Luxilus cornutus prey size selection in response to different habitat densities

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Abstract

Macrophytes are important components of aquatic habitats, increasing habitat complexity which benefits biodiversity and overall system stability. However, eutrophication, dredging, invasive species, climate change, and other anthropogenic changes to the environment can alter macrophyte assemblages in aquatic ecosystems. Changes in macrophyte density have the potential to alter foraging habits of predator populations, which could hold greater trophic consequences for the larger ecosystem. Cyprinids are prolific in freshwater systems and have the capacity to impact macroinvertebrate biodiversity. To test the effects of varying habitat density on Cyprinid prey size selectivity, I offered Anisoptera nymphs of three distinct size classes as prey items to *Luxilus cornutus* in treatments that simulated different macrophyte densities. I hypothesized that in highest density, *L. cornutus* would have high energy output while foraging in highest density and in lowest density, there would be a stronger predation risk causing *L. cornutus* to demonstrate less size selectivity. In intermediate density, both factors are moderate, allowing *L. cornutus* to be more selective. Few nymphs across all trials were consumed making it impossible to reject the null hypothesis. Habitat density (*P* = 0.665, *F* = 0.2, df = 1) and nymph size class (*P* = 0.345, *F* = 1.2, df = 2) were not significant nor were interactions between the two factors (*P* = 0.296, *F* = 1.4, df = 2). However, there is value in continuing to study Cyprinid models for foraging in varying habitat densities. A shift in lower-level predator foraging strategies in response to changing habitat density or structure can hold consequences for the greater aquatic system.
Introduction

In the wild, it is important for organisms to maximize the intake of energy while minimizing output. Successful individuals can match or exceed the amount of energy they expend in food and nutrient intake. Optimal foraging theory states that an organism will exhibit preferential foraging behavior that changes in response to a range of factors and environments (Altmann and Wagner 1978). Experiments on foraging behavior and the impact of factors on foraging behavior in a variety of species have been performed, including studies of the impacts prey abundance (Lacher et al. 1982), prey size (Werner and Hall 1974), and hunger and experience (Beukema 1968) on how animals choose to forage.

Macrophytes are important components of aquatic habitats that affect both the intraspecific and interspecific interactions of aquatic populations. Addition of a structural complexity such as macrophytes has been linked to higher plankton abundance (Basu et al. 2008) and diversity (Declerck et al. 2007). In shoreline macrophyte habitats, a high abundance of macroinvertebrates have been observed (Thomaz et al. 2000; Gregg and Rose 1985). High food availability and shelter from predators creates valuable nursing grounds. The largest percent of immature fish within aquatic systems are often observed within shoreline vegetation (Conrow et al. 2011; Floyd 1983).

However, human activity has led to the acceleration of eutrophication in aquatic systems through fertilizer runoff and other industrial processes (de Jonge et al. 2002). Decline of macrophytes have been observed in eutrophic bodies of water due to algal blooms inhibiting light penetration and increasing pH (Hough 1989; Jupp and Spence 1977; BoQiang et al. 2013). Climate change has implications for the dispersal and range of different aquatic plant species (Viana 1906; Alahuhta et al. 2011). Activities such as dredging in lakes cause changes in aquatic
macrophyte assemblages with little to no recovery of assemblages in deeper sections of lakes observed (Nichols 1984). Human activity has also facilitated the introduction and spread of various invasive species such as *Orconectes rusticus*, or rusty crayfish, in Wisconsin lakes whose presence have been tied to decreases in macrophyte biodiversity (Wilson 2002).

Changes to macrophyte cover in aquatic systems can have consequences for the trophic interactions since in a more dense and complex habitat where movement and sight is restricted, handling and search time of predators increase (Diehl and Kornijów 1998). In different vegetation densities, changes to foraging strategies (Savino and Stein 1989) and prey selectivity (Anderson 1984) have been observed in piscivorous fish species. However, few studies have been conducted on prey selectivity of mainly insectivore fishes including Cyprinids in response to different habitat complexity. Cyprinids are capable of exerting predation pressure affecting biodiversity of macroinvertebrate populations (Gilliam et al. 1989; Williams et al. 2003). A change in prey size preference in Cyprinids with changes in macrophyte density could have greater implications for macroinvertebrate community biodiversity.

Common shiners, *Luxilus cornutus* are a member of Cyprinidae that are prolific in cool, clear streams in the Northeastern and Midwestern portion of the United States (Fuller 2004). *L. cornutus* are omnivorous, feeding on both plant matter and aquatic insect larvae (Trial et al. 1983). Insects in the suborder Anisoptera are considered strong indicators of aquatic ecosystem health (Chovanec and Waringer 2005; 2011). Both adults and nymphs are important components of aquatic and terrestrial trophic systems as they are both voracious predators that feed upon smaller invertebrates and fish as nymphs and nuisance insect as adults and prey for many other organisms (Keller et al. 2007).
*L. cornutus* need to optimize energy intake while balancing predation risk while foraging for Anisoptera nymphs in habitats of variable density. Considering this, I hypothesized that foraging would be least selective in treatments of lowest and highest density. Theoretically, behavior would be most affected by predation risk in tanks of lowest density while foraging will be most affected by large handling and search time in tanks of the highest density. In tanks of intermediate density, I expected higher selectivity where the two factors affecting foraging was be relatively moderate.

**Methods**

*Study area and collection:*

Collection and trials were all conducted on University of Notre Dame Environmental Research Center (UNDERC) property consisting of approximately 30.35 km² on the border of Wisconsin and the Upper Peninsula of Michigan. *L. cornutus* used were collected from Tenderfoot Creek using minnow traps while anisoptera nymphs were collected from Morris Lake using dip nets. Both Tenderfoot Creek and Morris Lake have clear water and soft, muddy bottoms with macrophyte assemblages bordering the shoreline. While Tenderfoot Creek flows, it is a low-gradient stream with very slow flow in most sections. *L. cornutus* was abundant at Tenderfoot Creek and while Anisoptera nymphs were also present at Tenderfoot, their abundance at Morris ensured enough nymphs of each size class could be collected. Prior to trials, *L. cornutus* were kept in aerated 91-gallon cattle tanks filled with water taken from Tenderfoot Lake that were kept at ambient temperature in order to minimize possible stress of being placed
in a new environment. All specimens were collected June-July and trials were conducted and completed on July.

*Setup of trials:*

Five 10-gallon glass aquariums (50.8 x 25.4 x 30.5 cm) were filled with Tenderfoot Lake water. Tanks were aerated and kept at ambient temperature for the experimental duration. Window screen mesh was cut into 50.8 x 25.4 cm sheets that fit onto the bottom of the tanks. Dark green braided nylon cord was cut into 30 cm strands with melted ends to prevent fraying. The middle of each strand was tied onto the mesh with clear fishing string so that when the mesh was placed into the tanks, the two ends of the strands would float upwards to mimic submerged macrophytes. Each aquarium simulated a different density of vegetation: 0, 100, 200, 400, and 600 stems/m². Aquariums that would have had an uneven number of stems present had one more added to become even since each nylon cord created two artificial stems. In each tank, six rocks of comparable size and shape held down the mesh. The placement of rocks was recorded and kept consistent between tanks and trials.

*Trials:*

The first treatment tested was randomly selected using a random number generator and following trials were done sequentially. The sequence of trials was only interrupted if treatments needed to be repeated. Prior to the trial, *L. cornutus* were acclimated for approximately 1.5-2 hours. Anisoptera nymphs were categorized into three size classes: small (6-10 mm), medium (11-15 mm), and large (16-20 mm). For each trial three nymphs of each size class were selected and head width and total length in millimeters of each was recorded. After acclimation, nymphs
were added to the aquarium. After an hour, *L. cornutus* were removed and measured (TL:mm; mouth gape; mm). Remaining nymphs were then collected. Size classes of nymphs not recovered were recorded.

**Data analysis:**

Regression analysis was used to test the linear relationship between the measured length and gape of *L. cornutus*. Chi-squared test of independence was used to determine effect of nymph length on *L. cornutus* predation risk. One-way and two-way ANOVA was used to determine impact of vegetation density on average number of nymphs total and average number of nymphs per size class eaten respectively. Software used to run statistics was R version 3.4.4 (The R Foundation for Statistical Computing. Vienna, Austria).

**Results**

The five habitat density treatments were categorized numerically from one to five, one being the lowest stem density (0 stems/m²) and five being the highest (600 stems/m²). A total of 34 trials were run with seven trials for all treatments except for treatment two (100 stems/m²) which had only six trials. *L. cornutus* total length and gape width were found to have a positive relationship ($R^2 = 0.6919$, $P = 1.095 \times 10^{-9}$, df= 32; Figure 1).

Overall, only three of the provided 306 Anisoptera across all treatments and trials were consumed. *L. cornutus* showed no overall size preference across all treatments ($x^2 = 0.019802$, df= 2, $P = 0.99$; Figure 2). Habitat density ($P = 0.665$, F= 0.2, df= 1) and nymph size class ($P=$
were not significant with no significant interactions between the two factors ($P = 0.296$, $F = 1.4$, $df = 2$; Figure 3).

**Discussion**

With only three out of the 306 provided Anisoptera larvae eaten, it is impossible for me to reject my null hypothesis that habitat density has no impact on *L. cornutus* prey size selectivity.

Several factors may have accounted for the limited dataset. The process of trapping, holding for extended periods of time, and movement to a new setting prior to the trial may have stressed subjects, making them unwilling to eat. It is possible that two hours of acclimation was not sufficient or that trial time should have been longer. There is also the possibility that the unrecovered nymphs were consumed instead by the larger nymphs present in the tank as Odonate nymphs are known to prey on smaller Odonates.

Due to the constraints of location for trials, lighting was variable. There was an uncovered window in the room positioned so that the first and second treatments received more ambient light than other treatments. Previous studies have observed behavioral changes in Cyprinids in relation to variable light intensities (Cerri 1983) so inconsistent light conditions between treatments may have been a confounding factor.

The use of artificial stems and removable mesh in place of real plants and substrate was intended to ensure that all larvae remaining could be found. However, the mesh posed problems as well. While rocks were used to hold the mesh to the bottom of the aquarium, parts of the mesh were not flush to the ground, allowing a few nymphs to slip underneath.
My results indicated no obvious size distinction made by foraging *L. cornutus* in different habitat densities. Previous studies indicate that success of predators is dependent on their ability to adapt and modify their strategies in response to a change in habitat. Changes in selectivity are not the only strategy utilized by organisms when both energy output and predation risk must be balanced. Warfe and Barmuta observed southern pygmy perch (*Nannoperca australis*) switching from an ambush technique that minimized movement and thus possible detection by predators to actively searching for prey in denser habitats (2004). However, small largemouth bass (*Micropterus salmoides*) of a similar size range actively searched for prey throughout all densities and instead displayed higher selectivity in higher densities where movement was far more restricted (Anderson 1984).

Most studies conducted on foraging habits in relation to habitat density use mid- and upper-level predators as models (Warfe and Barmuta 2004; Anderson 1984; Dionne and Folt 1991). However, there exists a knowledge gap surrounding how changes in habitat density impacts Cyprinid foraging behavior. As low-level predators, Cyprinids such as *L. cornutus* have the potential to be more impacted by predation risk and may display stronger predation avoidance behavior. Their foraging behavior may not be comparable to larger predators such as Centrarchids but Cyprinids have the potential to significantly alter macroinvertebrate biodiversity in aquatic ecosystems (Gilliam et al. 1989; Williams et al. 2003). Macroinvertebrates such as Odonate nymphs are important components of aquatic trophic systems as predators and prey and a change in their abundance or distribution could have greater consequences for ecosystems (Keller et al. 2007).

Cyprinids can prove to be important models in future studies of foraging and habitat density. It would be valuable to look at other Cyprinids and perhaps compare foraging strategies
in different densities between species. Previous studies have also made distinctions between macrophyte density and structural complexity. Structural complexity often plays a larger role in increasing search time than density (Warfe and Barmuta 2004; Dionne and Folt 1991) and would be a valuable factor to study as well.

Human actions will continue to affect macrophyte assemblages with greater frequency and intensity as we continue to develop and expand. Assemblages will continue to see a loss of abundance and diversity with eutrophication from fertilizer and industrial runoff and the spread of invasive species (Hough 1989; Jupp and Spence 1977; BoQiang et al. 2013; Wilson 2002). Species makeup and distribution will continue to shift with climate change and dredging (Nichols 1984; Viana 1906; Alahuhta et al. 2011). It is integral that we look at not only upper-level, but also lower-level trophic interactions when assessing the consequences changes to macrophyte assemblages will have on the larger aquatic ecosystem.
**Figure 1:** *L. cornutus* total length to gape (mm). *L. cornutus* gape was wider on individuals with longer total length ($R^2 = 0.6919$, $P = 1.095 \times 10^{-9}$, df = 32).
Figure 2: The proportion of Anisoptera nymphs eaten to the total number of nymphs offered by size class across all treatments. Anisoptera size classes were defined by total length. “Small” were 6-10 mm, “Medium” were 11-15 mm, and “Large” were 16-20 mm. *L. cornutus* showed no preference between the three size classes of Anisoptera nymphs ($x^2= 0.019802$, df= 2, *P*= 0.99).
Figure 3: Anisoptera nymph size classes were defined by total length. “Small” were 6-10 mm, “Medium” were 11-15 mm, and “Large” were 16-20 mm. Treatments differed in stem density with one to five being 0, 100, 200, 400, and 600 stems/m$^2$ respectively. *L. cornutus* showed no preference between treatments ($P = 0.665$, $F = 0.2$, df = 1) or size class ($P = 0.345$, $F = 1.2$, df = 2). There was also no significant interaction between the two factors ($P = 0.296$, $F = 1.4$, df = 2).
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