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Oviposition behavior partitions aquatic landscapes along predation and nutrient gradients

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That individuals attempt to minimize the ratio of mortality risk/growth rate ($\mu/g$) when foraging within individual habitat patches is well established. Do species partition among spatially discrete communities embedded in complex landscapes in a similar manner? We investigated how 3 ovipositing species (2 Hyla treefrogs and a hydrophilid beetle, \textit{Tropisternus lateralis}) responded to simultaneous gradients of nutrients and predation risk. Species partitioned our experimental metacommunity primarily by reducing oviposition with fish. \textit{Tropisternus} positively responded to increased nutrients, but the effect decreased with increasing risk, as predicted by $\mu/g$ theory. Use of fish habitats by \textit{Tropisternus} was unrelated to breeding intensity. In contrast, \textit{Hyla} showed no nutrient response but oviposited with fish only on nights with high breeding activity. Behavioral responses to the spatial distribution of resources and risk among discrete patches generated substantial variation in habitats-specific colonization rates, which has been identified as a primary mechanism generating both community and metacommunity structure. 

\textbf{Key words:} colonization, dispersal, habitat selection, minimize $\mu/g$, oviposition, predation risk. [\textit{Behav Ecol} 19:552–557 (2008)]

How the movement of individuals, species, and materials across habitat boundaries affects ecological dynamics has become a major focus of research in ecology (Polis et al. 1997; Hanski 1999; Leibold et al. 2004; Loreau and Holt 2004). The importance of the metapopulation, metacommunity, and spatial subsidy themes in ecology collectively derives from the question of the independence, or interdependence, of what typically have been viewed as distinct communities. The metacommunity concept, for example, specifically examines how spatially discrete local communities are linked by dispersal and colonization of multiple species into larger functional units (Holyoak et al. 2005). Under this view, varying magnitudes of dispersal interact with internal community processes to produce both local community and metacommunity structure (Kneitel and Miller 2003). When emigration and immigration are driven by habitat selection rules, substantial variation in the degree to which communities are linked by dispersal can be generated by the nonrandom colonization of patches (Blaustein 1999; Morris 2003; Resetarits et al. 2005).

Habitat selection is a foraging process and is closely associated with optimal foraging and optimization theory (MacArthur and Franka 1966; Charnov 1976; Rosenzweig 1991; Sutherland et al. 2001). Habitat selection models based on ideal free theory (Fretwell and Lucas 1970) predict that individuals increase their fitness by nonrandomly choosing habitats for feeding or reproduction (Moody et al. 1996; Sutherland 1996; Kijlswa et al. 2003). The most suitable habitat patches with the highest fitness payoff are colonized first until density dependence sufficiently alters the fitness landscape and forces colonization of secondary habitat types (Fretwell and Lucas 1970; Rosenzweig 1991). Factors that determine habitat suitability are diverse and include resources, predators, habitat complexity, and abiotic factors, the relative importance of which vary within and among species (Sutherland 1996; Morris 2003). Habitat suitability may also be defined by ratios of factors, including combinations of resources and habitat-specific mortality risk (Moody et al. 1996).

Predators affect most decision-making processes of their prey (Lima and Dill 1990; Lind and Cresswell 2005). Incorporating predator-induced mortality into foraging models produces a general foraging rule, whereby individuals attempt to minimize ratios of mortality ($\mu$) to growth rate ($g$) (minimize $\mu/g$ rule; see Werner and Gilliam 1984; Benard 2004; McPeek 2004). The ecological and evolutionary consequences of utilizing this rule have become a dominant research paradigm in both behavioral and theoretical ecology, and extensive evidence documents the ability of numerous species to minimize $\mu/g$ ratios when foraging (for reviews, see Dill 1987; Sih 1987; Lima and Dill 1990; Lima 1998). However, tests of this model are often conducted within the spatial confines of a single community, where predators, prey, and resources spatially co-occur. Whether spatially discrete patches are selected based on their ratio of mortality costs to resource gains and how these interact to influence reproductive decisions are 2 gaps in our understanding of how resources and risks affect decision making and the resulting community and metacommunity structure (Lima and Dill 1990; Lima 1998).

The discrete boundaries of small freshwater habitats (e.g., ponds, tree holes, rock pools, etc.) are ideal for examining processes relating to habitat selection, dispersal, and metacommunities (Resetarits et al. 2005). These systems often comprised of isolated patches linked to each other and to the surrounding terrestrial matrix by species with complex life cycles. The fauna contains the larval stages of many organisms that are (semi)terrestrial as adults or that must leave the water to complete their life cycle (Merritt and Cummins 1984; Duellman and Trueb 1986; Wilbur 1997). Persistence of these species depends on seasonal invasion and oviposition by dispersing adults. The challenge to colonizing/ovipositing species is to match correctly their own capabilities, or those of their offspring, to aspects of the habitat (Rausher 1983;
Colonization and oviposition behavior strongly affect the assembly of individual aquatic communities and are key processes linking communities across habitat boundaries and spatial scales in complex landscapes (Binckley and Resaretars 2003; Eitam and Blaustein 2004; Resaretars et al. 2005). We conducted an experiment quantifying oviposition site selection of 3 species (2 treefrogs and an aquatic beetle) in response to variation in resources and predation risk. These species are excellent model organisms for investigating habitat selection given their abundance and ability to rapidly colonize both natural and experimental ponds (Binckley and Resaretars 2005, 2007). We specifically hypothesized that ovipositing females would accept a greater risk, as indicated by ovipositing in ponds with fish predators, when resource levels (and hence growth potential) are elevated, and we examine the results in the context of habitat selection theory and the emerging paradigms of metacommunity ecology.

MATERIALS AND METHODS

Study organisms

This experiment, conducted at the Naval Security Group Activity Northwest (NSGANW) in Chesapeake, VA, examined how predators and nutrient addition affected oviposition in 2 species of anurans and an aquatic insect. *Hyla chrysoscelis* and *Hyla squirella* are terrestrial treefrogs that deposit eggs in ponds as floating surface rafts (*H. chrysoscelis*) or as small clusters or strings (*H. squirella*) after heavy rains during the late spring and summer. Eggs hatch into herbivorous larvae that forage in their natal pond until metamorphosing into terrestrial juveniles (Wilbur 1997). For *Hyla*, oviposition site selection is a specific form of habitat selection in which adults choose habitat for their offspring (Resaretars and Wilbur 1989; Blaustein 1999; Binckley and Resaretars 2003; Kiflawi et al. 2003; Eitam and Blaustein 2004). These species are 2 of the most abundant anurans on the Coastal Plain of Virginia, where the elevation (ca. 3–4 m above sea level), topography, and precipitation generate numerous wetlands.

*Tropisternus lateralis* is an abundant hydrophilid beetle with obligate aquatic adults, larvae, and egg cases (Zalom et al. 1979; Resaretars 2001). Adult dispersal occurs after an obligate terrestrial pupal stage along the periphery of ponds, and adults disperse aerially to ponds for feeding (adults are grazers/scavengers) and reproduction (Zalom et al. 1979). Females lay conspicuous silken egg cases (3–4 mm) containing 12–24 eggs that hatch into predacious larvae (Zalom et al. 1979; Resaretars 2001). Egg deposition occurs at our study site from early April to October with most occurring from June to August (Binckley CA, unpublished data). Females deposit many egg cases in a season (Young 1958); however, this number and the time interval between oviposition events are unknown. It is not clear if adults specifically select ponds for oviposition or simply deposit egg cases in the same localities they select for feeding (see Scheirs et al. 2004).

Experimental design and analysis

We established 54 experimental ponds using cattle watering tanks approximately 1.65 m in diameter, 0.61 m in depth, and 1001 l total volume. These experimental ponds are rapidly colonized by natural populations of anurans and aquatic insects for oviposition (Binckley and Resaretars 2005; Resaretars et al. 2005). Ponds were arranged into 6 rectangular blocks of 9 pools each, and tanks within a block were spaced approximately 1.5 m apart. Blocks were located approximately 30 m apart in 2 fields surrounded by hardwood and pine forest. The 2 fields were 0.5 km apart, 4 blocks were located in one field and 2 in the other. We covered all ponds with tight-fitting fiberglass mesh lids (mesh diameter 2 mm) to prevent premature colonization by anurans and aquatic insects and filled them with water pumped from a nearby creek on 29–30 May 2001. Ponds were allowed to settle for 2 days before 0.75 kg of dried leaf litter (red maple *Acer rubrum*, sweet gum *Liquidambar styraciflua*, and lobolly pine *Pinus taeda* leaves and needles) and 1.01 aliquots of plankton collected from fishless temporary ponds were randomly added to each pool.

This experiment employed a 2-factor randomized complete block design for analysis of variance (ANOVA). Nine treatments were assigned randomly to the 9 ponds in each of the 6 blocks. Treatments consisted of crossing the nonorthogonal presence of 0, 3, or 6 adult *Enneaanthus obesus* (Centrarchidae) with 0, 10, or 20 g of nutrient addition (rabbit chow pellets, Forti-Diet). We did not quantify productivity, but periphyton and phytoplankton responded rapidly to nutrient addition and others have repeatedly demonstrated that comparable levels of nutrient addition strongly influence growth of larval anurans and aquatic insects (see Leibold and Wilbur 1992; Blaustein and Kotler 1993; Skelly et al. 2002).

*Enneaanthus obesus* is a known predator of hylid eggs and larva (Chalcraft and Resaretars 2004) and consumes a wide variety of larval and adult insects, including aquatic beetles (Graham 1986). During spring floods or hurricanes, *Enneaanthus* often invades the ephemeral wetlands used by *Hyla* and aquatic beetles for reproduction using an extensive network of natural creeks and drainage ditches (Binckley CA, personal observation). Thus, *Enneaanthus* is widespread and abundant at NSGANW, and densities used span the wide range of natural densities. Previous experiments have demonstrated that colonizing/ovipositing females of all 3 species avoid *Enneaanthus* (Binckley and Resaretars 2007).

Individuals of *E. obesus* were collected from small ponds at NSGANW using plastic minnow traps and were kept in 1000 l cattle watering tanks inoculated with zooplankton until the experiment began. Fish were stratified by body size among blocks, and average individual mass was 3.8 ± 1.3 g standard deviation (n = 162).

PREDATOR/NUTRIENT ADDITION COMBINATIONS WERE RANDOMLY ASSIGNED TO POOLS IN EACH BLOCK ON 31 MAY 2001, AND THE EXPERIMENT RAN UNTIL 20 AUGUST 2001. ON NIGHTS WITH HEAVY RAIN, WE PUSHED ALL LIDS DOWN INTO THE WATER TO PERMIT ACCESS BY THE NATURAL BREEDING POPULATIONS OF *Hyla* and *Tropisternus*. Screens eliminated physical interaction between fish and reproducing females and allowed ready collection of eggs and egg cases by simply raising the screens. The morning after rain events, we removed all eggs/egg cases, placed them in separate containers (one for each species at each pond), and did complete counts by hand for all 3 species. Ponds were recovered after collection of eggs/egg cases.

We examined the effect of block, nutrient addition, predator density, and the nutrient × predator interaction on 3 response variables: mean number of *H. chrysoscelis* eggs, *H. squirella* eggs, and *T. lateralis* egg cases. We performed univariate ANOVAs for each response variable using SAS for Windows version 8.0 with type III sums of squares and α = 0.05. This is a conservative analysis, but treating each night that any pond within any block received eggs as replicates to increase power did not change the results. In the absence of significant nutrient × predator interaction, all possible pairwise contrasts (thus nonorthogonal) among the levels of any significant main effect were examined (e.g., 0 vs. 3, 0 vs. 6, and 3 vs. 6 fish predators). All data were square root transformed (square root of Y + 0.5) before analysis. This normalized the data, but...
considerable heterogeneity of variances persisted given that fish means were at or near zero. This did not affect the overall conclusions; thus, we utilized the transformed analysis common for count data such as ours. Habitat selection theory predicts colonization of secondary habitat types as density increases in preferred patches (Fretwell and Lucas 1970; Sutherland 1996). We examined this prospect using regression of the number of eggs/egg cases deposited with fish (and low nutrients for T. lateralis) versus total number of eggs/egg cases for each night that oviposition occurred (e.g., number of data points equals the number of nights with oviposition). We did not analyze the H. chrysoscelis data given that reproduction occurred on only 4 nights.

Treating each pond as a single data point potentially masks any temporal changes in oviposition responses. Given that a time lag in the effect nutrients had on productivity and hence growth potential might have existed, we used correlation analysis to examine if our 3 species increased their proportional use of the 10- and 20-g treatments over the course of the experiment. We could not use repeated measures ANOVA as the number of eggs/egg cases deposited on each night of oviposition was sometimes only a single clutch resulting in zero values for most experimental units.

RESULTS

Hyla squirella bred on 9 nights depositing a total of 41 441 eggs, whereas 4 nights of H. chrysoscelis oviposition (all overlapping with H. squirella) produced 17 945 eggs. The 2 blocks located in the second field did not receive any treefrog eggs and were removed from analyses. For Hyla, only the block (H. squirella) and main effect of fish (both species) were significant in the ANOVAs (Table 1, Figure 1). There was no evidence that ovipositing treefrogs responded to one another (correlation between Hyla oviposition on night when both species reproduced, $r = -0.14, P = 0.71$), which is consistent with other observations (Binkley CA, unpublished data). Contrasts revealed that females avoided oviposition in experimental ponds containing Eunacanthus, as predator-free controls received significantly more eggs than those with 3 or 6 predators, which did not differ significantly from each other (Table 1, contrasts, Figure 1). Nutrient addition did not affect treefrog oviposition (Table 1, Figure 1) but resulted in rapid increase in growth as the experimental ponds and phytoplankton in the water column (Binkley CA, personal observation). H. squirella showed a classic, ideal free response to increasing oviposition activity, completely avoiding fish when oviposition activity was low (≤4000 eggs/night), but switching to use of both fishless and fish habitats at higher densities, with approximately 22% of eggs deposited with fish. The H. squirella data fit a 3-parameter sigmoidal function ($R^2 = 0.95, n = 9$; Figure 2).

A total of 405 T. lateralis egg cases were deposited on 8 nights of colonization/oviposition activity. In contrast to Hyla, T. lateralis females showed a strong response not only to fish but also to the gradient of nutrients (Figure 1). All effects were significant in the T. lateralis ANOVA including the 2-way fish × nutrient interaction term (Table 1). Significantly more egg cases were deposited in ponds with elevated nutrients, but these differences greatly decreased along the predator density gradient (Figure 1). Nutrient addition had the greatest effect in the absence of fish, caused a slight increase in the intermediate predator treatments, and had no effect at the highest predator level (Figure 1). Tropistorus lateralis females did not vary the proportion of egg cases deposited in patches with fish ($R^2 = 0.02, P = 0.72, n = 8$) or those without added nutrients ($R^2 = 0.10, P = 0.42, n = 8$) as the total number of egg cases increased (Figure 2).

For all 3 species, there was no evidence of proportional increase in use of ponds with added nutrients as the experiment progressed ($P > 0.18$ for all comparisons). This result held even when analyzing only the non-fish controls where most of the oviposition occurred ($P > 0.25$ for all comparisons). Thus, assessment of productivity was not affected by time.

DISCUSSION

How species behaviorally respond to variation in resources and risk has become a major focus of research in ecology (Brodin and Johansson 2004; McPeck 2004). Within the spatial confines of a single community, many species effectively minimize ratios of mortality to growth ($\mu/g$) (Werner and Gilliam 1984; Lima 1998). An important issue is whether spatially discrete communities comprising potential metacommunities are behaviorally partitioned using similar rules. Our study represents the logical extension of a general paradigm from behavioral ecology to the landscape scale. Evidence that variation in both resources and risk, as well as the application of the minimum $\mu/g$ rule, affects habitat selection at this scale further underscores the importance of incorporating habitat selection into our consideration of both.
community dynamics and the emerging paradigms of meta-community ecology (Holyoak et al. 2005; Leibold et al. 2004; Resetarits et al. 2005).

Our study contrasts responses in species with distinct differences in life history. Adult female *Hyla* spend little time at breeding sites, perhaps a single night/breeding season, and garner no resources from ponds. Predation risk to adults from fish predators is small, except in bodies of water containing fish much larger than *Enneacanthus*. Thus, adults appear to make choices based primarily on consequences for their offspring. This is supported by oviposition responses to other predators that pose no threat to adults (Resetarits and Wilbur 1989). In contrast, *Hyla* larvae are strongly affected by both variation in resources and risk of fish predation. Increased nutrients should potentially offset the growth cost of oviposition in fish-free ponds that have a strong competitive environment such that high-nutrient/no-fish pools should theoretically maximize larval performance. However, ovipositing treefrogs either are incapable of detecting variation in resources or simply do not respond. Responses were very strong to the presence of fish; distribution of *Hyla* eggs was thus exclusively driven by perceived predation risk through females applying

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**Figure 1**
Effects of predator density and nutrient addition on oviposition site selection. (A) Mean number of *Hyla squirella* eggs, (B) *Hyla chrysoscelis* eggs, and (C) *Tropisternus lateralis* egg cases (± 1 standard error) at the 3 nutrient addition levels for each level of predator density. The fish × nutrient interaction and main effect of nutrient addition were not significant for either treefrog species, whereas the presence of fish significantly reduced oviposition. For both *Hyla* species, controls received significantly more eggs than the predator treatments, which did not differ from each other. In contrast, all effects in the model were significant for *T. lateralis* as most egg cases were deposited at 10 and 20 g of nutrient addition when fish were absent and fewer at intermediate predator densities but not at the highest predator abundance.

**Figure 2**
The proportion of (A) *Hyla squirella* eggs and (B) *Tropisternus lateralis* egg cases in fish treatments versus the total number of eggs for each night when reproduction occurred. (C) The proportion of *T. lateralis* egg cases oviposited at the 0-g nutrient level versus the total number of egg cases oviposited for each night when reproduction occurred. *Hyla squirella* switches to using fish habitats as egg density increases, matching the expectations of the ideal free distribution. In contrast, *T. lateralis* displayed no pattern in the proportion of egg cases placed in fish or low-nutrient habitats with increased egg case density.
a “minimize mortality” rule (see also Eitam and Blaustein 2004; Rieger et al. 2004).

In the context of habitat selection, presence/absence of predatory fish provides reliable and immediate information concerning mortality, given the limited ability of fish to disperse among ponds. Absence of fish during the breeding season is a good indicator of long-term conditions, corresponding to a low probability that fish will invade later and thus obviate the fitness advantages of selecting fish-free ponds (Resetarits and Wilbur 1989; Resetarits 1996). The only observed concession to potential variation in resource availability was the observed increase in the proportion of eggs laid in pools with fish on nights when breeding activity was high (Figure 2), sensu the ideal free distribution (Fretwell and Lucas 1970; see Kiflawi et al. 2003). Adult Hyla avoid conspecific larvae (Resetarits and Wilbur 1989), and perhaps, intra-specific density is a more reliable, or accessible, cue to larval performance than nutrient concentrations. However, our species avoid ponds that are heavily shaded (Binckley and Resetarits 2007), and avoidance of fish predators, typically associated with longer hydroperiods, places many ovipositing species into highly productive, ephemeral water bodies (Odum et al. 1995; Palik et al. 2001) without specifically having to search for these sites.

In contrast, T. lateralis responded to both resources and predation risk as predicted by theory. More egg cases were deposited at higher nutrient levels in the absence of fish predators, and the importance of nutrient addition declined with increasing predator abundance (Figure 1). Female mosquitoes (Culiseta longiareolata) show a similar response, increasing oviposition in ponds with predatory tadpoles when aquatic resource levels are elevated (Blaustein and Kotler 1993). Yet, despite the significant response of adult T. lateralis to variation in nutrient levels, fish clearly dominate oviposition decisions (Table 1, Figure 1).

Both adult and larval T. lateralis are obligate aquatic, only leaving ponds for pupation and subsequent adult dispersal flights. However, because of differences in life history, pond conditions affect fitness by generating variation in both adult and larval growth and survival. Females may deposit egg cases in the habitats where they choose to feed (Mayhew 2001; Scheirs et al. 2004) or may specifically seek out sites most suitable for their offspring. Clearly, moving among sites specifically to increase egg/larval survivorship at the cost of adult performance elevates both metabolic and potential mortality costs.

Another informative contrast is the difference in observed responses to increased colonization/oviposition activity. Unlike H. squirella, adult T. lateralis relegates a highly variable proportion of total breeding activity to “poorer” habitats, unrelated to overall breeding activity (Figure 2). Thus, rather than following an ideal free distribution, the behavior of T. lateralis suggests 1) a random error rate by colonizing females, 2) population-level variation in colonization strategy, with certain females actually choosing fish or low-nutrient habitats, 3) a bet-hedging strategy by individual females that lay eggs in different habitat types on different nights, or 4) egg cases simply never reached critical density to induce habitat switching. It is unlikely that individual females laid egg cases in multiple ponds on a given night because they cannot readily redisperse after initially choosing a pond, even if that pond proves to be poor habitat (Resetarits 2001). Which mechanism drives the proportion of egg cases in habitats with predatory fish or low nutrients is unknown.

In its original context, body size was a key parameter in mass effects driven by lethal (differential mortality) versus nonlethal (habitat selection) determination of species distributions and abundances will be critical for developing a truly comprehensive and predictive understanding of both community and metacommunity structure. Just as behavioral assessment of the distribution of resources and risk among habitats within a single community is critical for determining the local distribution of organisms (Werner and Gilliam 1984; Lima 1998), behavioral assessment of resources and risk among discrete communities within larger metacommunities is likewise critical in determining the regional distribution of organisms.

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