

## CHOICE OF OVIPOSITION SITE BY GRAY TREEFROGS: THE ROLE OF POTENTIAL PARASITIC INFECTION

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**Abstract.** The role of potential infection by parasitic trematodes in the choice of oviposition site choice by gray treefrogs, *Hyla versicolor*, was examined in a randomized experiment using 25 experimental pools. Treatment pools containing low (five snails) or high (10 snails) densities of either infected or uninfected trematode vector *Pseudosuccinea columella* were compared with control pools containing no *P. columella*.

Treatments had significant effects on the number of gray treefrog eggs deposited in pools. Compared to control pools, fewer eggs were laid in all treatment pools, either because fewer pairs laid eggs, or fewer eggs were laid per visit. Pools containing infected *P. columella* also had fewer eggs deposited relative to pools containing uninfected *P. columella*.

The gray treefrog, *H. versicolor*, can discriminate between oviposition sites, based on the species present and the potential for infection. Choice of oviposition site can be a mechanism that determines the composition of ecological communities as well as influencing parental reproductive success. Our results emphasize the importance of disease agents in shaping patterns of distribution, and they underscore the importance of understanding how potential hosts may use behavior to mitigate infection risk.

**Key words:** anuran larvae; Cercariae; disease; egg-laying behavior; Hyla; oviposition site choice; pathogen-mediated; performance; *Pseudosuccinea columella*; snails; Trematode.

### INTRODUCTION

There are numerous examples of parasites that alter intermediate host behavior to secure transmission to their next host (e.g., Moore 1984, Moore 1995). Despite the obvious benefits of such behaviors, there are few documented cases of behavioral responses used by potential hosts to avoid infection (Hart 1990, Andersson 1994, Loehle 1995, Wedekind and Milinski 1996, Pfennig et al. 1998, Kiesecker et al. 1999b). If host fitness is strongly reduced by infection, there should be strong selective pressure to avoid situations that would promote infection. A potential host may be able to avoid being parasitized by avoiding consumption of infective prey, or avoiding areas that increase the risk of infection. As a corollary, many species may be able to protect offspring from potential infection by preferentially ovipositing in areas relatively free of pathogens.

Choice of oviposition site can strongly affect the reproductive success of many pond-breeding amphib-

ians (Halliday 1983, Resetarits and Wilbur 1989). Anurans are known to choose oviposition sites based on physical characteristics of sites (Seale 1982), interactions between female choice of oviposition site and the quality of a male's territory (Howard 1978) and biotic factors, such as the presence of predators and competitors (Resetarits and Wilbur 1989, Hopey and Petranka 1994, Petranka et al. 1994, Laurila and Aho 1997). In an experiment that manipulated the composition of artificial communities, female *Hyla chrysoscelis* discriminated between potential oviposition sites, based on species composition. In the latter study, patterns of oviposition reduced exposure of larvae to potential predators and competitors (Resetarits and Wilbur 1989). Despite its potential importance, the role of disease in the choice of oviposition site has not been previously studied.

Amphibians are host to a wide variety of pathogens that attack most developmental stages (e.g., Aho 1990, Sessions and Ruth 1990, Goater and Ward 1992). Pathogenic infection can result in considerable mortality of developing amphibian embryos and larvae (e.g., Worthylake and Hovingh 1989, Blaustein et al. 1994, Kiesecker and Blaustein 1995, Kiesecker and Blaustein 1997). Recent studies have demonstrated that ovipo-

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sition behavior can influence pathogen-mediated mortality of developing embryos, and modify larval performance (Kiesecker and Blaustein 1997, Kiesecker and Blaustein 1999).

In northeastern Connecticut, USA, larvae of the gray treefrog (*Hyla versicolor*) co-occur in temporary and permanent ponds with a snail (*Pseudosuccinea columella*) which is frequently infected with a digenetic trematode. Within the snail host, this parasite produces free swimming armatae cercariae that 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%, although ponds vary considerably in both the density of snails and prevalence of infection. Exposure to infected *P. columella* results in decreased growth and survival of gray treefrog larvae raised in artificial pond communities (J. M. Kiesecker and D. K. Skelly, unpublished manuscript). Given the effects of trematodes on gray treefrog larvae, we predicted that breeding adults avoid oviposition near infected *P. columella*.

We manipulated the presence of infected and uninfected snails, *P. columella*, in replicated experimental pools to determine the effect of this species on oviposition site choice by gray treefrogs. Two questions were addressed: Do gray treefrogs discriminate between potential oviposition sites based on the presence of snails, *P. columella* and (2) Does density or infection status of snails influence this choice?

#### METHODS AND MATERIALS

*Hyla versicolor* is the northern tetraploid member of the *H. chrysoscelis* complex distributed across much of eastern North America. This species breeds during late spring and summer in small ponds and temporary pools and is locally abundant at the Yale-Myers Forest, Tolland and Windham Counties, Connecticut, USA, where this experiment was conducted. The gray treefrog is the only summer-breeding treefrog at Yale-Myers and is known to breed freely in experimental pools. Eggs are laid in floating packets that are easy to distinguish from those of other summer-breeding anurans.

Our experiment took place in 25 artificial pools (plastic pools, 1.2 m in diameter and 120 L in volume) arranged in five blocks of five pools each. Pools within a block were ~1 meter apart. Blocks were located at least 35 m apart in an open marsh (French House Beaver Pond, FHBP). Plastic pools were floated within the marsh and secured in place with wooden stakes.

All pools were filled with pond water that was filtered through a 500- $\mu$ m dip net. We also added 20 g of dried leaf litter that was collected from the bottom of FHBP and two small tree branches that were leaned against the side of the pools to allow easy movement of adults in and out of pools.

#### EXPERIMENTAL DESIGN

We manipulated the presence of snails (infected or uninfected) and the density of snails (low, 5 snails, or high, 10 snails per pool) using a randomized-block design. The five treatment combinations were: a control with no snails ( $N = 5$ ), infected snails present at low density ( $N = 5$ ), uninfected snails present at low density ( $N = 5$ ), infected snails present at high density ( $N = 5$ ), and uninfected snails present at high density ( $N = 5$ ). Treatments were randomly assigned to pools within each block.

Snails were collected from ponds at the Yale-Meyers forest. All snails introduced were adult. Initial length, from the tip of the spire to the outermost edge of the aperture (mean in mm  $\pm$  1 SE) was  $15.3 \pm 0.27$  for the infected *P. columella* and  $14.9 \pm 0.42$  for the uninfected *P. columella*.

To determine the infection status of snails, each individual snail was screened 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 hours. The water from each tube was then removed and examined under a dissecting scope for the presence of cercariae. Presence of cercariae indicated infection. Those snails not shedding cercariae were examined on at least two occasions before they were considered to be uninfected. All infected snails used in this experiment shed armatae cercariae (Schell 1985).

The experiment began on 13 May 1998 and ran until 17 June 1998. Pools were checked every third day, except on one occasion when pools were left unchecked for five days. On each visit, we searched all pools and recorded the presence of any gray treefrog eggs. We removed eggs from the pools and placed them in plastic shoeboxes to be counted. On each visit we also removed any potential predators or competitors that might have colonized the pools. On day 12, 21 and 30 of the experiment all pools were drained, cleaned, and restocked. Pools were cleaned to help prevent the build-up of algae that might have prevented the detection of eggs, and to control for changes in snail density due to mortality.

#### STATISTICAL ANALYSES

Choice of oviposition sites by gray treefrogs in this experiment can be partitioned into several stages. First, breeding pairs may visit a pool (or several pools) in which to initiate oviposition. A female may deposit her entire egg complement in one pool or may distribute her clutch among several pools. Consequently, two variables were used to examine oviposition behavior: (1)

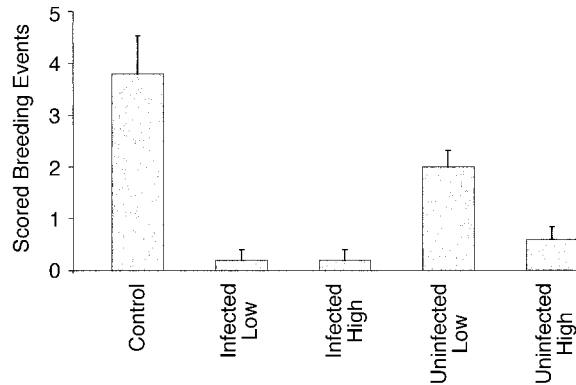


FIG. 1. Summary of the effects of *Pseudosuccinea columella* status (infected or uninfected) and density (low or high) on the number of *Hyla versicolor* breeding events. Bars indicate means plus 1 SE.

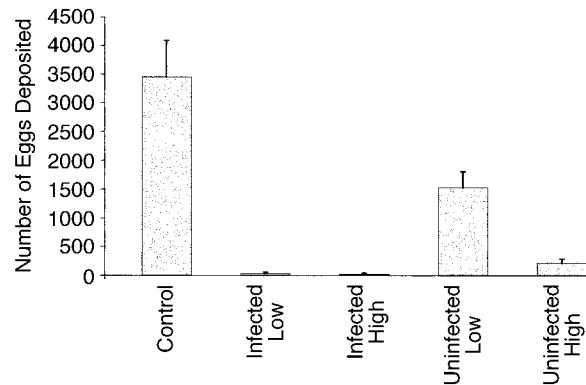


FIG. 2. Summary of the effects of *Pseudosuccinea columella* status (infected or uninfected) and density (low or high) on the mean number of *Hyla versicolor* eggs deposited. Bars indicate means plus 1 SE.

number of breeding events, defined as the total number of times a pool received any clutch or part of a clutch during the interval between censuses, and (2) eggs deposited, defined as the total number of eggs deposited within each pool.

Response variables were analyzed separately using analyses of variance (ANOVA). Tukey's (hsd) tests were used to compare treatment means where significant ( $P < 0.05$ ) differences were found with the ANOVA. A preliminary analysis indicated no significant block effect ( $F_{4,20} = 0.197$ ,  $P = 0.937$ ). Therefore, the block and error terms were pooled for remaining tests. All variables met parametric assumptions without transformation.

## RESULTS

There were a total of 34 oviposition events distributed over five out of the 11 intervals (3 d) between visits, yielding a total of 26 347 gray treefrog eggs. Based on a mean clutch size of ~1500 (Resetarits and Wilbur 1989), this activity represents ~18 clutches. Eggs of other amphibians were not found in the pools.

Treatment had a significant effect on both the number of breeding events ( $F_{4,20} = 15.44$ ,  $P < 0.001$ , Fig. 1) and the number of eggs deposited ( $F_{4,20} = 23.62$ ,  $P < 0.001$ , Fig. 2), indicating that breeding pairs could discriminate among treatments, resulting in a nonrandom distribution of eggs. For both the number of breeding events and number of eggs deposited, pairwise comparisons of treatment means with control means revealed significant (Tukey's hsd,  $P < 0.031$ ) differences between all treatments and the control. There were significant differences in the number of breeding events and number of eggs deposited in pools with infected snails, compared with pools containing uninfected snails. Pools with low densities of uninfected snails

received a greater number of breeding events when compared to either of the high or low density infected snail treatments (Tukey's hsd,  $P < 0.037$ ). There were no differences in the number of breeding events between the high density uninfected snail treatment and either the high or low density infected snails treatments (Tukey's hsd,  $P = 0.85$ ). In addition, pools with low densities of uninfected snails received higher numbers of deposited eggs, when compared to both infected snail treatments and the high density uninfected snail treatment (Tukey's hsd;  $P < 0.041$ ).

Control pools received 55.7% of the breeding events and 66.1% of the eggs deposited. In comparison, pools with uninfected snails received to 38.2% of the breeding events and 33.5% of the eggs deposited, while pools with infected snails received only 5.8% of the breeding events and 0.4% of the eggs deposited.

## DISCUSSION

Ecologists have long noted that species are specialized in their distributions. Relative to their local region, most species are found within a relatively narrow range of habitats and conditions (Futuyma and Moreno 1988). The potential causes for these patterns fall into two broad classes of mechanisms. In the first, species are eliminated from areas where they cannot to survive and reproduce. This "ecological sorting" mechanism has been a predominant theme in studies of a number of communities (e.g., Paine 1966, Tilman 1982). The second class of mechanisms concerns the placement of propagules. For many organisms, such as plants, the movement and final disposition of propagules is predominantly passive (although influenced by attributes of parent and propagule). However, in many animal species, breeding adults actively choose oviposition sites. In doing so, adults place their offspring into par-

ticular environmental contexts that can have profound impacts on their prospects for survival and growth. While this mechanism could be of equal importance to ecological patterns, it has received far less attention than evaluations of ecological sorting.

Studies of amphibians are a case in point. There has been a large number of studies aimed at understanding environmental factors that affect postoviposition performance of amphibians (reviewed by Wellborn et al. 1996). However, relatively few studies have focused on the factors that influence oviposition site choice in amphibians (Resetarits and Wilbur 1989, Hopey and Petranka 1994, Petranka et al. 1994, Laurila and Aho 1997). These studies have shown that ovipositing adults use environmental cues to evaluate potential breeding sites, and can respond by not laying eggs proximal to potential larval predators or competitors.

In this study, we found that selective oviposition behavior by adults extends to the avoidance of infection risk to offspring. Gray treefrogs laid few eggs in pools containing snails infected with a trematode known to infect tadpoles. A previous experiment in artificial ponds demonstrated that infection with trematodes can lead to a 40% decline in metamorphic mass and a 30% decline in survival to metamorphosis (J. M. Kiesecker and D. K. Skelly, *unpublished manuscript*). While the costs to larvae in natural ponds remain unquantified, our preliminary results suggest that the benefits of avoiding trematode infection could be significant.

Our experimental design allowed us to evaluate the possibility that adult treefrogs avoid snails regardless of their infection status. While we did find significant avoidance of uninfected snails, there was a stronger avoidance of infected snails. Because snails are potential competitors with anuran larvae (Holomuzki and Hemphill 1996, Lefcort et al. 1999) as well as vectors of infection, the interpretation of this pattern will require further study. Among several possibilities, the ovipositing tree frogs' avoidance of uninfected snails could reflect either an avoidance of potential competitors, or an imperfect ability to assess the infection status of snails.

The specific mechanisms that gray treefrogs used to detect potential infection is unknown. Snails could be readily seen within the ponds, suggesting that visual cues could be involved in their detection. However, it is difficult to detect cercariae with the naked eye, and thus unlikely that visual cues are used to detect differences between infected and uninfected snails. This suggests that chemical or mechanical cues may be the primary means for gray treefrog adults to detect infection. Chemical cues are known to play important roles in predator recognition of larval amphibians (e.g., Petranka et al. 1987, Kiesecker et al. 1996, Chivers and Smith 1998, Kiesecker et al. 1999a). In addition, recent

work has demonstrated that larval amphibians are capable of using chemical cues to detect infected conspecifics (Kiesecker et al. 1999b). However, chemically mediated detection of infection by adult amphibians has not been documented. Alternatively, cercariae may attack adult frogs, providing a clear indication of infection risk.

Over the last two decades, the study of amphibian communities has been dominated by the evaluation of a few factors (pond permanence, competition, and predation) in contributing to the sorting of species among ponds (e.g., Wellborn et al. 1996). Experiments have documented strong support for the roles of these factors in natural communities. However, it is becoming clear that other variables may have important roles. In this study, we have found that the risk of infection by a parasite may influence distribution patterns of an amphibian. Preliminary evidence suggests that adult behavior as well as the impact of disease on larval amphibians may contribute to these patterns.

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