site K

Site K is located toward the southern end of the Lake Los Carneros Mitigation Bank. It is a moist area suitable for riparian species. Willows and wild blackberries installed by SBCFCWCD thrive here. Rushes were noted in or near Site K. (Figure 136)



Figure 136. Rushes in or near Site K. Trautwein. February 7, 2022.

Highlights:

- Moist area supports healthy riparian vegetation installed by SBCFCWCD.

Recommendations:

- Continue to monitor Site K annually or every other year.

Site L

This site contains coast live oak trees, cottonwood trees, wild giant ryegrass, and coyote brush. This site appears to be transitional between riparian and upland. Given the drought, this site could transition to upland and become dominated by coyote brush and/or nonnative weeds such as mustard.

Recommendations:

- Continue to monitor Site L annually or every other year using aerial photos and field surveys.
- If riparian vegetation transitions to upland vegetation, plant upland species, control nonnatives that may colonize the area, and apply the .75:1 credit ratio for upland areas.
- Eradicate Cape ivy in this section and monitor for reinfestations.
- Eradicate exotic Cape smilax denoted by pink flags underneath large coast live oak tree on west wide of Site along main trail.²⁰⁵ (Figures 137 a and b)

²⁰⁵ The Seek App identified this species as Cape smilax.



Figure 137 a. and b. Nonnative Cape smilax under oak in Site L. Trautwein. February 7, 2022.

Sites M and N

These Sites are on the far west side of the bank. They support dense willow thickets with wild blackberry understory. They are well established but in fair condition given the drought.

Highlights:

- Wood rat nests were observed in this area indicating wildlife use in restored areas.

Recommendations:

- Continue to monitor Sites M and N annually or every other year using both aerial photographs and field surveys.
- If willows succumb to the drought, retain snags.
- If riparian vegetation transitions to upland vegetation, monitor to ensure native upland species colonize the area, plant upland species if necessary to maintain native plant cover and diversity native upland plant community, control nonnative weeds which may colonize the area, and apply the .75:1 credit ratio for upland areas.

Site O

Site O located south of the Dam is one of the largest sites in the Lake Los Carneros Mitigation Bank and also the most upland sites. It contains upland species, including sumac, blackberry, oak, , lemonade berry coyote brush, buckwheat (Figure 138), Encelia, Santa Barbara honeysuckle, coffeeberry, purple sage, and California sage, as well as riparian species including

sycamore. (Figure 139) Providing upland plant communities enhances the functionality of the adjoining riparian woodlands by allowing for use by a greater number of wildlife species.²⁰⁶ Overall plant health ranges from poor to good with riparian species closest to the Dam (northern portion of Site O) generally in the poor to fair range with some dead willows. (Figure 140) Some sycamores in this area are in good condition.

The western portion is almost solid coyote brush, which may not meet the CDFW success criteria related to percent cover by diverse native species.²⁰⁷

Aerial photos from as recently as 2019 showed good percent cover of natives e.g., 75-80%, however, it appears that since watering ceased during the current megadrought, some native plants have not fared well. The percent cover still appears to be in the range of 70-80% but lower in the northern portion of Site O. (Figures 141 and 145) Nonnative herbaceous plant communities are present in the northern portion of Site O and dominate the understory. Overall, Site O may be the least successful polygon in the Lake Los Carneros Mitigation Bank.

Highlights:

- Large number of upland species established.
- Presence of Santa Barbara honeysuckle, a California Rare Native Plant species.

Recommendations:

- Continue to monitor annually or every other year using aerial photos and field surveys to ensure riparian sites remain riparian and do not transition to native or nonnative upland plant communities because such a transition would signal long-term failure of mitigation.
- If riparian vegetation transitions to upland vegetation, monitor to ensure nonnative plants do not colonize the area, control nonnative species which may colonize the area, plant native upland plants to ensure species diversity, apply the .75:1 credit ratio for upland areas.
- Control invasive herbaceous understory species especially in northern portion of Site O.
- Replant upland species in open northern portions of Site O dominated by nonnative species.
- Increase upland plant diversity in western portion of Site O currently dominated by coyote brush.

²⁰⁶ SBCFCWCD, *Los Carneros Mitigation Bank Progress Report* at 2 (Summer 2021).

²⁰⁷ CDFW SAA Success Criterion 3.9(b) at 17.



Figure 138. Buckwheat in northern portion of Site O. Trautwein. February 7, 2022.



Figure 139. Upland plant community in northern portion of Site O including coyote brush, lemonade berry, and buckwheat, with nonnative mustard growing between native plants. Note dam in background. Trautwein. February 7, 2022.



Figure 140. Some willows have died due to apparent lack of water in the northern portion of Site O, including this one which grew around an irrigation line. Trautwein. February 7, 2022.



Figure 141. Northern portion of Site O closest to the dam contains open areas dominated by nonnative plants. Trautwein. February 7, 2022.

Lake Los Carneros Mitigation Bank Overall Highlights:

- Eradication of large number of large pampas grass and German ivy plants with no young pampas grasses or German ivy apparent.
- Large contiguous area of habitat created.
- Success of riparian trees and understory species, especially in southern polygons (Sites K, J, I, H, and D).
- Co-location of upland and riparian areas to increase habitat diversity and benefit birds and wildlife.

Lake Los Carneros Mitigation Bank Overall Recommendations:

- Continue to monitor annually or every other year using aerial photos and field surveys to ensure riparian sites remain riparian and do not transition to native or nonnative upland plant communities because such a transition would signal long-term failure of mitigation.
- If riparian vegetation transitions to upland vegetation, apply the .75:1 credit ratio for upland areas.
- Control invasive species including Shamel ash and Cape ivy.
- Monitor for other invasive species and control to prevent significant infestations, e.g., castorbean, pampas grass, and mustard.
- Retain Monterey cypress snags.
- Remove all irrigation lines once sites are established. Reinstall only if needed to prevent loss of riparian vegetation due to the megadrought. (Figure 142)



Figure 142. Irrigation lines are broken in some areas and should be removed to prevent plastic pollution. Trautwein. February 7, 2022.



Figure 143. Los Carneros Mitigation Banks site prior to removal of pampas grass. Google Earth. November 10, 2003.



Figure 144. Lake Los Carneros Mitigation Bank Polygon Map. Safter pampas grass removal and before maturation of plantings. SBCFCWCD. Circa 2009.

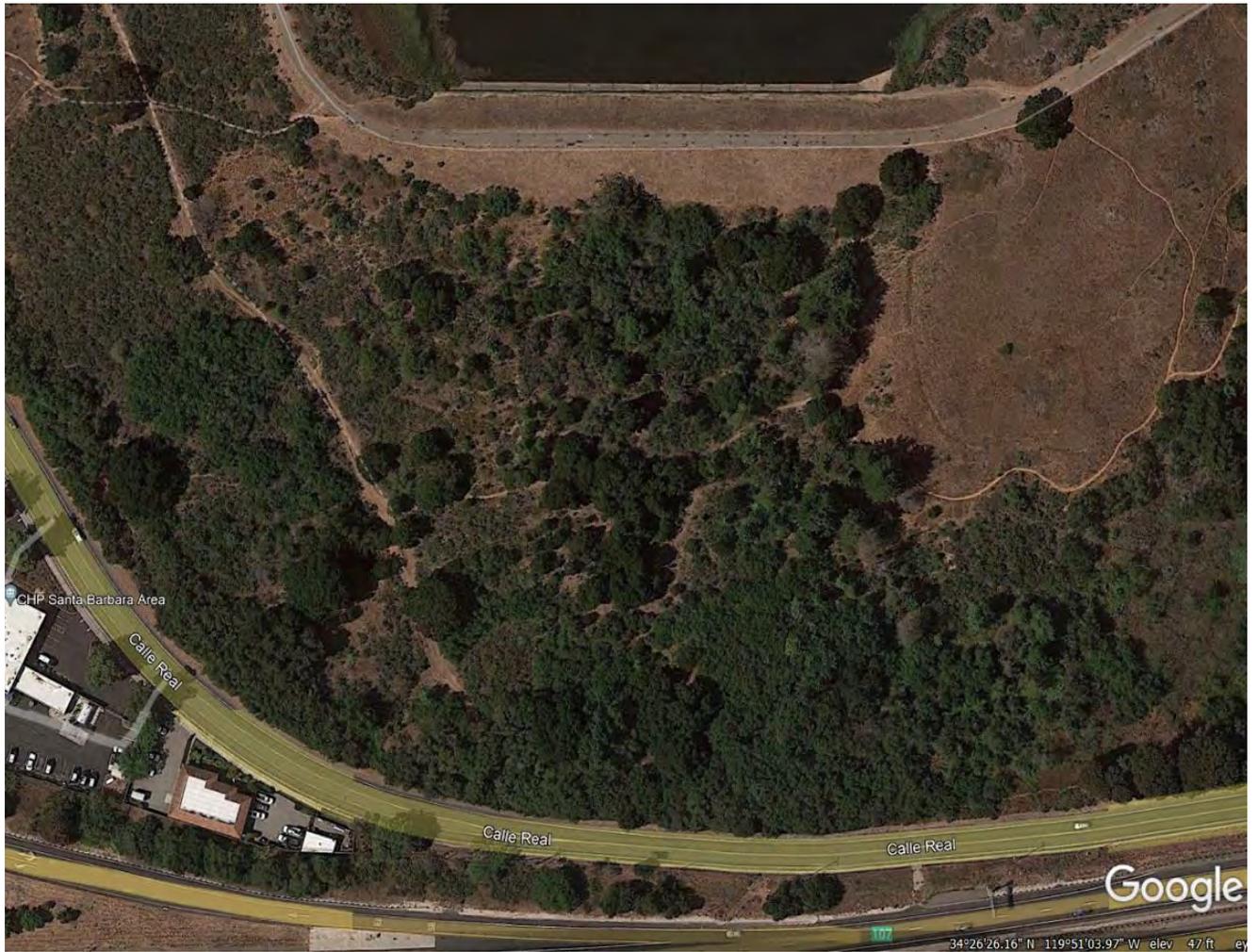


Figure 145. Los Carneros Mitigation Bank after maturation of plantings. Google Earth. August 2019.

VI. GLOBAL RECOMMENDATIONS FOR SBCFCWCD REVEGETATION

The following revegetation recommendations for SBCFCWCD and permitting agencies such as CDFW are designed to (1) ensure impacts to the quality and quantity of riparian vegetation are mitigated over the long-term through successful establishment of permanent riparian habitat by revegetation projects, (2) improve the success of riparian revegetation projects, and (3) enable more effective and quantitative monitoring and tracking of revegetation sites by providing measurable data relevant to CDFW's SAA success criteria, such as percent cover, plant survival rates, plant growth rates, and presence and percent cover of invasive species.²⁰⁸

- A. Mitigation Ratio. Discussion:** Observations indicate that 2015 SAA success criteria are not always met in the short term and long term and that compensatory mitigation is inadequate to offset or substantially lessen impacts.²⁰⁹ Specifically, observations documented herein demonstrate that a number of revegetation projects do not meet success criteria related percent cover, percent cover of herbaceous invasive understory, tree growth rates, and presence of woody nonnative vegetation over the short and long term. Success criteria also indicate that ratios should be greater than 1:1 for impacts which are not mitigated by implementing revegetation within a year.²¹⁰ Observations and review of SBCFCWCD Maintenance and Revegetation Reports indicate that mitigation for temporary impacts is not always implemented with a year, and/or is not successful the first year and requires replanting. Despite this, the SBCFCWCD appears to only credit mitigation for temporary impacts at 1:1. Furthermore, revegetation sites typically contain lower species diversity compared to adjacent natural habitats.

Recommendation: Given the failure of some revegetation sites to meet and maintain success with respect to the measurable criteria, the mitigation ratio for temporary impacts must be increased to ensure adequate mitigation. The ratio should also be increased because despite the requirement of Criterion 3.1 SBCFCWCD does not undertake mitigation at a ratio greater than 1:1 for revegetation projects occurring after the first growing season following impacts. Therefore, EDC recommends that CDFW and other agencies amend the SAA and other permits to increase mitigation ratio for temporary impacts (a) from 1:1 to 2:1 to account for failure to achieve and maintain success pursuant to the criteria, and (b) from 1:1 to 3:1 for revegetation projects successfully initiated later than the first growing season after the impact occurs.

- B. One-time Mitigation of Temporary Impacts Within Ten Years. Discussion:** Instream riparian habitat that is cleared typically recovers and creates important habitat within five years if not re-cleared. Currently the trigger for mitigating

²⁰⁸ CDFW SAA at 17.

²⁰⁹ Success Criterion 3.9(f) notably involves attainment "for the life of the project." CDFW (2015) at 17.

²¹⁰ Criterion 3.1 requires, "a 1:1 acreage replacement ration *if habitat is replaced the following growing season.*" (*Emphasis added.*) CDFW (2015) Criterion 3.1 at 15.

temporary impacts is ten years. However, when riparian habitat in the same location is re-cleared after a five-year period, new temporary vegetation and habitat impacts result, triggering the need for new mitigation in the form of revegetation.

Recommendation: Amend the SAA and other permits to require revegetation each time a temporary impact occurs in the same location after five years (i.e., reduce the permit trigger from ten years to five years).²¹¹ Keep the permit term at ten years unless it must match the permit trigger, in which case reduce the permit term to five years to match the mitigation trigger.

- C. In-kind Mitigation.** Discussion: Revegetation sites such as COVA are upland plant communities (e.g., Ceanothus, California sage, coyote brush, white sage, black sage, etc.) which do not mitigate loss of riparian habitat. Recommendation: Amend the SAA and other permits to not count upland revegetation sites as mitigation for loss of riparian species or to count upland revegetation sites at lower credit ratio, i.e., 50%.²¹²
- D. In-watershed Mitigation and Use of Lake Los Carneros Mitigation Bank.** Discussion: SBCFCWCD appropriately attempts to mitigate within the site of disturbance or within the same watershed whenever possible, but there is no decision tree or process for determining when onsite or in-watershed mitigation is infeasible. Recommendation: Amend the SAA and other permits to include a decision tree or enforceable process to ensure revegetation occurs in the watershed where impact occurs to the maximum extent possible.²¹³ Permitting agencies should develop an objective, quantitative method for determining when the Lake Los Carneros Mitigation Bank must be used. For example, a decision tree which includes mapping and assessing the feasibility of available onsite public and private revegetation locations, including landowner cooperation, could be used to determine when onsite or in-watershed revegetation is infeasible thereby triggering the use of the Lake Los Carneros Mitigation Bank.
- E. Increase Species Diversity.** Discussion: Revegetation projects often include a low species diversity compared to the adjacent native riparian habitats. Recommendation: Amend the SAA and other permits to increase diversity of species planted at revegetation sites.
- F. Credit Eradication of Invasive Nonnative Plant Species as Mitigation.** Discussion: Invasive plant species are spreading in San Jose Creek and other

²¹¹ CDFW (2015) Criterion 3.2 at 15.

²¹² The Lake Los Carneros Mitigation Bank only credits upland vegetation at a .75:1 ratio.

²¹³ Santa Barbara County (2008); *See e.g.*, 2020-2021 Maintenance and Revegetation Report at 37 stating that SBCFCWCD “may draw from the surplus restoration for the other watersheds in the Goleta Slough system.”

riparian habitats which are maintained by SBCFCWCD.²¹⁴ As discussed above, revegetation projects do not always successfully mitigate the impacts of the SBCFCWCD Annual Maintenance Program over the long term. Therefore, there should be a requirement for SBCFCWCD to eradicate or control specific invasive species, including but not limited to Shamel ash, castorbean, tamarix, and *Arundo donax*, to prevent their spread in areas subject to maintenance in a given year's Annual Plan. We acknowledge SBCFCWCD's statement that it is not funded specifically to restore habitat. However, successful restoration is required by resource agency permits and CEQA. Effective removal of invasive species would create additional areas for onsite revegetation and would help ensure successful mitigation to fulfill permit requirements.

Recommendation F1: Regulatory agencies should require SBCFCWCD to remove specified invasive species, including seedlings, saplings, and plants from the riparian corridor to the maximum extent feasible within areas subject to maintenance in each Annual Maintenance Plan to mitigate the effects of the Annual Maintenance Program.

In the absence of a requirement to remove invasive species as part of the Annual Maintenance Plan, there should be an effective incentive for SBCFCWCD to remove these species.

Recommendation F2: Regulatory agencies should credit effective eradication of exotic invasive species to SBCFCWCD's mitigation program when SBCFCWCD (1) documents removal of such species from sections of creek subject to maintenance, (2) successfully revegetates areas over one hundred square feet where invasive species are effectively removed, and (3) continues to remove and document removal of invasive species seedlings, saplings, and plants for five years following initial eradication. If invasive species are successfully eradicated over five years in a given area and the area is successfully revegetated pursuant to success criteria, the areas should count as 100% mitigation credit (i.e., one hundred square feet of eradication and revegetation should count as one hundred square feet of mitigation). Areas where over one hundred square feet of invasive plants are successfully eradicated must be revegetated. If areas where invasive species are successfully eradicated are less than one hundred square feet and not revegetated, the areas of eradication should count as 50% mitigation. For Recommendation F2 to be effective at improving the success of mitigation, long-term monitoring by SBCFCWCD and regulatory agencies must demonstrate that the targeted invasives are being effectively minimized and replaced with native species.

G. Increase Weed Control Efforts and Ensure Removal of Nonnative Trees in Revegetation Sites. Discussion: EDC observed significant levels of nonnative invasive woody vegetation and nonnative invasive herbaceous plants often over

²¹⁴ EDC, *Goleta Watersheds and Wildland Urban Interfaces: Enhancing Fire Safety and Riparian Forest Health at 178-187* (November 2021)

50-90% at revegetation sites even though Success Criterion 3.9(c) states, “no woody invasive species shall be present, and herbaceous invasive species shall not exceed five percent.”²¹⁵ SBCFCWCD stated that when it obtains approval for revegetation projects on private land, such as at COVA, landowners sometimes request them to preserve nonnative trees within the revegetation area, and SBCFCWCD has no option but to comply if it wants to plant on private land. EDC observed nonnative trees, including eucalyptus, yucca, and jacaranda retained in several revegetation sites located on private property, such as the COVA and La Goleta sites. This is problematic because these tree species shade revegetation sites and were observed spreading near the riparian habitat revegetation sites.

The SAA requires that invasive herbaceous species cover no more than five percent of revegetation sites. SBCFCWCD stated that invasive herbaceous species like oxalis are widespread in local watersheds and it would take significant resources to try to control them within revegetation sites, so SBCFCWCD tolerates these species and does not expend the resources necessary to remove these species to try to meet the success criterion for invasive herbaceous species.²¹⁶

Permitting agencies generally do not hold SBCFCWCD to the quantitative success criteria.²¹⁷ According to SBCFCWCD, it is better to track density and presence of multilayered canopies than percent survival when monitoring success of revegetation sites.²¹⁸ Part of the reason for this is because it is hard to count the number of surviving plants for species which grow by rhizome such as California rose and wild blackberry. SBCFCWCD would prefer to have Success Criterion 3(g) (percent survival) removed from the permits but the permitting agencies prefer to retain the criteria.²¹⁹

Recommendation G1: Retain all success criteria in the SAA.

Recommendation G2: Require increased weed control efforts at revegetation sites to fulfill CDFW Success Criterion 3.9(g) to achieve zero percent presence of invasive woody species and less than five percent cover of invasive herbaceous vegetation at revegetation sites as required under Success Criterion 3.9(g).

Recommendation G3: Permitting agencies should require SBCFCWCD to collaborate with landowners to ensure that nonnative and especially invasive woody species are removed from SBCFCWCD revegetation sites to the maximum extent possible before mitigation is accepted as successful. Retaining invasive or nonnative woody plants in revegetation sites should be prohibited to the maximum extent possible and accepted only as a last result when necessary to

²¹⁵ CDFW (2015) at 17.

²¹⁶ Raaf (2022).

²¹⁷ *Id.*

²¹⁸ *Id.*

²¹⁹ *Id.*

obtain landowner approval to conduct revegetation. The landowner’s requirement to retain nonnative plants must be demonstrated in writing to the permitting agencies in such situations, and SBCFCWCD must be required to prevent the spread of such plants

Recommendation G4: Track success pursuant to established success criteria in CDFW SAA and also track density and presence of multilayered canopies (i.e., groundcover, shrub layer, and tree canopy) as an additional measure of success.

Recommendation G5: Track success of plants which spread by rhizomes e.g., California rose and wild blackberry by percent cover rather than percent survival.

H. Improve Tracking and Reporting in Annual Maintenance and Revegetation

Reports: Discussion: The following recommendations are intended to ensure that the success of revegetation projects can be tracked pursuant to the CDFW SAA, which requires that, “each chapter includes a detailed discussion of data collection, ability to meet success criteria, monitoring activities... species used, and so forth.” The Maintenance and Revegetation Reports do not contain an assessment of specific success criteria used in the SAA, including percent survival,²²⁰ percent cover,²²¹ tree heights,²²² percent cover of invasive herbaceous vegetation,²²³ or presence of woody invasive nonnative species.²²⁴ Annual Maintenance and Revegetation Reports do not consistently set forth the species planted or the number of plants installed.²²⁵ SBCFCWCD assesses success qualitatively.²²⁶ SBCFCWCD can do a better job of counting the number of plants planted and surviving.²²⁷

Recommendations: The following recommendations are necessary for SBCFCWCD and CDFW and other permitting agencies to accurately measure success pursuant to permits including the CDFW SAA’s Success Criteria.

1. Map revegetation site boundaries using GPS coordinates at site boundaries to document precise locations. Include maps in each year’s Maintenance and Revegetation Report.
2. Demarcate boundaries of revegetation sites in the field using durable markers and replace markers if removed.
3. Consistently name or number each revegetation site.
4. Number all figures in Maintenance and Revegetation Reports.

²²⁰ The CDFW SAA requires “80% survival, by species, the first year and 100% survival thereafter and/or shall retain 75% cover after 3 years and 90% cover after 5 years for the life of the project.” Success Criterion 3.9(f) (Emphasis added.)

²²¹ *Id.*

²²² CDFW SAA Section 3.8 Criteria for Tree Replacement at 16 and Success Criterion 3.9(e) at 17.

²²³ CDFW SAA Success Criteria 3.9(c) at 17.

²²⁴ *Id.*

²²⁵ SBCFCWCD, *Maintenance and Revegetation Reports* (1993-1994 – 2020-21); *See also* Annual Plans (1993-94 – 2021-22).

²²⁶ Maureen Spencer, Operations Manager, SBCFCWCD, personal communication with Brian Trautwein, Environmental Analyst / Watershed Program Coordinator, EDC (January 2022.)

²²⁷ Raaf (2022).

5. Add photos of each site from set reference point locations in annual Maintenance and Revegetation Reports.
6. Document all species planted at each site.
7. Document number of plants of each species planted at each site for tracking pursuant to SAA Success Criterion 3.9(f).
8. Document and track survival rates annually (i.e., number and percent of installed plants surviving) pursuant to SAA Success Criterion 3.9(f).
9. Annually track year-to-year plant height and growth pursuant to SAA Section 3.8 Criterion for Tree Replacement.
10. Annually track percent cover of native species pursuant to SAA Success Criterion 3.9(f).
11. Annually track percent cover of herbaceous nonnative species present pursuant to SAA Success Criterion 3.9(c).
12. Annually track presence of woody invasive species pursuant to SAA Success Criterion 3.9(c).
13. Annually track general health and condition of plants installed.

I. SBCFCWCD Should Encourage Restoration Projects by Nonprofits.

Discussion: SBCFCWCD has an informal policy to prohibit nonprofit groups from conducting creek revegetation projects on County owned land or easements in order to preserve these areas for SBCFCWCD future revegetation projects.²²⁸

This precludes enhancement of the region's riparian habitat and habitat for listed species such as steelhead. There are many locations where nonprofits could enhance riparian vegetation if this policy were lifted. Moreover, such areas are not being used by the SBCFCWCD for revegetation.

Recommendation: Encourage and support nonprofit efforts to conduct voluntary habitat restoration projects along County-owned properties and easements. Consider allowing SBCFCWCD to credit a percentage of this restoration as mitigation, for example, if SBCFCWCD provides half the plants, labor, and maintenance then 50% of the revegetation area could be credited as mitigation.

J. Exclude Voluntary Community Restoration Projects. Discussion: The potential exists for SBCFCWCD to claim mitigation credit for a nonprofit group's creek revegetation projects when done in tandem with SBCFCWCD.

Recommendation: SBCFCWCD and permitting agencies should ensure that SBCFCWCD does not count voluntary restoration projects undertaken by nonprofit groups, Homeowners Associations, and community groups as mitigation for SBCFCWCD maintenance projects.²²⁹

²²⁸ Personal communication, Tom Fayram, former Deputy Director, SBCFCWCD (circa 1997).

²²⁹ Urban Creeks Council's joint project with SBCFCWCD at Las Vegas Creek counted as 50% mitigation for SBCFCWCD maintenance activities. Joint revegetation projects such as this must be tracked carefully to ensure the correct acreage is attributed to SBCFCWCD's revegetation program.

- K. No Double-counting Mitigation Projects.** Discussion: SBCFCWCD has revegetated areas where a private entity (COVA) was required to undertake revegetation to mitigate the impacts of COVA's clearing of riparian habitat. Recommendation: SBCFCWCD and permitting agencies must develop a method to ensure that revegetation projects are not double-counted as mitigation for both SBCFCWCD maintenance projects and private projects.²³⁰
- L. Annual Maintenance and Revegetation Reports Should Clearly Exclude Community Landscaping Projects.** Discussion: SBCFCWCD must clarify that SBCFCWCD does not take mitigation credit for community landscaping projects such as the La Goleta Condominiums Pollinator Garden (which includes nonnative and non-local plant species) as mitigation for SBCFCWCD's maintenance program. (See pages 131 - 132 above.) Recommendation: SBCFCWCD and permitting agencies should ensure that non-restoration community landscaping projects such as pollinator gardens are not credited as mitigation.²³¹
- M. Require Local Genotype Plants.** Discussion: In at least one instance, horticultural, non-local varieties of native plants were apparently used in a revegetation project credited as SBCFCWCD mitigation. Restoration practitioners and agencies generally require local genotype plants to protect the genetic integrity of local native plant populations.²³²
- Recommendation: CDFW and other permitting agencies permits should require plants installed to be from local genotypes grown from seeds or cuttings collected along the creek where the impact occurs whenever feasible, or within proximity (e.g., the Goleta Watershed) to protect the genetic integrity of local native plant populations.
- N. Increase the Monitoring and Maintenance Period to Ten Years.** Discussion: The current program's use of a five-year maintenance and monitoring period has resulted in unsuccessful revegetation projects as documented in this report. Revegetation projects may initially succeed when watered and weeded but often become overrun with exotics and thwarted by mortality (note percent covers and survival rates in plant tables above). The problem is likely to worsen given climate change-induced droughts.

²³⁰ See e.g., discussion of COVA mitigation projects being counted as SBCFCWCD mitigation projects.

²³¹ The pollinator garden planted by Kitson Nursery with the la Goleta Condominium residents includes a mixture of native and non-native flowering plants which appears to be a commercial seed mix rather than local native species.

²³² See e.g., City of Goleta, *Creek and Watershed Management Plan Strategy* 11.1 and Action 11.1.2 at 258 – 259 (November 2020); See also Belnap, Jayne, *Genetic Integrity: Why Do We Care? An Overview of the Issues* https://www.fs.fed.us/rm/pubs/int_gtr315/5_belnap.pdf (December 26, 2021); Ken Owens, Executive Director, Channel Islands Restoration, personal communication to Watershed Alliance of South Coast Organizations stating that most sycamore seedlings found in local creeks are hybrids with London plane trees, and to ensure local sycamore population's genetic integrity is maintained, propagation must occur using cuttings from heritage trees which predate importation of London plane trees to this region. (April 14, 2022).

Recommendation: The SAA and other permits should require maintenance and monitoring for a minimum of ten years and longer if needed to achieve the measurable success criteria in the SAA and other permits and to increase long-term success and effectively mitigate impacts.

- O. CDFW Inspections of Revegetation Sites.** Discussion: CDFW does not inspect all of the many revegetation sites and instead relies on SBCFCWCD’s Maintenance and Revegetation Reports.²³³

Recommendation: CDFW and all permitting agencies should visit each revegetation site and measure success pursuant to the measurable success criteria before signing off on revegetation sites as successful.

- P. Plant a More Upland Plant Palette for Climate Change Resiliency.**

Discussion: EDC has observed many local creeks that used to flow year-round have become intermittent due to the climate change-induced drought. Projections indicate our region will have a warmer climate with less frequent winter storms, a diminished water supply, and increasing reliance on groundwater, SBCFCWCD should consider planting oak riparian woodland plant communities where willow woodland and other riparian communities once thrived.

Appendix I

ACRONYMS

CDFW	California Department of Fish and Wildlife
CEQA	California Environmental Quality Act
COVA	Cathedral Oaks Village Association
DBH	Diameter at Breast Height
EDC	Environmental Defense Center
EIR	Environmental Impact Report
HOA	Homeowners Association
SAA	Streambed Alteration Agreement
SBCFCWCD	Santa Barbara County Flood Control and Water Conservation District
ROW	Right of Way
UCC	Urban Creeks Council

²³³ Rains (2021).



Toxicity of glyphosate to animals: A meta-analytical approach[☆]

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ABSTRACT

Glyphosate (GLY)-based herbicides (GBHs) are the most commonly applied pesticide worldwide, and non-target organisms (e.g., animals) are now regularly exposed to GLY and GBHs due to the accumulation of these chemicals in many environments. Although GLY/GBH was previously considered to be non-toxic, growing evidence indicates that GLY/GBH negatively affects some animal taxa. However, there has been no systematic analysis quantifying its toxicity to animals. Therefore, we used a meta-analytical approach to determine whether there is a demonstrable effect of GLY/GBH toxicity across animals. We further addressed whether the effects of GLY/GBH vary due to (1) taxon (invertebrate vs. vertebrate), (2) habitat (aquatic vs. terrestrial), (3) type of biological response (behavior vs. physiology vs. survival), and (4) dosage or concentration of GLY/GBH. Using this approach, we also determined whether adjuvants (e.g., surfactants) in commercial formulations of GBHs increased toxicity for animals relative to exposure to GLY alone. We analyzed 1282 observations from 121 articles. We conclude that GLY is generally sub-lethally toxic for animals, particularly for animals in aquatic or marine habitats, and that toxicity did not exhibit dose-dependency. Yet, our analyses detected evidence for widespread publication bias so we encourage continued experimental investigations to better understand factors influencing GLY/GBH toxicity to animals.

1. Introduction

Glyphosate (GLY) is the most commonly used herbicide worldwide, and its use is increasing (Benbrook, 2016; Maggi et al., 2019, 2020). Glyphosate targets plants by inhibiting the shikimate pathway, which is involved in synthesizing aromatic amino acids. Although this pathway is unique to plants and some microorganisms, recent work indicates that exposure to GLY-based herbicides (GBHs; a.k.a., GLY-based formulations or GBFs) may affect animals (Gill et al., 2017, 2018). Glyphosate-based herbicides, such as Roundup®, typically contain surfactants that help GLY penetrate plant tissue. Polyoxyethyleneamine (POEA) is a surfactant commonly found in GBHs, and POEA alone can exceed the effects of GBHs on non-target organisms, such as animals (Tsui and Chu, 2003; Howe et al., 2004; Brausch et al., 2007; reviewed in Gill et al., 2018; Klátyik et al., 2023). Therefore, it is important to understand animals' sensitivity to GLY alone relative to co-formulants of GBHs (i.e., surfactants and other adjuvants) to create safer formulations of GBHs in the future.

There is a rich history of investigation into the effects of GLY and

GBHs on organisms and ecosystems. For example, a Scopus search indicates there have been over 13,000 GLY-related papers published, and over 7000 papers published in the last decade alone. Only recently have researchers begun to formally review the sensitivity of animals to GLY, such as qualitative reviews of its effects across animals (Gill et al., 2018; Klátyik et al., 2023), and meta-analyzing GLY's effects on mortality in bees (Battisti et al., 2021) and on non-Hodgkin lymphoma in humans (Zhang et al., 2019). However, we lack a quantitative understanding of the effects of GLY and GBHs on animals, in general, as well as sub-lethal effects of GLY/GBH, which is important because other sub-lethal pollutants can influence populations and communities (Guedes et al., 2017; Saaristo et al., 2018; Sievers et al., 2019; Sherborne et al., 2020; Rastgar et al., 2022).

Meta-analyses allow for the statistical analysis of empirical data from independent experiments focused on a shared experimental goal or question thereby generating quantitative estimates of the studied effects. Meta-analyses have been commonplace in biomedical research for decades (DerSimonian and Laird, 1986; Stroup, 2000; Rosenberger et al., 2021), and they have recently been used to understand the

[☆] This paper has been recommended for acceptance by Philip N. Smith.

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ecotoxicological effects of pollutants (e.g., Xiao et al., 2023). Here, we used meta-analyses to determine whether there is a demonstrable effect of GLY/GBH toxicity across animals. In addition to this general goal, we had five specific questions.

- (1) Do animals exhibit a different sensitivity to GBH exposure relative to that of GLY alone? Here, we examined whether adjuvants in commercial formulations of GBHs increased toxicity for animals (sensu Tsui and Chu, 2003; Howe et al., 2004; Brausch et al., 2007; reviewed in Gill et al., 2018; Mesnage and Antoniou, 2018).
- (2) Are some animal taxa more vulnerable to GLY/GBH than other taxa? Here, we compared invertebrates to vertebrates given the distinct evolutionary histories and ecological roles of these two major taxa.
- (3) Does an animal's habitat type influence its sensitivity to GLY/GBH? Although GBHs are typically applied to terrestrial systems (reviewed in Benbrook, 2016), run-off and overspray exposes aquatic animals that can absorb GLY/GBH through their cutaneous and respiratory surfaces.
- (4) Do different biological responses vary in their sensitivity to GLY/GBH? For example, GLY/GBH may have less of an effect on mortality relative to sub-lethal traits, such as reproduction, growth, and behavior, because other pesticides and pollutants are often sub-lethal (Guedes et al., 2017; Saaristo et al., 2018; Sievers et al., 2019; Sherborne et al., 2020; Rastgar et al., 2022).
- (5) Are the effects of GLY/GBH dose-dependent whereby higher concentrations of GLY induce greater costs to animals?

Together, our meta-analytical approaches will clarify general patterns in animals' vulnerabilities to a broad-spectrum herbicide that is the most commonly applied pesticide in the U.S. and worldwide (Portier, 2020; Jarrell et al., 2020).

2. Materials and methods

2.1. Search method

Relevant papers to include in the meta-analysis were identified using the SCOPUS database to conduct keyword searches on articles published through 2022. The keywords “glyphosate AND taxon” were used where “taxon” was one of 9 target taxa from on a qualitative review of glyphosate toxicity in animals (Gill et al., 2018)— specifically, “amphibian*”, “annelid*”, “arthropod*”, “bird*”, “echinoderm*”, “fish*”, “mammal*”, “mollus*”, and “reptil*”. Studies were excluded for several reasons, including failure to quantify or report the amount of GLY or GBH exposure, exposure to a combination of GLY/GBH, and other factors, such as non-GLY/GBH herbicides (thereby making it impossible to determine the independent effects of GLY/GBH), lack of sufficient control group, mention (but not manipulation or quantification) of GLY/GBH, and examination of transgenerational effects of GLY/GBH. Inclusion criteria were studies: 1) testing for either GLY or GBH (or both) effects on animals; 2) with a control group that was not exposed to GLY or GBH; 3) with at least two replicates per treatment; and 4) containing study statistics, specifically a mean, standard deviation, and sample size. When study statistics were not explicitly reported in the text or tables of a paper, they were determined by extracting data from figures using WebPlotDigitizer (v.4.5, Pacifica, CA). This resulted in 121 articles with 1282 observations (Table S1; Supp. Mat.).

2.2. Recording of results

For each observation, the collected study statistics were converted into the standard effect size measure, Hedges' *g* (a.k.a., Hedges' *d*), which controls for bias associated with small sample size, unlike other metrics such as Cohen's *d* (Nakagawa and Cuthill, 2007; Borenstein

et al., 2009; Rosenberg et al., 2013; Del Re, 2015). The following were recorded for each observation: author-year of each publication, the type of exposure (i.e., GLY or GBH), the specific taxon for (e.g., *Danio rerio*), as well as its general taxon type (i.e., invertebrate or vertebrate), the habitat of each animal (i.e., terrestrial or aquatic/marine), the specific dependent variable (e.g., catalase activity or body mass), as well as one of six categories of biological response (survival; reproduction; growth/development/morphology; physiology [excluding sex steroids, which were included in the ‘reproduction’ sub-group]; behavior; population). Effect sizes were multiplied by -1 when the relationship between the dependent variable and fitness was negative. That is, an effect size larger than zero indicates a benefit of GLY/GBH to animals while a negative effect size indicates a cost of GLY/GBH to animals. The GLY/GBH exposure route was recorded as 1) internal (injection or ingestion); 2) external in the aquatic environment; and 3) other (e.g., application to the soil environment). The units for the concentration of GLY typically varied with exposure route (e.g., $\mu\text{g GLY/kg body mass vs. } \mu\text{g GLY/l of water}$). Therefore, concentration data for each exposure route were separately standardized to allow us to test whether effect size covaried with standardized GLY concentration.

2.3. Data analysis

All meta-analyses were conducted on Hedges' *g* values and standard errors of effect sizes using a random-effects restricted maximum likelihood method in SPSS (v.28, IBM Corp.), and two-tailed significance was determined at $\alpha = 0.05$. An omnibus test was performed on all data ($n = 1282$ observations) and included standardized GLY concentration (see above) as a covariate to examine whether effects were dose-dependent. Sub-group meta-analyses were also performed to address the questions in our study (i.e., effects of exposure type, taxon, habitat, and dependent variable; see above). For each meta-analysis, author-year was included as a study label to account for study-specific effects, and an Egger's test was conducted because it is particularly sensitive in identifying publication bias (Lin et al., 2018).

3. Results

Overall, GLY/GBH exposure was costly to animals (i.e., negative effect size: Fig. 1; Table 1), but there was no dose-dependency of GLY/GBH because standardized GLY concentration did not covary with effect size ($t = -0.40$; $P = 0.69$). Sub-group meta-analyses indicated that GLY/GBH toxicity was variable (Fig. 1; Table 1). Specifically, GBH (but not GLY) exposure was toxic to animals, and both vertebrates and invertebrates experienced costs due to GLY/GBH exposure. Further, animals in aquatic or marine (but not terrestrial) environments experienced GLY/GBH toxicity. Last, GLY/GBH exposure impaired reproduction, physiology, and behavior, but it did not affect survival, population dynamics, growth, development, or morphology (Fig. 1; Table 1). However, Egger's test results suggested widespread publication bias overall and in nearly 60% of sub-group analyses (Fig. 1; Table 1).

4. Discussion

Animals are increasingly exposed to GLY, which is the most commonly applied herbicide worldwide (Portier, 2020; Jarrell et al., 2020). Glyphosate is purportedly safe for animals because it targets plant- and microbe-specific biochemistry (i.e., the shikimate pathway: Leino et al., 2021), but systematic qualitative reviews indicate that GLY and GBHs may be toxic to animals (Gill et al., 2018; Klátýik et al., 2023). Meta-analyses have been used to quantify the costs of GLY/GBH to animals— however, to date, these approaches have limited their scope to particular traits in specific animal taxa (e.g., mortality in bees [Battisti et al., 2021], non-Hodgkin lymphoma in humans [Zhang et al., 2019], and micronuclei frequency in vertebrates [Ghisi et al., 2016]). Therefore, we meta-analyzed over 1200 observations of GLY/GBH exposure

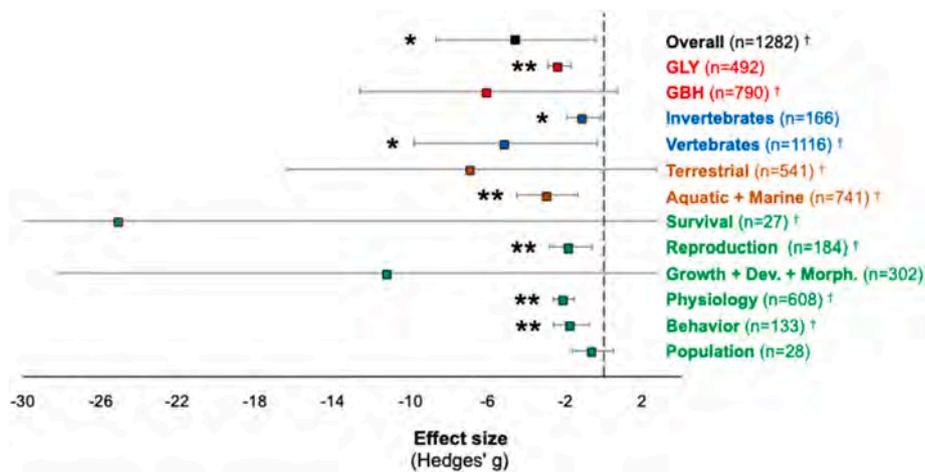


Fig. 1. Effects of glycosate (GLY) and GLY-based herbicide (GBH) exposure to animals. Effect sizes (Hedges' g) with post-mean and 95% confidence intervals (CIs) are shown for each data set (overall data set, as well as data sets for sub-groups). Negative values indicate costs of GLY/GBH. Sample sizes for each data set (i.e., number of observations) are shown parenthetically. The CIs for survival and for growth, development, and morphology (42 and 17, respectively) were too large to present. Asterisks indicate significant effect sizes (*P < 0.05; **P < 0.01), and daggers (†) indicate data sets with significant Egger's Test results (P < 0.05), which suggest publication bias.

Table 1

Meta-analyses results for overall effects (n = 1282 observations), as well as effects from sub-group meta-analyses. Daggers (†) indicate data sets with significant Egger's Test results (P < 0.05), which suggest publication bias.

	Z	P
Overall †	-2.2	0.030
Exposure type		
GLY	-7.6	<0.001
GBH †	-2.2	0.08
Taxon type		
Invertebrate	-2.3	0.021
Vertebrate †	-2.1	0.035
Habitat type		
Terrestrial †	-1.4	0.16
Aquatic or marine †	-3.6	<0.001
Type of biological response		
Survival	-1.2	0.24
Reproduction †	-3.1	0.002
Growth, development, and morphology	-1.9	0.20
Physiology †	-7.6	<0.001
Behavior †	-3.6	<0.001
Population	-1.0	0.31

for a range of dependent variables—from survival to physiology to behavior—across approx. 80 animal taxa. Our results provide general support that GLY is sub-lethally toxic to animals (Fig. 1; Table 1). Our results also indicate widespread publication bias, and biased data sets tended to indicate costs of GLY/GBH relative to non-biased data sets (71% vs. 40%, respectively; Fig. 1; Table 1). In sum, we found general support for GLY/GBH toxicity to animals, but we encourage researchers to publish non-significant findings to create a more balanced literature. Below, we discuss results related to our five specific questions (e.g., whether toxicity of GLY/GBH to animals is taxon- or trait-specific).

4.1. GBH may not be more toxic than GLY alone

Abundant empirical evidence indicates that the toxic effects of GBHs for animals may largely be due to adjuvants rather than GLY itself (reviewed in Ghisi et al., 2016; Mesnage and Antoniou, 2018; Martins-Gomes et al., 2022). For example, GBHs typically include surfactants that increase the penetration of GLY into plant tissue, and GBHs can be more toxic to animals than GLY alone (Richard et al., 2005; Mesnage et al., 2014; Janssens and Stoks, 2017). However, our meta-analyses indicate that GLY alone may be more toxic to animals

than GBH (Fig. 1), though issues related to publication bias make this result less definitive. Because animals lack the enzyme facilitating the shikimate pathway that is inhibited by GLY (5-enolpyruvylshikimate-3-phosphate synthase [EPSPS]), the costs of GLY alone may be due to GLY's effects on animal microbiomes because some microorganisms express EPSPS (Patterson et al., 2018; Vicini et al., 2019). For example, exposure to GLY alone can affect the gut microbiomes of some animals (Blot et al., 2019; Iori et al., 2020), and microbiome changes can alter endocrine and immune systems (Maddalon et al., 2021). Yet, independent of its influence on microbiomes, GLY alone may have a range of direct effects on animal cells based on *in vitro*, cell-line research, such as oxidative damage to lipids and nucleic acids (reviewed in Agostini et al., 2020). Cellular costs may be due to GLY's high affinity for divalent cations, particularly manganese, which is critical for the proper functioning of many animal enzymes (Samsel and Seneff, 2015). We encourage continued investigation into the cellular and microbiomic effects of GLY and GBHs for animals. Further, our results suggest that simply changing the proprietary blend of adjuvants in GBHs may not lead to safer outcomes for animals exposed to GBHs.

4.2. Vertebrates and invertebrates are both sensitive to GLY/GBH

Previous meta-analytical research has found toxic effects of GLY/GBH in specific taxa, such as bees or humans (e.g., Zhang et al., 2019; Battisti et al., 2021). However, major taxonomic groups, such as vertebrates and invertebrates, vary in their ecological roles and evolutionary histories, and they can also exhibit different tolerances to chemical pollutants (Lagadic and Caquet, 1998; Xin et al., 2015; Hughes et al., 2021). Invertebrates tend to be more sensitive to environmental contaminants, such as heavy metals and petrochemicals, relative to vertebrates (Sauer et al., 1997; Xin et al., 2015; Hughes et al., 2021). Yet, invertebrates' shorter generation times has allowed them to rapidly evolve resistance to other pesticides (Lenormand et al., 1999; Brengues et al., 2003). Our results indicate a similar toxicity to GLY/GBH for vertebrates and invertebrates (Fig. 1). However, we offer several caveats for this result because it contrasts with other ecotoxicological comparisons between invertebrates and vertebrates (e.g., Sauer et al., 1997; Xin et al., 2015; Hughes et al., 2021). First, our data set for vertebrates, but not for invertebrates, appeared to exhibit significant publication bias based on a significant Egger's Test result (Fig. 1; Table 1). Second, our data set for invertebrates was nearly 7-fold smaller than that for vertebrates (i.e., n = 166 vs. n = 1116 observations, respectively), even though there are

>20-fold more described invertebrate species relative to vertebrate species (IUCN, 2021). Invertebrates are alarmingly understudied and underappreciated in biodiversity research (Troudet et al., 2017; Eisenhauer et al., 2019), and taxonomic biases in publication and citation are common in several biological sub-disciplines (Bonnet et al., 2002; Clark and May 2002; Stahlschmidt, 2011; Rosenthal et al., 2017). Thus, we encourage continued investigation of GLY/GBH toxicity in invertebrates, particularly in light of the ethical considerations of vertebrate ecotoxicological research (Hughes et al., 2021), and we also hope for a more balanced, unbiased set of vertebrate publications related to GLY/GBH toxicity.

4.3. GLY/GBH may be particularly toxic to aquatic and marine animals

Pesticide risk analysis typically integrates an organism's level of exposure to a particular pesticide with the organism's sensitivity to the pesticide (e.g., Sørensen et al., 2020). Aquatic animals are exposed to GLY/GBH through diffuse contamination by leaching, drift, or runoff due to the overuse and poor disposal of GLY/GBH, which has led to its detection in groundwater and surface water (Reynoso et al., 2020; Mercurio et al., 2014; Feltracco et al., 2022). Yet, GLY and GBHs are generally applied to terrestrial environments for weed control (Kanissery et al., 2019) where most of the degradation is performed by soil microbes (reviewed in Castrejón-Godínez et al., 2021). Therefore, terrestrial animals may experience greater GLY/GBH exposure compared to aquatic or marine animals. Comparing the toxicities of pesticides for aquatic and terrestrial animals can be complicated (Chalew and Halden, 2009; Bueno and Cunha, 2020) because aquatic animals are likely exposed to GLY/GBH through more routes (ingestion, integumentary, and respiratory) relative to terrestrial animals (ingestion alone). Thus, aquatic sensitivity to pesticides may not correspond to terrestrial sensitivity (Adams et al., 2021). Our meta-analyses indicated that GLY/GBH exposure was toxic for aquatic/marine- but not terrestrial- animals (Fig. 1; Table 1), which suggests that terrestrial animals may not be at a higher risk for GLY/GBH toxicity even though they may have increased likelihood of exposure to GLY/GBH.

4.4. GLY/GBH toxicity for animals is sub-lethal

The toxicities of pesticides are often evaluated based on their lethality, including the effects of GLY/GBH on mortality in some animal taxa (e.g., Battisti et al., 2021). Yet, exposure to pesticides, including herbicides, and other pollutants often results in sub-lethal effects that can have a diversity of important population- and community-level effects (Techer et al., 2015; Guedes et al., 2017; Saaristo et al., 2018; Sievers et al., 2019; Sherborne et al., 2020; Rastgar et al., 2022). Therefore, understanding the by-stander costs of GLY/GBH use requires examining its effects across a range of biological responses of animals. Our meta-analyses demonstrate costs of GLY/GBH to animal physiology, behavior, and reproduction (Fig. 1; Table 1). Yet, GLY/GBH exposure only appeared to entail sub-lethal effects because it did not affect survival- however, we urge caution in this interpretation given our survival data set's small sample size ($n = 27$) and large variation in effect size (Fig. 1; Table 1). Likewise, the organismal effects of GLY/GBH on animals did not scale up to the population level in our study. On one hand, these results may suggest that GLY/GBH toxicity has relatively subtle ecological effects similar to other emergent features of environmental change. For example, artificial light at night (ALAN; i.e., ecological light pollution) is increasingly prevalent (Falchi et al., 2016; Kyba et al., 2017), and a recent meta-analysis indicated that ALAN similarly exerts effects at the organismal- but not population or community- level (Sanders et al. 2021). On the other hand, our data sets for survival and population effects may have been too small (i.e., $n = 27$ and $n = 28$, respectively), particularly given our population-level data set was derived from only four studies. Clearly, more work is required to better understand whether sub-lethal effects of GLY/GBH are truly

uncoupled from mortality and population-level effects.

4.5. GLY/GBH toxicity across animals may not be dose-dependent

Animals experience dose-dependent effects from many environmental contaminants (Tchounwou et al., 2004; Anderson et al., 2012; Hatef et al., 2013; Kim et al., 2020) whereby greater pollutant exposure increases toxicity. Likewise, there is within-study support that GLY/GBH exerts dose-dependent toxicity in animals, including humans (Benachour et al., 2007; Lanzarin et al., 2019; Dallegrave et al., 2007; Oliveira et al., 2007). Yet, our across-study meta-analysis indicated that GLY/GBH toxicity was not dependent on dose because standardized GLY concentration did not covary with effect size. Discrepancies between within- and among-study results have also been shown for the dose-dependency of ALAN's effects across animals (Sanders et al., 2021). On one hand, our results indicate that removing the potential by-stander costs of GLY/GBH may require eliminating GLY/GBH use entirely because even small applications can be toxic to animals. On the other hand, this result may be due to significant among-study variation in experimental design, and in the difficulty in quantifying "dose". To quantify dose, we standardized GLY concentration within each exposure route (e.g., injection or ingestion for terrestrial animals, or through cutaneous or respiratory surfaces for aquatic animals), but these routes likely vary in the efficiency by which GLY/GBH is absorbed by animals. Further, the duration or time-dependency of GLY/GBH exposure may also influence toxicity, which has been shown for other pollutants (e.g., Kouba et al., 2010) but is poorly understood for pesticides (Bommuraj et al., 2021). Experimental protocols could be designed to standardize tests of GLY/GBH toxicity to animals. For example, the Centers for Disease Control and the World Health Organization have each developed widely adopted methods to assess the sensitivity of insects to insecticides (reviewed in Richards et al., 2020).

4.6. Publication bias in GLY/GBH research in animals

Meta-analysis is a powerful tool that allows researchers to integrate results across studies (Sutton and Higgins, 2008) thereby informing important biomedical, pesticide use, and conservation decisions. Yet, like any other analysis, the value of a meta-analysis lies in the soundness of its data set, and publication bias can occur if significant findings are over-represented in the literature. A range of publication bias has been detected in meta-analyzed data sets (Kicinski et al., 2015), including in those associated with the COVID-19 pandemic (Mathur and VanderWeele, 2021; Rosenberger et al., 2021). Further, many meta-analyses fail to assess publication bias. For example, a review in 2014 indicated that only 31% of ecological meta-analyses tested for publication bias (Koricheva and Gurevitch, 2014), and 40% of systematic reviews and meta-analyses on COVID-19 data failed to test for publication bias (Rosenberger et al., 2021). Our meta-analyses indicated that GLY/GBH was toxic to animals, but we also detected publication bias in over 60% of our meta-analyzed datasets (Fig. 1; Table 1). The variability of potential bias among datasets hinders our understanding of GLY/GBH toxicity. For example, the GBH dataset did not indicate an effect of GBH toxicity and may have had publication bias, whereas the GLY data set indicated toxicity and was unbiased (Fig. 1; Table 1). One interpretation of these results is that scientists are over-reporting non-significant results from experiments using GBH. We encourage ecotoxicologists studying GLY/GBH and animal toxicity to report as many of their results as possible, including both significant and non-significant results, to better understand the effects of this increasingly common pollutant.

4.7. Future directions

Our results did not indicate a concentration-dependent effect of GLY/GBH toxicity; yet, there are other features of exposure intensity. For example, the duration of exposure to GLY/GBH can increase stress

biomarkers in invertebrates and vertebrates (e.g., [Guilherme et al., 2012](#); [Matozzo et al., 2018](#)). Unfortunately, the data used in our study did not lend themselves to detect duration-dependent effects across taxa given the range of different exposure routes and concentrations used among experiments. However, future work should explore the combined effects of GLY/GBH concentration and exposure duration among related taxa or those exposed to GLY/GBH through similar routes (e.g., aquatic animals).

The increasing presence of GLY/GBH is only one feature of global environmental change, and a multiple-stressor framework may inform the real-world toxicity of GLY/GBH for animals. For example, temperature and GLY/GBH toxicity can have additive or interactive effects on animals ([Gandhi and Cecala, 2016](#); [Silva et al., 2020](#); [Stahlschmidt et al., 2022](#); but see [Stahlschmidt and Vo, 2022](#); [Stahlschmidt et al., 2023](#)). However, the combined effects of GLY/GBH and other environmental factors (e.g., food limitation or exposure to other chemical pollutants) on animals are less understood and require further investigation.

Because GLY/GBH is typically applied to terrestrial environments to control weeds ([Kanissery et al., 2019](#)), bioremediation efforts may limit the transfer of GLY/GBH from terrestrial to aquatic environments. For example, a range of GLY-degrading microbes are highly effective at bioremediation of GLY-contaminated soil (reviewed in [Sviridov et al., 2015](#); [Zhan et al., 2018](#); [Zhang et al., 2022](#)). Yet, GLY is degraded to aminomethylphosphonic acid (AMPA), and AMPA alone can be toxic to a range of animals— from invertebrates to humans ([Mañas et al., 2009](#); [Iori et al., 2020](#); [Tresnakova et al., 2021](#); [Makris et al., 2022](#)). Thus, future meta-analytical approaches could focus on understanding the toxicity of AMPA to animals relative to that of GLY and GBH.

5. Conclusions

We systematically and quantitatively reviewed the toxicity of GLY/GBH to animals to characterize the effects of GLY and commercial GLY-based formulations within and across taxa. We analyzed over 1200 observations from over 100 studies across dozens of animal species, but confidence in our meta-analytical results was hampered by widespread publication bias in our data set ([Fig. 1](#); [Table 1](#)). Nonetheless, we conservatively demonstrate that GLY/GBH can be sub-lethally toxic for animals, particularly for animals in aquatic or marine habitats ([Fig. 1](#); [Table 1](#)). Our analyses did not indicate that toxicity varied due to dose (concentration) of GLY/GBH suggesting that simply reducing the amount of GLY/GBH per application may not limit its toxic effects on animals. The use of GLY/GBH will certainly persist, and potentially, increase— therefore, it is critical that we continue to investigate its risks to non-target organisms using both experimental and meta-analytical approaches.

CRedit authorship contribution statement

P.S. Evalen: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **E.N. Barnhardt:** Writing – review & editing, Methodology, Investigation. **J. Ryu:** Writing – review & editing, Investigation. **Z.R. Stahlschmidt:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are included as a supplemental table

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2024.123669>.

References

- Adams, E., Gerstle, V., Schmitt, T., Brühl, C.A., 2021. Co-formulants and adjuvants affect the acute aquatic and terrestrial toxicity of a cycloxydim herbicide formulation to European common frogs (*Rana temporaria*). *Sci. Total Environ.* 789, 147865 <https://doi.org/10.1016/j.scitotenv.2021.147865>.
- Agostini, L.P., Dettogni, R.S., Dos Reis, R.S., Stur, E., Dos Santos, E.V.W., Venterim, D.P., Garcia, F.M., Cardoso, R.C., Graceli, J.B., Louro, I.D., 2020. Effects of glyphosate exposure on human health: insights from epidemiological and in vitro studies. *Sci. Total Environ.* 705, 135808 <https://doi.org/10.1016/j.scitotenv.2019.135808>.
- Anderson, J.O., Thundiyil, J.G., Stolbach, A., 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. *J. Med. Toxicol.* 8 (2), 166–175. <https://doi.org/10.1007/s13181-011-0203-1>.
- Battisti, L., Potrich, M., Sampaio, A.R., De Castilhos Ghisi, N., Costa-Maia, F.M., Abati, R., Dos Reis Martinez, C.B., Sofia, S.H., 2021. Is glyphosate toxic to bees? A meta-analytical review. *Sci. Total Environ.* 767, 145397 <https://doi.org/10.1016/j.scitotenv.2021.145397>.
- Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., Séralini, G.E., 2007. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch. Environ. Contam. Toxicol.* 53 (1), 126–133. <https://doi.org/10.1007/s00244-006-0154-8>.
- Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* 28 (1), 3. <https://doi.org/10.1186/s12302-016-0070-0>.
- Blot, N., Veillat, L., Rouzé, R., Delatte, H., 2019. Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota. *PLoS One* 14 (4), e0215466. <https://doi.org/10.1371/journal.pone.0215466>.
- Bommuraj, V., Chen, Y., Birenboim, M., Barel, S., Shimshoni, J.A., 2021. Concentration- and time-dependent toxicity of commonly encountered pesticides and pesticide mixtures to honeybees (*Apis mellifera* L.). *Chemosphere* 266, 128974. <https://doi.org/10.1016/j.chemosphere.2020.128974>.
- Bonnet, X., Shine, R., Lourda, O., 2002. Taxonomic chauvinism. *Trends Ecol. Evol.* 17 (1), 1–3. [https://doi.org/10.1016/S0169-5347\(01\)02381-3](https://doi.org/10.1016/S0169-5347(01)02381-3).
- Borenstein, M., Hedges, L.V., Higgins, J.P.T., Rothstein, H.R., 2009. *Introduction to Meta-Analysis*, first ed. Wiley <https://onlinelibrary.wiley.com/doi/book/10.1002/9780470743386>.
- Brausch, J.M., Beall, B., Smith, P.N., 2007. Acute and sub-lethal toxicity of three POEA surfactant formulations to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* 78 (6), 510–514. <https://doi.org/10.1007/s00128-007-9091-0>.
- Brengues, C., Hawkes, N.J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., Manguin, S., Morgan, J.C., Hemingway, J., 2003. Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Med. Vet. Entomol.* 17 (1), 87–94. <https://doi.org/10.1046/j.1365-2915.2003.00412.x>.
- Bueno, M.R., Cunha, J.P.A.R.D., 2020. Environmental risk for aquatic and terrestrial organisms associated with drift from pesticides used in soybean crops. *An. Acad. Bras. Cienc.* 92 (Suppl. 1), e20181245 <https://doi.org/10.1590/0001-3765202020181245>.
- Castrejón-Godínez, M.L., Tovar-Sánchez, E., Valencia-Cuevas, L., Rosas-Ramírez, M.E., Rodríguez, A., Mussali-Galante, P., 2021. Glyphosate pollution treatment and microbial degradation alternatives, a review. *Microorganisms* 9 (11), 2322. <https://doi.org/10.3390/microorganisms9112322>.
- Chalew, T.E.A., Halden, R.U., 2009. Environmental exposure of aquatic and terrestrial biota to trichloro and trichloro-carban. *JAWRA Journal of the American Water Resources Association* 45 (1), 4–13. <https://doi.org/10.1111/j.1752-1688.2008.00284.x>.
- Clark, J.A., May, R.M., 2002. Taxonomic bias in conservation research. *Science* 297 (5579), 191–192. <https://doi.org/10.1126/science.297.5579.191b>.
- Dallegre, E., Mantese, F.D., Oliveira, R.T., Andrade, A.J.M., Dalsenter, P.R., Langeloh, A., 2007. Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Arch. Toxicol.* 81 (9), 665–673. <https://doi.org/10.1007/s00204-006-0170-5>.
- Del Re, A.C., 2015. A practical tutorial on conducting meta-analysis in R. *The Quantitative Methods for Psychology* 11 (1), 37–50. <https://doi.org/10.20982/tqmp.11.1.p037>.
- DerSimonian, R., Laird, N., 1986. Meta-analysis in clinical trials. *Contr. Clin. Trials* 7 (3), 177–188. [https://doi.org/10.1016/0197-2456\(86\)90046-2](https://doi.org/10.1016/0197-2456(86)90046-2).
- Eisenhauer, N., Bonn, A., Guerra C, A., 2019. Recognizing the quiet extinction of invertebrates. *Nat. Commun.* 10 (1), 50. <https://doi.org/10.1038/s41467-018-07916-1>.

- Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C.C.M., Elvidge, C.D., Baugh, K., Portnov, B. A., Rybnikova, N.A., Furgoni, R., 2016. The new world atlas of artificial night sky brightness. *Sci. Adv.* 2 (6), e1600377 <https://doi.org/10.1126/sciadv.1600377>.
- Feltracco, M., Barbaro, E., Morabito, E., Zangrando, R., Piazza, R., Barbante, C., Gambaro, A., 2022. Assessing glyphosate in water, marine particulate matter, and sediments in the Lagoon of Venice. *Environ. Sci. Pollut. Control Ser.* 29 (11), 16383–16391. <https://doi.org/10.1007/s11356-021-16957-x>.
- Gandhi, J.S., Cecala, K.K., 2016. Interactive effects of temperature and glyphosate on the behavior of blue ridge two-lined salamanders (*Eurycea wilderae*): glyphosate and temperature impacts on salamander behavior. *Environ. Toxicol. Chem.* 35 (9), 2297–2303. <https://doi.org/10.1002/etc.3398>.
- Ghisi, N.D.C., Oliveira, E.C.D., Prioli, A.J., 2016. Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta-analytic review. *Chemosphere* 145, 42–54. <https://doi.org/10.1016/j.chemosphere.2015.11.044>.
- Gill, J.P.K., Sethi, N., Mohan, A., 2017. Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. *Environ. Chem. Lett.* 15 (1), 85–100. <https://doi.org/10.1007/s10311-016-0585-z>.
- Gill, J.P.K., Sethi, N., Mohan, A., Datta, S., Girdhar, M., 2018. Glyphosate toxicity for animals. *Environ. Chem. Lett.* 16 (2), 401–426. <https://doi.org/10.1007/s10311-017-0689-0>.
- Guedes, R.N.C., Walse, S.S., Throne, J.E., 2017. Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science* 21, 47–53. <https://doi.org/10.1016/j.cois.2017.04.010>.
- Guilherme, S., Gaivão, I., Santos, M.A., Pacheco, M., 2012. DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – elucidation of organ-specificity and the role of oxidative stress. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 743 (1–2), 1–9. <https://doi.org/10.1016/j.mrgentox.2011.10.017>.
- Hatef, A., Alavi, S.M.H., Golshan, M., Linhart, O., 2013. Toxicity of environmental contaminants to fish spermatozoa function in vitro—a review. *Aquat. Toxicol.* 140–141, 134–144. <https://doi.org/10.1016/j.aquatox.2013.05.016>.
- Howe, C.M., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N., 2004. Toxicity of glyphosate-based pesticides to four north AMERICAN frog species. *Environ. Toxicol. Chem.* 23 (8), 1928. <https://doi.org/10.1897/03-71>.
- Hughes, S.A., Maloney, E.M., Bejarano, A.C., 2021. Are vertebrates still needed in routine whole effluent toxicity testing for oil and gas discharges? *Environ. Toxicol. Chem.* 40 (5), 1255–1265. <https://doi.org/10.1002/etc.4963>.
- Iori, S., Rovere, G.D., Ezzat, L., Smits, M., Ferrareso, S.S., Babbucci, M., Marin, M.G., Masiero, L., Fabrello, J., Garro, E., Carraro, L., Cardazzo, B., Patarnello, T., Matozzo, V., Bargelloni, L., Milan, M., 2020. The effects of glyphosate and AMPA on the mediterranean mussel *Mytilus galloprovincialis* and its microbiota. *Environ. Res.* 182, 108984 <https://doi.org/10.1016/j.envres.2019.108984>.
- IUCN. 2021. The IUCN Red List of Threatened Species. Version 2021.
- Janssens, L., Stoks, R., 2017. Stronger effects of Roundup than its active ingredient glyphosate in damselfly larvae. *Aquat. Toxicol.* 193, 210–216. <https://doi.org/10.1016/j.aquatox.2017.10.028>.
- Jarrell, Z.R., Ahammad, M.U., Benson, A.P., 2020. Glyphosate-based herbicide formulations and reproductive toxicity in animals. *Veterinary and Animal Science* 10, 100126. <https://doi.org/10.1016/j.vas.2020.100126>.
- Kanissery, R., Gairhe, B., Kadyampakeni, D., Batuman, O., Alferrez, F., 2019. Glyphosate: its environmental persistence and impact on crop health and nutrition. *Plants* 8 (11), 499. <https://doi.org/10.3390/plants8110499>.
- Kicinski, M., Springate, D.A., Kontopantelis, E., 2015. Publication bias in meta-analyses from the cochrane database of systematic reviews. *Stat. Med.* 34 (20), 2781–2793. <https://doi.org/10.1002/sim.6525>.
- Kim, J.-J., Kumar, S., Kumar, V., Lee, Y.-M., Kim, Y.-S., Kumar, V., 2020. Bisphenols as a legacy pollutant, and their effects on organ vulnerability. *Int. J. Environ. Res. Publ. Health* 17 (1), 112. <https://doi.org/10.3390/ijerph17010112>.
- Klátyik, S., Simon, G., Oláh, M., Mesnage, R., Antoniou, M.N., Zaller, J.G., Székács, A., 2023. Terrestrial ecotoxicity of glyphosate, its formulations, and co-formulants: evidence from 2010–2023. *Environ. Sci. Eur.* 35 (1), 51. <https://doi.org/10.1186/s12302-023-00758-9>.
- Koricheva, J., Gurevitch, J., 2014. Uses and misuses of meta-analysis in plant ecology. *J. Ecol.* 102 (4), 828–844. <https://doi.org/10.1111/1365-2745.12224>.
- Kouba, A., Buřič, M., Kozák, P., 2010. Bioaccumulation and effects of heavy metals in crayfish: a review. *Water, Air, Soil Pollut.* 211 (1–4), 5–16. <https://doi.org/10.1007/s11270-009-0273-8>.
- Kyba, C.C.M., Kuester, T., Sánchez De Miguel, A., Baugh, K., Jechow, A., Hölker, F., Bennie, J., Elvidge, C.D., Gaston, K.J., Guanter, L., 2017. Artificially lit surface of Earth at night increasing in radiance and extent. *Sci. Adv.* 3 (11), e1701528 <https://doi.org/10.1126/sciadv.1701528>.
- Lagadic, L., Caquet, T., 1998. Invertebrates in testing of environmental chemicals: are they alternatives? *Environ. Health Perspect.* 106 (Suppl. 2), 593–611. <https://doi.org/10.1289/ehp.98106593>.
- Lanzarin, G.A.B., Félix, L.M., Santos, D., Venâncio, C.A.S., Monteiro, S.M., 2019. Dose-dependent effects of a glyphosate commercial formulation – Roundup® UltraMax—on the early zebrafish embryogenesis. *Chemosphere* 223, 514–522. <https://doi.org/10.1016/j.chemosphere.2019.02.071>.
- Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., Puigbò, P., 2021. Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. *J. Hazard Mater.* 408, 124556 <https://doi.org/10.1016/j.jhazmat.2020.124556>.
- Lenormand, T., Bourguet, D., Guillemaud, T., Raymond, M., 1999. Tracking the evolution of insecticide resistance in the mosquito *Culex pipiens*. *Nature* 400 (6747), 861–864. <https://doi.org/10.1038/23685>.
- Lin, L., Chu, H., Murad, M.H., Hong, C., Qu, Z., Cole, S.R., Chen, Y., 2018. Empirical comparison of publication bias tests in meta-analysis. *J. Gen. Intern. Med.* 33 (8), 1260–1267. <https://doi.org/10.1007/s11606-018-4425-7>.
- Maddaloni, A., Galbiati, V., Colosio, C., Mandić-Rajčević, S., Corsini, E., 2021. Glyphosate-based herbicides: evidence of immune-endocrine alteration. *Toxicology* 459, 152851. <https://doi.org/10.1016/j.tox.2021.152851>.
- Maggi, F., La Cecilia, D., Tang, F.H.M., McBratney, A., 2020. The global environmental hazard of glyphosate use. *Sci. Total Environ.* 717, 137167 <https://doi.org/10.1016/j.scitotenv.2020.137167>.
- Maggi, F., Tang, F.H.M., La Cecilia, D., McBratney, A., 2019. PEST-CHEMGRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. *Sci. Data* 6 (1), 170. <https://doi.org/10.1038/s41597-019-0169-4>.
- Makris, K.C., Efthymiou, N., Konstantinou, C., Anastasi, E., Schoeters, G., Kolossa-Gehring, M., Katsonouri, A., 2022. Oxidative stress of glyphosate, AMPA and metabolites of pyrethroids and chlorpyrifos pesticides among primary school children in Cyprus. *Environ. Res.* 212, 113316 <https://doi.org/10.1016/j.envres.2022.113316>.
- Mañas, F., Peralta, L., Raviolo, J., García Ovando, H., Weyers, A., Ugnia, L., Gonzalez Cid, M., Larriva, I., Gorla, N., 2009. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol. Environ. Saf.* 72 (3), 834–837. <https://doi.org/10.1016/j.ecoenv.2008.09.019>.
- Martins-Gomes, C., Silva, T.L., Andreani, T., Silva, A.M., 2022. Glyphosate vs. Glyphosate-based herbicides exposure: a review on their toxicity. *Journal of Xenobiotics* 12 (1), 21–40. <https://doi.org/10.3390/jox12010003>.
- Mathur, M.B., VanderWeele, T.J., 2021. Estimating publication bias in meta-analyses of peer-reviewed studies: a meta-meta-analysis across disciplines and journal tiers. *Res. Synth. Methods* 12 (2), 176–191. <https://doi.org/10.1002/jrsm.1464>.
- Matozzo, V., Fabrello, J., Masiero, L., Ferraccioli, F., Finos, L., Pastore, P., Di Gangi, I.M., Bogiatti, S., 2018. Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: a case study with the mussel *Mytilus galloprovincialis*. *Environ. Pollut.* 233, 623–632. <https://doi.org/10.1016/j.envpol.2017.10.100>.
- Mercurio, P., Flores, F., Mueller, J.F., Carter, S., Negri, A.P., 2014. Glyphosate persistence in seawater. *Mar. Pollut. Bull.* 85 (2), 385–390. <https://doi.org/10.1016/j.marpolbul.2014.01.021>.
- Mesnage, R., Antoniou, M.N., 2018. Ignoring adjuvant toxicity falsifies the safety profile of commercial pesticides. *Front. Public Health* 5, 361. <https://doi.org/10.3389/fpubh.2017.00361>.
- Mesnage, R., Defarge, N., Spiroux De Vendômois, J., Séralini, G.-E., 2014. Major pesticides are more toxic to human cells than their declared active principles. *BioMed Res. Int.* 2014, 1. <https://doi.org/10.1155/2014/179691>–8.
- Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* 82 (4), 591–605. <https://doi.org/10.1111/j.1469-185X.2007.00027.x>.
- Oliveira, A.G., Telles, L.F., Hess, R.A., Mahecha, G.A.B., Oliveira, C.A., 2007. Effects of the herbicide Roundup on the epididymal region of drakes *Anas platyrhynchos*. *Reprod. Toxicol.* 23 (2), 182–191. <https://doi.org/10.1016/j.reprotox.2006.11.004>.
- Patterson, E.L., Pettinga, D.J., Ravet, K., Neve, P., Gaines, T.A., 2018. Glyphosate resistance and EPSPS gene duplication: convergent evolution in multiple plant species. *J. Hered.* 109 (2), 117–125. <https://doi.org/10.1093/jhered/esx087>.
- Portier, C.J., 2020. A comprehensive analysis of the animal carcinogenicity data for glyphosate from chronic exposure rodent carcinogenicity studies. *Environmental Health* 19 (1), 18. <https://doi.org/10.1186/s12940-020-00574-1>.
- Rastgar, S., Alijani Ardeshir, R., Segner, H., Tyler, C.R., Peijnenburg W, J.G.M., Wang, Y., Salati, A.P., Movahedinia, A., 2022. Immunotoxic effects of metal-based nanoparticles in fish and bivalves. *Nanotoxicology* 16 (1), 88–113. <https://doi.org/10.1080/17435390.2022.2041756>.
- Reynoso, E.C., Peña, R.D., Reyes, D., Chavarin-Pineda, Y., Palchetti, I., Torres, E., 2020. Determination of glyphosate in water from a rural locality in México and its implications for the population based on water consumption and use habits. *Int. J. Environ. Res. Publ. Health* 17 (19), 7102. <https://doi.org/10.3390/ijerph17197102>.
- Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Séralini, G.-E., 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ. Health Perspect.* 113 (6), 716–720. <https://doi.org/10.1289/ehp.7728>.
- Richards, S.L., Byrd, B.D., Reiskind, M.H., White, A.V., 2020. Assessing insecticide resistance in adult mosquitoes: perspectives on current methods. *Environ. Health Insights* 14, 117863022095279. <https://doi.org/10.1177/1178630220952790>.
- Rosenberger, K.J., Xu, C., Lin, L., 2021. Methodological assessment of systematic reviews and meta-analyses on COVID-19: a meta-epidemiological study. *J. Eval. Clin. Pract.* 27 (5), 1123–1133. <https://doi.org/10.1111/jep.13578>.
- Rosenberg, M.S., Rothstein, H.R., Gurevitch, J., 2013. Effect sizes: conventional choices and calculations. pp. 61–71 in *Handbook of Meta-analysis in Ecology and Evolution*, J. Koricheva, J. Gurevitch and K.L. Mengersen, eds. Princeton University Press: Princeton, NJ.
- Rosenthal, M.F., Gertler, M., Hamilton, A.D., Prasad, S., Andrade, M.C.B., 2017. Taxonomic bias in animal behaviour publications. *Anim. Behav.* 127, 83–89. <https://doi.org/10.1016/j.anbehav.2017.02.017>.
- Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M., Arnold, K.E., 2018. Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proc. Biol. Sci.* 285 (1885), 20181297 <https://doi.org/10.1098/rspb.2018.1297>.
- Samsel, A., Seneff, S., 2015. Glyphosate, pathways to modern diseases III: manganese, neurological diseases, and associated pathologies. *Surg. Neurol. Int.* 6 (1), 45. <https://doi.org/10.4103/2152-7806.153876>.

- Sanders, D., Frago, E., Kehoe, R., Patterson, C., Gaston, K.J., 2021. A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution* 5 (1), 74–81. <https://doi.org/10.1038/s41559-020-01322-x>.
- Sauer, T.C., Costa, H.J., Brown, J.S., Ward, T.J., 1997. Toxicity identification evaluations of produced-water effluents. *Environ. Toxicol. Chem.* 16 (10), 2020–2028. <https://doi.org/10.1002/etc.5620161006>.
- Sherborne, N., Galic, N., Ashauer, R., 2020. Sublethal effect modelling for environmental risk assessment of chemicals: problem definition, model variants, application and challenges. *Sci. Total Environ.* 745, 141027 <https://doi.org/10.1016/j.scitotenv.2020.141027>.
- Sievers, M., Hale, R., Parris, K.M., Melvin, S.D., Lanctôt, C.M., Swearer, S.E., 2019. Contaminant-induced behavioural changes in amphibians: a meta-analysis. *Sci. Total Environ.* 693, 133570 <https://doi.org/10.1016/j.scitotenv.2019.07.376>.
- Silva, L.C.M., Daam, M.A., Gusmao, F., 2020. Acclimation alters glyphosate temperature-dependent toxicity: implications for risk assessment under climate change. *J. Hazard Mater.* 385, 121512 <https://doi.org/10.1016/j.jhazmat.2019.121512>.
- Sørensen, P.B., Kjær, C., Wiberg-Larsen, P., Bruus, M., Strandberg, B., Rasmussen, J.J., Damgaard, C.F., Larsen, S.E., Strandberg, M., 2020. Pesticide risk indicator for terrestrial adult stages of aquatic insects. *Ecol. Indic.* 118, 106718 <https://doi.org/10.1016/j.ecolind.2020.106718>.
- Stahlschmidt, Z.R., 2011. Taxonomic chauvinism revisited: insight from parental care research. *PLoS One* 6 (8), e24192. <https://doi.org/10.1371/journal.pone.0024192>.
- Stahlschmidt, Z.R., Vo, C., 2022. Spatial bet hedging, thermal trade-offs and glyphosate: crickets integrate multivariate information during oviposition. *Anim. Behav.* 185, 105–112. <https://doi.org/10.1016/j.anbehav.2022.01.007>.
- Stahlschmidt, Z.R., Whitlock, J., Vo, C., Evalen, P., D, B., 2022. Pesticides in a warmer world: effects of glyphosate and warming across insect life stages. *Environ. Pollut.* 307, 119508 <https://doi.org/10.1016/j.envpol.2022.119508>.
- Stahlschmidt, Z.R., Choi, J., Choy, B., Perez, P.L., Whitlock, J., 2023. A simulated heat wave—but not herbicide exposure—alters resource investment strategy in an insect. *J. Therm. Biol.* 116, 103670 <https://doi.org/10.1016/j.jtherbio.2023.103670>.
- Stroup, D.F., 2000. Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 283 (15). <https://doi.org/10.1001/jama.283.15.2008>, 2008.
- Sutton, A.J., Higgins, J.P.T., 2008. Recent developments in meta-analysis. *Stat. Med.* 27 (5), 625–650. <https://doi.org/10.1002/sim.2934>.
- Sviridov, A.V., Shushkova, T.V., Ermakova, I.T., Ivanova, E.V., Epiktetov, D.O., Leontievsky, A.A., 2015. Microbial degradation of glyphosate herbicides. *Appl. Biochem. Microbiol.* 51 (2), 188–195. <https://doi.org/10.1134/S0003683815020209> (Review).
- Tchounwou, P.B., Centeno, J.A., Patlolla, A.K., 2004. Arsenic toxicity, mutagenesis, and carcinogenesis – a health risk assessment and management approach. *Mol. Cell. Biochem.* 255 (1/2), 47–55. <https://doi.org/10.1023/B:MCBL.00000007260.32981.b9>.
- Techer, D., Milla, S., Fontaine, P., Viot, S., Thomas, M., 2015. Acute toxicity and sublethal effects of gallic and pelargonic acids on the zebrafish *Danio rerio*. *Environ. Sci. Pollut. Control Ser.* 22 (7), 5020–5029. <https://doi.org/10.1007/s11356-015-4098-2>.
- Tresnakova, N., Stara, A., Velisek, J., 2021. Effects of glyphosate and its metabolite AMPA on aquatic organisms. *Appl. Sci.* 11 (19) <https://doi.org/10.3390/app11199004>, 9004.
- Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., Legendre, F., 2017. Taxonomic bias in biodiversity data and societal preferences. *Sci. Rep.* 7 (1), 9132. <https://doi.org/10.1038/s41598-017-09084-6>.
- Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* 52 (7), 1189–1197. [https://doi.org/10.1016/S0045-6535\(03\)00306-0](https://doi.org/10.1016/S0045-6535(03)00306-0).
- Vicini, J.L., Reeves, W.R., Swarthout, J.T., Karberg, K.A., 2019. Glyphosate in livestock: feed residues and animal health. *J. Anim. Sci.* 97 (11), 4509–4518. <https://doi.org/10.1093/jas/skz295>.
- Xiao, C.-H., Meng, X.-Z., Li, B.-X., Gao, H.-W., 2023. A systematic review and meta-analysis of pollutants in environmental media. *Environ. Sci. Pollut. Control Ser.* 30 (53), 113205–113217. <https://doi.org/10.1007/s11356-023-30347-5>.
- Xin, Z., Wencho, Z., Zhenguang, Y., Yiguo, H., Zhengtao, L., Xianliang, Y., Xiaonan, W., Tingting, L., Liming, Z., 2015. Species sensitivity analysis of heavy metals to freshwater organisms. *Ecotoxicology* 24 (7–8), 1621–1631. <https://doi.org/10.1007/s10646-015-1500-2>.
- Zhan, H., Feng, Y., Fan, X., Chen, S., 2018. Recent advances in glyphosate biodegradation. *Appl. Microbiol. Biotechnol.* 102 (12), 5033–5043. <https://doi.org/10.1007/s00253-018-9035-0>.
- Zhang, L., Rana, I., Shaffer, R.M., Taioli, E., Sheppard, L., 2019. Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: a meta-analysis and supporting evidence. *Mutation Research/Reviews in Mutation Research* 781, 186–206. <https://doi.org/10.1016/j.mrrev.2019.02.001>.
- Zhang, W., Li, J., Zhang, Y., Wu, X., Zhou, Z., Huang, Y., Zhao, Y., Mishra, S., Bhatt, P., Chen, S., 2022. Characterization of a novel glyphosate-degrading bacterial species, *Chryseobacterium* sp. Y16C, and evaluation of its effects on microbial communities in glyphosate-contaminated soil. *J. Hazard Mater.* 432, 128689 <https://doi.org/10.1016/j.jhazmat.2022.128689>.



Larval mosquito management and risk to aquatic ecosystems: A comparative approach including current tactics and gene-drive *Anopheles* techniques

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Abstract Genetic engineering of mosquitoes represents a promising tactic for reducing human suffering from malaria. Gene-drive techniques being developed that suppress or modify populations of *Anopheles gambiae* have the potential to be used with, or even possibly obviate, microbial and synthetic insecticides. However, these techniques are new and therefore there is attendant concern and uncertainty from regulators, policymakers, and the public about their environmental risks. Therefore, there is a need to assist decision-makers and public health stewards by assessing the risks associated with these newer mosquito management tactics so the risks can be compared as a basis for informed decision making. Previously, the effect of gene-drive mosquitoes on water quality in Africa was identified as a concern by stakeholders. Here, we use a comparative risk assessment approach for the effect of gene-drive mosquitoes on water quality in Africa. We compare the use of existing larvicides and the proposed genetic techniques in aquatic environments. Based on our analysis, we conclude that the tactic of gene-drive *Anopheles* for malaria management is unlikely to result in risks to

aquatic environments that exceed current tactics for larval mosquitoes. As such, these new techniques would likely comply with currently recommended safety standards.

Keywords Risk assessment · Larvicide · Culicidae · Anopheline · Malaria · Mosquito control

Introduction

For the past 20 years, malaria in much of sub-Saharan Africa has primarily been managed by indoor residual treatments of insecticides, long-lasting insecticidal bednets, and artemisinin-based combination therapy (WHO 2020; Zhou et al. 2020). Although these tactics have been remarkably successful in lowering malaria deaths, these gains are threatened by resistance, persistence, and resurgence. Consequently, the World Health Organization (WHO) has called for the research, development, and use of alternative tactics for malaria management to maintain and improve on the successes in recent years (Derua et al. 2019; WHO 2020; Zhou et al. 2020; Antonio-Nkondjio et al. 2021).

Existing and new technologies for mosquito and malaria management pose benefits and risks to human health and ecosystems. Genetically engineered mosquitoes represent a promising tactic for reducing human suffering from malaria. This technology includes gene-drive approaches that suppress

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populations of specific mosquito species (often referred to as population suppression strategies), such as *Anopheles gambiae* (sensu lato), the vectors of *Plasmodium* spp., the pathogen that causes malaria. Another approach known as population modification does not reduce mosquito populations, but, rather, it limits the ability of mosquitoes to transmit *Plasmodium* spp. but otherwise does not intentionally affect the mosquitoes (Bier 2021).

Currently, research and development of a gene-drive system for population suppression using the the *doublesex* locus (*dsxF^{CRISPRh}*) has shown promise in experiments with caged, laboratory populations of *An. gambiae* (Kyrou et al. 2018; Connolly et al. 2021; Hammond et al. 2021). Other forms of gene drive are also being researched, including integral gene drives, daisy-chain gene drives, and toxin-antidote recessive embryo (TARE) drives (Nash et al. 2018; Noble et al. 2019; Champer et al. 2020).

The techniques currently being researched that suppress or modify populations of *An. gambiae* have the potential to be used with or even possibly obviate microbial and synthetic organic insecticides. However, these technologies are new and therefore there is attendant concern from opinion leaders, regulators, policymakers, and the general public about their environmental risks (Scudellari 2019; Teem et al. 2019; Connolly et al. 2021). Consequently, there is a pressing need to assist decision-makers and public health stewards by objectively assessing the risks associated with relevant mosquito management tactics so that the risks can be compared to each other as a basis for informed decision making (United Nations 2020).

The optimal way to accomplish this is by using the science-based framework of risk assessment (NRC 1983, 1996, 2009), specifically comparative risk assessment. The purpose of comparative risk assessment is to qualitatively and quantitatively compare different environmental risks for the purpose of improved decision-making (e.g., Peterson and Arntzen 2004; Peterson and Shama 2005; Peterson 2006; Peterson et al. 2006; Davis et al. 2007; Davis and Peterson 2008; Schleier et al. 2008; Schleier and Peterson 2013; Raybould and Macdonald 2018).

In workshop exercises associated with the use of gene-drive mosquitoes in Africa for malaria management, participants identified general protection goals and possible pathways of harm (Roberts et al. 2017; Teem et al. 2019; Connolly et al. 2021). In particular,

the groups identified human and animal health, biodiversity, and water quality as major protection goals. Consequently—as one example—it is imperative to understand and communicate the risks of mosquito management tactics to aquatic environments and water quality, including risks to people and other non-target organisms. Therefore, our scope in this paper is to discuss these risks focusing on stressor identification and effects assessment of using gene-drive mosquitoes for malaria management compared to existing non-gene-drive larviciding tactics (i.e., tactics directed at larval mosquitoes). We define “water quality” broadly as that which includes the abiotic and biotic characteristics that determine its suitability for a particular purpose, including consumption by people and other animals (USNOAA 2021).

Approach and risk characterization

For the purposes of this paper, we define risk assessment as a formalized basis for the objective evaluation of risk in which assumptions and uncertainties are considered and presented (NRC 1983, 1996, 2009; National Academies of Sciences, Engineering, and Medicine 2016; WHO 2017). Both human-health and ecological risk can be described in quantitative terms as a function of effect (in many cases “toxicity”) and exposure (NRC 1983). Risk assessment, therefore, is arguably the most established, robust, and science-based method available to estimate risk. Consequently, it is a powerful tool for evidence-based societal decision-making.

Risk assessment typically uses a tiered modeling approach extending from deterministic models (tier 1) based on conservative assumptions to probabilistic models (tier 4) using refined assumptions (SETAC 1994). Conservative assumptions in lower-tier assessments represent overestimates of effect and exposure; therefore, the resulting quantitative risk values typically are conservative and err on the side of safety.

Although terminology may vary, risk assessments typically follow these steps: (1) problem formulation, (2) analysis phase, and (3) risk characterization (NRC 1983, 1996, 2009; SETAC 1994; EFSA 2010; National Academies of Sciences, Engineering, and Medicine 2016; EFSA et al. 2020). The problem formulation establishes the goals, breadth, and focus of the assessment, the analysis phase has an effects

assessment and an exposure assessment, and the risk characterization is a consideration of the joint property of effect and exposure to determine risk or what additional data are needed to calculate risk or refine risk estimates (USEPA 1998a). The effect assessment often includes an identification of the stressor and dose–response or density–response relationships. A stressor (also referred to as a hazard) is the entity that has the inherent ability to cause harm, whether it be a substance, organism, or activity.

On first glance, the risk assessment framework may not seem well aligned with this particular system and question because gene-drive mosquitoes for malaria management are still in research and development stages. Therefore, there is little to no experiential information on potential stressors, effects, and exposure. However, the stepwise nature of risk assessment allows for a logical process whereby risk issues can be presented, compared, and considered (Peterson and Arntzen 2004; Wolt et al. 2010; Raybould and Macdonald 2018; Raybould et al. 2019; Romeis et al. 2020). In addition, genetically engineered *Aedes aegypti* mosquitoes have been produced, assessed for risks, approved by regulatory agencies, and commercially used (Harris et al. 2011; MCTI-CTNBio 2014; Carvalho et al. 2015; USFDA 2016; USEPA 2020a, b), although the techniques and modes of action are different than what is being developed for gene-drive *An. gambiae*. This demonstrates, however, that risk assessment and regulatory approaches are amenable to genetically engineered mosquitoes. This paper will explore via a comparative, qualitative risk assessment framework the risks of using existing larvicides versus those of gene-drive mosquitoes to aquatic environments.

Conduct of the assessment

The fact that there currently are no gene-drive systems for malaria management that are sufficiently advanced to be presented to regulatory authorities presents fundamental constraints on the thoroughness of risk assessments that can be done. For example, the inherent ability of a genetically engineered protein to cause harm is not yet known for a gene-drive *An. gambiae*. However, the framework is still valuable because we can focus on the problem formulation and effect assessment (especially stressor identification)

(Connolly et al. 2021) and engage in initial comparisons to currently used larvicidal tactics.

By focusing on problem formulation and effects, we can identify potential primary and secondary effects, which are important concepts in ecological risk assessment. We define a primary effect as the stressor acting directly on a receptor. The USEPA (1998a) also terms this a “direct effect”. A secondary effect is when the direct response on a receptor becomes a stressor to another receptor (usually another life stage, species, or abiotic entity). The USEPA (1998a) also terms this an “indirect effect.”

Previous scoping and problem formulation work on gene-drive mosquitoes has identified potential primary and secondary effects (Roberts et al. 2017; Teem et al. 2019; Connolly et al. 2021) (Fig. 1). Obviously, there will always be limited knowledge of secondary effects posed by a stressor because the possibilities could represent a virtually uncountable number. However, scientifically reasonable and probable secondary effects are a much lower and practically manageable number. Regardless, the concept of primary and secondary effects is important for our purposes because we are dealing with stressors that can be shown to have no or very low inherent toxicity to non-target organisms, including humans. This is especially germane to gene-drive mosquitoes because not only will the engineered proteins most likely be inherently non-toxic to non-target organisms, but they will also most likely be produced by the mosquitoes and will be very low concentrations in the environment.

Comparative risk assessment

An obvious advantage of comparative risk assessment is that we can evaluate if the new tactic (in this case, gene-drive mosquitoes) has the potential to pose increased risk compared to current tactics (in this case, larvicides). Although obvious, this ability is underused, but is particularly powerful because it allows risk to be evaluated within the context of existing management systems for pests. Comparative risk assessment is also fundamental as a starting point in the safety assessment of genetically engineered organisms, termed “substantial equivalence” (Codex Alimentarius Commission 2003). Furthermore, this concept is embedded in the safety standard suggested by James et al. (2020), which recommends that gene-drive mosquitoes should be released in the field only

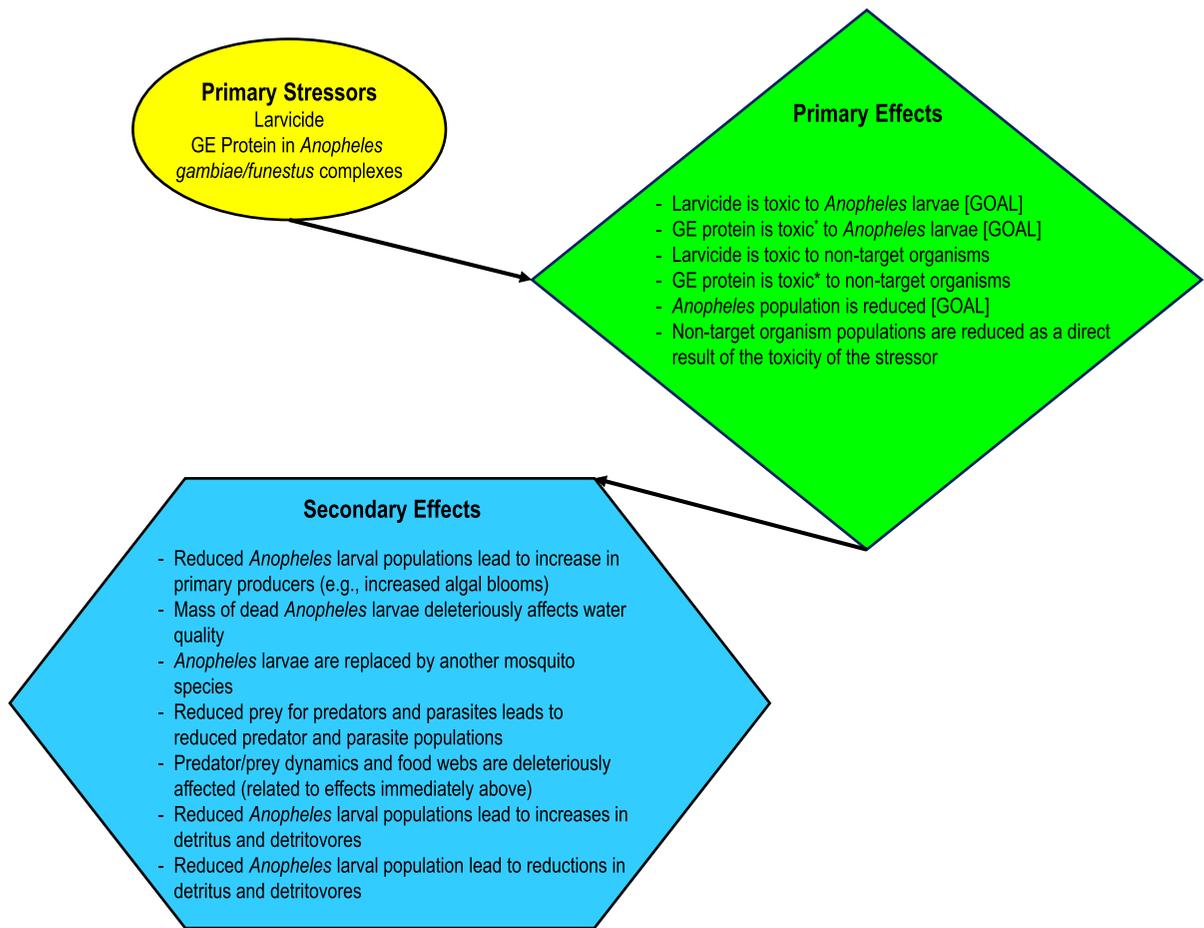


Fig. 1 A conceptual map of stressors, primary effects, and secondary effects associated with larvicides and genetically engineered mosquitoes for malaria management in sub-Saharan Africa. *denotes the hypothetical case that the genetically

engineered protein is toxic to both the target larvae and non-target organisms even though all current projects suggest that the protein will not be toxic

if they “...will do no more harm to human health than wild-type mosquitoes of the same genetic background and no more harm to the ecosystem than other conventional vector control interventions.”

Larvicides as the comparator

Because larvicides are the comparator in this assessment, some background on this mosquito management tool is warranted. When used according to product labels, current larvicides will deleteriously affect some aquatic non-target organisms (discussed in detail below). However, these effects most likely will not produce unacceptable risks according to current regulatory thresholds (USEPA 1991, 1998b, 2006).

This is because of the regulatory distinction between effects on individuals and populations. In most cases, there will be no effects on populations even though there might be effects on individuals, but there is some evidence of secondary effects on non-target populations with repeated use (Hershey et al. 1998; Lawler 2017; Brühl et al. 2020).

As mentioned above, the effects assessment in the analysis phase of a risk assessment identifies if a stressor has the inherent ability to cause harm. For conventional larvicides, this is a relatively straightforward process because the stressor is a known toxin and the toxic mode of action is well understood and studied as well as the doses necessary to cause morbidity and mortality (Fig. 2). However,

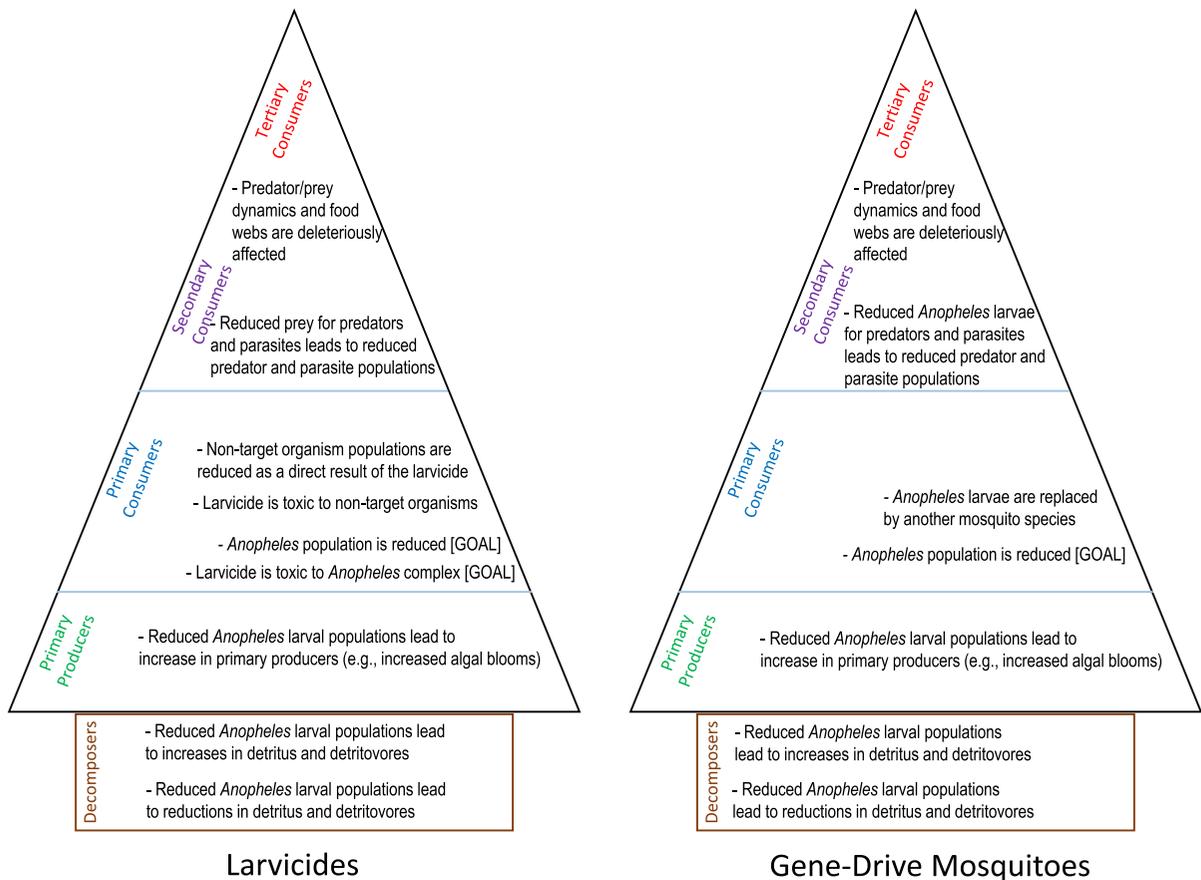


Fig. 2 Potential primary and secondary effects of larvicides (left) and gene-drive mosquitoes (right) associated with trophic levels for aquatic ecosystems. Secondary and tertiary consumers are grouped together because the effects would apply to both levels. The “Gene-Drive Mosquitoes” graphic assumes

that the active protein is not toxic to non-target organisms. The effects would apply mostly to *Anopheles coluzzii* and *An. funestus* because they are the only species that occupy semi-permanent and permanent water bodies

for gene-drive mosquitoes, the transgene may encode proteins that cannot be identified as causing “harm” to any other organism except for the intended effect on the target organism. This fact challenges the notion that complete risk assessments are needed, or can even be done, for some of these products. This is because if there is no inherent ability of the protein to cause harm (i.e., stressor) to any other organism, there is conceptually no need (country-specific regulatory requirements notwithstanding) to engage in the stepwise risk assessment process in which estimates of exposure to the stressor are compared to dose–response relationships (Peterson and Arntzen 2004). Risk assessment traditionally relies on estimating or using actual environmental exposures to the stressor and comparing those to effects to arrive

at a characterization of risk—as has been the case for larvicides. For these new tactics such as gene-drive mosquitoes, the problem formulation, stressor identification, and effect assessment arguably will be more important (Fig. 2) to the final estimate of risk.

The purpose of our paper is to comparatively examine issues associated with the risks to water quality from current vector management tactics and from gene-drive mosquito tactics. Although gene-drive mosquito systems for malaria management are still in research and development stages with several engineered genes being investigated, it is highly likely that the resulting proteins will not have conventional insecticidal properties. As such, they should not impose risks on water quality and non-target aquatic organisms that are greater than current larvicides. Indeed, the risks might

be appreciably lower (Fig. 2). However, it is important to stress that in most cases gene-drive mosquitoes will be used within an existing Integrated Pest Management (IPM) system for malarial mosquito management (WHO 2017, 2020). Therefore, multiple tactics such as larvicides and gene-drive mosquitoes will be used concurrently and assume that implementation within an IPM system has as a goal ensuring that risks from all tactics are acceptable.

A notable difference between current larvicides and gene-drive mosquitoes is that the mosquitoes (stressor) can multiply in the environment (up to the point at which total population numbers decline over time, which is the purpose of the population suppression gene-drive tactic). This attribute should be part of the risk assessment, but its uniqueness should not be construed as necessitating a separate risk assessment. Indeed, and arguably, risk assessments primarily should be based on the effects of and exposure to the product, not the process by which the product was produced.

Choice of larvicides

Unlike gene-drive mosquitoes, there is a relatively large amount of data on toxicity and exposure for conventional larvicides. This is because of global regulatory requirements for chemical and biological pesticides as well as years of commercial use after the pesticides have been registered (WHO 2013, 2017). In this paper, we discuss the larvicides methoprene, *Bacillus thuringiensis israelensis*, and *Lysinibacillus sphaericus* (= *Bacillus sphaericus*) to provide examples of risk issues associated with current products. Although there are other larvicides, such as monomolecular films, pyriproxyfen, spinosad, diflubenzuron, temephos, and novaluron, we will not evaluate these out of concerns for brevity, because they are either not currently used for mosquito management in Africa, or because they are unlikely to be used in the near future (Choi et al. 2019; Derua et al. 2019). Similarly, we will not evaluate biological controls, such as larvivorous fish.

Target species: the *Anopheles gambiae* and *Anopheles funestus* species complexes

Knowledge of habitat and food preferences of the two main species complexes of malaria mosquitoes in

Africa is essential because the ecology of these species is critical to understand when assessing primary and especially potential secondary effects. Although we focus on the *An. gambiae* complex for most of this paper, because of the current status of gene-drive research and development, we also are including the *An. funestus* complex because of its importance in malaria transmission and possible future targeting efforts.

Four primary malaria vectors belong to two main mosquito complexes in Africa. The *An. gambiae* complex is comprised of nine species (Sinka et al. 2012; Barrón et al. 2019) and the *An. funestus* complex has 13 species (Ogola et al. 2018). Three of the most important vectors occur within the *An. gambiae* complex (or *An. gambiae* sensu lato (s.l.)): *An. gambiae* sensu stricto (s.s. or S-form), *An. arabiensis*, and *An. coluzzii* (M-form).

Larvae of *An. gambiae* and *An. arabiensis* exploit similar habitats. Both species prefer small, sunlit, temporary, vegetation-free habitats, which are common during the rainy season (Githeko et al. 1996; Gimnig et al. 2001; Koenraadt et al. 2004). Although both anopheline species develop quickly in warm water, a strategy which prevents desiccation in their ephemeral habitats, *An. arabiensis* is better adapted to hot, dry conditions (Githeko et al. 1996), developing approximately one week faster than *An. gambiae* (Schneider et al. 2000). However, the eggs and first instars of both species are relatively resistant to desiccation (Beier et al. 1990; Koenraadt et al. 2003).

Larvae of these two species adapt quickly to temporary, anthropic habitats. During the rainy season, human-made breeding sites include temporary pools created during construction (Khaemba et al. 1994), borrow pits, drinking water vessels, and tire ruts (Gimonneau et al. 2012; Etang et al. 2016). During the dry season, preferred anthropic habitats include brick-making pits (Carlson et al. 2004) and permanent dams (Khaemba et al. 1994). Other production sites consist of early-season rice fields without well-developed vegetation and wells.

Anopheles coluzzii and *An. funestus* are also primary malaria vectors in Africa, and they exploit very different habitat types than *An. gambiae* and *An. arabiensis*. Larvae of these species are associated with large, permanent, complex, and stable habitats (Etang et al. 2016). They are commonly found in water bodies dominated by floating plants, overhanging

vegetation, and algae and are tolerant of shade (Gimnig et al. 2001; Gimonneau et al. 2012). Preferred habitat includes slow-moving water along rivers and natural ponds (Gimnig et al. 2001), as well as water bodies related to anthropogenic activities such as mature rice fields. The rate of development for *An. coluzzii* is slower, but this species exhibits strong predator-avoidance behavior, an important strategy because predators are more common in the permanent, complex habitats where they occur (Gimonneau et al. 2010).

Abundance of *An. coluzzii* and *An. funestus* peaks during and immediately after the rainy season (Gimonneau et al. 2012), and Kudom (2015) documented that *An. coluzzii* larvae can coexist with *An. gambiae* in temporary habitats such as footprints and tire tracks during this period. However, they are sustained throughout the dry season by breeding in permanent water bodies with high levels of organic material (Kudom 2015). In fact, populations of many anophelines increase early in the dry season, when larval habitats are more stable and less prone to flooding (Kweka et al. 2012, 2015). Warm, ephemeral pools tend to have greater exposure to sunlight, which supports the growth of microorganisms and provides an important food resource for foraging larvae (Minakawa et al. 1999; WHO 2013; Kweka et al. 2015).

Larvicides: methoprene

Methoprene is a chemical that mimics the juvenile hormone of certain insects. It hinders normal maturation of early mosquito instars, and, therefore, larvae that consume methoprene are unable to reach adulthood (USEPA 1991, 2006). Application timing of methoprene is critical; it works best when the insects are at earlier developmental stages (Gordon and Burford 1984) because late instars, pupae, and adults are not affected.

Methoprene degrades quickly in soil, groundwater, exposed water, and vegetation. Half-lives in water range from 30 h in clean water to 60 to 70 h in sewage. As much as 80% will degrade within 13 days after application (USEPA 1991).

The ecotoxicology of methoprene is reviewed thoroughly by Lawler (2017), and therefore we will only summarize here. Methoprene is practically non-toxic to terrestrial vertebrates and amphibians (USEPA

1991; Lawler 2017). However, fish are susceptible to methoprene exposure at relatively high concentrations that exceed application rates for mosquito management (Brown et al. 1998, 2002; Smith et al. 2003; Hurst et al. 2007); it is moderately toxic to rainbow trout, *Oncorhynchus mykiss*, and bluegill sunfish, *Lepomis macrochirus*.

Methoprene is classified as highly toxic to the planktonic crustacean *Daphnia magna*. It has adverse effects on freshwater amphipods, *Gammarus* sp. (Breaud et al. 1977), lobster (Walker et al. 2005), blue crab, *Callinectes sapidus* (Horst and Walker 1999), fiddler crab (Stueckle et al. 2008), shrimp (Brown et al. 1998; Wirth et al. 2001; Ghekiere et al. 2007), a mayfly species, *Callibaetis pacificus*, non-biting midges (Chironomidae), and a dytiscid beetle, *Laccophilus* sp. (Norland and Mulla 1975).

In a long-term study on experimental ponds where each site was treated at three-week intervals six times over a season, Hershey et al. (1998) concluded that methoprene had a negative effect on aquatic insect predators at treated sites. These impacts were considered to be both direct and indirect through food and interaction webs, as the chemical acted to cause mortality to the predator populations, but also decreased the availability of prey. Pinkney et al. (2000) observed that methoprene applied to experimental ponds had no significant impact on non-target arthropods compared to control treatments.

In a reasonable worst-case (i.e., tier-1) risk assessment, Davis (2007) found that acute and chronic exposures to methoprene did not exceed USEPA regulatory levels of concern for *Daphnia magna*, bluegill sunfish, or rainbow trout. In a review focused on environmental safety, Lawler (2017) concluded that the rates of methoprene used for mosquito management have no detectable effects on the majority of freshwater and marine invertebrates evaluated. Further, Lawler (2017) stressed the important distinction between outcomes from laboratory toxicological studies (i.e., effects) and field studies and actual environmental exposures (i.e., risk).

Larvicides: *Bacillus thuringiensis israelensis*

Bacillus thuringiensis (Bt) is a soil bacterium. Its insecticidal property is the result of a crystalline by-product (endotoxin) of sporulation that affects an insect's microvillar lining when consumed (Mittal

2003). The insecticide most likely creates an infection court for secondary infection by other bacteria that are common in the insect's midgut (Broderick et al. 2006) as well as other toxic mechanisms (Caccia et al. 2016). Bt is a highly regarded insecticide because its many strains target specific insect species or narrow groups of insects. Consequently, it is well known that Bt endotoxins are practically non-toxic to mammals, fish, and birds (Mittal 2003) and they break down quickly in the environment (USEPA 1998b).

Bacillus thuringiensis israelensis (Bti) is the strain of Bt that is used for mosquito management. Bti is practically non-toxic to mammals, birds, and fish (Mittal 2003) and is not persistent (Hajaj et al. 2005), although it is toxic to some aquatic receptors, including non-biting midges (Chironomidae). Ali (1981) found that applications of Bti to experimental ponds significantly lowered numbers of non-target chironomids. At the highest treatment rate of 4,000 g/ha, there was a 54 to 92% reduction in chironomid abundance. In golf-course ponds at a treatment of 3,000 g/ha, there was a 30 to 67% chironomid reduction, but numbers returned to pre-treatment levels 14 days after treatment (Ali 1981). Charbonneau et al. (1994) found that although Bti caused high mortality of chironomids in a laboratory, a much lower and statistically non-significant mortality was observed in the field. Similarly, Duchet et al. (2015) did not observe any effects on two chironomid species and Lagadic et al. (2016) observed no immediate or long-term effects on chironomid community structure after application of Bti.

However, a series of recent studies in Europe suggest repeated use of Bti has secondary deleterious effects on predators (Jakob and Poulin 2016; Poulin and Lefebvre 2018), primarily through reducing chironomid populations. Allgeier et al. (2019) and Brühl et al. (2020) observed significant reductions in adult chironomid emergence rates after Bti applications in mesocosm and field studies. In a microcosm experiment, Bordalo et al. (2021) also observed deleterious effects on stream benthic invertebrates, including chironomids. It is important to note that in many of these studies, the location evaluated received 30 to 50 aerial Bti applications per year, an exceptionally high frequency of application for Bti. However, WHO (2013) has recommendations that include a maximum of 24 applications per year.

Two formulations of Bti had no effect on non-target invertebrates, including the amphipod *Hyalella azteca*, in test ponds that had a Bti concentration of 100 mg/L (Gharib and Hilsenhoff 1988). Milam et al. (2000) found that treatments of Bti were much more damaging to *An. quadrimaculatus* than sentinel species, including *Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*, and *Pimephales promelas*. In a laboratory assay, Olmo et al. (2016) observed dose–response toxicity in two copepod and three cladoceran species. Hershey et al. (1998) conducted a large-scale study using 27 experimental ponds in Minnesota, USA. The focus of their study was to determine the impact of multiple aerially applied direct applications of granular methoprene and Bti on non-target invertebrates. Bti and methoprene significantly lowered numbers of chironomids, tipulids, ceratopogonids, and brachycerans in treatment ponds. Disruption of food webs and interaction webs was hypothesized to have occurred in many of these reductions because predators seemed to decline with prey. However, populations rebounded in the years after the treatments. Niemi et al. (1999) found changes in insect diversity in Bti-treated ponds, and reduced total insect numbers in ponds treated with both methoprene and Bti. Lawler et al. (1999) found that Bti and methoprene had no measurable impact on sentinel amphipods in ephemeral mangrove swamps on Sanibel Island, Florida, USA when treated with Bti granules at 5.6 kg/ha and a methoprene liquid formulation applied at 10.65 ml AI/ha for the control of *Aedes taeniorhynchus*. Davis and Peterson (2008) did not observe any overall deleterious effects on non-target arthropods in a field experiment with a single application of Bti.

Ecological effects have been noted for Bti used for black fly and mosquito management. Merritt et al. (1989) observed few changes in indices used to measure treatment effects of Bti used for black fly management in a Michigan river. Drift samples taken at a control and treatment site did not differ for chironomids, baetids, gammarids, or hydropsychids, but there were some treatment effects on perlid stoneflies and elmids beetles. Similar results were observed in 10 stream trials measuring stream insect density of selected taxa (Lawler 2017). Molloy (1992) observed that Bti applied for black fly control within a New York stream affected filter-feeding chironomids, but not surface-dwelling or tube-dwelling members of

the same family. Caddisflies and mayflies showed no positive or negative response to Bti treatments.

Larvicides: *Lysinibacillus sphaericus*

Lysinibacillus sphaericus (= *Bacillus sphaericus*) is a soil bacterium that has a similar insecticidal action as Bti (Mittal 2003). For *L. sphaericus*, the insecticidal agent is in the spore cell wall and is a by-product of spore production (Mittal 2003). When the agent is consumed by the mosquito larva, it degrades the lining of the midgut. The insecticide is more effective against *Anopheles* and *Culex* species than *Aedes* species (Mittal 2003), and it remains more active in eutrophic waters than Bti (Lawler 2017).

Brown et al. (2004) found no toxicity to non-target Australian fauna including the fish *Pseudomugil signifier* and the shrimp *Leander tenuicornis*. Merritt et al. (2005) observed similar results in a three-year study in two habitats in which 138 invertebrate taxa were exposed to *L. sphaericus*. Results indicated few impacts on taxa categorized into functional groups.

Secondary effects: larvicides and gene-drive mosquitoes

Although all substances are toxic depending on the dose, it is clear that proteins expressed in a gene-drive system to suppress or modify mosquito populations for malaria management would not be similar to larvicidal active ingredients. They would most likely be practically non-toxic to non-target organisms and would challenge the current situation with pesticides that there are deleterious effects other than those caused by a reduction in the population of the target population. Further, as proteins expressed in mosquito larvae, they would almost certainly be expressed at environmental concentrations that are orders of magnitude lower than conventional larvicides (Connolly et al. 2021).

Consequently, the focus in most cases would be on the secondary effects associated with population suppression of the target organism (in this case, species in the *An. gambiae* or *An. funestus* complex). It is important to note that the goal of both conventional larvicides and the gene-drive systems discussed here is to lower the population of the pest mosquito to reduce malaria (Fig. 1). Indeed, that is the point of the management tactic unless the focus is population

modification. In the following paragraphs, we discuss secondary effects that apply to both current and gene-drive approaches.

Immature mosquitoes as food for predators

One secondary effect of population suppression is the potential reduction of beneficial species that feed on the larvae and pupae of *An. gambiae* (sensu lato) (Fig. 1). Many invertebrate species and larvivorous fish feed on the aquatic larval and pupal life stages of mosquitoes (Service 1977; Ohba et al. 2010; Dida et al. 2015). Predatory invertebrates may be responsible for as much as 90% of the mortality of immature mosquitoes in certain aquatic habitats (Service 1971, 1973, 1977). In the wetlands of western Kenya, Ohba et al. (2010) found that 54.2% of 330 potential predators had ingested immature stages of *An. gambiae*, including Odonata larvae (70.2%), Hemiptera (62.8%), Amphibia (41.7%), and Coleoptera (18%).

However, there is little evidence that aquatic predators rely solely on immature mosquitoes for survival. Rather, larval and pupal stages of mosquitoes serve as one of many food sources for predators. After an extensive literature review of *An. gambiae* predation in Africa, Collins et al. (2019) suggested that no predators have been found to be closely associated or dependent on *An. gambiae* larvae, and that this mosquito complex is probably not an essential part of any ecosystem food web. Roberts et al. (2017) concurred, suggesting the loss of *An. gambiae* from a particular aquatic habitat is unlikely to cause ecological harm, even though many invertebrates and fish prey on this species. Likewise, Derua et al. (2018) found that long-lasting microbial larvicides (Bti and *L. sphaericus*), which reduce immature populations of *An. gambiae* and *An. funestus*, have no ecologically significant impact on the abundance or diversity of non-target invertebrates or vertebrates in the western highlands of Kenya.

Another important consideration for ecological risk is that in sub-Saharan Africa two of the three primary malaria vectors prefer small, ephemeral, sunlit water bodies that do not support predator populations (Carlson et al. 2004; Diabate et al. 2005; Gimonneau et al. 2010, 2012). Aquatic predators typically require more time to develop than mosquito larvae, and therefore occur in more permanent habitats (Kindlmann and Dixon 1999; Terhorst et al. 2010). Therefore,

mosquito larvae in ephemeral habitats such as hoof prints or road ruts exhibit higher survival because there are fewer predators (Munga et al. 2006). The seasonality of *An. gambiae* combined with the ephemeral nature of its larval habitats likely results in predation that is limited to opportunistic generalist predators (Collins et al. 2019), and does not disproportionately and adversely affect any specific non-target species. Overall, the current weight of evidence suggests that a reduction in *An. gambiae* and closely related mosquito larvae most likely would have a negligible impact on predator abundance. Moreover, the species complex does not seem to play a key role in ecosystems (Collins et al. 2019; Connolly et al. 2021).

Effects on the food of larval mosquitoes

Another secondary effect of population suppression could be an increase in algal blooms (including toxic algal blooms), which might adversely affect wildlife. Algae and other primary producers are important larval food sources for anopheline mosquitoes (Connolly et al. 2021). Kaufman et al. (2006) suggested that algal biomass on water surfaces is important for larval development of *An. gambiae*, and Gimnig et al. (2002) found that *An. gambiae* larval grazing reduced algal biomass and abundance in an experiment using an artificial habitat with rainwater seeded with cow dung. The presence of algal mats also serves as an attractant for ovipositing *Anopheles* females (Bond et al. 2005). Both *An. gambiae* and *An. funestus* have been positively associated with algae (Minakawa et al. 1999; Gimnig et al. 2001), despite their different habitat preferences. However, this association may also reflect the growth of inedible algal forms, such as filamentous green algae, which is indigestible for most invertebrates (Martin and Kukor 1984). Studies linking reductions in *An. gambiae* larvae to increases in algal blooms might be irrelevant because habitat used by this species is temporary and may not support healthy communities of primary producers (Teem et al. 2019). However, larvae of *An. coluzzii* and *An. funestus* occur in more complex, permanent habitats (Gimnig et al. 2001; Gimonneau et al. 2012) and might play a greater role in reducing algal blooms. Regardless, a decline in mosquito larvae would not affect toxic algal blooms because the cyanobacteria that comprise these blooms are toxic to many animals, including mosquito larvae, so cyanobacteria

would not be reduced through feeding (Marten 2007; Connolly et al. 2021).

Bacteria, protozoa, and other primary producers may serve as secondary food sources for mosquito larvae and therefore may be affected by reduced numbers of larvae. Gimnig et al. (2002) suggested that if algal resources are depleted, *An. gambiae* larvae will feed on available bacteria, but bacterial abundance was not significantly affected. Östman et al. (2008) found that protozoan densities and diversity increased dramatically after floodwater mosquito populations were significantly reduced by Bti treatments.

Somewhat related to the food and detritus issue is the secondary effect of numerous dead *An. gambiae* larvae having a deleterious effect on water quality. To our knowledge, there have been no studies of this for current larvicides. Gene-drive population suppression would reduce the population, resulting in increasingly fewer larvae and therefore negate specific concerns about water quality due to extensive larval mortality. Conversely, with a larvicide, there would be dead larvae in the water and concentrations of the larvicide each time it is used.

Effects of engineered proteins and nucleic acids

Another potential secondary effect is that dead gene-drive mosquito larvae will differentially contaminate the water compared to non-gene-drive larvae. Based on the techniques currently being investigated, it is unlikely that the DNA, RNA, or proteins responsible for population suppression in gene-drive mosquitoes would negatively affect water quality any more than non-gene-drive mosquitoes. Of course, the engineered proteins responsible for the desired effect in the gene-drive mosquitoes would be assessed for fundamental toxicity and allergenicity as is currently done with transgenic products, with positive toxicity or allergenicity likely leading to a regulatory rejection (EFSA 2010; EFSA et al. 2020; Connolly et al. 2021). Given the likely impact of the population suppression strategies, which would be to reduce the production of offspring (i.e., larvae), the “contamination” due to gene-drive larvae would be less than that of non-gene drive larvae, or gene-drive larvae from population modification strategies. However, in none of the larval types would the effect of the “contamination” be any greater than that of non-genetically engineered mosquitoes in the environment.

Niche replacement

A substantive reduction of larval *An. gambiae* populations could also result in an ecological niche opening up for other vector species that transmit malaria or other diseases. Studies have documented mosquito management which reduced populations of anopheline mosquitoes in East Africa and resulted in higher densities of other species, likely because of preferential elimination of adults and consequently population reduction (Gillies and Smith 1960; Gillies and Furlong 1964; Bayoh et al. 2010). *Anopheles gambiae* is the most efficient vector of malaria (Lindsay et al. 1998), in part because it has a very effective biological response to competition. It reduces its larval developmental time in the presence of competitors without an increase in larval mortality or a reduction in body size, but the effect depends on water volume (Paaajmans et al. 2009). This strategy results in higher competitive success compared to *An. arabiensis* or *An. coluzzii*, which share aquatic habitats with *An. gambiae* but have lower rates of malaria transmission. Therefore, any reduction in *An. gambiae* abundance should translate to reduced risk of malaria, since the competitors most likely to replace it are not as efficient vectors.

The *An. gambiae* complex is comprised of many morphologically indistinguishable species, which means hybridization potentially occurs. If gene flow between species includes the gene construct of gene-drive mosquitoes, malaria transmission may be further reduced, as naïve species in the complex are exposed and eventually genetically modified (Roberts et al. 2017). Under such conditions, this management tactic should result in fewer inputs over time, including potentially requiring fewer larvicide applications. In addition, McArthur et al. (2014) determined that gene-drive *An. gambiae* larvae have the same mortality rate as wild-type larvae, suggesting there should not be an increase in the accumulation of phenotypes in the environment.

Conclusion

Because of workshops with stakeholders that identified concerns about aquatic environments and water quality, we have used a comparative qualitative risk assessment approach for aquatic environments

(Roberts et al. 2017; Teem et al. 2019; Connolly et al. 2021). We conclude that the tactic of gene-drive *An. gambiae* for malaria management is unlikely to result in risks to aquatic environments that exceed current larviciding tactics. Although these systems currently are in research and development stages, it is likely that the resulting proteins will not have insecticidal properties that are mechanistically similar to current larvicides. As such, they should not impose risks on water quality and non-target aquatic organisms that are greater than current larvicides. In fact, the risks might be lower (Fig. 2). Our conclusions directly relate to the important regulatory concept of “substantial equivalence” (Codex Alimentarius Commission 2003). Furthermore, they are consistent with the recommended safety standard of James et al. (2020), who recommend that gene-drive mosquitoes should be released only if they “...will do no more harm to human health than wild-type mosquitoes of the same genetic background and no more harm to the ecosystem than other conventional vector control interventions.”

It is important to reiterate, however, that in most cases gene-drive mosquitoes will be used within an existing IPM system. Consequently, IPM tactics such as larvicides and gene-drive mosquitoes will be used concurrently and regulators will need to ensure that risks from all tactics are acceptable.

Traditionally, risk assessment relies on estimating or using actual environmental exposures to the stressor and comparing those to effects to arrive at a quantitative characterization of risk. However, for gene-drive mosquitoes, the problem formulation, stressor identification, and effect assessment may be more important to the final risk estimate (Fig. 2), especially in these early days when there is no body of experiential use data.

Mosquito and malaria management should always use IPM. This approach is also referred to as Integrated Mosquito Management (IMM) and Integrated Vector Management (IVM) when concerned with mosquito vector management. IPM is a comprehensive approach to managing pests that is economically and ecologically sustainable (Peterson et al. 2018). Although using multiple tactics and integrating those tactics are not an absolute requirement for a successful, sustainable IPM program, they are commonly a feature of IPM. The concept of ecological sustainability includes resistance by the pest to the management

tactic, and, therefore, an overall goal of IPM is to manage resistance. This is especially salient because management tactics such as contemporary synthetic insecticides, biological insecticides, and gene-drive approaches obviate long-term issues of broad-spectrum toxicity and environmental residuality of pesticides. Consequently, because resistance development by pests is arguably the most significant contemporary risk with management tactics (Peterson et al. 2018), the discovery and development of new tactics is critical to long-term management success (WHO 2020). Provided initial regulatory safety assessments and field applications and monitoring are successful, gene-drive mosquitoes will undoubtedly be an important tactic within IPM programs for malaria management.

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References

- Ali A (1981) *Bacillus thuringiensis* serovar. *israelensis* (ABG-6108) against chironomids and some nontarget aquatic invertebrates. *J Invertebr Pathol* 38:264–272
- Allgeier S, Kästel A, Brühl CA (2019) Adverse effects of mosquito control using *Bacillus thuringiensis* var. *israelensis*: Reduced chironomid abundances in mesocosm, semi-field and field studies. *Ecotoxicol Environ Saf* 169:786–796
- Antonio-Nkondjio C, Doumbe-Belisse P, Djamouko-Djonkam L et al (2021) High efficacy of microbial larvicides for malaria vectors control in the city of Yaounde Cameroon following a cluster randomized trial. *Sci Rep* 11:17101. <https://doi.org/10.1038/s41598-021-96362-z>
- Barrón MG, Paupy C, Rahola N et al (2019) A new species in the major malaria vector complex sheds light on reticulated species evolution. *Sci Rep* 9:14753. <https://doi.org/10.1038/s41598-019-49065-5>
- Bayoh MN, Mathias DK, Odiere MR et al (2010) *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malar J* 9:62. <http://www.malariajournal.com/content/9/1/62>
- Beier JC, Copeland R, Oyaro C et al (1990) *Anopheles gambiae* complex egg-stage survival in dry soil from larval development sites in western Kenya. *J Am Mosq Control Assoc* 6:105–109
- Bier E (2021) Gene drives gaining speed. *Nat Rev Genet*. <https://doi.org/10.1038/s41576-021-00386-0>
- Bond JG, Arredondo-Jimenez JI, Rodriguez MH et al (2005) Oviposition habitat selection for a predator refuge and food source in a mosquito. *Ecol Entomol* 30:255–263
- Bordalo MD, Machado AL, Campos D et al (2021) Responses of benthic macroinvertebrate communities to a Bti-based insecticide in artificial microcosm streams. *Environ Pollut* 282:117030. <https://doi.org/10.1016/j.envpol.2021.117030>
- Breaud T, Farlow J, Steelman C, Schilling P (1977) Effects of insect growth-regulator methoprene on natural populations of aquatic organisms in Louisiana intermediate marsh habitats. *Mosq News* 37:704–712
- Broderick NA, Raffa KF, Handelsman J (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proc Natl Acad Sci* 103:15196–15199
- Brown MD, Thomas D, Kay B (1998) Acute toxicity of selected pesticides to the Pacific blue-eye, *Pseudomugil signifer* (Pisces). *J Am Mosq Control Assoc* 14:463–466
- Brown MD, Carter J, Thomas D, Purdie DM, Kay BH (2002) Pulse-exposure effects of selected insecticides to juvenile Australian crimson-spotted rainbowfish (*Melanotaenia duboulayi*). *J Econ Entomol* 95:294–298
- Brown MD, Watson T, Carter J, Purdie DM, Kay BH (2004) Toxicity of VectoLex (*Bacillus sphaericus*) products to selected Australian mosquito and nontarget species. *J Econ Entomol* 97:51–58
- Brühl CA, Després L, Frör O, Patil CD, Poulin B, Tetreau G, Allgeier S (2020) Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti). *Sci Total Environ*. 724:137800
- Caccia S, Di Lelio I, La Storia A et al (2016) Midgut microbiota and host immunocompetence underlie *Bacillus thuringiensis* killing mechanism. *Proc Natl Acad Sci USA* 113:9486–9491. <https://doi.org/10.1073/pnas.1521741113>
- Carlson JC, Byrd BD, Omlin FX (2004) Field assessments in western Kenya link malaria vectors to environmentally disturbed habitats during the dry season. *BMC Public Health* 4:33. <https://doi.org/10.1186/1471-2458-4-33>
- Carvalho DO, McKemey AR, Garziera L et al (2015) Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. *PLoS*

- Negl Trop Dis 9:e0003864. <https://doi.org/10.1371/journal.pntd.0003864>
- Champer J, Lee E, Yang E et al (2020) A toxin-antidote CRISPR gene drive system for regional population modification. *Nat Commun* 11:1082. <https://doi.org/10.1038/s41467-020-14960-3>
- Charbonneau CS, Drobney RD, Rabeni CF (1994) Effects of *Bacillus thuringiensis* var. *israelensis* on nontarget benthic organisms in a lentic habitat and factors affecting the efficacy of the larvicide. *Environ Toxicol Chem* 13:267–279
- Choi L, Majambere S, Wilson AL (2019) Larviciding to prevent malaria transmission. *Cochrane Database Syst Rev*. <https://doi.org/10.1002/14651858.CD012736.pub2>
- Codex Alimentarius Commission (2003) Codex Alimentarius - Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants
- Collins CM, Bonds JAS, Quinlan MM, Mumford JD (2019) Effects of the removal or reduction in density of the malaria mosquito, *Anopheles gambiae* on interacting predators and competitors in local ecosystems. *Med Vet Entomol* 33:1–15. <https://doi.org/10.1111/mve.12327>
- Connolly JB, Mumford JD, Fuchs S, Turner G, Beech C, North AR, Burt A (2021) Systematic identification of plausible pathways to potential harm via problem formulation for investigational releases of a population suppression gene drive to control the human malaria vector *Anopheles gambiae* in West Africa. *Malar J* 20:170
- Davis RS, Peterson RKD (2008) Effects of single and multiple applications of mosquito insecticides on nontarget arthropods. *J Am Mosq Control Assoc* 24:270–280
- Davis RS, Peterson RK, Macedo PA (2007) An ecological risk assessment for insecticides used in adult mosquito management. *Integr Environ Assess Manag* 3:373–382. <https://doi.org/10.1002/ieam.5630030308>
- Davis RS (2007) An ecological risk assessment for mosquito insecticides. Thesis. Montana State University. Bozeman, Montana
- Derua YA, Kahindi SC, Mosha FW et al (2018) Microbial larvicides for mosquito control: Impact of long lasting formulations of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* on non-target organisms in western Kenya highlands. *Ecol Evol* 8:7563–7573. <https://doi.org/10.1002/ece3.4250>
- Derua YA, Kweka EJ, Kisinza WN et al (2019) Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. *Parasites Vectors* 12:426. <https://doi.org/10.1186/s13071-019-3683-5>
- Diabate A, Dabiré RK, Kim EH et al (2005) Larval development of the molecular forms of *Anopheles gambiae* (Diptera: Culicidae) in different habitats: a transplantation experiment. *J Med Entomol* 42:548–553
- Dida GO, Gelder FB, Anyona DN et al (2015) Presence and distribution of mosquito larvae predators and factors influencing their abundance along the Mara River, Kenya and Tanzania. *Springerplus* 4:136. <https://doi.org/10.1186/s40064-015-0905-y>
- Duchet C, Franquet E, Lagadic L, Lagneau C (2015) Effects of *Bacillus thuringiensis israelensis* and spinosad on adult emergence of the non-biting midges *Polypedilum nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in coastal wetlands. *Ecotoxicol Environ Saf* 115:272–278
- EFSA (2010) Guidance on the environmental risk assessment of genetically modified plants. *EFSA J* 8:1879. <https://doi.org/10.2903/j.efsa.2010.1879>
- EFSA, Naegeli H, Bresson J et al (2020) Adequacy and sufficiency evaluation of existing EFSA guidelines for the molecular characterisation, environmental risk assessment and post-market environmental monitoring of genetically modified insects containing engineered gene drives. *EFS2* 18. <https://doi.org/10.2903/j.efsa.2020.6297>
- Etang J, Mbida Mbida A, Ntonga Akono P et al (2016) *Anopheles coluzzii* larval habitat and insecticide resistance in the island area of Manoka, Cameroon. *BMC Infect Dis* 16:217. <https://doi.org/10.1186/s12879-016-1542-y>
- Gharib A, Hilsenhoff W (1988) Efficacy of 2 formulations of *Bacillus thuringiensis* var. *israelensis* (H-14) against *Aedes vexans* and safety to non-target macroinvertebrates. *J Am Mosq Control Assoc* 4:252–255
- Ghekiere A, Fockedeey N, Verslycke T, Vincx M, Janssen CR (2007) Marsupial development in the mysid *Neomysis integer* (Crustacea: Mysidacea) to evaluate the effects of endocrine-disrupting chemicals. *Ecotoxicol Environ Saf* 66:9–15
- Gillies M, Furlong M (1964) An investigation into the behaviour of *Anopheles parensis* Gillies at Malindi on the Kenya coast. *Bull Entomol Res* 55:1–16
- Gillies M, Smith A (1960) The effect of a residual house-spraying campaign in East Africa on species balance in the *Anopheles funestus* group. The replacement of *A. funestus* Giles by *A. rivulorum* Leeson. *Bull Entomol Res* 51:243–252
- Gimnig JE, Ombok M, Kamau L, Hawley WA (2001) Characteristics of larval anopheline (Diptera: Culicidae) habitats in western Kenya. *J Med Entomol* 38:282–288
- Gimnig JE, Ombok M, Otieno S et al (2002) Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. *J Med Entomol* 39:162–172. <https://doi.org/10.1603/0022-2585-39.1.162>
- Gimonneau G, Bouyer J, Morand S et al (2010) A behavioral mechanism underlying ecological divergence in the malaria mosquito *Anopheles gambiae*. *Behav Ecol* 21:1087–1092. <https://doi.org/10.1093/beheco/arq114>
- Gimonneau G, Pombi M, Choisy M et al (2012) Larval habitat segregation between the molecular forms of the mosquito *Anopheles gambiae* in a rice field area of Burkina Faso, West Africa. *Med Vet Entomol* 26:9–17. <https://doi.org/10.1111/j.1365-2915.2011.00957.x>
- Githeko AK, Service MW, Mbogo CM, Atieli FK (1996) Resting behaviour, ecology and genetics of malaria vectors in large scale agricultural areas of Western Kenya. *Parassitologia* 38:481–489
- Gordon R, Burford IR (1984) Effects of methoprene, a juvenile hormone analogue, on the larval and pupal stages of the yellow fever mosquito, *Aedes Aegypti*. *J Insect Physiol* 30:279–286
- Hajaij M, Carron A, Deleuze J, Gaven B, Setier-Rio M-L, Vigo G, Thiéry I, Nielsen-LeRoux C, Lagneau C (2005) Low persistence of *Bacillus thuringiensis* serovar *israelensis*

- spores in four mosquito biotopes of a salt marsh in southern France. *Microb Ecol* 50:475–487
- Hammond A, Pollegioni P, Persampieri T et al (2021) Gene-drive suppression of mosquito populations in large cages as a bridge between lab and field. *Nat Commun* 12:4589. <https://doi.org/10.1038/s41467-021-24790-6>
- Harris AF, Nimmo D, McKemey AR et al (2011) Field performance of engineered male mosquitoes. *Nat Biotechnol* 29:1034–1037. <https://doi.org/10.1038/nbt.2019>
- Hershey AE, Lima AR, Niemi GJ, Regal RR (1998) Effects of *Bacillus thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecol Appl* 8:41–60
- Horst MN, Walker AN (1999) Effects of the pesticide methoprene on morphogenesis and shell formation in the blue crab *Callinectes sapidus*. *J Crustac Biol* 19:699
- Hurst TP, Kay BH, Ryan PA, Brown MD (2007) Sublethal effects of mosquito larvicides on swimming performance of larvivoracious fish *Melanotaenia duboulayi* (Atheriniformes: Melanotaeniidae). *J Econ Entomol* 100:61–65
- Jakob C, Poulin B (2016) Indirect effects of mosquito control using *Bti* on dragonflies and damselflies (Odonata) in the Camargue. *Insect Conserv Divers* 9:161–169. <https://doi.org/10.1111/icad.12155>
- James SL, Marshall JM, Christophides GK et al (2020) Toward the definition of efficacy and safety criteria for advancing gene drive-modified mosquitoes to field testing. *Vector-Borne Zoonotic Dis* 20:237–251. <https://doi.org/10.1089/vbz.2019.2606>
- Kaufman MG, Wanja E, Maknoja S et al (2006) Importance of algal biomass to growth and development of *Anopheles gambiae* larvae. *J Med Entomol* 43:669–676
- Khaemba BM, Mutani A, Bett MK (1994) Studies of anopheline mosquitoes transmitting malaria in a newly developed highland urban area: a case study of Moi University and its environs. *East Afr Med J* 71:159–164
- Kindlmann P, Dixon AAF (1999) Generation time ratios—determinants of prey abundance in insect predator–prey interactions. *Biol Control* 16:133–138
- Koenraadt CJM, Paaijmans KP, Githeko AK et al (2003) Egg hatching, larval movement and larval survival of the malaria vector *Anopheles gambiae* in desiccating habitats. *Malar J* 2:20. <http://www.malariajournal.com/content/2/1/20>
- Koenraadt CJM, Githeko AK, Takken W (2004) The effects of rainfall and evapotranspiration on the temporal dynamics of *Anopheles gambiae* s.s. and *Anopheles arabiensis* in a Kenyan village. *Acta Trop* 90:141–153. <https://doi.org/10.1016/j.actatropica.2003.11.007>
- Kudom AA (2015) Larval ecology of *Anopheles coluzzii* in Cape Coast, Ghana: water quality, nature of habitat and implication for larval control. *Malar J* 14:447. <https://doi.org/10.1186/s12936-015-0989-4>
- Kweka EJ, Zhou G, Munga S et al (2012) Anopheline larval habitats seasonality and species distribution: a prerequisite for effective targeted larval habitats control programmes. *PLoS ONE* 7:e52084. <https://doi.org/10.1371/journal.pone.0052084>
- Kweka EJ, Munga S, Himeidan Y et al (2015) Assessment of mosquito larval productivity among different land use types for targeted malaria vector control in the western Kenya highlands. *Parasites Vectors* 8:356. <https://doi.org/10.1186/s13071-015-0968-1>
- Kyrou K, Hammond AM, Galizi R et al (2018) A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat Biotechnol* 36:1062–1066. <https://doi.org/10.1038/nbt.4245>
- Lagadic L, Schäfer RB, Roucaute M, Szöcs E, Chouin S, de Maupeou J, Duchet C, Franquet E, Le Hunsec B, Bertrand C, Fayolle S, Francés B, Rozier Y, Foussadier R, Santoni J-B, Lagneau C (2016) No association between the use of Bti for mosquito control and the dynamics of non-target aquatic invertebrates in French coastal and continental wetlands. *Sci Total Environ* 553:486–494
- Lawler SP (2017) Environmental safety review of methoprene and bacterially-derived pesticides commonly used for sustained mosquito control. *Ecotoxicol Environ Saf* 139:335–343. <https://doi.org/10.1016/j.ecoenv.2016.12.038>
- Lawler SP, Jensen T, Dritz D, Wichterman G (1999) Field efficacy and nontarget effects of the mosquito Larvicides temephos, methoprene, and *Bacillus thuringiensis* var. *israelensis* in Florida mangrove swamps. *J Am Mosq Control Assoc* 15:446–452
- Lindsay SW, Parson L, Thomas CJ (1998) Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* sensu stricto and *An. arabiensis*, using climate data. *Proceedings: Biol Sci* 265:847–854
- Marten GG (2007) Larvicidal algae. *AMCA Bulletin* No 7 23:177–183
- Martin MM, Kukor JJ (1984) Role of mycophagy and bacteriophagy in invertebrate nutrition. *Current Perspectives in Microbial Ecology: Proceedings of the Third International Symposium on Microbial Ecology*, Michigan State University, 7–12 August 1983 257–263
- McArthur CC, Meredith JM, Eggleston P (2014) Transgenic *Anopheles gambiae* expressing an antimalarial peptide suffer no significant fitness cost. *PLoS ONE* 9:e88625. <https://doi.org/10.1371/journal.pone.0088625>
- MCTI-CTNBio (2014) Technical Opinion no. 3964/2014: Request for Opinion on Commercial Release of Genetically Modified Microorganism. National Biosafety Technical Commission, Brasilia, Brasil
- Merritt R, Walker E, Wilzbach M et al (1989) A broad evaluation of BTI for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. *J Am Mosq Control Assoc* 5:397–415
- Merritt RW, Lessard JL, Wessell KJ et al (2005) Lack of effects of *Bacillus sphaericus* (VECTOLEX®) on nontarget organisms in a mosquito-control program in southeastern Wisconsin: a 3-year study. *J Am Mosq Control Assoc* 21:201–212. [https://doi.org/10.2987/8756-971X\(2005\)21\[201:LOE OBS\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2005)21[201:LOE OBS]2.0.CO;2)
- Milam C, Farris J, Wilhide J (2000) Evaluating mosquito control pesticides for effect on target and nontarget organisms. *Arch Environ Contam Toxicol* 39:324–328
- Minakawa N, Mutero CM, Githure JI et al (1999) Spatial distribution and habitat characterization of anopheline

- mosquito larvae in western Kenya. *Am J Trop Med Hyg* 61:1010–1016
- Mittal P (2003) Biolarvicides in vector control: challenges and prospects. *J Vector Borne Dis* 40:20–32
- Molloy D (1992) Impact of the black fly (Diptera: Simuliidae) control agent *Bacillus thuringiensis* va israelensis on chironomids (Diptera: Chironomidae) and other nontarget insects - results of 10 field trials. *J Am Mosq Control Assoc* 8:24–31
- Munga S, Minakawa N, Zhou G et al (2006) Association between land cover and habitat productivity of malaria vectors in western Kenyan highlands. *Am J Trop Med Hyg* 74:69–75
- Nash A, Urdaneta GM, Beaghton AK et al (2018) Integral gene drives for population replacement. *Biol Open* bio.037762. <https://doi.org/10.1242/bio.037762>
- National Academies of Sciences, Engineering, and Medicine (2016) *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*. National Academies Press, Washington, D.C.
- Niemi GJ, Hershey AE, Shannon L et al (1999) Ecological effects of mosquito control on zooplankton, insects, and birds. *Environ Toxicol Chem* 18:549–559. <https://doi.org/10.1002/etc.5620180325>
- Noble C, Min J, Olejarz J et al (2019) Daisy-chain gene drives for the alteration of local populations. *Proc Natl Acad Sci USA* 116:8275–8282. <https://doi.org/10.1073/pnas.1716358116>
- Norland RL, Mulla MS (1975) Impact of Altosid on selected members of an aquatic ecosystem. *Environ Entomol* 4:145–152. <https://doi.org/10.1093/ee/4.1.145>
- NRC (1983) *Risk assessment in the federal government: managing the process*. National Academy Press, Washington, D.C.
- NRC (1996) *Understanding risk: information decisions in a democratic society*. National Academy Press, Washington, D.C.
- NRC (2009) *Science and decisions: advancing risk assessment*. National Academies Press, Washington, D.C.
- Ogola EO, Fillinger U, Ondiba IM et al (2018) Insights into malaria transmission among *Anopheles funestus* mosquitoes, Kenya. *Parasites Vectors* 11:577. <https://doi.org/10.1186/s13071-018-3171-3>
- Ohba S-Y, Kawada H, Dida GO et al (2010) Predators of *Anopheles gambiae* sensu lato (Diptera: Culicidae) larvae in wetlands, western Kenya: confirmation by polymerase chain reaction method. *J Med Entomol* 47:783–787
- Olmo C, Marco A, Armengol X, Ortells R (2016) Effects of *Bacillus thuringiensis* var. israelensis on nonstandard microcrustacean species isolated from field zooplankton communities. *Ecotoxicology* 25:1730–1738. <https://doi.org/10.1007/s10646-016-1708-9>
- Östman Ö, Lundström JO, Persson Vinnersten TZ (2008) Effects of mosquito larvae removal with *Bacillus thuringiensis israelensis* (Bti) on natural protozoan communities. *Hydrobiologia* 607:231–235. <https://doi.org/10.1007/s10750-008-9387-z>
- Paaijmans KP, Huijben S, Githeko AK, Takken W (2009) Competitive interactions between larvae of the malaria mosquitoes *Anopheles arabiensis* and *Anopheles gambiae* under semi-field conditions in western Kenya. *Acta Trop* 109:124–130. <https://doi.org/10.1016/j.actatropica.2008.07.010>
- Peterson RK (2006) Comparing ecological risks of pesticides: the utility of a Risk Quotient ranking approach across refinements of exposure. *Pest Manag Sci* 62:46–56. <https://doi.org/10.1002/ps.1126>
- Peterson RKD, Arntzen CJ (2004) On risk and plant-based biopharmaceuticals. *Trends Biotechnol* 22:64–66. <https://doi.org/10.1016/j.tibtech.2003.11.007>
- Peterson RKD, Shama LM (2005) A comparative risk assessment of genetically engineered, mutagenic, and conventional wheat production systems. *Transgenic Res* 14:859–875. <https://doi.org/10.1007/s11248-005-1411-8>
- Peterson RKD, Macedo PA, Davis RS (2006) A human-health risk assessment for West Nile virus and insecticides used in mosquito management. *Environ Health Perspect* 114:366–372. <https://doi.org/10.1289/ehp.8667>
- Peterson RKD, Higley LG, Pedigo LP (2018) Whatever happened to IPM? *Am Entomol* 64:146–150. <https://doi.org/10.1093/ae/tmy049>
- Pinkney AE, McGowan PC, Murphy DR et al (2000) Effects of the mosquito larvicides temephos and methoprene on insect populations in experimental ponds. *Environ Toxicol Chem* 19:678–684. <https://doi.org/10.1002/etc.5620190320>
- Poulin B, Lefebvre G (2018) Perturbation and delayed recovery of the reed invertebrate assemblage in Camargue marshes sprayed with *Bacillus thuringiensis israelensis*: Bti impact on reed invertebrates. *Insect Science* 25:542–548. <https://doi.org/10.1111/1744-7917.12416>
- Raybould A, Macdonald P (2018) Policy-led comparative environmental risk assessment of genetically modified crops: testing for increased risk rather than profiling phenotypes leads to predictable and transparent decision-making. *Front Bioeng Biotechnol* 6:43. <https://doi.org/10.3389/fbioe.2018.00043>
- Raybould A, Holt K, Kimber I (2019) Using problem formulation to clarify the meaning of weight of evidence and biological relevance in environmental risk assessments for genetically modified crops. *GM Crops Food* 10:63–76. <https://doi.org/10.1080/21645698.2019.1621615>
- Roberts A, de Andrade PP, Okumu F et al (2017) Results from the workshop “Problem Formulation for the Use of Gene Drive in Mosquitoes”. *Am J Trop Med Hyg* 96:530–533. <https://doi.org/10.4269/ajtmh.16-0726>
- Romeis J, Collatz J, Glandorf DCM, Bonsall MB (2020) The value of existing regulatory frameworks for the environmental risk assessment of agricultural pest control using gene drives. *Environ Sci Policy* 108:19–36. <https://doi.org/10.1016/j.envsci.2020.02.016>
- Schleier JJ, Peterson RKD (2013) A refined aquatic ecological risk assessment for a pyrethroid insecticide used for adult mosquito management. *Environ Toxicol Chem* 32:948–953. <https://doi.org/10.1002/etc.2126>
- Schleier JJ, Davis RS, Shama LM et al (2008) Equine risk assessment for insecticides used in adult mosquito management. *Hum Ecol Risk Assess Int J* 14:392–407. <https://doi.org/10.1080/10807030801934812>
- Schneider P, Takken W, McCall PJ (2000) Interspecific competition between sibling species larvae of

- Anopheles arabiensis* and *An. gambiae*. *Med Vet Entomol* 14:165–170
- Scudellari M (2019) Self-destructing mosquitoes and sterilized rodents: the promise of gene drives. *Nature* 571:160–162. <https://doi.org/10.1038/d41586-019-02087-5>
- Service MW (1971) Studies on sampling larval populations of the *Anopheles gambiae* complex. *Bull World Health Organ* 45:169–180
- Service MW (1973) Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of predators by the precipitin test. *Bull Entomol Res* 62:359–369
- Service MW (1977) Ecological and biological studies on *Aedes cantans* (Meig.) (Diptera: Culicidae) in southern England. *J Appl Ecol* 14:159–196
- SETAC (1994) Final report: Aquatic Risk Assessment and Mitigation Dialogue Group. SETAC Press, Pensacola, Fla
- Sinka ME, Bangs MJ, Manguin S et al (2012) A global map of dominant malaria vectors. *Parasites Vectors* 5:69. <https://doi.org/10.1186/1756-3305-5-69>
- Smith DG, Wilburn C, McCarthy RA (2003) Methoprene photolytic compounds disrupt zebrafish development, producing phenocopies of mutants in the sonic hedgehog signaling pathway. *Mar Biotechnol* 5:201–212. <https://doi.org/10.1007/s10126-002-0062-5>
- Stueckle TA, Griffin K, Foran CM (2008) No acute toxicity to *Uca pugnax*, the mud fiddler crab, following a 96-h exposure to sediment-bound permethrin. *Environ Toxicol* 23:530–538. <https://doi.org/10.1002/tox.20363>
- Teem JL, Ambali A, Glover B et al (2019) Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: results from four regional consultations 2016–2018. *Malar J* 18:347. <https://doi.org/10.1186/s12936-019-2978-5>
- Terhorst CP, Miller TE, Levitan DR (2010) Evolution of prey in ecological time reduces the effect size of predators in experimental microcosms. *Ecology* 91:629–636
- United Nations (2020) Report of the ad hoc technical expert group on risk assessment. United Nations Environment Programme, Montreal, Canada
- USEPA (1991) Reregistration eligibility document isopropyl (2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate (referred to as methoprene). U.S. Environmental Protection Agency, Washington, D.C
- USEPA (1998a) Guidelines for ecological risk assessment. U.S. Environmental Protection Agency, Washington, D.C
- USEPA (1998b) *Bacillus thuringiensis*. U.S. Environmental Protection Agency, Washington, D.C
- USEPA (2006) Larvicides for mosquito control. U.S. Environmental Protection Agency, Washington, D.C
- USEPA (2020a) Summary of the Data and Information Related to Vectorial Capacity Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E). Memo from Amanda A. Pierce to Eric W. Bohnenblust. U.S. Environmental Protection Agency, Washington, D.C
- USEPA (2020b) Human Health and Environmental Risk Assessment for the New Product OX5034 Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; New Active Ingredient) Protein, a DsRed2 Protein Variant (DsRed2-OX5034; New Inert Ingredient), and Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 *Aedes aegypti*. U.S. Environmental Protection Agency, Washington, D.C
- USFDA (2016) Finding of No Significant Impact (FONSI) In Support of a Proposed Field Trial of GE male *Aedes aegypti* mosquitoes of the line OX513A In Key Haven, Monroe County, Florida Under an investigational new animal drug exemption. U.S. Food and Drug Administration, Washington, D.C
- USNOAA (2021) Water quality describes the condition of the water, including chemical, physical, and biological characteristics, usually with respect to its suitability for a particular purpose such as drinking or swimming. In: U.S. National Oceanic & Atmospheric Administration. <https://floridakeys.noaa.gov/ocean/waterquality.html>. Accessed 9 Sep 2021
- Walker A, Bush P, Wilson T et al (2005) Metabolic effects of acute exposure to methoprene in the American lobster, *Homarus americanus*. *J Shellfish Res* 24:787–794
- WHO (2017) The evaluation process for vector control products. World Health Organization, Geneva
- WHO (2020) World Malaria Report 2020: 20 Years of Global Progress and Challenges. World Health Organization, Geneva
- WHO (2013) Larval source management: a supplementary measure for malaria vector control: an operational manual. World Health Organization, Geneva
- Wirth EF, Lund SA, Fulton MH, Scott GI (2001) Determination of acute mortality in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and methoprene exposure. *Aquat Toxicol* 53:9–18. [https://doi.org/10.1016/S0166-445X\(00\)00157-0](https://doi.org/10.1016/S0166-445X(00)00157-0)
- Wolt JD, Keese P, Raybould A et al (2010) Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Res* 19:425–436. <https://doi.org/10.1007/s11248-009-9321-9>
- Zhou G, Lo E, Githeko AK et al (2020) Long-lasting microbial larvicides for controlling insecticide resistant and outdoor transmitting vectors: a cost-effective supplement for malaria interventions. *Infect Dis Poverty* 9:162. <https://doi.org/10.1186/s40249-020-00767-3>

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