



Acute mortality in California tiger salamander (*Ambystoma californiense*) and Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*) caused by *Ribeiroia ondatrae* (Class: Trematoda)

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ABSTRACT

In early September 2019, a morbidity and mortality event affecting California tiger salamanders (*Ambystoma californiense*) and Santa Cruz long-toed salamanders (*Ambystoma macrodactylum croceum*) in late stages of metamorphosis was reported at a National Wildlife Refuge in Santa Cruz County, California, U.S.A. During the postmortem disease investigation, severe integumentary metacercarial (Class: Trematoda) infection, associated with widespread skin lesions, was observed. Planorbid snails collected from the ponds of the refuge within seven days of the mortality event were infected with *Ribeiroia ondatrae*, a digenetic trematode that can cause malformation and death in some amphibians. We suggest sustained seasonal high-water levels due to active habitat management along with several years of increased rainfall led to increased bird visitation, increased overwintering of snails, and prolonged salamander metamorphosis, resulting in a confluence of conditions and cascading of host-parasite dynamics to create a hyper-parasitized state.

1. Introduction

1.1. Amphibian decline

North America harbors approximately 230 species of amphibians, including 90 anuran species (frog and toads) and about 190 urodele species (salamander and newts), representing the highest diversity of salamanders in the world (AmphibiaWeb, 2021). With regards to the worldwide amphibian decline (Stuart et al., 2004; Wake and Vredenburg 2008) the United States is no exception with reports that up to 42 percent of the species in the U.S.A. are listed as threatened or declining (Stuart et al., 2004; Bradford, 2005; Grant et al., 2016). Habitat loss, climate change, increased disease risk, and invasive species, alone or in synergism, can cause direct mortality and sublethal effects with individual and population-level consequences, and are important to consider in disease investigations (Blaustein et al., 2011; Hof et al., 2011; Grant et al., 2016).

Most suspected disease agents credited with extirpations or

extinctions in amphibians have been fungal (Longcore et al., 1999; Fisher et al., 2012; Scheele et al., 2019) or viral (Jancovich et al., 1997; Earl et al., 2016). The role of helminth parasites in amphibian mortality events or population declines is not as well studied or understood (Tinsley, 1995; Fernández-Loras et al., 2011). Parasite life cycles, especially the complex life cycles of trematodes, make discerning the role of helminth infections in population declines much more difficult as both adult and larval stages can cause disease and can respond to environmental changes differently and independently. Deleterious effects can be manifested as reduced fecundity, less recruitment into adult populations, stunting, or other difficult to measure manifestations. Other factors such as herbicides (Rohr et al., 2008), which alter habitats and intermediate host environments, and invasive species (Sinsch et al., 2018), which can amplify or act as a “sink” for parasites, can make the role of parasites more difficult to discern.

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1.2. Ellicott slough National Wildlife Refuge

The U.S. Fish and Wildlife Service National Wildlife Refuge System provides one the largest networks of managed protected areas for vulnerable fish and wildlife species in the world and is essential for conservation efforts. The Ellicott Slough National Wildlife Refuge (ESNWR) (https://www.fws.gov/refuge/Ellicott_Slough/Ellicott_SloughNWR.html) in Santa Cruz County, California, U.S.A., was established in 1975 to protect the endangered Santa Cruz long-toed salamander (SCLTS, *Ambystoma macrodactylum croceum*) and supports two of the 24 known breeding populations for this species. The California red-legged frog (*Rana draytonii*) and the California tiger salamander (CTS, *Ambystoma californiense*) were federally listed as a threatened species in 1996 and 2004, respectively, and their protection added to the mission of the ESNWR. The ESNWR manages four natural and constructed ponds to provide SCLTS and CTS optimal breeding habitat and conditions for metamorphosis, as well as to counteract anticipated effects of climate change such as drought. In early September 2019, a morbidity and mortality event affecting salamanders in late stages of metamorphosis of the CTS and SCLTS was noted in the Ellicott and Prospect ponds (36–55°18' N 121–50°13' W). Prospect Pond was constructed in 1996 by refuge staff with reconstruction in 2012 to add sodium bentonite, a natural sealer to increase water retention as a longer hydroperiod and larger body size is correlated with increased recruitment into the adult population (Tejedo and Reques, 1994; Denver et al., 1998; Searcy et al., 2015). The pond basin is 67 m × 12.5 m with an average of 92 cm of water. With increased water retention both CTS and SCLT breeding occurred yearly since 2014. Ellicott Pond is a natural pond and remnant of a slough. The pond is shallow, approximately 1 ha in surface area with a basin of 260 m × 68 m. Ellicott Pond did not reliably retain water every year until 2014 when a 30 m × 9 m subbasin was created to increase water retention. The ponds are in proximity with each other; Ellicott Pond located 370 m to the north of Prospect Pond. We present an investigation of the cause of death, including the environmental conditions, snail community, and relevant abiotic parameters at the time. We use the results of this unusual case to highlight the importance of considering a system-wide response (including parasites and hosts) to alteration and timing of water levels in management of urodelans in the face of anticipated droughts and changing rainfall patterns that may be exacerbated with climate change.

2. Methods

2.1. History

In early September 2019, a morbidity and mortality event affecting CTS and SCLTS in late stages of metamorphosis was noted in the Ellicott and Prospect ponds with an estimated number of deaths of 25 CTS and 10 SCLTS. Approximately 10,000 SCLTS breeding adults were at risk, however, the CTS population had not been estimated recently (Biosearch Associates, 2015). Observed clinical signs of disease in both species included lethargy, generalized pallor, and sores near the mouth, anus, and proximal limbs. Unlike in previous years, both ponds were still holding water at this time of year and the water was discolored brown and had abundant planorbid and lymnaeid snails. Water levels and chemistry were within normal ranges with a maximum depth of 0.73 m, a surface temperature of 22.2 °C, pH 6.85, and oxygen saturation at 35.2%. Refuge biologists collected and submitted salamander carcasses to the U.S. Geological Survey National Wildlife Health Center (NWHC), Madison, Wisconsin, U.S.A. for necropsy and cause-of-death determination.

2.2. Post-mortem examination

Seventeen CTS and 9 SCLTS were submitted either chilled, frozen, or preserved in 70% ethanol. Six suitable CTS carcasses were selected for

post-mortem examination compromising two of each type of preservation. All 9 SCLTS were examined grossly and 5 were considered unsuitable for postmortem examination due to post-mortem autolysis. If in suitable postmortem condition, tissue samples from heart, lung, liver, kidney, spleen, gastrointestinal tract, and cross sections of the head, tail and feet were collected, fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) for histopathological examination (Luna, 1968).

2.3. Microparasite/pathogen analysis

Liver from one freshly submitted carcass was cultured on tryptic soy agar with 5% sheep blood and incubated at 24 °C for detection of bacteria. All submitted animals were tested for *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal) by quantitative real-time polymerase chain reaction (qrt-PCR) using post-mortem skin swabs as described in Blooi et al. (2013, 2016). Testing for Ranavirus (genus *Ranavirus*; family Iridoviridae) was performed on pooled samples of liver, kidney and/or spleen by a qrt-PCR (Leung et al., 2017) and/or virus isolation in the Zebrafish-cell line ZF4 (ATCC®, CRL-2050™) (Ip et al., 2016) depending on type of preservation.

2.4. Macroparasite characterization

Metacercariae were dissected from the gills and base of the limbs from each of one frozen and one ethanol-fixed frozen CTS and two frozen SCLTS. Metacercariae were not in suitable condition for detailed morphological study nor infection studies. Metacercariae were lysed and DNA extracted using Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, California) per manufacturer instructions. The metacercariae were molecularly characterized using the partial sequences of the 28S rRNA gene (Tkach et al., 2016), *COI* mitochondrial gene (Van Steenkiste et al., 2015), and internal transcribed spacer (*ITS-2*) gene (Reinitz et al., 2007) with modifications according to Huver et al. (2015). PCR products were analyzed on a 2% agarose gel, excess primers and nucleotides removed from the PCR products using 1 µl ExoSap-IT (Applied Biosciences Affymetric Inc., Santa Clara California), then DNA was sequenced (Functional Biosciences, Madison Wisconsin, using ABI 3730 xl DNA sequencer automated DNA sequencing instrument; Applied Biosystems, Foster City, California), aligned and trimmed using SeqMan Pro (DNA Star Lasergene17, DNASTAR, Madison, Wisconsin). Molecular analyses were conducted with MEGA X (Kumar et al., 2018) where sequences were aligned with sequences from GenBank (www.ncbi.nlm.nih.gov/genbank/) using MUSCLE and manually trimmed. Maximum likelihood phylogenetic relationships among each of the gene sequences were calculated based on the Hasegawa-Kishino-Yano plus G (HKY + G) model with 500 bootstrap replications based using MEGA X (Kumar et al., 2018). Cluster analyses were performed by using the unweighted pair group method with arithmetic mean (UPMGA) and neighbor joining algorithms. Statistical support for groupings was estimated by using bootstrap analysis (Kumar et al., 2018).

To document intermediate hosts a visual inspection of shoreline was conducted on Sept. 30, 2019, within 7 days following the salamander mortality. *Planorbella* sp. (n = 15) and Lymnaeid gen. sp. (n = 13) from Prospect Pond and *Planorbella* sp (n = 30) from Ellicott Pond were sent live via overnight courier to NWHC then placed in commercial spring water with supplemental incandescent light for 12–18 h. Snails were identified using taxonomic keys (Burch, 1982; Dillon, 2019) with identifications corroborated by sequencing of partial *COI* mitochondrial gene (Folmer et al., 1994) (PX1645 and PX1646; GenBank: OK187263, OK187254). A subsample of snails shedding cercariae over the next 2–3 days were crushed and examined for larval trematodes using Normarski differential interference contrast microscopy (Olympus BX50F microscope, Olympus, Center Valley, Pennsylvania) to observe movement and taxonomically relevant characters. A subset of larval stages was

photographed (CMOS camera with SPOT 5.2 digital imaging software-Spot Imaging, Sterling Heights, Michigan), lysed, DNA extracted, and sequenced as outlined above. All parasites, hosts and associated DNA were archived in the Parasitology Division, Museum of Southwestern Biology, University of New Mexico, Albuquerque (MSB: Host:24584–24652 and MSB:Para:32142–32203; 32206) (Keller and Cole, 2021).

3. Results

3.1. Post-mortem examination: gross and histological findings

Gross and histological evaluations were based on suitable carcasses of fair to good postmortem condition (6 CTS, 9 SCLTS). All animals of both species were in late stages of metamorphosis as they were older than Harrison Stage 46 with extremities partially developed, and external gills and tail fins still present (Harrison, 1969). Body condition was considered fair to good based on presence and size of the internal fat bodies, which was indicative of a fast-acting disease process. Sex was not determined. Gross lesions were restricted to the integument and consisted of irregular areas of variable size (3 mm–4 cm), with roughening and occasional pale tan discoloration interpreted as dermal erosions and ulcers with crust formation. Lesions were observed all over the body but most commonly around the eyes, nares, gular fold, limb- and tail bases, and the cloacal opening in both CTS and SCLTS (Fig. 1). Metacercariae were often noted closely adjacent to or centrally located in gross lesions and most easily seen using dissection microscopes up to 10× magnification. In general, lesions were less prominent in the SCLTS than CTS, though the small body size and dark pigmentation could have obscured lesions. Examination of internal organs was unremarkable in all animals. Metacercarial cysts were most abundant in the ocular cavity, oral cavity, gill arch, and within dorsal skin around the tail base and thus corresponded to the gross lesions (Fig. 1). Histologic evaluation was performed on 6 CTS and 3 SCLTS with autolysis impairing the examination of all individuals to variable degrees. Microscopically, all animals had a severe metacercarial infection, with metacercariae either attached to epithelial surfaces within necrotic debris, intraepithelial, or within the subepithelial fibrous tissue associated with a variable degree of histiocytic to granulocytic inflammation and skin erosions and ulcers (Fig. 2A). Metacercariae with minimal surrounding inflammatory reactions were also present. Metacercarial cysts were most abundant around the eye socket, gill arch, dorsal tail base, and oral cavity, corresponding to the gross lesions (Fig. 1). Gill filaments were often thickened as a result of high numbers of metacercariae and corresponding inflammation (Fig. 2B). Two CTS also had evidence of a suspect septicemia, with increased numbers of granulocytes within hepatic sinusoids occasionally invading hepatic cords. Other examined organs were unremarkable. Considering the severe, multifocal metacercarial encystment with corresponding significant inflammation and lack of other disease processes, trematodiasis was determined to be the cause of death in all animals examined.

3.2. Microparasite/pathogen detection

All 26 carcasses were tested for Bd and Bsal. Bd was detected by qrt-PCR on skin swabs of 8 CTS. Bsal was not detected by qrt-PCR. None of the ten carcasses tested for Ranavirus were positive by virus isolation (4 SCLT, 4CTS) and/or direct qrt-PCR (1 SCLT, 6 CTS) on pooled liver, kidney and/or spleen. Bacterial liver culture performed on one of the six CTS yielded growth of *Aeromonas* sp. with no further identification of species.

3.3. Macroparasite characterization

Cercariae collected from *Planorbella* sp. (n = 4 from Prospect, n = 3 from Ellicott) with an esophageal diverticula along with other minor characteristics such as shape of the excretory system and granules were consistent with species of *Ribeiroia* (Beaver, 1939, Fig. 3). Sequences of the partial 28S rRNA gene from the cercariae and metacercariae were identical with zero base substitutions (Tamura et al., 2004; Kumar et al., 2018). GenBank BLAST® (<https://www.ncbi.nlm.nih.gov/genbank/>) of partial 28S rRNA gene sequences from one metacercaria (PX1386; GenBank OK188967) infecting CTS and 25 cercariae (PX1415; GenBank OK171108) from *Planorbella* sp. were similar to adult specimens of *R. ondatrae* (KT956956) with only 1 base pair difference (1014/1015 bp) and used in construction of a phylogenetic tree (Fig. 4) supporting identification of *Ribeiroia*-like cercariae from *Planorbella* sp. and metacercariae from salamanders as *R. ondatrae*. Partial sequences of ITS 2 rRNA (270bp) gene and partial mitochondrial COI gene (595 bp) from cercariae and metacercariae had fewer publicly available sequences in GenBank and were not used in the analysis but were deposited in GenBank (Table 1).

Prospect Pond also had *Planorbella* sp. and Lymnaeid gen. sp. infected with cercariae of *Australapatemon* sp. (Dubois, 1968) (PX1409-1411). *Echinoparyphium* sp. (Fried et al., 1998) cercariae (PX1397, PX1407) and Echinostomatid metacercariae (PX1396) were recovered from Lymnaeid gen. sp. with identifications based on morphology and sequencing of partial 28S rRNA and partial COI mitochondrial genes.

4. Discussion

Ribeiroia ondatrae is a digenetic trematode that uses planorbid snails as a first intermediate host, fish and amphibians as second intermediate hosts, and primarily piscivorous birds or mammals (*Ondatra zibethicus*) as final or definitive hosts (Beaver, 1939; Taft et al., 1993). Since the 1990s, research has shown *R. ondatrae* can cause malformations in a wide range of amphibians in North America when exposed to cercariae at specific developmental stages during metamorphosis (Sessions and Ruth, 1990; Johnson et al., 1999; Schotthoefer et al., 2003). The present study sheds a different light on *R. ondatrae*, where infection led to widespread acute mortality instead of malformations in salamanders in late-stage metamorphosis of two conservation-priority species. Direct mortalities related to *R. ondatrae* have been reported primarily in

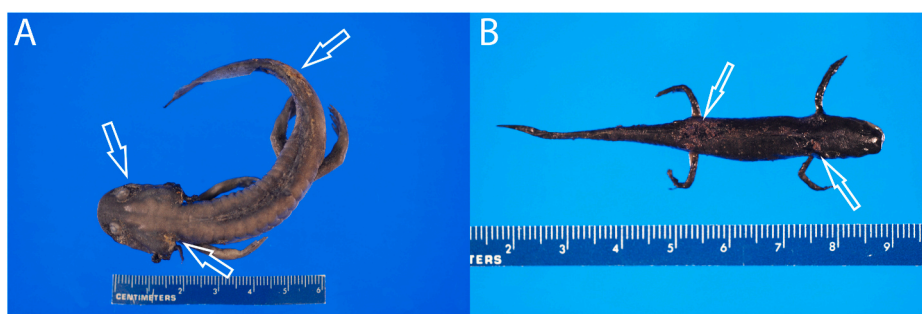


Fig. 1. Gross photographs of ethanol-fixed salamanders in late-stage metamorphosis from a mortality event in the Ellicott Slough National Wildlife Refuge (ESNWR) in Santa Cruz County, California, U.S.A. showing integumentary lesions. (A) California tiger salamander (*Ambystoma californiense*) exhibiting an extensive roughening of the skin with multifocal ulceration around the eyes, gular fold, and dorsal tail (arrows). (B) Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*) with ulceration and crust formation on the gular fold and base of the tail (arrow).

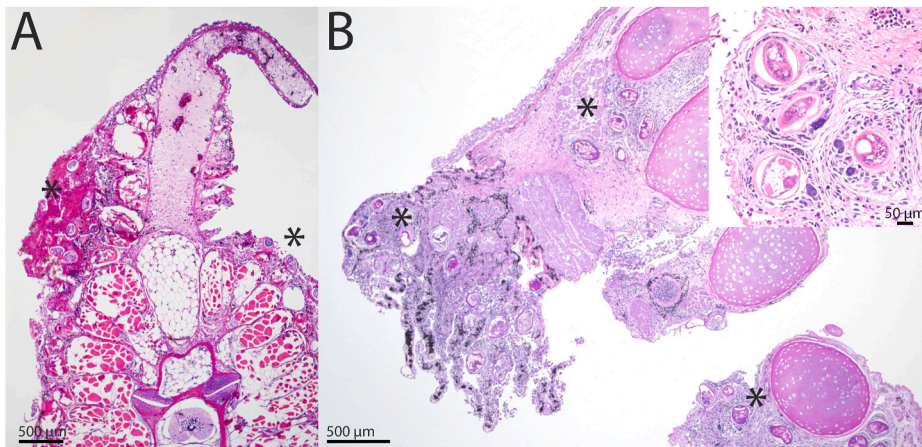


Fig. 2. Photomicrograph of California tiger salamanders (*Ambystoma californiense*) in late-stage metamorphosis from a mortality event in the Elliott Slough National Wildlife Refuge in Santa Cruz County, California, U.S.A. (A) Cross-section of dorsal tail showing widespread ulcerative dermatitis with superficial serocellular crust formation and intralesional metacercariae (asterisk) (H&E). (B) Metacercariae (asterisk) associated with mixed cellular to granulomatous inflammation widespread in the gills and subcutis (PAS). Inset: Encysted metacercariae are surrounded by mixed-cellular to granulomatous to infiltrate (H&E).



Fig. 3. *Ribeiroia ondatrae* cercaria from *Planorbella* sp. collected from Elliott Slough National Wildlife Refuge, Santa Cruz County, California, U.S.A.

experimental settings with susceptibility to and outcome of trematode infection correlated to infection density and timing of exposure relative to the stage of metamorphosis (Johnson et al., 2001; Schotthoefer et al., 2003; Dare et al., 2006). Experimental infections of anurans in early stages of metamorphosis more often result in a lethal outcome rather than malformations, indicating phenology of parasite and amphibians could determine the outcome (Schotthoefer et al., 2003). Past research has focused primarily on the development of *R. ondatrae*-induced malformations in frogs with less attention to infection in urodeleans such as salamanders and newts (Sessions and Ruth, 1990; Johnson et al., 2006).

In the present case, gross and microscopic lesions consisting of skin ulcerations and inflammation corresponded in location with large numbers of metacercarial cysts; skin lesions associated with encystment have been reported before in experimental (Schotthoefer et al., 2003) and wild (Johnson et al., 2001, 2002, 2006) amphibians. Consistent with observed gross lesions in anurans, *R. ondatrae* encystment sites were reported primarily at the base of the limbs and to a lesser extent the posterior coelom, nares, and gular folds, but expanded across the body with higher infection levels (Johnson et al., 2002, 2004; Stopper et al., 2002; Roberts and Dickinson, 2012). Similarly, decreased survival of amphibian larvae have been reported with increased parasite exposure (Schotthoefer et al., 2003; Roberts and Dickinson, 2012). Salamanders

examined herein, had gills so heavily infected that moving the gills of fixed specimens caused large numbers of metacercariae to break free, reminiscent of grape clusters. Evidence of a concurrent septicemia was seen histologically in two animals, and *Aeromonas* sp., a known facultative-pathogenic bacteria of amphibians, was cultured from one of those animals (Hird et al., 1981; Schadich and Cole, 2010; Pessier, 2018), although a post mortem bacterial overgrowth cannot be ruled out. We concluded the underlying cause of death of the CTS and SCLTS was the large number of invading cercariae, which penetrated the epidermis and then encysted as metacercariae. Dermal disruption by invading cercariae can rapidly be fatal due to secondary septicemia or metabolic imbalances as the amphibian skin has critical physiological function in water absorption, osmoregulation, and respiration (Pessier, 2002).

Ribeiroia ondatrae is likely endemic to California (Johnson et al., 2002) and likely was present at these sites prior to the mortality event, as low-level malformations have been noted in Pacific tree frogs (*Pseudacris regilla*) for many years (Reeves et al., 2013) and all hosts necessary for *R. ondatrae* to complete its life-cycle (planorbid snails, amphibians fish and waterbirds) regularly occur in the refuge ponds (Beaver, 1939; Taft et al., 1993). We hypothesize that a variation in hydrologic conditions during the previous few years led to the severe manifestation of disease via a late season crowding phenomenon and prolonged hydroperiod in the ESNWR ponds.

Density of *R. ondatrae* can be positively correlated to the abundance of *Planorbella* sp., the first intermediate host of *R. ondatrae* (Johnson et al., 2004; Blaustein et al., 2011; Orlofske et al., 2018), and high numbers of snails were observed by ESNWR biologists in the weeks leading up to the observed salamander mortality. The Elliott and Prospect ponds usually dry up in late July or August; however, there was heavy rainfall during water year 2019 (October 1, 2018–September 30, 2019 [<https://water.ca.gov/Water-Basics/Glossary>]) and was 113% of the 30 y average (https://www.cnrfc.noaa.gov/monthly_precip_2019.php) as recorded at the Watsonville, California water works station (WVIC1) 6000 m east of the ponds. Those permanent to semi-permanent aquatic habitats likely facilitated both higher input of trematode eggs from infected definitive hosts (waterbird use) and continued survival of the snails, leading to a higher parasite reproductive potential the spring and summer of 2019 (Roberts and Dickinson, 2012). Brown et al. (1988) showed that more permanent water bodies had more diverse bird visitation and were more productive with regards to snail fecundity than semi-permanent or vernal ponds. Huspeni and Lafferty (2004) reported that in recently restored tidal salt marsh trematode prevalence and species richness increased and was directly related to increased use of the site by birds. Likewise, Poulin (2011) noted parasite infection rates in snails were determined primarily via definitive host rather than snail dynamics. Additionally, the snails examined for this investigation were

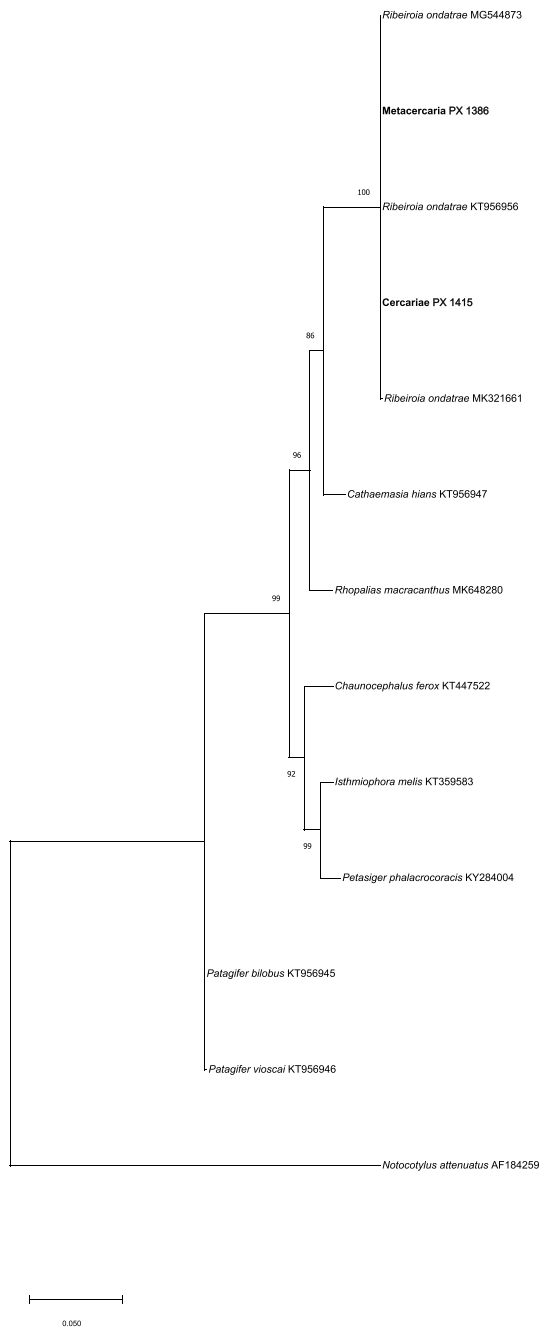


Fig. 4. Molecular phylogenetic analysis by Maximum Likelihood method based on the Hasegawa-Kishino-Yano plus G model with 500 bootstrap replications based on partial 28S rRNA gene sequences of *Ribeiroia ondatrae* metacercariae from California tiger salamanders (*Ambystoma californiense*), cercariae from *Planorbella* sp. and sequences of *R. ondatrae* publicly available in GenBank with *Notocotylus attenuatus* as an outgroup. Tree is drawn to scale with branch lengths measure in the number of substitutions per site. The analysis involved 13 nucleotide sequences. All positions with less than 95% site coverage were eliminated. There was a total of 1189 positions in the final dataset.

0.9 cm and larger, and therefore could avoid predation by invertebrates, the main regulators of smaller systems devoid of molluscivorous fish such as the ESNWR ponds (Johnson et al., 2002; Chase, 2003). Other factors associated with increased planorbid snail population, such as eutrophication and low oxygen levels, were not identified in the ESNWR systems (Lodge et al., 1987; Johnson and Chase, 2004). Finally, one of the triggers for salamander metamorphosis is decreasing water volumes (Semlitsch and Wilbur, 1988) and the holding of water volumes most

Table 1

Sample number, gene sequenced (28S rRNA, partial COI mitochondrial or ITS2) and GenBank voucher number from parasites or hosts from Ellicott Slough National Wildlife Refuge, California, U.S.A. submitted to U.S. Geological Survey National Wildlife Health Center.

Sample	28S rRNA	partial COI	ITS2
PX1386	OK188967	OK188993	OK239684
PX1388	OK188965	OK210381	OK239683
PX1390	NS ^a	OK210492	NS
PX1396	NS	OK206702	NS
PX1397	OK239681	NS	NS
PX1400	OK174408	OK188790	OK239682
PX1407	OK184707	NS	NS
PX1409	OK184705	OK187264	NS
PX1410	NS	OK257879	NS
PX1411	OK284407	OK257861	NS
PX1415	OK171108	OK171723	OK172583
PX1645	NS	OK187263	NS
PX1646	NS	OK187254	NS

^a NS Not sequenced.

likely suspended or slowed metamorphosis, thus increasing the window of susceptibility of the salamanders to infectious cercariae.

All of these factors may have produced the “perfect storm” involving continued snail and parasite abundance with salamanders in a sustained susceptible stage of metamorphosis. In addition, infection with *R. ondatrae* may also delay metamorphosis in some amphibians such as the Pacific tree frog (Romansic et al., 2011) and long-toed salamander (*Ambystoma macrodactylum*) (Johnson et al., 2006), as energy resources are hypothesized to shift away from development to skin repair. As suggested by McDevitt-Galles et al. (2020) interaction of parasite and host phenology and synchronization of infectious *R. ondatrae* cercariae with vulnerable anuran life stages affects the frequency and severity of infections. Similar relationships/influences could exist at Ellicott and Prospect Ponds resulting in mortalities as we report.

Although studies have modeled effects of parameters such as temperature increase and pond desiccation on parasite-host dynamics (Paull and Johnson, 2018), the converse, such as increased permanence of vernal or semi-permanent ponds and its effect on host-parasite dynamics in the short term, has not been examined. Various stressors (including parasites and other disease agents) undoubtedly have played a role in the evolution of life history traits (Stearns, 2000). Escape from parasites via use of vernal or semi-permanent ponds along with a shorter period of metamorphosis may be evolutionarily important for these species of salamanders.

5. Conclusions

The conservation of endangered and threatened species, like CTS and SCLTS, often warrants active habitat management and habitat creation. In 2012, after several years of drought, and the inability of Prospect Pond to hold water, the ESNWR excavated and rebuilt the pond. Prospect Pond reconstruction was considered an essential management action by the U.S. Fish and Wildlife Service critical to maintaining suitable breeding habitat for these species and resulted in successful breeding of CTS and SCLTS within 3 years post-reconstruction. A few years later, Ellicott Pond was also deepened, facilitating ponding with the management objective of enhancing salamander breeding. Engineered ponds, like Prospect, have drain infrastructure included in design plans to create an “ephemeral” condition to allow active management if competing or predatory species such as invasive fish, bull frogs, or crayfish become established in the ponds. The ability to actively manage pond hydrology can help in the conservation of the threatened and endangered salamanders and red-legged frogs on the ESNWR. Following the 2019 salamander mortality event Prospect Pond is drained annually, and water is not held year to year. This case highlights a possible set of

interacting factors to consider when planning and monitoring active habitat management to avoid potentiation of parasite transmission and disease.

Declaration of competing interest

The authors declare no competing interests or non-financial interests. We have no conflicts of interest to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2021.10.008>.

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