PREDATORY LEECHES (HIRUDINIDA) MAY CONTRIBUTE TO AMPHIBIAN DECLINES IN THE LASSEN REGION, CALIFORNIA

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ABSTRACT—Researchers have documented precipitous declines in Cascades Frog (*Rana cascadae*) populations in the southern portion of the species' range, in the Lassen region of California. Reasons for the declines, however, have not been elucidated. In addition to common, widespread causes, an understanding of local community interactions may be necessary to fully understand proximal causes of the declines. Based on existing literature and observations made during extensive aquatic surveys throughout the range of *R. cascadae* in California, we propose that a proliferation of freshwater leeches (subclass Hirudinida) in the Lassen region may be adversely affecting *R. cascadae* populations. Leeches may affect *R. cascadae* survival or fecundity directly by preying on egg and hatchling life stages, and indirectly by contributing to the spread of pathogens and secondary parasites. In 2007, we conducted focused surveys at known or historic *R. cascadae* breeding sites to document co-occurrences of *R. cascadae* and leeches, determine if leeches were preying on or parasitizing eggs or hatchlings of *R. cascadae*, and identify the leech species to establish whether or not they were native to the region. We found *R. cascade* at 4 of 21 sites surveyed and freshwater leeches at 9 sites, including all sites with *R. cascadae*. In 2007 and 2008, the predatory leech *Haemopis marmorata* frequented *R. cascadae* egg masses, was observed probing or tearing at eggs on 24 occasions, and was 10 times more common in 1-m^2 plots centered on egg masses than in similar plots without egg masses. Six species of leech were identified from the Lassen region, only 3 of which have been documented from the region prior to this study. Given our documentation of a diverse freshwater leech fauna in direct association with a precipitously declining species, we believe a better understanding of the biology and ecology of this poorly studied taxon is needed, in addition to studies of the effects of freshwater leeches on survival and recruitment of co-occurring amphibians.

Key words: amphibian decline, California, Cascades Frog, *Haemopis marmorata*, Lassen, pathogen, predation, *Rana cascadae*

Unidentified processes threaten 48% of rapidly declining amphibian species worldwide, resulting in more unexplained declines than any other taxon (Stuart and others 2004). Our understanding of large-scale threats such as disease (Berger and others 1998; Pieter 2006; Rachowicz and Briggs 2007), contaminants (Hayes and others 2006; Relyea 2009), and introduced predators (Kats and Ferrer 2003; Vredenburg 2004; Pope 2008) has greatly improved in recent years. Advances in our understanding of the causes of amphibian declines in protected regions of the western United States, however, have not satisfactorily explained continuing declines of Cascades Frog (*Rana cascadae*) populations from the Lassen region of northern California, the southern-most portion of the species' range, where only a handful of small populations remain (Fellers and others 2008). The decline was brought to the attention of researchers and managers in 1993 (Fellers and Drost 1993), but the causes remain a mystery. Researchers are currently testing leading theories of decline including disease (amphibian chytridiomycosis), pesticide drift, and habitat modification. In addition to these widespread threats, an understanding of local community interactions may be necessary to fully understand proximal causes of the declines of *R. cascadae* in the Lassen region. Conservation efforts aimed at preventing extirpation of *R. cascadae* are unlikely to succeed unless the specific causes of the declines are identified.

While conducting extensive surveys for *R. cascadae* throughout California, we made several observations of then-unidentified freshwater leeches in the littoral zone of amphibian habitats in the Lassen region, but not in the Klamath Mountains where *R. cascadae* populations are doing relatively well (Welsh and others 2006). This led us to consider the ecological relationship between leeches and *R. cascadae*. We propose that a proliferation of freshwater leeches (subclass Hirudinida) in the Lassen region may be affecting *R. cascadae* population declines. Freshwater leeches, including parasitic and predatory species, may affect *R. cascadae* survival or fecundity directly by preying on egg (Chivers and others 2001) and hatchling life stages; and indirectly, by contributing to the spread of pathogens (Raffel and others 2006) and secondary parasites (Sawyer 1986). Little is known about the distribution of leeches in the western United States, and from the few reports of their distribution around the Lassen region, it is unclear which species naturally occur there. Although detailed records regarding this neglected taxon in California are unavailable, anecdotal evidence suggests that a proliferation of leeches in the Lassen region corresponds roughly in time with *R. cascadae* population declines.

Here, we outline evidence of a strong ecological link between the predatory leech, *Haemopis marmorata*, and *R. cascadae* in the Lassen region. Our objectives were to: 1) identify occurrences of freshwater leeches in *R. cascadae* habitat in the Lassen region; 2) observe direct interaction between leeches and *R. cascadae* during the breeding season, when frogs should be most vulnerable to predation and parasitism by aquatic leeches; and 3) determine what leech species are present and whether or not they are native to the region.

METHODS

In 2007, we sampled 9 sites known to support extant populations of *R. cascadae* in the Lassen region (defined here as lands within a 50 km radius of Lassen Peak), 4 sites with recent or historical occurrences of *R. cascadae*, and 8 sites with appropriate habitat that did not have a known historical occurrence. Land stewardship included Lassen National Forest, Lassen Volcanic National Park, and private timberlands. Site selection was based primarily on existing *R. cascadae* distribution datasets (Fellers and others 2008).

We conducted field surveys between 25 April and 18 May during the period when *R. cascadae* breeding occurred (with 1 exception, Duck Lake, surveyed on 27 June). On the 1st visit to each site we walked the shoreline and shallow parts of ponds, lakes, streams, and wet meadows, conducting a visual encounter survey (Crump and Scott 1994) to identify presence, species, and life stage of amphibians, and presence of leeches. Free-living leeches, as well as parasitic forms between feeding bouts, can be found attached to substrates such as rocks, logs, and vegetation (Klemm 1982) or swimming in the water column. In addition to carefully scanning shallow water for free-swimming leeches and amphibians, we also examined woody debris, rocks, vegetation, and other substrate that could provide cover for leeches or amphibians, and occasionally dragged a dip net through vegetation or along the substrate. Amphibians were captured briefly when needed to confirm species identification.

Leeches were collected and preserved by relaxation by gradually adding 70% ethanol to a small container of water containing the leech until its movement ceased; then fixed in 10% formalin to preserve coloration (Klemm 1985); and transferred to 70% ethanol for storage and preservation. We identified leeches in the laboratory with a dissecting microscope, using primarily the keys and descriptions by Klemm (1985) and other published materials (for example, Sawyer 1986; Kutschera 1988; Hovingh and Klemm 2000; Smith 2001; Siddall and Bowerman 2006). To avoid unintentionally spreading disease between populations of amphibians, all gear and equipment that came in contact with the aquatic habitat during surveys was decontaminated using diluted quaternary ammonia before being transported between watersheds.

Repeat Surveys

At the 2 sites (Carter Meadow and Old Cow Creek) where *R. cascadae* egg masses were detected during the initial visit, we made 3

TABLE 1. Summary of sites surveyed and survey results; including the presumed status of *Rana cascadae* prior to the survey, the type of habitat present, the drainage basin or hydrologic unit (CWP 2008) in which the site lies; and a summary of leech and amphibian survey results.

Location	RACA[1] status	Habitat type[2]	Hydrologic unit	Leeches[3] present	Amphibians present
Colby Creek	extant	S, WM	Butte Creek	HAMA	PSRE, RACA
Willow Creek	extant	S, WM	Butte Creek	-	PSRE
Carter Mdw.	extant	S, WM	Eastern Tehama	HAMA	AMMA, PSRE, RACA
Deer Creek Mdw.	extirpated	S, WM	Eastern Tehama	ERLA, HAMA	PSRE
Gurnsey Ck. Mdw.	extirpated	S, WM	Eastern Tehama	-	PSRE
Beaver ponds	historic range	S, P	Feather River	-	AMMA, PSRE
Isolated pond	historic range	P	Feather River	-	AMMA, PSRE
Butt Creek	extant	S, WM	Feather River	-	AMMA, PSRE
Warner Valley	extant	S, WM	Feather River	ERPU	PSRE, RACA
Juniper Lake	extant	L	Feather River	ERPU[4]	-
Calwaters 11352	extant	P	Feather River	-	AMMA
Juniper Lk. outlet	historic range	L	Feather River	-	AMMA, PSRE
Juniper Lake inlet	extirpated	S, P, L	Feather River	-	-
Indian Lake	historic range	L	Feather River	-	-
Calwaters 11222	historic range	P	Feather River	ERPU	-
Calwaters 11318	extant	P	Feather River	-	-
Duck Lake	historic range	L	Pit River	ERPU, PLPA	AMMA, PSRE
Old Cow Creek	extant	S, WM	Whitmore	HAMA	PSRE, RACA, TAGR
Huckleberry Lake	historic range	L	Whitmore	-	-
Huckleberry Mdw.	historic range	S, WM	Whitmore	-	-
Snow Pond	extirpated	P	Whitmore	ERPU, GLCO, HEST	AMMA, PSRE, TAGR

[1] AMMA = *Ambystoma macrodactylum*, PSRE = *Pseudacris regilla*, RACA = *Rana cascadae*, TAGR = *Taricha granulosa*.
[2] S = stream, WM = wet meadow, P = pond, L = lake.
[3] ERPU = *Erpobdella punctata*, ERLA = *Erpobdella lahontana*, GLCO = *Glossiphonia complanata*, HEST = *Helobdella stagnalis*, PLPA = *Placobdella papillifera*, HAMA = *Haemopis marmorata*.
[4] Erpobdellid leeches were found in Juniper Lake, and erpobdellid leech cocoons were found at one unnamed pond near Juniper Lake (Calwaters 11222). These may have been *Erpodella punctata* or *Erpobdella lahontana*, but no voucher specimens were collected from either site.

subsequent visits in 2007 to observe direct interactions between leeches and *R. cascadae* egg masses. We visited each egg mass and noted the health and development stage (Gosner 1960) of the eggs. We also noted if any leeches were seen within 0.5 m of the egg mass.

Timed Leech Counts

During one of the repeat surveys at Old Cow Creek, on 14 May 2007, we conducted 10 min focused counts of leeches at four 1-m^2 plots centered on *R. cascadae* egg masses and 4 adjacent plots without egg masses. The plots without egg masses were selected non-randomly to minimize differences in water depth, flow, vegetation, and other habitat characteristics among plots. Each leech seen within the plot during a 10-min observation period was counted and its behavior noted.

In 2008, we re-visited Old Cow Creek on 21 May and 5 June, and made similar observations as in 2007 at 5 additional plots with and without egg masses. To test whether leeches were more abundant in plots with egg masses than plots without we used a one-tailed *t*-test for 2 samples with unequal variance, and $\alpha = 0.05$.

RESULTS

We surveyed a variety of habitats, including ponds, lakes, wet meadows, and streams, and identified 6 species of freshwater leeches from 9 sites in the Lassen region (Table 1). Species identified included *Helobdella stagnalis*, *Erpobdella punctata*, *Erpobdella lahontana*, *Placobdella papillifera*, *Haemopis marmorata*, and *Glossiphonia complanata* (Table 2). We encountered *Haemopis marmorata* most often and found it in higher numbers and densities than the other species. However, we found *H. stagnalis* to be abundant at Snow Pond in Lassen Volcanic National Park where it was sympatric with *G. complanata* and *E. punctata*. *Erpobdella punctata* and *P. papillifera* were abundant in Duck Lake.

TABLE 2. Description of leeches found in the Lassen region including family, species, feeding habits, known distribution, and additional notes.

Family	Species	Feeding habits	Known distribution	Notes
Erpobdellidae	*Erpobdella punctata*	opportunistic predator and scavenger, (Sawyer 1970); found as phoronts on amphibians (Khan and Frick 1997)	common throughout most of the US and Canada, esp. the Great Lakes region (Klemm 1985)	associated with organically enriched sites (Klemm 1985) and polluted waters (Sawyer 1986)
Erpobdellidae	*Erpobdella lahontana*	presumed to be similar to *Erpobdella punctata*	previously known only from the Lahontan sub-basin of the Great Basin (Hovingh and Klemm 2000, Hovingh 2004)	first record outside of the Great Basin
Glossiphoniidae	*Glossiphonia complanata*	predatory, often on snails and other soft-bodied invertebrates	common and widely distributed in northern half of US and Canada (Klemm 1985)	perhaps more appropriately called *Glossiphonia elegans* in North America due to dissimilarities with European species (Siddall and others 2005)
Glossiphoniidae	*Helobdella stagnalis*	predatory (Sawyer 1986); also parasitic on amphibians (Platt and others 1993) and humans (JE Stead, pers. obs.)	cosmopolitan, prevalent in northern North America (Moser and others 2006)	perhaps more appropriately called *Helobdella modesta* in North America due to dissimilarities with the European species (Siddall and others 2005)
Glossiphoniidae	*Placobdella papillifera*	parasitic (Davies and and Wilkialis 1982)	widely distributed (Klemm 1985); has been found from Alaska to southern California and New Mexico	conflicting reports regarding preferred host but may prefer mammals; known to parasitize humans
Hirudinidae	*Haemopis marmorata*	opportunistic predator and scavenger (Moser and others 2006); eats tadpoles (Riggs and Ulner 1983)	widely distributed in North America and sometimes locally common (Klemm 1985)	commonly observed probing *R. cascadae* egg masses at Old Cow Creek

Of the 21 sites visited, we found amphibians at 14 sites (Table 1). Amphibian species included Long-toed Salamander (*Ambystoma macrodactylum*), Northern Pacific Treefrog (*Pseudacris regilla*), Rough-skinned Newt (*Taricha granulosa*), and *R. cascadae*. We found *R. cascadae* at 4 sites (Colby Creek, Carter Meadow, Warner Valley, and Old Cow Creek), and *R. cascadae* egg masses at 2 sites (Carter Meadow and Old Cow Creek).

We found leeches at all sites where we found *R. cascadae* (Fig. 1). Leech specimens from these sites were identified to either *H. marmorata* or *E. punctata*, both of which are known to be opportunistic predators (Table 2). *Haemopis marmorata* was observed at both sites where *R. cascadae* egg masses were found and in the same pools as the egg masses. At Carter Meadow we only observed 1 *H. marmorata* on the first of the 4 visits, and although it was present within 1 m

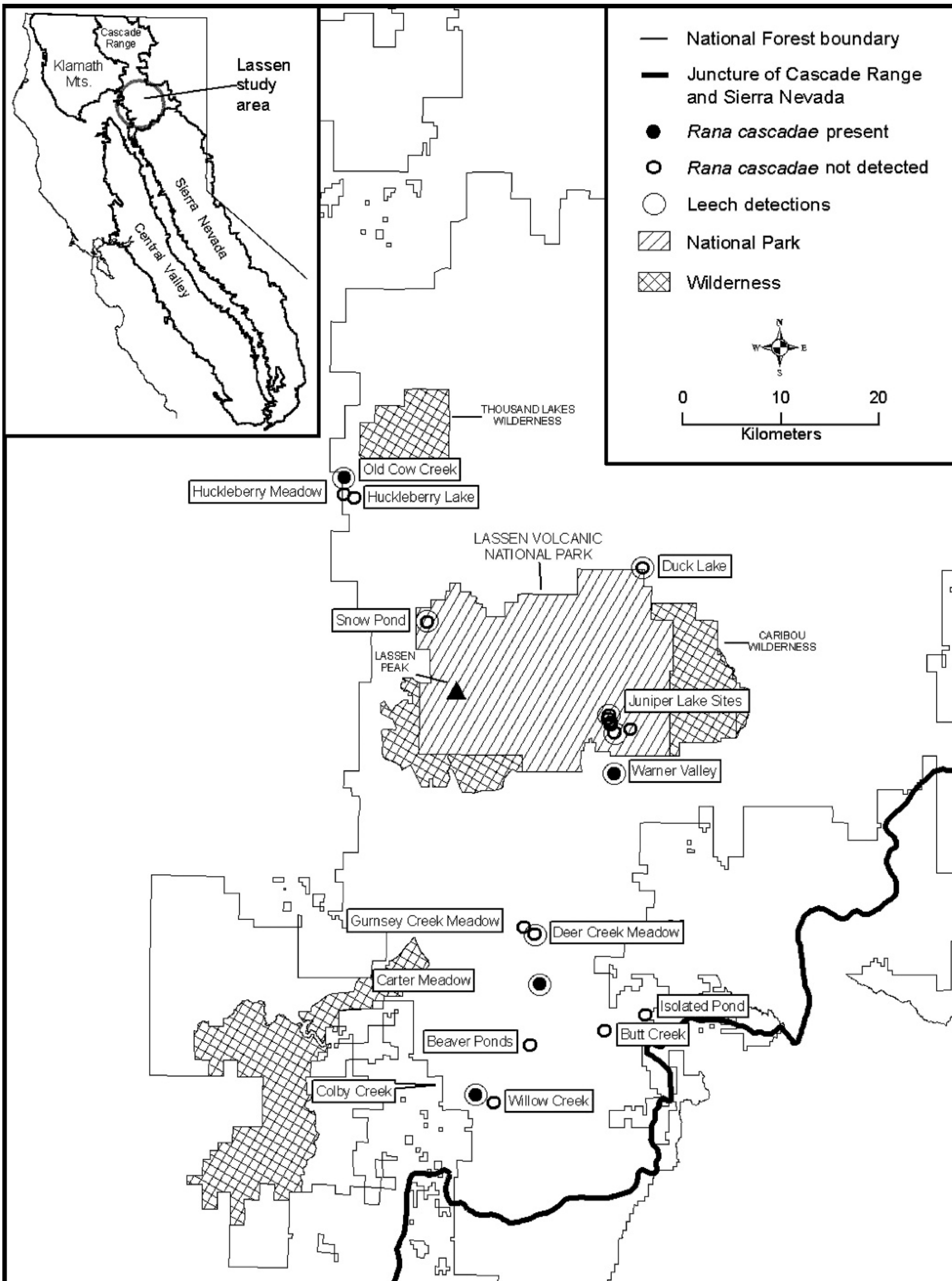

FIGURE 1. Map of sites surveyed, with sites where *Rana cascadae* was found depicted with filled circles, sites where *R. cascadae* was not found depicted with open circles, and leech presence indicated by a halo around the circle.

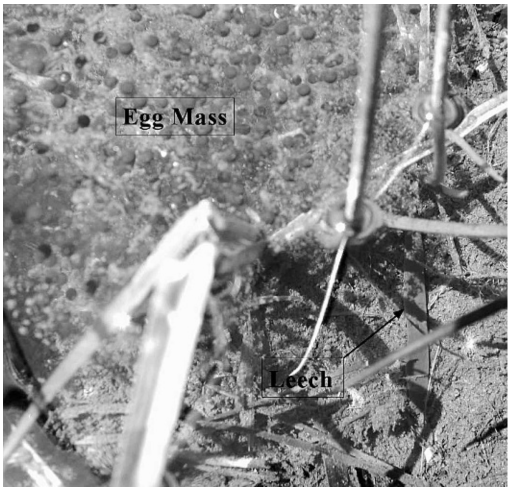

FIGURE 2. Photograph of a predatory leech (*Haemopis marmorata*) probing the underside of a *Rana cascadae* egg mass at Old Cow Creek (photographed and digitally enhanced [contrast increased to improve clarity] by JE Stead).

of a cluster of *R. cascadae* egg masses, no direct interactions were observed. At Old Cow Creek, however, we observed between 20 and 100 *H. marmorata* (at each visit), during 3 of 4 visits in 2007. *Haemopis marmorata* also was abundant (>20 individuals seen) at Deer Creek Meadows among *P. regilla* eggs and larvae.

We observed direct interactions between *H. marmorata* and *R. cascadae* egg masses in 2007 and 2008 at Old Cow Creek. On 24 recorded occasions, we observed *H. marmorata* approach an egg mass and begin probing the eggs (Fig. 2). On another occasion, approximately 80 *H. marmorata* were observed swimming against light flow in a meadow channel toward recently deposited *R. cascadae* egg masses. In 2008, we observed 3 *H. marmorata* aggressively swimming through an egg mass using their bodies and mouths to dislodge individual eggs (video available at http://www.youtube.com/watch?v=5HtpwvXJPzE). During the timed counts, *H. marmorata* were 10 times more abundant at plots centered on *R. cascadae* egg masses ($\bar{x} = 10.2$, $s_{\bar{x}} = 2.2$) than at plots that did not contain egg masses ($\bar{x} = 1$, $s_{\bar{x}} = 0.6$; $t_9 = 2.26$, $P = 0.001$). At the end of the 2007 observations most of the egg masses at Old Cow Creek had begun hatching, and we noted that there were not as many hatchlings in the vicinity of the egg masses as there were empty eggs.

DISCUSSION

Five of the 6 leech species that we identified from the Lassen region are primarily predatory (Table 2; Sawyer 1970; Riggs and Ulner 1983; Sawyer 1986; Moser and others 2006). The 6th species, *P. papillifera*, is parasitic (Davies and and Wilkialis 1982). In addition to being predatory, *H. stagnalis* is also parasitic on amphibians (Platt and others 1993). The most abundant leech we detected, *H. marmorata*, is primarily nocturnal (Sawyer 1986) and an opportunistic predator and scavenger.

Of the species we identified, 3 (*G. complanata*, *H. stagnalis*, and *H. marmorata*) are recorded in the published record of leeches that occur in California (Klemm 1985). We are not aware of previously published accounts of leeches specifically occurring in the Lassen region, but leeches are fairly ubiquitous in lentic waters of western North America and may be overlooked or go unrecorded. According to unpublished accounts (JD DeMartini, Humboldt State University, CA, pers. comm.; KB Lunde, UC Berkeley, CA, pers. comm.), 3 of the species that we encountered (*H. stagnalis*, *H. marmorata*, and *E. punctata*) have been found in the Lassen region previously, 3 (*P. papillifera*, *E. lahontana*, and *G. complanata*) have not, and 1 (*E. lahontana*) is the 1st record outside of the Great Basin (P Hovingh, University of Utah [retired], Salt Lake City, UT, pers. comm.).

Given the poor historic record of the distribution of leeches in California, we traced anecdotal sources for information on the occurrence and possible spread of leeches in the Lassen region. This evidence suggests that proliferation of some highly visible leech species in the Lassen region corresponds roughly in time with a decline in *R. cascadae* populations. For example, 1 angler fished Lassen Volcanic National Park every summer since 1948 (J Busher, US Forest Service [retired], CA, pers. comm.). Based on his recollection, it was approximately 1960 when he first noticed leeches in the park, in a small pond he had fished year after year. Within the same decade he noticed leeches in Indian Lake, and by the 1970s he commonly found them in Horseshoe Lake. Now large freshwater leeches can be found throughout much of the park (JE Stead, pers. obs.). Similar anecdotes have been repeated by other long-time park visitors during the course of casual conversations (JE Stead, pers. comm.). Although it could be coincidence, *R. cascadae* declines were first documented shortly after this apparent proliferation of leeches.

Leech introductions are not uncommon, have repeatedly resulted in the establishment of resident alien populations (Siddall and others 2005), and once established leeches can actively colonize new habitats (Pennak 1989). For example, the widespread *Helobdella europaea* was first described in Germany but is actually native to South America (Siddall and Budinoff 2005). Other documented introductions include the European Duck Leech (*Theromyzon tessulatum*), now found in Chile; the North American *Placobdella multilineata*, now found in Asia; *Hirudinaria manillensis*, endemic to the Philippines and Malaysia, introduced to the Caribbean; and the now globally widespread *Barbronia weberi*, native to south Asia. Leeches have been sold by bait shops to anglers in Redding, CA (approximately 15 km west of the Lassen region), and a commercial business in Minnesota annually shipped 15 tons of leeches, including the widely distributed *H. marmorata*, throughout the United States (Enger 1997). These and similar practices could have resulted in widespread introductions.

Our findings of overlapping occurrences of *R. cascadae* and predatory leeches (*H. marmorata* and *E. punctata*), and direct contact by *H. marmorata* on amphibian egg masses suggest that predatory leeches, particularly *H. marmorata*, may be preying on *R. cascadae* in the Lassen region. Although we did not directly observe eggs or larvae being eaten, leeches in the families Glossiphoniidae and Erpobdellidae are known to prey on *R. cascadae* egg masses in Oregon (Chivers and others 2001), and *H. marmorata* is known to eat tadpoles (Riggs and Ulner 1983). At Old Cow Creek, we observed *H. marmorata* actively probing eggs on many occasions, and in 1 case individual eggs were dislodged from egg masses. Predation may have occurred from underneath the egg masses, out of our view, at night, or later in egg development when the protective gelatinous layer of the egg begins to break down. The low number of hatchlings observed around spent egg masses at the end of the study at Old Cow Creek is consistent with our belief that predation occurred.

In addition to direct egg predation, predatory leeches may elicit behavioral responses in *R. cascadae*, such as early hatching or early metamorphosis that may further reduce fitness (Chivers and others 2001; Relyea 2001). Hatching early in response to the presence of egg predators may reduce predation while in the egg stage, but larvae that hatch early would have reduced mobility and sensory abilities and may be at higher risk from additional predators that prey on larval amphibians. Post-metamorphic amphibians also may alter behavior in the presence of predatory leeches. During 2004 and 2005, *Placobdella burresonae* leeches were unusually prevalent at a pond in Oregon (Siddall and Bowerman 2006). Adult *R. pretiosa* abandoned the pond in those years shortly after breeding and juveniles left immediately following metamorphosis (J Bowerman, Sunriver Nature Center, OR, pers. comm.). This behavior was noted as atypical because *R. pretiosa* are usually found foraging at that pond all summer. Similar avoidance of leech-infested sites in the Lassen region could cause *R. cascadae* to move into suboptimal habitat.

Disease is now believed to play a larger role than previously thought in catastrophic amphibian declines (Vredenburg and Wake 2007), and elucidating mechanisms requires an understanding of reciprocal interactions among hosts and parasites (Johnson 2006). Although understudied, both predatory and parasitic leeches can vector disease-causing organisms among amphibian populations, and invasion by disease-causing organisms can have a profound effect on native species (Mack and others 2000). Predatory leeches often serve as intermediate hosts for digenetic trematodes and nematodes (Sawyer 1986), which can cause amphibian malformations (Johnson and others 2004), particularly in synergy with other environmental stressors (Kiesecker 2002). Parasitic leeches play a major role in transmission of blood parasites such as *Trypanosoma diemyctili* to amphibians (Mock 1987, Barta and Desser 1989). Field evidence also indicates that *Placobdella picta*, a parasitic leech, vectors a fungal pathogen (*Ichthyophonus* sp.) to amphibians that may cause mass mortality within populations (Raffel and others 2006). If *H. marmorata* carries amphibian pathogens, observed contact with *R. cascadae* egg masses could result in rapid transmission of pathogens to egg or hatchling life stages. The relatively sudden decline of *R. cascadae* in the Lassen region is compatible with a disease hypothesis (Fellers and others 2008), and the potential for *H. marmorata* and other leeches in the Lassen region to vector pathogens among amphibians warrants investigation.

While we have observed common, direct interactions between *H. marmorata* and *R. cascadae* egg masses, the technique we used for observing leech-egg mass interactions was insufficient to document rates of predation. Predation rates may best be quantified in a laboratory or controlled field experiment, or by examination of leech guts from leeches caught around egg masses. Nevertheless, in just a short period of time we documented a diverse leech fauna in the Lassen region in close association with the native amphibians. The biology and ecological impacts of these leeches deserve significantly more attention than they have received previously. While this investigation lends only limited correlative support to our hypothesis that freshwater leeches are affecting *R. cascadae* populations in the Lassen region, we have established a baseline of knowledge from which this hypothesis, as well as the diversity, distribution, and ecology of leeches, can be further investigated.

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