Determination of the functional response of *Orconectes propinquus* for ephemeroptera nymphs of the family Heptageniidae

Brian Argus
bargus@nd.edu
Mentor: Ashley Baldridge
BIOS 35502: Practicum in Field Biology
7/19/10

Abstract

Crayfish native to North America are commonplace in many freshwater ecosystems across the United States and play complex roles within them, as they do not fall into a specific trophic level. Due to their diet's wide range, crayfish play a key role in energy flow and transformation within lakes. Describing an organism's feeding rate based on prey density, also known as its functional response, is a common method used to characterize an organism's role within its ecosystem. Here, I performed feeding experiments using the crayfish *Orconectes propinquus* and one of its preferred prey items, ephemeroptera nymphs, at differing densities to generate its functional response in the presence and absence of the aquatic macrophyte *Potamogeton amplifolius*. Results showed that crayfish exhibit a Type I functional response in both vegetated and non-vegetated conditions, contrary to what was expected. Further studies are necessary to confirm or refute my results, as the relationship between crayfish feeding behavior and habitat complexity is critical to the stability of freshwater ecosystems.

Introduction

Crayfish native to North America, which represent nearly 75% of crayfish species worldwide, are commonplace in many freshwater ecosystems across the United States, particularly in the southeast (Lodge et al. 2000). Their roles in aquatic ecosystems are complex, as they do not fall into a specific trophic level because of their broad diets, which include algae, aquatic vascular macrophytes, detritus, invertebrates, fish eggs, and carrion (Hobbs 1993, Momot 1995). Because their food sources span multiple trophic levels, crayfish play a key role in energy flow and transformation within lakes (Momot et al. 1978) and understanding how crayfish feeding habits affect lake ecosystems is paramount to developing a more complete knowledge of freshwater food webs.

A common method used to analyze an organism's role in an ecosystem's food web is to describe its functional response, which is the relationship between predator feeding rate and prey density (Solomon 1949, Holling 1959, 1965). This relationship is expressed graphically in the form of one of three unique curves. Type I curves have a constant slope and are associated with organisms that consume prey at a rate proportional to the rate at which they encounter it. Type II curves always have a decreasing slope and are characteristic of organisms that take time to ingest and capture their prey. Type III curves are sigmoid and correspond to organisms that display

learned behavior (Real 1977), meaning that as prey density increases the organism becomes more efficient at detecting, capturing, and/or handling prey. Examples of these curves are shown in Figure 1 and general equations for Type II and III curves are as follows:

$$\text{Type II } f = F*A/(G+A)$$

$$\text{Type III } f = F*A^n/(G+A^n),$$

where f represents the organism's feeding rate, F its resource-utilization efficiency, G its affinity for the prey, A the density of prey available, and n the number of encounters the organism must have before reaching maximum predation efficiency (Real 1977). All three types eventually reach upper asymptotes for feeding rate (f), at which point any further increase in density elicits no response.

Functional responses are determined by many factors such as encounter rates, capture rates, and handling time (Holling 1966), all of which are components of the variables within the general equations presented above. These, in turn, can be affected by: alternative prey choices (Acre & Johnson 1979), other predators, relative prey size (Aljetlawi et al. 2004), and substrate (Hildrew and Townsend 1977). Alternative prey choices most directly affect a predator's affinity (G) for the initial prey item, as Acre & Johnson demonstrated in experiments using damselfly naiads given *Daphnia* and *Simocephalu* in complementary densities. They found that naiads tended to prefer whichever prey was available at higher density and that as densities changed naiads displayed switching behavior between prey items, which was particularly apparent in starved naiads. Relative prey size most directly affects a predator's resource-utilization efficiency (F) by affecting capture and handling rates, as Aljetlawi et al. found in experiments altering isopod predator size and amphipod prey size. They observed that smaller predators' handling times increased with an increase in prey size, whereas larger predators' handling times

were not affected by change in prey size. Substrate primarily affects encounter rates by providing refuge for prey, thus increasing search and capture costs. This was demonstrated by Hildrew and Townsend, who found that as habitat complexity increased, predation of stonefly larvae by caddisfly larvae decreased. Real also described the general effects of habitat complexity in terms of n, F, and G, shown in Figure 2. For instance, as habitat complexity increases, a predator's resource-utilization efficiency (F) decreases due to an increase in search costs, possibly due to the prey gaining new sources of refuge.

There is little quantitative data describing functional response curves for crayfish. In this experiment, I performed a series of feeding trials to elucidate the functional response curves of *Orconectes propinquus* for aquatic mayfly larvae (Order Ephemeroptera, Family Heptageniidae) under differing substrate conditions, specifically the presence or absence of vegetation. *O. propinquus*, also known as the northern clearwater crayfish, is prevalent throughout the Midwest, invasive to Tenderfoot lake, and a close relative of to *O. ructicus* and *O. virilis*, making it a good model system for the *Orconectes* genus. The feeding habits of *O. propinquus* have also been described in an observational study performed by Capelli (1980) at Trout Lake, in Vilas County, Wisconsin. In this study, Capelli found that crayfish diets were seasonal and the most seasonally dependent prey was Heptageniidae mayflies, which at the lowest and highest points were found in <15% and 55-90% of stomachs sampled, respectively. The large variance in frequency was primarily caused by seasonal variation in mayfly density and size due to their life cycle. This result suggests that mayfly are a preferred prey choice of *O. propinquus* when present at sufficient densities and sizes, which may be due to decreasing search costs and increased gains. Other findings, such as the observation that crayfish depend more heavily on animal than plant

material for growth (Hill et al. 1993), likely play a role in making mayfly a preferred prey species.

I hypothesized that the crayfish would display a Type II response curve for mayflies under cobble substrate and a Type III curve upon the addition of vegetation. This is because the Type II response curve is often referred to as the invertebrate curve (Real 1977) and has been shown to shift to a Type III with decreasing encounter rates (Hassel 1977), which increasing habitat complexity is known to cause (Hildrew and Townsend 1977). Since crayfish are known to reduce habitat heterogeneity by severely decreasing the biomass and diversity of vascular macrophytes (Dorn and Wojdak 2004, Lodge et al. 1997), studying their functional response for a preferred prey item by varying substrate complexity will help characterize their role in freshwater food webs.

Materials and Methods

I hand collected *O. propinquus* and Heptageniidae ephemeroptera larvae from Tenderfoot lake just offshore from the wetlab and stored them in separate aerated holding tanks containing lake water. Crayfish were fed a diet of shrimp pellets when not involved in trials. Only Form II males with carapace length 20-25mm were used. I also collected cobble, sand, and *Potamogeton amplifolius* from the same location to be used as substrate.

To construct mesocosms, I evenly distributed a mixture of cobble and sand along the bottoms of 0.13 m^2 tanks. Tanks were wrapped on 3 sides with black plastic 15.24-17.78cm high to prevent interference from adjacent tanks while leaving one side open for observation. For non-vegetated trials, tanks were filled with 16.4-19.7L of water from Tenderfoot Lake. For vegetated trials, tanks were filled with 26.2-32.8 L of water, also from Tenderfoot Lake. To determine the natural density of *P. amplifolius* in the lake, I counted all stems in $\frac{1}{2} \text{ m}^2$ quadrats. I used the average as the target density for the vegetated tanks. Only the top portion of *P. amplifolius* was

used to obtain plant matter with similar leaf distribution and size. Individual stalks ranged in length from 14-26cm, overall plant mass per tank from 8.39-19.56g, and the number of leaves from 4-5. I cleared each stalk of most alternative prey sources, mainly snails and large egg masses, and proceeded to anchor each to a rock using rubber bands, making sure that at least 1 leaf was near the substrate. 4 stalks were evenly distributed within each tank in two staggered rows. Each set of stalks were used in two consecutive trials for logistical ease. Because the vegetated trials were conducted during the beginning of a crayfish molt transition and crustacea decrease feeding prior to molting (Lipcius and Herrnkind 2004), these crayfish were monitored for 48hrs post trial to ensure they were not premolt.

For each trial, a single crayfish was starved for 24-48 hrs before being given access to prey. I introduced mayflies at set densities of 23, 39, 62, 78, or 116 per m^2 30 minutes prior to crayfish introduction to allow the mayflies to colonize the tank. 5 replicates of each density were performed for each substrate type for a total of 50 trials, 25 vegetated and non-vegetated. The densities 39, 78, and 116 were selected based on pilot trials, while the densities 23 and 62 were added mid-study. After the addition of a starved crayfish, a screen was taped on top of the tank to prevent emergent mayflies, if any, from escaping. Trials began between 9:00-11:45pm and were run for 12-14hrs. After 12-14hrs, crayfish were removed and mayflies collected and massed. Feeding rate was defined in two ways: as the number of mayflies consumed per trial and as the mass consumed per trial. Mayflies were massed prior to trials, with mass ranges: 0.06-0.08g for density 23; 0.11-0.14g for density 39; 0.17-.0.21g for density 62; 0.23-0.28g for density 78, and 5, 0.38-0.41g for density 116. Individual mayflies ranged from 0.01-0.05g to represent the size distribution of the population in Tenderfoot Lake.

A nonlinear model corresponding to a Type II functional response curve was fit to both

number and mass consumed vs. density. I then performed box plots of number consumed vs. density across vegetated treatments to determine normality, followed by general linear model to determine the relationship between curves for number consumed and mass consumed vs. density. Density 116 was removed from this analysis due to the possibility that the non-vegetated crayfish had reached satiation because using points beyond this point, where density no longer affects feeding rate, would have skewed the data. Linear regressions on both curves were then used to confirm the validity of results from the nonlinear model. Standard one-tailed two-sample t-tests with Bonferroni correction α levels were used to compare density 116's across treatments for number and mass consumed as well to compare number and mass consumed in density 116 and 78 for vegetated treatments.

To explore the possibility that crayfish in vegetated trials were foraging on minute eggs on the undersides of *P. ampliofolius* leaves I ran trials, again 12-14 hrs, in smaller tanks using only one anchored leaf per crayfish. Seven replicates were run with crayfish and seven without. Qualitative analysis was performed by taking pictures of the eggs/leaves before and after to ascertain if crayfish were foraging on them.

Results

Fitting a nonlinear model corresponding to a Type II functional response curve to both number and mass consumed vs. density revealed that it was mostly likely a Type I curve instead of a Type II. Further analysis using box plots on number consumed vs. density showed that the data was normal, allowing for the use of simple linear regressions. For non-vegetated trials, after density 116 was removed to eliminate the non-linear effect caused by satiation, a linear regression proved the presence of a Type I curve for both number (Slope = 0.0985 R^2 = 0.6227, $F_{1,18}$ = 29.7019, P < 0.0001) and mass consumed (Slope = 0.0028, R^2 = 0.6451, $F_{1,18}$ = 32.7223, P < 0.0001). Vegetated trials including density 116 also exhibited a Type I curve for both

number (Slope = 0.0439, R^2 = 0.4829, $F_{1,23}$ = 21.4796, P = 0.0001) and mass consumed (Slope = 0.0012, R^2 = 0.4958, $F_{1,23}$ = 22.6159, P = 0.0001). Regressions for number and mass consumed are shown in Figures 3&4, respectively. A general linear model for number consumed, excluding density 116, revealed that density was significant ($F_{1,36}$ = 24.0106, P < 0.0001), presence or absence of vegetation was marginally significant ($F_{1,36}$ = 3.6643, P = 0.0636), and there was a significant interaction between vegetation and density ($F_{1,36}$ = 14.6561, P = 0.0005). A general linear model for mass consumed, excluding density 116, showed similar results for density ($F_{1,36}$ = 28.2581, P < 0.0001) and the interaction term between vegetation and density ($F_{1,36}$ = 13.3158, P = 0.0008), but the presence or absence of vegetation became nonsignificant ($F_{1,36}$ = 2.2829, P = 0.1395). Overall, this data showed a Type I functional response curve that was significantly depressed upon addition of vegetation.

One-tailed two-sample t-tests with Bonferroni corrections revealed that there was a significant difference in number (t_8 = -4.4174, P = 0.0022) and mass consumed (t_8 = -3.8775, P = 0.0046) across the vegetated 78 and 116 densities, but no significant difference for either number (t_8 = 0.9949, P = 0.3489) or mass (t_8 = 1.6890, P = 0.1297) consumed across the 116 density.

Visual examination of before and after photographs of the leaves showed that in tanks with a crayfish present, the majority of eggs were consumed. The most striking evidence came from leaves that had eggs only one half of their underside. The half that had eggs was often severely damaged, whereas the half without eggs was relatively untouched.

Discussion

The result that crayfish exhibit a Type I functional response curve for mayfly in both vegetated and non-vegetated conditions was counter to my prediction (Figures 3&4), particularly since Type I functional response curves are generally associated with filter feeders (Real 1977). This is because in a linear functional response, the only variable affecting feeding rate (*f*) is the

density of prey, leaving one with the simple equation $f = mx + b$, where m and b are experimentally derived constants. Since crayfish are not filter feeders, but rather active foragers, their functional response curve should factor in the various costs of foraging, including but not limited to: risk of predation, energetic search, capture, and handling costs. A Type I functional response curve does not accurately represent these costs because foraging is not quantified. Assuming that my results do not accurately reflect the actual functional response curve between crayfish and mayflies, the question becomes why.

Hassel et al. (1977) demonstrated that predators that previously displayed Type II curves could exhibit a Type III curve when the number of encounters with prey was decreased. This could be achieved in a variety of ways, including decreasing prey density, increasing relative prey:predator size, increasing the size of the experimental confines, and/or increasing refugia by increasing habitat complexity. They posited that by reducing functional response experiments to preferred prey species in relatively small and simplistic "universes" for logistical ease, many studies have elicited a Type II response not because it is what occurs naturally, but rather because the experimental design was unintentionally biased. More specifically, the bias stemmed from artificially high encounter rates for reasons previously described, which then led to decreased search costs.

Extending Hassel et al.'s hypothesis further, I suspect that if the number of encounters with prey was increased enough, the associated search cost may be reduced to such a degree that it becomes negligible. This would, in theory, cause a predator that actively forages to exhibit a Type I functional response. I hypothesize that an artificial increase in encounter rates due to a smaller experimental "universe" and preferred prey may have occurred in my experiment, ultimately leading to a Type I functional response for vegetated and non-vegetated trials. Studies

examining mayfly density in lakes with similar characteristics to Tenderfoot are scarce, making inferences based off of the densities used in my experiment difficult due to lack of comparison. Densities from stream studies are not likely to be reliable due to both the dramatic differences in mayfly habitat and observed densities (Casey 1987, Ward & Berner 1980).

The differences between the curves for vegetated and non-vegetated trials were more straightforward. As I expected, the increase in substrate complexity decreased predation, presumably through increased search costs to the crayfish, thus depressing the curves for both number and mass consumed vs. density, as shown in Figures 3&4. The lack of a shift in the type of response curve may be attributable to possible experimental design errors discussed earlier. It is also reasonable to state that, for vegetated trials, I needed to run higher densities to establish a satiation cutoff. With the data collected, I am only able to describe a portion of the curve because I do not have densities that show satiation, and therefore cannot make any definitive statements. While this is true, I believe that because the linear regression showed such a strong fit for both number and mass consumed, the curve is likely Type I.

One finding that occurred during trials may suggest that the actual depression is less than that observed. I observed that most leaves were heavily coated in minute eggs or egg sacs. These were present on virtually all *P. amplifolius* leaves to some degree, and the logistics of removing them without damaging the plant material were such that their removal was deemed unnecessary. I noticed, however, that after the first round of vegetated trials the vast majority of eggs were no longer present on the undersides of leaves and that some crayfish were found on the *P. amplifolius* at the end of trials when it was daylight. Qualitative comparisons of before and after photographs strongly suggest that the crayfish did indeed forage on the eggs. Why crayfish, if they were functioning under a Type I response and thus had negligible search costs, would

switch prey items is up for debate. The most likely explanation seems to be that the eggs were a more preferred prey source, possibly having higher G and/or F values, while being at a high enough density to where search cost is negligible yet again.

The effect of having an alternative prey item present was presumably negated for the second set of trials run with the same stalks, as most of the egg matter had already been removed. Density 15 likely would have been affected the greatest because all 5 replicates were run on the second night of trials for that set of stalks. This may explain the significant difference in both number ($t_8 = -4.4174$, $P = 0.0022$) and mass ($t_8 = -3.8775$, $P = 0.0046$) consumed between densities 78 and 116 found after running one-tailed two-sample t-tests with a Bonferroni correction. I can only speculate as to the effects of egg foraging because the vegetation trials contained two variables as long as eggs were present: 1) the increase in substrate complexity, and 2) the presence of an alternative prey item.

However, the data from the vegetated density 116, which presumably had no egg interference for any replicates, would be comparable to that of the non-vegetated trials. Standard one-tailed two sample t-tests with a Bonferroni correction showed that there was no significance between the two groups for number consumed ($t_8 = 0.9949$, $P = 0.3489$) and marginal nonsignificance for mass consumed ($t_8 = 1.6890$, $P = 0.1297$).

To confirm that *O. propinquus* exhibits a Type I functional response curve for mayflies, or observe a Type II or III curve as I would expect, I have identified several modifications to improve the experimental design for future studies. Trials should be run within as close a time frame as logistically possible to prevent seasonal changes in behavior, sunlight, and temperature from influencing results. My trials, due to time and capacity constraints, were spread over the course of 26 days, during which temperature fluctuations, particularly on the night of June 30th

when the air temperature dropped to 10°C, may have affected behavior. In addition to using the densities used here, the densities of 155 and 194 individuals per m^2, which were planned but unable to be completed due to time constraints, should also be used. Vegetation would be cleaned of all possible alternative prey items to prevent interference, and the use of multiple plant species to mimic the benthic community would provide more realism. Larger "universes" should be considered as well, along with alternative, less preferred prey items.

Since crayfish alter their environments by dramatically decreasing the overall biomass and diversity of vascular macrophytes (Dorn and Wojdak 2004, Lodge et al. 1997), thus decreasing habitat complexity, they may create a positive feedback loop for their success as a predator by decreasing the costs associated with foraging. As such, the relationship between crayfish feeding behavior and habitat complexity is critical to the understanding of their role(s) in freshwater food webs.
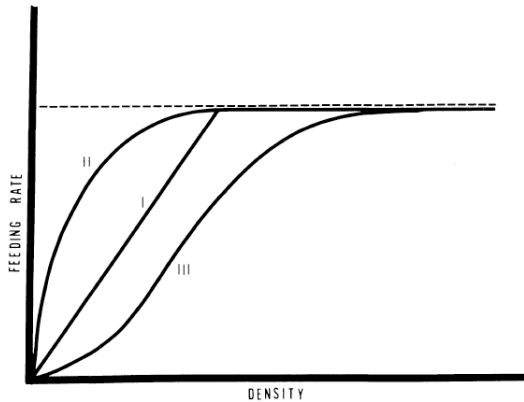
Acknowledgements

References

1. Akre, B.G. and D.M. Johnson. 1979. Switching and sigmoid functional response curves by damselfly naiads with alternative prey available. *Journal of Animal Ecology* 48: 703-720.
2. Aljetlawi, A.A., E. Sparrevik, and K. Leonardsson. 2004. Prey–predator size-dependent functional response: derivation and rescaling to the real world. *Journal of Animal Ecology* 73:239-252.
3. Capelli, G.M. 1980. Seasonal variation in the food habits of the crayfish *Orconectes propinquus* (Girard) in Trout Lake, Vilas County, Wisconsin, U.S.A (Decapoda,

Astacidea, Cambaridae). *Crustaceana* 38:1:82-86.

4. Casey, R.J. 1987. Diel periodicity in density of Ephemeroptera nymphs on stream substrata and the relationship with drift and selected abiotic factors. *Canadian Journal of Zoology* 65:2945-2952.

5. Dorn, N. and J. Wojdak. 2004. The role of omnivorous crayfish in littoral communities. *Oecologia* 140:1:150-159.

6. Hassel, M.P., J.H. Lawton, and J.R. Beddington. 1977. Sigmoid functional responses by invertebrate predators and parasitoids. *Journal of Animal Ecology* 16:1:249-262.

7. Hildrew, A.G. and C.R. Townsend. 1977. The influence of substrate on the functional response of plectrocnemia conspersa (Curtis) larvae (trichoptera: polycentropodidae). *Oecologia* 31:1:21-26.

8. Hill, A., D. Sinars, and D. Lodge. 1993. Invasion of an occupied niche by the crayfish *orconectes rusticus*: potential importance of growth and mortality. *Oecologia* 94:3:303-306.

9. Hobbs, H.H. III. 1993. Trophic relationships of North American freshwater crayfishes and shrimps. *Milwaukee Public Museum Contribution to Biology & Geology* 85:1-110.

10. Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. *Canadian Entomologist* 91:385-398.

11. Holling, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. *Memoirs of the Entomological Society of Canada* 45:5-60.

12. Holling, C.S. 1966. The functional response of invertebrate predators to prey density. *Memoirs of the Entomological Society of Canada* 48:1-86.

13. Lipcius, R.N. and W.F. Herrnkind. 2004. Molt cycle alterations in behavior, feeding and diel rhythms of a decapod crustacean, the spiny lobster *Panulirus argus*. *Marine Biology*. 68:3:241-252.

14. Lodge, D.M. and J.G. Lorman. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. *Canadian Journal of Fisheries and Aquatic Sciences*. 44:591-597.

15. Lodge, D., C. Taylor, D. Holdich, and J. Skurdal. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. *Fisheries* 25:8:7-20.

16. Momot, W.T. 1995. Redefining the role of crayfish in aquatic ecosystems. *Review of Fisheries Science* 3:33-63.

17. Momot, W.T., H. Gowing, and P.D. Jones. 1978. The dynamics of crayfish and their role in ecosystems. *The American Midland Naturalist* 99:1:10-35.

18. Real, L.A. 1977. The kinetics of functional response. *The American Naturalist* 111:978:289-300.

19. Solomon, M.E. 1949. The natural control of animal populations. *The Journal of Animal Ecology* 18:1:1-35.

20. Ward, J.V. and L. Berner. 1987. Abundance and altitudinal distribution of ephemeroptera in a rocky mountain stream. *Advances in Ephemeroptera Biology*. Plenum Press, New York and London: 169–177.

This paper is meant to provide general information on past work at UNDERC.
However, because this report is not published in a peer-reviewed journal, we cannot guarantee the accuracy of any data collected.

Argus, 13

Figure 1: The Basic Functional Response Curves. These are the three basic types of functional response curves as presented by Real. Type I curves are linear and are associated with organisms that consume prey at a constant rate proportional to prey density. Type II curves have a decreasing slope and are associated with organisms that take time to capture and ingest their prey. Type III curves are sigmoid and are associated with organisms that display learned behavior. (Real 1977)
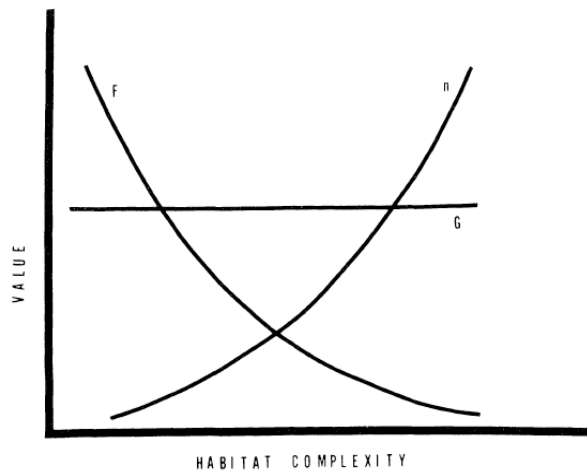
Figure 2: The Relationship Between Resource-Utilization Efficiency (F), Predator Affinity (G), and the Number of Encounters to Ensure Maximum Predatory Efficiency (n) to Habitat Complexity. This figure, from Real 1977, describes how the 3 variables F, G, and n are affected by habitat complexity.
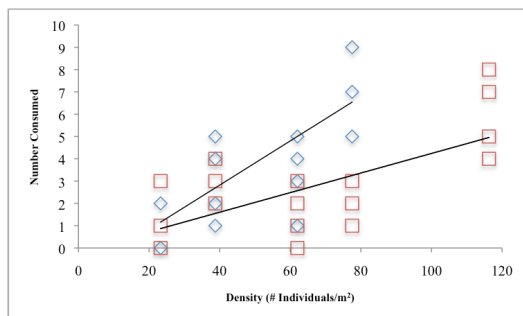
Figure 3: Ephemeroptera Number Consumed vs. Density Under Vegetated and Non-vegetated Conditions. Simple linear regressions were used to characterize the curves for both vegetated (Slope = 0.0439, $R^2 = 0.4829$, $F_{1,23} = 21.4796$, P = 0.0001) and non-vegetated (Slope = 0.0985 $R^2 = 0.6227$, $F_{1,18} = 29.7019$, P < 0.0001) treatments (n=5 for each density level). Density 116 was removed from analysis for non-vegetated trials due to satiation. Both regressions show a clear Type I functional response curve.
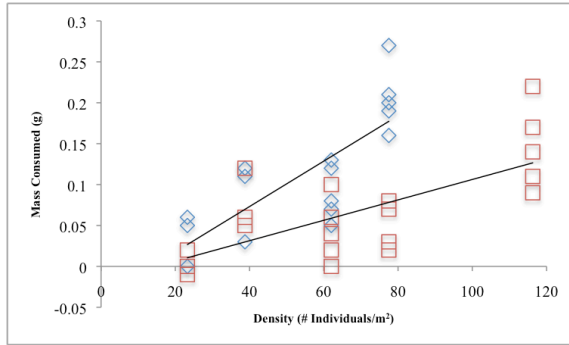
Figure 4: Ephemeroptera Mass Consumed vs. Density Under Vegetated and Non-Vegetated Substrate. Five replicates of each density were performed. Simple linear regressions were used to characterize the curves for both vegetated (Slope = 0.0012, R^2 = 0.4958, $F_{1,23}$ = 22.6159, P = 0.0001) and non-vegetated (Slope = 0.0028, R^2 = 0.6451, $F_{1,18}$ = 32.7223, P < 0.0001) treatments (n=5 for each density level). Density 116 was removed from analysis for non-vegetated trials due to satiation. Both regressions show a clear Type I functional response curve.