The Threat of Trematodes to the California Newt (*Taricha torosa)*

Steven Ly

ABSTRACT

In North America, high incidences of malformed amphibians have led to studies into the causes and ecological implications of this phenomenon on already threatened amphibian populations. Current research suggests that the trematode *Ribeiroia ondatrae* is responsible for inducing malformations in many amphibian hosts. My research investigates whether *Ribeiroia* induces malformations in the California newt, *Taricha torosa,* and whether these malformations contribute to newt population declines. This study utilizes field data and preserved biological specimens of California newts collected from 2007 to 2009 at Hog Lake in Hopland, CA. Partitioning of the lake into two sides and experimental elevation of trematode density in one side of the pool allowed for investigation of these hypotheses. Linear regression of the average number of newt malformations to the average parasite load per year yielded an R^2 of 0.73, supporting a positive correlation between parasite levels and malformation prevalence. Though a significant difference was found in parasite load between the experiment and control sides of the lake ($p < 0.0001$), a significant difference was not found in malformation proportions ($p = 0.4495$). The linear regression slope of average newt density over time was higher for the control side ($b = -0.01970, \pm 0.01497$) than the experiment ($b = -0.22853, \pm 0.05099$) indicating that newt mortality was higher in the experiment side. The difference in mortality cannot be attributed to a difference in malformation proportions, but it could be explained by the difference in parasite load, suggesting that the increased mortality could be due to direct exposure to infection. Overall, it is impossible to say with certainty whether or not Ribeiroia trematodes pose a significant threat to *Taricha torosa* newts.

KEYWORDS

Ribeiroia ondatrae, trematode infection, amphibian malformations, predation, incomplete limb regeneration

INTRODUCTION

Since as early as the 1970s, the world has experienced a significant decline in its amphibian populations (Stuart et al. 2004). It has been found that worldwide, 32.5% of all amphibian species are threatened (vulnerable, endangered, or critically endangered) and 43.2% of all amphibian species are experiencing population declines (Stuart et al. 2004). Furthermore, the rate of amphibian declines has been increasing in recent years (Alford and Richards 1999). Proposed explanations for these declines include habitat loss, pesticide use, climate change (Alford and Richards 1999), fungal diseases such as chytridiomycosis (Rachowicz et al. 2006), and parasites (Johnson and Lunde 2005). Amphibians species are ecologically important as mid-level vertebrate predators; consuming a large variety of insect species (including pest species such as mosquitoes) and serving as prey to many species of reptiles, fish, birds, and mammals, allowing energy and nutrients to be transferred from small invertebrates to larger predators (Davic and Welsh 2004). Likewise, their amphibious lifestyle acts to transfer energy and matter from aquatic habitats to terrestrial ones (Davic and Welsh 2004). Amphibians are also important to humans as an indicator species, commonly used in metrics for determining ecosystem health and integrity (Davic and Welsh 2004; Marcot and Hayden 2001).

In North America, there has been a dramatic increase in reports of malformed amphibians: amphibians with missing or extra digits and/or limbs (Johnson et al. 1999). Studies into the cause of these malformations have demonstrated that infection by the trematode *Ribeiroia ondatrae* is responsible for these developmental abnormalities in many species across the United States (Johnson et al. 1999; Johnson et al. 2002; Johnson et al. 2006). A commonly suggested hypothesis for why trematodes induce malformations in their amphibian hosts is that it enhances the amphibian's susceptibility to predation by the definitive hosts of the trematodes (see Fig. 1), thus increasing the transmission rate of trematodes from one host to another (Johnson et al. 2004). Prior studies seem to support this hypothesis, but there still remains much to learn about this complex biotic interaction (Johnson and Lunde 2005). Although it has been shown that *Ribeiroia* is responsible for inducing malformations in many species of amphibians (Johnson et al. 2004), whether or not such malformations are responsible for increasing predation and thus host-transmission has yet to be firmly established. This hypothesis is not a novel one, as previous studies have shown that parasites are capable of manipulating the physiology and

behavior of their intermediate hosts in order to advance to their definitive hosts (Berdoy et al. 2000; Kunz and Pung 2004).

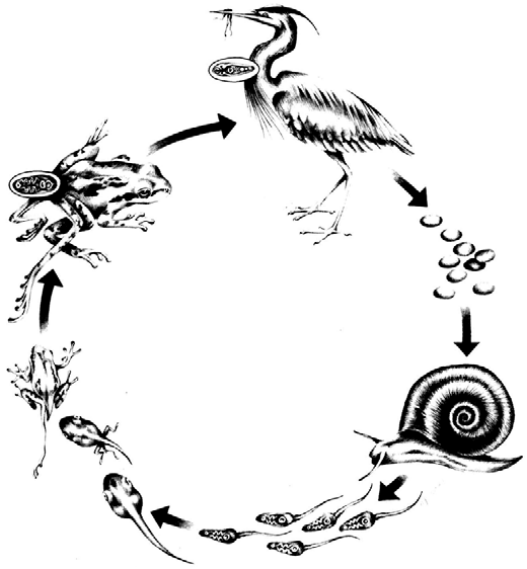

Figure 1. Life cycle of *Ribeiroia* trematodes. The first stage of life begins in planorbid snails, the first intermediate host, where the trematodes produce and release cercariae to infect their second intermediate hosts, larval amphibians and freshwater fishes. Consumption of the second intermediate hosts by the definitive hosts, birds and mammals, allows the cercariae to mature into metacercariae. Parasite eggs are produced in the definitive hosts and released through their feces to infect snails (Johnson et al. 2004).

One of the known host species of *Ribeiroia* trematodes is *Taricha torosa*, the California newt. *T. torosa* newts are one of the host species for which the biological and ecological effects of *Ribeiroia* trematode infection have not yet been studied. Currently, *T. torosa* newts are listed as a California Special Concern species (Jennings and Hayes 1994) because of the introduction of non-native predators, such as mosquitofish and crayfish (Gamradt and Katz 1996), and exposure to ultraviolet-B radiation (Anzalone et al. 1998). My research aims to determine if *Ribeiroia*-induced malformations also pose a risk to populations of *T. torosa* newts.

My hypotheses are that *Ribeiroia* trematodes induce malformations in *Taricha torosa* larvae and that such malformations lead to an increase in *Taricha* mortality levels. I used a combination of three years of monitoring data at a site known to support high infection levels in Pacific chorus frogs (*Pseudacris regilla*). In particular, I determined if *Ribeiroia* trematodes can induce malformations in *T. torosa* newts. Second, I determined if trematode exposures levels

corresponded to differential rates of survivorship. This research is pertinent because little is known about trematode-host interactions in newts or salamanders. If my findings support my hypotheses, they will also provide more support for the host-transmission hypothesis of trematode-induced malformations in amphibians.

METHODS

My research focuses on a laboratory analysis of preserved California newt samples collected from Hog Lake (39.0317N, 123.0789W) between 2007 and 2009. Field data and biological samples of amphibians were collected by Kevin B. Lunde, a UC Berkeley graduate student from the Department of Environmental Science, Policy, and Management. Kevin's work examines the effects of *Ribeiroia* trematodes on populations of the Pacific chorus frog (*Pseudacris regilla)* at Hog Lake, located at the Hopland Research and Extension Center in Hopland, CA. Prior to 2008, Hog Lake was split into two sides, a control side (Eastern half) and an experimental side (Western half), using a large partition constructed of plastic tarp. Data and sample collecting began at Hog Lake in 2007 using random net sweeps to collect biological samples (preserved in 95% ethanol solution) and randomized 1 m^2 transects to collect population estimates on both sides of the lake. Because amphibian eggs generally hatch late in the spring, samples were collected throughout the summer to capture amphibians as they developed into adulthood. In the beginning of 2009, an experiment was conducted by releasing trematode-infected snails into the western half of Hog Lake ("experimental side"). The eastern half was not altered and thus serves as the "control". Data and specimen collection on both sides of the lake continued throughout the summer of 2009. Data collected from the control and experimental sides before and after the introduction of trematodes will allow me to determine whether or not trematodes are inducing deformities in California newts, and whether these deformities, or parasites in general, have a negative effect on newt survival rates.

Three sets of preserved samples (each 'set' of data is composed of 5 sweeps from the experimental and 5 from the control side of the lake on a given day) were collected in 2007, two sets in 2008, and three sets in 2009, along with population estimates using transects. I collected data from the preserved samples on the number of newts per sample, the length of each individual in the sample (head to cloaca, in millimeters), and the number and extent of malformations present in each individual (e.g., number of missing or extra digits or limbs).

Trematodes and malformation prevalence

To test my hypothesis that *Ribeiroia* trematodes can induce malformations in newts, I analyzed the regression of the average number of newt malformations per year to the average number of trematodes found per Pacific chorus frog per year. I am using the average number of trematodes per Pacific chorus frog as a proxy for infection in *T. torosa* because dissection data on newts for previous years was not available. I am assuming that the average number of trematodes per Pacific chorus frog will be indicative of overall parasite load in the lake for that given year side of the lake each year. This analysis will determine if a correlation exists between the proportion of newt malformations and the general parasite load of the lake.

If a correlation between parasite load and malformation prevalence can be established, causation can then be determined if 1) the half-lake experiment results in significantly higher parasite levels in the experiment side over that of the control and 2) the malformation rate of the experiment side is significantly elevated over that of the control as well. A significant difference in parasite load was evaluated using a t-test comparing the average number of *Ribeiroia* trematodes per Pacific chorus frog between the two sides of the lake in 2009, the year the experiment was conducted. A significant difference in malformation rates was evaluated using a chi-square test between the two sides of the lake.

Trematodes and newt mortality

To test my second hypothesis, that trematode-induced abnormalities increase newt mortality, I compared the linear regression slopes of average newt density over time between the two sides of the lake. Because I am expecting newt densities in 2009 to decrease for the experimental side but remain relatively unaffected for the control side, I expect that the slope of the linear regression line for the experimental side will be lower than that of the control side. I will use 95% confidence intervals for the slope estimate of the control and experimental sides to determine if the difference is statistically significant.

RESULTS

Trematodes and malformation prevalence

Testing for a correlation between the proportion of malformed *T. torosa* newts to the average number of *Ribeiroia* parasites per Pacific chorus frog showed a significant relationship between the two variables, $R^2 = 0.73$, $F(1,4) = 11.22$, $p = 0.029$ (Fig. 2). Overall, higher numbers of *Ribeiroia* in Pacific chorus frogs correlates with higher proportions of malformation in *Taricha torosa*.

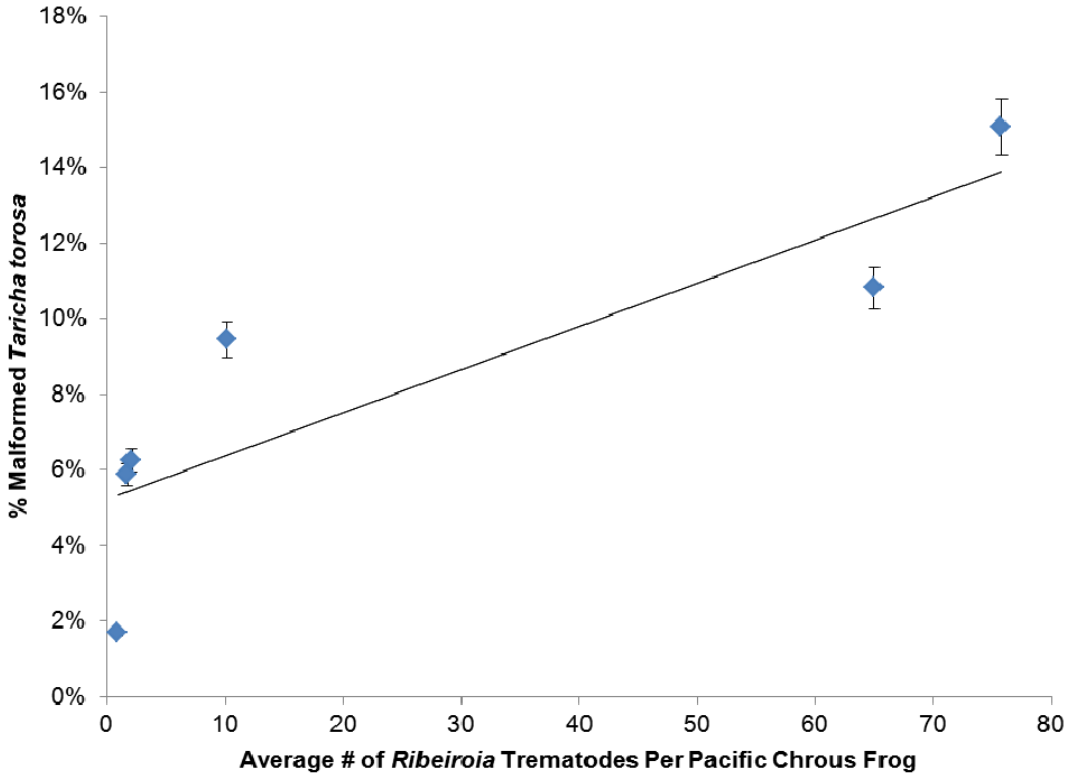

Figure 2. Linear regression between percent Malformed *Taricha torosa* and average number of *Ribeiroia* per Pacific chorus frog. 95% confidence intervals are shown for the average number of *Ribeiroia* per Pacific chorus frog.

The parasite load in the experiment side ($M = 1.67$, $SD = 8.77$) was found to be significantly higher than that of the control ($M = 9.51$, $SD = 8.54$), $t(90) = 4.3668$, $p < 0.0001$ (Fig. 3). However, the malformation rate in the experiment side (9.43%) was not found to be significantly higher than that of the control (5.88%), χ^2 (1, $N = 157$) = 0.5719, $p = 0.4495$ (Fig. 4).

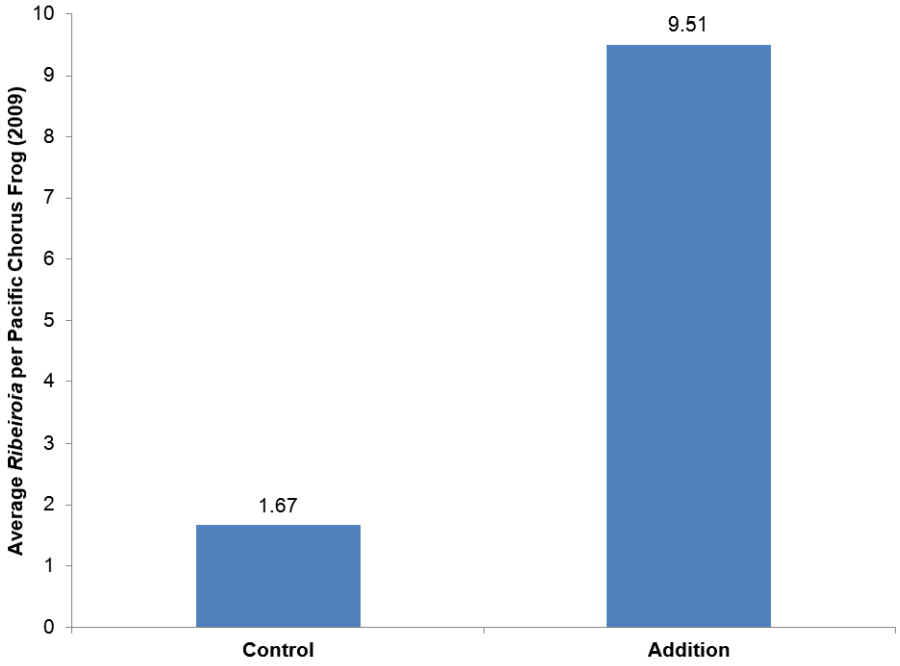

Figure 3. Average number of *Ribeiroia* per Pacific chorus frog in control and experiment sides of Hog Lake in 2009 ($t(90) = 4.3668$, $p < 0.0001$).

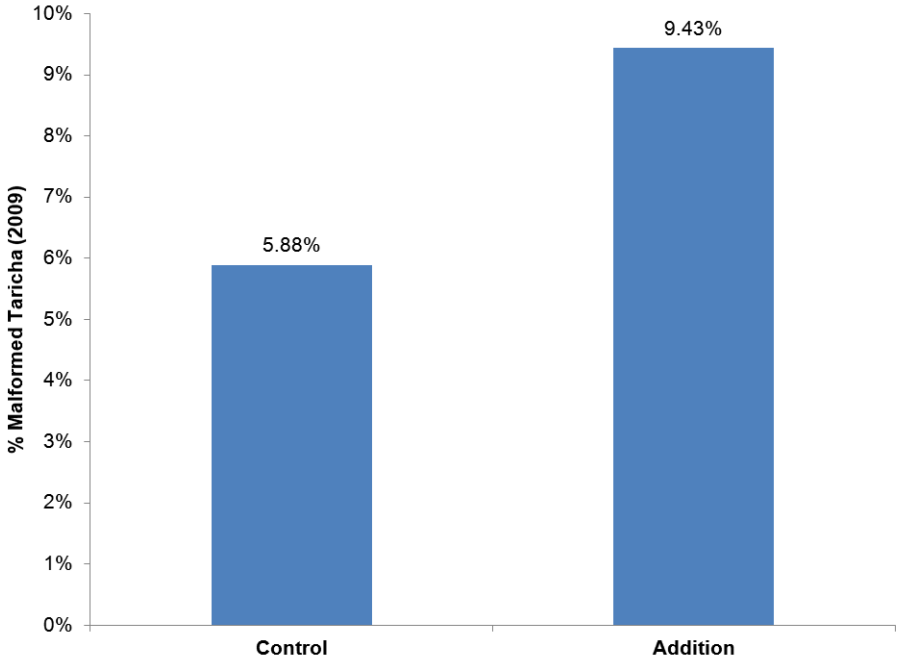

Figure 4. Percent malformed *T. torosa* newts in control and experiment sides of Hog Lake in 2009 (χ^2 (1, N = 157) = 0.5719, p = 0.4495).

Trematodes and newt mortality. The slope of the linear regression line for average *Taricha* density over time in 2009 (Fig. 5 & 6) was more negative for the experiment side (b = -0.22853, 95% CI: -0.27952, -0.17754) than the control side (b = -0.01979, 95% CI: -0.03476, -0.00482). The difference was statistically significant because the 95% confidence intervals do not overlap the other estimate (Fig. 7).

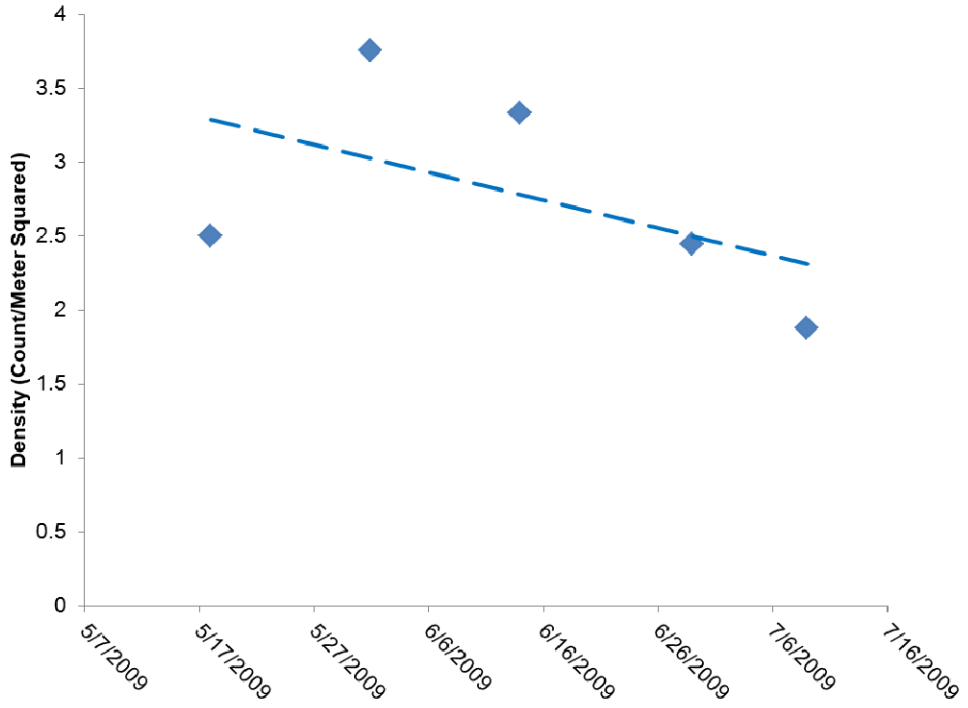

Figure 5. Linear regression of *T. torosa* newt density over time, control side (2009).

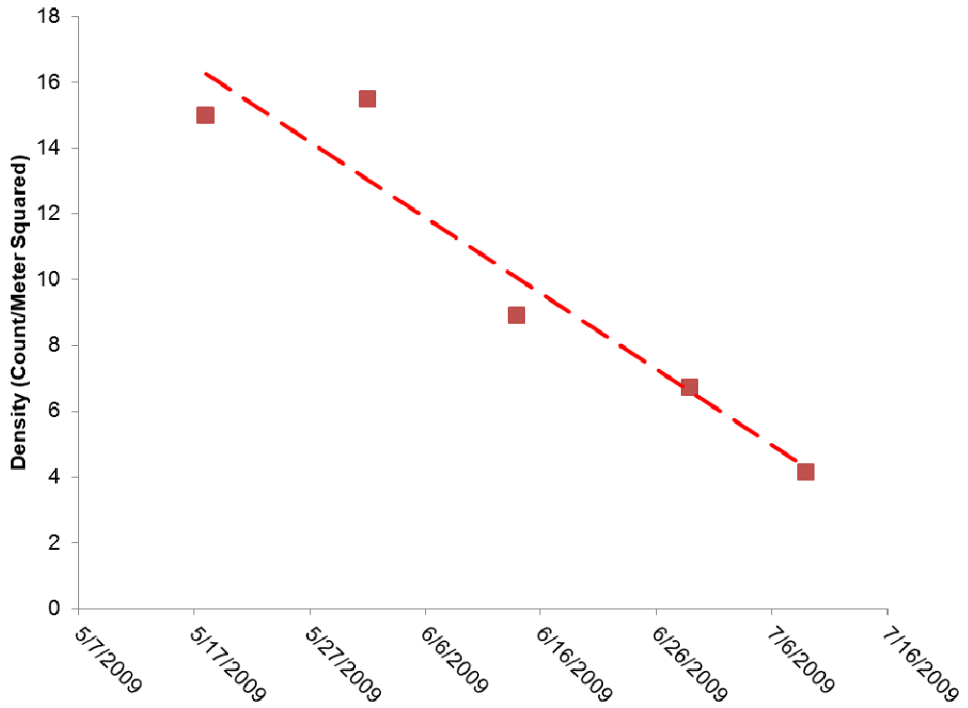

Figure 6. Linear regression of *T. torosa* newt density over time, addition side (2009).

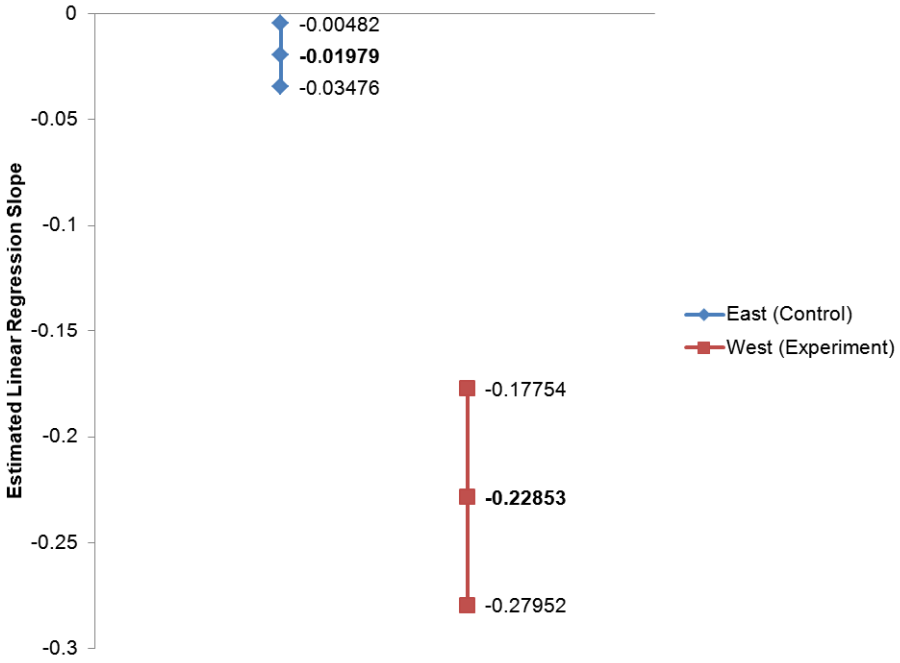

Figure 7. Comparison of the 95% confidence intervals of the linear regression slopes comparing *T. torosa density* between the control and experiment sides (2009).

DISCUSSION

My research examined whether or not *Ribeiroia* trematodes can induce malformations in *T. torosa* newts and whether or not these malformations or parasite exposure are responsible for increasing mortality levels in *T. torosa* newt populations. Although a positive correlation was found between parasite levels and malformation prevalence, causation between the two variables could not be established. An increase in mortality level could not be attributed to increased malformation prevalence, but it may be a result of direct infection.

Trematodes and malformation prevalence

A significant positive correlation exists between the parasite load of the pool and the prevalence of newt malformations. These results, however, should be taken with caution because the test assumes independence of all points and this analysis involves the same sites that are temporally correlated. Although the experiment was able to significantly raise the parasite levels in the experiment side over that of the control side, this difference failed to produce a statistically significant increase in malformation levels. Taken together, these two analyses suggest that higher *Ribeiroia* levels may lead to a slight increase in malformation prevalence. However, the lack of statistical significance of the experiment fails to support my hypothesis that *Ribeiroia* trematode infection leads to malformations in *T. torosa* newts. In future work, larger sample sizes are needed to establish a difference for these small effect sizes.

Trematodes and newt mortality

The slope of the linear regression line of newt density over time is lower in the experimental side, indicating that the control population had higher survivorship rates. This result confirms my initial hypothesis that the mortality rate of the experimental side would be higher as a result of higher malformation rates from the increased parasite load. This difference, however, was not due to malformations, but may have occurred as a result of direction infection by parasites, which was found to be significantly higher in the experiment side.

My examination of the newts in this study determined that a vast majority of observed malformations involved missing digits and/or limbs. Another study on malformations in populations of salamanders found similar results, with malformations typically consisting of missing digits or limbs, even in the absence of *Ribeiroia* (Johnson et al. 2006). However, the study also found that in the presence of *Ribeiroia*, the majority of malformations were of a developmental nature, such as extra digits and limbs. It also found that salamanders exposed to

both limb injuries and trematode infection were 3-5 times more likely to have an abnormality than either factor alone, suggesting that *Ribeiroia* trematodes possibly induce a synergistic effect in regards to malformations. However, a study conducted in the eastern United States found low levels of amphibian malformation were not caused by *Ribeiroia* and thus other causes of abnormalities remain elusive (Blaustein and Johnson 2003; Skelly et al. 2007).

A prior study reported that the deformities most commonly found in wild amphibians were missing digits and limbs, possibly resulting from selective predation (Ballangee and Sessions 2009). Though amphibians are capable of regenerating lost limbs, regeneration does not occur in all cases and sometimes results in incomplete regeneration (Ballangee and Sessions 2009). Thus, the absence of limbs or digits in *T. torosa* newts could be attributed to predation by aquatic predators (Bowerman et al. 2010), such as dragonfly nymphs (Ballangee and Sessions 2009) or even as a result of conspecific predation by other newts (Johnson et al. 2006). A prior study monitoring abnormality patterns in California amphibians found that the highest rate of abnormalities occurred in species of *Taricha torosa*, with abnormality rates ranging from 15 to 50% (Johnson et al. 2001), possibly indicating that *T. torosa* newts are highly susceptible to digit and limb loss.

Examining the host-transmission hypothesis in regards to newts suggests that the hypothesis would not seem to hold in the specific case of newts. The skin of adult *T. torosa* newts contains tetrodotoxin, an incredibly potent toxin also found in Japanese puffer fish, and the only predator capable of safely consuming *T. torosa* newts are garter snakes (Brodie 1968). Very few records of avian predation on newts have been reported, but limited observations have led to the death of the avian predator soon after (Mobley and Stidham 2000). Because the definitive hosts of *Ribeiroia* trematodes are birds and mammals (Johnson et al. 2004), which are highly susceptible to even minute amounts of tetrodotoxin (Brodie 1968), it would seem counterproductive for *Ribeiroia* trematodes to infect *T. torosa* larvae, as this would prevent the trematode from completing its life cycle in a suitable definitive host.

Although my findings indicate that *Ribeiroia* trematodes are likely to have biological and ecological effects on newt larvae, certain limitations in the study design prevent us from making that a firm conclusion. For one, the estimates of parasite levels in the lake were based on dissection data on Pacific chorus frogs. It is a definite possibility that *Ribeiroia* trematodes affect *T. torosa* larvae and Pacific chorus frogs at very different rates because *Ribeiroia* might

prefer chorus frogs over newts. Furthermore, the positive correlation between parasite load and malformation prevalence was based only on three years' worth of data, so collecting data for a longer time frame would more strongly support an observed correlation. My results also showed that malformation rates increased in the experiment side relative to the control, but that the difference was not statistically significant. However, with larger sample sizes, the statistical power of the test would be strengthened to detect a difference of only 5% in malformation prevalence.

While my findings show a relationship between *Ribeiroia* trematodes and *T. torosa* newts, the absence of definitive trematode hosts that prey on *T. torosa* newts as a result of their toxicity suggests that the host-transmission hypothesis may not be applicable to the specific case of *T. torosa* newts. Because it is so evolutionarily maladaptive for *Ribeiroia* trematodes to infect *T. torosa* newts, we might expect it to be rare in this genus. However, a field survey of *T. granulosa* found infection intensity of 12.7, much higher than the < 1 in *T. torosa* (Johnson et al. 2002). Further research into this connection must take care to distinguish malformations that can be due to selective predation and improper limb regeneration, such as missing digits and limbs, from those due to developmental abnormalities, such as excess digits and limbs. For the time being, it is impossible to say with certainty whether or not *Ribeiroia* trematodes pose a considerable threat to populations of *Taricha torosa.*

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