LIFE HISTORY TRAITS OF THE MIRROR SHINER, *NOTROPIS SPECTRUNCULUS*, IN WESTERN NORTH CAROLINA

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

By

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March 2012

ACKNOWLEDGEMENTS

I’d like to thank my committee members for their guidance and support. I’d also like to thank the Biology Department and Graduate School for the funding they provided for me to travel and present this work.

Many thanks are due to the number of people who helped me in the field completing this project.

Finally, I’d like to thank my parents for believing in me through the bad times and celebrating with me through the good.

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ABSTRACT

LIFE HISTORY TRAITS OF THE MIRROR SHINER, *NOTROPIS SPECTRUNCULUS*, IN WESTERN NORTH CAROLINA

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I investigated the life history of *Notropis spectrunculus* (Mirror Shiner) using seven monthly collections at each of four locations in the Tennessee River drainage in western North Carolina (Hominy Creek, Pigeon River, and two sites on the Tuckasegee River). Specimens were collected by seining and examined to identify age, growth, reproductive patterns, and feeding habits. The largest *Notropis spectrunculus* male collected was 71 mm standard length (SL) and 2.39 g weight. The largest female collected was 77 mm SL and 2.96 g weight. Sexual maturity occurred at approximately one year of age. The oldest specimens collected were age three, and both males and females were collected of this age. Spawning occurred in late spring and early summer with 13-331 mature oocytes (mean = 115.53, SD = 75.36) and male breeding coloration was present in specimens collected in May, June, and July. Gut contents consisted mainly of insect fragments, primarily Coleoptera and Diptera. Fish were found to be inhabiting water 0.5 to 0.75 m deep with sandy substrate, directly below flow-disrupting stream objects.

INTRODUCTION

One of the side effects of human development and expansion is pollution. At times, the cost of progress has been the wide-scale destruction of both terrestrial and aquatic habitats. Efforts to restore aquatic habitats can be difficult for several reasons. Moving water carries pollution away from the source (León et al. 2001, Lowrance et al. 1985), either making identifying the source of the problem difficult or decreasing the public concern near the source. Further, bodies of water have sometimes been thought of as too big to fail (Clover 2006).

Human activity has had a number of effects on aquatic systems. These include agricultural runoff from farms into rivers (Wang et al. 1997, Boesch et al. 2001), runoff from roads (Drapper et al. 2000, Krein and Schorer 2000), the diversion of rivers for human activities (Pearlstine et al. 1985), the disruption of natural high water events that allow for the natural movement and succession of organisms in an ecosystem (Ritter et al. 1999), the eutrophication of the Laurentian Great Lakes (Chapra and Robertson 1977, Beeton 1965), the bleaching of coral reefs (Glynn 1991, Hoegh-Guldberg 1999), the introduction of innumerable alien species (Roberts 1990, Whitfield et al. 2002), and overfishing (Jackson et al. 2001, Rotschild et al. 1994, Clover 2006), among other problems. All contribute to the disruption of natural hydroperiod regimes and/or cause harm to the health of aquatic communities.

The Pigeon River, part of the Tennessee River watershed in North Carolina and Tennessee, is one such degraded system. A kraft paper and pulp mill exists in the city of Canton, NC, which lies near the headwaters of the Pigeon River. Construction of the mill began in 1906 and in 1907, the mill, operated as the Champion Fibre Company (later named Champion Paper Products), secured permission from the state of North Carolina to avoid prosecution for any pollution they would expel into the river (Bartlett 1995). The mill diverted flow and polluted the river with effluent and artificially heated water, destroying the natural flora and fauna and tainting the color and smell of the Pigeon River downstream into Tennessee (Bartlett 1995). In 1982, the state of Tennessee sued the state of North Carolina and the EPA to force the mill to clean up its effluent, resulting in the EPA taking the permitting authority of the state of North Carolina (Bartlett 1995). The mill underwent over $300 million in renovations and improvements between 1988 and 1994 to clean the water it was releasing (Bartlett 1995). The mill, now operating as Blue Ridge Paper Products, Inc., a subsidiary of the Evergreen Packaging Group, has decreased the amount of color and dioxins they release and the volume of water they use (Paul Dickens, Blue Ridge Paper Products, Personal Communication, Bartlett 1995). Although the mill has significantly reduced its water usage and the chemical pollutants it releases into the river, two low-head dams on the Pigeon River located at the mill in Canton may prevent natural recolonization below the mill from populations of fish from above the mill (LaVoie 2007) and issues remain concerning the color and temperature of discharged water from the mill (Hyatt 2010). Recolonization from below the mill is also impossible due to Walters Dam (operated by Progress Energy Carolinas) and the reservoir created by the dam, which extends up the river for approximately 8-km, creating Waterville Lake. Progress Energy diverts much of water from the river below the dam for approximately 19-km to the Walters Hydroelectric Plant near the Tennessee-North Carolina state line at Waterville, NC, creating a large de-watered section (North Carolina Wildlife Resource Commission 2005).

The Pigeon River Recovery Project, jointly supported by the University of Tennessee, the Tennessee Valley Authority, the Tennessee Department of Environment and Conservation, the North Carolina Division of Water Quality, Blue Ridge Paper Products, and the North Carolina Wildlife Resources Commission, has been trying to reintroduce fish species thought to have been extirpated from the Pigeon River downstream of Canton in an effort to restore naturally reproducing populations (Joyce Coombs, University of Tennessee at Knoxville, Personal Communication). The project has had success establishing reproducing populations of some of the 24 target species, such as the Banded Darter (*Etheostoma zonale*), the Tennessee Shiner (*Notropis leuciodus*), the Saffron Shiner (*Notropis rubricroceus*), the Bigeye Chub (*Hybopsis amblops*), the Gilt Darter (*Percina evides*), the Telescope Shiner (*Notropis telescopus*), the Silver Shiner (*Notropis photogenis*), and the Highland Shiner (*Notropis micropteryx*). However, attempts to reintroduce other species, including the Mirror Shiner *(Notropis spectrunculus*) [described by Cope, 1868] have thus far failed.

Since 2004, over 6000 *N. spectrunculus* individuals have been relocated to habitat below the mill in Canton, NC (Joyce Coombs, University of Tennessee at Knoxville, Personal Communication). However, unlike other species of fish reintroduced by the project, none of these individuals have been recovered in surveys conducted later in the year of the release. The lack of success in recapturing this species, relative to the success in recapturing other reintroduced species, suggests they may be experiencing severe mortality. However, very little is known about the biology of *N. spectrunculus* and so it is difficult to speculate on possible reasons for the apparent failure of the reintroduction.

Notropis spectrunculus is a stream-dwelling fish of the family Cyprinidae found in tributaries of the watershed of the Tennessee River. *Notropis spectrunculus* are believed to inhabit deep pools just below riffles (Joyce Coombs, University of Tennessee at Knoxville, Personal Communication) and rocky pools and runs (Etnier and Starnes 1994). Breeding coloration in males has been observed from mid-May to late-June but Etnier and Starnes (1994) state that the biology of *N. spectrunculus* remains generally unreported. Etnier and Starnes also note that according to Mayden (1989), *N. spectrunculus* is most closely related to the Mimic Shiner (*Notropis volucellus*) [described by Cope, 1865].

Much of the activity and behavior of *N. spectrunculus* has been assumed to be similar to that of the better known species, *N. vollucellus*. *Notropis volucellus* is found in most watersheds throughout the eastern United States. Its life span is three years and it reaches sexual maturity during the first year. Tubercles – small growths on the head that male fish use in competition for mates – have been reported from late May through early October (Etnier and Starnes 1994), indicating that *N. volucellus* spawn throughout much of the year. Johnson and Dropkins (1995) reported that the majority of the diet of *N. volucellus* is chironomids, and their peak feeding time is midnight.

The Rainbow Shiner, another related species, may provide insights into the life history of *N. spectrunculus* is, *Notropis chrosomus*. *Notropis chrosomus* is found in the same habitat as *N. spectrunculus. Notropis chrosomus* is insectivorous, preying primarily on Chironomids, Diptera, and Collembola. Spawning has been observed in April, May, and June. Sexual maturity occurs at one year of age and two years is the maximum age of the species (Holder and Powers 2010).

Finally, looking at the biology of a sympatric congeneric species of *N. spectrunculus* – *Notropis leuciodus*, the Tennessee Shiner – can help provide further insights into the potential life history of *N. spectrunculus*. Etnier and Starnes (1994) *Notropis leuciodus* has a breeding season that peaks in May and June. Similarly to other *Notropis* spp., the fish shows little tolerance for reservoirs. Spawning has been observed over Central Stoneroller (*Campostoma anomalum*), and River Chub (*Nocomis micropogon*), nests (Outten 1961, 1962, and Etnier and Starnes 1994).

As noted in Etnier and Starnes (1994), *Notropis spectrunculus, N. leuciodus, N. chrosomus,* and *N. volucellus* all belong to the subgenus *Hydrophlox*. Members of this subgenus often spawn over the nests of other fish species and feed on terrestrial and aquatic insects. Breeding individuals of most species lack tubercles but normally exhibit red or yellow mating coloration. *Notropis volucellus* and *leuciodus* occur sympatrically with *N. spectrunculus* in western North Carolina river systems. *Notropis chrosomus* occurs with *N. spectrunculus* in a few streams in southwest Tennessee (Etnier and Starnes 1994). This sympatry suggests niche partitioning between *Notropis* spp., but the extent of partitioning is not known (Schoener 1974, Ross 1996, Etnier and Starnes 1994).

Collecting more information about *N. spectrunculus* could be crucial to the reintroduction efforts of the species. Therefore, I have described some basic life history traits of *N. spectrunculus* such as age, growth rate, diet, fecundity, and habitat use.

METHODS

I attempted to find several sites with *N. spectrunculus* populations on several different rivers so that I could ensure my results weren't skewed by river-specific characteristics. With help from the North Carolina Division of Water Quality, I was able to identify sites where *N. spectrunculus* had been found in enough numbers to ensure my ability to catch enough specimens each month for this study while not depleting the population.

I sampled candidate study sites with a 5-m seine until I caught ten individuals or until I had sampled for an hour and a half. If ten individuals were captured, they were sacrificed and constituted a sample. If an hour and a half of sampling passed before ten individuals were captured, the site was determined to not have enough individuals to make monthly sampling a viable option, and I released the captured individuals and attempted to locate another site. Sites were found at four locations (Figure 1): the Pigeon River upstream of Canton, Hominy Creek, and the Tuckasegee River at East Laport and at Wilmot (Table 1). Due to changing river conditions, a specific location at each site was not sampled each trip. Deep eddies immediately below riffle areas were instead sampled on each visit, sometimes as much as 30 m from the previous sample site.

Field sampling was undertaken once a month, with a minimum of two weeks between sampling events. I followed the approach in Yanchis (1993) and sampled with a 5-m seine with 3-mm mesh size until ten fish had been caught or an hour and a half of sampling was complete. Captured fish were sacrificed by an overdose of tricaine methanesulfonate, MS-222, and immediately placed in an ice chest. In addition, the specific location where sampling occurred in the river was noted, as was any breeding coloration of *N. spectrunculus.*

At each sampling event, the specific location in the stream where specimens were captured was noted. The substrate type at each collection location and water depth at the nearest USGS gage was also noted. Water depth, water temperature and distance to in-stream obstacles such as riffles or boulders at each site was measured. This allowed for a comparison to be made between river characteristics where *N. spectrunculus* was found. To obtain a better idea of which microhabitats *N. spectrunculus* occupy and in which microhabitats they cannot be found, a 4-mile stretch of the Tuckasegee River was sampled, noting presence of *N. spectrunculus,* substrate type, water depth, and distance to flow-disrupting objects was noted at each location. Fourteen sites were sampled with this method. One site – the Tuckasegee River at Wilmot – was surveyed using a surveyor's level to provide a detailed example of *N. spectrunculus* habitat.

After returning from the field, the fish were removed from the ice chest and placed in a freezer for preservation for future dissection. In the laboratory, specimens were slowly warmed to room temperature in a warm water bath. Notes on coloration were made and standard length (SL) and wet weight was noted (to the nearest mm and nearest 0.01 g, respectively). An incision was made anteriorly from the anus to the pelvic girdle into each specimen. An incision was then made dorsally from this first incision in order to allow access to the internal organs of the fish for observation. Fish were sexed by visual inspection of gonads, except in small fish with poorly developed gonads, where sex could not be reliably determined. A Chi-square goodness of fit test was run to test for departure from a 1:1 sex ratio. Gonadosomatic indices (GSI), the ratio of gonad weight to body weight, were computed by removing the gonads and weighing them to the nearest 0.01 g. The GSI was then be computed according to the formula: GSI= gonad weight (g)/body weight (g) x100 (Crim and Glebe 1990). Eggs were removed from the body cavity and were counted. An ANOVA followed by a Tukey HSD test was used to compare GSI between months. An ANOVA was run to test for a significant relationship between egg count and size, excluding outlying values. Finally, the alimentary canal of each fish was removed and contents were inspected using a dissecting microscope. Macroinvertebrates found inside were identified to order. The presence of detritus and material containing chloroplasts was also noted. I tested for differences in number of fish observed with a diet item among sites by using a G-test of homogeneity (Sokal and Rohlf 1981).

Age was determined by inspection of monthly length-weight scatterplots for all fish caught. Distinct clusters of points were interpreted to represent different ages. Age, determined in this way, was added to month caught after birth month to determine age in months of specimens (Holder and Powers 2010). Birth month was determined by analyzing GSI, breeding coloration, and the first presence of young-of-year in my samples.

To determine if a sex-dependent growth rate existed, the statistical significance of the interaction term in an ANOVA relating standard length to age and sex was tested. Similarly, pair-wise analyses including collection site was used to test for differences in growth rates among sites. Holm’s sequentially rejective method was used to control experiment-wise error rates (Holm 1979).

Length at age was fit to a von Bertalanffy growth model:

$\mathrm{E[L|t]} = L_\infty(1 - e^{-K(t-t_0)})$, where E[L|t] is the expected or average length at age t, L_∞ is the asymptotic average length, K is the Brody growth rate coefficient (units are year^{-1}), and t_0 is a modeling artifact that represents the age when the average length was zero (the model was fit using the FSA package for R, Ogle 2011). All statistical analyses were computed using R (version 2.14.1, www.r-project.org) or Microsoft Excel (version 2010).

Figure 1. Map showing locations of field sites in western North Carolina where *N. spectrunculus* were captured. Sites are identified with black stars. North is toward the left of the page. From west to east, sites are Wilmot, East Laporte, Canton, Candler. River systems upon which sampling occurred are highlighted. Map courtesy of Google (© 2011).

Table 1. Table of field sites where *N. spectrunculus* were captured in western North Carolina showing locations and characteristics. Stream discharge data courtesy of the USGS (historical data collected from www.usgs.gov).

Site	Pigeon River at Canton	Tuckasegee River at East Laport	Tuckasegee River at Wilmot	Hominy Creek at Candler
Latitude	35°31’31”N	35°17’49”N	35°24’14”N	35°32’07”N
Longitude	82°50’25”W	83°08’52”W	83°18’47”W	82°41’37”W
River Width at Field Site	Approx 30 m	Approx 30 m	Approx 40 m	Approx 5 m
Average Discharge in 2010	381.7 cfs	502.1 cfs	1007 cfs	N/A
Upstream Use	Agriculture	Agriculture, Residential	Agriculture, Residential, Paper Mill	Sparse Residential

RESULTS

I collected and dissected 238 individuals of *Notropis spectrunculus*. All fish were captured over sandy substrate, in pools 0.5 to 1.0-m in depth where the beginning of the pool was 1.0 m or less in distance to a water-flow altering obstacle in the river. 81 individuals were sexed – 47 were male and 34 were female. This sex ratio was not significantly different from a 1:1 ratio (P= 0.15). All individuals able to be sexed were age 1+ years. The largest individual was a female 77 mm SL and 2.96 g total weight. The largest male was 71 mm SL and 2.39 g total weight. However, the oldest individual was a 46 month old male. The oldest female was 37 months old. Weight increased with standard length (r^2= 0.91) (Figure 2).

The maximum age of fish observed was 3 years. This appears to be the same for both males and females, as at least one individual of each sex was found in their third year. Mortality rate was consistent for age classes 0 through 2. Only 5 individuals reached their age 3, or 2.1% of total specimens. Survivorship fit a weighted catch-curve estimate of mortality (Figure 3), as described by Maceina and Bettoli (1998). Catch data from year 3 was excluded from the estimate due to low catch numbers. I found a mortality rate of 36.7% using this method. However, mortality rates calculated in this fashion assume a stable age distribution, which may be unlikely for these sampled populations.

I found no differences between male and female growth (P= 0.40, F= 0.70, df= 1). Statistical significance was found among growth rates at the different sites in three of the comparisons (Table 2). The growth rate at the Hominy Creek site was significantly higher than that at Canton and Wilmot. Further, the growth rate at East Laporte was also significantly higher than that at Canton. The growth rate at Hominy Creek appeared to be the highest, although it was not significantly different from that at East Laporte. The growth rate at Canton appeared to be lower than that at Wilmot. The growth rate from all sites combined was 1.05 mm/month.

The fit of length at age to a Von Bertalanffy Growth Model (Figure 4) suggests an asymptotic average standard length of 91 mm and a Brody growth rate of 0.0246 year^{-1}. The growth model was fit using data from all sites combined to produce a more general growth curve for the species rather than for a particular location.

Ovigerous females had 13 – 331 oocytes (Figure 5). Ovigerous females in their 23rd month had an average of 133 oocytes. Females in their 24th month had an average of 95 eggs. Females in their 25th month had an average of 70 eggs. The lone 37 month old female with oocytes had 331. Oocyte count was not size dependent (P= 0.63, F= 0.23, df= 1).

Individual GSIs ranged from 2.26 to 19.23. Average GSI was significantly higher in May than in April and June but not July (Figure 6). No other comparisons were significant (Table 3).

One hundred and twenty seven individuals (53% of those examined) contained food. Of these, 80 contained detritus, 22 contained Diptera, 20 contained Coleoptera, 14 contained Ephemeroptera, 4 contained Hemiptera, 9 contained Hymenoptera, 1 contained Lepidoptera, 7 contained Megaloptera, 11 contained Odonata, 10 contained Trichoptera, and 16 contained material containing chloroplasts (Figure 7). I found no significant difference in frequency of occurrence of diet items between any of the sites by using a pairwise G- test (Table 4). For the G-test, all observations that were not part of Coleoptera, Diptera, or Detritus were combined into an "Other" category.

All specimens were captured in water ranging from 0.5 to 0.75 m in depth (Figures 8 and 9). As water level changed, *N. spectrunculus* moved to maintain this microhabitat. Furthermore, the locations where specimens were captured were all immediately downstream (less than 1.0 m) from a riffle or rock in the stream that disrupted streamflow. All pools in which specimens were collected had a sandy substrate. Sites that lacked a sandy substrate, a pool beneath a flow-disrupting object, or water 0.5 to 0.75 m in depth also lacked *N. spectrunculus* (Table 5). Water temperature was consistently lower at the Hominy Creek and East Laporte sites than at Canton and Wilmot (Figure 10).

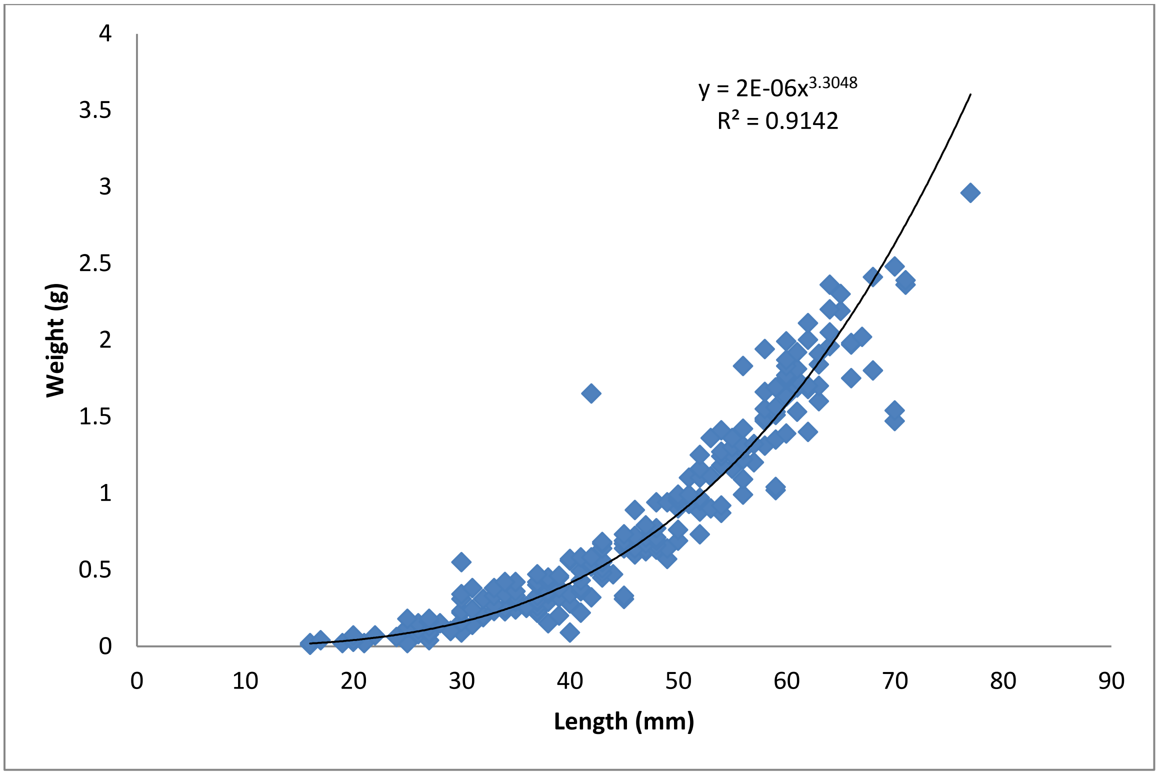

Figure 2. Length – weight power function of all *N. spectrunculus* examined. Specimens were captured in western North Carolina from April – November 2010.

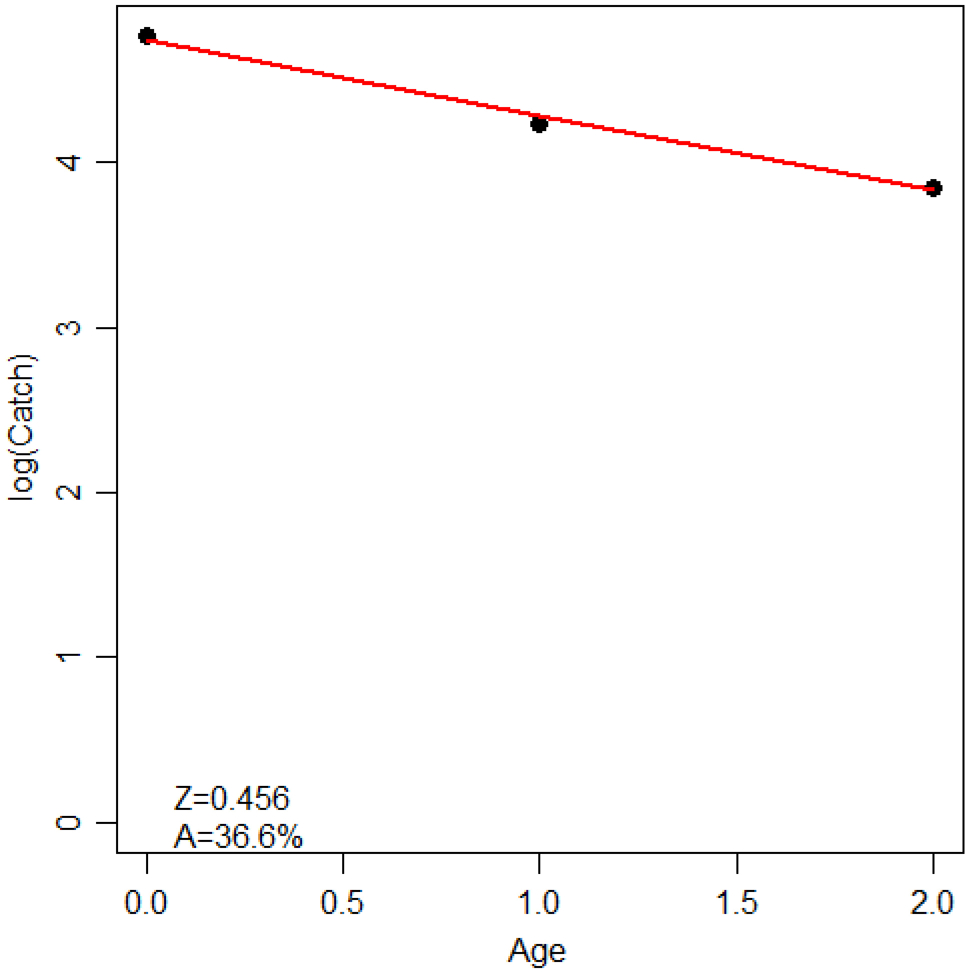

Figure 3. Catch-curve regression showing mortality of *N. spectrunculus* in western North Carolina. Variable A is yearly mortality rate and variable Z is instantaneous mortality rate. Graph obtained using the FSA package for R (Ogle 2011).

Table 2. A table showing P-values of pairwise ANCOVAs comparing growth rate of *N. spectrunculus* between sites. Significant P-values were found by protecting experiment-wise error rates using the Holm sequentially rejective method. Results with a P-value of less than 0.05 are noted with an asterisk.

	Hominy Creek	Wilmot	Canton
East Laporte	F= 165.4 with 3, 97 df P= 0.126	F= 67.9 with 3, 116 df P= 0.0273	F= 75.8 with 3, 120 df P< 0.001*
Hominy Creek		F= 130.9 with 3, 109 df P< 0.001*	F= 164.6 with 3, 113 df P< 0.001*
Wilmot			F= 46.3 with 3, 132 df P= 0.135

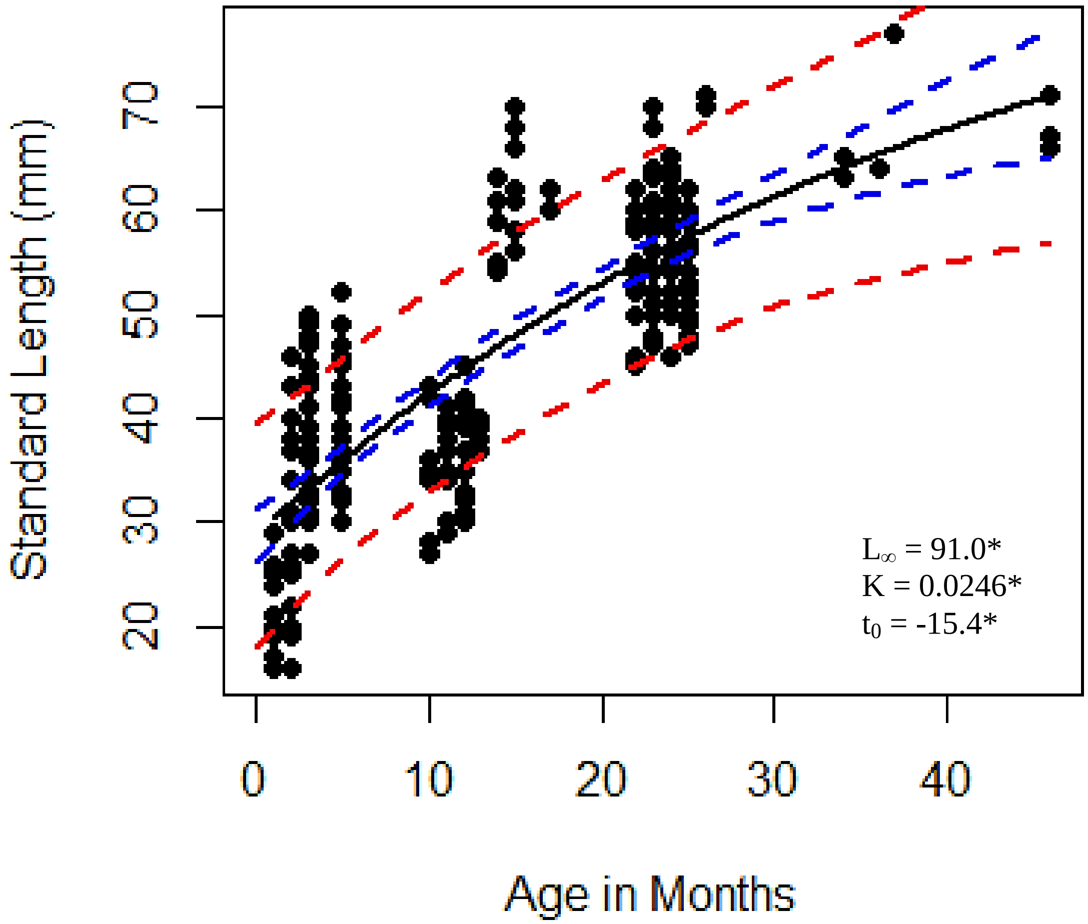

Figure 4. Fitted line plot for the a Von Bertalanffy Growth Model for *N. spectrunculus* with approximate 95% bootstrap confidence intervals shown as blue dashed lines and 95% bootstrap prediction bounds shown as red dashed lines. Confidence intervals and prediction bounds based on 1000 bootstraps. Statistically significant parameters denoted by an asterisk.

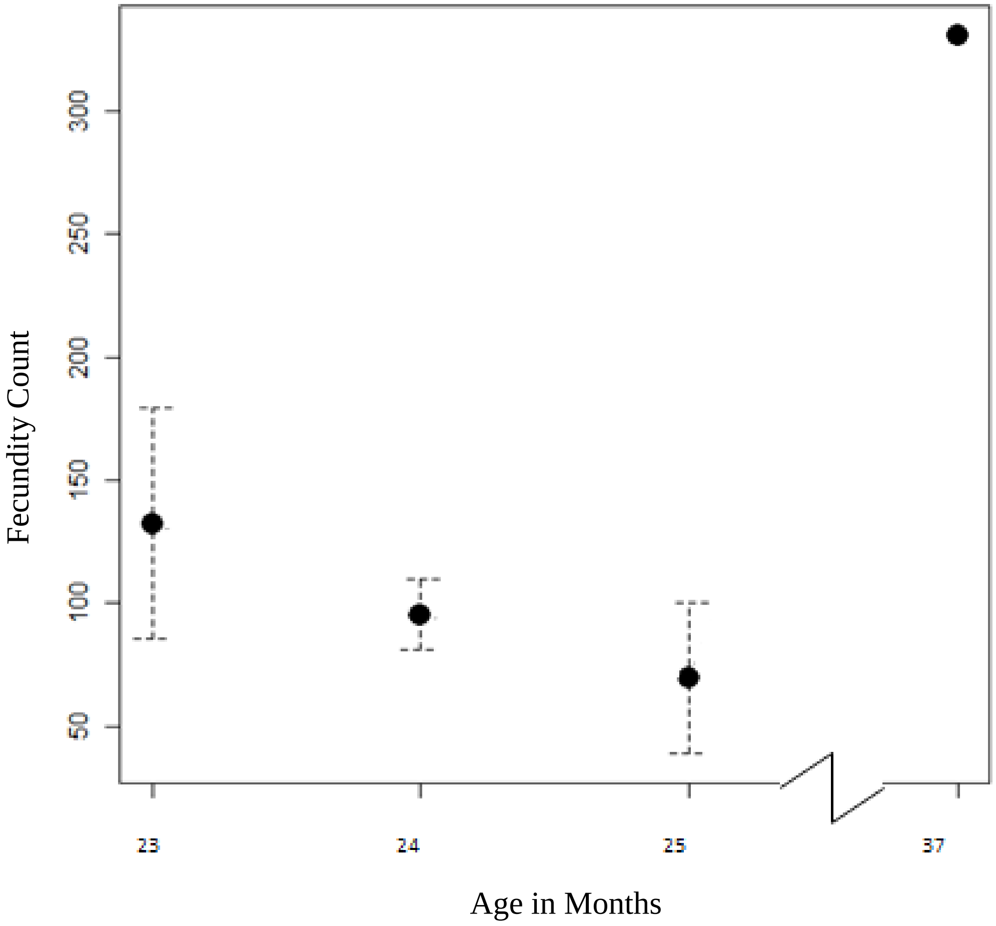

Figure 5. Mean egg count of *N. spectrunculus* ± 95% CI plotted by age.

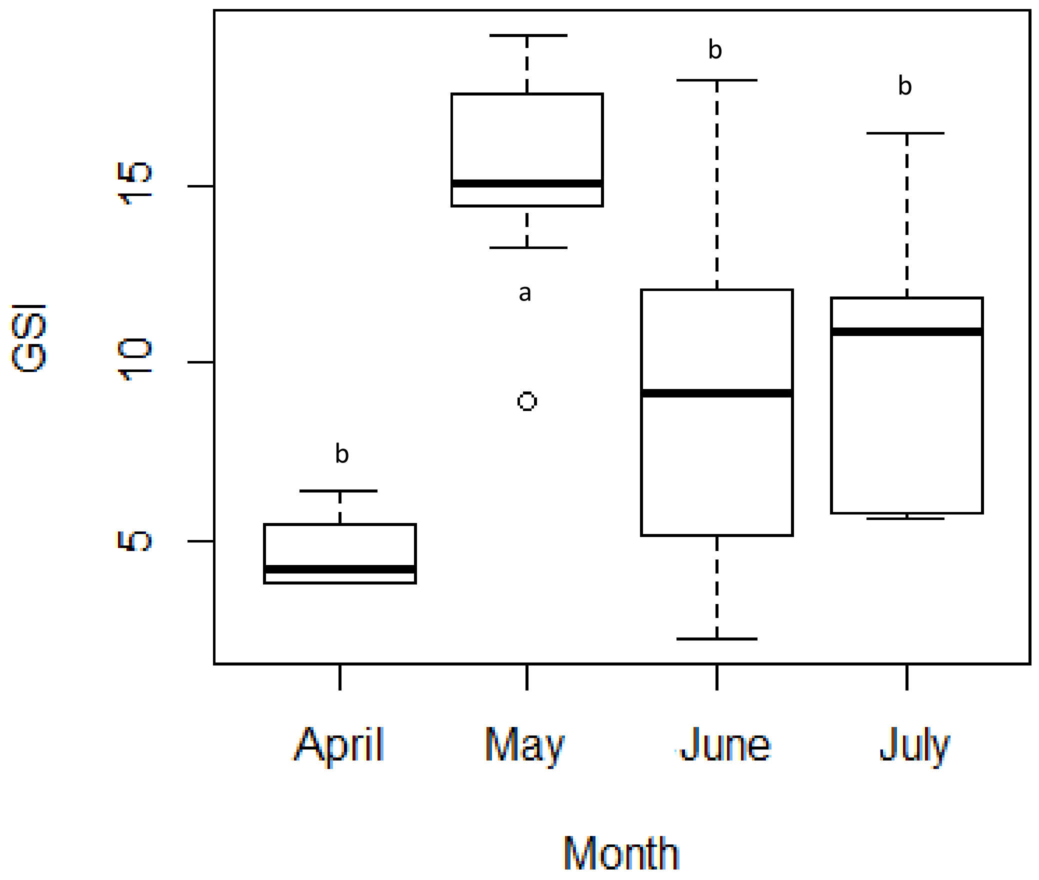

Figure 6. Boxplots of GSI of female *N. spectrunculus* by month with outliers (those farther than 1.5X the interquartile range) shown as circles. Boxplots with the same letter denote comparisons of means that did not differ significantly.

Table 3. Table showing results of Tukey HSD comparing GSIs of female *N. spectrunculus* by month followed by 95% confidence interval of difference followed by average difference. Results with a P-value of less than 0.05 are marked with an asterisk.

	May	June	July
April	P< 0.001* 4.07-17.3 10.7	P= 0.31 -2.53-11.5 4.46	P= 0.21 -2.01-12.9 5.47
May		P= 0.02* 0.718-11.7 6.21	P= 0.11 -0.905-11.3 5.20
June			P= 0.97 -7.54-5.52 -1.01

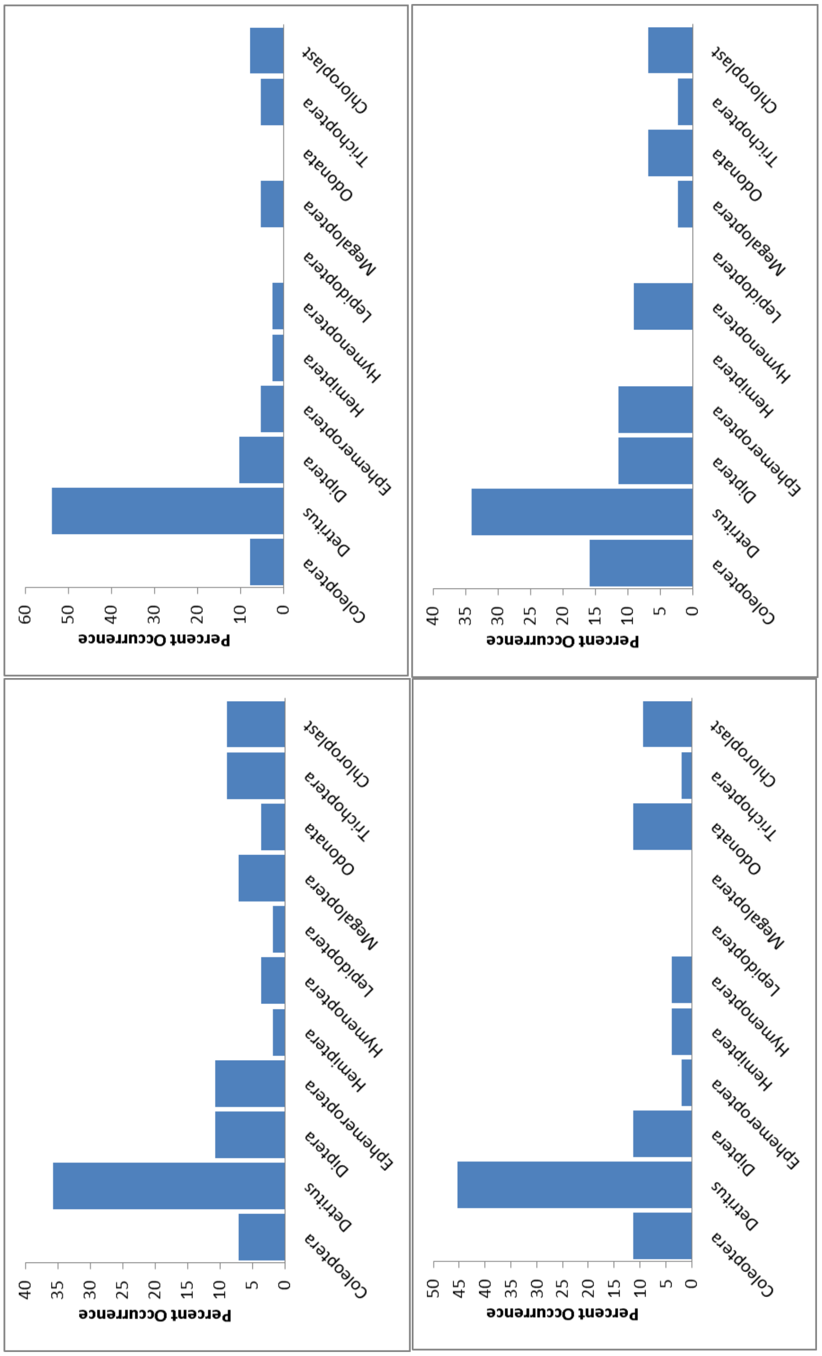

Figure 7. Percent occurrence of gut contents in *N. spectrunculus* specimens. Macroinvertebrates are identified to order. Sites are Canton, Hominy, East Laporte, and Wilmot, clockwise from figure in upper right corner of page.

Table 4. P-values from G-tests comparing number of *N. spectrunculus* observed with diet items between sites in western North Carolina.

	Hominy Creek	Wilmot	Canton
East Laporte	P= 0.55 Df= 3	P= 0.46 Df= 3	P= 0.29 Df= 3
Hominy Creek		P= 0.70 Df= 3	P= 0.29 Df= 3
Wilmot			P= 0.85 Df= 3

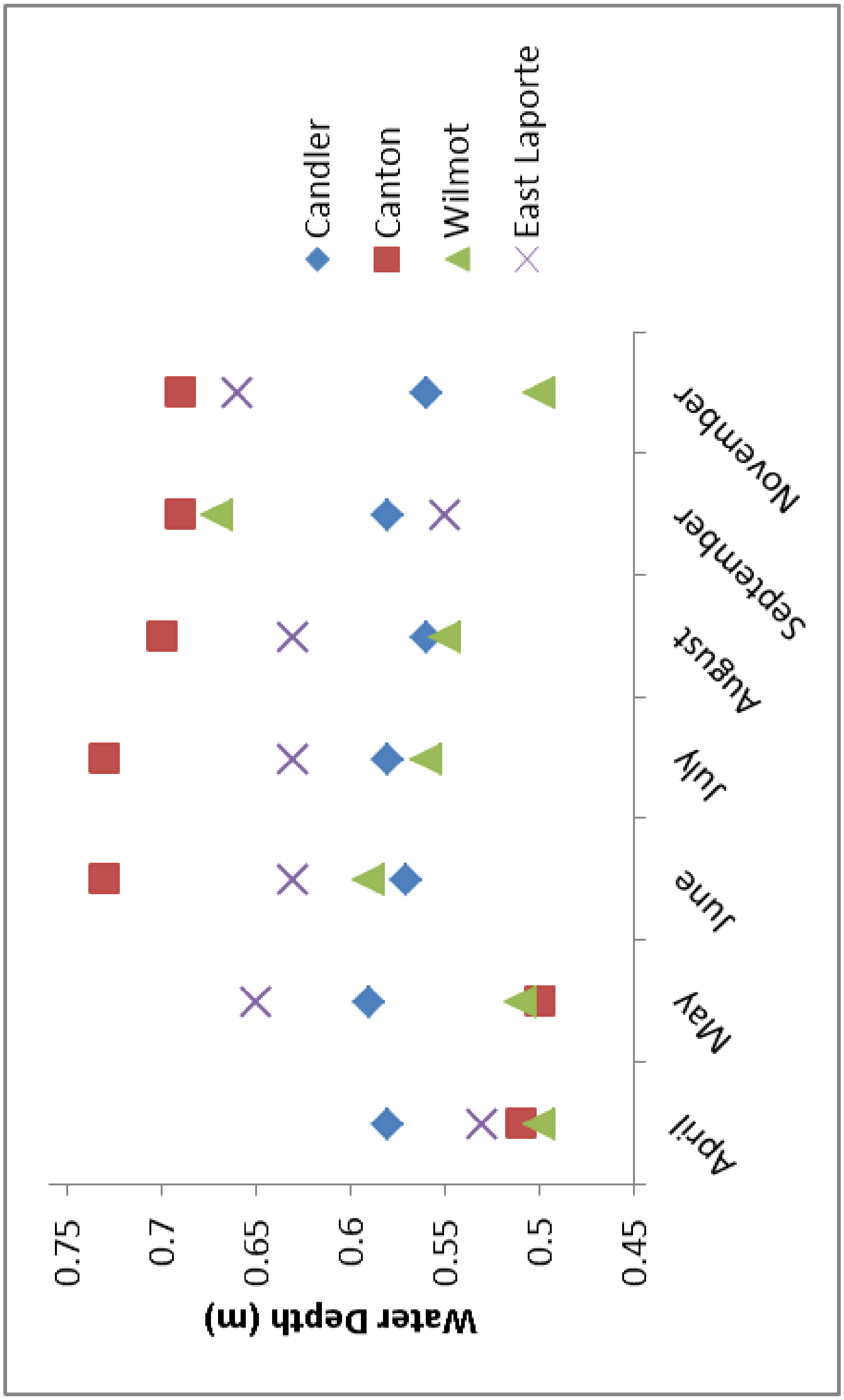

Figure 8. Water depth (m) at sites where *N. spectrunculus* were captured varied monthly over the course of the study.

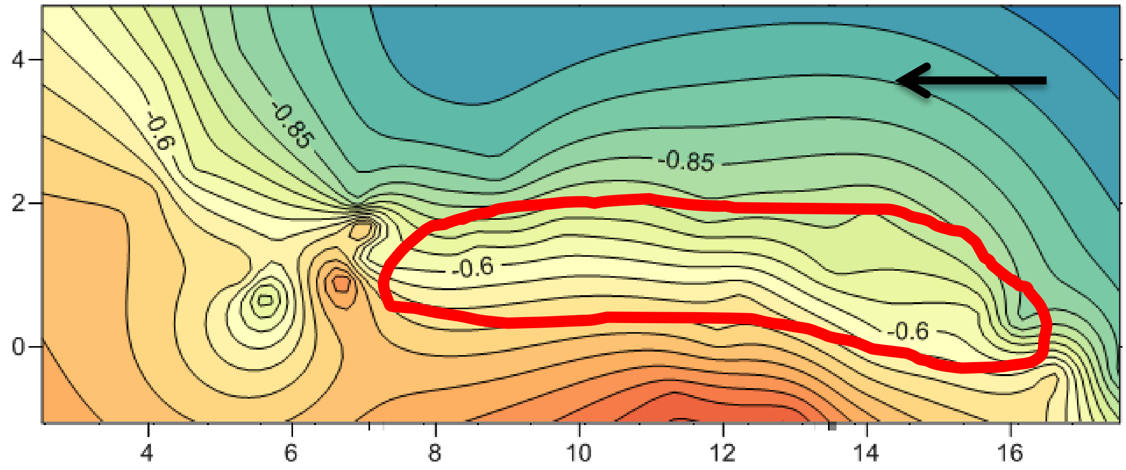

Figure 9. Bathymetric map of Wilmot field site. *N. spectrunculus* were only caught within the area encircled by the red line. The X-axis represents meters upstream from the end of the pool, the y-axis represents meters towards middle of stream. The contour lines are meters below the water surface to the bottom of the river, in 0.05 meter increments. The arrow represents direction of flow.

Table 5. Stream conditions at sites where *N. spectrunculus* were found or were not found while sampling the Tuckasegee River. Each row represents a different sampling location.

Substrate Type	Water Depth (cm)	Distance to Flow-Disrupting Object	Were *N. spectrunculus* present?
Sand	27	<1m	No
Sand	53	<1m	Yes
Sand	63	<1m	Yes
Sand	17	>1m	No
Cobble	12	<1m	No
Cobble	44	>1m	No
Cobble	37	>1m	No
Cobble	40	>1m	No
Cobble	43	>1m	No
Cobble	56	>1m	No
Cobble	39	>1m	No
Boulder	134	>1m	No
Boulder	75	>1m	No
Boulder	83	>1m	No

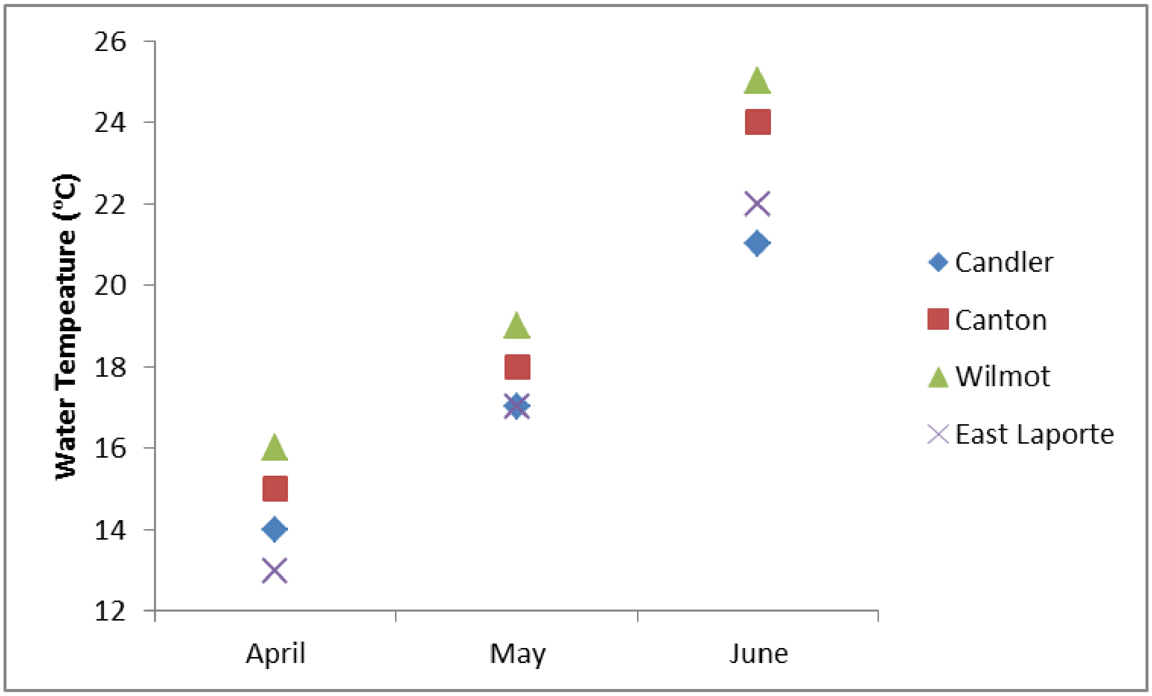

Figure 10. Water temperature at sites where *N. spectrunculus* were captured varied monthly during the study.

DISCUSSION

A small survivorship to maximum age is not uncommon for shiners (Holder and Powers 2010). This could be due to individuals recruiting into a size class that is heavily preyed upon by larger fish or due to an energetic investment in reproduction that proves fatal after the breeding season, among other possible reasons. Many *Notropis* spp. have a maximum lifespan of three years, although *N. leuciodus* and *N. rubricroceus* have been reported to live to a maximum of five years (Clayton 2000, as cited in Holder and Powers 2010; Outten 1958).

The field site on Hominy Creek is the location with the least amount of disturbance upstream and the one with the coldest water. East Laporte had the second coldest water temperature during the study and is also the second least-disturbed location. As Table 1 shows, there is significant agricultural use upstream of the Canton site and a paper mill upstream of the Wilmot site. Given that growth rate was higher at the colder and less disturbed sites, it seems that *N. spectrunculus* growth depends on environmental factors. No difference existed between male and female growth rate. Pooled growth rate was similar to that of *Notropis chrosomus* (1.35 mm/month) (Holder and Powers 2010).

The highest GSI value was collected in May. Given that the GSI in May was statistically higher than in April and June, combined with an observation of intense breeding coloration in males in May, with less in April and June, means that peak breeding season for *N. spectrunculus* likely occurs in May. Breeding activity likely starts in April and runs through the middle of the summer, probably ending in July. Specimens collected late in the year were beginning to mature, but lacked developed eggs. This indicates a single breeding season. No spawning behavior was observed, but closely related species are nest associates with *Semotilus* spp. (Etnier and Starnes 1994). GSI values were comparable to those of *N. chrosomus* (Holder and Powers 2010). Breeding of *N. spectrunculus* is comparable to *N. leuciodus*, with a single breeding season peaking in spring to early summer (Etnier and Starnes 1994). This is also comparable to other *Notropis* spp., such as *N. lutipinnis, N. nubilus,* and *N. rubricroceus* (Boschung and Mayden 2004, Clayton 2000, as cited in Holder and Powers 2010; Fowler et al. 1984, Outten 1958).

Egg count for individuals in year two decreased as the year went on, although not significantly. The egg count for the year three female collected appears to be higher than the counts for those collected in year two, however only one year-three individual was collected. No ovigerous year one individuals were collected. Egg count was similar to what was found by McAulliffe and Bennett (1981) in *N. leuciodus*, who found up to 286 eggs per individual.

The predominant food items were Diptera, Coleoptera, and Ephemeroptera. A large number of individuals were found containing detritus (33.8% of individuals containing food). It is unlikely that this large amount of detritus is an indication that *N. spectrunculus* are detritivores. The large amount of detritus simply represents mostly digested, and thus unidentifiable, food in the guts of specimens. The observation that all food choices were found to be occurring in percentages of fish indicates that *N. spectrunculus* are opportunistic drift-feeders. The lack of a significant difference in diet among sites further corroborates this hypothesis. Diet was similar to *N. leuciodus* – primarily aquatic and terrestrial insects. Both *N. leuciodus* and *N. spectrunculus* are hypothesized to be opportunistic feeders (Clayton 2000, as cited in Holder and Powers 2010).

Finally, at all four sites, the location at which *N. spectrunculus* were captured varied through time based on water depth. Furthermore, *N. spectrunculus* were only captured over sandy substrates in pools just below an in-stream flow disturbing object. Therefore, I conclude that *N. spectrunculus* are a habitat specific fish. Fish, and *Notropis* spp. specifically, have been shown to exhibit habitat-specific choice tendencies (Aadland 1993, Wall et al. 2004).

This habitat specificity could also explain the high survivorship from year 1 to year 2 and food choice in *N. spectrunculus*. Since the fish only lives in one specific type of habitat, they must wait for food to come to them instead of seeking out food items. Furthermore, the fish is likely well adapted for survival in their preferred microhabitat. Therefore, those individuals who survive their difficult first year are likely to survive through to their third year. This high survivorship into year 2 appears especially important for *N. spectrunculus* since no year 1 individuals were collected with eggs. *Notropis* spp. about which more is known, such as *N. chrosomus* and the Ozark Minnow (*N. nubilus*) appear to reach sexual maturity at one year of age, although *N. lutipinnis* and *N. rubricroceus* do not reach maturity until two years of age (Holder and Powers 2000).

CONCLUSIONS

Notropis spectrunculus seem to be similar to other *Notropis* species. They have comparable diet, fecundity, total size and growth rate, and GSI. However, they exhibit habitat specificity. This habitat choice means that the fish may be very patchily distributed. Therefore, the Pigeon River Recovery Project may have reintroduced *N. spectrunculus* into a stretch of the Pigeon River that is lacking in proper habitat or they may have not sampled the appropriate microhabitat to discover surviving transplants. More work is being done to better classify habitat where *N. spectrunculus* is found and where it is not found. The damming of the river at Canton may have altered the particle sizes of transported sediment, reducing the sandy habitat in pools that this species needs to survive. In order to reintroduce *N. spectrunculus* successfully, stretches of the Pigeon River with several sandy-bottom pools immediately downstream of in-stream flow-disrupting objects should be used. Also, the stretch of river designated for reintroduction of *N. spectrunculus* should have several sandy bottom pools of different depths to ensure proper habitat for the fish throughout river height changes.

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