2017

Evidence Of Chemical Camouflage In Pirate Perch (Aphredoderus Sayanus); Avoiding Detection By Colonizing/Ovipositing Prey Through Chemical Crypsis

Tyler Breech
University of Mississippi

EVIDENCE OF CHEMICAL CAMOUFLAGE IN PIRATE PERCH (*APHREDODERUS SAYANUS*); AVOIDING DETECTION BY COLONIZING/OVIPOSITING PREY THROUGH CHEMICAL CRYPSIS

Master of Science Degree

University of Mississippi

Department of Biology

Tyler Breech

13 May 2017

ABSTRACT

Prey adaptations, such as avoidance of predators, larval life history traits, and competition abilities, are potentially important in shaping community and metacommunity structure. One prey adaptation observed in aquatic ecosystems is the ability of prey to detect the presence of fish through water or air via kairomones released by fish. Non-visual camouflage, or non-visual crypsis, is a trait of a signal sender that hinders the receivers' ability to determine the presence or location of the sender. The pirate perch, *Aphredoderus sayanus*, presents an interesting potential example of chemical camouflage. The objectives of this study were to: 1) determine if ammonia is a kairomone detected and avoided by ovipositing species, 2) determine the possibility of a chemical cue masking ability in the pirate perch, and 3) assess the potential for chemical crypsis. These objectives were examined through measurement of oviposition preferences by mosquitoes, tree frogs, and beetles. In the study there was no evidence of a masking capability in pirate perch, and ammonia was not the detected chemical cue used by mosquitoes. Higher densities of pirate perch showed a trend of a negative impact on oviposition preference in both mosquitoes and tree frogs. The results indicated pirate perch are chemically camouflaged likely through low cue production to avoid detection.

TABLE OF CONTENTS:

LIST OF TABLES:

LIST OF FIGURES:

CHAPTER 1

INTRODUCTION

In the coevolutionary arms race between predators and prey, predators evolve new "weapons" or adaptations for consumption of prey, and prey consequently evolve "escape" mechanisms (Thompson 2013). Prey typically evolve defensive adaptations more rapidly than predators evolve prey hunting adaptations. This is attributed to the "lunch versus life principle;" if a predator fails to catch prey, it only loses out on its lunch, but if prey fails to escape a predator, it loses its life (Thompson 2013). There is thus a higher cost, or selective pressure, on prey to adapt in response to predators than predators to adapt in response to prey (Thompson 2013).

Prey adaptations, such as avoidance of predators, larval life history traits, and competition abilities, are potentially important in shaping community and metacommunity structure (Wellborn et al 1996). One prey adaptation observed in aquatic ecosystems is the ability of prey, (e.g tree frogs, beetles, and mosquitoes) to detect the presence of fish through water or air via kairomones released by fish (Bronmark and Hansson 2000; Wisenden 2000; Binckley and Resetarits 2005; Silberbush and Blaustein 2008; Resetarits and Binckley 2013a; Eveland et al 2016). Kairomones are cues that are beneficial to the receiver but not the sender. Chemoreception allows prey species to maximize fitness through individual survival or offspring survivorship by avoiding ponds containing fish (Resetarits 2001; Eveland et al 2016). The energy expenditure involved in avoiding ovipositing where predators are detected is the only

form of parental care exhibited by many tree frogs, beetles, and mosquitoes (Resetarits and Wilbur 1989; Rieger et al 2004; Binckley and Resetarits 2005; Resetarits and Binckley 2009; Resetarits and Binckley 2013b). Predator detection is particularly important in aquatic beetles that typically disperse only once in their lifetime because the end point of dispersal becomes both their own foraging patch as well as the habitat patch for the early life stages of their offspring (Zalom et al. 1979; Zera and Denno 1997).

Chemoreception works by the same method as other forms of communication; a sender sends a signal, whether it be visual, auditory, chemical, and a receiver receives the signal and interprets the information from that signal. However, there are various ways in which the process can be altered by sender, receiver, or even environment, including attempted camouflage of a signal by the sender. Ruxton (2009) defines non-visual camouflage, or non-visual crypsis, as a trait of a signal sender that hinders the receivers' ability to determine the presence or location of the sender. This differs from mimicry, where a sender has traits that attempt to fool the receiver into misidentifying the sender, and hiding, where the sender does not emit a signal (Ruxton 2009). While camouflage is most often studied in a visual context, more emphasis has been placed on other sensory modalities in recent years (Breed et al. 1992; Atema 1995; Bronmark and Hansson 2000; Ruxton 2009). For example, chemical camouflage has been shown in ant species (Breed et al. 1992) and potentially other invertebrate species (Akino et al. 2004; Portugal and Trigo 2005; Raffa et al. 2007). Others have shown potential evidence, or suggested the possibility, of camouflage in other detectable signals such as sound (Belwood and Morris 1987; Rednondo and De Reyna 1988), electrical signals produced by fish (Stoddard and Markham 2008), and even wake trails left by animals moving through the water (Ruxton 2009). In the aquatic environment, chemoreception is likely an important sensory system utilized for

predator detection. However, there is some evidence of chemical camouflage by signal senders (Fishlyn and Phillips 1980), as well chemical signature hiding (Atema 1995; Brown et al. 1995) in response to the use of chemical cues by many species.

The pirate perch, *Aphredoderus sayanus* (Gilliams) presents an interesting potential example of chemical camouflage. In previous studies, prey species have failed to avoid pirate perch when ovipositing. (Resetarits and Binckley 2013a). The pirate perch is a small nocturnal freshwater fish that rarely grows larger than 14.5 cm. It is the closest living relative of the cave fishes, and is the only species in its family Aphredoderidae (Ross 2001). The species is widespread in the United States, inhabiting much of the area east of the Mississippi River. It is known to eat a wide array of invertebrate species, as well as other fish, tadpoles, and crustaceans (Shepherd and Huish 1978; Ross 2001). Given its predatory preferences, it is surprising that there are no known examples of ovipositing prey species actively avoiding the pirate perch's presence, including prey with the ability to detect, and accurately avoid, chemical signatures of other fish predators (Resetarits and Binckley 2013a, unpub. data). In fact, it has been suggested that the pirate perch is a chemical "ghost" and is able to mask its chemical signature in the environment (Resetarits and Binckley 2013a).

The specific fish kairomone(s) detected by ovipositing prey species is(are) currently unknown, although one chemical that ovipositing mosquitoes respond to have been identified in a predatory aquatic beetle (Silberbush et al. 2010). In aquatic environments, the common fish waste product is ammonia, with a lower percentage of waste excreted as urea (Forster and Goldstein 1969; van Waarde 1983; Engin et al 2013). Given the elevated ammonia concentrations in bodies of water containing fish, ammonia has the potential to be a major component of the fish kairomone signal detected by ovipositing species.

Many of the fish species avoided by ovipositing organisms are small, highly active fishes, such as western mosquitofish (*Gambusia affinis)* and golden topminnows (*Fundulus chrysotus)* (Eveland et al 2016; unpub. data). Since activity and metabolic rates are intrinsically linked, these fishes most likely have high metabolic rates. Since ammonia production is linked to metabolic rate and feeding rate, these fishes likely also have higher ammonia excretion rates (Brett 1972; Brett and Zala 1975; Schalles and Wissing 1976; van Waarde 1983). Additionally, other avoided fishes that are not highly active are often heavily carnivorous (Resetarits lab unpub. data), which leads to a higher ammonia rate due to protein breakdown (van Waarde 1983; Brunty el al. 1997). This suggests that ammonia may be an important component in detecting chemical signatures.

Observations of pirate perch from multiple sources indicate that it moves little during the day (pers. obs.; Bohenek pers. comm.). Therefore, pirate perch may have a lower metabolic rate and ammonia excretion rate compared to other fish species. Studies comparing metabolic rates of epigean (surface) and hypogean (below ground, or cave) fishes usually indicate a lower metabolic rate in hypogean fish (Huppop 1986), although other studies have reported the opposite results in some species (Huppop 1986; Passow et al. 2015). This lowered metabolic rate is likely an adaptation to deal with reduced food availability, but could also be advantageous in low dissolved oxygen environments (Huppop 1986; McCue 2010). Because pirate perch are the closest relative of cave fish, it is possible that pirate perch have a decreased metabolic rate. Lower metabolic and ammonia excretion rates could reduce the potential for detection by prey species and may account for chemical camouflage in pirate perch. Even if the detected kairomone is not ammonia, a lower metabolic rate could result in lower kairomone production. Alternatively, pirate perch could be camouflaged by a secondary masking compound. This could

occur through a compound excreted by pirate perch that either blocked reception of the chemical cue by chemoreceptors in the prey, or degraded the detected compound before reception by ovipositing species took place. Although masking chemicals have not yet been verified to exist in a vertebrate system, they would be beneficial if the detected cue was an unavoidably produced metabolic by-product.

The objectives of this study were to: 1) determine if ammonia is a kairomone detected and avoided by ovipositing species, 2) determine the possibility of a chemical cue masking ability in the pirate perch, and 3) assess the potential for chemical crypsis.

I hypothesized that ammonia was the chemical cue detected by prey species when ovipositing, and these species avoid ammonia levels above a certain threshold. I predicted mosquitoes would avoid laying eggs in pools with experimentally manipulated high ammonia concentrations. I thus hypothesized pirate perch chemically camouflage themselves by producing low levels of ammonia. I predicted that if cue levels increased due to greater pirate perch density, that a minimum detection threshold may be reached leading to a significant repellent effect on oviposition. Additionally, I predicted that pirate perch would not be able to camouflage other fish species. I tested ammonia effects on oviposition, as well as the potential for masking other fishes' presence and increased density effects on oviposition, using naturally occurring mosquitoes. I also tested the effects of increased density of pirate perch on tree frog oviposition preferences and beetle colonization preferences.

CHAPTER 2

METHODS

Ammonia as a chemical cue

To determine if ammonia is an important chemical cue used by ovipositing prey to detect predators, I tested the effect of ammonia on oviposition preference of mosquitoes. This was tested with ammonia treatments in a circular array of pools in a small field at the University of Mississippi Field Station (UMFS). A single wading pool 0.914 m in diameter was placed in the center of an open field ("center pool") and filled with water to 7 cm below the rim. One half kilogram of leaf litter and 20g of rabbit chow were added immediately following filling to stimulate the growth of bacteria and attract mosquitoes. This center pool was only used to attract mosquitoes for oviposition, and was tightly covered with window mesh above the water level to prevent oviposition by mosquitoes in it. Around the center pool, 8 30-L black, rectangular pools were placed equidistant from each other 5 m from the center pool. One tenth of a kilogram of leaf litter was placed in each rectangular pool immediately after filling to serve as a nutrient base. Treatments alternated in adjacent pools, resulting in four replicates of each treatment. Treatments consisted of either 7 mol/L ammonia in methanol or pure methanol. Naturally occurring ammonia levels in fishless pools varied between 3-5 mg/L in previous pools from a separate experiment, so ammonia treatments were maintained near 11 mg/L, as measured by a YSI ammonia probe, which was the average level in fish pools from the same previous experiment. Ammonia was diluted in methanol, thus methanol was used as a control, and was

added to control treatments in an amount equal to ammonia treatments. Mosquito egg rafts were removed and counted every day for four days, and the ammonia checked daily to ensure maintenance as close to 11 mg/L as possible. The egg raft count data was analyzed using an analysis of variance (ANOVA).

Masking ability and increased density

The potential of pirate perch to camouflage other predatory fish species and the effect of increased pirate perch density on mosquito oviposition preferences were determined with a method similar to Eveland et al (2016). Six arrays of four wading pools 0.914 m in diameter, each pool containing 0.1 kg of leaf litter and two holding cages, designed to hold and predation by the separate fish species used in the treatments, were placed in a straight line array in an open field at UMFS one meter from the forest edge. The holding cages were plastic pots 30.48 cm in diameter closed at the top with window screen and two side windows cut out and covered with window screen to allow for water flow through the cage. Each pool in an array was randomly assigned one of four treatments; no fish, three pirate perch, one mosquitofish (*Gambusia affinis* Baird & Girard), or both fish species treatments. Previous experiments have shown one mosquitofish to have significant negative impact on mosquito oviposition preference, and three pirate perch were used to test the increased density effects of pirate perch on mosquito oviposition preference. Each pool in an array was separated by two and a half meters from center to center, and the end pools of different arrays were separated by 15 m from edge to edge (Fig. 1). Each pool was filled with unchlorinated ground water from a nearby well until overflowing, and after filling, fish were randomly assigned and placed in separated holding cage by species. Daily counts and removal of mosquito egg rafts in the pool were conducted each

morning for five consecutive days. Data were analyzed using an ANOVA, and significance was examined using a Tukey's HSD post-hoc test.

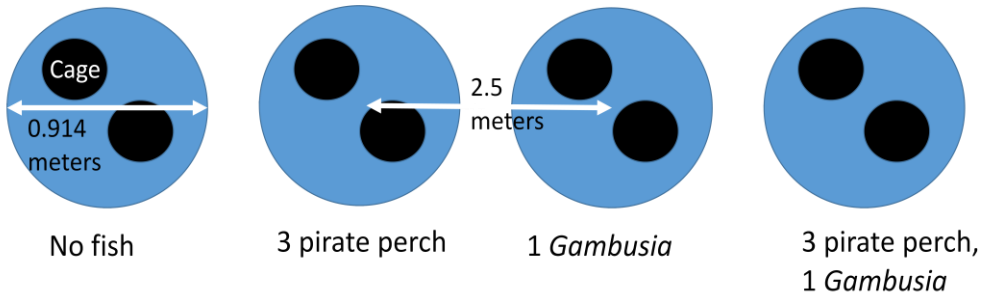

Figure 1: Schematic of a single block in an experiment testing for possible masking effects and density effects on mosquito oviposition. The same experimental design and methods were used for another masking effect experiment, except treatments were No fish, one golden topminnow, one pirate perch, and Both species.

The same methods were used in a second experiment designed to test potential masking ability of pirate perch with another species, golden topminnows (*Fundulus chrysotus* Günther), as well as demonstrate the non-detection (or non-avoidance) of pirate perch by mosquitoes. Golden topminnows have been shown in previous lab experiments to have a significant repellent effect on mosquito oviposition when present. This provided a comparison of mosquito oviposition preference in a low density treatment, as well as further insight into the potential camouflaging abilities in the pirate perch. The four treatments in the second experiment were no fish, one pirate perch, one golden topminnow, or one pirate perch and one golden topminnow. Data were analyzed in the same manner.

Density effects on tree frogs and beetles

The effects of increased density of pirate perch on oviposition habits of tree frogs and colonization of beetles were measured using cattle tanks. Four arrays of three cattle tanks 1.83 m in diameter were set up in a line no closer than 1.5 m from the forest edge in an open field at UMFS. Three treatments were randomly assigned to each block of three pools: no fish (Fishless treatment), 2 pirate perch (Low density treatment), and 12 pirate perch (High density treatment) (Fig. 2).

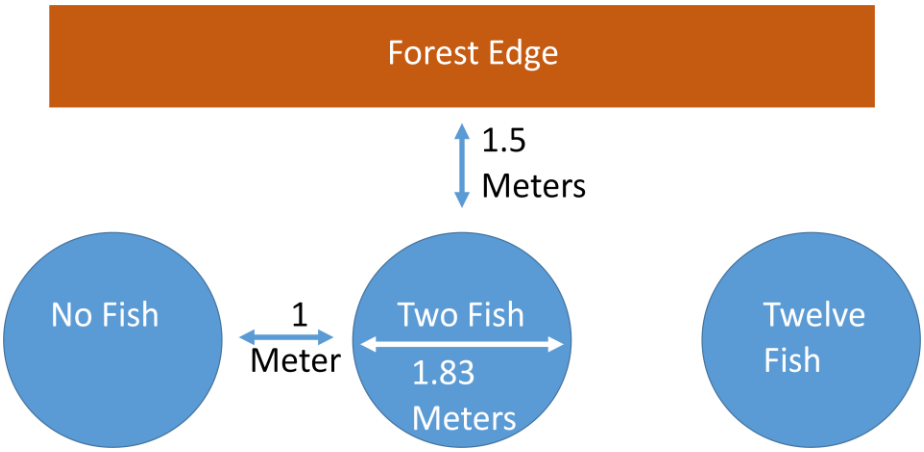

Figure 2: Schematic of a single block in an experiment testing for treefrog oviposition and beetle colonization responses to varying densities of pirate perch.

The two pirate perch treatment had a biomass greater than 15 g, and the 12 pirate perch treatment had a biomass greater than 50 g to account for biomass effects in each treatment in each block. Fish biomass per liter has been suggested to have an effect on oviposition preference, as so was accounted for to remove this confounding factor. Each tank had 1 kg of leaf litter added and was filled with unchlorinated ground water to 5 cm below the rim. To maintain water level, each tank had a stand pipe to allow for excess water to drain, and every tank was refilled to the starting level every two weeks as needed. The filled pools were covered in window screen which was then submerged below the water level. The tanks were checked for

frog eggs daily. Any eggs were removed from the tanks, photographed in a limited plane to eliminate depth of field problems, and placed in a natural, fishless pond. The photographs were processed in ImageJ to quantify the number of eggs laid. The technique used has been previously quantified and tested, and has been found to be within ~95% accuracy as hand counting eggs, while saving exponential amounts of time. Beetles were collected once a week from on top of the submerged screen tops, sorted, and identified as close to species as possible. Frog egg data were analyzed using an ANOVA, using a square root transformation on count data to approach normality; paired comparisons used Tukey's HSD post-hoc test. Beetle assemblage response was tested using a PERMANOVA on species abundance data. Total beetle numbers, as well as data for two specific families (*Hydrophilidae and Dytiscidae*), were analyzed using separate ANOVAs.

CHAPTER 3

RESULTS

Ammonia as a chemical cue

In the ammonia detection experiment, a total of 404 mosquito egg rafts were collected from both treatments over the course of four days; 216 from ammonia treatments and 188 from control pools (Fig. 3).

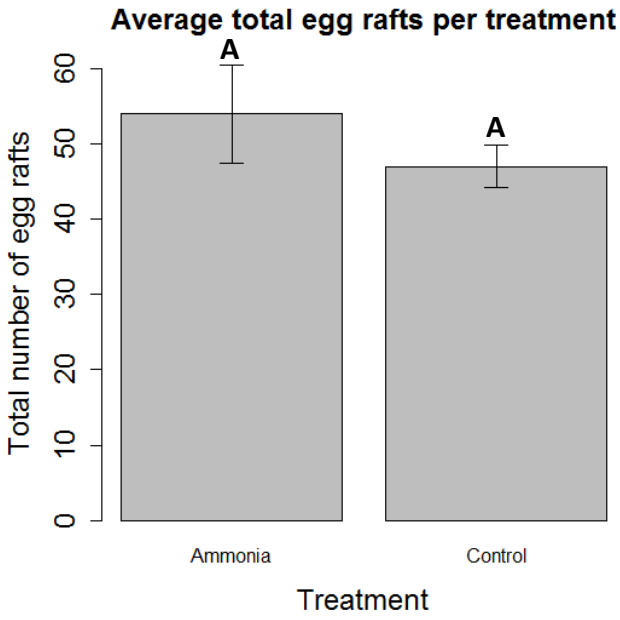

Figure 3: Average total number of mosquito egg rafts in Ammonia and Control pools. Ammonia used was 7 mol/l ammonia in methanol, so Controls contained an equal volume of methanol. There was no significant difference between treatments (p=0.339). Error bars represent standard deviation.

An ANOVA showed no significant difference in the number of mosquito egg rafts between the treatments ($F_{(1,6)}$=0.99, p=0.36) (Table 1). Thus, ammonia does not affect mosquito oviposition site preference.

Table 1: ANOVA table for effect of ammonia on mosquito oviposition.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	1	98	98.000	0.9866	0.3589
Residuals	6	596	99.333		

Masking ability and increased density

In the first masking ability treatment experiment, a total of 2654 mosquito egg rafts were collected from all treatments over the course of six days; 1367 from the fishless treatments (Fishless), 969 from 3 pirate perch (PP3), 179 from *Gambusia* treatments (Gambusia), and 139 from pirate perch + *Gambusia* treatments (Both) (Fig. 4).

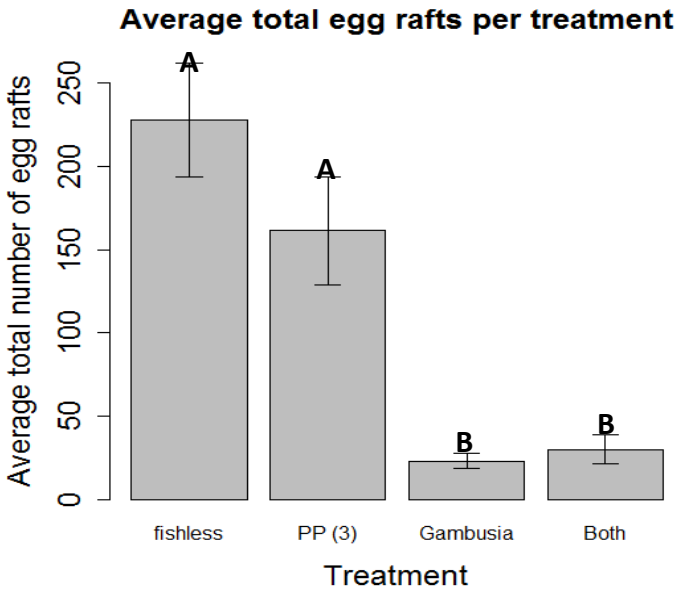

Figure 4: Average total number of mosquito egg rafts per treatment pool. Treatments were: Fishless=No fish, PP(3)=Three pirate perch, Gambusia=One mosquitofish, Both=Both three pirate perch and one mosquitofish. Error bars represent standard deviation.

An ANOVA indicated a difference among treatments ($F_{(3,15)}$=17.52, p<0.0001, Table 2), and Tukey's post-hoc tests revealed Fishless had a marginally significant higher number of egg rafts than PP3 (p=0.094), and significantly higher egg raft numbers than Gambusia (p<0.0001), and Both (p<0.0001). PP3 had significantly more egg rafts than Gambusia (p=0.0004) and Both (p=0.0008) as well. Gambusia and Both did not differ significantly (p=0.99). There was no evidence of masking capabilities in pirate perch on *Gambusia*, as there was no difference between Gambusia and Both. There was a marginally significant effect of increased density of pirate perch on mosquito oviposition site preference as compared to fishless treatments with the trend being mosquitoes avoiding the 3 pirate perch treatment.

Table 2: ANOVA and Tukey's post hoc test table for the first density and masking effect on mosquito oviposition experiment.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	183028	61009	29.7519	1.466e-06 ***
Block	5	38877	7775	3.7918	0.02026 *
Residuals	15	30759	2051		

Comparison	P adj
Fishless-Both	0.0000092
Gambusia-Both	0.9929964
PP (3)-Both	0.0007705
Gambusia-fishless	0.0000060
PP (3)-fishless	0.0941180
PP (3)-Gambusia	0.0004651

In the second masking ability treatment experiment, a total of 3432 mosquito egg rafts were collected over six days; 1225 from fishless treatments (Fishless), 1075 from 1 pirate perch (PP), 590 from golden topminnow (FC), and 542 from Combined (Fig. 5).

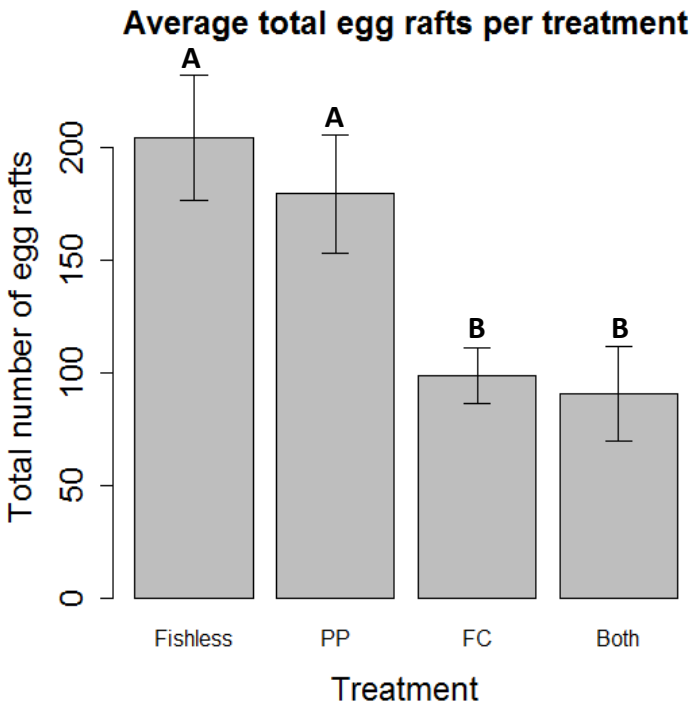

Figure 5: Average total number of mosquito egg rafts per treatment pool. Treatments are as follows: Fishless=No fish, PP=One pirate perch, FC=One golden topminnow, Both=Both one pirate perch and one golden topminnow. Error bars represent standard deviation.

An ANOVA showed a significant difference between treatments ($F_{(3,15)}$=22.90, p<0.0001, Table 3), and a Tukey's post-hoc test revealed Fishless received significantly more mosquito egg rafts than FC (p<0.00001) and Both (p<0.00001). Fishless and PP were not significantly different (p=0.47). PP received significantly more egg rafts than FC (p=0.0012) and Both (p=0.0005). FC and Both did not significantly differ (p=0.96). There was no evidence of pirate perch being able to mask golden topminnows, as Combined and FC did not significantly differ. There was also no difference between Fishless and PP, providing further evidence that a single pirate perch are undetected by mosquitoes when the mosquitoes are moving to oviposit.

Table 3: ANOVA and Tukey's post hoc test table for the second masking effect on mosquito oviposition experiment.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	3	58910	19636.6	22.896	7.484e-06 ***
Block	5	48733	9746.7	11.364	0.0001135 ***
Residuals	15	12865	857.7		

	P adj
FC-Both	0.9638515
Fishless-Both	0.0000360
PP-Both	0.0005046
Fishless-FC	0.0000813
PP-FC	0.0012404
PP-Fishless	0.4735978

Density effects on tree frogs and beetles

A final total of 36,754 tree frog eggs, nearly all *Hyla chrysoscelis*, were collected over 63 days from all tanks in the experiment; 4679 total from high density treatments (High), 21,227 total from low density treatments (Low), and 10,848 total from no fish treatments (None) (Fig. 6).

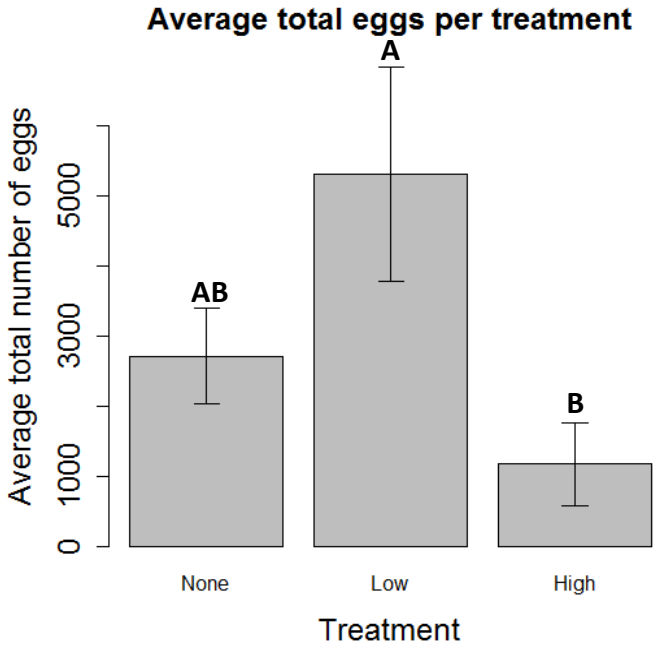

Figure 6: Average total number of tree frog eggs in each treatment pool of a density experiment. High treatments had 12 pirate perch, Low had 2 pirate perch, and None had no fish in it. Error bars represent standard deviation.

An ANOVA indicated significant differences between treatments ($F_{(2,9)}$=4.60, p=0.042, Table 4), and Tukey's post-hoc tests revealed High received significantly fewer frog eggs than Low density (p=0.034), but did not differ from None (p=0.28). Low and None were not significantly different (p=0.37). Low was attractive compared to High, but did not differ significantly from None. High and None did not differ significantly, which indicated that Low was the preferred habitat by ovipositing tree frogs.

Table 4: ANOVA and Tukey's post hoc test table for frog egg data after square root transformation.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	3471.0	1735.49	4.6064	0.04192 *
Residuals	9	3390.8	376.76		

	P adj
Low-High	0.0343284
None-High	0.2889243
None-Low	0.3721861

We collected 1415 specimens from 32 different species of beetles over the course of the experiment. A PERMANOVA determined there was a significant effect of block on beetle assemblages (p=0.001), but not treatment (p=0.526). An NMDS plot was created which visually showed no species sorting trends by treatment (Fig. 7).

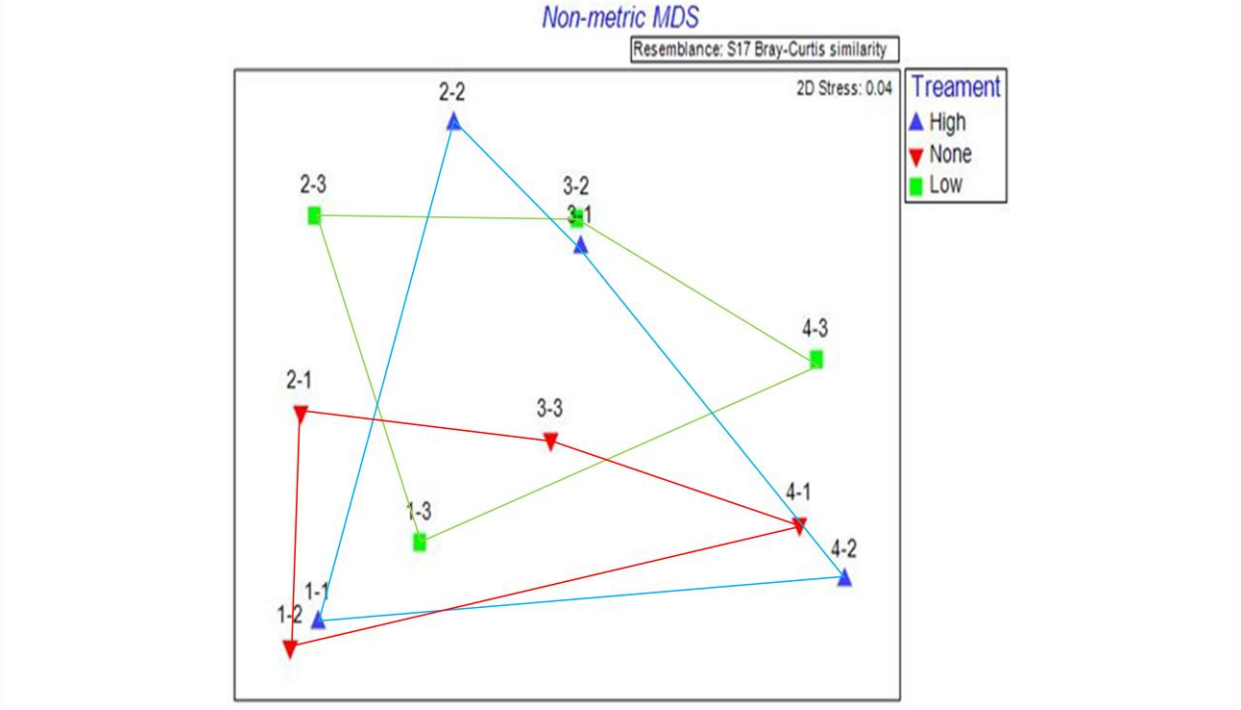

Figure 7: NMDS plot of beetle species in the same experiment as the tree frog eggs, with polygons drawn for each treatment.

Separate ANOVAs did not indicate any significant differences between treatments in total beetle abundances ($F_{(2,6)}$= 3.6505, p=0.0917931, Fig. 8, Table 5), Hydrophilidae abundances ($F_{(2,6)}$= 2.1241, p=0.200686, Table 5), or Dytiscidae abundances ($F_{(2,6)}$= 0.9212, p=0.44784, Table 5). These results indicated beetles did not have a colonization preference among treatments.

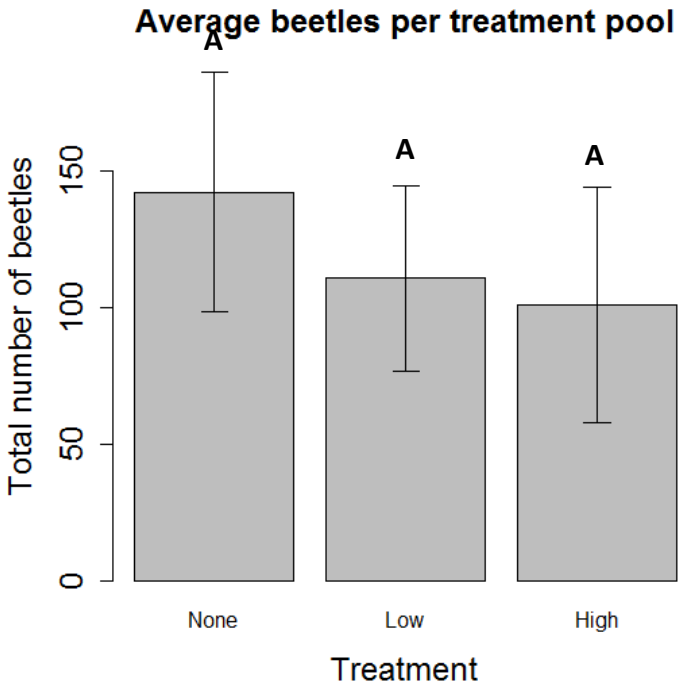

Figure 8: Average total beetles collected from each treatment pool. Error bars represent standard deviation.

Table 5: ANOVA tables for beetle abundance data, Hydrophilidae abundance data, and Dytiscidae abundance data.

Total	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	3753	1876.3	3.6505	0.0917931
Block	3	56166	18722.1	36.4243	0.0003024 ***
Residuals	6	3084	514.0		

Hydrophilidae	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	2542	1271.1	2.1241	0.200686
Block	3	42391	14130.3	23.6129	0.001011 **
Residuals	6	3591	598.4		

Dytiscidae	Df	Sum Sq	Mean Sq	F value	Pr(>F)
TRT	2	179.17	89.58	0.9212	0.44784
Block	3	1444.25	481.42	4.9503	0.04613 *
Residuals	6	583.5	97.25		

CHAPTER 4

DISCUSSION

Ammonia is a common excretory waste product in aquatic environments, especially in freshwater, where water conservation is not a physiological issue for most organisms. The hypothesis that this common excretory product could be the chemical cue detected by mosquitoes was not supported. There was no significant difference between control pools and experimental ammonia pools; even though ammonia treatment pools were maintained significantly above fishless pool background ammonia levels, ammonia treatments actually received more mosquito egg rafts than control pools. Ammonia is not the cue detected or avoided by mosquitoes, although it may be part of a cocktail of chemicals that together make the cue, which is often suggested (Boriss et al. 1999; Bronmark and Hansson 2000). The lack of detection, or perhaps lack of avoidance, of ammonia likely results from the fact that low levels of natural ammonia are generated in waters even without fish; thus the cue is probably too common to be used to an evolutionary advantage.

Pirate perch are also unable to mask other fish species from mosquito detection. Neither mosquitofish (*G. affinis*) nor golden topminnows (*F. chrysotus*) were masked from detection by the mosquitoes by the close proximity of a pirate perch in the same pools, and thus the hypothesis that pirate perch have a generalized masking chemical was rejected. However, pirate perch appeared to be detected and avoided by mosquitoes at higher densities, but not at low

densities. This provides evidence that pirate perch likely have a form of chemical camouflage that involves a reduced chemical signature rather than a masking chemical. If each fish produced a small amount of chemical cue, one fish could remain undetected, but the additive effect of the cues would surpass the detection threshold causing avoidance by ovipositing mosquitoes, as observed in the experiment. If a masking chemical was being produced, each fish would produce enough masking chemical to camouflage its chemical cue, and therefore the fish would not be detected even at high density. Another possibility, though unlikely, is that pirate perch act similarly to predatory northern pike (*Esox lucius*). Pike leave their foraging grounds to defecate, as minnows they prey upon can detect alarm cues from excreted minnow skins and therefore avoid areas where pike defecate (Brown et al. 1995). In a similar fashion, pirate perch could defecate during the day while ovipositing species are not laying eggs, thus allowing the cues to dissipate before the nightly egg laying process. However, this is unlikely, as the pools and water were not changed in any way, and thus the cues would likely have built up even for a single pirate perch, causing an avoidance response in ovipositing mosquitoes. There was no avoidance of a solitary pirate perch in this experiment, nor in previous studies (Resetarits and Binckley 2013a; unpub. data).

Tree frog oviposition preferences were much different than anticipated, and superficially different from the mosquito response. In accordance with gaining the highest possible fitness, which includes increasing offspring survival, I predicted that the High treatment should have significantly lower numbers of eggs than either of the other treatments. Low and None were expected to have similar egg numbers, as the Low density would have fish not likely to be detected. However, High and None did not differ significantly, although High did have roughly half the egg numbers as None. Surprisingly, Low had a significantly higher number of eggs than

High, and although not statistically significantly more, it did have a much higher egg count than the optimal habitat of None. This could be explained in two different ways.

The explanation that the fish are chemically camouflaged by low production of chemical cue likely still holds true. If pirate perch produce low amounts of chemical cue, those in Low would not be detected; they produce too little cue for tree frogs to detect and avoid them. The window mesh used ($1.3mm^2$) was not fine enough to prevent colonization of pools by nearly microscopic copepods and other zooplankton, nor to prevent dragonfly and small beetle eggs from dropping through the screen to the bottom portion of the pool and hatching. However, the pirate perch in Low would be preying upon these organisms in the pool, such as copepods and dragonfly naiads. This top down effect would result in reduction of primary consumers and would increase the food base, especially in phytoplankton and periphyton, and decrease predation rate on tadpoles from naiads and potentially other invertebrate predators. While nutrient level modifications has been experimentally shown to not affect oviposition site choice (Binckley and Resetarits 2008), specific alterations of levels of periphyton and plankton have not been tested. More importantly, adult tree frogs have also been experimentally shown to be very good at predator detection during oviposition site choice (Resetarits and Wilbur 1989; Resetarits and Binckley 2003; Binckley and Resetarits 2008; Binckley and Resetarits 2013). The increased food base and decreased detected predator population should certainly be more attractive than the None treatment. In addition to the Low treatment, this would occur in the High treatment, but at the high density the fish cues would be detected, similarly to the mosquito results, and those pools avoided. This is a possible explanation for the results, but the data to confirm this explanation were not collected during the experiment.

Alternatively, pirate perch could be completely undetected by tree frogs in the experiment. In that case, in Low treatments the primary production increased and other predators besides pirate perch decreased, and therefore Low became the preferred habitat for tree frog oviposition over both High and None. This could give rise to the observed pattern in tree frog oviposition preferences where Low received the highest numbers of frog eggs, but we would also expect None and High to receive close to the same numbers of frog eggs. This explanation would not be transferrable to mosquito data interpretation. The mosquitoes avoided high density treatments, showing they could detect pirate perch in high enough densities.

Beetles did not significantly differ among treatments, and an NMDS plot did not suggest trends in beetle species sorting (Fig. 7). There were no distinct clusters for any treatment. Block had a significant effect on beetle abundance, as well as on abundance of Hydrophilids and Dytiscids, while there was only a marginally significant effect of treatment on beetle abundance. The trend was a lower abundance of beetles when fish were present, which correlated with previous studies demonstrating beetle avoidance of predators (Resetarits 2001; Binckley and Resetarits 2005).

Chemical signals in aquatic environments are poorly studied (Bronmark and Hansson 2000). Chemical mimicry and camouflage in aquatic environments are just beginning to receive in depth attention, and there are likely cases of chemical mimicry and camouflage yet to be discovered (Bronmark and Hansson 2000; Ruxton 2009). Even when detected, chemical signal modulation is unlikely to be fully understood until pertinent chemical compounds responsible for the observed patterns are discovered. Even now, only a select few compounds involved in certain aquatic chemical signaling systems have been identified (Boriss et al. 1999; Bronmark and Hansson 2000; Silberbush et al. 2010). The current study has revealed evidence of a system

of chemical camouflage in pirate perch (*A. sayanus)*. Low densities of pirate perch were undetected, or avoided, by mosquitoes, and seemingly attractive, or non-inhibitory at least, to tree frogs, while higher densities were avoided by both ovipositing organisms. The camouflage system likely functions by a low amount of detectable cue being produced, rather than a masking chemical. In addition, it has been shown that ammonia is not the detected chemical cue alone, if it plays a part in the cue at all. This evidence gives impetus for further studies into exactly what cue(s) are being detected by ovipositing organisms, and how pirate perch produce so little as to remain undetected.

LIST OF REFERENCES:

Adams, G. L., and Johnson, J. E. (2001). Metabolic rate and natural history of Ozark cavefish, Amblyopsis rosae, in Logan Cave, Arkansas. *Environmental Biology of Fishes*, *62*(1–3), 97–105. http://doi.org/10.1023/A:1011812922841

Akino, T., Nakamura, K. and Wakamura, S. (2004). Diet induced chemical phyomimesis by twig-like caterpillars of *Biston robustum*. *Chemoecology*. *14*, 165-174.

Atema, J. (1995). Chemical signals in the marine environment: Dispersal, detection, and temporal signal analysis. *Proc. Natl. Acad. Sci.* 92, 62-66.

Belwood, J. and Morris, G. (1987). Bat predation and its influence on calling behavior in Neotropical katydids. *Science*. *238*, 64-67.

Binckley, C. A., and Resetarits, W. J. (2008). Oviposition behavior partitions aquatic landscapes along predation and nutrient gradients. *Behavioral Ecology*, *19*(3), 552–557. http://doi.org/10.1093/beheco/arm164

Binckley, C. A, and Resetarits, W. J. (2005). Habitat selection determines abundance, richness and species composition of beetles in aquatic communities. *Biology Letters*, *1*(3), 370–4. http://doi.org/10.1098/rsbl.2005.0310

Boriss, H., Boersema, M., and Wiltshire, K. (1999). Trimethylamine induces migration of waterfleas. *Nature*. *398*, 382.

Breed, M., Snyder, L., Lynn, T., and Morhart, J. (1992). Acquired chemical camouflage in a tropical ant. *Animal Behavior*. *44*, 519-523.

Brett, J. R. (1972). The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. *Respiration Physiology*, *14*(1–2), 151–170. http://doi.org/10.1016/0034-5687(72)90025-4

Brett, J.R. and Zala, C.A. (1975). Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (Oncorhynchus nerka) under controlled conditions. *J. Fish. Res. Board Can*. *32*, 2479-2486.

Bronmark, C., and Hansson, L. (2000). Chemical communication in aquatic systems: an introduction. *Oikos*. *88*, 103-109.

Brown, G., Chivers, D., and Smith, R. (1995). Localized defecation by pike: a response to labeling by cyprinid alarm pheromone? *Behav. Ecol. Sociobiol. 36*, 105-110.

Brunty, J. L., Bucklin, R., Davis, J., Baird, C. D., and Nordstedt, R. (1997). The Influence of Feed Protein Intake on Tilapia Ammonia Production. *Aquacultural Engineering*, *16*(3), 161–166. http://doi.org/10.1016/S0144-8609(96)01019-9

Clarke, A., and Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, *68*(5), 893–905. http://doi.org/10.1046/j.1365-2656.1999.00337.x

Cui, Y., and Liu, J. (1990). Comparison of energy budget among six teleosts—II. Metabolic rates. *Comparative Biochemistry and Physiology Part A: Physiology*, *97*(2), 169–174. http://doi.org/10.1016/0300-9629(90)90165-O

Engin, K., Tufan Eroldoğan, O., Özşahinoğlu, I., Asuman Yilmaz, H., and Mumoğullarinda, P. (2013). Diurnal ammonia and urea excretion rates in European sea bass, Dicentrarchus labrax fed diets containing mixtures of canola and cotton seed oil at two different ambient temperature. *Journal of Thermal Biology*, *38*(8), 588–596. http://doi.org/10.1016/j.jtherbio.2013.10.004

Eveland, L., Bohenek, J., Silberbush, A., and Resetarits, W.J. (2016). Detection of Fish and Newt Kairomones by Ovipositing Mosquitoes. In *Chemical Signals in Vertebrates*, 247-259. http://doi.org/10.1007/978-0-387-73945-8

Fishlyn, D. and Phillips, D. (1980). Chemical camouflaging and behavioural defenses against predatory seastar by three species of gastropods from the surfgrass *Phyllospadix* community. *Biol. Bulletin*. *158*, 34-48.

Forster, R.P. and Goldstein, L. (1969). Formation of excretory products. In *Fish Physiology*, Vol. 1, 313-350. Edited by W.S. Hoar and D.J. Randall. Academic

Huppop, K. (1986). The role of metabolism in the evolution of cave animals. *The National Speleological Society Bulletin*. *47*(2), 136-146.

McCall, P.J. (2002). Chemoecology of oviposition in insects of medical and veterinary importance. In: M. Hilker and T. Meiners (eds.). *Chemoecology of Insect Eggs and Egg Deposition*. 265-290.

McCue, M. D. (2010). Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comparative Biochemistry and Physiology – A Molecular and Integrative Physiology*, *156*(1), 1–18.

Passow, C. N., Greenway, R., Arias-Rodriguez, L., Jeyasingh, P. D., and Tobler, M. (2015). Reduction of Energetic Demands through Modification of Body Size and Routine Metabolic Rates in Extremophile Fish. *Physiological and Biochemical Zoology*, *88*(4), 371–383.

Portugal, A. and Trigo, J. (2005). Similarity of cuticular lipids between a caterpillar and its host plant: a way to make prey undetectable for predatory ants? *J. Chem. Ecol. 31*, 2551-2561.

Raffa, K., Hobson, K., LaFontaine, S. and Aukema, B. (2007). Can chemical communication be cryptic? Adaptations by herbivores to natural enemies exploiting prey semiochemistry. *Oecologia*. *153,* 1009-1019.

Randall, D. J., and Wright, P. A. (1987). Ammonia distribution and excretion in fish. *Fish Physiology and Biochemistry*, *3*(3), 107–120.

Rednondo, J. and De Reyna, L. (1988). Locatability of begging calls in nesting altricial birds. *Animal Behavior. 36,* 653-661.

Resetarits, W. J. (2001). Colonization under threat of predation: Avoidance of fish by an aquatic beetle, Tropisternus lateralis (Coleoptera: Hydrophilidae). *Oecologia*, *129*(1), 155–160. http://doi.org/10.1007/s004420100704

Resetarits, W. J., and Binckley, C.A. (2013a). Is the pirate really a ghost? Evidence for generalized chemical camouflage in an aquatic predator, pirate perch Aphredoderus sayanus. *The American Naturalist*, *181*(5), 690–9. http://doi.org/10.1086/670016

Resetarits, W. J., and Binckley, C.A. (2013b). Patch quality and context, but not patch number, drive multi-scale colonization dynamics in experimental aquatic landscapes. *Oecologia*, *173*(3), 933–946. http://doi.org/10.1007/s00442-013-2644-5

Resetarits, W. J., and Binckley, C.A. (2009). Spatial contagion of predation risk affects colonization rate and community structure in experimental landscapes. *Ecology 90*, 869-876.

Resetarits W.J. and Wilbur H.M. (1989) Choice of oviposition site by Hyla chrysoscelis: role of predators and competitors. *Ecology. 70,* 220–228.

Rieger, J. F., Binckley, C. A., and Resetarits, W. J. (2004). Larval performance and oviposition site preference along a predation gradient. *Ecology*, *85*(8), 2094–2099. http://doi.org/10.1890/04-0156

Ross, S. T. (2001). The Inland Fishes of Mississippi. University Press of Mississippi, Jackson.

Ruxton, G. (2009). Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. *Phil. Trans. R. Soc. B. 364*, 549-557.

Schalles, J. F. and Wissing, T. E. (1976). Effects of dry pellet diets on the metabolic rates of bluegill (*Lepomis macrochirus*). *J. Fish. Rrs. Bd Can. 33*, 2443-2449.

Shepherd, M. E., and Huish, M.T. (1978). Age, growth, and diet of the pirate perch in a coastal plain stream of North Carolina. *Transactions of the American Fisheries Society. 108,* 457–459.

Silberbush, A., and Blaustein, L. (2008). Oviposition habitat selection by a mosquito in response to a predator: are predator-released kairomones air-borne cues? *Journal of Vector Ecology : Journal of the Society for Vector Ecology*, *33*(1), 208–211. http://doi.org/10.3376/1081-1710(2008)33

Silberbush, A., Markman, S., Lewinsohn, E., Bar, E., Cohen, J. E., and Blaustein, L. (2010). Predator-released hydrocarbons repel oviposition by a mosquito. *Ecology Letters*, *13*(9), 1129–1138. http://doi.org/10.1111/j.1461-0248.2010.01501.x

Stevens, M. and Merilaita, M. (2009). Animal camouflage: current issues and new perspectives. *Phil. Trans. R. Soc. B. 364*, 423-427.

Stoddard, P. and Markham, M. (2008). Signal clocking by electric fish. *Bioscience*. *58*, 415-425.

Thompson, J. (2013). *Relentless Evolution*. The University of Chicago Press, Chicago, IL.

Van Waarde, A. (1983). Review: Aerobic and Anaerobic Ammonia Production by Fish. *Comp. Biochem. Physiol.*, *74b*(4), 675–684.

Wellborn, G., Skelly, D., and Werner, E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst*. *27*, 337–63

Wisenden, B.D. (2000). Olfactory assessment of predation risk in the aquatic environment. *Philos. T. R. Soc. B. 35*, 1205-1208.

Zalom, F. G., Grigarick, A.A. and Way. M. (1979). Seasonal and diel flight periodicities of rice field Hydrophilidae. *Environmental Entomology, 8*, 938–943.

Zera, A. J., and Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology, 42*, 207–230.

Zhou, L., and Boyd, C. E. (2015). An assessment of total ammonia nitrogen concentration in Alabama (USA) ictalurid catfish ponds and the possible risk of ammonia toxicity. *Aquaculture*, *437*, 263–269. http://doi.org/10.1016/j.aquaculture.2014.12.001

VITA

Education:

B.S. cum laude in History and Secondary Education Certificate, Lycoming College 2012

Presentations:

- Thesis Proposal Seminar: “Can pirate perch chemically camouflage their presence?”
- University of Mississippi Annual Research Symposium Poster Session: “Evidence of chemically camouflaged pirate perch”
- Ecological Society of America 2017: "Evidence of Chemical Camouflage in Pirate Perch"