Parasitic Copepod (*Lernaea cyprinacea*) Outbreaks in Foothill Yellow-legged Frogs (*Rana boylii*) Linked to Unusually Warm Summers and Amphibian Malformations in Northern California

Sarah J. Kupferberg[1,2], Alessandro Catenazzi[2], Kevin Lunde[3], Amy J. Lind[4], and Wendy J. Palen[5]

How climate change may affect parasite–host assemblages and emerging infectious diseases is an important question in amphibian decline research. We present data supporting a link between periods of unusually warm summer water temperatures during 2006 and 2008 in a northern California river, outbreaks of the parasitic copepod *Lernaea cyprinacea*, and malformations in tadpoles and young of the year Foothill Yellow-legged Frogs (*Rana boylii*). Relative to baseline data gathered since 1989, both 2006 and 2008 had significantly longer periods when daily mean water temperatures exceeded 20°C compared to years without copepod outbreaks. Infestation varied spatially in the watershed, as prevalence increased concomitantly with temperature along a 5.2 km longitudinal transect. At breeding sites of *R. boylii* with copepods in 2006, infestation ranged from 2.9% of individuals upstream to 58.3% downstream. In 2008, copepods were absent from the most upstream sites and infested up to 28.6% of individuals sampled at downstream locations. Copepods most frequently embedded near a hind limb or the cloaca. Among individuals with parasites in 2006, 26.5% had morphological abnormalities compared to 1.1% of un-infested individuals. In 2008 when the infestation peak occurred late in development (post Gosner stage 39), abnormalities were not associated with copepod infestation. In both years, recently metamorphosed frogs with copepods were, on average, slightly smaller than those not infested. These occurrences represent a sudden increase in local prevalence atypical for this river ecosystem. Previously we had only once seen copepods on amphibians (on non-native Bullfrogs, *Rana catesbeiana*), six km further downstream. Pacific Chorus Frogs, *Pseudacris regilla*, which co-occur with *R. boylii* in shallow near shore habitats were not used as hosts. The data suggest that increasing summer water temperatures, decreased daily discharge, or a combination of both, promote outbreaks of this non-native parasite on an indigenous host, and could present a threat to the long-term conservation of *R. boylii* under the flow regime scenarios predicted by climate change models.

WITH increased attention on parasitism and disease as threats to biodiversity, there is a need to identify the pathogens and parasites which pose significant risks (Daszak et al., 2000; Smith et al., 2006) and determine which ecological factors may be drivers of disease spread (Plowright et al., 2008). Warming and changes in precipitation anticipated with global climate change are predicted to exacerbate the impact of parasites and pathogens in aquatic ecosystems (Marcogliese, 2001). Increased air and water temperatures can enhance the breeding of vector organisms (Freed et al., 2005), increase overall population growth rates of a pathogen (Woodhams et al., 2008) or accelerate transmission rates by causing proliferation of infective stages (Freed et al., 2005; Poulin, 2006). Amphibians with narrow thermal niches are particularly at risk from warming, and are vulnerable to multiple stressors including the spread of virulent infectious diseases, such as chytridiomycosis (Berger et al., 1998; Muths et al., 2003; Scherer et al., 2005; Wake and Vredenburg, 2008), which may be expanding through space and time independent of warming (Lips et al., 2008). The role of pathogens and parasites in amphibian declines, however, is not limited to chytridiomycosis (Pessier, 2002; DiRosa et al., 2007; Picco and Collins, 2008), and as Pounds et al. (2007) state, ''the rogues' gallery of amphibian pathogens that benefit from climate change is only just beginning to be assembled.''

Accordingly, our intention in this paper is to alert biologists to a crustacean parasite particularly sensitive to temperature. We report the prevalence of a copepod, *Lernaea cyprinacea*, affecting larval (Fig. 1A, B) and recent post-metamorphic stages (Fig. 1C, D) of the river breeding Foothill Yellow-legged Frog, *Rana boylii*. We hypothesize that the outbreaks associated with warm and dry conditions in the South Fork Eel River of northern California foreshadow a consequence of the climate change predictions across the range of *R. boylii* in California and Oregon. Stream temperatures will likely be influenced through increased ambient summer air temperatures and reduced discharges (Kiparsky and Gleick, 2003; Snyder et al., 2004; Webb et al., 2008), two factors that control the heating of water as it flows downstream. General sensitivity analyses of a process-based, basin-scale stream temperature model developed for the South Fork Eel indicate that reductions in flow may have the greatest impacts on stream temperature (Allen, 2008).

Lernaea cyprinacea, commonly referred to as Anchor Worm, is a Eurasian species that has become globally widespread. Despite its Latin name, it does not limit itself to cyprinid fishes for hosts. There are several reports, both historical and recent, of *L. cyprinacea* using amphibians as hosts in North America (Haderlie, 1950; Baldauf, 1961; Tidd, 1962; Hoffman, 1967), South America (Martins and Souza, 1996; Alcalde and Batistoni, 2005), and Asia (Ming, 2001).

[1] Questa Engineering, P.O. Box 70356, 1220 Brickyard Cove Road, Suite 206, Pt. Richmond, California 94807; E-mail: skupferberg@gmail.com. Send reprint requests to this address.
[2] Department of Integrative Biology, University of California Berkeley, Berkeley, California 94720; E-mail: acatenazzi@gmail.com.
[3] Department of Environmental Science, Policy, and Management, University of California Berkeley, Berkeley, California 94720; E-mail: klunde@nature.berkeley.edu.
[4] USDA Forest Service, Sierra Nevada Research Center, 1731 Research Park Drive, Davis, California 95618; E-mail: alind@fs.fed.us.
[5] Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada; E-mail: wpalen@sfu.ca.
Submitted: 18 January 2008. Accepted: 16 March 2009. Associate Editor: M. J. Lannoo.
© 2009 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CH-08-011

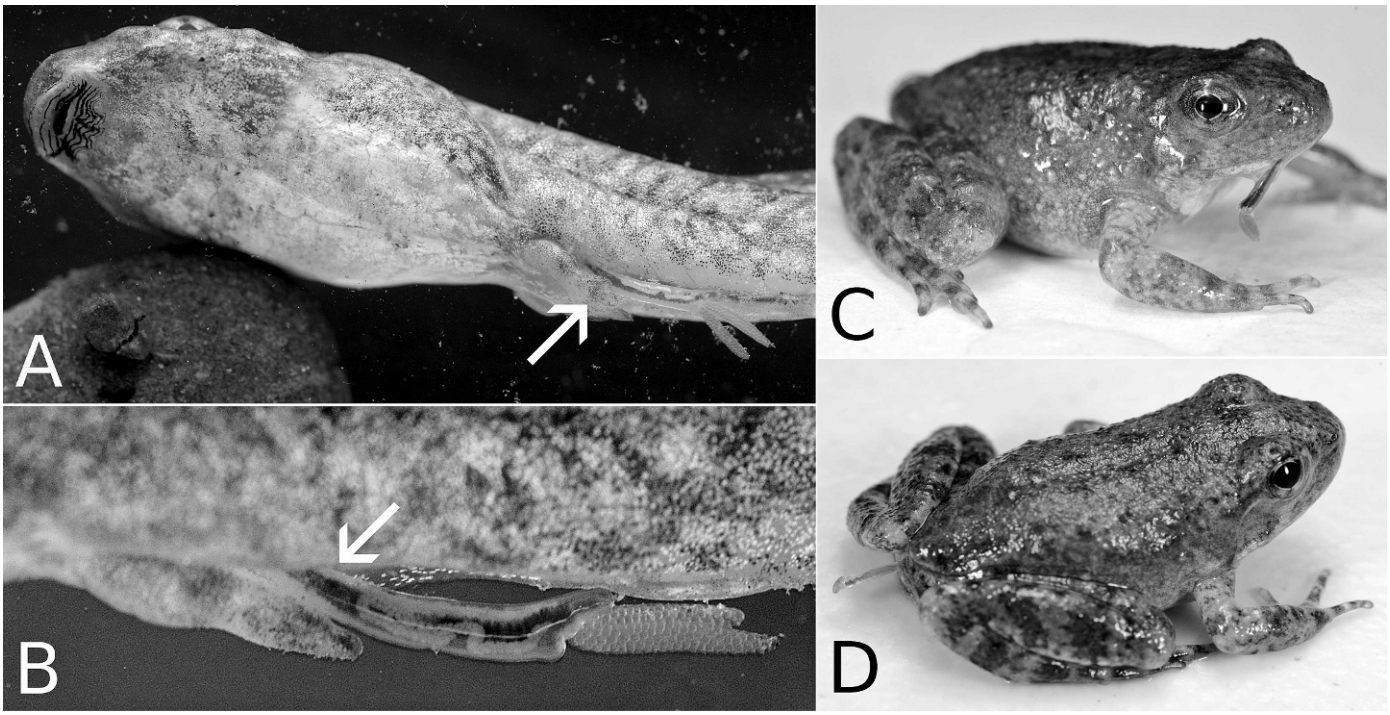

Fig. 1. (A) Adult female copepod parasite (approx. 7 mm long) *Lernaea cyprinacea* on a tadpole of *Rana boylii* with arrow indicating the insertion site near the base of a developing hind limb. (B) A close-up view of the copepod's abdomen with arrow indicating its egg sacs. Copepods on recently metamorphosed frogs embedded on the throat (C) and near the cloaca (D).

Parasitism by *L. cyprinacea* is generally a problem in aquaculture facilities and in the aquarium trade (Hoffman, 1999), where infestation can be potentially lethal or pathogenic when secondary microbial infections occur at the sites of lesions. Parasitism by *L. cyprinacea* can, however, be a wildlife conservation issue because of its negative effects on the condition of sensitive native fish (Durham et al., 2002; Bond, 2004; Hoffnagle et al., 2006) and possibly amphibians (Ming, 2001) when it becomes established in the wild.

There are approximately 10,000 species (in 187 families) in the Copepoda subclass of Crustacea, and almost half of these species are either parasitic or live in association with other aquatic animals (Bowman and Abele, 1982; Huys and Boxhall, 1991). Like most parasitic copepods, *L. cyprinacea* has a direct life cycle involving only one host, and transmission occurs via free swimming nauplii and the first copepodid larval stage. Adult females carry their eggs in sacs outside their body (Fig. 1B), from which they release nauplii. *Lernaea cyprinacea* is highly thermophilic (Marcogliese, 1991) and can develop from mature eggs to the first parasitic stage in as little as 4–8 days (Al-Hamed and Hermiz, 1973). The first larval stage is taken up by fish in the gills or by a tadpole through the spiracle (Bird, 1968). The first through fifth copepodid stages are localized within the tadpole's mouth and branchial chambers (Shields and Tidd, 1963). Copepodids attach by their maxillipeds and remain sessile, eating epithelial tissue and underlying connective tissue (Shields and Tidd, 1974) until the fifth larval stage when copulation occurs. Post-copulation, females undergo another molt, travel along the integument of the tadpole, and penetrate the tadpole's tissue with their heads. A transformation then occurs in which the body of *L. cyprinacea* lengthens (up to approx. 1 cm) and the cephalothorax develops into an anchor shaped process (Bird, 1968). Penetration sites can be anywhere, but most often near the juncture between tail and body, and a number of tadpole tissues (e.g., liver, lung, spinal cord) can be damaged (Tidd, 1962). As much of this previous work examining copepod effects on tadpoles has been conducted in the laboratory, our focus here is to document the *in situ* environmental conditions associated with parasitic copepod outbreaks, spatial patterns of prevalence, and the coincidence between the presence of copepods and morphological abnormalities in an otherwise robust population of frogs.

MATERIALS AND METHODS

We conducted parasite surveys along 5.2 km of the South Fork Eel River (SF Eel) on the University of California's Angelo Coast Range Reserve, Mendocino Co., California (39.733°N, 123.65°W). Study of *R. boylii* began there in 1989 with ongoing full-reach censuses of frog breeding established in 1992. Along the study reach, there are five perennial tributaries, and several more ephemeral drainages, inhabited by juvenile and adult *R. boylii*. Spawning, however, occurs only at historic breeding sites along the margins of the SF Eel. The sites are geomorphologically distinct locations (i.e., depositional areas, such as cobble bars or pool tail outs) with the most populous sites clustered near (within one riffle-pool sequence) tributary confluences (Kupferberg, 1996). Mean (±1 s.e.) inter-site distance is 118.8 ± 11.4 m (n = 44). The extent of individual movement among sites is not known, but we assume the collection of frogs using these breeding sites constitutes a meta-population.

We sampled frogs for copepods in 10% of the study reach (approximately 520 m) during 2006 and 2008. No copepods were observed on frogs in 2007. In 2006, search efforts were spatially concentrated and in 2008, search efforts were

spatially dispersed, but we sampled similar total lengths of river each year. On 17–18 August 2006, we visited the near-shore habitats of three breeding sites, at the upstream, midpoint, and downstream extent of the reach. One month later, as part of a mark and recapture demographic study, juvenile *R. boylii* were captured over three days, 16, 17, and 21 September, along a 460 m segment near the midpoint of the 5.2 km reach. Captured individuals received daily batch marks using Visible Implant Elastomer (Northwest Marine Technologies, Seattle, WA). To determine what proportion of the population was examined for copepods, we estimated the abundance of recently metamorphosed frogs for the three-day survey period using program CAPTURE (Otis et al., 1978; White et al., 1982). An estimator was not available for the best-fit model, so the model with the next highest ranking was used. Between 13 August and 7 September 2008, we visited 13 breeding sites. The relationship between parasite prevalence and distance was evaluated using linear regression. Prevalence data from 2008 were transformed by taking the arcsin square root of the proportion infested at each of the 13 breeding sites sampled. In both years we measured tadpole and frog snout-to-urostyle length (SUL) to the nearest mm, and visually inspected for ectoparasites and morphological abnormalities. In 2008, we also inspected Pacific Chorus Frogs, *Pseudacris regilla*.

To describe environmental conditions in the river, we calculated mean daily discharge (m^3 sec^{-1}) for August, and the number of summer days with mean water temperature ≥20°C from data collected hourly at the most upstream extent of the study reach. Stream gaging and thermal monitoring began in 1989 at a retired USGS gaging station re-established by M. E. Power and W. Dietrich. Not all years have complete data sets due to occasional equipment failures. The gaging station defines the starting point of the 5.2 km study reach where breeding censuses and parasite surveys occurred. Along with the abiotic factors, we calculated annual density of clutches of eggs (#/km) and compared values for 2006 and 2008 to the longer term means of ''no copepod'' years (1992–2005, 2007) using t-tests. To detect whether a gradient in water temperature exists along the length of the study reach, we placed iButton (Maxim Integrated Products, Sunnyvale, CA) temperature recorders at seven locations, between 25 August and 7 September 2008. Temperature was measured in 0.5°C increments every two hours, with an accuracy of ± 1°C. We calculated the two-week average of daily mean temperature and assessed the relationship with distance downstream using linear regression.

We investigated whether copepod infestation could be associated with the occurrence of abnormalities. For 370 individuals sampled in 2006 (332 were post-metamorphosis) and 411 in 2008 (159 were post-metamorphosis), we compared the frequency of abnormalities among animals infested by *L. cyprinacea* to the frequency of abnormalities in un-infested animals with G-tests of independence. We did not confirm the designation of ''un-infested'' by dissection or other independent method. Parasites may have been shed prior to our observations. Host rejection of *L. cyprinacea* by tadpoles of *R. pipiens* has been observed via a process involving extensive formation of connective tissue to encapsulate the anchor process of the copepod (Shields and Goode, 1978).

In 2008, we inspected abnormal *R. boylii* for trematodes, along with a random subset of normal individuals to determine if the parasite *Ribeiroia ondatrae* might be associated with the observed malformations. However, due to the protected status of *R. boylii* in California, collections were limited. To reliably conclude the presence and abundance of trematodes, we supplemented necropsy data of *R. boylii* with dissections of normal and abnormal *P. regilla* that had developed in the same reach of the SF Eel. *Pseudacris regilla* is present at breeding sites of *R. boylii*, does not have any special protections, and is susceptible to infection by *R. ondatrae* (Johnson et al., 1999). Groups of 7–8 metamorphs of *P. regilla* were collected at two-week intervals during August and September 2008. Following euthanasia in MS-222, frogs were inspected for all trematode metacercariae using a stereo dissection scope. Metacercariae were counted, removed, excysted, and identified using a compound microscope.

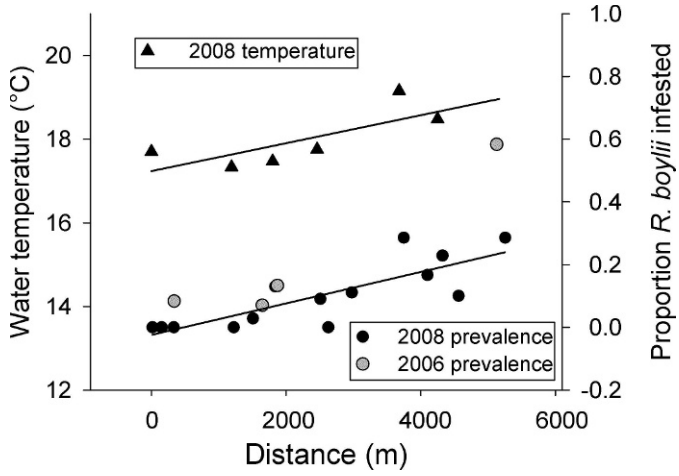

Fig. 2. Prevalence of parasitic copepod infestation in 2006 and 2008, and daily mean water temperature over 14 days between August and September 2008 along 5.2 km of the South Fork Eel River, Mendocino Co., California. For distance sampled, zero is at the most upstream end of the study reach.

RESULTS

The overall prevalence (# of infested individuals/total # inspected) of copepod infestation on *R. boylii* was 9.2% (34 cases/370 individuals in 520 m) in 2006, and 10.5% (43/411 individuals) in 2008. None of 75 inspected *P. regilla* had copepods. Copepod prevalence varied spatially (range 7.0 to 58.3% in 2006, 0 to 28.6% in 2008), and the angular transformed proportion of infested individuals increased significantly with distance downstream along the 5.2 km of river sampled ($R^2 = 0.68$, $F = 23.05$, df = 1,12, $P < 0.001$; Fig. 2). Daily mean water temperature similarly increased with distance ($R^2 = 0.70$, $F = 11.7$, df = 1,6, $P = 0.02$; Fig. 2).

The population estimate, based on the 2006 mark and recapture effort, was 622 ± 24.3 recently metamorphosed frogs, which corresponds to a density of 1.35 ± 0.05 individuals per m of river. Our capture rates of 0.73 and 0.78 frogs per m in 2006 and 2008, respectively, indicate that we were inspecting about half the animals present at our sampling sites, assuming similar densities between years.

The outbreaks of copepods were associated with three unusual conditions: high water temperature (in 2006 and 2008); drought induced low flow (2008); and high densities of tadpoles (Fig. 3). In 2006, *R. boylii* had the greatest reproductive output in 17 years of record, with the number

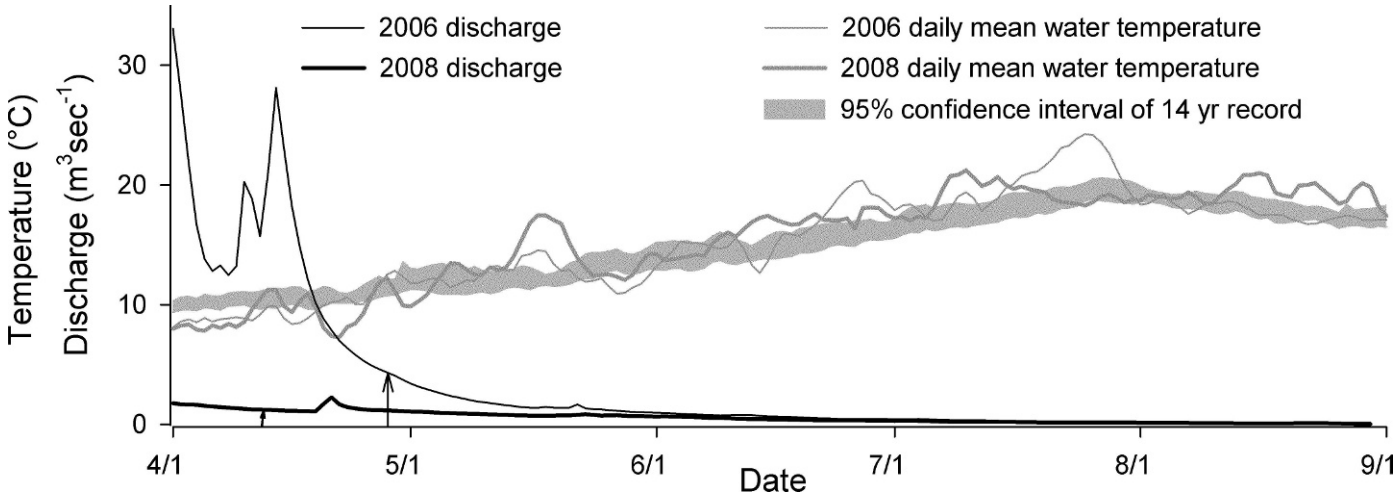

Fig. 3. Daily mean water temperature and flow conditions during the summers of 2006 and 2008 when outbreaks of *Lernaea cyprinacea* occurred. Confidence interval (shaded gray area) is based on the distribution of temperatures for that date over 14 years with complete records (1990–1998, 2001, 2003–2005, 2007) when copepods were not observed on amphibians. Arrows indicate the discharge when frogs began breeding (earlier in 2008, later in 2006).

of clutches deposited in the study reach significantly greater than average (Table 1). Additionally, there were significantly more days when the mean daily water temperature was above 20°C in 2006 and 2008, compared to years without copepod outbreaks (14 years with complete temperature data sets: 1990–1998, 2001, 2003–2005 and 2007). In 2008, reproductive output was again high, and extremely low flow conditions contributed to high concentrations of tadpoles in near shore habitats.

The abundance (# of parasites/host) was uniformly a single embedded copepod in 2008. In 2006, only three individuals had two copepods, with the remainder having a single parasite. Copepod prevalence differed among Gosner stages in infested tadpoles and froglets in both 2006 ($G = 13.85$, df = 2, $P = 0.001$) and 2008 ($G = 7.79$, df = 1, $P = 0.05$). The highest prevalence in 2006 occurred at earlier Gosner stages than in 2008 (Fig. 4A). Copepods were most frequently embedded near the base of a hind limb or the cloaca (Fig. 4B). Morphological abnormalities were also most frequent in the hind limbs (Table 2), but were not generally common in the whole sample of *R. boylii* (3.42% in 2006, and 2.68% in 2008). Of those infested with copepods in 2006, 26.5% had abnormalities while only 1.1% of un-infested animals had abnormalities. The association between limb abnormalities and copepod infestation was highly significant ($G = 30.4$, df = 1, $P < 0.001$). In 2008, however, no association was observed ($G = 0.8$, df = 1, $P > 0.5$). We also found un-infested *P. regilla* missing a hindlimb (one case each in 2006 and 2008) and infested, but morphologically normal, Pacific Giant Salamander (*Dicamptodon ensatus*): one dead in 2006, one alive in 2007.

In both years recently metamorphosed frogs with copepods were slightly smaller than those without copepods embedded (Fig. 4C). These differences approach statistical significance ($F = 3.02$ 2, df = 1, 485, $P = 0.08$) in a two-way analysis of variance of ln(SUL). Differences in size at metamorphosis were significant between years ($F = 10.97$, df = 1, 485, $P = 0.001$), and there was no copepod × year interaction ($F = 0.07$, df = 1, 485, $P = 0.8$).

The community of trematode parasites observed in 14 *R. boylii* and 26 *P. regilla* recent metamorphs is summarized in Table 3. *Ribeiroia ondatrae* was identified in only one of the *R. boylii* and was absent in all *P. regilla*. The one infected *R. boylii* had a single metacercarial cyst, and had normal morphology. Echinostomes were common in the kidneys of both species, though four times more abundant in *R. boylii* ($P < 0.01$). *Echinostoma* sp. infection intensity was quite variable; for example, two *R. boylii* suffered a burden of more than 700 cysts while others had none. *Manodistomum* sp. was significantly more abundant in *P. regilla* metamorphs ($P < 0.05$). Other larval trematodes known to infect amphibian larvae, e.g., *Fibricola* sp., *Alaria* sp., *Clinostomum* sp., *Auridistomum* sp. (Sutherland, 2005), were not encountered. In the field, while inspecting for copepods, we observed the monogenean trematode, *Gyrodactylus* sp., on the tails of many tadpoles of *R. boylii*.

Table 1. Environmental Conditions Associated with Outbreaks of Parasitic Copepods on *Rana boylii* (2006 and 2008), as Well as Summers When Copepods Were Not Observed. Asterisks indicate the significance of one-sample t-tests comparing the mean of non-copepod years to the 2006 and 2008 individual values, using the Bonferroni adjusted (for six tests) critical value of $P < 0.0083$.

Environmental factor n = # of years	years w/o copepods (mean ± s.e.)	2006	2008
No. days mean water temperature > 20°C ($n = 14$)	8.57 ± 2.3	16*	15*
Mean daily discharge for August (m^3 sec^{-1}, $n = 18$)	0.168 ± 0.018	0.195	0.110*
R. boylii density (clutches/km, $n = 15$)	100.3 ± 6.5	176.9*	134.0*

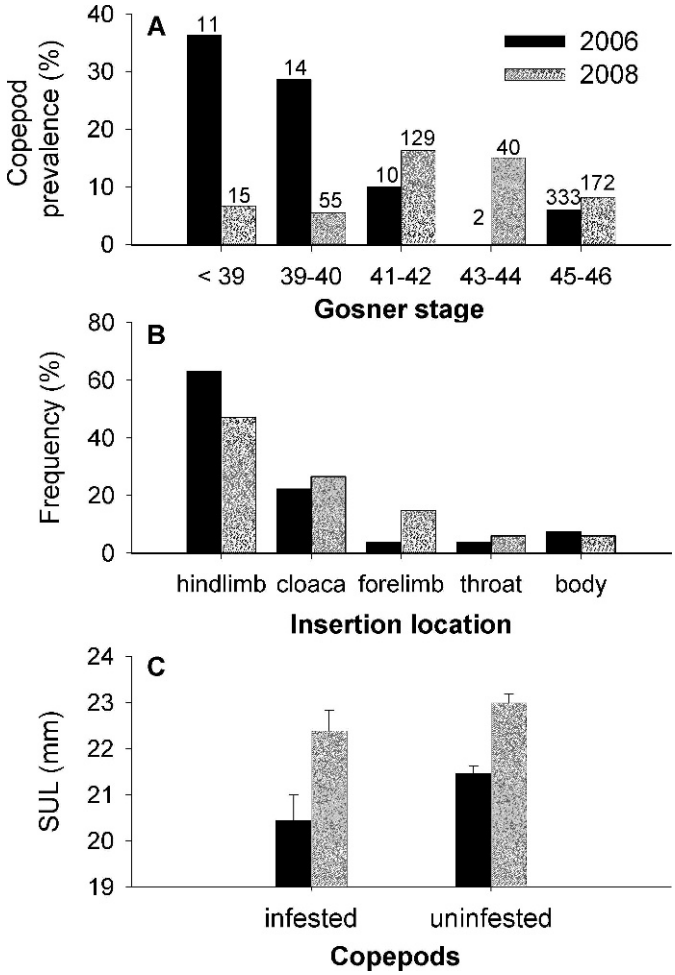

Fig. 4. Prevalence of infestation by *Lernaea cyprinacea* (A), insertion points (B), and snout–urostyle length (SUL) of Gosner stage 46 individuals (C), among tadpoles of *Rana boylii* in 2006 (dark bars) and 2008 (light bars). Numbers above bars indicate total number of individuals examined.

DISCUSSION

The outbreak of *L. cyprinacea* in the SF Eel was associated with periods of warm water temperatures, declining discharge, and shrinking pool sizes, conditions which are typical for infestations on fish in other rivers (Adams, 1984; Medeiros and Maltchik, 1999). In addition to the warm water temperatures, host density was high because there were record high numbers of egg masses of *R. boylii* and conditions for survival to hatching were excellent. The two main sources of embryonic mortality, scour and stranding (Kupferberg, 1996), were largely absent as there were no spates sufficient to scour egg masses and river stage declined gradually to keep eggs inundated. In late July 2006, there was an abnormally hot period when fish kills were observed. In 2008, there were also several warm periods when mean daily water temperatures exceeded the upper bounds of the 95% confidence interval for the mean of that date, although not as extreme as in 2006. We hypothesize that tadpoles may have been both directly stressed by periods of high temperatures and indirectly affected because warmth favors the development, reproduction, and fecundity of *L. cyprinacea* (Shields, 1978). Extremely low discharge and concomitantly slow current velocities in the river may also have enhanced transmission of copepod nauplii, as has been observed for the free-swimming stages of the myxozoan parasite responsible for whirling disease in salmonids (Hallett and Bartholomew, 2008).

The spatial pattern of higher copepod prevalence at the downstream end of the study reach may indicate an invasion front as well as reflect the gradient in thermal conditions (i.e., cooler water upstream). We observed these parasites 14 years earlier (21 September 1992) on non-native amphibians six km downstream of the study reach. In a pool with dense aggregations of first year Bullfrog tadpoles (*R. catesbeiana*), five tadpoles with *L. cyprinacea* embedded at the hind limb buds were collected and identified via dissection (M. Poteet and Kupferberg, unpubl.). Adult copepods were not seen on amphibians in the intervening years despite

Table 2. Relative Frequency of Abnormality Types in Tadpoles and Metamorphs of *Rana boylii* (Total Individuals Examined: $n = 370$ in 2006, 411 in 2008).

Abnormality type	Description	*n* 2006	*n* 2008	%
Hind limb				
Amelia	Missing leg	3	3	25
Apody	Absence of a foot	1	2	12.5
Brachymelia	Abnormally short limb	1	—	4.2
Femoral projection	Bony projection	2	—	8.3
Hemimelia	Partial/complete absence of distal part of leg	1	1	8.3
Hyperextension	Rigid flexure of a joint	1	—	4.2
Polymelia	Extra leg	1	1	8.3
Syndactyly	Fused digits	1	—	4.2
Fore limb				
Amelia	Missing arm	—	1	4.2
Apody	Absence of a hand	1	1	8.3
Other				
Missing eye		1	1	8.3
Misshapen snout		—	1	4.2
∑ Abnormal individuals		*13*	*11*	*100*

Table 3. Trematode Parasite Community within *Rana boylii* and *Pseudacris regilla* from the South Fork Eel River. Sample size (*n*) is number of amphibians inspected via necropsy (collected between 15 August and 15 September 2008). The first number is infection prevalence (# infected frogs/# inspected frogs), while the number in parenthesis is parasite abundance (mean # metacercarial cysts per inspected frog). Listed *P*-value of non-parametric Wilcoxon rank-sum test compares trematode abundance between the two species.

Type of trematode	*Rana boylii* (*n* = 14)	*Pseudacris regilla* (*n* = 26)	*P*
Ribeiroia ondatrae	7.1% (1)	0%	n.s.
Echinostoma spp.	100% (222.8)	84.6% (49.6)	0.004
Manodistomum spp.	57.1% (6.5)	92.3% (10.7)	0.040
Unidentifiable cyst	7.1% (1)	3.8% (1)	

active use of the Angelo Coast Range Reserve by ecologists. As noted during annual fish snorkeling surveys (begun in 1988 by M. E. Power, pers. comm.), however, copepods have been continuously present on Roach (*Lavinia symmetricus*), and in 2006 and 2008 were abundant on juvenile Steelhead *(Oncorhynchus mykiss)* as well as Roach (Palen, pers. obs.).

It is possible, that during the intervening years larval *L. cyprinacea* have been present in tadpole gills, but inconspicuous to field researchers. In years with 'normal' temperatures, *R. boylii* might rid themselves of the ectoparasitic copepodids in the branchial chamber if gill resorption preceded *L. cyprinacea* reaching sexual maturity and transforming to mesoparasitic embedding adults. This hypothesis is supported by observations of 15 tadpoles collected in 2006 and held in captivity as part of a swimming study (Kupferberg et al., 2008). Tadpoles maintained for one week at 18°C with several water changes did not reveal any parasites when examined at 5–10× magnification. After completing swimming trials, four of 15 SF Eel tadpoles transferred to a warmer facility (21–23°C) had adult parasites embedded near a hind limb within three days. Tadpoles from two other rivers did not develop infestations. After removing the infested individuals and chilling the water ≤19°C, no further incidences of copepods embedding occurred and all 11 tadpoles successfully metamorphosed. Of the four with copepods, two survived. One could not bend its left hind limb and died six weeks post-metamorphosis. The other lacked visible abnormalities but was small and died three months later. This trend of infested frogs being smaller was consistent in the wild at the SF Eel, and likely has negative consequences as size at metamorphosis is a correlate of over-winter survival and fitness in amphibians (Smith, 1987; Semlitsch et al., 1988; Altwegg and Reyer, 2003).

We have not established a cause and effect relationship between the growth of a copepod's anchor process and malformation of a limb, although insertion is most common near the hind limb (Tidd, 1962; Shields and Tidd, 1963), and copepods have previously been suspected to play a role in hind limb abnormalities (Ming, 2001). The predominance of inguinal attachment is consistent with a fluid dynamics model illustrating that the region is a dead zone in flow around a tadpole's body where hind limbs can grow without increasing drag forces (Liu et al., 1996, 1997). High-speed videography observations confirm that echinostomatoid cercaria attack tadpoles in that region (Taylor et al., 2004) even while tadpoles twist, turn, and swim. Despite the propensity to embed near hind limbs, almost three-fourths of infested *R. boylii* in 2006 did not exhibit abnormalities and the association did not recur in 2008. We do not suspect widespread involvement of the trematode parasite *Ribeiroia ondatrae* at the SF Eel, but cannot eliminate it as a possibility. One *R. boylii* was found with a cyst in 2008; however, that individual was morphologically normal. The absence in *P. regilla* also indicates that *Ribeiroia* is not common at the SF Eel. Infection by *Ribeiroia* can lead to severe limb malformations (Johnson et al., 1999, 2002; Johnson and Sutherland, 2003; but see discussion in Lannoo, 2008 for a critical review) with likely mechanisms including limb bud rotation when metacercariae encyst (Sessions, 1999; Stopper et al., 2002). Although *Ribeiroia* has been shown through laboratory and field studies to induce fore- and hind limb malformations (reviewed by Blaustein and Johnson, 2003), its absence at wetlands with high prevalence (>5%) of malformations is clear evidence that other causes are important factors (Meteyer et al., 2000; Lannoo et al., 2003; Skelly et al., 2007, Lannoo, 2008).

Other possible causes include the direct effects of heat stress on the development of hind limb abnormalities (Dournan et al., 1998) and asymmetries (Alford et al., 2007). Although the etiology of the abnormalities we observed is not known, we can rule out a wide range of anthropogenic factors that have been implicated in causing abnormalities, ranging from acidification, radioactive pollution, heavy metals, and agricultural chemicals (reviewed by Oullet, 2000; Lannoo et al., 2003; Lannoo, 2008). Surface and ground water pollutants are not likely in the remote and relatively pristine mixed-conifer forests within the Angelo Coast Range Reserve. Downwind drift of aerial pesticides is unlikely given the site's close proximity to the Pacific Ocean and prevailing wind direction from the northwest. Also, the <3% overall limb abnormality prevalence is still within an overall baseline of 0–3% and thus might not represent any deviation from normal genetic or developmental errors and injury.

A possible explanation for the lack of consistent association between copepods and abnormalities is that the parasite's effects may be stage specific. Experiments with tadpoles of *Rana pipiens* and *Ribeiroia ondatrae* illustrate that timing of infection can alter malformation outcomes (Schotthoefer et al., 2003). Tadpoles were exposed to cercariae at the pre-limb-bud (Gosner stages 24 and 25), limb-bud (Gosner stages 27 and 28), or paddle (Gosner stages 31–33) stages of development. At metamorphosis, only tadpoles infected at the limb-bud stage displayed a high (16%) malformation rate, whereas less developed tadpoles suffered significant mortality. When cercariae encysted after the paddle stage, neither limb development nor tadpole survival was influenced. At the SF Eel in 2008, tadpole breeding began more than two weeks earlier than in 2006, and warm temperatures in May accelerated development, such that many tadpoles' limbs had already completed formation at the peak of the copepod outbreak in August. In 2006, infestation was more prevalent in earlier develop-

mental stages relative to 2008. The better body condition (i.e., larger size) in 2008 may also have contributed to a different response to infestation. An explicit investigation of the relationship between the timing and location of an adult female copepod's penetration site, the condition of the tadpole host, and the potential teratogenetic effects on limb formation is needed.

Parasites are but one of many threats to *R. boylii*, which has disappeared from 54% of its historic sites (Davidson et al., 2002; Lind, 2005). Potential causes for decline are diverse, but absence from historic localities is more common in close proximity to large dams (Lind, 2005). Dam-associated risk factors to this species, whose entire life cycle is completed in fluvial habitats, include the scouring of egg masses by large magnitude dam releases (Lind et al., 1996) and predation by invasive species that flourish in waterways subject to flow diversion and regulation (Hayes and Jennings, 1988; Moyle and Light, 1996; Marchetti et al., 2004). Dams can also alter downstream thermal regime, but the extent of impacts on *R. boylii* has not been quantified, and whether dams would increase the risk of parasite outbreaks is not clear. Temperatures can be either raised or lowered during dam operation. When outfalls are at low elevation on a dam, water is drawn from the hypolimnion of the upstream reservoir, and the resulting temperatures downstream are often colder than they would be naturally and thus create a refuge from thermophilic parasites (Hoffnagle et al., 2006). Alternatively, the retention of water behind a dam can result in artificially low summer base flow discharges, causing unnaturally warm downstream temperatures. Stressors may also originate outside a population's watershed, such as the downwind drift of organophosphate pesticides (Davidson, 2004; Sparling and Fellers, 2007). At present, however, observations within regulated watersheds and manipulative field experiments implicate the direct effects of flow alteration, particularly aseasonal pulsed releases of water, as a proximal cause of *R. boylii* decline (Kupferberg et al., 2008). If copepod parasitism or the direct effect of thermal stress on *R. boylii* at the un-regulated SF Eel did result in low recruitment, we would not detect a population-level response until the females of the 2006 and 2008 cohorts reach reproductive maturity and their egg masses are counted in future censuses. Thus, the conservation implications of the parasites reported here remain an open question and an active area of research within our group.

ACKNOWLEDGMENTS

This research was supported by grants from the Public Interest Energy Research Program of the California Energy Commission and the Division of Water Rights of the State Water Resources Control Board through the Pulsed Flow Program of the Center of Aquatic Biology and Aquaculture of UC Davis (Kupferberg and Lind); the National Center for Earth-surface Dynamics, a National Science Foundation Science Technology Center (Palen); the Swiss National Science Foundation (post-doctoral fellowship for Catenazzi); the Environmental Protection Agency STAR Fellowship, and the National Science Foundation Graduate Research Fellowship Program (Lunde). L. Gray, L. Fraser, J. Smythe, and her environmental studies class from the College Preparatory School (Oakland, CA) helped with field work. N. Nieto helped with parasite identification. We thank the University of California Natural Reserve System for maintaining the Angelo Coast Range Reserve as a protected site for basic research, and M. Power, P. Steel, and C. Bode for logistical help, environmental data and analysis, and egg mass censusing. This research was approved by the UC Davis Animal Care and Use Committee (Protocol 06-12357), the UC Berkeley Animal Care and Use Committee (Protocol R132-0809), and the California Department of Fish and Game (Permits SC-008820 to Kupferberg, SC-001608 to Lind, and SC-008784 to Lunde).

LITERATURE CITED

Adams, A. M. 1984. Infestation of *Fundulus kansae* (Garman) (Pisces: Cyprinodontidae) by the copepodid *Lernaea cyprinacea* Linnaeus 1758, in the South Platte River, Nebraska. American Midland Naturalist 112:131–137.

Alcalde, L., and P. Batistoni. 2005. *Hyla pulchella cardobae* (Cardoba treefrog). Parasitism. Herpetological Review 36:302.

Alford, R. A., K. S. Bradfield, and S. J. Richards. 2007. Global warming and amphibian losses. Nature 447:E3–E4.

Al-Hamed, M., and L. Hermiz. 1973. Experiments on the control of anchor worms (*Lernaea cyprinacea*). Aquaculture 2:45–51.

Allen, D. M. 2008. Development and application of a process-based, basin-scale stream temperature model. Unpubl. Ph.D. diss., University of California, Berkeley.

Altwegg, R., and H.-U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

Baldauf, R. J. 1961. Another case of parasitic copepods on amphibians. Journal of Parasitology 47:195.

Berger, L., R. Speare, P. Daszak, D. E. Green, A. A. Cunningham, C. L. Goggin, R. Slocombe, M. A. Ragan, A. D. Hyatt, K. R. McDonald, H. B. Hines, K. R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95:9031–9036.

Bird, N. T. 1968. Effects of mating on subsequent development of a parasitic copepod. Journal of Parasitology 54:1194–1196.

Blaustein, A. R., and P. T. J. Johnson. 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment 1:87–94.

Bond, N. R. 2004. Observations on the effects of the introduced parasite *Lernaea cyprinacea* on a lowland population of a small native Australian fish, Mountain Galaxias (*Galaxia olidus*). Victorian Naturalist 121: 194–198.

Bowman, T. E., and L. G. Abele. 1982. Classification of the recent Crustacea, p. 1–27. *In*: The Biology of the Crustacea, Vol. 1. Systematics, the Fossil Record, and Biogeography. D. F. Bliss (ed.). Academic Press, New York.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 287:443–449.

Davidson, C. 2004. Declining downwind: amphibian population declines in California and historical pesticide use. Ecological Applications 14:1892–1902.

Davidson, C., H. B. Shaffer, and M. R. Jennings. 2002. Spatial test of pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. Conservation Biology 16:1588–1601.

DiRosa, I., F. Simoncelli, A. Fogotti, and R. Pascolini. 2007. The proximate cause of frog declines? Nature 447:E4–E5.

Dournan, C., A. Bautz, H. Membre, M. Lauthier, and A. Collenot. 1998. Expression of hindlimb abnormalities under rearing temperature effects during the larval development of the salamander *Pleurodeles waltl* (urodele amphibian). Development Growth and Differentiation 40:555–565.

Durham, B. W., T. H. Bonner, and G. R. Wilde. 2002. Occurrence of *Lernaea cyprinacea* on Arkansas River Shiners and Peppered Chubs in the Canadian River, New Mexico and Texas. Southwestern Naturalist 47: 95–98.

Freed, L. A., R. L. Cann, M. L. Goff, W. A. Kuntz, and G. R. Bodner. 2005. Increase in avian malaria at upper elevation in Hawai'i. Condor 107:753–764.

Haderlie, E. C. 1950. Parasites of the fresh-water fishes of northern California. Unpubl. Ph.D. diss., University of California, Berkeley.

Hallet, S. L., and J. L. Bartholomew. 2008. Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. Parasitology 135:371–384.

Hayes, M. P., and M. R. Jennings. 1988. Habitat correlates of the distribution of California red legged frog (*Rana aurora draytonii*) and Foothill Yellow-legged Frog (*Rana boylii*): implications for management, p. 144–158. *In*: Management of Amphibians, Reptiles, and Small Mammals in North America. R. C. Szaro, K. E. Severson, and D. R. Patton (tech. coords.). General Technical Report RM 166. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado.

Hoffman, G. L. 1967. Parasites of North American Freshwater Fishes. University of California Press, Berkeley.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes. Second edition. University of California Press, Berkeley.

Hoffnagle, T. L., A. Choudhury, and R. A. Cole. 2006. Parasitism and body condition in Humpback Chub from the Colorado and Little Colorado rivers, Grand Canyon, Arizona. Journal of Aquatic Animal Health 18:184–193.

Huys, R., and G. A. Boxhall. 1991. Copepod Evolution. The Ray Society, London.

Johnson, P. T. J., K. B. Lunde, E. G. Ritchie, and A. E. Launer. 1999. The effect of trematode infestation on amphibian limb development and survivorship. Science 284:802–804.

Johnson, P. T. J., K. B. Lunde, E. M. Thurman, E. G. Ritchie, S. N. Wray, D. R. Sutherland, J. M. Kapfer, T. J. Frest, J. Bowerman, and A. R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infestation linked to amphibian malformations in the western United States. Ecological Monographs 72:151–168.

Johnson, P. T. J., and D. R. Sutherland. 2003. Amphibian deformities and *Ribeiroia* infestation: an emerging helminthiasis. Trends in Parasitology 19:332–335.

Kiparsky, M., and P. Gleick. 2003. Climate change and California water resources: a survey and summary of the literature. California Energy Commission Report 500-04-073.

Kupferberg, S. J. 1996. Hydrologic and geomorphic factors affecting reproduction of the Foothill Yellow-legged Frog (*Rana boylii*). Ecological Applications 6:1332–1344.

Kupferberg, S. J., A. J. Lind, J. Mount, and S. Yarnell. 2008. Pulsed flow effects on the Foothill Yellow-legged Frog (*Rana boylii*): Integration of empirical, experimental and hydrodynamic modeling approaches. Final Report. CEC-500-2009-002. California Energy Commission, Public Interest Energy Research Group, Sacramento, California.

Lannoo, M. J. 2008. Malformed Frogs: The Collapse of Aquatic Ecosystems. University of California Press, Berkeley.

Lannoo, M. J., D. R. Sutherland, P. Jones, D. Rosenberry, R. W. Klaver, D. M. Hoppe, P. T. J. Johnson, K. Lunde, C. Facemire, and J. Kapfer. 2003. Multiple causes for the malformed frog phenomenon, p. 233–262. *In*: Multiple Stressor Effects in Relation to Declining Amphibian Populations. G. Linder, S. Krest, D. Sparling, and E. Little (eds.). ASTM International, West Conshohocken, Pennsylvania.

Lind, A. J. 2005. Reintroduction of a declining amphibian: determining an ecologically feasible approach for the foothill yellow-legged frog (*Rana boylii*) through analysis of decline factors, genetic structure, and habitat associations. Unpubl. Ph.D. diss., University of California, Davis.

Lind, A. J., H. H. Welsh, Jr., and R. A. Wilson. 1996. The effects of a dam on breeding habitat and egg survival of the foothill yellow-legged frog (*Rana boylii*) in northwestern California. Herpotological Review 27:62–67.

Lips, K. R., J. Diffendorfer, J. R. Mendelson, III, and M. W. Sears. 2008. Riding the wave: reconciling the roles of disease and climate change in amphibian declines. PLoS Biology 6:441–454.

Liu, H., R. J. Wassersug, and K. Kawachi. 1996. A computational fluid dynamics study of tadpole swimming. Journal of Experimental Biology 199:2139–2151.

Liu, H., R. J. Wassersug, and K. Kawachi. 1997. The three dimensional hydrodynamics of tadpole locomotion. Journal of Experimental Biology 200:2807–2819.

Marchetti, M. P., P. B. Moyle, and R. Levine. 2004. Alien fishes in California watersheds: characteristics of successful and failed invaders. Ecological Applications 14:587–596.

Marcogliese, D. J. 1991. Seasonal occurrence of *Lernaea cyprinacea* on fishes in Belews Lake, North Carolina. Journal of Parasitology 77:326–327.

Marcogliese, D. J. 2001. Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79:1331–1352.

Martins, M. L., and F. L. D. J. Souza. 1996. Experimental infestations of *Rana catesbeiana* Shaw tadpoles by copepodids of *Lernaea cyprinacea* Linnaeus (Copepoda, Lernaeidae). Revista Brasileira de Zoologia 12:619–625.

Medeiros, E. S. F., and L. Maltchik. 1999. The effects of hydrological disturbance on the intensity of infestation of *Lernaea cyprinacea* in an intermittent stream fish community. Journal of Arid Environments 43:351–356.

Meteyer, C. U., I. K. Loeffler, J. F. Fallon, K. A. Converse, E. Green, J. C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole, and J. G. Burkhart. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. Teratology 62:151–171.

Ming, L. T. 2001. Parasitic copepods responsible for limb abnormalities? Froglog 46:3.

Moyle, P. B., and T. Light. 1996. Fish invasions in California: Do abiotic factors determine success? Ecology 77:1666–1670.

Muths, E., P. S. Corn, A. P. Pessier, and D. E. Green. 2003. Evidence for disease-related amphibian decline in Colorado. Biological Conservation 110:357–365.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:1–135.

Ouellet, M. 2000. Amphibian deformities: Current state of knowledge, p. 617–661. *In*: Ecotoxicology of Amphibians and Reptiles. D. W. Sparling, G. Linder, and C. A. Bishop (eds.). Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, Florida.

Pessier, A. P. 2002. An overview of amphibian skin disease. Seminars in Avian and Exotic Pet Medicine 11:162–174.

Picco, A. M., and J. P. Collins. 2008. Amphibian commerce as a likely source of pathogen pollution. Conservation Biology 22:1582–1589.

Plowright, R. K., S. H. Sokolow, M. E. Gorman, P. Daszak, and J. E. Foley. 2008. Causal inference in disease ecology: investigating ecological drivers of disease emergence. Frontiers in Ecology and the Environment 6:420–429.

Poulin, R. 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132:143–151.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. L. Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sánchez-Azofeifa, C. J. Still, and B. E. Young. 2007. Pounds et al. reply. Nature 447:E5–E6.

Scherer, R. D., E. Muths, B. R. Noon, and P. S. Corn. 2005. An evaluation of weather and disease as causes of decline in two populations of boreal toads. Ecological Applications 15:2150–2160.

Schotthoefer, A. M., A. V. Koehler, C. U. Meteyer, and R. A. Cole. 2003. Influence of *Ribeiroia ondatrae* (Trematoda: Digenea) infection on limb development and survival of northern leopard frogs (*Rana pipiens*): effects of host stage and parasite-exposure level. Canadian Journal of Zoology 81:1144–1153.

Semlitsch, R. D., D. E. Scott, and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69:184–192.

Sessions, S. K. 1999. Morphological clues from multilegged frogs: Are retinoids to blame? Science 284:800–802.

Shields, R. J. 1978. Procedures for the laboratory rearing of *Lernaea cyprinacea* L. (Copepoda). Crustaceana 35: 259–264.

Shields, R. J., and R. P. Goode. 1978. Host rejection of *Lernaea cyprinacea* L. (Copepoda). Crustaceana 35:301–307.

Shields, R. J., and W. M. Tidd. 1963. The tadpole as a host for larval *Lernaea cyprinacea*. Journal of Parasitology 49:43.

Shields, R. J., and W. M. Tidd. 1974. Site selection on hosts by copepodids of *Lernaea cyprinacea* L. (Copepoda). Crustaceana 27:225–230.

Skelly, D. K., S. R. Bolden, L. K. Freidenburg, N. A. Freidenfelds, and R. Levey. 2007. *Ribeiroia* infestation is not responsible for Vermont amphibian deformities. EcoHealth 4:156–163.

Smith, D. C. 1987. Adult recruitment in chorus frogs: effect of size and date at metamorphosis. Ecology 68:344–350.

Smith, K. F., D. F. Sax, and K. D. Lafferty. 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology 20:1349–1357.

Snyder, M. A., L. C. Sloan, and J. L. Bell. 2004. Modeled regional climate change in the hydrologic regions of California: a CO_2 sensitivity study. Journal of the American Water Resources Association 40:591–601.

Sparling, D. W., and G. Fellers. 2007. Comparative toxicity of chloropyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. Environmental Pollution 147:535–539.

Stopper, G. F., L. Hecker, R. A. Franssen, and S. K. Sessions. 2002. How trematodes cause limb deformities in amphibians. Journal of Experimental Zoology 294:252–263.

Sutherland, D. 2005. Parasites of North American frogs, p. 109–123. *In*: Amphibian Declines: The Conservation Status of United States Species. M. J. Lannoo (ed.). University of California Press, Berkeley.

Taylor, C. N., K. L. Oseen, and R. J. Wassersug. 2004. On the behavioural response of *Rana* and *Bufo* tadpoles to echinostomatoid cercariae: implications to synergistic factors influencing trematode infections in anurans. Canadian Journal of Zoology 82:701–706.

Tidd, W. M. 1962. Experimental infestations of frog tadpoles by *Lernaea cyprinacea*. Journal of Parasitology 48:870.

Wake, D. B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences of the United States of America 105:11466–11473.

Webb, B. W., D. M. Hannah, R. D. Moore, L. E. Brown, and F. Nobilis. 2008. Recent advances in stream and river temperature research. Hydrological Processes 22:902–918.

White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture–recapture and removal methods for sampling closed populations. Los Alamos National Laboratory LA-8787-NERP.

Woodhams, D. C., R. A. Alford, C. J. Briggs, M. Johnson, and L. A. Rollins-Smith. 2008. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. Ecology 89:1627–1639.