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# Threatened amphibians: trends in distribution and abundance at Elkhorn Slough National Estuarine Research Reserve 1997-2023

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## AUTHOR AFFILIATION

Hemingway, Laursen, and Reis contributed to this report as volunteers. All other authors conducted this work as staff for Elkhorn Slough National Estuarine Research Reserve.




## ABOUT THE ELKHORN SLOUGH TECHNICAL REPORT SERIES

The mission of the Elkhorn Slough Foundation and the Elkhorn Slough National Estuarine Research Reserve is conservation of estuarine ecosystems and watersheds, with particular emphasis on Elkhorn Slough, a small estuary in central California. Both organizations practice science-based management, and strongly support applied conservation research as a tool for improving coastal decision-making and management. The Elkhorn Slough Technical Report Series is a means for archiving and disseminating data sets, curricula, research findings or other information that would be useful to coastal managers, educators, and researchers, yet are unlikely to be published in the primary literature.

## Contents



# Summary


Amphibian populations were monitored for over twenty-five years at the Elkhorn Slough National Estuarine Research Reserve. Eight native and one introduced species were reported from the area, including breeding Santa Cruz long-toed salamanders, an endangered species with a very limited range. The pond breeding amphibian species use the watershed's freshwater (or slightly brackish) wetlands; well water addition to key aquatic breeding sites supported successful breeding of listed amphibian species in these wetlands. Creation of additional wetlands provided new aquatic habitats, replacing those that had become too saline for successful breeding. A network of small wildlife-watering troughs on the Reserve also provided aquatic refuges. In addition, the surrounding protected grasslands and woodlands provide vital upland habitat.

Monitoring efforts have varied in intensity and scope over this period. From 1997-2014 California red-legged frogs were the focus of intensive study. Regular annual dip net surveys began in 2015 to detect wetland breeding of California red-legged frogs, California tiger salamanders, and Santa Cruz long-toed salamanders. Other monitoring and detections occurred more opportunistically over the entire period.

Data from the first years reveal abundant, broadly distributed breeding populations of California red-legged frogs (CRLF). In later years, distribution and abundance both declined dramatically. Fewer aquatic breeding habitats were available in the later years due to decreased freshwater during drought. Trematode-induced malformations and chytrid disease were identified in Reserve populations, and some Reserve wetlands received polluted agricultural run-off. The role of these and other factors in causing the observed decline is unknown.

California tiger salamanders (CTS) and Santa Cruz long-toed salamanders (SCLTS) were sporadically detected over the years, breeding in wetlands in some years while not in other years. SCLTS and CTS were found breeding in the same Reserve wetlands as CRLF. Many of the SCLTS breeding sites and some of the CTS sites are in former estuarine habitats managed as freshwater impoundments; these sites often had salinities that were too high to allow breeding, resulting from a combination of water control structure failure and decreased freshwater inputs due to drought.

Ongoing monitoring of rare amphibians plus management to provide suitable wetland breeding habitat for them will continue as a priority in helping these rare amphibians to continue thriving in the wetlands at Elkhorn Slough Reserve.

# Introduction

A variety of native amphibians are found on Elkhorn Slough National Estuarine Research Reserve, many of them breeding in aquatic habitats (freshwater wetlands and the Reserve's wildlife-watering troughs) while using a variety of aquatic and surrounding terrestrial habitats for foraging and refuge. Species include the California red-legged frog (*Rana aurora draytonii*), Pacific chorus frog (*Pseudacris regilla*), California tiger salamander (*Ambystoma californiense*) and Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*). Several species of terrestrial salamanders are also found on the Reserve – Monterey ensatina (*Ensatina montereyensis*), Gabilan Mountain slender salamander (*Batrachoseps gavilanensis*), and Arboreal salamander (*Aneides lugubris*). The native California toad (*Anaxyrus boreas halophilus*) and non-native American bullfrog (*Lithobates catesbeianus*) were reported to occur in the area.

From 1997 to 2004 Reserve wetlands and wildlife-watering troughs ("guzzlers") were consistently monitored by researchers and Reserve staff to track adult populations, larval production, recruitment and phenology of both native and non-native frog species. Amphibian monitoring emphasized frogs, both because these were easy to detect with non-invasive surveys and out of concern for California red-legged frog populations. In addition, the presence of other associated native and non-native aquatic species, vegetative cover, pathogens, water levels, and water quality were measured. From 2005-2014 Reserve wetlands were monitored most years with varying effort. From 2005-2007, A. D'Amore conducted a mark and recapture study of adult CRLF to track the population and movement of frogs between wetlands. During 2004-2006, D'Amore conducted intensive larval wetland surveys and nighttime surveys of CRLF. Starting in 2015, dipnet surveys for larval amphibians of all three listed species were conducted annually in Reserve wetlands by research staff with the help of volunteers. In addition, the presence of aquatic invertebrates, vegetative cover, and water quality measurements were

recorded. Incidental sightings of amphibians in other places and times were recorded, and as of 2018, amphibian use of cover boards were monitored. Numerous researchers and volunteers have taken part in monitoring, with varying intensity and scope for more than twenty-five years. This report summarizes those various monitoring efforts and describes management of Reserve wetlands.

# Focal species

## *California red-legged frog*

One of the largest native frogs in the western United States, the California red-legged frog (CRLF) has been listed under the Endangered Species Act as threatened within its remaining range in California since 1996 (USFWS 2002a). Historically, the CRLF was found in 46 counties ranging from Marin to northern Baja California, Mexico, and inland counties from Shasta County southward. CRLF currently exists in 23 of its original counties (USFWS 2002a). Coastal populations in central California are considered the most abundant and stable, while southern and inland species are considered most threatened (USFWS 2002a). Potential threats to the subspecies include elimination or degradation of habitat, disease, and predation by non-native aquatic species such as bullfrogs (*Lithobates catesbeianus*) (BF) and mosquitofish (*Gambusia affinis*) (Webb et al. 1997; Lawler et al. 1999; USFWS 2002a). CRLF require habitat that includes aquatic breeding areas, such as ponds, freshwater marshes, lagoons, and streams surrounded by a mixture of riparian and upland habitat (Allen and Tennant 2000). Breeding from November through April, CRLF are thought to use surrounding upland habitat throughout the year as corridors between aquatic habitats. Wetland permanence can have a significant impact on CRLF survival and on the presence of non-native predators and competitors. Seasonal wetlands that dry by the end of summer hold water just long enough for CRLF to complete development; bullfrogs need permanent water to complete their two-year breeding cycle (D'Amore et al. 2010). Thus, management plans developed to help enhance CRLF populations include management of water levels in wetlands and reduction in presence of exotic species (Scott et al. 2002; D'Amore et al. 2009, 2010).

## *Santa Cruz long-toed salamander*

The Santa Cruz long-toed salamander (SCLTS), discovered in 1954, inhabits a narrow range, occurring only in southern Santa Cruz and northern Monterey counties within 12 km of the coastline. Twenty-four breeding sites -17 in Santa Cruz County and 7 in Monterey County - have been documented for SCLTS (USFWS 2009). These salamanders breed in wetlands and migrate to adjacent upland scrub and woodland areas during the nonbreeding season, habitats that exist in relatively few areas along the central coast of California. SCLTS appear to prefer shallow wetlands that dry-down annually (USFWS 1999). SCLTS breed in these wetlands during the rainy season and spend much of the rest of their time underground in burrows of small mammals and in root systems of chaparral and coast live oak, Monterey pine, willow, cattail, and bulrush (USFWS 2009). Santa Cruz long-toed salamanders were classified as endangered in 1967 by the Fish and Wildlife Service, with threats similar to those of CRLF. Following an assessment by USFWS of the current status of SCLTS, and given concerns of anticipated sea level rise, the most urgent need is to focus on the populations in Monterey County, where most breeding sites are below current sea level in historic estuarine habitats managed as freshwater impoundments, at risk currently due to periodic water control structure failure, and at increasing risk in the future

as preventing seawater from re-entering former estuarine habitats will become increasing difficult. Therefore, priorities include improving present breeding sites and identifying new locations for migration or translocation beyond the historic saltmarsh footprint that will allow for future sustainability (Camara et al. 2019).

### *California tiger salamander*

California tiger salamanders (CTS) have a discontinuous distribution in west-central California. In the coastal counties the species ranges from Sonoma County south to Santa Barbara County; CTS is also found in the Central Valley and surrounding foothills. Agriculture and urban development have eliminated this species from many areas of its former range (Stebbins 2003). Populations in Sonoma and Santa Barbara County are endangered (USFWS 2000, 2002b), Monterey County populations are threatened (USFWS 2004). CTS typically live in open woodland or grassland habitats in small mammal burrows (e.g., California ground squirrel and Botta's pocket gopher) throughout most of the year (Trenham 2001). Eggs are laid singly on submerged plant stems, twigs, and leaves, mostly in vernal pools or shallow wetlands (sometimes in permanent wetlands), and adults spend a short time in breeding sites. Hybridization with the introduced Barred tiger salamander (*Ambystoma tigrinum*) is of great concern to conservation of the CTS, potentially leading to loss of native genetic diversity and extinction (Riley et al. 2003).

### *Pacific chorus frog*

Pacific chorus frogs (PCF) are commonly seen in much of California including at the Reserve. As an important food source for CRLF, SCLTS, and CTS, Pacific chorus frog abundance is relevant to threatened amphibian populations (USFWS 1999, 2002a, 2002b). PCF inhabit a variety of habitats, including woodlands, grasslands, chaparral and farmland, but are primarily found in low foliage near water (Stebbins 2003).

## Reserve amphibian wetlands

### *Overview*

Historical documents and maps from the late 1800s and early 1900s indicate that today's Elkhorn Slough Reserve's land included streams and springs – some may have been seasonal, others appear to have been perennial. Reserve wetlands also appear to have included wet meadows and willow groves, and there are two 20$^{th}$ century accounts that local streams would sometimes be blocked by hillside erosion, creating wetlands. Today, the streams run only during rain events, and the springs are gone. Currently there are eighteen created wetlands on the Reserve (Fig. 1). The wetlands can be grouped in terms of both suitability as amphibian habitat and breeding as well as by vulnerability to salinity increase as related to elevation of wetland and long-term sea level rise (Table 1).

Eleven wetlands are in former estuarine habitat and are separated from saltwater habitats by berms or other obstructions to tidal influence. These include Upper and Lower Cattail swale, and the three Rookery-complex wetlands, Upper and Lower Barn wetlands, Five Fingers wetland, Howell wetland and Minhoto Wetland. Strawberry wetland, directly downstream of Howell, has fluctuating salinity, and it holds freshwater due to a road serving as a berm, and a blocked culvert under the road. An additional seven wetlands were created in higher areas (Minhoto Reservoir,

Midden, Rana, Tonii, Visitor Center, Hix and Long Valley) and plans for future wetlands are underway. As of December 2023, two freshwater ponds have been partly constructed in uplands on the Reserve's Minhoto parcel; these will be completed and two more will be built at the site by the end of 2024.

Twenty guzzlers (mostly installed in the 1980s) were scattered throughout the Reserve to provide freshwater to wildlife (Fig. 2). All except one are approximately 1.5 meters long by 1.0 meter wide, with a maximum depth of 0.75 meters, sloping upwards on one side, providing shallow water. Guzzlers were buried in the ground with the top edge approximately flush with the ground. Some of the more recently installed guzzlers have rain catchment systems.

## *Amphibian monitoring*

Since 1997 monitoring of varying intensity has been carried out by researchers and staff assisted by volunteers) (see Appendix for further details). From 1997-2000 D. Reis, (lead) conducted CRLF monitoring efforts to determine distribution, relative abundance and key breeding sites of frogs at the Reserve. From 2001-2013, A. D'Amore and V. Hemingway, (leads) continued monitoring CRLF and they tracked changes in their population over time. From 2005-2007, A. D'Amore conducted a mark and recapture study, PIT-tagging adult CRLF to track movement between wetlands and determine survivorship, sex ratios and relative male and female body size (D'Amore 2007). Additionally, during 2004-2006, D'Amore conducted intensive larval wetland surveys and nighttime surveys of CRLF. Data were also collected on the other amphibian species and habitat parameters. In 2003, data was obtained from student researcher W. Savage who documented the presence of SCLTS in two of the Reserve's wetlands and monitored their populations. From 2015-2023, S. Fork, (lead) conducted annual spring dipnetting surveys for larval amphibians. Aquatic invertebrates and plants were recorded (see Appendix). From 2020-2023, S. Fork and I.M. Laursen conducted nighttime surveys for CRLF (calling and eyeshine) and visual egg mass (day) surveys were conducted at key Reserve wetlands.

Due to concerns about increasing salinity at Lower Cattail swale (a known breeding site for SCLTS), in 2018-2019 and 2019-2020, 20-30 cover boards were placed above that wetland during the rainy season and salamanders found migrating to the wetland were relocated to nearby Upper Cattail swale. Additionally, 24 cover boards were placed around the perimeter of Upper Cattail beginning in 2019 and checked weekly during the rainy season for incoming breeding SCLTS and then again from mid-June to late-July for metamorph SCLTS leaving the wetland. Also beginning in 2019 drift fences/pitfall traps were installed adjacent to Upper Cattail and were checked each fall for incoming breeding SCLTS and outgoing summer metamorphs.

Beginning in 2016, wetlands were sampled for invertebrates and plants during spring amphibian monitoring. Using aquatic insect nets and dipnets, invertebrate abundances were estimated, based on number caught and identified to family, genus (or species); aquatic plants were identified to genus (or species). Photo vouchers of invertebrates and plants were archived. A summary of invertebrates and aquatic plants present in wetlands is found in the Appendix.

Environmental variables, including water depth, dry down date, pH and salinity, were also monitored with variable intensity in different periods.

## *Management strategies to support amphibian populations*

*Addition of freshwater.* Four 2500 gallon capacity storage tanks were installed in 2014 to catch and store rainwater to fill Lower Cattail during drought years (Table 2). In 2017, a rainwater catchment and filtration system were constructed for the Visitor Center wetland, the same year that the wetland was excavated and lined. Most of the other wetlands were fed solely by groundwater (unlined wetlands) or rainwater. Since around 2004, in lower-rainfall years, attempts were made to add sufficient well water to Main Rookery and Rana to keep these wetlands wet through summer for amphibian metamorphosis, but in drier years the well could not sustain this effort and only Rana and Swimming Pool Rookery wetlands were kept filled. From the 1980s – 2023, the guzzlers were fed by a combination of freshwater tap/rainwater catchment systems and kept full throughout the year, though there were periodic failures of individual ones.

*Exclusion of saltwater*. Various former tidal wetlands were managed as freshwater impoundments, and efforts were made to prevent saltwater from entering. Historically, the Cattail swales were a tidal wetland area, possibly with low salinity due to high seasonal freshwater input, but by the late 1980s had become a degraded wetland with hypersaline conditions. Around 1987 an existing dike was reinforced and a water control structure (tide gate) was installed at Lower Cattail swale to reduce saltwater intrusion. Excavated sediments from Upper Cattail were used to create a dike between the two swales; Upper Cattail served as a sediment catch basin above Lower Cattail. At Main Rookery, where salinity has been elevated since fall 2017, a new culvert installed in 2019 at the base of the levee replaced the partially-repaired leaky and corroded culvert (that was allowing saltwater into the wetland). Other than the Cattail swales and the Rookery wetlands, barriers to keep saltwater out were not actively managed for the other wetlands occupying former estuarine habitat (Upper and Lower Barn, Five Fingers, Howell and Minhoto wetlands); these barriers will eventually fail, and these wetlands are likely to revert to estuarine habitats. In fact, in summer 2022, the culvert at Lower Cattail started to rust, and on some high tides salt water leaked through this corroded pipe, bypassing the slide gate, and flowed directly into Lower Cattail.

*Wetland creation.* Several wetlands were created in areas above sea level to establish new wetland habitat protected from saltwater - Rana (2003), Howell (2015), Visitor Center (2017), Long Valley (2020) and Hix (2021), on ESF land adjacent to ESNERR's Howell parcel.

*Wetland dredging.* One other wetland (Upper Cattail) originally created to trap eroded sediment from upstream agriculture, filled with sediment over the years since construction and was dredged (and lined) in summer 2015.

*Wetland lining.* Several wetlands were lined as a strategy to ensure some water was held throughout the breeding period. Liners consist of a synthetic EPDM non-petroleum based material plus a top layer of woven geotextile material used to protect the liner from puncture and allow substrate adherence. Wetlands lined in summer 2015 included a part of Lower Cattail, as well as Upper Cattail, Middle Rookery, Swimming Pool Rookery and Rana; Visitor Center was lined in 2017 and Long Valley was lined in 2020.

*Vegetation enhancement of wetlands and guzzlers.* Vegetation has been managed in and around the wetland and guzzlers, with the goals of removing potentially harmful non-native species and enhancing growth of natives that provide refuge against predation for amphibians. At Rana and Swimming Pool Rookery, cattail (*Typha latifolia*) and bur reed (*Sparganium eurycarpum*) were thinned to maintain areas of open water; willows (*Salix sp*.) were pruned around Upper Cattail to reduce shading. Routine maintenance of the guzzlers during 1997-2004 included removal of dense algal mats and planting of fennel pondweed (*Stuckenia pectinatus*). Since 2004 guzzlers have been kept filled with water and sporadically with aquatic plant management.

*Wetland margin vegetation and surrounding understory vegetation.* Vegetation has been managed around the wetland margin for amphibian refuge and egg laying substrate while adjacent understory plants were managed with the goals of removing selected non-native species and enhancing growth of natives that provide refuge against predation for amphibians. Spikerush (*Eleocharis macrostachya*) was planted along the margins of Upper Cattail swale and Visitor Center as an egg laying substrate for amphibians. Rush (*Juncus sp.)* was planted around the perimeter of Rana and Middle Rookery. Additional species planted around Visitor Center wetland include gumplant (*Grindelia stricta*), creeping wildrye (*Elymus triticoides*), seep monkey flower (*Diplacus guttatus*), blackberry (*Rubus ursinus*), California wild rose (*Rosa californica*) and *Juncus sp*. Native understory plants including silverweed (*Potentilla sp.*), willow (*Salix sp.*), goldenrod (*Euthamia sp.*), coffeeberry (*Rhamnus sp.*), and rush *(Juncus sp.)* were planted around the perimeter of Swimming Pool Rookery.

*Adjacent trees.* Trees growing around wetlands have been managed to improve habitat for amphibians in wetlands. Much of the vegetation management has focused on the Rookery complex wetlands. From 1993 through 1996 about fifty Monterey pine trees from Point Lobos were planted around the vicinity of the wetlands to replace dying older trees; by 2009 most of those planted pines have since died due to many years of nesting by rookery birds. Removal of eucalyptus (*E. globulus*) has been a primary focus of recent vegetation management on the Reserve. In fall 2019, the eucalyptus trees adjacent to Rookery complex were removed and in 2021 and 2022 eucalyptus trees were removed just north of Upper Cattail, in Owl Canyon.

*Control of invasive predators.* Management activities to reduce numbers of invasive mosquitofish and bullfrogs - both predators of amphibians - was a high priority. Reserve staff worked with Monterey County Mosquito Abatement, emphasizing the importance of not stocking mosquitofish in Reserve wetlands. The management strategy for non-native bullfrogs (*Lithobates catesbeianus)* (BF) was early detection and control.

*Agricultural inputs reduction and monitoring.* Monitoring activities were employed to track nutrient levels in several wetlands; agricultural land upstream of one of the wetlands was targeted for land acquisition. From 2003 - 2004 nitrate, ammonia and phosphate were measured periodically at Lower Cattail; from 2005 - 2023 nitrate, ammonia and phosphate were measured monthly at Lower Cattail and Main Rookery.

*Captive breeding to increase population size.* To increase the small population size and genetic diversity of SCLTS (on the Reserve and elsewhere in North Monterey County), a USFWS-funded captive propagation program of SCLTS at UC Santa Cruz was launched in 2020. In

2020-2021 and 2021-2022, SCLTS from Upper Cattail were captured (by S. Fork and I.M. Laursen) and brought to the rearing facility to be maintained with adults from other populations in large aquatic breeding tanks. Larvae were reared and the resulting young-of-year salamanders released in several wetlands in the watershed, including Long Valley, Swimming Pool Rookery and Upper Cattail.

# Results

## *Overview of amphibian diversity on the Reserve*

A total of eight native (and one non-native) amphibian species were reported on the Reserve. Of aquatic breeding amphibians present, Pacific chorus frogs were the most common, with evidence of them occurring in 13 of 17 surveyed wetlands, often at high abundance. Ten wetlands had evidence of breeding by CRLF, CTS and/or SCLTS during the breeding season for at least one year, as noted by the presence of egg masses, tadpoles (or, in the case of CRLF), calling males; those wetlands without breeding signs include Long Valley, Minhoto Reservoir (Minhoto wetland was not surveyed), Tonii and Visitor Center. Pacific chorus frogs also occurred in all guzzlers at least once during the 1998-2004 study period and had successful reproduction in most guzzlers during these observations. Adult and juvenile CRLF were observed in most of the guzzlers during 1998-2004. Western toads were only seen on a few occasions - three adults were seen near Lower Barn wetland (2002); tadpoles were observed in spring of 2004 in Strawberry wetland (D. Reis and A. D'Amore, pers. com.) and near Long Valley wetland in spring 2023 (A. Woolfolk and M. Paul, pers. com.).

Of the remaining amphibians (all terrestrial for their entire life cycle), Gabilan Mountain Slender salamanders *(Batrachoseps gavilanensis)* were the most widespread and abundant species found under a variety of natural and artificial cover. Monterey Ensatina salamanders *(Ensatina eschscholtzii eschscholtzii)* were also seen periodically, as well as one record of an arboreal salamander *(Aneides lugubris)* under a coverboard.

## *Temporal and spatial patterns of habitat use by the three focal species*

The presence of the focal amphibian species varied over the years of observation during 1998-2023, with sporadic detection of CTS and SCLTS and a general decline in CRLF detections (Table 3). In particular, in recent years, CRLF were observed in fewer wetlands than in the earlier years (Table 4).

CRLF - Adults and tadpoles were seen at nearly all of the Reserve wetlands at some time during 1998-2023, most notably in the early years, with fewer detections in drought years (Table 3; Fig. 3). In the early years with above average rainfall, CRLF were observed in more wetlands, while during the later drought years CRLF were detected in fewer wetlands (Fig. 4). Since 2018 one or more lifestages (eggs, tadpoles, young-of-the-year frogs and adults), were regularly seen in Rana. The Reserve population has declined significantly since the early years. In 1997 and 1998, the maximum number of adults observed monthly from Lower Cattail Swale, Midden, and the Rookery wetlands combined was 195 and 366, respectively. By 2004, however, the population was dramatically lower, and has continued to decline over the years. From 2015-2018 tadpoles and adults were observed in only a few wetlands (Lower and Upper Cattail and Rana). In 2019 at Upper Cattail, more than 50 young-of-the-year (YOY) CRLF were observed under coverboards along the wetland's waterline; in 2023 at least 50 YOY CRLF were seen in the water. Egg

masses were observed during surveys at Swimming Pool Rookery (2021 and 2022) and Rana (2021-2023) and Upper Cattail (2021).

SCLTS - Adults and juveniles were found sporadically on the Reserve; larval SCLTS were found in Main Rookery, Lower Cattail, Upper Cattail and Howell. The first observation of SCLTS was in 1993 and a few adults and juveniles were observed again from 1998 - 2002. Larvae were first observed in Lower Cattail and Main Rookery in 2002. Larval SCLTS were found in Lower Cattail in 2003 - 2005, 2011, 2015, 2016 and 2023. In 2019, more than 100 larvae were found in Howell. At Upper Cattail during each rainy season from 2019 - 2023, breeding adults were seen underneath cover boards placed around the wetland's perimeter (and at Lower Cattail in 2019). Adult and juvenile SCLTS were captured in pitfall traps in 2020, 2021 and 2022. Larval SCLTS were found in Upper Cattail in spring of 2020, confirming breeding there for the first time; larval SCLTS were again seen in 2022 and 2023.

CTS - Larvae were found sporadically since 2015 in a few of the wetlands - Lower Cattail (2015), Rana (2015 and 2017) and Howell (2016 and 2018). Adult salamanders were observed along roads just outside of the Reserve boundary on rainy nights in fall 2018 and 2019. At Upper Cattail, a juvenile CTS was captured in December 2020 in a pitfall trap and a male CTS was found under a coverboard in December 2022. Adults were seen in a nearby private wetland along Strawberry Road in November 2022.

## *Trends in potential stressors to amphibian populations*

Water levels and wetland dry down - Overall, during the early part of the study period (1997-2004) many wetlands remained wet through the amphibian breeding season; a majority of these years had about average or above average rainfall (Fig. 3). Indeed, rainfall total in 1998 (July 1, 1997 - June 30, 1998) was one of the highest on recent record, with 1000 mm of rain (UC-IPM, 2023). But during the later study period, from about 2007 - 2018, fewer wetlands remained full through the breeding season (when drought conditions were more common), particularly during the southwest North American "megadrought" of 2020-2021 (Williams et al. 2022). Stored rainwater for Lower Cattail swale was insufficient in keeping the swale wet through the breeding season, other than the small lined depression. At Visitor Center, the catchment system kept the wetland full through the breeding season each year. From 2019 - 2023 well water added to Rana and Swimming Pool Rookery kept these wetlands full through the breeding season. 2023 was an exceptionally wet year, with over 700 mm of precipitation (Fig. 3).

Salinity issues in wetlands below sea level - Attempts to keep saltwater out of the managed freshwater impoundments (Lower Cattail and Main Rookery) were only moderately successful (Fig. 4a, b). The water control structure at Lower Cattail was engineered so that the tide gate held freshwater in the wetland. Lower Cattail remained mostly freshwater and yearly average salinity measurements from 2005 - 2017 ranged from 0.4 – 2.7 ppt until about 2018 (Fig. 4a), when average yearly salinity increased to over 23 ppt. Subsequent repairs to the slidegate to prevent saltwater intrusion were completed in December 2019. In summer 2022 the culvert began to corrode, forming holes in the pipe that allowed saltwater to bypass the repaired slidegate and flow directly into Lower Cattail on some high tides. In 2023, the above average rainfall significantly decreased the average salinity at Lower Cattail to 1.6 ppt. Yearly average salinity

from 2005 - 2017 at Main Rookery varied from 0.2 – 3.9 ppt and jumped to 18 ppt by 2018 (Fig. 4b) In 2023, the above average rainfall significantly decreased the average salinity at Main Rookery to 0.2 ppt.

Invasive predators - All wetlands and guzzlers were stocked with mosquitofish (*Gambusia affinis*) at some point in the past, with the intent of controlling mosquito populations. The mosquitofish populations were very high in some of the continuously wet wetlands, including in Howell and Swimming Rookery. At present, however, none of the Reserve wetlands have mosquitofish and since fall 2018 no guzzlers have had mosquitofish. As of March 2020 Mosquito Abatement has agreed to minimize mosquito control activities of freshwater Reserve wetlands. Unlike other areas in the Elkhorn Slough watershed, bullfrogs have not heavily invaded the Reserve, and, in fact bullfrogs were only detected at the Rookery complex. Their population was successfully controlled during a concentrated effort from 2001-2004 to eradicate them. Since around 2008 no individuals have been seen or heard nor breeding observed at the Reserve.

Amphibian pathologies - Between 1997 - 2008, a few studies documented the presence of pathogenic organism infecting the Reserve's amphibians, including the trematode (*Ribeiroia ondatrae*), which causes limb malformations, and chytrid fungus (*Batrachochytrium dendrobatidis*) (*Bd*), a skin disease that can be fatal in susceptible individuals. Hemingway (2015) investigated the impact of *Bd* on a community of three co-occurring larval species, CRLF, PCF and BF, in Reserve wetlands and what environmental conditions favor *Bd* success (see Appendix for details).

The parasitic trematode *Ribeiroia ondatrae* was found in PCF larvae with limb malformations at Lower Cattail in 2003 and 2004 by V. Hemingway and V. McKenzie. V. Hemingway observed *Bd* in BF, CRLF and PCF. Infected CRLF were predominantly mid- to late-stage tadpoles and had low mortality. BF had higher prevalence and intensity of *Bd* infection than CRLF and PCF tadpoles. (See Appendix on further details of chytrid in Reserve amphibians.) Other pathogens observed in Reserve amphibians, include several species of opportunistic fungi. In 2000, V. Hemingway observed *Saprolegenia ferax* infections of PCF egg masses in Main Rookery. This fungus can cause mortality in infected amphibian embryos (G. Padgett-Flohr, pers. com.). In 2003 PCF, CRLF and SCLTS larvae from Lower Cattail were observed with hemorrhages, loss of pigment and edema. In 2004, PCF and Western toad larvae from Strawberry wetland, with similar symptoms were found to be infected with the fungus *Serratia fonticola*, an opportunistic infection that occurs as a result of another stressor on affected amphibians (G. Bradley, pers. com.).

Agricultural pollution - Average yearly nitrate, ammonia and phosphate varied over the time period sampled (Fig. 5a-f). At Lower Cattail, yearly averages of nitrate were 1 mg/L or lower, but peaked at about 4 mg/L in 2010 and again in 2021 (Fig. 5a). Ammonia levels were around 5 mg/L or lower most years except in 2020 (46 mg/L) and 2021 (26 mg/L) (Fig. 5b); phosphate levels were less than 1 mg/L during the sampling period (Fig. 5c). At Main Rookery, average annual nitrate levels were highly variable, the highest levels occurred in 2016 (17 mg/L) and 2020 (21 mg/L) (Fig. 5d). Ammonia levels varied widely, with peak values in 2015 (13 mg/L),

2020 (24 mg/L) and 2021 (14 mg/L) (Fig. 5e). Phosphate levels were 2 mg/L most years except in 2020 (17 mg/L) (Fig. 5f).

# Discussion

## *Reserve lands support threatened amphibians*

Elkhorn Slough National Estuarine Research Reserve provides important habitat for threatened and endangered amphibians. It offers 1700 acres of protected habitat including wetlands with plenty of emergent vegetation and riparian forest and surrounding upland habitat including oak woodland, chaparral and grasslands. The Reserve and its wetlands provide a wide variety of food sources for CRLF, CTS and SCLTS, including an array of aquatic and terrestrial invertebrates and Pacific chorus frogs. In an unusual situation, two wetlands (Lower Cattail and Howell) have hosted all three listed amphibian species in certain years. CRLF were extremely abundant and widespread in the network of Reserve wetlands during wet years and benefitted from the wildlife watering troughs in dry years. SCLTS have more extensive protected upland habitat on the Reserve than in most other portions of this endangered species' small range, and Reserve wetlands are among the southernmost regular breeding sites for the species. CTS, when present, were often extremely abundant.

## *Stressors to reserve amphibian populations and effectiveness of management strategies to mitigate them*

Multiple, interacting factors are likely challenging the Reserve's threatened amphibian populations, leading to decline of CRLF over the monitoring period and low current numbers of SCLTS and CTS. Here we review some of the potential contributing factors and evaluate the effectiveness of management strategies employed to address them.

Breeding habitat scarcity in drought years

One important factor limiting many amphibians is available aquatic breeding habitat in drought years (Walls et al. 2013; Cayuela et al. 2016; Kissel et al. 2019). In order to complete development, CRLF require wetlands that stay wet through August, while CTS and SCLTS need wetlands that stay wet through at least mid-June. The effect of drought was especially apparent on CRLF populations on the Reserve. During 1997 and 1998 (El Niño years) an extensive network of wetlands remained wet through the summer and a record number of young-of-year CRLF were observed plus hundreds of juvenile and adult frogs. By contrast, during years experiencing drought conditions (2001-2004, 2007-2009, 2012-2017 and 2020-2022) many wetlands dried up in early summer, and CRLF reproduction was observed in fewer Reserve wetlands. Unlike the previous few years, 2023 was another above-average rainfall year with an abundance of young-of-year CRLF and SCLTS at key Reserve wetlands.

The Reserve's management strategies to mitigate drought impact show some benefit. Guzzlers clearly provide aquatic refuges for CRLF, and likely food resources in the form of PCF that use them regularly. Supplementation with well water and/or wetland liners have enabled some of the smaller wetlands to remain wet through summer in years where they would have dried down without such action. Removal of eucalyptus trees adjacent to wetlands, as accomplished at the Reserve's Rookery wetlands and Upper and Lower Cattail swale should also increase water levels, since eucalypts are known to use substantial amounts of groundwater (Fritzsche et al.

2006; Rodríguez-Suárez et al 2011). While such measures undoubtedly are beneficial in supporting breeding in these small wetlands, their effect is minor in comparison with that of weather cycles - they cannot duplicate the benefit of having extensive networks of wetlands available as occurs in wet years.

Saltwater leakage into former estuarine wetlands

Saltwater intrusion into wetlands is an increasing threat to amphibians that use wetlands for breeding (e.g. Karraker et al. 2010; Walls et al. 2013). CRLF eggs and pre-hatchling larvae, for example, do not survive with prolonged exposure to salinity of 4.5ppt (Jennings and Hayes 1990). Many of the breeding sites for listed species on the Reserve are former estuarine wetlands that were diked to exclude saltwater and managed as freshwater impoundments. As the dikes and water control structures age, they become increasingly prone to failure, allowing tidal water to return to former salt marshes. Since 1997, 6 out of 17 of these former salt marshes have reverted from freshwater back to salty conditions. One management strategy is to attempt to repair leaking berms and water control structures. Repairs to berms and tide gates have addressed saltwater leakage, reducing the amount of saltwater intrusion with varying success. Salt accumulated in wetlands needs to be flushed from the sediments, which is not always feasible during drought years. Given repeated failure in the past years, and the increasing pressure from accelerated sea-level rise, continuing to manage these wetlands for freshwater does not appear to be a viable strategy, and there is a risk that attempting to do so creates a sink rather than a source for listed amphibian populations. An alternative strategy is to create new wetlands well above sea level within close proximity to the estuarine impoundments, providing the amphibians safer alternative breeding sites. Creating large new wetlands is challenging, due to permitting, concerns about habitat conversion, and availability of freshwater. But creation of small wetlands adjacent to threatened breeding sites appears to be a feasible strategy. Our observations suggest it may take some years before they are discovered and used by breeding amphibians. Although Rana was originally constructed in 2003, CRLF and CTS were observed breeding in the wetland only beginning in 2016. It may be some years before Visitor Center wetland, constructed in 2017, has breeding CRLF, CTS, or SCLTS.

Agricultural pollution

Nitrates and other agrochemicals have been shown to have detrimental effects on amphibians (e.g. Schuytema et al., 1999; Ortiz-Santaliestra et al. 2006; Adelizzi et al. 2019; Borumand-Fumany et al. 2023). Toxic levels of nitrate were reported in amphibians exposed to less than 2 mg/L (Marco, et al., 1999). At sub-lethal doses of nitrate, effects can range from decreased survival and growth (e.g. Hamer et al. 2004; Ortiz et al. 2004; McLean and Rodgers 2023), reduced predator avoidance (Ortiz-Santaliestra 2010; Polo-Cavia et al. 2016) and compromised immune system and increase in susceptibility to other diseases (e.g. Peltzer et al. 2008). Most of the Reserve wetlands did not directly receive agricultural run-off except at Main Rookery complex and Cattail swales. Acquisition of the property upstream of the Cattail swales was motivated out of concern of maintaining amphibian breeding habitat. Prior to Elkhorn Slough Foundation's purchase in 2007 of the property above Cattail swales, during drought years, agricultural nutrients built up in the soil above Upper Cattail and were released into the swales during periods with heavy run-off, allowing for nutrient loading in water bodies and great fluctuations in nutrient levels. Since 2007, the agricultural run-off decreased but it is unclear whether this was a demographic benefit to amphibians or not. Nitrate-based and phosphate

fertilizers applied to soils in agricultural fields may be a contributing stressor to Reserve amphibians, but we have no concrete evidence that this is the case.

Amphibian pathologies

Another threat impacting amphibians is pathogenic disease, including infections by parasites, fungi and viruses (e.g. Kiesecker, 2011; Price et al., 2014; Fisher and Garner, 2020). Two pathogens of amphibians, the trematode parasite *Ribeiroia ondatrae* and the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) have been detected in amphibians living in Reserve wetlands, during the study period (Hemingway, 2015), but we have no reason to believe it is main causal factor for low population sizes.

The parasitic trematode *Ribeiroia ondatrae* was found in PCF larvae with limb malformations at Lower Cattail in 2003 and 2004; CRLF and SCLTS from the wetland also had limb malformations, but it is unknown whether these amphibians were infected with *Ribeiroia* trematodes. Chytrid (*Batrachochytrium dendrobatidis*) (*Bd*), a fatal fungal pathogen that breaks down keratinized parts in amphibians (Berger et al., 1998, Daszak et al., 1999), has been confirmed infecting several amphibian species sampled at the Reserve.

Elevated levels of nutrients can play a role in amphibian pathologies, such as triggering a cascading sequence of increased algal growth and proliferation of aquatic snails that are intermediate hosts of trematodes which infect amphibians. Acquisition and restoration of property upstream of the Cattail swales dramatically reduced agricultural runoff, but it is unclear whether the net effect on population was beneficial; still we have seen very few amphibian malformations since. Following recommendations of Hemingway (2015) to reduce the incidence of chytrid, we manage aquatic vegetation to maintain habitat complexity and thin out marginal vegetation to increase sunning sites for CRLF and PCF in wetlands; it is unclear whether these management strategies are successful since regular monitoring of chytrid presence has not continued.

Non-native predators

Bullfrogs are known to pose significant threats to various threatened amphibian species (e.g. D'Amore et al. 2009; Blaustein et al., 2020; McDevitt-Galles et al. 2022). The Reserve successfully conducted early detection and eradication of bullfrogs when they appeared, a strategy that was much more effective than attempting control bullfrogs after populations have become widespread. Mosquitofish may also pose a threat to larval stages of amphibians (e.g. Lawler et al., 1999; Klop-Toker et al., 2018). Through dialogue and collaboration with the Monterey County Mosquito Abatement, the Reserve prevented stocking by mosquitofish on Reserve wetlands in recent years. These measures related to invasive species control should help support the listed amphibians, but are not likely the major contributors to the native amphibians' population trends relative to the major drivers of limited availability of breeding habitat.

Low population sizes

Amphibian population declines leading to low populations is a widespread phenomenon (e.g. Drost and Fellers, 1996; Laurance et al. 1996; Lips 1998). Population declines of imperiled amphibian species can lead to decrease in genetic variation and less adaptability of the species to a changing environment (e.g. Allentoft and O'Brien 2010; Hardy et al. 2021). Santa Cruz long-

toed salamanders in particular are considered to be at such low population size that successful reproduction is difficult. A USFWS-funded captive propagation program at UC Santa Cruz of SCLTS (captured from several locations in North Monterey County, including at Upper Cattail) has been launched to increase population size and genetic diversity of the salamanders. It is too early to know whether the captive breeding will be successful for increasing the Reserve's SCLTS population sizes, but the strategy seems promising.

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**Table 1.** Aquatic habitats on Elkhorn Slough National Estuarine Research Reserve (listed from north to south. Wetland origin: agricultural basin (A), excavated in upland (U), sediment catch basin (S), impounded tidal wetland (T), other (O);( †enhanced as amphibian habitat, ††likely built as swimming pool in early 1900s). Salinity: averages of at least 4 samples, generally taken from both summer and winter between 2015-2022; salinity definitions: freshwater < 0.5 ppt (bright green highlight); brackish (3 categories), oligohaline, 0.5-5.0 ppt (light green highlight), mesohaline, 5.1-18.0 ppt: yellow highlight (5.1-6.5ppt) and orange highlight 6.6-18ppt) and polyhaline, 18.1-30.0 ppt; saltwater ≥ 30 ppt; *sites with a single measurement, **CRLF tadpole salinity threshold ≤6.5ppt, (Reis, 1999). Elevation (NAVD88): <2.3 m = tidal elevations susceptible to tidal influences if levees or water control structure break, leak or are overtopped (orange highlight); 2.3 – 3.2 m = upland elevations that are susceptible to becoming tidal with expected sea level rise (yellow highlight); >3.2 m = upland elevation (bright green highlight). Wetland dry down (before end of August): always wet = A (bright green highlight), rarely dry down = B (yellow highlight), often dry down = C (orange highlight). Species documented (at least once in the wetland): California red-legged frog, CRLF; California tiger salamander, CTS (**seen under pond-margin coverboard); Santa Cruz long-toed salamander, SCLTS. (Minhoto wetland, Hix and Long Valley are not shown).

| Name | Origin | Size (hectares) | Elevation (m) | Salinity average (range), ppt | Wetland dry down | Presence of listed amphibian species |
|---|---|---|---|---|---|---|
| Tonii | U | <0.01 | 9.5 | 0.1 (0-0.3) | C | |
| Strawberry | T | 2.39 | < 2.1 | 3.5 (0.2-10) | C | CRLF |
| Howell | T | 0.04 | < 2.1 | 3.9 (0.16-13) | B | CRLF, CTS, SCLTS |
| Rana | U | 0.01 | 5 | 0.6 (0.07-6) | B | CRLF, CTS |
| Swimming Pool Rookery | O†† | 0.01 | 4 | 1.56 (0.16-10) | B | CRLF |
| Middle Rookery | T† | 0.81 | < 2.1 | 2.1 (0-8) | C | CRLF |
| Main Rookery | T | 0.81 | < 2.1 | 5.9 (0.1-25) | C | CRLF, SCLTS |
| Midden | U | 0.01 | 9 | 0.8 (0-2) | C | CRLF |
| Lower Cattail | T† | 0.77 | < 2.1 | 7.5 (0.03-34)** | A | CRLF CTS SCLTS |
| Upper Cattail | S† | 0.04 | 2.7 | 0.3 (0-3) | A | CRLF CTS‡ SCLTS |
| Lower Barn | T | 1.13 | < 2.1 | 4.6 (0.3-16.7) | A | CRLF |
| Upper Barn | T | 0.85 | < 2.1 | 2.4 (0.3-8) | C | CRLF |
| Visitor Center | U | 0.04 | 35 | 0.9 (0.3-2) | B | |
| Five Fingers | T | 0.56 | < 2.1 | 0.1* | C | CRLF |
| Minhoto Reservoir | A | 0.56 | 20 | 1.3* | A | |

**Table 2.** Management strategies of wetlands at Elkhorn Slough Reserve.

| Pond | saltwater exclusion | freshwater addition | Agricultural input reduction | Lining | Vegetation management |
|---|---|---|---|---|---|
| Lower Cattail | levee and tide gate | rain catchment | yes | partial | |
| Main Rookery | levee and tide gate | | | | |
| Middle Rookery | | | | X | |
| Rana | | well water | | X | cattail thinning, native planting |
| Swim Pool Rookery | | well water | | X | burr reed thinning, native planting |
| Upper Cattail | levee and tide gate | | yes | X | willow trimming, native planting |
| Visitor Center | | rain catchment | | X | native planting |

**Table 3.** Amphibians found at focal wetlands on Elkhorn Slough Reserve. Present (one or more life stage observed - E=egg, L=larvae, Y=YOY/metamorph, A=adult/juveniles) (green); absent (blue); not surveyed (no color); before wetland construction (black). Notes: Adult SCLTS at Upper Cattail (2020) found 8' from wetland; SP Rookery (Swimming Pool Rookery).

| | Lower Cattail | | | Upper Cattail | | | Main Rookery | | | SP Rookery | | | Rana | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CRLF | CTS | SCLTS | CRLF | CTS | SCLTS | CRLF | CTS | SCLTS | CRLF | CTS | SCLTS | CRLF | CTS | SCLTS | |
| 1997 | LYA | | | ELYA | | | LA | | | A | | | | | | 1997 |
| 1998 | LA | | | ELYA | | | ELA | | | A | | | | | | 1998 |
| 1999 | LYA | | | ELA | | | LA | | | | | | | | | 1999 |
| 2000 | YA | | | ELA | | | A | | | | | | | | | 2000 |
| 2001 | A | | | A | | | A | | | A | | | | | | 2001 |
| 2002 | EA | | L | | | | LA | | L | A | | | | | | 2002 |
| 2003 | LYA | | LA | A | | | A | | | A | | | | | | 2003 |
| 2004 | ELA | | LA | | | | | | | LA | | | | | | 2004 |
| 2005 | ELYA | | | E | | | A | | | LYA | | | | | | 2005 |
| 2006 | ELYA | | | ELYA | | | LA | | | LYA | | | | | | 2006 |
| 2007 | A | | | A | | | | | | LYA | | | | | | 2007 |
| 2008 | | | | A | | | | | | ELA | | | | | | 2008 |
| 2009 | A | | | | | | | | | A | | | | | | 2009 |
| 2010 | YA | | | A | | | | | | | | | | | | 2010 |
| 2011 | A | | | | | | | | | | | | | | | 2011 |
| 2012 | A | | | | | | | | | | | | | | | 2012 |
| 2013 | | | | | | | | | | | | | | | | 2013 |
| 2014 | | | | | | | | | | | | | | | | 2014 |
| 2015 | | L | L | | | | | | | | | | | | | 2015 |
| 2016 | | | L | A | | | | | | | | | | L | | 2016 |
| 2017 | | | | L | | | | | | | | | | L | | 2017 |
| 2018 | | | A | A | | | salty | salty | salty | A | | | A | | | 2018 |
| 2019 | salty | salty | A (salty) | LYA | | YA | | | | LYA | | | | | | 2019 |
| 2020 | salty | salty | salty | YA | Y | LYA | salty | salty | salty | LYA | | | YA | | | 2020 |
| 2021 | salty | salty | salty | ELYA | | YA | salty | salty | salty | ELYA | | | ELYA | | | 2021 |
| 2022 | salty | salty | salty | LYA | A | LA | salty | salty | salty | ELYA | | | ELYA | | | 2022 |
| 2023 | L | | L | LY | | LYA | LA | | | A | | | ELYA | | | 2023 |

**Table 4. Red-legged frogs (CRLF) found at wetlands of Elkhorn Slough Reserve.** Present (one or more life stage observed) (green); absent (grey), prior to wetland construction (black), not surveyed (no color). Threats: D (premature drydown, before end of August), P (agricultural pollution), S (excessive salinity). Note: SP Rookery (Swimming Pool Rookery).

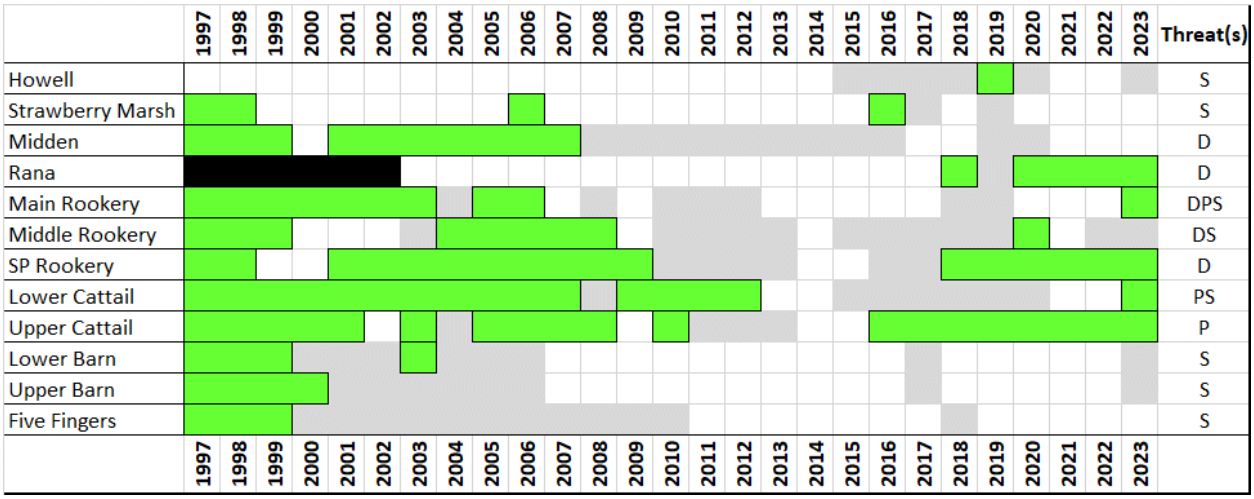


**Figure 1.** Reserve wetlands at Elkhorn Slough Reserve. Wetland color coding: orange, impounded tidal wetland (susceptible to tidal influences if levee or water control structure fails or is overtopped), blue, wetlands located in upland. (Minhoto Reservoir and wetlands are not shown).

**Figure 2.** Wildlife watering troughs (guzzlers) at Elkhorn Slough Reserve.

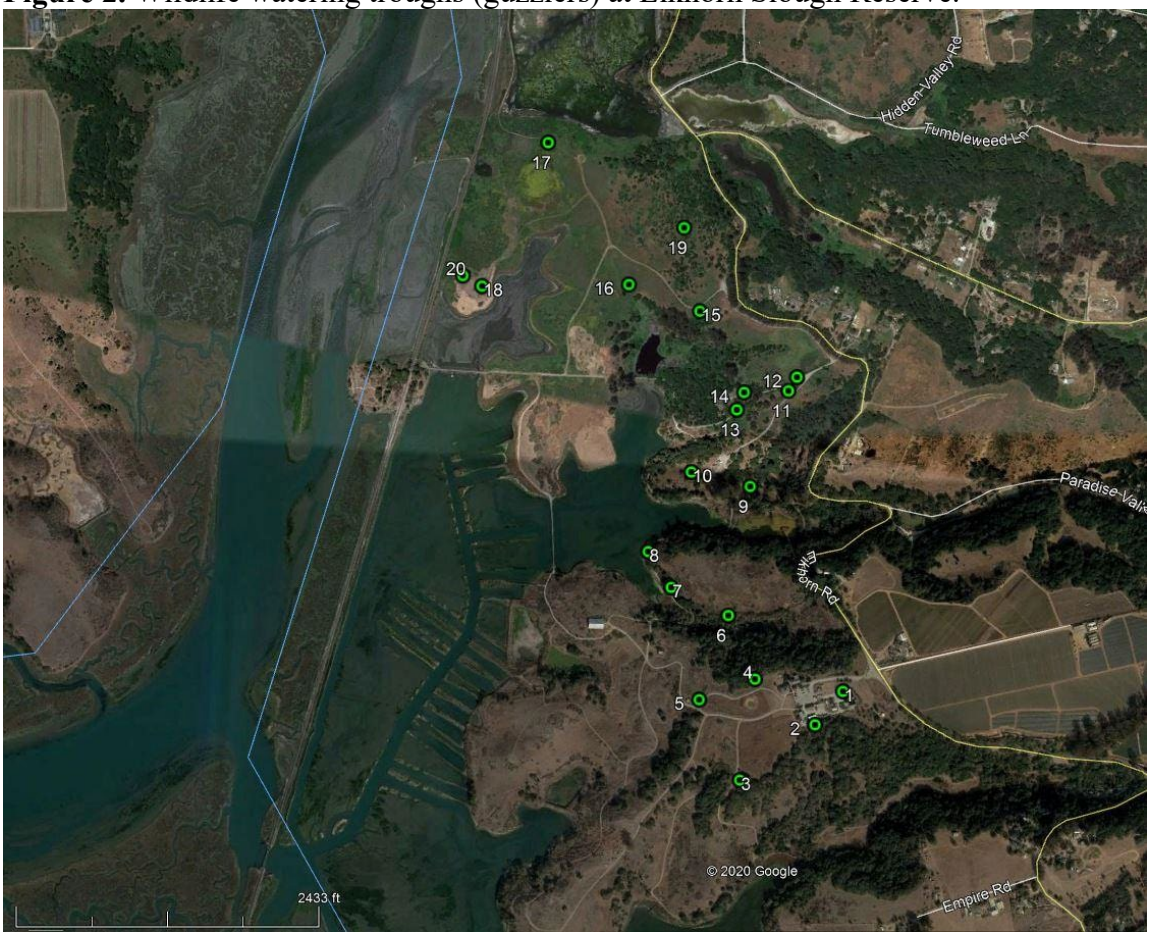


**Figure 3.** Rainfall totals for Elkhorn Slough Reserve 1997-2023. (Source: SWMP Caspian weather station; 1999-00 total from Castroville, Ca weather station, UC-IPM website).

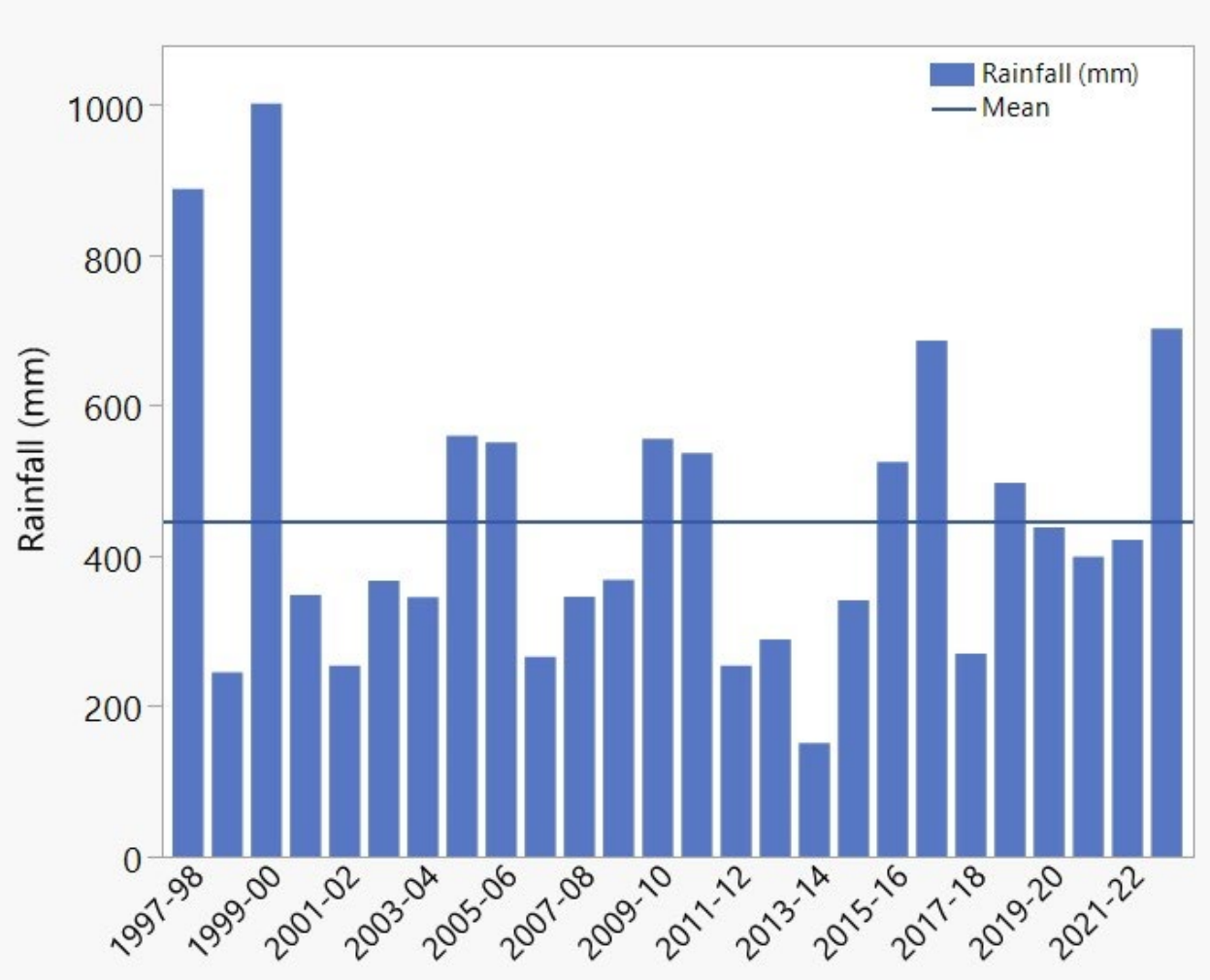


**Figure 4.** Salinity (ppt), annual average from monthly samples, for Elkhorn Slough Reserve wetlands 2005-2023. a, Lower Cattail; b, Main Rookery.

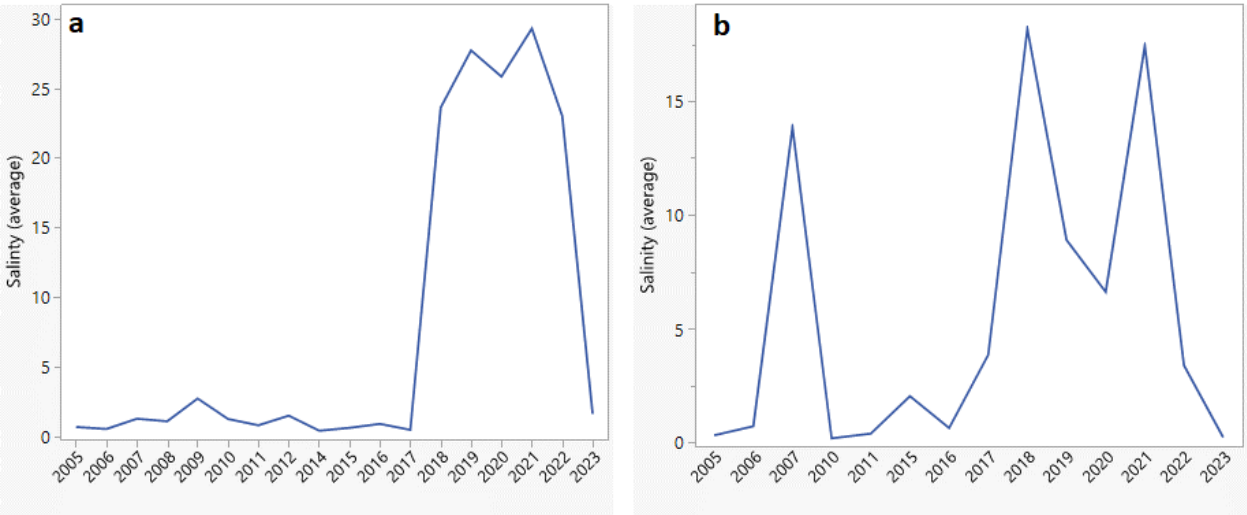
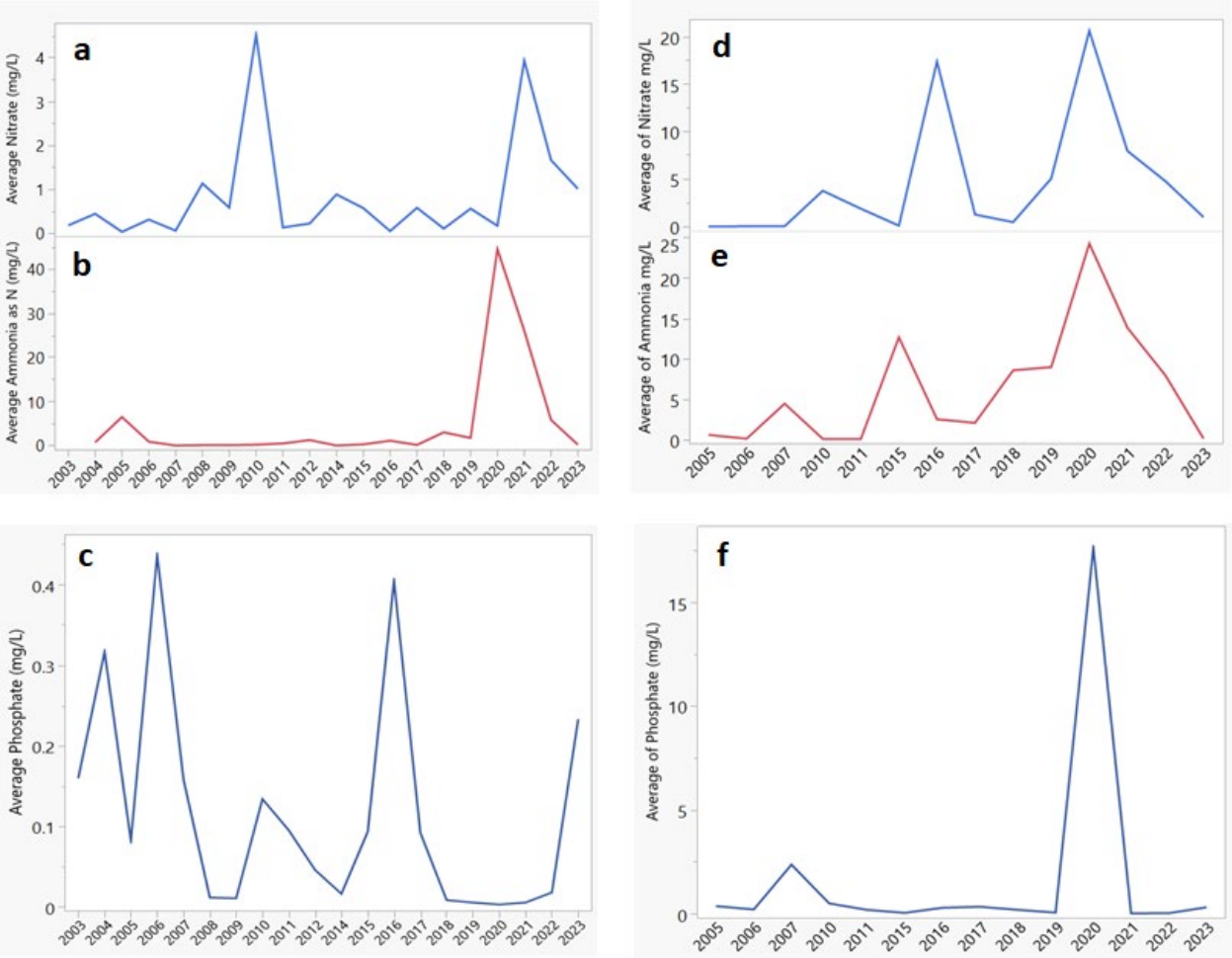


**Figure 5.** Nitrate, ammonia and phosphate (mg/L), annual average monthly samples, for Elkhorn Slough Reserve wetlands 2003-2023. a - c, Lower Cattail; d - f. Main Rookery.




# Appendix

**Amphibian population monitoring methods**

Efforts at population monitoring varied over the years and can be loosely subdivided into three time periods.

*1997-2000*

Led by D. Reis (assisted by volunteers), monitoring efforts focused on CRLF and included determining distribution, relative abundance and key breeding sites of CRLF on the Reserve, and tracking changes over time. Data were also collected on other amphibian species and habitat parameters. Amphibians were monitored using modified versions of the Visual Encounter Survey (VES) and Audio Strip Transects (ATS) (Crump and Scott 1994). Wetlands were surveyed at least once per year, using a combination of daytime and nighttime surveys. Wetlands were dipnetted twice during this time period, most wetlands in 1997 and a subset of wetlands in

1999. Guzzlers were also visually monitored on a regular basis to document observed life stages of CRLF.

*2001-2014*
Led by A. D'Amore and V. Hemingway (assisted by volunteers), efforts again focused on CRLF, following a similar monitoring protocol as those of Reis to determine distribution, relative abundance, key breeding sites, as well as continuing with data collection on other amphibian species and habitat parameters. N. D'Amore visited wetlands regularly from 2004 - 2006 as part of an intensive mark and recapture study of PIT-tagged CRLF (D'Amore 2007). From 2008 - 2014 most Reserve wetlands were visited by V. Hemingway to check for signs of breeding by CRLF, as well as document other amphibians observed. Additionally, led by W. Savage in 2003, the presence of SCLTS was documented in two Reserve wetlands and populations were monitored. Guzzlers were also visually monitored for CRLF with varying effort over this time period.

*2015-2023*
From 2015-2023, annual spring dipnetting surveys for CTS, SCLTS and CRLF larval amphibians were conducted by Reserve staff (assisted by volunteers) at Reserve wetlands. Aquatic vegetation and invertebrates were identified; counts were made of invertebrates. Beginning in 2016, water parameters were periodically determined, using a sonde to measure pH, dissolved $0_2$, salinity, turbidity and temperature.

**Wetland overview**
**Lower Cattail swale** Since 1997, observers saw or heard CRLF in Lower Cattail swale most years through 2012 and not again until 2023. CTS were observed in Lower Cattail in 2015; SCLTS were observed from 2003-2005, in 2011 and 2015 and 2016, 2018 and 2023. During 2015 - 2017 dipnetting surveys, an abundance of Pacific chorus frog larvae and many different kinds of aquatic invertebrates (backswimmers, diving beetles, dragonfly nymphs, daphnia, giant water bugs, etc.) were observed in Lower Cattail. In early 2018 salinity levels began to increase and the wetland had a distinct orange appearance. Our 2018 dipnetting survey revealed no amphibians and only a few aquatic invertebrates (dragonfly nymphs and a few backswimmers), but salinity was over 10ppt. The tide gate was repaired in Dec 2019 which kept saltwater out until it failed again in summer 2022. In 2023, with abundant rainfall, dipnet seine net surveys revealed a few CRLF and SCLTS larvae, plus a diverse aquatic invertebrate community and salinity was less than 1ppt.

**Upper Cattail swale** Originally acting as a sediment catch basin above Lower Cattail swale, Upper Cattail swale was dredged and lined in 2015. Signs of breeding by CRLF (calling males, egg mass and larvae) were observed many years since beginning in 1997. CRLF tadpoles were observed in 2016 and 2017 dipnetting survey and juveniles were observed in fall 2017; no CRLF were observed in 2018, but tadpoles, juveniles and adults were observed again from 2019 - 2023. Upper Cattail swale is higher in elevation than Lower Cattail swale and salinity is not an issue. Pacific chorus frog larvae as well as aquatic invertebrates (backswimmers, diving beetles and water scavenger beetles) were found in abundance in Upper Cattail swale since beginning monitoring for them in 2016. SCLTS adults were found at the wetland since 2019 and juveniles since 2020. SCLTS larvae were documented for the first time in 2020 and again in 2022 and

2023 in Upper Cattail swale. One young-of-year CTS was found in a pitfall trap near the wetland in 2020; a male CTS was found under a coverboard along the wetland margin in December 2022.

**Rookery complex wetlands - Main, Middle, and Swimming Pool** Rookery complex wetlands are an odd assortment of wetlands - varied both in characteristics and history. Main Rookery, separated from the slough by a levee, is the biggest of the three wetlands. During 1997-2006 signs of Red-legged frog breeding were observed 5 of 10 years. No evidence of CTS breeding has been observed in the wetland but 7 SCLTS larvae were discovered during dipnetting in 2002. Dipnetting surveys in 2016 and 2017 revealed abundant Pacific chorus frog tadpoles. Like Lower Cattail swale, Main Rookery wetland has lately become increasingly salty as leaks are apparently developing within the levee. Salinity measurement during spring 2018 dipnetting was a whopping 13ppt! Despite the saltier conditions, we still found a variety of aquatic invertebrates, including water scavenger beetles, water boatmen, dragonfly and damselfly nymphs but no amphibians. In 2023 a large CRLF tadpole (with 4 legs) was found, confirming breeding in the wetland, along with an abundant and diverse community of aquatic invertebrates.

Middle Rookery a shallow wetland lined in 2015, had breeding CRLF from 1997-1999 and 2005, plus juvenile and adult frogs in 2004 and 2006-2008 and 2022. No CRLF or SCLTS larvae were seen in Middle Rookery, but in our yearly dipnetting surveys, we typically catch many Pacific chorus frog larvae plus many aquatic invertebrates (diving beetles, backswimmers, boatmen, and water striders). Swimming Pool Rookery wetland, also lined in 2015, is unique among wetlands at Elkhorn Slough. As the name suggests, this wetland was originally built as a swimming pool more than 50 years ago. Surrounding eucalyptus trees and a few scattered Monterey pines shaded the wetland for years keeping the wetland several degrees cooler; an oily sheen was sometimes present on the surface possibly due to leaching of eucalyptus debris in the wetland. In fall 2019 most of the surrounding eucalyptus trees were removed thus improving conditions at Swimming Pool Rookery. Red-legged frogs were seen in Swimming Pool Rookery many years since 1997 and breeding signs (males calling or tadpoles) noted in 1997, 1998 and 2003-2008. After seeing only one juvenile frog in 2009, no CRLF were observed at this wetland until 2018, when we finally saw one juvenile red-legged frog. From 2018-2022, adult, juvenile, and/or young-of-year CRLF were seen; egg masses were again observed in 2021 and 2022, but not in 2023. From 2019-2022 CRLF tadpoles were observed in the wetland; no CRLF tadpoles were observed in 2023. No CTS or SCLTS were recorded from Swimming Pool Rookery, though aquatic invertebrates are abundant, particularly dragonfly larvae and diving beetles.

**Rana Wetland** Created in 2003 and lined in 2015, Rana wetland (just east of the Rookery wetlands), occupies a natural depression near the junction of North Marsh service road and South Marsh loop. This wetland is completely unshaded, surrounded by grasses and scattered coyote brush. Three CTS larvae were first discovered in Rana wetland in 2016, just a year after the wetland's construction. In 2017, 9 CTS larvae were caught by seining. In spring 2018, a single juvenile CRLF was observed for the first time. In 2020 juvenile and adult CRLF were observed. Since 2021, all stages (eggs, larvae, juveniles and adults) CRLF were seen in the wetland each year. No SCLTS were observed in Rana wetland, but Pacific chorus frog tadpoles and aquatic invertebrates are typically abundant.

**Howell and Strawberry wetlands** These wetlands are found on the south side of Strawberry Rd, about a mile east of Elkhorn Road and are separated from Strawberry Marsh by a road and crushed culvert; the road is at tidal elevation and is overtopped occasionally. After removal of accumulated trash from years of private ownership, Howell wetland was scraped and lined in 2015. Located not far above the level of the slough, Howell is separated from Strawberry wetland by an earthen levee and the levee is at tidal elevation. Howell averaged higher salinity (5 ppt) than other Reserve wetlands, with the (recent) exception of Lower Cattail swale. Despite issues with salinity, Pacific chorus frog tadpoles and aquatic invertebrates are typically abundant at Howell, as well as occasional sticklebacks and abundant mosquitofish (non-native). Several CTS larvae were found in 2015 and 2018, and a few CRLF tadpoles were found in 2019. In April 2019 more than 100 SCLTS larvae were found in Howell. In June 2019, 15 SCLTS larvae were transferred to Upper Cattail swale out of concern that Howell's salinity exceeded 5ppt and approaching a potential tolerance limit of SCLTS larvae. From 2020 - 2022, the salinity remained too high to support amphibian breeding; however, with abundant rainfall in 2023 the salinity was <1ppt and supported a diverse invertebrate community but no listed amphibian were found.

**Visitor Center wetland** This wetland was excavated and lined in 2017. Water from rainfall (or from parking lot runoff and filtered through a bioswale) fills the wetland. Pacific chorus frogs and aquatic invertebrates were found in abundance since spring 2018. No listed amphibian species were observed since beginning dipnet surveys in 2018.

**Long Valley wetland** The newest wetland was excavated and lined in 2020 and receives seasonal runoff that flows into the nearby wet meadow. Pacific chorus frogs and aquatic invertebrates were found in dipnet surveys beginning in 2021, but no listed amphibians were found.

**Five Fingers and Minhoto Reservoir** These wetlands were rarely checked for amphibians. Adult CRLF were last seen at Five Fingers in 1999. Water from rainfall or an agricultural well (1-5ppt) fills the Minhoto Reservoir. (Minhoto wetlands were not surveyed).

**Upper and Lower Barn wetlands** Signs of breeding by CRLF were observed at Upper and Lower Barn wetlands in 1997 and 1998 and in 2003 in Lower Barn. Pacific chorus frogs were seen in Upper Barn and rarely in Lower Barn wetland. Most years, Upper and Lower Barn wetlands were too salty to support amphibian breeding.

**Tonii pond** Other than chorus frog tadpoles, no other amphibians were observed in Tonii pond, since it was built in 2014.

**Midden pond** Signs of breeding by CRLF were observed in Midden pond (a small cement basin) from 1997 – 2004, but it is not likely breeding was successful due to the pond's small size. Signs of breeding by chorus frogs were occasionally observed in Midden.

**Natural history of Reserve wetlands**

*Aquatic macroinvertebrates*

A variety of aquatic invertebrates were observed during annual dipnetting surveys of the Reserve's wetlands, including crustaceans (ostracods, cladocerans, amphipods), insects (dragonfly and damselfly nymphs), diving and scavenger beetles, backswimmers, water boatman, giant water bugs, midge larvae (chironomids, chaoborids) and mosquito larvae. Other invertebrates found in wetlands include leeches, bryozoans and a variety of snails (lymnaeid, planorbids and physa).

*Aquatic and emergent vegetation*
A variety of native aquatic and emergent plants are found in Reserve wetlands (Table A1). Plants found in wetlands include several species of pondweeds, (*Potamogeton*, *Ceratophyllum, Stuckenia, Zannichella*), and several species of the multicellular green algae (*Chara and NitellaI*) as well as filamentous green algae. Two other native aquatic plant species are periodically found in the Reserve's freshwater wetlands, including Azolla and Lemna or duckweed (Lemnaceae). A non-native, water-milfoil (*Myriophyllum spicatum*), that had been found in Middle Rookery and Swimming Pool Rookery in the past has not been present since spring 2020. Emergent plants include aquatic knotweed (*Persicaria amphibia*), tule (*Schoenoplectus californicus*), cattail (*Typhus latifolia*), Broadfruit bur reed *(Sparganium eurycarpum*) and Spikerush (*Eleocharis macrostachya*).

**Amphibian pathologies**
Prior to 2003, there was no consistent monitoring for amphibian pathologies. During population monitoring in 1997, ten dead CRLF young-of-the-year from a total of 182 individuals in Lower Cattail swale were reported. In April of 2000, *Saprolegnia ferax*, a soil-based fungus associated with fisheries was identified from Pacific chorus frog eggs in the Main Rookery. This fungus typically causes mortality in infected amphibian embryos. In 2002, another fungus was observed in PCF egg masses in Guzzlers 6 and 9. Although a positive identification was not made on this fungus, it was determined not to be *Saprolegenia ferax* (G. Padgett-Flohr, pers. com.).

On 15 July 2003, seven vigorous CRLF larvae from Midden pond were observed with hemorrhages, loss of pigment, and edema. Of the seven inspected (under permit of D. Reis), six appeared to be losing pigment in their skin and were translucent in regions, three had hemorrhaging in the gill region, two had edema, and two appeared to be swollen around the oral papillae and losing pigment from tooth rows. Inspections of tadpoles revealed similar symptoms in PCF, SCLTS, and CRLF larvae in Lower Cattail swale. In 2004, PCF and Western toad larvae with similar symptoms were observed in Strawberry wetland, although many of them were moribund unlike those observed the previous year, which were still active. Samples of the larvae from Strawberry wetland were sent to Dr. Greg Bradley at the University of Arizona Veterinary Laboratory for testing. These tests revealed an opportunistic infector, *Serratia fonticola*, causing the hemorrhaging and edema. Dr. Bradley indicated that there was likely another stressor on the infected amphibians that is allowing the opportunistic infection.

Qualitative notes reveal malformations were apparently rare between 1997 and 2000; D. Reis would have detected them if they were common. On 25 July 2003 an intensive sampling of amphibians in Lower Cattail swale yielded data on percentage of malformations in three amphibian populations there – which were remarkably common. All malformations were observed in larval and young-of-the-year amphibians, and none in adult amphibians.

Malformations observed included extra and missing limbs and digits, skin webbings, shortened limbs, and replicate long bones in hind limbs, all consistent with those described as caused by the trematode *Ribeiroia ondatrae* (Sessions et al. 1999, Johnson et al. 1999). Dissections of the freshwater snail, *Planorbidae*, in 2003 did not allow for a positive identification of *Ribieroia ondatrae*. Three PCF larvae with malformations were dissected in 2003 and revealed hundreds of trematode metacercaria encysted in their limb bud regions, but a positive identification again could not be made. In 2004, malformations were again observed in larval amphibians in Lower Cattail swale. Dissections of *Planorbidae* and PCF with V. McKenzie allowed for a positive identification of *Ribieroia ondatrae*. Malformations were not observed in amphibians from any other wetlands on the Reserve in either year.

The pathogenic chytrid fungus, *Batrachochytrium dendrobatidis,* has been confirmed in the Reserve's amphibian populations as well. Individuals from Guzzler 9 and Midden pond tested positive for this pathogen in 2003 and 2004; chytrid was detected in Swimming Pool Rookery, and Upper Cattail swale in 2007.

**Mosquitofish**

In the past introduced *Gambusia,* or mosquitofish, were added to wetlands in hopes of reducing mosquito populations without the use of chemicals. In winter 2004, UCSC researchers, Mike Booth and Nina D'Amore, removed mosquitofish from half the guzzlers. In the absence of mosquitofish, mosquito populations remained tiny in the guzzlers (D'Amore pers. com.) Mosquitofish were observed to eat and molest amphibian eggs and larvae in wetlands that contain few invertebrates, (i.e. little complexity in the food web). Under these conditions, it was found that CRLF tadpoles suffered more injuries in the presence of mosquitofish and weighed a third less on average at metamorphosis (Lawler et al. 1999). This type of habitat is present on the Reserve in Midden pond and the guzzlers, although CRLF have rarely bred in the guzzlers. In March 2004, mosquitofish were observed consuming eight CRLF larvae as they emerged from an egg mass. Another CRLF larvae was observed being consumed by mosquitofish in this same wetland in May. Although predation by mosquitofish on CRLF in Midden is disturbing, it is not believed to be the case in the Reserve's other wetlands where the food web is more complex and mosquitofish may feed on preferred invertebrates.

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**Table A1. Aquatic and emergent plants found at Elkhorn Slough Reserve wetlands** (*nonnative; †P. pusillus or foliosus; ††planted during 2018-2019).

| | Middle Rookery | Swimming Pool Rookery | Rana | Tonii | Upper Cattail | Visitor Center |
|---|---|---|---|---|---|---|
| **Aquatic plants** | | | | | | |
| *Ceratophyllum demersum* | x | x | | | x | |
| *Chara braunii* | x | | | | | |
| *Chara globularis* | x | | | | | x |
| *Chara* sp. | | | | | | |
| *Najas guadalupensis* | | | | | x | x |
| *Potamogeton foliosus* | | | | | | x |
| *Potamogeton* sp.† | | x | | | | x |
| *Potamogeton pusillus* | | | x | | x | |
| *Stuckenia pectinatus* | x | | x | | | |
| *Zannichella palustris* | x | | | | | |
| **Emergent Plants** | | | | | | |
| *Baccharis douglasii* | | x | | | | |
| *Cyperus eragrostis* | | | x | x | x | |
| *Eleocharis macrostachya††* | | | | | x | x |
| *Juncus balticus††* | | | x | | | |
| *Juncus patens††* | | | x | | | |
| *Juncus* sp.†† | | x | | x | | x |
| *Lythrium hyssopifolium** | | | | | | x |
| *Persicaria punctata* | x | x | | | x | |
| *Sparganium eurycarpum* | x | x | | | | |
| *Typha latifolia* | | | x | | | |