The Ecology of Fear: Oviposition and Colonization in Aquatic Systems

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THE ECOLOGY OF FEAR: COLONIZATION AND OVIPOSITION IN AQUATIC SYSTEMS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

THE ECOLOGY OF FEAR: COLONIZATION AND OVIPOSITION IN AQUATIC SYSTEMS

By Leeanna Theresa Pletcher, Master of Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2008

Major Director: Dr. James Vonesh
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Amphibians and aquatic invertebrates have complex life histories that link aquatic and terrestrial food webs. It has been suggested that amphibian reproduction is an important source of carbon to some aquatic systems. This process of energy flow may be shaped by shifts in habitat selection in response to predators. We hypothesized that predators decrease colonization and oviposition of prey, reducing active inputs. Thus predation risk is expected to shift the relative amounts of active and passive subsidies. We manipulated the presence of fish predators in aquatic mesocosms. Results suggest hylid
treefrog eggs and hydrophilid beetles were less abundant in predator treatments. This difference in oviposition and colonization translated into small reductions in calories and ash free dry mass of active inputs. However, passive allochthonous inputs were more than double active amounts and variable, therefore relative amounts of active and passive inputs did not differ across the levels of predation risk.
CHAPTER 1 Introduction

Many species of amphibians and insects have complex life cycles in which adults disperse from terrestrial habitats to aquatic areas for reproduction. This life history movement from land to water for colonization and oviposition links aquatic and terrestrial food webs (Knight et al. 2005). Movement of organisms across the aquatic-terrestrial habitat boundary can represent important subsidies to the receiving habitat (Baxter et al. 2005, Regester et al. 2006). Subsidies are organisms, nutrients, or detritus that cross habitat boundaries and are consumed, and these allochthonous inputs can affect food web structure (Polis et al. 1997). This can happen when one habitat type is more productive than the other; a classic example is when ocean currents and waves bring rich biomass to beach shores and the raft of biomass feeds crabs and birds (Polis and Hurd 1996). Colonization and oviposition bring energy and organic matter that can have consequences for receiving food webs. The effect of the input depends on what resources are already available in the aquatic habitat; other allochthonous inputs, autochthonous production, and food habits can affect the significance of inputs from organisms with complex life cycles.

Aquatic predators play an important role in determining abundance of organisms in aquatic habitats affecting community composition. Presence of predatory fish may be the strongest biotic feature determining suitability of aquatic sites for species with complex life cycles (Resetarits 2001). Predators have a lethal effect when they consume organisms,
but predators also have non-lethal effects as organisms change their traits (e.g. behavior) in response to perceived predation risk. To disentangle the lethal and non-lethal effects of a predator, a caged predator is used to represent fear - predation risk without consumption. Past studies have assumed or concluded that lethal effects of predators are primarily responsible for shaping aquatic community composition. Non-lethal effects of a predator may be just as important as lethal effects (Preisser et al. 2005) and are considerably less studied than lethal effects.

Predator induced shifts in habitat selection are a well known non-lethal effect in aquatic systems. In certain aquatic beetles, merely the chemical cues (kairomones) of a predator can trigger avoidance behavior (Resetarits 2001). Some mosquitoes, frogs, and beetles can detect and avoid colonizing habitats occupied by predators (Resetarits and Wilbur 1989, Petranka and Fakhoury 1991, Blaustein and Kotler 1993, Binckley and Resetarits 2005). These predators also affect reproductive decisions of organisms. Theory predicts that a parent will avoid ovipositing if there is high larval vulnerability to a predator (Blaustein et al. 2004). In some aquatic beetles, 50% fewer larvae were oviposited in pools with a caged fish treatment compared to a no predator treatment (Brodin et al. 2006). Squirrel treefrog females travel through the landscape to find pools where fish predators are absent, showing 93% fish avoidance in ovipositen (Binckley and Resetarits 2002). Organisms have a means of recognizing which breeding grounds are favorable and which are not and this habitat selection behavior affects inputs from eggs to aquatic habitats.
Passive subsidies, like leaf litter, are brought by wind or water and move involuntarily. In contrast, active subsidies involve behavioral choices of habitat selection. In salamander breeding migrations to vernal ponds, energy gleaned from the terrestrial habitat is brought to otherwise unproductive systems. Vernal pools generally are too shady to have high algal production, so salamander eggs and larvae are important active subsidies (Regester et al. 2006). In one study, eggs and metamorphs of ambystomatid salamanders represent a net subsidy of 630 g ash-free dry mass per year to a fishless vernal pool complex (Regester et al. 2006). These same amphibians showed avoidance of fish; following initial fish invasion egg mass numbers decreased about 68% (Petranka and Holbrook 2006). Predators can alter subsidies by consuming nutrients, detritus or organisms that would otherwise move across habitat boundaries (Baxter et al. 2004). For example, fish eat insect larvae, reducing the number of adult insects that emerge during metamorphosis. Spider abundance depends on the number of flying insects caught in webs, so fish can affect adjacent terrestrial food webs (Baxter et al. 2004). Predators may have a similar effect on active inputs from invertebrates; if a predator is present, fewer invertebrate species colonize thus less biomass is present (Abjornsson et al. 2002). Thus, aquatic predators may reduce active subsidies, measured in energy flow and organic matter input, into aquatic habitats brought by organisms with complex life histories from terrestrial to aquatic ecosystems. Subsidies from oviposition have been quantified in only a few studies (Regester et al. 2006).

We hypothesized that the presence of a predator will decrease active inputs from the terrestrial system by organisms with complex life cycles. Conversely, passive
allochthonous inputs should be unaffected by predation risk. Thus predation risk should shift the relative importance of these active and passive aquatic inputs. These inputs represent potential subsidies and shift in ratio of input types would be important to consumers in the aquatic food web. A shift the relative importance of active and passive inputs would change the resources available to different trophic levels.

Our goals for this experiment were to 1) test whether the presence of predators alters oviposition by treefrogs and colonization by a diverse community of aquatic insects; 2) quantify predator-induced shifts in energy and organic matter as potential changes in subsidies; 3) evaluate the relative amount of energy and organic matter from active and passive sources to determine if changes in colonization and oviposition are important to aquatic systems.
CHAPTER 2 Methods and Analysis

Field Experiment

To test the effects of aquatic predators on terrestrial inputs to aquatic habitats, we manipulated the presence of free roaming and caged fish in aquatic mesocosms and quantified colonization, oviposition, and passive allochthonous inputs. Thirty mesocosms were placed in two spatial blocks in old fields at the Virginia Commonwealth University Walter and Inger Rice Center for Environmental Life Sciences (W77°12’30.5”, N37°19’56.32”) and the U.S. Fish and Wildlife Service Harrison Lake National Fish Hatchery (W77°11’17.85”, N37°20’29.92”) in Charles City County Virginia, USA. Replicates were placed > 20 m apart to ensure mesocosm independence (Resetarits 2005). Both old fields were approximately 1,700 m² and surrounded by forests dominated by loblolly pine (Pinus taeda), white oak (Quercus alba) and sweetgum (Liquidambar styraciflua).

The mesocosms were Rubbermaid© black plastic 1135.6 L stock tanks 1.7 m diameter, 63.5 cm in depth, with 2 m² of water surface area. Mesocosms were filled with water from a hatchery pond at Harrison Lake June 5 - 15th. Water was filtered through 20 X 20 mesh size (20 openings per inch Phiferglass© no-see-um mesh, Phifer Incorporated, Tuscaloosa Alabama USA) to prevent introduction of larger organisms. Dried mixed oak and pine needle leaf litter (300 g mesocosm⁻¹) was added to the water (Binckley and
Resetarits 2003) and allowed to sit for 4 days. The water temperature of 18 randomly
selected mesocosms was recorded for part of the experiment using data loggers (Water
Temp Pro v2 HOBO®). Dissolved oxygen and temperature were measured with a
handheld meter (Model 85, YSI Incorporated Yellow Springs Ohio USA). Water levels
were visually monitored and water was added to maintain levels. Before the start of the
experiment on June 30th, mesh covers prevented colonization of mesocosms. The
experiment lasted 50 days.

Treatments were 3 free-swimming blue-spotted sunfish, *Enneacanthus gloriosus*
(Centrarchidae), 3 caged fish or an empty cage (control) and were randomly assigned to 10
replicates. The treatments are named free roaming predator, caged predator, or no predator.
The density of 3 fish per mesocosm reflected a density typically observed in the coastal
plain of Virginia and this density has been shown to elicit shifts in habitat selection in
some aquatic taxa (Rieger et al. 2004, Resetarits 2005). Predator cages were constructed of
black plastic trashcans with both ends cut open and covered with mesh. Cages allowed
chemical cues from fish to enter the mesocosm but prevented consumption of colonists
larger than 1 mm. Adult male fish were caught with dip nets at Harrison Lake in dense
vegetation. Length of fish was measured at the start of the experiment and only fish > 40
mm standard length were used (mean 44.02 ± 0.46 mm). All replicates were fed 1 large
pinch of Brine Shrimp Aquarium Flake Food (Ocean Star International, USA) weekly.
Fish were added to mesocosms 2 days before the start of the experiment and any fish that
died were replaced. Our experimental design included a free roaming predator treatment to
test hypotheses not reported here. This treatment was necessary to examine the effects of free roaming predators on community assembly after colonization.

Treefrog oviposition was quantified by visually examining mesocosms for eggs after nights with rainfall, because *Hyla* breeding is highly correlated with rain events (Ritke et al. 1992); this method has been used effectively in previous studies (Rieger et al. 2004). Eggs and recent hatchlings (Gosner stages 1-20; Gosner, 1960) were transferred into white trays and digital photographs of egg clutches were taken, then eggs were returned to the mesocosm. A subsample of treefrog eggs was collected and frozen for later analysis. Handling, collection, and euthanasia of amphibian eggs followed standard operating procedures and guidelines (ARMI 2001, ASIH 2004). To accurately estimate the total number of eggs deposited in each mesocosm, all images were analyzed on Image J imaging software (version 1.38x National Institutes of Health, USA) using the automatic particle counting application. A subset of 16 images was also hand counted to estimate bias and standardize Image J automatic counts using regression.

Sampling for invertebrates and passive allochthonous inputs was done by draping mesh across the mesocosm on June 30th and pushing it down to sit about 6 inches under the water’s surface. Mesh covers have been used successfully in a number of studies (Binckley and Resetarits 2003, Resetarits 2005). After 2 or 3 days, the mesh was raised out of the water and inputs were removed, placed into a bag and frozen. About half of the replicates were sampled on July 2nd, the other half July 3rd.
Laboratory Methods

Invertebrates were removed from the leaf litter, grouped to morphospecies, and identified using a dissecting microscope. Length was measured using digital calipers (Mitutoyo Digimatic, Aurora IL, USA) and a voucher specimen for each morphospecies was preserved in 70% isopropyl alcohol. Dry weight was averaged from a haphazard sample of 3 individuals in most morphospecies. In less common morphospecies, we weighed only 1 individual. For morphospecies in which only one individual was present, these dry weights were estimated based on length mass regressions from other taxa. Dry weight was measured by placing invertebrates in a drying oven at 100 ºC for >24 hours and then weighed to the nearest 0.1 mg. Passive allochthonous inputs were sorted into 4 categories: deciduous leaf litter, pine needles, woody stems and bark, or terrestrial invertebrates. Dry weight was measured for the 4 categories after samples were placed on filter paper in a drying oven at 100 ºC for >24 hours then weighed to the nearest 0.01 g.

I quantified inputs using both ash-free dry mass (AFDM) and calorimetry. We included both methods to have a more complete evaluation of the composition of inputs. AFDM measures organic content of the subsidy (Regester et al. 2006). Calorimetry measures the number of calories per gram of each input type. Organic matter content was calculated by quantifying dry mass, AFDM, and percent organic matter. Each sample was dried for 24 hrs. at 100°C, weighed, placed in an Isotemp Muffle Furnace (550 Series, Model 14, Fisher Scientific, Pittsburgh, PA, USA), and heated at 550°C for 2 hrs. The samples were allowed to cool for 20 minutes then re-weighed to the nearest 0.1 mg to calculate ash weight. AFDM was calculated as AFDM = sample weight – ash weight.
Percent organic matter was calculated as \( \% \text{OM} = \frac{\text{AFDM}}{\text{sample weight}} \times 100 \). We ashed treefrog eggs and then estimated total AFDM from egg counts in photographs. For invertebrates, AFDM was determined for a subsample of 4 morphospecies selected based on abundance and length, and 3 individuals of each morphospecies were ashed. Regression analysis was used to estimate percent organic matter for all other invertebrates using total length. For all inputs, percent organic matter was multiplied by biomass (dry mass) per mesocosm to estimate input.

Energetic content of collected treefrog eggs, invertebrates and passive allochthonous inputs were quantified using oxygen bomb calorimetry. A Parr 6725 Semi-micro Calorimeter (Parr Instrument Company Moline, Illinois USA) was used to obtain gross heat in cal/g. Dried material was ground with a mortar and pestle and re-dried for 24 hrs, formed into pellets with a pellet press (Parr No. 2811), weighed to the nearest 0.1 mg and combusted. Calorimetry procedures followed Official Methods of Analysis (AOAC 2006). Treefrog eggs could not be identified to species, so mesocosms were classified as containing either gray treefrog tadpoles, green treefrog tadpoles or both species by observing the distinct phenotypes of tadpoles in mesocosms as they developed through the season. Dry weight of treefrog eggs was measured by placing eggs on filter paper in a drying oven at 100°C for 24 hours; three groups of dried eggs were weighed to the nearest 0.1 mg and divided by number of eggs. A mean of the 3 estimates for calories per gram was multiplied by the mean weight per egg to get mean calories per egg. The standard error for calories per egg was calculated using the exact formula for variance of products.
Four invertebrate taxa were used to estimate energetic content as described in the procedure for AFDM.

**Statistical Analysis**

We examined the distribution of the data using Q-Q plots, histograms, and we used boxplots to determine outliers (SPSS 14.0 for Windows, SPSS Inc., Chicago, Illinois, USA). Treefrog egg data was not normally distributed, so we log(x + 1) transformed the data, which improved normality for most data. We arc sin square root transformed percent active inputs. In cases where data remained non-normal after transformations, we proceeded with analysis because ANOVA is robust to moderate violations of the assumption of normality (Cottingham et al. 2005). The equal variances assumption of ANOVA was tested using Levene’s test. Two replicates, mesocosms 19 and 26, were omitted from the treefrog egg analysis because one was run over by a tractor, and in the other fish died and we not rapidly replaced before an oviposition night. However, these mesocosms were fine on the first sampling date for invertebrates and passive allochthonous inputs, so all 30 replicates were used for those analyses.

Abundance, calories and AFDM from treefrog eggs, and abundance of chironomids eggs, were tested for treatment, block and time effects using a repeated measures ANOVA, since multiple dates were measured for each mesocosm, the mesocosms were not independent. Repeated measures tests were subsequently run for pairs of treatments to determine where significant differences lay. Multiple comparisons of treatments represent *a priori* planned comparisons and were not adjusted for *post-hoc* multiple comparisons.
Treatment and block effects on total aquatic invertebrate abundance and abundance of invertebrate families and orders were tested using ANOVA. For comparisons of treefrog eggs, invertebrates and allochthonous inputs, per day input amounts were calculated, because these inputs were collected over different time scales. Treatment and block effects on calories and g AFDM were tested using ANOVA. We assessed differences in average temperature over the duration of the experiment using an ANOVA. To compare dissolved oxygen between treatments, we factored out temperature as a variable and reported the mean oxygen saturation per treatment per date. All tests were conducted using SYSTAT (SYSTAT for Windows version 11.0, San Jose, California, USA) at $\alpha = 0.05$. 
CHAPTER 3 Results

Amphibian Response

The Cope’s gray treefrog (*Hyla chrysoscelis*) and the green treefrog (*Hyla cinerea*) were observed ovipositing in the mesocosms at Harrison Lake. Only *H. chrysoscelis* was observed at the Rice Center. These two species oviposited ~ 39,000 eggs in the mesocosms on seven oviposition nights July 2\textsuperscript{nd} – August 18\textsuperscript{th}. Seventy-five percent of mesocosms received eggs (21/28). The regression equation number of eggs = 1.16(image J automatic count) + 43.29 was used to assign final number of treefrog eggs laid. The presence of predators significantly decreased the amount of amphibian eggs laid (Fig. 1A; $F_{1,15} = 3.665$, $P = 0.041$). Caged fish predators reduced the mean number of treefrog eggs by 70% compared to control ($F_{1,15} = 4.596$, $P = 0.049$). Free roaming fish predators marginally reduced the mean number of treefrog eggs by 57% compared to control ($F_{1,17} = 4.349$, $P = 0.052$). Caged predator and free roaming predator treatments were not different ($F_{1,15} = 0.008$, $P = 0.929$). There was a significant effect of site on the number of eggs laid ($F_{1,24} = 7.060$, $P = 0.014$); about three times more eggs were laid at the Rice Center than Harrison Lake. There was also a significant effect of date on the number of eggs laid ($F_{6,144} = 5.425$, $P < 0.001$); the most eggs were laid in late July. There was no interaction between treatment and sampling date ($F_{12,144} = 0.852$, $P = 0.597$).
To examine how the strength of predator avoidance varied over the duration of the experiment, we calculated a log response ratio \((L)\), \(L = \log(\text{mean eggs in no predator}) - \log(\text{mean eggs in caged predator})\), for each date that oviposition was observed \((n = 7)\) and then regressed \(L\) against date (Fig. 2). There was a significant relationship between predator avoidance and date; over the course of the experiment the effect of predation risk decreased \((F_{1,5} = 9.072, P = 0.030; r^2 = 0.65, \text{intercept: } 1.96 \pm 0.47 \text{ slope: } -0.06 \pm 0.02)\) and in August predators were no longer avoided. A log response ratio was also calculated for no predator and free roaming predator (Fig. 2); there was a relationship \((r^2 = 0.50, \text{intercept: } 1.95 \pm 0.74, \text{ slope: } -0.07 \pm 0.03)\), however the regression was only marginally significant for the relationship between predator avoidance and date \((F_{1,5} = 4.928, P = 0.077)\). A log response ratio was also calculated for caged predator and free roaming predator. The slope was close to zero \((r^2 = 0.01, \text{intercept: } -0.02 \pm 0.86, \text{ slope: } -0.01 \pm 0.03)\) and the regression was not significant, so there was no relationship between predator avoidance and date \((F_{1,5} = 0.061, P = 0.815)\). This pattern suggests that treefrogs responded to caged and free roaming predator treatments in a similar way.

**Aquatic Invertebrate Response**

Forty morphospecies of invertebrates colonized the mesocosms, the majority of which were in families Hydrophilidae, Dytiscidae, Corixidae and Notonectidae. The most commonly encountered invertebrates were dytisids; *Laccophilus fasciatus rufus* was found in 90% of mesocosms during the first sampling period \((27/30)\). Common invertebrates were *Notonecta irrorata*, *Paracymus* sp., and *Berosus* sp. The most abundant invertebrate
was *Paracymus* sp. with a total of 341 individuals colonizing within 72 hours (Table 1). There were 5 rare morphospecies, for which only 1 individual was found.

Total aquatic invertebrates marginally varied between treatments \( (F_{2, 24} = 2.897, P = 0.075; \text{Fig. 3A}) \). There was no interaction between site and treatment for total invertebrates \( (F_{2, 24} = 0.961, P = 0.397) \). Hydrophilidae showed significant habitat colonization differences due to predation risk \( (F_{2, 24} = 4.124, P = 0.029; \text{Fig. 3B}) \). A significant difference was seen in *post hoc* tests between the no predator and caged predator \( (F = 8.190, P = 0.011) \) and free roaming predator and caged predator treatments \( (F = 4.752, P = 0.045) \). Caged fish predators reduced hydrophilid beetles by 55%, and for one morphospecies, *Tropisternus* sp., caged fish predators decreased the mean abundance by 60%. Free-roaming fish predators reduced hydrophilid beetles by 20%. This difference between treatments was not seen for Dytiscidae \( (F_{2, 24} = 1.467, P = 0.251) \) or Corixidae \( (F_{2, 24} = 1.567, P = 0.229) \). When grouped at the order level, coleopterans showed marginally significant habitat colonization differences \( (F_{2, 24} = 3.202, P = 0.059) \). This pattern may be driven by hydrophilids. When grouped at the order level, hemipterans did not show colonization differences between treatments \( (F_{2, 24} = 0.671, P = 0.521) \).

Eggs from chironomids (Diptera), beetles (Coleoptera), and dragonflies (Odonata) were observed, showing that many organisms reproduced in the mesocosms. Chironomid egg mass numbers did not differ between treatments \( (F_{2, 26} = 0.540, P = 0.589) \). Numbers of egg masses decreased over time, with the peak mean at 16 egg masses per day per mesocosm at Harrison Lake on July 2\textsuperscript{nd}. The peak came later at the Rice Center, with a mean of 4 egg masses per day per mesocosm over a month later. There was a significant
effect of time \( (F_{3, 78} = 7.481, P < 0.001) \) and an effect of time on site \( (F_{3, 78} = 5.94, P = 0.001) \) on number of chironomid eggs oviposited in mesocosms. Beetle egg cases were quantified for *Tropisternus* spp. egg cases only. The most were laid in free-roaming predator treatments, however there was not a significant difference among treatments \( (F_{2, 26} = 1.828, P = 0.181) \). Dragonfly eggs were not quantified, as they were found attached to passive allochthonous inputs on only a few occasions. These eggs and dead adult dragonflies were added to the terrestrial insect portion of passive allochthonous inputs.

**Passive Allochthonous Inputs**

The total allochthonous inputs were not significantly different between treatments \( (F_{2, 26} = 1.634, P = 0.215) \). We did not anticipate a treatment effect on passive allochthonous inputs such as the amount of wood and bark, pine needles, deciduous leaf litter, or terrestrial invertebrates falling into mesocosms. Pine needles \( (F_{2, 26} = 0.651, P = 0.530) \), deciduous leaf litter \( (F_{2, 26} = 2.378, P = 0.113) \), and terrestrial invertebrates \( (F_{2, 26} = 0.681, P = 0.515) \) were similar between treatments (Fig. 4). However, we saw a significant increase in the amount of wood and bark in the free roaming treatment \( (F_{2, 26} = 3.437, P = 0.047) \). *Post-hoc* examination of these inputs showed that the wood pattern was primarily driven by 3 outlier free roaming predator replicates. When the largest outlier was removed, the difference in wood and bark was no longer significant \( (F_{2, 25} = 2.219, P = 0.130) \). This outlier remained dropped in all subsequent analyses and figures. The highest mass (g per day) of allochthonous input was pine needles, followed by deciduous leaf
litter, then woody materials. Terrestrial invertebrates contributed very small amounts of mass.

**Energetic and Organic Matter Qualities of Inputs**

In general, active inputs have higher mean energy content than passive inputs (mean ± 1SE: 5897 ± 62 cal/g and 5016 ± 136 cal/g respectively). Notonectids had the highest caloric content (Fig. 5). Treefrog eggs contributed a total of ~ 106,000 calories to all mesocosms over the experiment. *Hyla chrysoscelis* eggs contained 2.58 ± 0.11 calories per egg. A mix of *H. cinerea* and *H. chrysoscelis* eggs contained 3.38 ± 0.14 calories per egg. There was great range in the caloric content of dead terrestrial invertebrates falling into mesocosms (5626 ± 287 cal/g); this high variation may be because these inputs include moths, bees, spiders, dragonfly eggs, and terrestrial beetles, a diverse group. Deciduous leaves and woody material had the lowest caloric content, about 4680 cal/g (Fig. 5). The percent organic matter of inputs varied between 91 - 97% (Fig. 5). Hydrophilids, pine needles and wood and bark had high organic content ~ at 97 %, while treefrog eggs had the lowest percent organic content (*H. cinerea* and *H. chrysoscelis* mix had a mean of 91.58% organic matter; Fig. 5). Because we used 4 representative taxa to estimate the calorie and organic matter content of all aquatic invertebrates, we used the linear regression formulas: $\text{cal/g} = 41.10(\text{length mm}) + 5466.90$ and $\% \text{ organic matter} = 0.002(\text{length mm}) + 0.94$.

Overall, there was greater mass of passive allochthonous inputs relative to active inputs. Passive allochthonous inputs thus provided the greatest amount of calories to the
aquatic system, followed by aquatic invertebrates; treefrog eggs provided the least amount of calories. There was more organic matter input from passive allochthonous sources than active colonizers in all treatments (Fig. 6B). Hyla eggs represented <1 - 2% of all organic matter inputs (0.006 – 0.019 g AFDM per day). Treefrog oviposition resulted in 16 g AFDM being deposited over the entire experiment. In contrast, an average day’s worth of pine needles deposited 11 g AFDM, leaves 9 g AFDM, wood and bark 5 g AFDM, aquatic insects 5 g AFDM and insects 0.6 g AFDM.

**Effects of Predators on Energy and Organic Matter Inputs**

A repeated measures test on calorie input over time showed that the difference in amount of energy from Hyla eggs was marginally significant between treatments ($F_{2,24} = 3.014, P = 0.068$; Fig. 1B). However, the difference in the mean of calories from all input types was not significantly different between treatments ($F_{2,23} = 0.590, P = 0.562$; Fig. 6A). The mean of calories from total aquatic invertebrates was not significantly different between treatments ($F_{2,26} = 2.426, P = 0.108$; Fig. 3C). When considering calories from all input types combined, the mean calorie input for the free roaming fish predator was the highest (6608 ± 2139 cal/day), followed by the mean calorie input for the no predator treatment (4579 ± 1424 cal/day), the mean calorie input for the caged predator treatment was the smallest (4084 ± 1526 cal/day). The proportions of active energy input relative to the total of active and passive inputs were not significantly different (Fig. 6A; $F_{2,24} = 0.778, P = 0.471$ arc sin square root transformed data) between treatments. The proportion
of active energy input was less than half of the total of active and passive input (means 0.28 - 0.42; Fig. 6C).

A repeated measures test on organic matter input over time showed that the difference in g AFDM from *Hyla* eggs was marginally significant between treatments ($F_{2, 24} = 3.015, P = 0.068$). However, the difference in the mean of organic matter from all input types was not significantly different between treatments ($F_{2, 23} = 0.647, P = 0.533$). When looking at organic matter from all input types combined, the mean input for the free roaming fish predator was the highest ($1.28 \pm 0.42$ g AFDM/day), followed by the no predator treatment ($0.85 \pm 0.27$ g AFDM/day), and the mean input for the caged predator treatment was the smallest ($0.77 \pm 0.30$ g AFDM/day). The proportion of active organic matter input relative to the total of active and passive inputs was not significantly different among treatments (Fig. 6 B; $F_{2, 24} = 0.739, P = 0.488$ arc sin square root transformed data). The proportions of active organic matter inputs were less than half of the total of active and passive inputs (means 0.25 - 0.38; Fig. 6C).

**Abiotic Variables**

Mesocosm abiotic characteristics were generally not different between treatments. The mean oxygen saturations were similar between treatments but declined over the course of the experiment (Table 1). Mean oxygen saturations were highest (~45%) on June 25th a few days before the start of the experiment. On July 12th and September 5th the mean percent oxygen saturation dropped to ~30%. Temperature was monitored continuously from July 21st in 18 mesocosms every 5 minutes throughout the experiment using loggers.
The mean temperature from July 22\textsuperscript{nd} – September 5\textsuperscript{th} was not significantly different between treatments ($F_{2, 15} = 1.796$, $P = 0.200$).
CHAPTER 4 Discussion

A predator induced difference in colonization of *Hyla* treefrogs translated into small differences in energy and organic matter inputs between the no predator and caged predator treatments. We also observed habitat selection in some aquatic invertebrates; there was a significant difference in hydrophilid beetles caused by the presence of fish. Our research supports other studies that show predator-induced habitat selection from caged fish predators in treefrogs (Binckley and Resetarits 2002) and hydrophilid beetles (Resetarits 2001). This indicates a consistent response in these taxa. We observed a weaker predator avoidance effect (71%) compared to effect sizes seen in *Hyla squirella*, a closely related species (93%) (Binckley and Resetarits 2002). Predator avoidance was not seen in most invertebrate organisms, indicating that other factors, not fish predation, controlled habitat selection for these organisms. We were able to quantify predator-induced shifts in energy and organic matter using calorimetry and ash free dry mass. Fear caused by the presence of predators reduced colonization and oviposition of some taxa, slightly reducing active inputs measured as calories and g AFDM.

Furthermore, we found that predator avoidance in treefrogs decreased with time. Although not directly measured, competition could affect habitat selection, as seen in other studies. Our findings supported the idea that oviposition would switch back to replicates with fish when controls (no predator replicates) are densely filled with conspecifics (Binckley and Resetarits 2003). Competition of larvae for food resources would be higher
in dense environments. The log response ratio of the no predator: caged predator treatment has a less steep slope than the log response ratio of the no predator: free roaming predator treatment (Fig. 2), indicating the lethal effect diminished more than the non-lethal effect. This change in predator avoidance over time could also be due to colonization by other predators, such as dragonfly larvae that were observed in most mesocosms. We did observe morphological changes (long red tails) in tadpoles in no predator replicates. Treefrog tadpoles reared with predatory dragonfly larvae are known to differ in shape and color from tadpoles reared in the absence of predators; these phenotypes are induced by cues present when dragonflies prey on Hyla tadpoles (McCollum and Leimberger 1997).

Few studies compare animal subsidies to passive allochthonous inputs. Our results indicate that on a per gram basis, active animal inputs contained higher calories and percent organic matter than passive allochthonous inputs. We were able to quantitatively compare inputs over a short period of time. It has been suggested that amphibian reproduction is an important source of carbon to some aquatic systems. We wanted to know if active inputs represent a sizable fraction of calories and organic matter. We found that compared to allochthonous inputs, the amount of calories and organic matter from active colonizers is small. Thus, shifts in the active inputs due to predator avoidance do not affect enough biomass to have a large effect on energy flux to aquatic systems. The effect of habitat selection on active inputs was swamped by passive inputs, which were at least twice as large as active inputs and showed no significant difference between treatments. Our finding supports the idea that amphibian eggs correspond to a small portion of all yearly inputs compared to leaf litter (Regester et al. 2006). The importance of organisms
that choose to avoid aquatic systems with predators seems to depend on its context among other allochthonous inputs and autochthonous production. In our aquatic system, the proportion of active inputs out of all inputs did not change between treatments. This suggests that predators do not change the relative importance of active versus passive inputs, as we hypothesized. This result could also be due to high variation of passive inputs (standard errors are ~ 40% of mean) which change the proportion of passive to active inputs. The differences in proportion are driven by differences in amounts of passive inputs. We were able to evaluate the relative amount of energy and organic matter from active and passive sources and we determined that changes in colonization and oviposition did not significantly affect the ratio of these potential resources to our aquatic system.

In terms of quantifying the inputs of organic matter, we may be slightly underestimating allochthonous amounts. Since the mesocosm walls were ~ 0.6 m above ground level, we only allowed direct inputs (vertical movement) and not lateral inputs (horizontal movement). Direct allochthonous inputs were ~ 90% of inputs to forested beaver ponds compared to 10% from lateral inputs (Naiman et al. 1986). So, we can assume that direct passive allochthonous inputs are the dominant passive allochthonous input. To put amphibian contributions into context, a previous study used leaf litterfall estimates from forested beaver ponds. Energetic inputs of salamander eggs in vernal pools represented up to 5 - 15% of direct allochthonous inputs and up to 16 - 240% of lateral inputs to these beaver ponds (Regester et al. 2006). In our study, carbon inputs from treefrog eggs represented 1 - 2% of our observed direct allochthonous inputs. We thus found a smaller carbon input from treefrogs than of other amphibian species previously
studied, but we are using a passive allochthonous input estimate generated within the same system.

Treefrogs and aquatic beetles strongly avoid oviposition in closed canopy areas (Binckley and Resetarits 2007). Open canopy areas likely have less passive allochthonous inputs than forest. Our passive allochthonous input rate is less than other estimates from interior forest, reflecting distance to the forest edge. Placement of mesocosms in the field was 20 m or greater from the tree line, thus spatial location influences leaf litter inputs, affecting the ratio of passive to active inputs. Our initial input of 300 g leaf litter (~ 1450 kcal) represents 25 - 40% of annual leaf litterfall amounts to a forested stream in Virginia’s Coastal Plain (Smock 1997).

This experiment was conducted over a relatively short time frame, and experiments in which subsidies are studied over the long-term might give a clearer picture (Baxter et al. 2005). Gray treefrogs (*Hyla versicolor*) breed April – August (Martof 1980), so we most likely missed breeding events in April, May and June because our study was conducted in July and August. This likely caused an underestimation of their seasonal contribution. Considering that there is a highly seasonal impact of amphibian reproduction and beetle colonization, we would expect to see the differences when we did the experiment. The most important input to pools may be leaves in autumn, whereas in spring active inputs may be most important. It was better to have a long estimate for treefrog eggs because they are laid after rain in a highly pulsed manner. We assume leaf litter fell at a more even rate during the summer, although this could also be influenced by storm events.
Amphibian eggs of *Rana*, *Acris* and *Hyla* provided 2 - 3 mg C/m²/day to a fishless pond (Seale 1980). This input can be compared to our no predator treatment by converting percent carbon to percent organic matter (g AFDM) by multiplying carbon by two (personal comment Bukaveckas). This converts to ~ 4 - 6 mg OM/m²/day. We observed 9 mg OM/m²/day in the no predator treatment; thus our control treatment received comparable organic matter inputs from amphibian eggs compared to a natural small pond. Caged predator and fish predator treatments received less input at 2 - 4 mg OM/m²/day respectively. Ambystomatid salamanders added ~ 15 – 61 mg OM/m²/day to vernal pools (Regester et al. 2006), which was 2 – 7 times higher than our observed amphibian input. Overall, carbon inputs from amphibians to our experimental system were of a similar magnitude to those found in natural systems, suggesting that our results can be generalized to natural ecosystems.

The quality analyses of inputs yielded similar results to previously published values. The amount of calories per egg that we found was similar to other *Hyla* that deposit eggs in water (Crump and Kaplan 1979). We observed similar caloric values of leaves (4404 - 4958 cal/g) as found in another study using the same calorimeter (*Acer* = 4490 and *Quercus* = 4880), but with a slightly higher standard error (Schroeder 1977). The slight difference in variation could be due to multiple species of tree leaves and deciduous material in our samples. Treefrog eggs contained approximately 46% carbon, this value was also reported for Ambystomid egg clutches (Regester and Whiles 2006). Similar quality values suggest that other studies could extrapolate the effect of predators to calories
and carbon simply by using published values of inputs, which would assist in understanding the magnitude and importance of predation on energy and carbon fluxes.

Lethal and non-lethal predators have similar effects on energy and organic matter inputs into aquatic systems. The two predator treatments had overall the same affect on total active calories and total active organic matter inputs, but their effects on colonization and oviposition were not the same. One unexpected result was that the caged predator treatment tended to have a stronger habitat selection effect than the free-roaming predator. This effect was significant for hydrophilid beetles. Another study found the caged predator treatment had an effect on invertebrates similar to no predator treatments, while the free roaming predator reduced species richness and biomass (Abjornsson et al. 2002). Their observed effect was due to direct predation on invertebrates, which was prevented in our study by the mesh covers that effectively protected colonists from consumption. Thus, a better experimental method is needed to determine lethal effects on invertebrate colonization.

We studied small open canopy pools which have more sunlight than vernal pools, thus, internal autochthonous production is a source of carbon and calories and should be compared to active inputs. A previous study in an open pond showed that amphibian oviposition was a comparatively minor source of allochthonous organic matter, when evaluated against autotrophic production (Seale 1980). In our study, we did not directly measure algal production, and dissolved oxygen experiments to estimate production and respiration were unsuccessful. The dissolved oxygen measurements tell us a little about autochthonous production. Oxygen saturation was very low, suggesting mesocosms were a
heterotrophic environment where respiration dominates, as is typical for vernal pools. This may be due to 300 g of leaves added at initiation of the experiment.

What is the relationship between allochthonous inputs and subsidies? Thinking about subsidies in terms of the food web, inputs are only subsidies if they change the receiving food web. Leaves decompose, so they are consumed and become subsidies. Any input that is directly eaten by an aquatic predator becomes a subsidy. Only the fraction of eggs that does not survive to metamorphosis is a subsidy. Metamorphic frogs could indeed be exporting aquatic energy to terrestrial habitats. Aquatic invertebrates that do not leave, stay in the system and are consumed are true subsidies. Decomposing eggs subsidize different levels of the food web than eggs eaten by an aquatic predator. Since we did not determine the fate of most inputs, we can only consider them a potential subsidy.

Despite finding that amphibian eggs represent only a small percent of all inputs, we focused on organic matter quantity, not quality. As we have shown, the quality of the input in terms of calories and AFDM was different depending on its source. The food web may use some inputs faster and more efficiently than other sources. For example, a study of egg lability found that salamander eggs decay rapidly, compared to slowly degrading vegetation (Regester and Whiles 2006). So, despite the fact that treefrog eggs make up a small percentage of overall inputs, they may be consumed faster, representing a high quality input to the food web in the summer months. What is the effect of this active subsidy on the community? Members of the family Dytiscidae are known to readily consume frog eggs (Henrikson 1990). Would aquatic systems be a less diverse community
without these labile amphibian inputs? The effects of highly seasonal amphibian subsidies is largely untested (Regester et al. 2006).

This study helps quantify the relative importance of inputs from amphibian reproduction and invertebrate colonization versus other inputs. Although it has been speculated that these active inputs contribute a significant source of organic matter and energy, our study shows that in open pool aquatic systems, passive inputs like leaf litter contribute larger amounts. So, the effect of predators, although large in some systems, did not significantly affect overall inputs to open pools. The broader significance is that certain habitats may be more affected by predators, such as those that receive fewer passive inputs (i.e. aquatic systems with less shoreline). Future studies could assess longer time periods and more complete carbon budgets for open pool systems, to understand how carbon moves across the land-water ecosystem boundary facilitated by animals with complex life histories.
Literature Cited


- Allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. The American Naturalist 147:396.


### Table 1. Morphospecies of Aquatic Invertebrates at the Rice Center and Harrison Lake in 2007.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family Name</th>
<th>Scientific Name</th>
<th>Authority</th>
<th>Abundance</th>
<th>Total Length (mm)</th>
<th>Voucher Number</th>
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Table 2. Mean percent oxygen saturation (± SE) for mesocosms at the Rice Center and Harrison Lake

<table>
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<tr>
<th>Date</th>
<th>Treatment</th>
<th>No Predator</th>
<th>Caged Predator</th>
<th>Free Roaming Predator</th>
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<td>46.1 ± 1.6</td>
<td>45.2 ± 1.7</td>
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<tr>
<td>7/12/2007</td>
<td>29.3 ± 2.8</td>
<td>29.5 ± 1.9</td>
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<td>9/5/2007</td>
<td>30.3 ± 1.8</td>
<td>28.7 ± 1.9</td>
<td>29.8 ± 1.8</td>
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FIGURE LEGENDS

**Figure 1.** Effects of predators on mean number and calories of *Hyla* eggs. A) Mean number of eggs per mesocosm per treatment was of 2 different species of treefrog because we saw both in some tanks. NONE mesocosms got more eggs early on. Treatments are NONE = no fish (n = 10), CAGED = 3 caged fish (n = 8), and FISH = 3 free roaming fish (n = 10). Standard error bars are 1 SE. Repeated measures showed a significant difference in eggs ($F_{1, 15} = 4.596, P = 0.049$) of log (x + 1) transformed data. Outliers were dropped. B) Mean calories per mesocosm per treatment from *Hyla* eggs.

**Figure 2.** The relationship between *Hyla* predator avoidance and date. As the experiment progresses, the effect of predation risk decreases and the effect size gets smaller. Treatments are N = no fish (n = 10), C = 3 caged fish (n = 8), and F = 3 free roaming fish (n = 10). The log response ratio of the no predator: caged predator treatments (dashed line) has a less steep slope than the log response ratio of the no predator: free roaming predator treatments (solid line), indicating the lethal effect diminished more than the non-lethal effect ($r^2 = 0.645$, intercept: $1.960 \pm 0.475$ slope: $-0.057 \pm 0.019$). The regression was significant for the relationship between predator avoidance and date ($F_{1, 5} = 9.072, P = 0.030$). The regression was only marginally significant for the relationship between predator avoidance and date of the no predator: free roaming predator treatments ($F_{1, 5} = 4.928, P = 0.077$).
**Figure 3.** Mean hydrophilid abundance, mean of invertebrate morphospecies per day and mean calories of aquatic invertebrates. **A)** Aquatic invertebrate colonization 2 - 3 days after initiation of the experiment. Shown is a mean of the total of all morphospecies per mesocosm per treatment. The total morphospecies was not significantly different between treatments ($F = 2.897, P = 0.075$). Treatments are NONE = no fish (n = 10), CAGED = 3 caged fish (n = 10), and FISH = 3 free roaming fish (n = 10). **B)** Hydrophilid beetle colonization 2 - 3 days after initiation of experiment was greater in NONE treatment ($F = 4.124, P = 0.029$). Shown is a mean of the total of hydrophilid morphospecies per mesocosm per treatment. A significant difference was seen in post hoc tests between NONE and CAGED ($F = 8.190, P = 0.011$) and FISH and CAGED ($F = 4.752, P = 0.045$). **C)** Mean calories per mesocosm per treatment from aquatic invertebrates.

**Figure 4.** Passive allochthonous inputs over 3 predator treatments. Mean mass (g day$^{-1}$ mesocosm$^{-1}$) and 1 standard error are reported. We think the variation seen in WOOD represents natural variation in material falling into mesocosms. Treatments are NONE = no fish (n = 10), CAGED = 3 caged fish (n = 10), and FISH = 3 free roaming fish (n = 9); one outlier was removed. PINE is pine needles, LEAF is deciduous leaf litter, WOOD is woody material, thick stems and bark, and INVERT are terrestrial invertebrates like spiders moths and bees. We consider these materials to be passive allochthonous inputs because they fell into mesocosms and were not actively choosing to fall. Inputs were collected for 2 - 3 days from initiation of the experiment, and divided by # of days to get g day$^{-1}$. The total input
is the sum of the 4 input types and the ANOVA of the log (x + 1) transformed data was not significantly different over the treatments ($F_{2, 26} = 1.634$, $P = 0.215$).

**Figure 5.** Calorie and organic matter content of active and passive inputs. Calories (cal g$^{-1}$) and organic matter content (% organic matter) showed different patterns. Samples from randomly selected replicates were analyzed ($n = 3$). Material was collected for 2 - 3 days from initiation of the experiment. *Notonecta irrorata*, *Laccophilus fasciatus*, *Berosus infuscatus* and *Tropisternus collaris striolatus* are common adult aquatic invertebrates that colonized the mesocosms. Gray treefrog eggs and a mix of gray and green treefrog eggs were analyzed separately. The caloric content of active inputs (habitat selecting organisms) was higher than the passive allochthonous inputs.

**Figure 6.** Active versus passive inputs per day per treatment. **A)** Active inputs bring fewer calories than passive ones. Passive calories are at least twice the amount of active calories. There was more variation in the amount of passive calories, however there was not a significant difference between treatments. **B)** This pattern also was seen in active versus passive g AFDM ash free dry mass. **C)** The proportion active out of total calories and organic matter does not change with predation risk.
Figure 1. Effects of predators on mean number and calories of *Hyla* eggs.
Figure 2. The relationship between *Hyla* predator avoidance and date.
Figure 3. Mean hydrophilid abundance, mean of invertebrate morphospecies per day and mean calories of aquatic invertebrates.
Figure 4. Passive allochthonous inputs over 3 predator treatments.
Figure 5. Calorie and organic matter content of active and passive inputs.
Figure 6. Active versus passive inputs per day per treatment.
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