SEASONAL ACTIVITY IN DWARF WATERDOGS (*NECTURUS PUNCTATUS*):
RISK MANAGEMENT OR RESOURCE ACQUISITION?

A thesis presented to the faculty of the Graduate School at Western Carolina University
in partial fulfillment of the requirements for a degree of Master of Science in Biology.

By

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ABSTRACT

SEASONAL ACTIVITY IN DWARF WATERDOGS (*NECTURUS PUNCTATUS*):

RISK MANAGEMENT OR RESOURCE ACQUISITION?

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Predators can have non-consumptive effects (NCE) on prey populations in addition to normal consumptive effects. One of these NCE may be changes in patterns of behavior to reduce predation risk. Aquatic salamanders of the genus *Necturus* exhibit seasonal activity patterns, with observations of *Necturus* peaking during mid-winter. Predator avoidance has been suggested as an explanation for cool season activity; however, another hypothesis is that increased *Necturus* activity during winter is related to increased prey availability in leaf packs.

To test the willingness of juvenile dwarf waterdogs to forfeit a foraging opportunity in the presence of different predation threats, I constructed three aquatic raceways. Three juvenile *Necturus* along with live blackworms (*Lumbriculus varigaetus*) were added to one of two chambers in each raceway. After foraging behavior by all *Necturus* was observed, I added either a *Rana clamitans* tadpole (non-predator control), an adult *Necturus* (gape-limited conspecific predator), or a crayfish (non-gape-limited predator) to the first chamber in each raceway. I removed the barrier detaining the juvenile waterdogs and recorded the distance of each from the treatment chamber every two minutes for a total of 14 min.

I also sampled *Necturus* and macroinvertebrates monthly from leaf packs in the South Fork of the Edisto River near Windsor, SC. The purpose of this field study was to examine the relationship between waterdog abundance in leaf packs and the abundance of macroinvertebrate prey as an alternative hypothesis for seasonal activity.

Treatment had no effect on waterdog behavior during lab trials. *Necturus* density was positively correlated with macroinvertebrate density and maximum stream discharge rate during the previous 30 days in partial correlation analyses, as well as with a maximum previous discharge/macroinvertebrate abundance interaction. My results suggest dwarf waterdogs may not be abandoning foraging areas during summer to avoid predators. Instead, increase in discharge rate may act as a cue to stimulate waterdogs to leave their summer refugia and move into leaf packs when prey density is at its peak.

INTRODUCTION

Relationships between predators and their prey can be very complex, and the mechanics of these interactions are sometimes not well understood. Changes in prey population dynamics related to the presence or abundance of their predators are often attributed to the consumption of that prey. However, non-consumptive effects (NCE) can be just as important to understanding these relationships and are often overlooked (Peckarsky et al. 2008), particularly if the NCE occurs in the same direction as the consumptive effect. Even many of the classic examples of predator-prey interactions (Estes and Palmisano 1974; Carpenter and Kitchell 1988; Krebs et al. 2001; Peterson and Vucetich 2001) can be at least partially explained by NCE (Peckarsky et al. 2008).

One means by which NCE may affect predator-prey dynamics is the alteration of prey behavior (Lima and Dill 1990), an aspect of "the Ecology of Fear" (Brown et al. 1999). Knowledge of predation risk may cause prey to increase vigilance, or modify/reduce activity to lower the risk of mortality (Madison et al. 1999; Ferrari et al. 2008; Roberts and Liebgold 2008; Maia et al. 2009.) The reduction in prey activity itself may make prey seem more scarce; however, these NCE can have other more negative effects on prey populations.

Predator avoidance seldom comes without a cost, some of which may be suboptimal foraging, reduced growth (Petranka 1989; Madison et al. 1999; Nelson et al. 2004) and increased physiological stress (Boonstra et al. 1998; Breves and Specker 2005; Remage-Healey et al. 2006). This can have lasting effects on fecundity for individuals (Sinclair et al. 2001), and effects on fitness may even transcend generations (Stephan and

Krebs 2001).

Prey, therefore, are under selective pressure to regulate activity to reduce NCE by balancing the contrasting demands of predator avoidance and acquisition of resources (Lima and Dill 1990; Madison et al. 1999; Robert and Thompson 2007; Werner and Hall 1988). If NCE are to be minimized, simple detection of a predator is not enough. More information about the predator needs to be obtained to avoid "false alarms" and unnecessary NCE. This means developing the ability to not only detect a predator's presence within a patch, but to also identify the type of predator and the relative level of threat that particular individual may impose.

If a specific predator is a risk, then prey may generalize that perception of risk to other predator species, with more intensely experienced threats leading to a wider generalization of predator recognition (Ferrari and Chivers 2009). Generalization of predator recognition, or the Predator Recognition Continuum Hypothesis (Ferrari et al. 2007), generally follows a taxonomic gradient, with predators more closely related to the species the prey is familiar with being more likely to elicit a response in the prey (Ferrari et al. 2008; Ferrari and Chivers 2009). This type of predator recognition is often learned and can begin very early in development, even within the egg as with *Rana sylvatica*, the wood frog (Mathis et al. 2008; Ferrari and Chivers 2009), and possibly aquatic crustaceans (Blaustein 1997). This generalized recognition does not necessarily protect prey from all predators, however, particularly when prey are naïve to an exotic predator species (Epp and Gabor 2008; Gall and Mathis 2010).

Chemoreception is used by many species to detect predators and assess predation risk (Robert and Thompson 2007), with amphibians being no exception (Kiesecker et al.

2003; Mathis 2003; Orizaola and Brana 2003; Gonzalo et al. 2006; Zimmer et al. 2006; Gonzalo et al. 2008). Chemoreception is, in fact, the most common sense used in predator detection in amphibians (Kats and Dill 1998). Both terrestrial and aquatic amphibians react negatively to odors of predators and distressed conspecifics in experimental studies (Madison et al. 1999; Epp and Gabor 2008; Ferrari and Chivers 2009).

Predator avoidance has been suggested to influence patterns of seasonal activity in some aquatic salamander species of the genus *Necturus* (Neil 1963; Shoop and Gunning 1967; Braswell and Ashton 1985). *Necturus* (family Proteidae) contains a small group of permanently aquatic salamanders commonly called "waterdogs" or "mudpuppies". All members of this family are neotenic, and thus retain many larval traits throughout life, including external gills, a caudal fin, and a lack of eyelids (Petranka 1998.) Another synapomorphy of the family is a reduction of skeletal elements, specifically parts of the cranium and in the number of digits on both the fore and hind feet. *Necturus* have only four digits on each foot, as opposed to most other salamanders which possess four digits on each forefoot and five digits on each hind foot.

Members of the genus *Necturus* can be found in eastern North America in a variety of aquatic habitats, but are most diverse in the southeastern USA where at least 5 species inhabit streams, rivers, lakes, and swamps of varying sizes. Fish and crayfish are presumed to be predators of waterdogs, but predation has been poorly documented (Petranka 1998). Predatory warm water fish are known to be more active during spring and summer months (Snedden et al. 1999; Jepsen et al. 1999; Hunter and Maceina 2008) and could negatively affect foraging behavior in *Necturus* during this period. There is evidence to suggest that the Neuse River waterdog, *Necturus lewisi*, may produce skin

secretions that make it less palatable to predators (Brandon and Huheey 1985).

The dwarf waterdog (*Necturus punctatus*) inhabits small to medium sized streams and associated aquatic habitats in the Atlantic Coastal Plain and lower Piedmont from southeastern Virginia to south-central Georgia (Petranka 1998). Meffe and Sheldon (1987) found dwarf waterdogs to be most abundant in slower, deeper portions of first to third order blackwater streams during their study of western South Carolina populations. The diet of *N. punctatus* consists mostly of small aquatic invertebrates, including oligochaetes, ephemeropterans, chironomids, and crayfish (Meffe and Sheldon 1987). The remains of an unidentified salamander were also found in the gut of one individual, possibly a result of cannibalism. Of 50 animals sampled, 54% had empty stomachs (Meffe and Sheldon 1987). This is similar to another diet study (Folkerts 1971) in which 9 of 20 adults collected also had empty digestive tracts.

Many aspects of the dwarf waterdog's natural history are still unknown, particularly aspects of its growth, survivorship/mortality, and reproductive behavior. No nests have been found, but oviposition sites are thought to be similar to those of other species of *Necturus*, with females laying eggs in a single layer on the underside of a log, rock, or other object partially embedded in the stream bottom (Petranka 1998). The diel activity patterns of *N. punctatus* have not been investigated, but dwarf waterdogs seem to be mostly nocturnal, only foraging away from cover in darkness or dim light (pers. observations.) It is possible that the diel rhythms of *Necturus* also reflect avoidance of diurnal predators. Individuals seem to be most active during the cooler months when they aggregate in leaf packs (Brimley 1924; Martof et al. 1980; Beane et al. 2010), but it is not known if this cool season peak in activity is related to predator avoidance, resource

acquisition, or other factors.

I conducted an experiment on predator avoidance behavior in the lab, as well as a field study on the seasonal ecology of the aquatic leaf packs inhabited by this species, to compare the following two hypotheses related to the apparent cool season activity peak in *Necturus*: 1) Predators cause behavioral changes resulting in forfeiture of foraging opportunities and causing potential NCE for *N. punctatus*, and 2) Seasonal activity of *N. punctatus* is related to foraging opportunities in leaf packs.

METHODS

Predator Avoidance Behavior Experiment

To measure the behavioral reaction and potential forfeiture of foraging opportunities in juvenile waterdogs caused by different predation threats, I used raceway behavior trials. I exposed foraging juvenile waterdogs (individuals < 75mm SVL) to three treatments representing different levels of predation threat: A green frog tadpole (*Rana clamitans*), a White River crayfish (*Procambarus acutus*), and an adult conspecific. The tadpole, being considered a benign organism incapable of causing any serious harm to a healthy waterdog, represented a control. The crayfish and the adult waterdog both represented predator treatments: one a non-gape-limited and easily distinguishable predator (the crayfish), and the other a gape-limited, less easily distinguishable predator (the adult waterdog.) I confirmed that the species of crayfish used was a potential predator of *N. punctatus* by conducting feeding trials prior to the raceway experiment. All treatments, including the control, will simply be referred to as "predators" or "predator treatments" even though the tadpole treatment was not meant to function as such.

I first constructed three 3 m raceways using sections of 11.4 cm wide vinyl home guttering and complimentary gutter end caps. I trimmed each gutter section to a length of 300 cm and saved the trimmings for future use in constructing barrier screens. By removing the rubber seal supplied with the end caps, I was able to slide the caps fully onto both ends of the gutter sections. I used aquarium-safe silicone sealant to attach and seal the caps. The raceways were allowed to sit empty for 24 h to permit the silicone to set and cure. Because the weight and outwards pressure produced by being filled would

have caused the sidewalls of the raceways to bow outwards and become deformed, I ran

four 95 mm diameter lag bolts transversely through the sidewalls near the top

approximately every 60 cm along each raceway, then secured those using nuts and

washers to prevent distortion or damage to the raceways during and after filling.

I used the trimmings from the ends of the gutters to construct six removable

barriers that fit neatly inside the raceways. Two layers of screen material (one of 50 mm

galvanized mesh and one of nylon window screen material) were cut to fit the cross-

sectional, hexagonal shape of the gutter trimmings. I affixed both screens inside the

gutter trimmings using monofilament fishing line so that they formed double-layered

barriers that prevented direct contact of juvenile waterdogs with any of the predators, but

would still allow odors, vibrations, and some visual cues to pass through.

During November 2011 through January 2012 I collected adult and juvenile dwarf

waterdogs and crayfish from the South Fork of the Edisto River and the Savannah River

Site in Aiken Co., SC by use of dip netting and baited funnel traps in streams. Green frog

tadpoles were collected from a small pond at the Jackson Co. Recreational Center in

Cullowhee, NC by dip netting, as well. All animals were maintained indoors in water-

filled, 12.7 L plastic containers, and were fed a diet of either live blackworms

(*Lumbriculus varigaetus*) in the case of the *Necturus* (also occasionally the crayfish), or

sinking algae tablets (in the case of both the tadpoles and crayfish). All animals were

fasted for at least two days prior to being used in trials to reduce the probability of

unintended chemical cues being released in their excretions during the experiment.

I conducted behavior trials during February 2012. Prior to the start of each set of

trials, I cleaned all three raceways using a 9% solution of household bleach (NaClO) to

remove residual odors from previous uses. The entire length of the raceway was sprayed

with the bleach solution and wiped with paper towels until clean and dry. I then arranged

raceways parallel to one another with the ends aligned. Once arranged, I added 4 drops

of aquarium dechlorinator ($Na_2S_2O_3$ solution) to each raceway and filled them to a depth

of 7 cm using tap water. I stretched two tape measures along the raceways in the spaces

between them to serve as references for the locations of juvenile waterdogs during the

trial. I then placed two of the screen barriers in each raceway to prevent animal

movement: one at 15 cm from the starting end to create a chamber to house a predator

and prevent direct contact between them and the juvenile waterdogs, and another at 25

cm which functioned as a gate to temporarily hold the juvenile waterdogs near the

starting line before the trial began.

To control for variation in behavioral response caused by the relative sizes of the

predators and the juvenile waterdogs that were paired in trials, I ranked all animals used

in the experiment by size. Juvenile waterdogs were ranked as groups of three similar

sized individuals. I ranked the predators individually, since only one at a time of each

type was to be used in any single trial. During trials, I paired groups of juvenile

waterdogs with their respectively ranked predators (i.e. the largest three waterdogs with

the largest predator, etc.)

To begin each set of trials, I moved a group of three juvenile waterdogs into the

chamber between the two screen barriers in each raceway. I then placed an open 20 mL

plastic vial containing 1 g of live blackworms on the bottom of each raceway in the

center of the chamber containing the juvenile waterdogs. The vial of blackworms was

upright and covered with screen to prevent the worms from escaping the vial and moving

away from the intended location. Afterwards, I dimmed the lights and allowed the

animals some time to adjust to the raceway environment, detect the blackworms, and

resume normal foraging behavior (usually 5-10 min.) As soon as the waterdogs appeared

adjusted to their environs and interested in the blackworms, I added a single predator of

one of the three treatment types to the adjacent chamber at the beginning each raceway

and lifted the barriers that had been preventing the juvenile waterdogs from moving down

the raceway. I then recorded the locations of the three juvenile waterdogs in each

raceway at 10 sec into the trial, and then every 2 min afterwards for the next 14 min. At

the end of the trial, all animals were removed and returned to their containers before

pumping the water out of the raceways by using a small electric aquarium pump. I

repeated this entire process for a total of 11 times, with all three predator treatments being

run simultaneously each time (n=11 of each of three treatments). No animals were used

more than once and all treatments were assigned randomly to the 3 raceways during each

set of trials. I conducted the experiment in an indoor laboratory at Western Carolina

University, under dim lighting, at approximately 22°C.

Statistical Analysis of Behavior Experiment Data

I analyzed behavioral trial data with SAS 9.2 using a split plot full repeated

measures ANOVA blocked by trial set ($\alpha = 0.05$) to test for differences in the movement

of waterdogs away from the predator stimuli and, consequently, the foraging opportunity.

I also compared the variances of distance measurements within trials among treatments to

test for differences in activity level between treatments (i.e. whether animals were more

or less sedate in the presence of different treatment stimuli) using a Friedman's rank sum test in R 2.15.2.

Leaf Pack Sampling

During November 2011-April 2012 I sampled leaf packs in the South Fork of the Edisto River in Aiken Co., SC to evaluate seasonal changes in invertebrate abundance and assemblage composition that may affect waterdog behavior and abundance. Once per month I collected macroinvertebrates and vertebrates from leaf packs in the river upstream of the public boat ramp at the SC Hwy 53 (State Park Rd) river crossing (N33°33'20", W81°29'01").

Each month, I made 10 sweeps through leaf packs with a dip net (hoop size approximately 20x55 cm, 3 mm mesh net) and identified and counted all macroinvertebrates and vertebrates found therein. All sweeps were approximately 1.5 m long. Before collecting the 10 sweeps, I measured water temperature using two thermometers (one alcohol, one mercury; the average between these two measurements later being used in statistical analysis). Gage height and discharge rate data were obtained from measurements taken by the USGS gage station on the South Fork Edisto River at Denmark, SC (the nearest gage downstream of the study area, approximately 37 km).

To reduce the effect of sampling on the leaf pack communities, I reduced the number of sweeps taken from each pack after the first month. All 10 sweeps were from one leaf pack (site) in November 2011. In subsequent months I never took more than 5 sweeps per leaf pack, and visited a minimum of two leaf packs to obtain 10 sweep

samples. Macroinvertebrate and vertebrate communities were similar between leaf packs during any sampling event, so I considered differences between monthly samples related to the exact leaf packs used in each sample to be minimal.

After completing each sweep, I euthanized all collected vertebrates in the field by placing them in a 10% (by volume) ethanol (ETOH) solution before preservation in either 70% ETOH or 10% formalin. All macroinvertebrates were placed directly in 80% ETOH for preservation as soon as they were picked from the swept up leaf pack material. The total number of waterdogs captured during the sweeps was noted.

To compare leaf pack assemblages among monthly samples, I first identified, sorted, and counted all insects collected during the monthly sampling events to order. Other individual macroinvertebrates representing less numerous taxa in the samples were often identified only to the level of class.

I also collected larval waterdogs (0^+ age class, 2011 cohort) for measurement to track growth of the 2011 cohort over the active season. I recorded man-hours spent during collecting. An attempt was made to collect at least 10 larvae each month, however only 9 were obtained in the November 2011 and the April 2012 samples. Immediately following euthanasia, I measured the snout-vent (SVL) and total length (TL) of the larvae collected to the nearest 1mm and recorded their mass to the nearest 0.01g.

Statistical Analysis of Leaf Pack Data

I square root transformed all count data (*Necturus* and macroinvertebrates) before analysis to correct for heteroscedasticity. To analyze the relationships between the numbers of *Necturus* collected (in 10 sweep samples), total number of macroinvertebrates

collected, and maximum discharge during the 30 days prior to each monthly sample, I ran a multiple regression analysis in R 2.15.2 with *Necturus* abundance as the dependent variable and total macroinvertebrates and maximum previous discharge as the independent variables (as well as an interaction between the independent variables). I also ran that same linear model, but instead of using total macroinvertebrate abundance I substituted the total abundance of macroinvertebrate taxa explicitly listed by Meffe and Sheldon (1987) as being present in the diet of *N. punctatus* in South Carolina. Preliminary analyses indicated that water temperature and current discharge rate were not significantly correlated with *Necturus* abundance, so these variables were not considered further.

RESULTS

Predator Avoidance Behavior Results

Predator presence had no significant effect on waterdog movement away from foraging opportunities during lab behavior trials. Juvenile *Necturus* neither moved farther away from (Table 1, Fig. 1), nor changed their level of activity (Friedman X^2 = 2.36, p = 0.307; Fig. 2) in the presence of any of the three predator treatments. The effects of trial set and time of measurement on distance, however, were significant (Table 1), with distance of juvenile waterdogs from the predator increasing with time of measurement.

Leaf Pack Sampling Results

Leaf pack macroinvertebrate community composition varied throughout the study, with the greatest taxa richness (11) occurring in the January 2012 sample and the greatest total number of individuals (219) being collected in February 2012 (Table 2). *Necturus* abundance was significantly positively correlated with maximum discharge rate during the previous 30 days (R^2 = 0.89, p = 0.0276; Fig. 3), and macroinvertebrate abundance (R^2 = 0.86, p = 0.0280; Fig. 3) in partial correlation analyses, as well as with a total macroinvertebrate abundance/maximum previous discharge interaction term (R^2 = 0.82, p = 0.035; Fig. 3).

Although Meffe and Sheldon's (1987) prey taxa reached their three highest relative (proportional) abundances in the same three months as for the highest *Necturus* abundances (January-March 2012; Table 2) in this study, *Necturus* abundance was not

significantly correlated with prey taxa, maximum previous discharge interaction, or their interaction (all $R^2 \leq 0.50$, all $p \geq 0.100$). This model did not, however, include the "unidentified insects" category used in their diet analysis, which undoubtedly included taxa I collected and included in total macroinvertebrate abundance.

While no significant relationship was apparent between current discharge rate, water temperature, and *Necturus* abundance in preliminary analyses, winter 2011/2012 was quite warm when compared to previous years (the lowest water temperature measurement being 11° C at the end of December 2011, compared to a measurement of 3° C I recorded at the same time the previous year). This may indicate water temperature measurements during winter 2011/2012 period may not reflect normal seasonal cycles for that site.

Necturus Length, Mass, and Size Distribution

The mean SVL of 2011 cohort larval *Necturus* increased from 22.9 mm in mid-November 2011 to 30.6 mm by the end of April 2012 (Fig. 4). Mean mass increased from 0.22 g to 0.74 g over this same period (Fig. 4). Ln (larval SVL) was significantly correlated with ln(mass) ($R^2 = 0.7900$, $p < 0.001$; Fig. 5).

Timing of peak abundance varied among size (age) classes of waterdogs (Fig. 6). I collected adult animals (> 75 mm SVL) only in November 2011 – January 2012, and even then in relatively low numbers (maximum of six individuals in November). No adult *Necturus* were detected in regular monthly 10 sweep samples in November – January (but were collected during additional dip netting efforts), or at all in February – April. The few adults were collected during additional dip-net sampling efforts to collect

animals for the predator avoidance behavior experiment. These individuals were not, therefore, included in any of my analyses involving monthly counts of waterdogs.

Young-of-the-year and intermediate age class animals seemed to differ in their timing of peak abundance, as well (Fig. 6). Individuals of the 2011 larval cohort (< 35 mm) did not reach peak density until the end of April/beginning of May (the last of my sampling efforts). I could not, therefore, determine when this particular age group would have retired from shallow leaf packs to their summer refugia. However, I was unable to detect *Necturus* of any size given similar efforts as early as the beginning of June the previous year (2011).

Intermediate sized animals (35 – 75 mm SVL; likely 2^+ to 5^+ year old cohorts) more closely followed the trend of seasonal activity for total *Necturus* abundance, with peak density occurring in the February 2012 sample, then becoming undetectable by the end of April 2012.

DISCUSSION

The results of my behavioral experiment and field sampling data do not support the hypothesis that *Necturus* abandon foraging areas simply because of increased predation risk. Given that juvenile dwarf waterdogs seem to be equally reluctant to move away from foraging opportunities in the presence of larger conspecifics, crayfish, and benign controls demonstrates the potential for prioritization of resource acquisition over reduction of predation risk. Juvenile waterdogs only moved approximately 100-125 cm away from predator treatments (on average; Fig. 1), while the ability to move nearly 300 cm away from these treatments was present in all trials. Although foraging behavior was often disrupted for one or more individuals in each trial, juvenile waterdogs never fully abandoned the foraging opportunity by retreating to (and remaining at) the opposite end of the raceway. This likely occurs as long as sufficient prey is present and waterdogs have yet to reach the point of satiation at which time any benefit of further foraging and prey consumption no longer outweighs the cost of exposure to avoidable predation risk.

Seasonal trends in abundance in leaf packs differed among size classes of *Necturus*. These ontogenetic changes in density of cohorts/cohort groups in leaf packs may reflect different strategies for taking advantage of seasonal resource availability. It is possible these differences in seasonal activity patterns are related to different selective pressures on size classes. For instance, if growth or predator avoidance is at a premium for smaller animals while mating/reproduction is more important for adults, different seasonal behavior patterns may develop for each of those groups. Adult *N. punctatus* may aggregate in leaf packs for only a short period in the fall and early winter to feed, but

then move to other more sheltered habitats (nest sites) to reproduce. However, sampling efforts better suited to collecting larger animals (such as the use of backpack electrofishers or baited traps) might be needed to assess the seasonal activity patterns of adult *N. punctatus* with more certainty.

When considered together, my lab and field data imply that waterdogs may move into leaf packs to feed during winter because of increased macroinvertebrate prey density at that time, regardless of potential predation risk in those areas. As prey density decreases with the coming of leaf pack decomposition in spring, waterdogs move away from these habitats, as there is no longer enough prey present to justify the inherent risk of spending time foraging away from more substantial refugia, such as burrows in the stream bank, under submerged woody debris and other solid cover objects, or in the stream bed itself. While this predator avoidance behavior could result in NCE for individual dwarf waterdogs, the overall net effect on fitness of abandoning foraging areas will be positive if prey availability is below the threshold at which fitness benefits from foraging reward are outweighed by the fitness costs of predation risk.

This is somewhat similar to the findings of Robert and Liebgold (2008). They propose a hypothesis that terrestrial salamanders may climb plants (a less efficient foraging strategy because of lower prey density on plants) to avoid predators only *after* having obtained a certain minimal amount of prey while foraging on the forest floor. This occurs despite the ground being an environment holding a higher risk of predation by predaceous arthropods, shrews, and larger salamanders. After adequate amounts of prey have been consumed, salamanders continue to forage to satiation. However, they are more willing to accept the NCE of pursuing prey in a less efficient microhabitat. Similar

tradeoffs between maximizing resource acquisition and mitigating predation risk have

been documented in a variety of taxa, including ungulates (White et al. 2011), bony fish

(Werner and Hall 1988), and insect pollinators (Llandres et al. 2011).

The shift in prioritization of foraging and predator avoidance can occur over the

course of a single night for terrestrial *Plethodon cinereus* in the above mentioned study

by Roberts and Liebgold (2008), however, as prey availability and predation risk change

seasonally in leaf packs, similar changes in the previous mentioned priorities may take

months in the case of stream dwelling *Necturus*. It is possible that various environmental

cues such as water temperature, discharge rate, weather conditions, etc. signal to

waterdogs when conditions are conducive to foraging and prey density has surpassed the

minimal reward/risk threshold in leaf packs. Maximum discharge rate during the 30 days

prior to the sample date may act as one of these cues, as it was significantly correlated

with waterdog abundance in our study. I observed on at least one occasion (April 17,

2011) movement of larval waterdogs into streamside leaf packs apparently in response to

increased discharge rate. After a large rain event leading to an unusually sharp rise in

stream flow, I captured many 0^+ age class animals in recently inundated areas, most of

which had been dry only 24 hrs prior. Although relatively few aquatic invertebrates were

collected in these recently inundated sites, I collected many terrestrial oligochaetes, ants,

and beetles that may serve as surrogate prey motivators to entice juvenile *Necturus* to

these areas. These sites could also serve as refuge from excessive current and waterdogs

may use them to reduce the risk of washout during flood events.

One potential complication of the relationship between waterdog and

macroinvertebrate prey densities is the gape limitation of the individual waterdogs used

in the analysis. I collected *Necturus* ranging in size from 20 to nearly 75 mm SVL during

this study. The gape sizes of these animals likely differed considerably, with the largest

Necturus being able to consume nearly any of the macroinvertebrates collected, and the

smallest being limited to only the smallest prey items. The gape size of each size class

(cohort) of *N. punctatus* also would have changed with growth, causing the size of prey

consumed to shift accordingly from month to month (in this case, sample to sample).

Individuals of all sizes were pooled for analysis in this study; however, changes in gape

size should probably be considered in future investigations of *Necturus* movement and

activity relative to prey availability or consumption.

One possible interpretation of the relationship of *Necturus* numbers and this

interaction term is that each of these variables represents a different level of Tinbergen's

(1963) explanations for behavior. Pulses in stream flow are the cue, or proximate cause,

for movement of waterdogs into leaf packs, whereas macroinvertebrate prey abundance is

the reward, or ultimate (adaptive) cause for this behavior. Both of these stimuli work

together in eliciting a behavioral response, in this case, migration to winter foraging

areas, with the response to each stimulus dependent on the other stimulus. The

correlation between these two causes may be related to at least two processes: 1)

Macroinvertebrates may move into leaf packs to feed on the newly fallen leaves and

detritus in fall and winter, coincidentally the time when stream flow tends to increase,

and 2) Invertebrate prey may also be carried by increased current during and shortly after

rain events and passively end up settling in the same areas as fallen leaves.

The relationship of *Necturus* abundance to the interaction term could also be

explained by the fact that macroinvertebrate abundance dropped during the March 2012

sample following a period of intense flooding (peaking at 792 cubic feet per second, the highest rate recorded in the 2011-2012 winter season) during the weeks prior to the sample. It may be that more moderate pulses in stream discharge stimulate waterdogs to move into leaf packs without dislodging invertebrates from these areas, whereas the flow levels seen in early March 2012 are detrimental to the accumulation of invertebrates in leaf packs while having little to no negative effect on waterdog accumulation (i.e. waterdogs may be able to burrow into the substrate and, therefore, be less prone to being displaced).

In summary, the cool season activity peak of *N. punctatus* in leaf packs does not appear to be a simple example of predator avoidance strategy, but also an example of timing activity to coincide with periods most conducive to resource acquisition. While freshwater predatory fish may be more active with warmer water temperatures in spring and summer (Snedden et al. 1999; Jepsen et al. 1999; Hunter and Maceina 2008), this study demonstrates that an increase in macroinvertebrate prey density during winter could also explain seasonal movements in *N. punctatus*. As the number of dwarf waterdogs collected in leaf packs closely follows both prey density and pulses in stream flow, and animals did not differentially flee to avoid predator and non-predator stimuli in behavior trials, a case can be made for resource acquisition as a driver of seasonal activity in *Necturus* over predator avoidance.

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FIGURES AND TABLES

Table 1. Repeated measures ANOVA of juvenile *Necturus* distance from predator treatments and foraging opportunity.

Effect	DF	F-value	p-value
Trial Set	10	3.40	0.0095
Time	1	99.7	<.0001
Treatment	2	1.15	0.3341
Time*Treatment	2	1.45	0.2501

Table 2. Summary of macroinvertebrate counts by sample and taxon. Approximate proportions of each sample represented by respective taxa are in parentheses. "Prey Taxa" includes the sum of counts for taxa (indicated by *) listed by Meffe and Sheldon (1987) as being present in the guts of *N. punctatus* from Savannah River Site, SC.

|	November	December	January	February	March	April
Amphipoda	-	6(0.06)	6(0.04)	11(0.05)	12(0.16)	13(0.13)
Bivalva	-	-	2(0.01)	6(0.03)	-	1(0.01)
Coleoptera	-	-	1(0.01)	-	12(0.16)	21(0.21)
Decapoda*	5(0.07)	2(0.02)	2(0.01)	4(0.02)	-	2(0.02)
Diptera*	-	-	13(0.08)	20(0.09)	19(0.26)	4(0.04)
Ephemeroptera*	3(0.04)	-	11(0.07)	61(0.28)	17(0.23)	-
Hemiptera	-	-	-	-	3(0.04)	13(0.13)
Megaloptera	3(0.04)	1(0.01)	8(0.05)	6(0.03)	-	-
Odonata	38(0.57)	16(0.16)	40(0.25)	26(0.12)	9(0.12)	47(0.47)
Oligochaeta*	2(0.03)	2(0.02)	8(0.05)	36(0.16)	-	-
Plecoptera	16(0.24)	70(0.72)	65(0.41)	46(0.21)	1(0.01)	-
Trichoptera	-	-	4(0.03)	3(0.01)	1(0.01)	-
Prey Taxa	10(0.15)	4(0.04)	34(0.21)	121(0.55)	36(0.49)	6(0.06)
Total Inverts	67	97	160	219	74	101
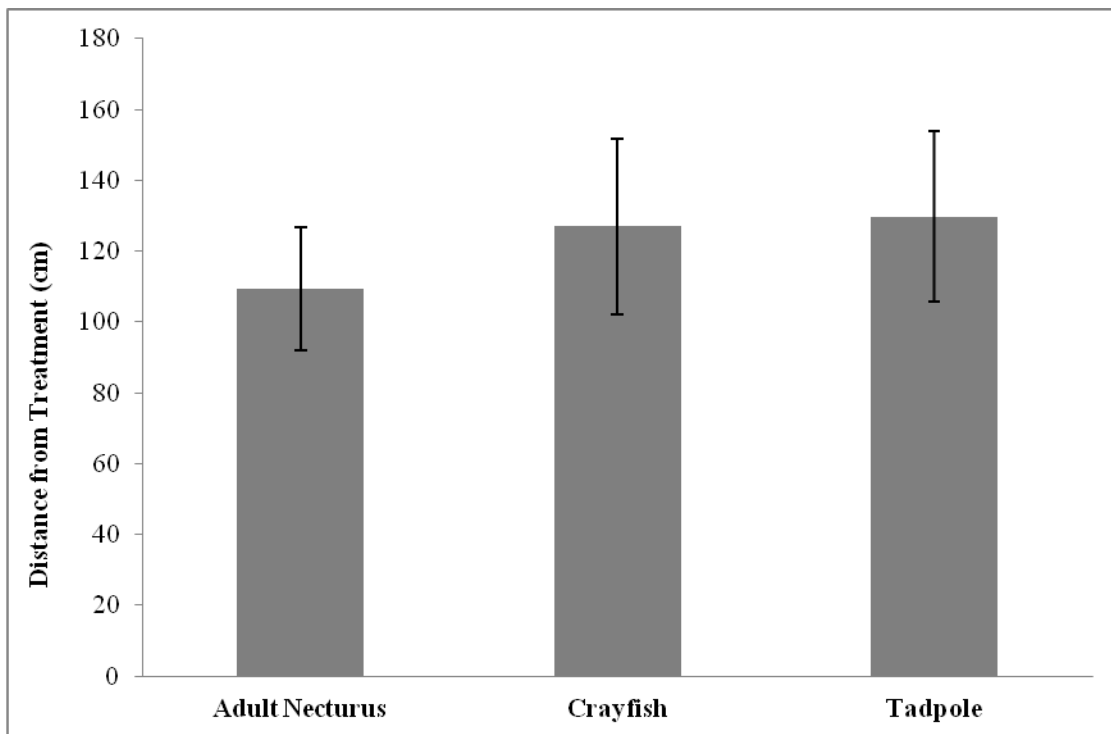
Figure 1. Mean distance measurement of juvenile *Necturus* from forage opportunity and treatment stimuli during predator avoidance behavior trials. Error bars represent 95% CI.
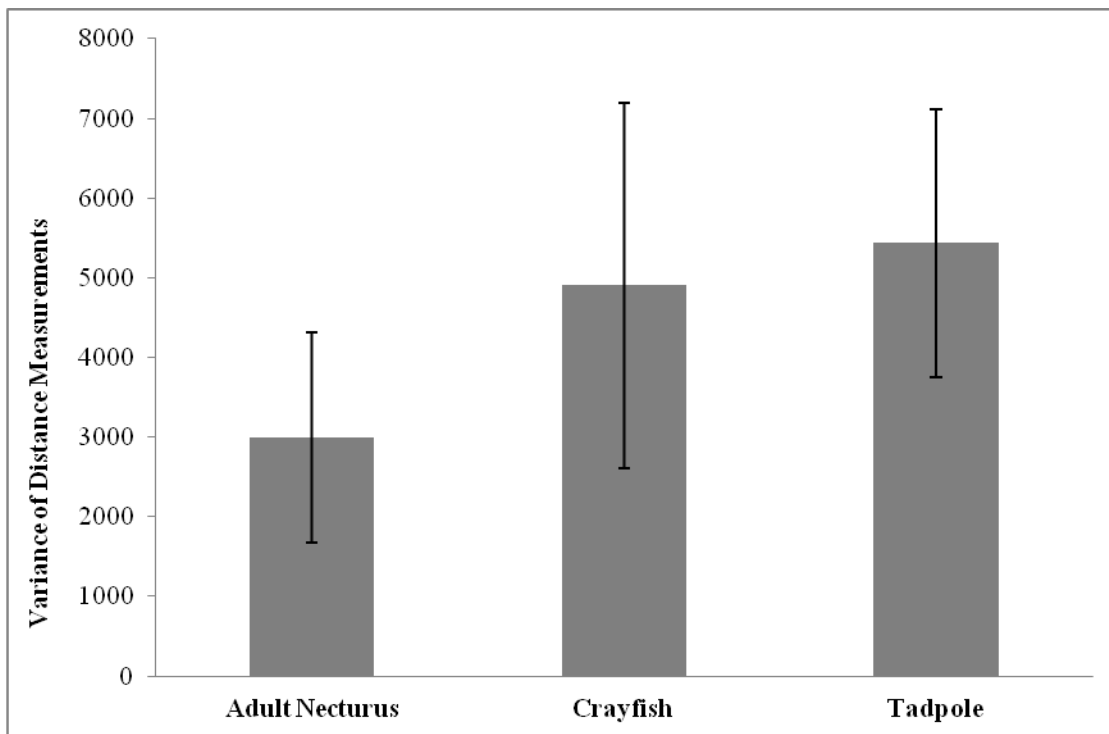
Figure 2. Mean variances of distance measurements for each trial by treatment. Error bars represent 95% CI.
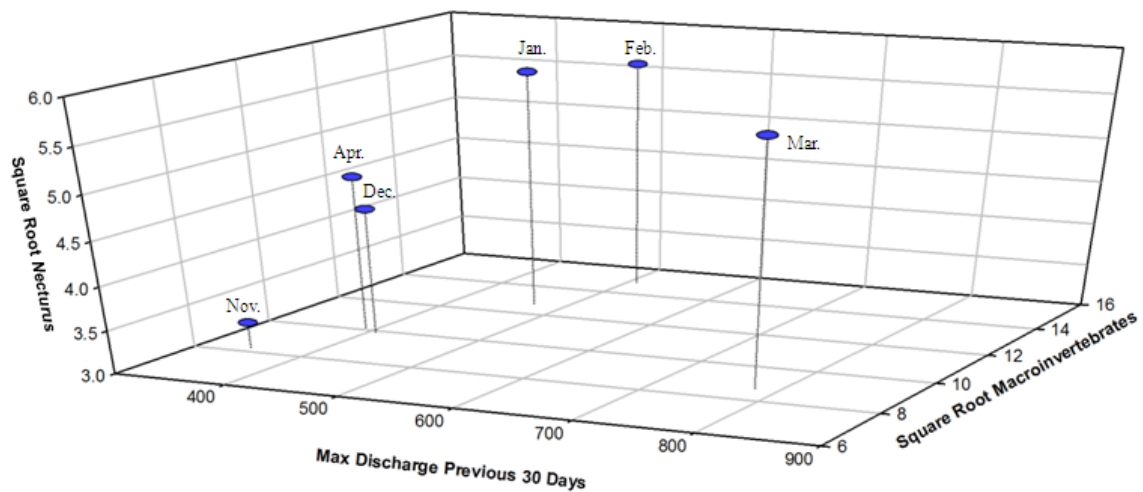
Figure 3. Three dimensional scatter plot of square root *Necturus* abundance, square root total macroinvertebrate abundance, and maximum discharge rate (cfs) during the 30 days prior to the sample date.
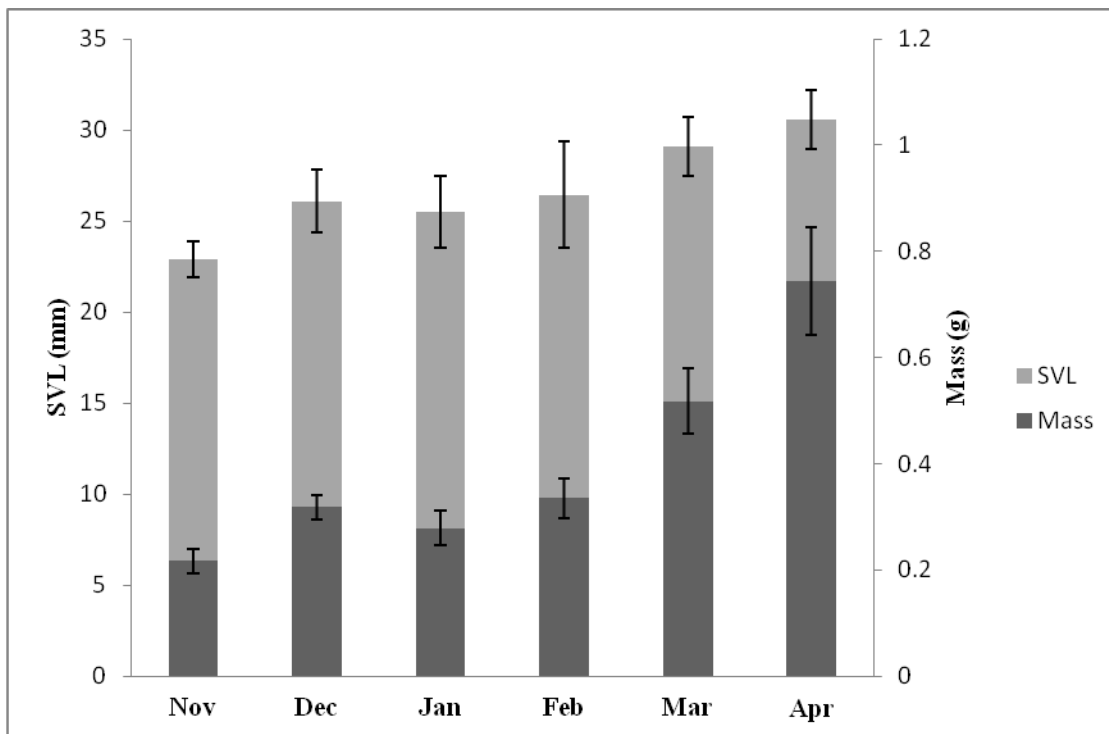
Figure 4. Larval *Necturus* mean snout-vent length (SVL) and mass by sample month.
Note: SVL is represented by the entire height of the bars. Error bars represent 95% CI.
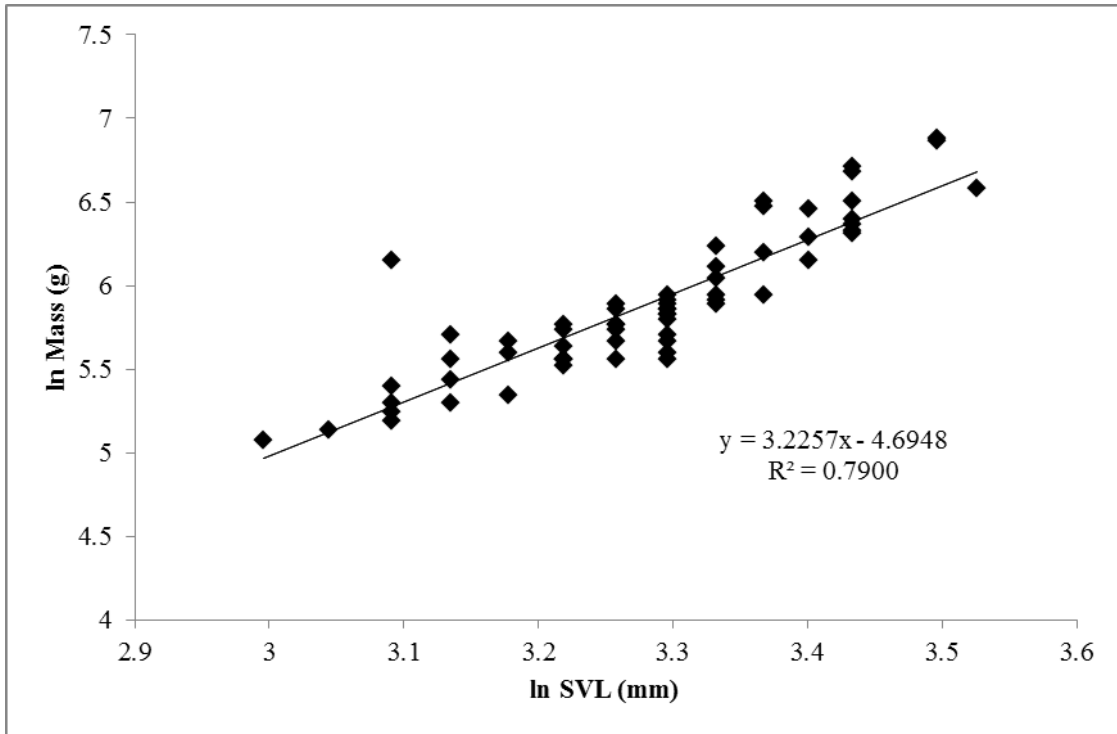
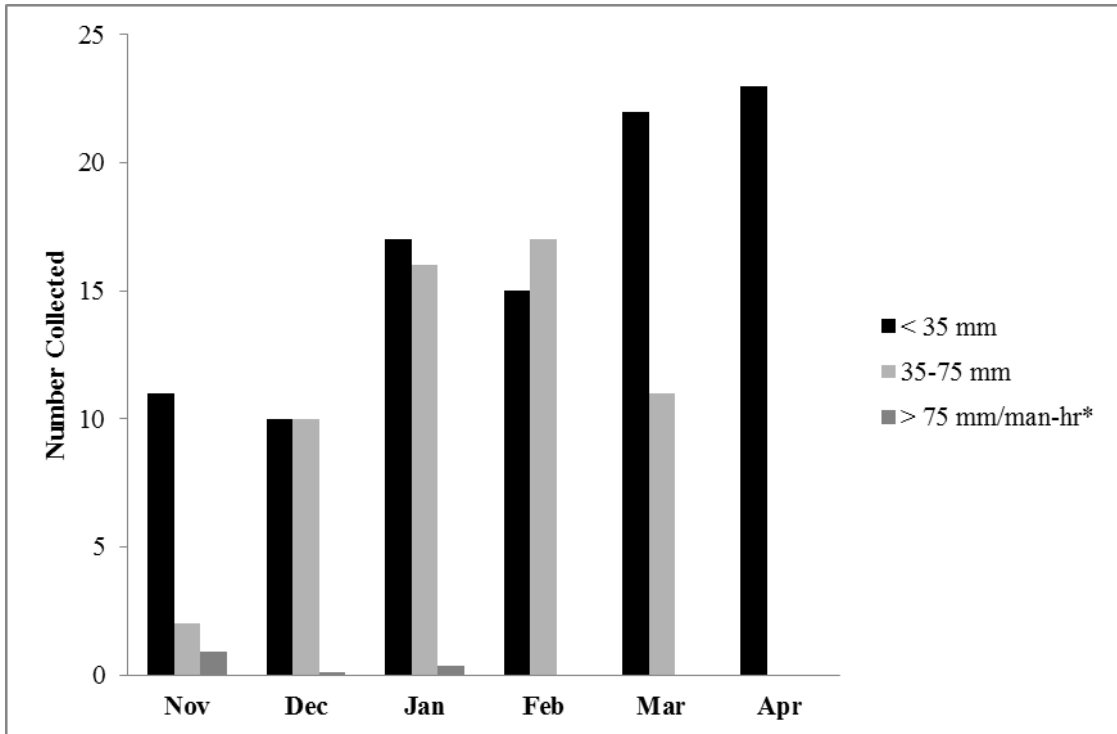Figure 5. Relationship between snout-vent length (SVL) and mass. Note: SVL and mass are natural log transformed.

Figure 6. Seasonal trends in abundance in different size classes of *N. punctatus*. Note: Adult animals (indicated by *) were not collected in regular 5 or 10 sweep samples. The numbers of animals collected for this group are from additional sampling efforts, presented as number captured per man-hour, and, therefore, not directly comparable to numbers of the other two size classes represented.