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EFFECTS OF DISEASE AND POND DRYING ON GRAY TREE FROG GROWTH, DEVELOPMENT, AND SURVIVAL

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Abstract. Pathogens have important effects on host growth, behavior, and population dynamics. Nevertheless, the impact of parasitic infection on host populations may be strongly context dependent. For example, the outcome of host–pathogen interactions may be subject to change based on the level of abiotic stress experienced by the host. In northeastern Connecticut, USA, larvae of the gray treefrog (*Hyla versicolor*) co-occur in temporary and permanent ponds with a snail (*Pseudosuccinea columella*) that is frequently infected with a digenetic trematode (*Telorchis* sp.) whose free-swimming cercariae subsequently infect *H. versicolor* tadpoles. Field collections of *H. versicolor* tadpoles suggest that the prevalence of infection by trematodes can be as high as 71%. We measured survival, mass, and time to metamorphosis of larval gray treefrogs in an artificial pond experiment designed to determine how infection with trematode cercariae and the stress of pond drying interact to influence gray treefrog performance. We exposed gray treefrog tadpoles to infected snails, uninfected snails, or no snails, within artificial ponds that were either permanent or subjected to a 49-d drying regime. The presence of infected *P. columella* had strong negative effects on the performance of gray treefrog larvae. However, this effect depended on whether ponds were temporary or permanent. When tadpoles were exposed to infected snails in temporary ponds, survivorship to metamorphosis decreased by ~30%, and mass at metamorphosis was reduced by 40%. The presence of infected *P. columella* had no detectable effect on larval performance of gray treefrogs in permanent ponds. Assays of infection level (mean number of cysts per individual) indicate that temporary pond animals were exposed to higher rates of infection. In contrast, uninfected snails had very little effect on *H. versicolor* in either temporary or permanent pools. There was, however, a small increase in time to metamorphosis for *H. versicolor* in permanent pools that were exposed to increased densities of uninfected snails. Our results indicate that an endemic parasite can have large effects on the performance of its native host, but that these effects may be strongly context dependent. Given this strong context dependence, an important challenge is to assay these effects within the natural environment.

Key words: amphibian; disease; hydroperiod; Hyla versicolor; *pathogen-mediated population dynamics; parasites; population regulation;* Pseudosuccinea columella; *snails; tadpoles; trematode.*

INTRODUCTION

Pathogens and parasites have a variety of deleterious effects on individual hosts, including decreased growth, reduced development, and behavioral modifications that may ultimately result in increased mortality (e.g., Dobson and Hudson 1986, Minchella and Scott 1991, Dobson and Crawley 1994, Poulin 1994). Parasites may also affect the size of host populations by reducing rates of host reproduction and by increasing rates of host mortality (Anderson 1978, Anderson and May 1978, Hudson et al. 1998, Tompkins and Begon 1999). Nevertheless, it has been hypothesized that the impact of parasitic infection on host populations may be strongly context dependent (Gulland 1995). The impact of infection on the host will depend upon the virulence of the parasite, the rate of infection, and the resistance of the host to infection. These parameters can be modified by a number of factors, such as overcrowding and nutritional stress, that also can interact with each other. In fact, previous studies suggest that the degree of differential mortality suffered by infected hosts depends not only on the particular host–parasite association under study, but also on the kind and level of abiotic stress experienced by the host (for example see Esch et al. 1975, Camp et al. 1982, Sousa and Gleason 1989). Thus, predicting the impact of a particular pathogen on host population dynamics will depend on the context of the host–pathogen interaction.

The purpose of this study is to experimentally evaluate the impact of an endemic macroparasite on its native host, and to distinguish conditions that may influence the outcome of the interaction. We used a natural system consisting of a digenetic trematode (*Telorchis* sp.) that infects a freshwater snail (*Pseudo-*

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succinea columella), and subsequently releases free swimming cercariae that then infect larvae of the gray treefrog (*Hyla versicolor*). In northeastern Connecticut, these species commonly co-occur in temporary and permanent ponds.

Amphibians are well suited for studies of host impacts for at least two reasons. First, the diversity of documented parasites found in amphibian hosts is extremely high (e.g., Bardsley and Harmsen 1973, Shields 1987, Aho 1990, Goater et al. 1993, Kiesecker and Blaustein 1997). Within populations of amphibians, prevalence of infection by parasite species can commonly exceed 50% (Rankin 1939, Bardsley and Harmsen 1973, Smyth and Smyth 1980, Aho 1990, Hunter et al. 1990, McAllister and Conn 1990, Schorr et al. 1990, Gruia-Gray and Desser 1992). Multiple infections are not uncommon and burdens from individual parasites are often extremely high (Dronen 1977, Crump and Pounds 1985, Shields 1987, Sessions and Ruth 1990). Second, the role of disease is of particular relevance to the understanding of amphibian population dynamics. Disease has been implicated as a possible factor in the declines and disappearances of several species around the world (Blaustein et al. 1994, Kiesecker and Blaustein 1995, Laurance et al. 1996, Kiesecker and Blaustein 1997, Berger et al. 1998). Unfortunately, with very specific exceptions (e.g., Goater and Ward 1992, Kiesecker and Blaustein 1999), ecologists know relatively little about the impacts of disease on amphibians.

We evaluated the impact of trematode infection by manipulating the presence and condition (infected or uninfected) of snails in artificial ponds constructed from 1000-L cattle watering tanks. Similar artificial ponds have been used to explore the impacts of a variety of factors, including competition and predation on the performance of larval amphibians (e.g., Wilbur 1987). For this study, the use of artificial ponds allowed us to measure tadpole performance while manipulating exposure to snails and the pathogen. Additionally, the artificial pond arena allowed us to manipulate hydroperiod. Our three study species are distributed among permanent ponds and those that may dry each summer. Other studies have found that shorter hydroperiods can exacerbate the impact of other interactions (e.g., interspecific competition, Wilbur 1987). We wanted to determine whether hydroperiod might act as a stressor that could enhance the impact of disease.

Study system

There are ~9000 species of trematodes, most of which are vertebrate parasites as adult worms (Schmidt and Roberts 1989). Trematodes in the Subclass Digenea usually undergo complex life cycles involving multiple intermediate hosts. Digenetic trematodes are quite common in freshwater habitats where numerous species infect anuran larvae as intermediate hosts (Schell 1985). Overlap in the life cycles of anurans and trematodes typically occurs during the tadpole stage that is infected with free-swimming cercariae.

Many digenetic trematodes infect three successive host species, although there is considerable variation in the generalized life cycle presented here. Eggs of the trematode exit through the feces of the definitive host and are either consumed by the first intermediate host (typically a snail), or hatch into free-living miracidial larvae that penetrate and infect snails. During the time the snail is infected, the trematode typically undergoes an embryonic amplification that results in the production of thousands of cercariae. Cercariae emerge from the snail, seek, penetrate and form a metacercarial cyst in the second intermediate host (e.g., tadpoles). The parasite's life cycle is completed when the infected secondary intermediate host is ingested by the definitive host (e.g., water snakes, *Natrix* spp.), where sexual reproduction is possible for the trematode.

The lymnaeid snail *P. columella* is widely distributed throughout North America (Jokinen 1983) and is the most common pond-breeding snail observed at the Yale-Myers Forest, Tolland and Windham Counties, Connecticut, U.S.A., where this work was carried out. This species breeds in both temporary and permanent ponds during late spring and early summer (Jokinen 1983), and undergoes an annual life cycle with the young snails aestivating on vegetation in the late summer and fall. At Yale-Myers, all infected *P. columella* have been observed to shed armatiae cercariae (*Telorchis* sp.: Schell 1985). Under laboratory conditions these cercariae will penetrate into gray treefrog tadpoles (J. M. Kiesecker, *unpublished data*).

Hyla versicolor is the northern tetraploid member of the *H. chrysoscelis* complex, which is widely distributed across eastern North America. In Connecticut this species breeds in late spring and summer in temporary and permanent ponds (Klemens 1993). In ponds at Yale-Myers, the distribution of gray treefrogs broadly overlaps with snails, including *P. columella,* that host trematode parasites. Field surveys of tadpole infections indicate that infection with *Telorchis* sp. metacercariae is quite common, with >50% of animals containing at least one metacercariae (J. M. Kiesecker, *unpublished data*). Given the high density of snails (0.02–0.15 snails/L) and high prevalence (>75%) of infected snails at several temporary and permanent sites, we hypothesized that infection with cercariae could be an important factor that could influence the growth and survival of gray treefrogs.

Methods and Materials

We evaluated the interactive impacts of pond drying, presence of the snail *Pseudosuccinea columella,* and trematode infection status of snails on growth, development, and survival to metamorphosis of gray treefrogs. Our experiment was conducted in 16 artificial ponds (190-cm diameter × 61 cm deep cattle watering tanks) located at the Yale-Myers Forest. Following an

established protocol (e.g., Morin 1983, Wilbur 1989, Werner and Anholt 1996), on 1 May 1998 we added ~1000 L of well water (depth of 54 cm), 25 g of rabbit chow, 300 g of oak leaves, and 1 L of phytoplankton that was filtered through a 153-μm plankton net to each pond. Ponds were allowed to sit undisturbed for 48 h, at which time we added 1 L of zooplankton, collected from five natural ponds. Ponds were covered with screen lids (48% shade cloth) in order to prevent colonization by frogs, invertebrates, and other organisms. The screen lids also retained metamorphosed amphibians until they could be collected and processed in order to examine their mass at, time to, and survival to metamorphosis.

Experimental design

We manipulated the presence of snails (infected or uninfected) and the drying times of ponds (temporary, dry in 49 d; or permanent) using a fully factorial design. The six treatment combinations were: temporary control with no snails ($n = 2$); permanent control with no snails ($n = 2$); temporary, infected snails present ($n = 3$); temporary, uninfected snails present ($n = 3$); permanent, infected snails present ($n = 3$); permanent, uninfected snails present ($n = 3$). The ponds were placed in two rows along north–south axis so that all ponds would receive equivalent sunlight. Treatments were randomly assigned to ponds within three spatial blocks. The end blocks (1 and 3) contained six ponds with all six treatments represented, while the middle block (2) contained only four ponds, and did not contain the two control treatments.

On 19 May 1998, we added snails, *P. columella* (either 10 infected or 10 uninfected), to each of 12 designated ponds. All snails introduced were adult. Initial length, from the tip of the spire to the outermost edge of the aperture (mean length in mm ± 1 SE) was 15.5 ± 0.08 for the infected *P. columella*, and 16.1 ± 0.13 for the uninfected *P. columella*.

In order to determine infection status of snails, each individual was screened in order to assess the presence of trematode infection, following the protocol described in Blankespoor and Reimink (1998). Snails were suspended in 2 mL of water placed in individual 20-mL centrifuge tubes, and exposed to intense fluorescent light on a 12L:12D cycle for 48 h. The water from each tube was then removed and examined under a dissecting microscope for the presence of cercariae. Those snails not shedding cercariae were screened on at least two occasions before they were considered to be uninfected. All infected snails used in this experiment shed armatiae cercariae (Schell 1985). All tadpoles used in this experiment were exposed to cercariae emanating from snail hosts that had been infected in natural ponds having populations of *H. versicolor*.

On 22 May 1998, 50 hatchling *H. versicolor* (Gosner stage 24, Gosner 1960) were added to each pond. Hatchlings from six separate clutches of *H. versicolor* were mixed prior to addition. Counted hatchlings were randomly assigned to each pond, and all introductions were made within 1 h. This protocol ensured that initial densities did not vary among ponds or treatments. Clutches were either collected from Cheri's Pond located in the Yale-Myers forest, or were collected from artificial breeding sites (120-L plastic pools, 120 cm in diameter) filled with well water, that were placed at the Yale-Myers Research facility.

In order to simulate pond drying due to loss of water through seepage, ponds in the temporary regime were drained. Water was siphoned from ponds using a 15-cm funnel that was covered with fiberglass screening. To simulate conditions in temporary ponds (Wilbur 1987), ponds were drained on a 49-d schedule. The 49-d schedule was designed to drop water levels at a rate comparable to drying patterns of ponds at Yale-Myers Forest (D. K. Skelly and J. M. Kiesecker, *unpublished data*). All ponds started the experiment at a depth of 54 cm. Water level was lowered by 3.5 cm twice a week from each temporary pond. Once the first metamorph was observed, ponds were checked daily and all metamorphs (front limb emergence; Gosner stage 42, Gosner 1960) were removed, and we recorded mass (to the nearest mg) at, and time (in days) to, metamorphosis. Temporary ponds contained 5 cm of water on the target drying date (9 July 1998). At that time the pond was carefully searched for gray treefrog tadpoles, metamorphs, and snails. All animals were removed and counted. Tadpoles and metamorphs were brought back to the lab, weighed to the nearest mg, and preserved for later determination of Gosner developmental stage (Gosner 1960) as well as staining for metacercarial cysts. On the same day, we also removed five gray treefrog tadpoles from each permanent pond, in order to compare the number of metacercarial cysts between treatments.

With one exception, water level of permanent ponds was not manipulated. Ponds were left to fluctuate naturally, and depth ranged between 47 cm and 54 cm during the course of the experiment. However, it was necessary on one occasion to lower the water to prevent overflow due to heavy rainfall. Once the first metamorph was observed, we monitored the ponds daily and removed individuals from the pools as they metamorphosed (front limb emergence; Gosner stage 42, Gosner 1960), recording mass (to the nearest mg) at, and time (in days) to, metamorphosis. We terminated the experiment after all tadpoles had either metamorphosed or died.

On 18 June 1998 (day 28), all ponds were examined, and we recorded the number of *P. columella* egg masses deposited on the sides of the ponds by infected and uninfected snails. After the last metamorph was removed from the permanent ponds, we drained the ponds, carefully searched the contents, and recorded the number of snails in each pond. During the experiment we made regular measurements of water tem-

perature in all ponds. During each measurement, the probe tip was located 10 cm below the water surface and moved back and forth until the reading stabilized. From 25 May until 30 July of 1998 each pond was visited on seven separate days between 0700 and 1030.

Assessing infection

We assessed the level of infection in tadpoles by clearing and staining tadpoles and metamorphs, and searching for metacercariae. When animals were removed from the ponds they were fixed in 10% buffered formalin for 24 h, rinsed in distilled water, and post-fixed in 70% ETOH. Specimens were then stained with Alcian blue, and cleared with methyl salicylate as described by Sessions and Ruth (1990). Subsequently, each animal was examined under a dissecting microscope and the number of metacercarial cysts was counted as an index of parasite load (number of cysts per individual).

Statistical analyses

We used multivariate analysis of variance (MANOVA) to evaluate the effects of independent factors, including snails (infected, uninfected, or absent), and hydroperiod (temporary or permanent), on the dependent variables, which were mean mass at metamorphosis, mean survival (number reaching metamorphosis), and mean time to metamorphosis (Tabachnick and Fidell 1989). After MANOVA, we used Bonferroni-adjusted univariate analysis of variance (ANOVA) on each response variable to interpret patterns uncovered by the MANOVA. Tukey's hsd tests were used to compare treatment means where significant ($P < 0.05$) differences were found with the ANOVA. ANOVA was also used to test for the effect of snails (infected or uninfected), and hydroperiod (temporary or permanent), on the number of snail egg masses deposited. An additional analysis comparing the final number of snails used a comparable ANOVA structure. We used a Student's *t* test to compare the mean number of metacercarial cysts of animals from the infected/temporary treatment to those of the animals removed from the infected/permanent treatments on 9 July 1998 (day 49). Because individuals in ponds were not independent from one another, all variables were analyzed as pond means. In order to meet the assumptions of the ANOVA, data on survival to metamorphosis were arcsine-transformed before the analysis. All other variables were analyzed in their raw form, meeting parametric assumptions without transformation.

Results

The results of our MANOVA revealed strong main effects of hydroperiod (temporary or permanent) and snail treatments on tadpole performance (Table 1). In addition, there was a strong interaction between hydroperiod and snail treatments. In the following section we use univariate tests (ANOVA) and post hoc comparisons (Tukey's hsd tests) to interpret patterns of response of size, developmental time, and survival due to our manipulations.

TABLE 1. Results of MANOVA for overall effects of pond hydroperiod (temporary or permanent) and snails (infected, uninfected, or absent) on *Hyla versicolor* survival, growth, and time to metamorphosis, and ANOVAs for each response variable. Response variables are mass at metamorphosis (mass), time to metamorphosis (time), and proportion reaching metamorphosis (survival).

Variables	*F*	*P*
MANOVA		
Constant	761.468	<0.001
Hydroperiod	75.850	<0.001
Snails	5.399	0.003
Hydroperiod × snails	7.312	0.001
ANOVAs		
Mass		
Hydroperiod	0.104	0.754
Snails	5.447	0.025
Hydroperiod × snails	6.456	0.016
Time		
Hydroperiod	160.767	<0.001
Snails	3.840	0.048
Hydroperiod × snails	15.470	0.001
Survival		
Hydroperiod	225.514	<0.001
Snails	12.786	0.002
Hydroperiod × snails	11.973	0.002

Note: Significance level for univariate tests is 0.0125 (Bonferroni-adjusted for three response variables).

Gray treefrog tadpoles responded to the conditions in temporary ponds by initiating metamorphosis more than 30 d faster than in permanent ponds (Table 1, Fig. 1). Despite this response, survival to metamorphosis was sharply lower in temporary ponds (28% vs. 74%).

Gray treefrogs in temporary ponds were significantly affected by snail presence for each of the response variables measured (Table 1, Fig. 1). Gray treefrogs that emerged from temporary ponds containing infected snails were 40% smaller than were their counterparts in temporary ponds containing uninfected snails or no snails (Tukey's hsd $P < 0.007$). Similarly, tadpoles in temporary ponds containing infected snails took 11 d longer to reach metamorphosis, when compared to control or uninfected snail treatments (Tukey's hsd $P < 0.024$). Finally, the number of animals reaching metamorphosis before drying was also lower for those *H. versicolor* exposed to infected snails, compared to uninfected snail or control treatments (Tukey's hsd $P < 0.002$). Among animals in the temporary pond treatments, only 8.6% of *H. versicolor* exposed to infected snails completed metamorphosis before the 49-d drying time, compared to 39% among tadpoles exposed to uninfected snails or no snails. We found no difference in survival, time to, or mass at metamorphosis between control and uninfected snail treatments (Tukey's hsd $P > 0.56$).

In marked contrast to patterns seen in temporary ponds, we found little impact of snail treatments on

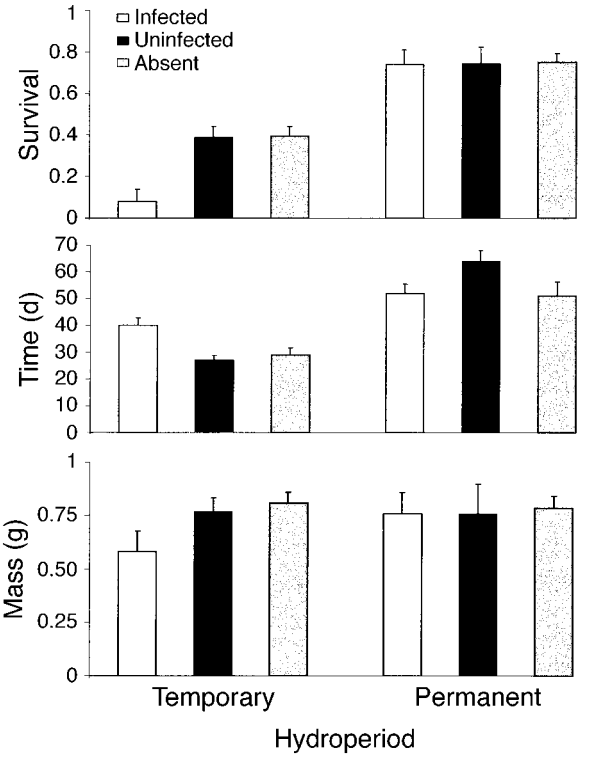

FIG. 1. Summary of the effects of hydroperiod (temporary or permanent) and *Psudosuccenea columella* (infected, uninfected, or absent) on the survival (proportion reaching metamorphosis), time (time to metamorphosis), and mass (mass at metamorphosis) of *Hyla versicolor*. Symbols indicate means +1 SE.

tadpoles in permanent ponds. Neither mass at, nor survival to metamorphosis was affected by snail presence (Table 1, Fig. 1). There was, however, a small but significant difference in time to metamorphosis among the snail treatments in the permanent regimes (Table 1, Fig. 1). Time to metamorphosis was greatest for *H. versicolor* in the permanent ponds that were exposed to uninfected snails, compared to either the control or infected snail treatments (Tukey's hsd $P < 0.028$). There was no difference in time to metamorphosis between control and infected snail treatments in the permanent ponds (Tukey's hsd $P = 0.83$).

There were also differences between the infected/temporary and infected/permanent treatments, in the mean number of metacercarial cysts ($t = 2.772$, $P = 0.049$; mean number of metacercariae ±1 SE = temporary, 8.75 ± 3.1; permanent, 2.67 ± 1.62). The majority (97%) of all tadpoles exposed to infected snails were found to have ≥1 cyst. In addition, 67% of animals in the temporary/infected treatment were found to ≥4 cysts. Infection with >4 cysts occurred in only 5% of animals in the permanent/infected treatment. No cysts were found in any of the animals from the uninfected snail or control treatments.

We monitored water temperature in ponds on several

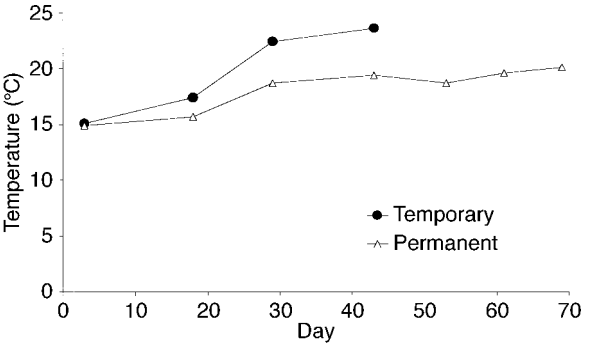

FIG. 2. Water temperature (°C) of temporary and permanent ponds taken during the course of the experiment. Symbols indicate means.

days during the experiment. While both temporary and permanent ponds warmed during the course of the experiment, temporary ponds were warmer than permanent ponds (Fig. 2). This difference became apparent during the third week of the experiment, when temporary ponds were ~4° C warmer than permanent ponds.

Observations of snail abundance within ponds indicated strong differences between infected and uninfected snail treatments. Snail reproduction, as indexed by the number of egg masses deposited on the tank walls, was lower for infected than for uninfected snail treatments (Table 2, Fig. 3a). Within temporary ponds, snail egg masses had not hatched before drying was completed, and thus there was no detectable difference in the final number of snails between the infected and uninfected treatments (Fig. 3b). However, the subsequent hatching and recruitment of snails in permanent, uninfected ponds led to a large increase in density within this treatment (Table 2, Fig. 3b).

DISCUSSION

In this study we have shown that the presence of an endemic, locally abundant parasite can have large impacts on the performance of an amphibian. These effects depend, however, on the environmental context

TABLE 2. Results of ANOVA for overall effects of pond hydroperiod (temporary or permanent) and snails (infected, or uninfected) on number of *Psudosuccenea columella* egg masses and final number of *P. columella*.

Source	df	*F*	*P*
Number of *P. columella* egg masses			
Hydroperiod	1	0.061	0.812
Snail	1	19.112	0.002
Hydroperiod × snail	1	0.752	0.411
Error	8		
Final number of *P. columella*			
Hydroperiod	1	44.930	<0.001
Snail	1	47.204	<0.001
Hydroperiod × snail	1	31.201	0.001
Error	8		

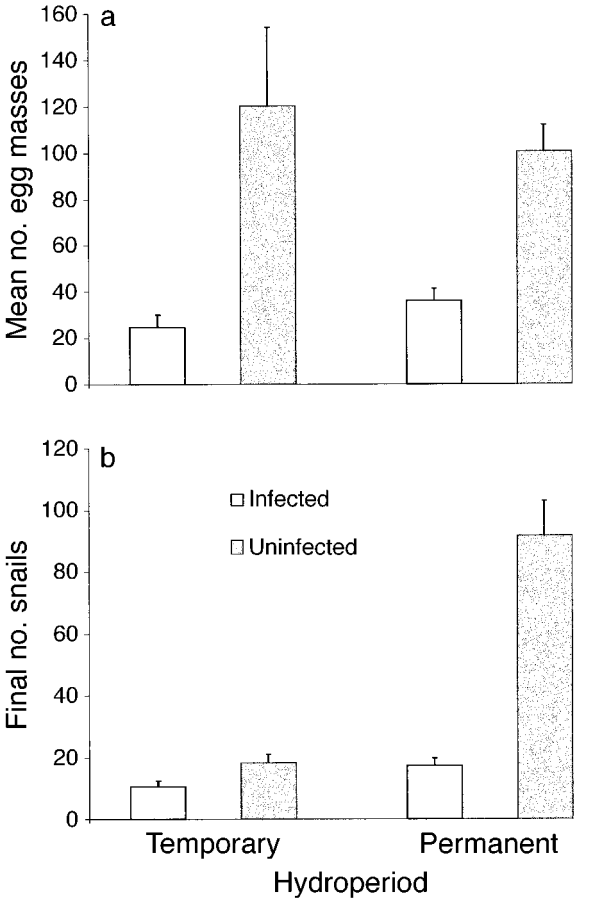

FIG. 3. (a) Number of *Psudosuccenea columella* egg masses deposited (measured on day 28), and (b) final number of snails. Symbols indicate means +1 SE.

for the interaction. Relative to controls, we found that in temporary ponds, gray treefrog tadpoles that were exposed to infected snails grew 40% more slowly, and that 30% fewer reached metamorphosis. Subsequent examinations confirmed that virtually every tadpole within the infected snail treatment in temporary ponds became infected. These results suggest that infection can be associated with a large cost to host performance. In contrast, we found that the impacts of the same disease organism were negligible when tadpoles were raised in a permanent environment. In the following discussion we consider two questions. First, how does infection by trematode cercariae impose a cost on tadpole hosts? And second, why might such costs be dependent on pond hydroperiod?

The measured response to infected snails included reduced growth and survivorship; however, results of our experiment revealed little if any mortality directly attributable to infection. Tadpoles in temporary ponds exposed to infected snails survived well until the ponds dried. Nevertheless, infection appeared to be associated with greatly reduced rates of growth and development. These effects of infection are not unlike previously documented impacts of competition and predation risk on tadpoles. In all of these cases, tadpoles in temporary ponds can become susceptible to mortality through pond drying, when the effects of reduced resources or facultative responses to predation risk result in reduced rates of growth and development (e.g., Skelly 1996). However, in the case of infection, we know very little of the mechanisms that lead to such reductions. Costs of infection can be related to the direct impact of the pathogen on host tissues, or to the response of the host to the pathogen. In the first case, invading cercariae are known to cause tissue damage during infection (e.g., Krist and Lively 1998). Subsequently, damaged tissues can become necrotic. In addition to the cost of repairing damage to tissues, such injuries could impair tissue function (e.g., Zukikhani and Rau 1998). Finally, the immune response elicited by cercarial infection could be costly (Lloyd 1995).

In addition to observing that infection can have large effects on tadpole performance, we found that such costs can be relaxed in a different environmental context. There was no effect of infected snails on tadpole performance in permanent ponds. Given the indirect pathway to infection-related mortality, we might have expected reduced survivorship to be restricted to temporary ponds. However, rates of growth and development also were not associated with infected snails in permanent ponds. In part, the lack of effect may be due to lower rate of transmission. Rates of encystment were roughly one-third of those observed among temporary pond tadpoles. The cause for lower rates of infection is difficult to deduce, but could be associated with differences in spatial scale and temperature between temporary and permanent treatments. As the experiment progressed, the volume of temporary ponds declined, and the distances separating snails and tadpoles would, of necessity, become increasingly constrained. Because cercariae have limited swimming powers, confinement of tadpoles and snails in a smaller volume may have facilitated transmission of cercariae (Lysne et al. 1998, Sousa and Grosholz 1991). In addition, the development and subsequent release of cercariae are influenced by temperature (e.g., Taskinen 1998). Increased temperature in temporary ponds may have promoted the shedding of more cercariae into the water column.

Our results indicate that the potential influence of trematode infection on gray treefrog populations will likely be the result of changes in the growth rates of infected individuals. Rates of growth and development are crucial features of the population ecology of amphibians that breed in temporary ponds, because amphibians must reach a certain minimum size in order to metamorphose before pond drying (Wilbur and Collins 1973). In addition, infection may also induce secondary effects as a result of changes in developmental time and mass at metamorphosis, which can influence individual fitness, and thus may ultimately have effects at the population level. Extending the larval period can

result in increased exposure to aquatic predators, and can affect the postmetamorphic stage by leaving juvenile amphibians inadequate time to store fat for winter survival (e.g., Morin 1983, Woodward 1983, Wilbur 1987). Smaller size at metamorphosis can decrease both survival and reproductive success in the terrestrial environment (Morin 1983, Woodward 1983, Skelly 1996).

Although our experiments were conducted in artificial ponds, they suggest an important role for pathogens in influencing growth rates of amphibian larvae and the potential for population regulation. However, it is unclear whether our ponds provide conditions that would result in infection levels that are similar to natural environments. For example, as the density of cercariae increases, tadpoles may be able to use behavior to mediate exposure (Kiesecker et al. 1999). Behavioral avoidance mechanisms may not be possible in artificial ponds, while cercariae may be able to disperse freely within the ponds. In addition, our results suggest that we should expect to see stronger impacts of disease in temporary ponds than in permanent natural ponds. However, the extreme context dependence seen in artificial ponds also suggests that differences in temperature and spatial scale in natural ponds could have a strong effect on both rates of transmission and the impact of infection on amphibian larvae. Field observations and experiments are needed before the relationship between results from artificial ponds and patterns in the natural environment can be fully ascertained.

In contrast to the strong effects of infected snails, uninfected snails had little effect on *H. versicolor* in either temporary or permanent pools. There was however, a small increase in time to metamorphosis for *H. versicolor* in permanent pools exposed to uninfected snails. One hypothesis for this increased developmental time is competition of tadpoles with juvenile snails. Overlap in resource use can be strong between tadpoles and snails and may result in strong competition (e.g., Holomuzki and Hemphill 1996). Tadpoles in the uninfected/permanent treatment were exposed to high densities of hatchling snails, resulting from reproduction by adult snails that were stocked into ponds at the start of the experiment. Reproduction by *P. columella* depended on their infection status. In ponds where snails were uninfected, reproduction was high, relative to infected snail ponds. This can likely be attributed to the effects trematode infection has on the reproduction of host snails (e.g., Sousa 1983, Sorensen and Minchella 1998). Infection can damage reproductive tissue, resulting in sterilization, or may divert energy that could otherwise have been invested in gametes (e.g., Zukikhani and Rau 1998).

Our results indicate that an endemic, locally abundant parasite can potentially influence population regulation under stressful situations that are commonly experienced by host amphibians. These results stress the importance of understanding the context-dependent nature of host–pathogen interactions. The challenge for future studies will be to identify how common these types of synergistic effects are, and to determine how sensitive host–pathogen interactions are to changes in conditions.

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