LIFE HISTORY OF A CLEAR STREAM MINNOW: GROWTH, REPRODUCTION, HABITAT USE, AND DIET OF *NOTROPIS BOOPS* (BIGEYE SHINER) IN TRIPLETT CREEK.

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A Thesis

Presented to

the Faculty of the College of Science

Morehead State University

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Matthew T. Fossett

July 6, 2019

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LIFE HISTORY OF A CLEAR STREAM SHINER GROWTH, REPRODUCTION, HABITAT USE, AND DIET OF *NOTROPIS BOOPS* (BIGEYE SHINER) IN TRIPLETT CREEK.

Matthew T. Fossett
Morehead State University, 2019

Director of Thesis: _____
David J. Eisenhour Ph.D.

Notropis boops, the Bigeye Shiner (Gilbert), occupies clear, rocky streams in much of the Midwest, but population have declined in many areas. Despite its recognition as a sensitive species, little life-history data have been published, data needed to make conservation management decisions. I studied growth, reproduction, microhabitat use, and diet of *Notropis boops* for ten months in 2017 in Triplett Creek in northeast Kentucky. Sampling occurred at monthly intervals during the ten months. Growth and age were evaluated by length-frequency analysis, corroborated by scale annuli. The reproductive cycle was assessed by seasonal changes in tuberculation, gonad development, and computation of gonadosomatic indices (GSI). The diet was determined via identification and enumeration of stomach contents. Visual snorkel surveys of 1 m^2 plots were used to establish sites of presence or absence for microhabitat analysis. Microhabitat variables included substrate composition, water depth, rate of flow, and presence of cover. *Notropis boops* averaged 38.2 mm standard length (SL) at 12 months and 52.2 mm SL at

24 months. It is a short-lived fish, living a maximum of 3 years. Sexually mature individuals appeared in collections from 18 May to 3 July and were 39.28-54.47 mm SL and averaged 46.89 mm SL. Sexually mature *N. boops* were in the 1 and 2+ age classes. The number of age 2+ *Notropis boops* rapidly declined during the summer and fall months, suggesting few individuals live past 2 years. Females had 120-259 (mean=177) advanced mature to ripe ova per ovary. Analysis of gonadosomatic indices suggests spawning occurs late May through early August. Few young-of-the-year (YOY) *Notropis boops* were captured during samples in late summer to early fall, perhaps due to multiple large flood events in 2017 disrupting recruitment of young. *Notropis boops* occupy calm or slow-moving water immediately adjacent to faster current, over heterogeneous substrates predominantly of sand and cobble, often with *Justicia americana* (Water Willow). The diet of *N. boops* is predominantly made up of chironomid midge (30% by biomass). The second largest group of food items came from the adult terrestrial insects Formicidae, and Ichneumonidae (25.8% by biomass). This suggests that *N. boops* is plucking prey from both the benthos and surface. The microhabitat location varies, depending on flow conditions, supporting the idea that diverse, heterogeneous habitats over long stream stretches, are needed. *Notropis boops* is a short-lived species, thus disturbances to spawning or recruitment of juveniles, particularly over a multi-year period, could result in sharp population declines.

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Acknowledgements

Table of Contents

List of Tables

List of Figures

Introduction

Study species

Notropis boops, the Bigeye Shiner (Gilbert 1884), is a common species that inhabits the highlands of Kentucky, Tennessee, Ohio, Indiana, Illinois, and northern Alabama as well as the Ozark and Ouachita highlands of Missouri, Arkansas, and Oklahoma (Page and Burr 2011). *Notropis boops* is a bentho-pelagic minnow (family Cyprinidae), a member of the *Notropis texanus* (Weed Shiner) species group and is most closely related to *Notropis xaenocephalus* (Coosa Shiner) (Burr and Dimmick 1983). They occupy large creeks to small rivers in warm, clear semi-permanent/permanent pools with rocky, silt-free substrates. They are slender, relatively small (up to 90 mm total length (TL)), with characteristically large eyes, longer than the snout, and a clear stripe above a black stripe that runs along the entire silver side, including both lips. They have a large, terminal mouth, the rear edge of which almost reaches the eye (Figure 1) (Page and Burr 2011).

In a review of the recent records of *N. boops* occurrence in Ohio, researchers noticed a decline in these populations (Rice et al. 1998). Also, a review of imperiled species in Illinois found that there were declines in the northern ends of *N. boops'* range (Herket 1992). Both studies attributed this to heavy siltation from agricultural/construction runoff, and habitat destruction from channelization. Populations at the northern and southern ends of the range are imperiled, but overall the species is stable, or slowly declining (Herket 1992). *Notropis boops* is listed as critically imperiled in Mississippi, endangered in Illinois, and has been proposed to be listed as threatened in Kansas (Haslouer et al. 2005, Illinois Endangered Species Protection Board 2015, Mississippi Natural Heritage Program 2018).
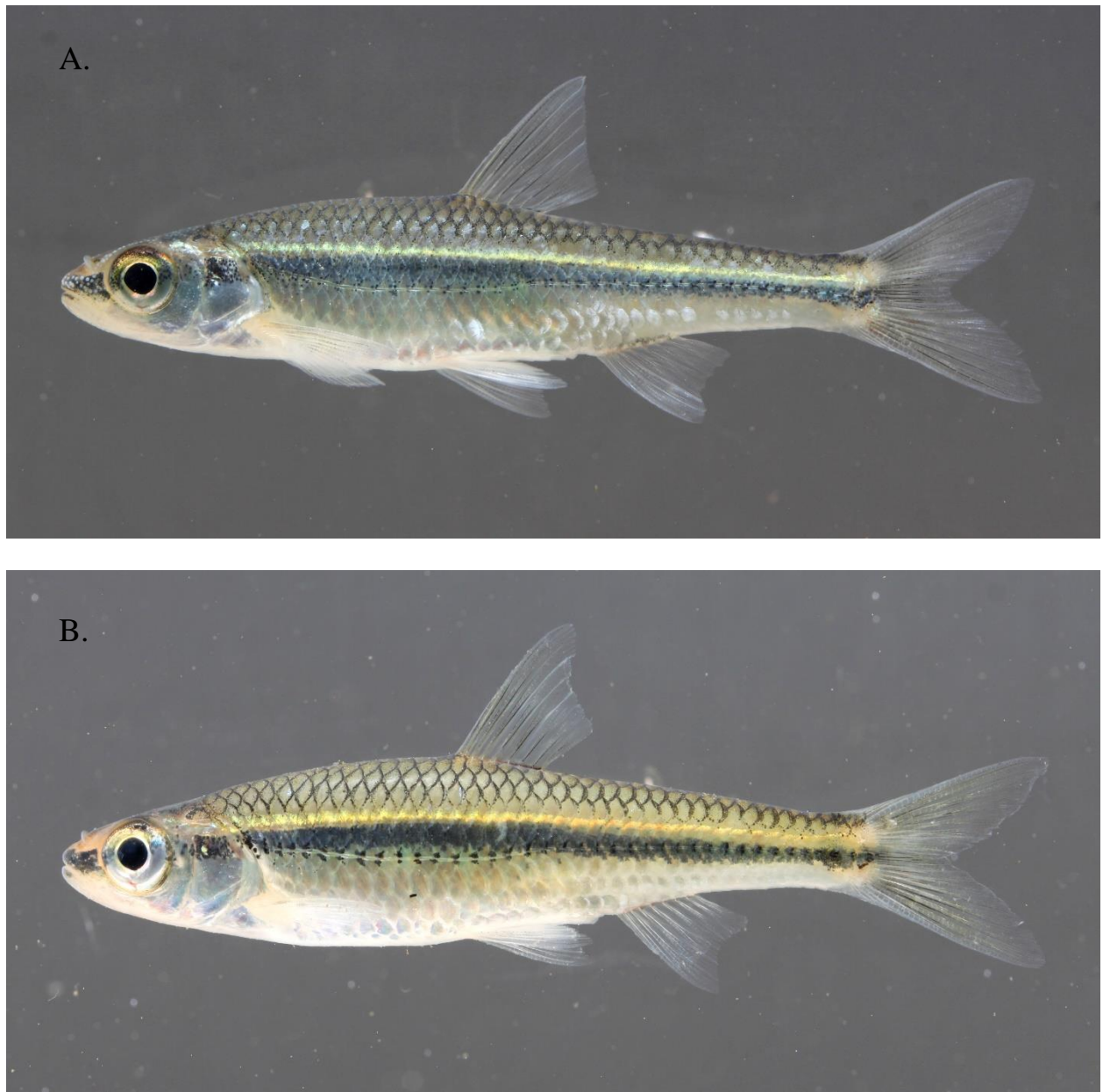
Figure 1. A. Male *N. boops* in spawning condition, 56 mm SL, captured 19 May 2017. B. Female *N. boops*, 56 mm SL, captured 19 May 2017.

Literature review of life history

Most of the literature on the biology of this fish is from anecdotal observations reported in state fish books (e.g., Trutman 1981, Etnier and Starnes 1993, Pflieger 1997), rather than quantitative studies. There is one formal life-history study of *Notropis boops*, it is a study of the reproductive biology of *N. boops* in Brier Creek, Oklahoma (Lehtinen and Echelle 1979). This study found that in this southern population the fish has an extended spawning season, from April – August. They also indicate that this fish reaches sexual maturity by its second summer and few live to spawn in a third summer. In Missouri, spawning for *N. boops* was reported to occur from June to August, based on the presence of tuberculate males (Pflieger 1997). A re-description of the species found that *N. boops* shows little to no sexual dimorphism (Burr and Dimmick 1983). Anecdotal reports of habitat use state that *N. boops* inhabit upland streams, often limestone bottomed, with substrates of clean gravel or sand. They can tolerate short instances of siltation, but typically only occur in streams with clear water (Clay 1975, Trautman 1981, Etnier and Starnes 1993, Pflieger 1997, Smith 2002). A mesocosm study (Schaefer 2001) examined the importance of riffles as barriers to movement in three cyprinid species: *Campostoma anomolum* (Central Stoneroller), *Cyprinella venusta* (Blacktail Shiner), and *N. boops*. *Notropis boops* showed aversion to faster currents with the lowest rate of movement of the target species, even with the threat of predation. The diet items of *N. boops* have not been reported. However, it is regarded as an invertivore that captures prey drifting in the water column, and swims to the surface reportedly even jumping out of the water to capture prey (Trautman 1981, Smith 2002).

Rational

Life-history studies examine aspects of an organisms' life that influence survival, at the individual, population, or species level. Life-history studies of fishes commonly examine reproductive biology, including spawning, fecundity of individuals, and timing of sexual maturity. Additional topics of life-history studies include foraging habits, habitat use, growth, aging, and distribution (Jolly and Powers 2008, Holder and Powers 2010, Hodgskins et al. 2016). Most life history studies of fishes have been conducted on species of economic value (e.g. *Micropterus salmoides* (Largemouth Bass), *Micropterus dolomieu* (Smallmouth Bass), *Micropterus punctulatus* (Spotted Bass), (Johnson et al. 2009) or those of conservation interest (e.g. *Clinostomus funduloides* (Rosyside Dace), Hazelton and Grossman 2009). More recently the trend has shifted towards understanding the life history traits of small-bodied stream fishes, which make up the bulk of biodiversity in lotic ecosystems.

Life history traits can vary geographically for freshwater fishes; this variation can be a response to the local environment, with latitude being one of the most important factors affecting this variation (Vinyoles et al. 2010, Grabowska et al. 2012). Understanding the ecological requirements of a species is an important part of making management decisions for that species. There is also a growing focus on ecosystem management and restoration, thus requiring basic biological information for many species in an ecosystem. Many studies of imperiled species focus on the microhabitat variables where target species occur (Jorden et al. 2007, Albanese et al. 2011). The most important microhabitat variables for fish species consist of current velocity, substrate composition, presence of cover, and water depth (Murphy and Willis 1996). This life history information, vital for making conservation and management decisions, is sparse for our

target species, which has been noted as a species sensitive to siltation and channelization (Herket 1992, Rice et al. 1998).

Objectives

This study examined four aspects of the life history of *N. boops* in Triplett Creek in 2017. Specifically, this study identified microhabitat variables that influenced the presence of *N. boops*. Second it examined the diet of *N. boops*. Third, this study examined the growth rate and longevity of *N. boops*. Finally, this study examined the reproductive biology of this species, including documenting of age at maturity, seasonal changes in reproductive activity, and fecundity. This study aimed to provide quantitative data that are needed for making conservation and ecosystem management decisions.

Materials and Methods

Study site description

The life history of *N. boops* was studied at two geographically proximate sites in the mainstem of Triplett Creek, in the Licking River watershed in Rowan County, in eastern Kentucky. The first site was 200 m downstream of the KY 519 crossing (Figure 2), at the mouth of Dry Creek (38.16701º N, -83.43771º W). The second site was downstream of the first, at a sharp bend in the stream along US 60 (Figure 3), 300 m downstream of the mouth of Mill Creek (38.16446º N, -83.44552º W). At each site I established a sampling reach of approximately 100 m. Triplett Creek is a large creek, with a drainage area of 172.49 km^2 above the first site. The mean wetted width of our sites was 13.26 m. Substrate composition was varied, and included gravel-bottomed riffles, bedrock runs, sand bars, gravel bars, and pools with cobble substrate.

Stream clarity varied throughout the year depending on flow conditions. During this study water temperatures averaged 17.6 ºC, conductivity averaged 185.3 µs/cm, and pH averaged 7.63. The immediate riparian vegetation along both banks was of shrubs and young trees; beyond that was grassy fields and suburban development. Both sites were downstream of the city of Morehead.

Figure 2. Photo of the upper study site, showing Dry Creek (upper right of photo), entering the mainstem of Triplett Creek.

Figure 3. Photo of the top of the reach at the second, downstream site showing a bed of *Justicia americana* (bottom left) where *N. boops* occurred.

Habitat assessment

Macrohabitat variables (water temperature, dissolved oxygen, conductivity, and pH) were measured using a Data Sonde (Yellow Springs Instrument, model 660). Microhabitat variables were measured at 145, 1 m^2 points (hereafter designated "plots") after a visual survey of *N. boops* was completed. Microhabitat variables included substrate composition, flow, depth, and presence of cover. A visual survey was conducted to obtain plots of presence of *N. boops*, where microhabitat variables could be measured. The visual survey began by quantifying snorkeling visibility via lateral Secchi (Cialdi and Secchi 1865) distance. Lateral Secchi distance was measured by moving a Secchi disk away from the snorkeler until the white and black was indistinguishable (Murphy and Willis 1996); this was done to the left and right of the snorkeler, as the snorkeler faced upstream and the two measurements were averaged. The visual survey protocol involved two snorkelers (or three if the stream width was wider than 10 m) moving upstream in parallel lanes, with each lane about 4-5 m wide. Snorkelers scanned left and right keeping adjacent snorkelers in view to minimize recounting of target species (Murphy and Willis 1996, Jorden et al. 2007). When *N. boops* were observed a weighted flag was dropped to indicate a plot of presence. The number of *N. boops*, and any other fish species present within 1 m radius of *N. boops* observation were relayed to a record keeper on the shore. Visual surveys were conducted 2 June, 12 June, 26 August, and 4 September of 2017.

Microhabitat data were collected at each weighted flag dropped during the visual survey, which marked plots where *N. boops* were present. Plots of absence were established along 10 transects, arrayed at 10 m intervals at each site. Microhabitat data were collected at a single plot on each transect, randomly chosen at either 25%, 50%, or 75% of the distance from the left bank, with the requirement that the plot had to be at least 1 m from any weighted flag, which signified

presence of *N. boops* (Figure 4) (Osier and Welsh 2007, Gibbs et al. 2014). Data collected during

the microhabitat assessment generated 38 plots with *N. boops* and 107 plots without *N. boops*.

Microhabitat was assessed at each of these plots within a PVC 4x4 $1m^2$ grid placed on the center

of the plot (Figure 5). Dominant substrate in each of the 16 squares of the grid was categorized,

using a modified Wentworth's scale (Wentworth 1922). These data were used to generate three

substrate variables: mean particle size, substrate variation, and percent composition of each

substrate category (Albanese et al. 2011). Depth was recorded using a meter stick at each corner

of the grid and at the center of the grid. From these data mean depth and depth variation were

computed for each microhabitat plot. Stream flow was measured at each corner of the grid and

the center of the grid using a calibrated flowmeter (Swoffer, model 2100), and mean flow and

flow variation for each plot were computed. Finally, I judged if coarse woody debris or emergent

vegetation ample enough to provide cover was present in the plot. A principal component

analysis (PCA) was conducted using the 14 microhabitat variables, and scatterplots were

produced using Minitab 18. Examination of variable loadings and scatterplots of principal

component scores identified potentially meaningful microhabitat variables; these variables were

further analyzed with a Kruskal-Wallis rank test performed in Minitab 18. Kruskal-Wallis, a

nonparametric test, was used because after a Shapiro-Wilks test the data did not meet the

assumptions of normality that a parametric hypothesis test requires (Minitab 18).
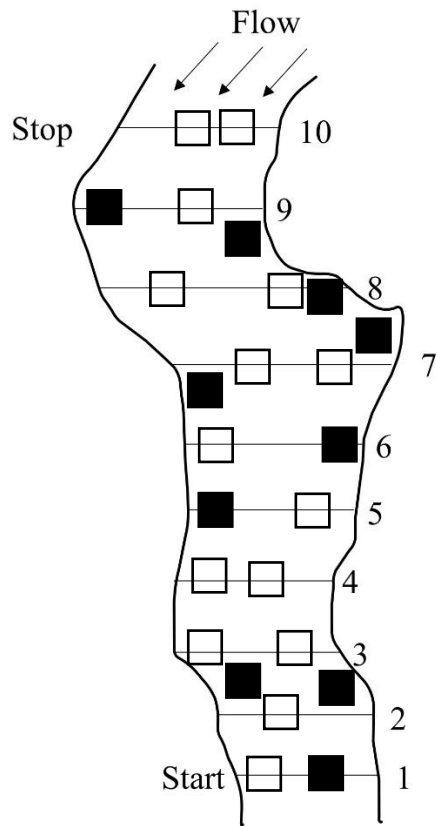
Figure 4. Diagram of methodology of segmenting sites into transects for microhabitat assessment. Square indicates a possible array of sampled plots, with dark squares indicating plots with *N. boops* present, established during the snorkeling survey and open squares indicating plots without *N. boops*, established along transects.

Figure 5. Measuring the microhabiatat variables at a plot of presence at the downstream site. A bed of *Justicia americana* is along the shore.

Age and growth

From April of 2017 until November of 2017 monthly seining was done in at least one of the two sites capturing specimens to evaluate (1) age and growth, (2) reproductive cycle, and (3) diet of *N. boops*. Captured *N. boops* were retained briefly in buckets; individuals were measured (standard length (SL)) and released. About 10 individuals from each sampling period were euthanized in MS222, then preserved in 10% formalin for laboratory analysis. Once in the lab these samples were transferred to 45% isopropanol and catalogued and vouchered in the Morehead State University Collection of Fishes.

Collected SL data from field measurements were used to produce length frequency histograms, which were used to generate age classifications based on the distribution of SLs of sampled individuals (Lehtinen and Echelle 1979). These age classifications were confirmed using scale annuli counts performed on preserved specimens. Scale annuli counts were conducted by removing five scales from the area just posterior and dorsal to the operculum of each fish. Scale annuli were then counted using a dissecting microscope with a glass base (Murphy and Willis 1996). Using the age classification data, and the movement of the modes of the age class distributions, parameters for a von Bertalanffy growth curve were produced using the FSA package in R. A growth curve was produced using the nlsTools and FSA packages in R (von Bertalanffy 1938, Murphy and Willis 1996, Jolly and Powers 2008, Baty et al. 2015, R Core Team 2017, Ogle et al. 2019).

Dietary analysis

Diet of *N. boops* was assessed by removing and opening the gastrointestinal tract and flushing the contents into a watch glass with 45% isopropanol. The contents were then identified to lowest taxonomic level possible (usually family) (Merritt et al. 2008) and enumerated under an Olympus SZ-ST dissecting scope. The number of each prey taxon per fish was recorded giving a percent composition by number. The number of individuals in each taxonomic group was recorded to produce a list of frequency of occurrence (Murphy and Willis 1996). Due to the small size and macerated nature of prey items, directly weighing the stomach contents was unfeasible. Instead length was measured using a calibrated ocular micrometer, and biomass was computed using a standard length-mass relationship equation $M = aL^b$ (Benke et al. 1999), where M = computed mass, *a* and *b* are constants, and L = length measurement (mm).

Reproduction

Gonad maturation was categorized using a scale developed for use on oviparous teleost fish with modifications for both annual and multiple spawners (Núñez and Duponchelle 2009). Testes were categorized as immature, maturing, ripe, or spent. Ovaries were categorized as immature, maturing, advanced maturation, ripe, spent, or resting (Núñez and Duponchelle 2009). Extent of tuberculation in males was noted, to help determine spawning season. All specimens were eviscerated, then eviscerated specimens and gonads were allowed to air-dry for 30 seconds and weighed (to the nearest 0.001 grams). This allowed for the computation of a gonadosomatic index (GSI; (gonad weight/eviscerated weight)*100) (Bean et al. 2010). To assess general fecundity ovaries were opened and ripe ova were counted. Diameters of ripe ova, mature

oocytes, and maturing oocytes were measured using a calibrated ocular micrometer (Holder and Powers 2010).

Results

Associated species

During monthly sampling by seining, the three species most often captured with *N. boops* were *Luxilus chrysocephalus* (Striped Shiner)*, Notropis photogenis* (Silver Shiner)*,* and *Pimephales notatus* (Bluntnose Minnow)*. Cyprinella spiloptera* (Spotfin Shiner)*, Semotilus atromaculatus* (Creek Chub)*, Notropis buccatus* (Silverjaw Minnow)*, Lepomis megalotis* (Longear Sunfish)*, Notropis rubellus* (Rosyface Shiner)*, Percina maculata* (Blackside Darter) and *C. anomalum* less commonly were captured with *N. boops*. During snorkeling survey *L. chrysocephalus,* and *P. notatus* were most commonly observed in association with *N. boops* (Table 1).

Table 1. Percent occurrence of species with *N. boops* during visual survey.

Associated Species	% Occurrence With *N. boops*
Luxilus chrysocephalus	81
Pimephales notatus	58
Lepomis megalotis	32
Micropterus dolomieu	13
Cyprinella spiloptera	10
Notropis rubellus	10
Percina maculata	10
Etheostoma blennioides	6
Etheostoma caeruleum	6
Hypentelium nigricans	6
Semotilus atromaculatus	6
Etheostoma flabellare	3
Etheostoma nigrum	3
Fundulus catenatus	3
Lythrurus umbratilis	3
Percina caprodes	3

Age and growth

Examination of a length-frequency histogram of all specimens captured during 2017 (Figure 6) along with comparisons to published data for other closely related cyprinids (Lehtinen and Echelle 1979, Jolly and Powers 2008, Bean et al. 2010) showed that at least three age classes were present; age class 0 (0-12 months; ≤ 30 mm SL), age class 1 (12-24 months; 31-45 mm SL), and age class 2+ (>24 months; ≥ 46 mm SL). The designation 2+ indicates that there may be some individuals in this group older than 2 years, but none older than 3. The limited number of large individuals captured did not allow for confident age class assignment of older individuals. The few large individuals captured suggest there is a high rate of mortality for age 2+ *N. boops*. Young of year (YOY) appeared in samples from August through October; however, we only captured seven YOY.

A histogram of individuals captured in May, June, and July 2017 gives a clear picture of age class assignments (Figure 7). Here a distinct mode for age class 1 individuals can be seen in May, and a mode for age class 2+ individuals can be seen in June. The months May through July were used to obtain average SL at 12 and 24 months of age. At 12 months *N. boops* averaged 38 mm SL, and at 24 months they averaged 52mm SL.

The growth rate of *N. boops* was determined from curves fitted to weight and length measurements at ages estimated from length frequency analysis and scale annuli counts. There was no statistically significant difference seen between males and females in growth rate, length, or eviscerated weight (Figure 8), consistent with other published observations (Burr and Dimmick 1983). This allowed for male and female data to be pooled (Figure 9) in subsequent analyses. A von Bertalanffy growth equation was determined using the equation $L_t=67.4(1-e^{-1(t-0.33)})$ (von Bertalanffy 1939), and a growth curve was produced of expected standard length over

three years (Figure 10). Many individuals captured during this survey were age class 1. Comparison of length frequency data from May to October indicated that Age 1 individuals grew about 10 mm SL over five months (Figures 6 and 11). The largest individual captured and released was 62 mm SL. The largest *N. boops* captured and preserved for analysis was a female 55.6 mm SL, with an eviscerated weight of 2.193 g.
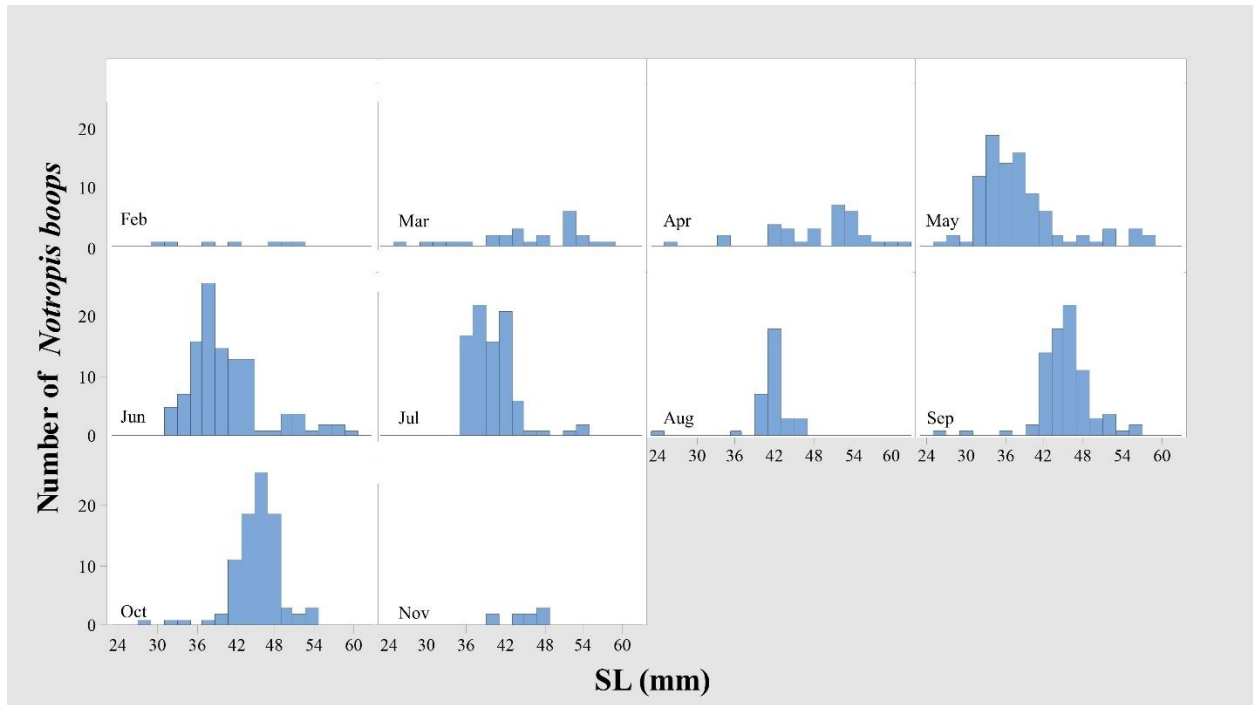
Figure 6. Histograms of length frequencies for all captured *N. boops* (N=567) from February through November of 2017 (Minitab 18 2017).
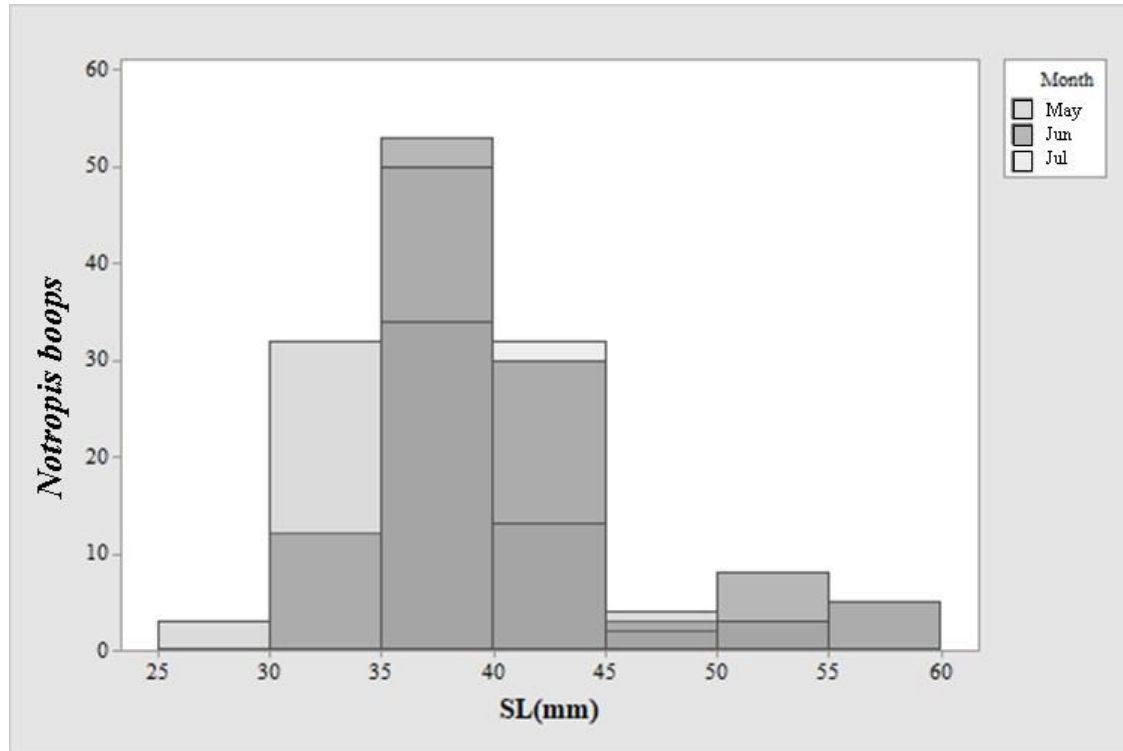
Figure 7. Overlaid length frequency data from May, June, and July (N=292). Specimens 24-45 mm SL corresponds to age class 1, while those 45-60 mm SL corresponds to age class 2+.
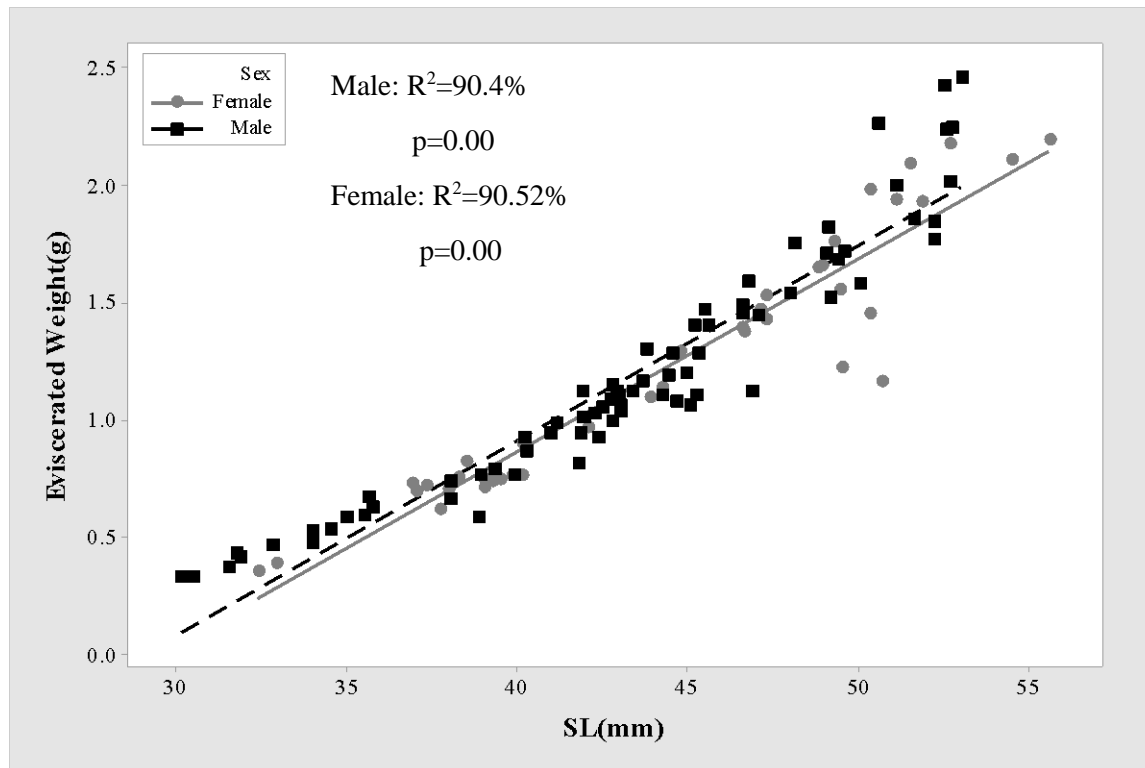
Figure 8. The relationship of SL to eviscerated weight for male (N=78) and female (N=49) *N. boops.*
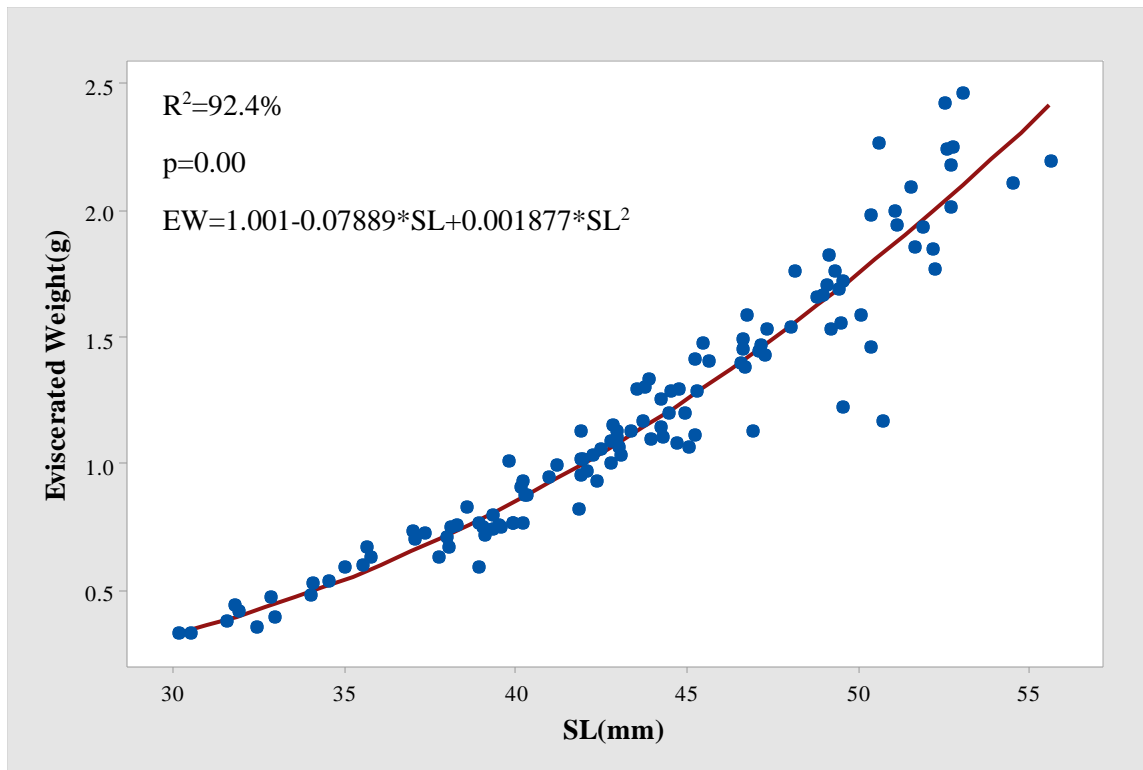
Figure 9. Length-weight relationships of N=127 male and female *N. boops*.
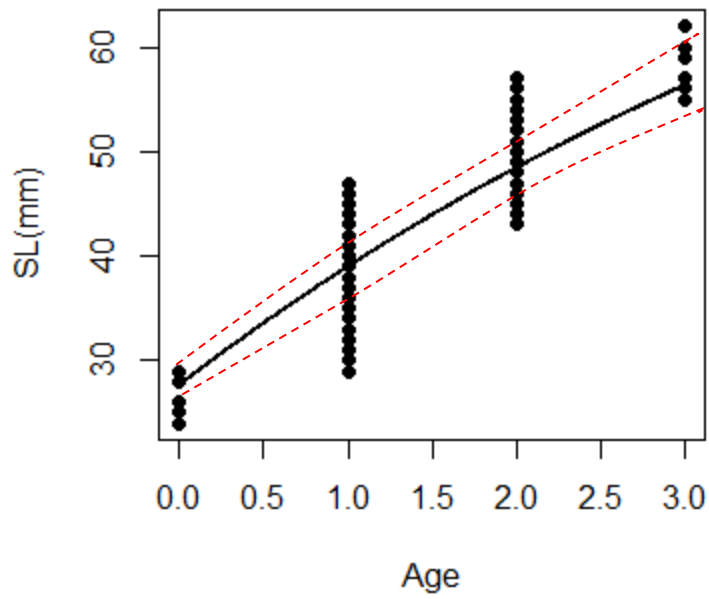
Figure 10. Stacked dot plot of lengths at one-year intervals from 0 to 3 years. Curve was derived from a von Bertalanffy growth equation $L_t=67.4(1-e^{-1(t-0.33)})$ (von Bertalanffy 1939) red dashed line indicates 95% confidence intervals.
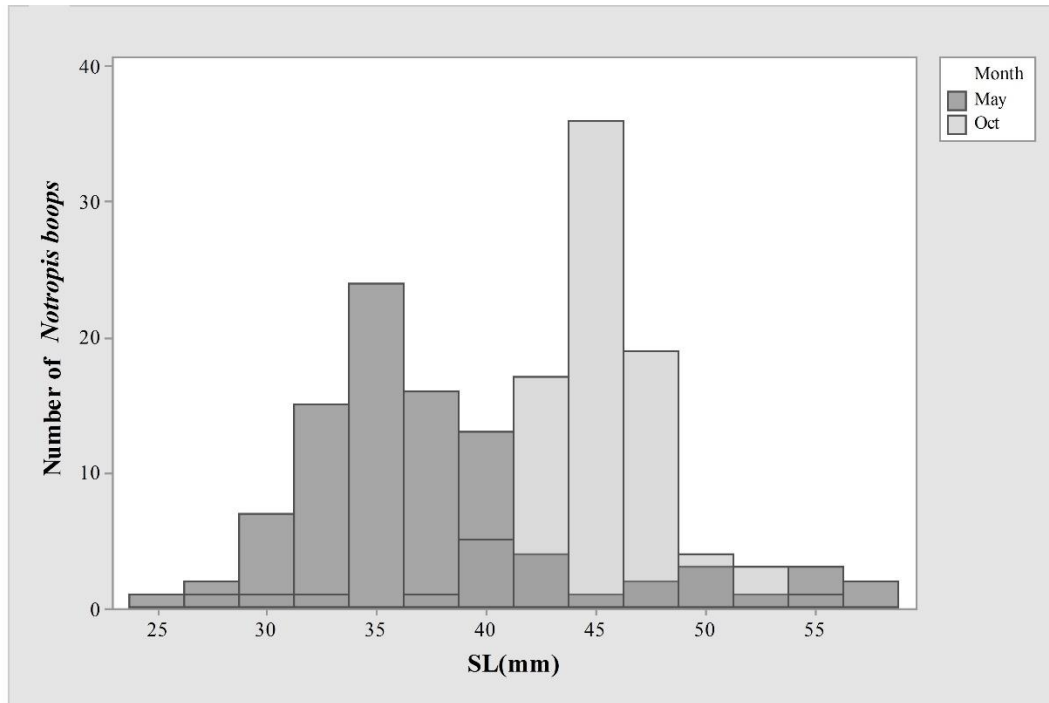
Figure 11. A histogram of length frequencies of May and October. Each of the peaks are age 1 individuals, indicating age 1 *N. boops* grew about 10 mm SL over 5 months.

Male reproductive cycle

Ripe testes were long, opaque white to creamy yellow, and contained many finger-like projections of various sizes. Immature testes, from small age 1 individuals were elongate threadlike structures, transparent to translucent, and with fewer, smaller finger like projections visible only under 60x magnification.

Gonadosomatic indices varied considerably among age class 1 individuals; however, there was a general trend towards increased GSI with increased SL from samples taken May-July (Figure 12). Examination of GSI showed an increase from February – April with two peaks in May and July; followed by a drop in August – November (Figure 13). Mature males captured in May, June, and July had well-developed tuberculation dorsal and posterior to the eyes, mandible, and on the front of snout (Figure 14). The dorsal surface of the head contained small tubercles densely distributed. The ventral surface of the head, preopercle, operculum, and gill membranes had small tubercles with a sparse distribution. There was minute tuberculation on the pectoral and dorsal fin rays. No tuberculate males were captured after the July sampling event. *Notropis boops* males showed no change in coloration during breeding apart from a light creamy coloration at the base of dorsal and pelvic fins, as noted in the literature (Burr and Dimmick 1983). Only a small percentage, 27%, of age class 1 males captured during the breeding season were in spawning condition, as evidenced by the amount of tuberculation and maturity of testis (Table 2). All age class 1 males in spawning condition were over 41 mm SL by June of 2017. All age class 2+ males were in spawning condition (Table 3), indicating that most of the spawning is carried out by the age class 2+ males.
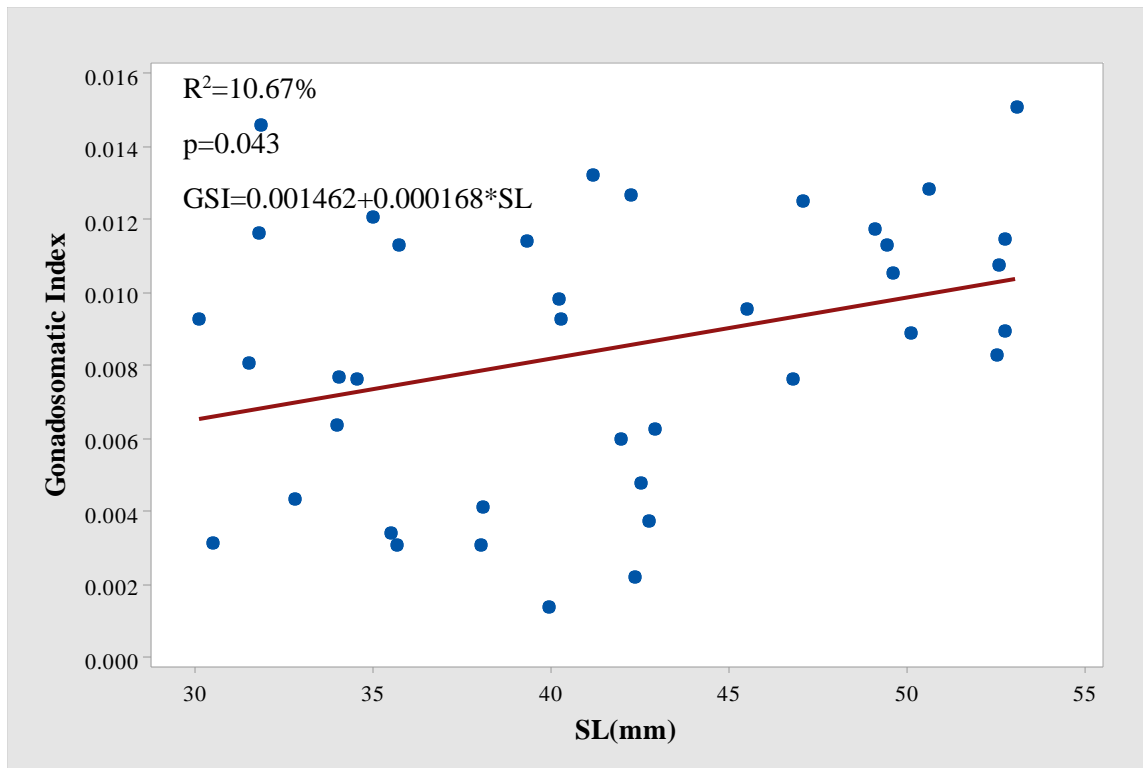
Figure 12. A scatterplot of GSI values versus SL (mm) from the months May through August, 2017 for mature and immature male *N. boops* (N=38). There is a slight positive correlation between increased SL and GSI value.

Figure 13. Gonadosomatic index (GSI) values for males (N=78) from Triplett Creek, 2017. Black dots indicate age class 2+ individuals, grey dots indicate age class 1 individuals.

Figure 14. Head of tuberculate male *N. boops* in spawning condition 56 mm SL, captured 19 May 2017.

Table 2. Reproductive condition of age class 1 and 2+ male individual *N. boops* during spawning season.

Age Class 1				Age Class 2+			
Date	SL	GSI	Gonad Maturity	Date	SL	GSI	Gonad Maturity
18 May 2017	31.87	1.456	Immature	18 May 2017	52.5	0.826	Ripe
18 May 2017	34.02	0.766	Immature	18 May 2017	53.02	1.504	Ripe
18 May 2017	34	0.630	Immature	18 May 2017	52.57	1.072	Ripe
18 May 2017	30.14	0.923	Immature	18 May 2017	49.55	1.050	Ripe
18 May 2017	31.78	1.163	Immature	18 May 2017	52.7	1.143	Ripe
18 May 2017	31.52	0.802	Immature	18 May 2017	46.75	0.757	Ripe
18 May 2017	32.81	0.431	Immature	18 May 2017	45.47	0.950	Ripe
2 Jun 2017	35.64	0.301	Maturing	2 Jun 2017	49.42	1.128	Ripe
2 Jun 2017	42.92	0.623	Ripe	7 Jun 2017	47.06	1.248	Ripe
2 Jun 2017	30.5	0.309	Immature	7 Jun 2017	50.56	1.281	Ripe
3 Jul 2017	42.23	1.266	Ripe	7 Jun 2017	52.72	0.891	Ripe
3 Jul 2017	41.95	0.595	Ripe	7 Jun 2017	46.79	1.719	Ripe
3 Jul 2017	41.16	1.320	Ripe	3 Jul 2017	50.05	0.884	Ripe
3 Jul 2017	39.32	1.141	Maturing	3 Jul 2017	49.07	1.174	Ripe
3 Jul 2017	34.99	1.205	Immature				
3 Jul 2017	38.05	0.405	Maturing				
3 Jul 2017	35.5	0.336	Immature				
18 Aug 2017	42.35	0.216	Spent				
18 Aug 2017	40.21	0.976	Maturing				
18 Aug 2017	39.91	0.132	Maturing				
18 Aug 2017	42.73	0.369	Spent				

Female reproductive cycle

Advanced mature and ripe ovaries of *N. boops* were greatly enlarged, had a deltoid shape, and were filled with orange to yellow oocytes, surrounded by smaller resting white oocytes. Immature ovaries were much smaller in size, deltoid in shape, and contained translucent to yellow or cream-colored immature oocytes. Mean mature oocyte count was 177 per ovary (Table 4). Age class 2+ females showed distended abdomens, but no other secondary sex characteristics were apparent.

Gonadosomatic indices for females sharply increased from May to June then rapidly decreased by August. Three of the age class 2+ individuals captured in late June to July had lower GSI values and oocyte counts than those in May and early June; these were most likely individuals that had already spawned. The remaining ripe oocytes in these individuals could indicate a second spawning event in the season sometime in late June to August. By mid-August GSI values indicated that spawning had concluded (Figure 15). An individual captured in August had an average of 52 advanced mature to ripe oocytes per ovary; these might have been in the process of reabsorbing prior to overwintering. There was a positive correlation between SL and number of ripe oocytes (R^2=36.9%; p = 0.083) (Figure 16). Like the males, most females in spawning condition were age class 2+ with only a small percentage of age class 1 (18%) females at spawning condition during the spawning season. Age class 1 females with ripe oocytes were all above 39 mm SL and were mature at the same time as the mature age class 2 individuals (Figure 15). Similar to the males, there was a positive correlation between SL and GSI values from samples taken May-July (Figure 17).
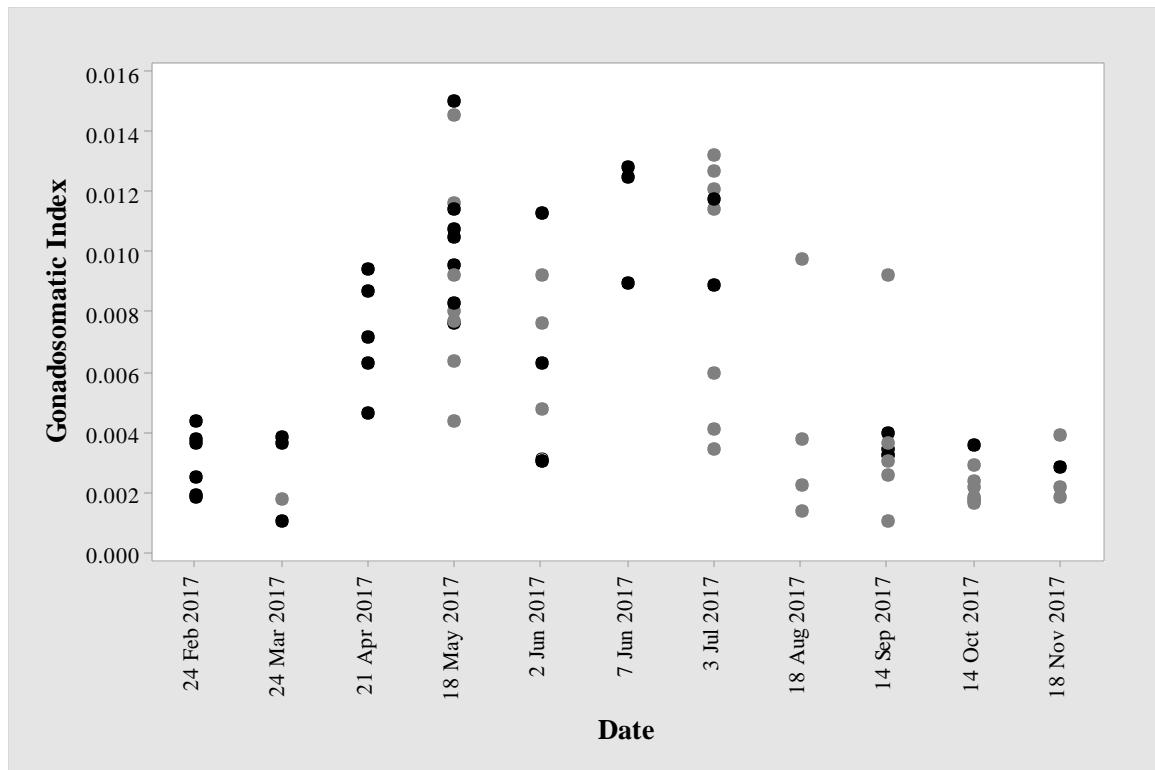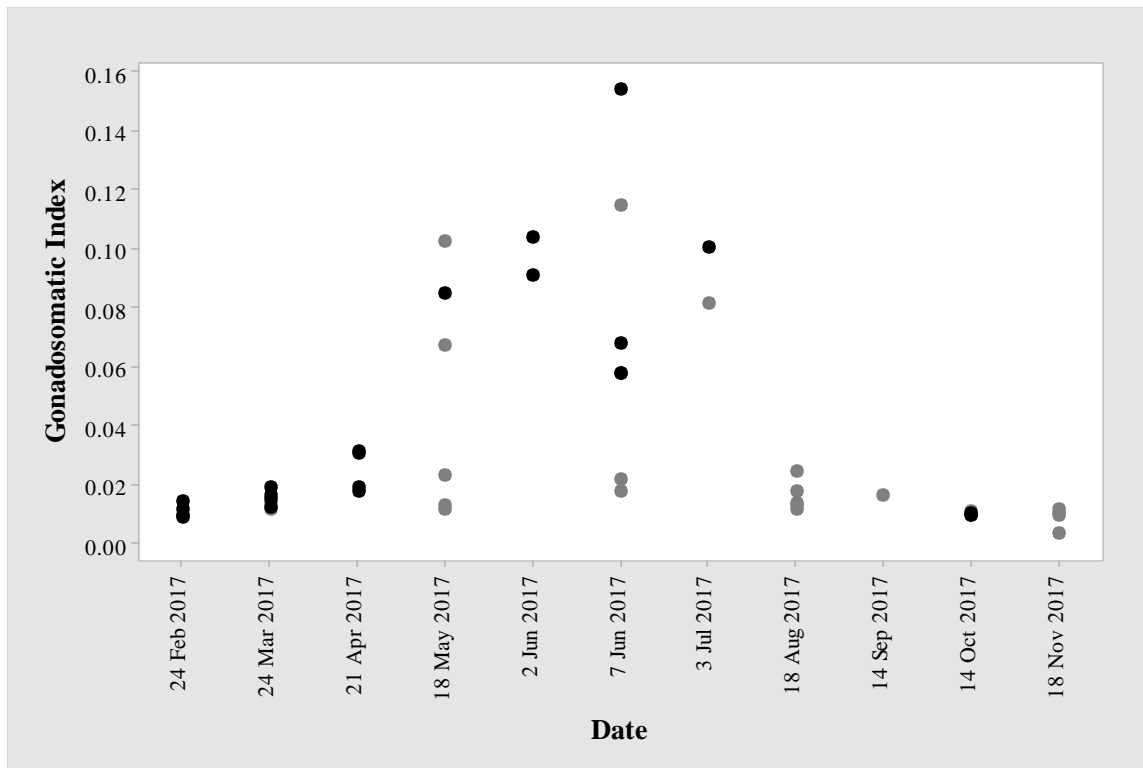
Figure 15. Gonadosomatic index (GSI) values for females (N=49) from Triplett Creek, 2017. Black dots are age class 2+, and grey dots are age class 1.

R²=36.9%

p=0.83

Number of eggs=220.3+10.31*SL
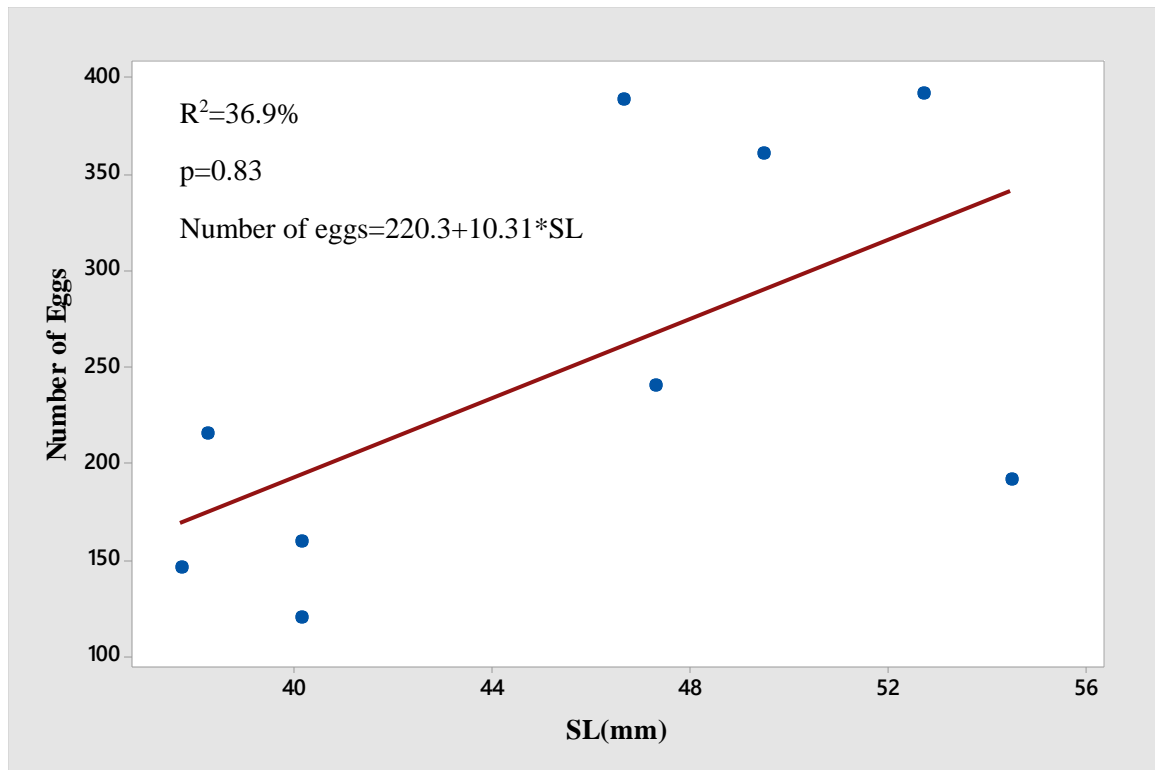
Figure 16. A scatterplot of mean number of mature eggs per ovary versus SL(mm). There is a positive correlation between SL and mean mature eggs per ovary.

R^2=11.1%

p= 0.130

GSI= -0.03911+0.002303*SL
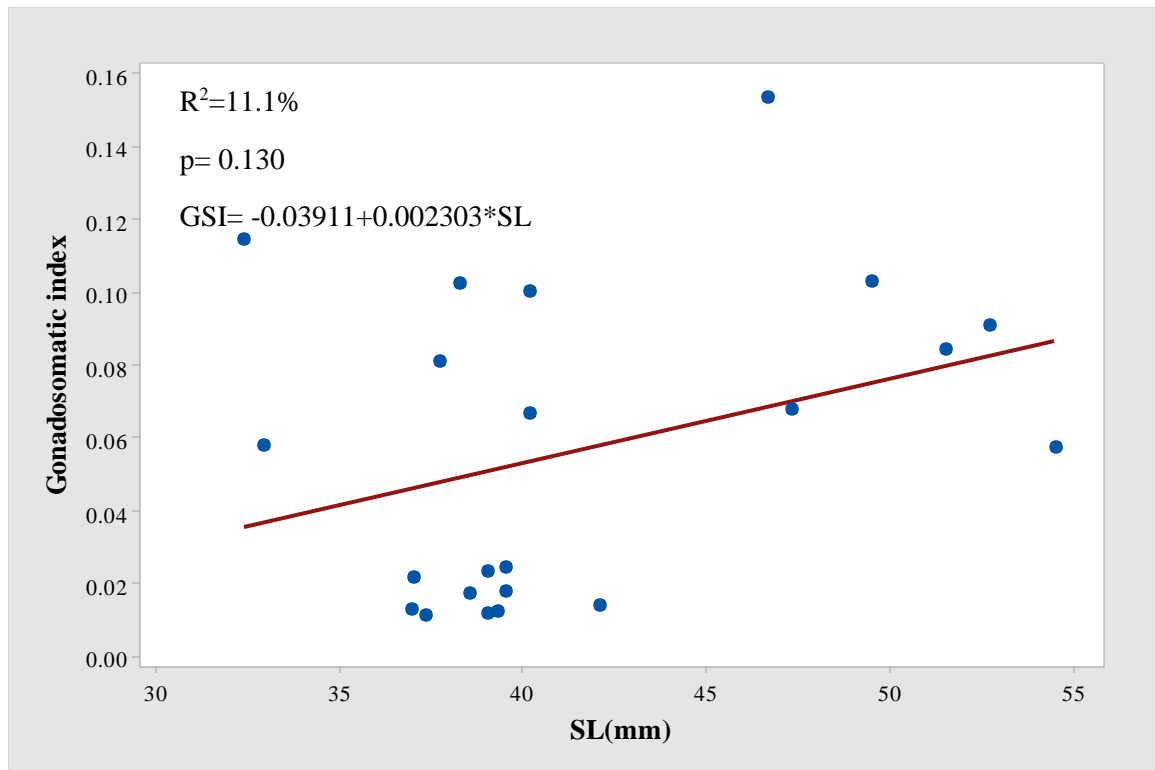
Figure 17. A scatterplot of GSI values versus SL(mm) for the months of May through August, 2017 for mature and immature female *N. boops*. There is a positive correlation between SL and GSI values.

Table 3. Gonadosomatic indices and average number of ripe ova per ovary (GSI) for 12 female
N. boops collected 21 April – 18 August 2017 from Triplett Creek, Kentucky.

SL (mm)	Date	Age	Number of Ripe Ova	GSI
51.12	21 April 2017	2+	140	0.869
38.27	18 May 2017	1	215	10.212
40.15	18 May 2017	1	120	6.659
51.51	18 May 2017	2+	259	8.421
49.47	2 June 2017	2+	212	10.303
52.69	2 June 2017	2+	246	9.053
54.47	7 June 2017	2+	188	5.703
46.65	7 June 2017	2+	189	15.334
47.28	7 June 2017	2+	196	6.737
37.73	3 July 2017	1	145	8.065
40.17	3 July 2017	1	159	10
39.28	18 August 2017	1	52	1.226

Spawning behavior

Spawning of *N. boops* was not observed during visual snorkel surveys throughout the spawning season of *N. boops*. *Notropis boops* was not associated with a mass of spawning *N. rubellus* observed on 15 May, 2018.

Habitat utilization

Eigenvector analysis of the correlation matrix showed that the first two principle components account for 36% of the variation in the data (Table 4). A PCA of 14 microhabitat variables in 145 plots indicated that *N. boops* is associated with microhabitats containing live vegetation (American Water-Willow, *Justicia americana*), heterogenous depth, and a mixed substrate of gravel, pebble, and cobble (Minitab 18) (Figure 5, 18, Table 4).

Kruskal-Wallis rank tests were performed on the seven microhabitat variables identified as most important in the PCA. Statistical significance was set at α = 0.007, by using the Bonferroni correction for multiple hypothesis tests (Bonferroni 1936). The presence of vegetation and higher % cobble were the only two variables significantly different between plots with and without *N. boops* (Table 5). *Notropis boops* were found in quiet water adjacent to flowing water, over a substrate of mixed cobble and sand, with heavy cover within two meters of site of presence. Cover was predominantly thick beds of *Justicia*, but also included dense woody cover in the form of root masses and submerged, fallen trees.

Table 4. Eigenvectors (loadings), proportions of variance, and eigenvalues for the first two principal components of a PCA of 14 microhabitat variables.

Variable	PC1	PC2
Eigenvalue	3.0385	2.0212
Proportion of Variance	0.217	0.144
Cumulative Variance	0.217	0.361
Depth mean	-0.049	-0.424
Depth variation	0.135	0.398
Flow mean	-0.217	0.277
Flow variation	0.244	0.099
Sub mean	-0.363	0.329
Sub variation	0.460	0.072
% silt	0.078	-0.226
% sand	0.449	0.009
% gravel	0.341	-0.059
% pebble	0.090	-0.210
% cobble	-0.306	-0.223
% bedrock	-0.144	0.460
woody	0.194	-0.175
vegetation	0.211	0.249
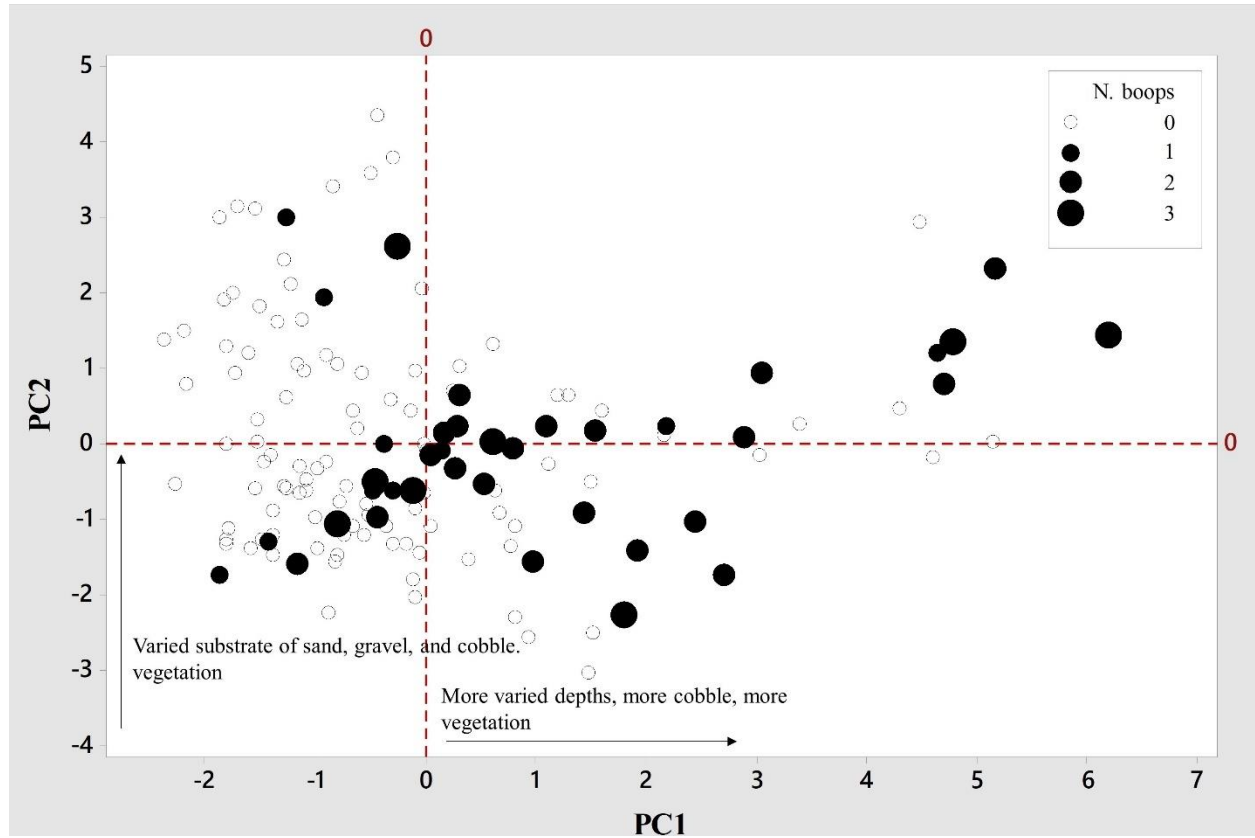
Figure 18. Scores for 145 plots with and without *N. boops,* from 14 microhabitat variables collected.

Table 5. Results of Kruskal-Wallis rank test performed on the seven variables identified by a PCA as the most important in discriminating microhabitat plots with and without *N. boops*. Two variables (vegetation and %cobble) were statistically different between these groups (α=0.007).

Variables	H-Value	DF	P-Value
Vegetation	25.25	1	0
Substrate mean	69.31	50	0.037
Substrate variation	106.1	84	0.052
% sand	17.37	10	0.067
% cobble	41.25	17	0.001
% gravel	9.21	10	0.512
Flow mean	83.58	86	0.554

Diet

Of the 125 *N. boops* examined, 104 had food items in their gut. There were at least 16 families of insects in the stomachs of *N. boops*. The highest level of diversity in an order was Diptera (five families); Ephemeroptera, Coleoptera, and Trichoptera each had two families represented. Only insects were identified in this study; no other invertebrates were seen in the stomachs of *N. boops*.

Immature dipterans and ephemeropterans made up the majority of the diet accounting for 82.2% of gut contents, by number of individuals (Table 7). Chironomid larvae accounted for 51% of the dipterans. Despite having a very small biomass chironomid larvae still made up the largest percentage of the biomass consumed at 30.9% (Figure 19). There were Formicidae present in the diet in the spring and early summer, making up 17% of the biomass in the diet of specimens sampled. All hymenopterans present only accounted for 2.44% by count alone, but 20% of the biomass. All dipterans made up 79.98% of the gut content in sampled fish by count, and 42.43% by biomass. Coleopterans only accounted for 1.09%. Only ephemeropteran nymphs were present, and as stated above they composed 11.78% of the gut contents by count and 22.2% by biomass. Tricopteran larvae were present but in small numbers, only making up 1.75% of the gut contents by count, and 4% by biomass (Figure 19). Larger prey items such as heptageniid mayflies, coleopterans, or hymenopterans were only found in large age 1 individuals, and age 2+ individuals, indicating a limit on prey size available to age 1, and YOY individuals. By count only 9.7% of the diet was made up of terrestrial insects, although by biomass they accounted for 26.8% by biomass.

Many (52% N=125) of the stomachs sampled contained parasitic cestodes. Infections ranged from light, with only a small fraction of a stomach contained proglottids, to heavy, where

a stomach was distended with proglottids. Attempts to isolate a scolex for identification were unsuccessful. In addition, there were many debris items found in stomachs sampled; such as sand, pebbles, very small pieces of wood, and various plant materials. These items were probably part of trichopteran cases or debris unintentionally consumed during foraging.

Table 6. Table of gut contents showing frequency of occurrence, percent composition by number, number of *N. boops* containing taxa, and percent of *N. boops* containing taxa.

Organism	Frequency of occurrence	% Composition by Number	# of *N. boops* containing taxa	% *N. boops* with taxa
Insecta				
Coleoptera				
Dytiscidae	1	0.12	1	0.8
Elmidae	2	0.24	2	1.6
unidentified adults	6	0.73	3	2.4
Diptera				
Ceratopogonidae(larvae)	15	1.83	4	3.2
Ceratopogonidae(adult)	5	0.61	3	2.4
Chaoboridae	9	1.10	4	3.2
Chironomidae(larvae)	419	51.04	83	66.4
Chironomidae(adult)	40	4.87	14	11.2
Culicidae(pupae)	16	1.95	6	4.8
Simuliidae	75	9.14	15	12
unidentified larvae	47	5.72	9	7.2
unidentified pupae	5	0.61	2	1.6
unidentified adults	28	3.41	7	5.6
Ephemeroptera				
Isonychiidae	47	5.72	16	12.8
Heptageniidae	22	2.68	14	11.2
unidentified nymphs	25	3.05	13	10.4
Hemiptera			1	0.8
unidentified adults	1	0.12		
Hymenoptera				
Formicidae	16	1.95	5	4
Ichneumonidae	3	0.37	2	1.6
unidentified adults	1	0.12	1	0.8
Megaloptera				
Sialidae	2	0.24	2	1.6
Odonata				
Calopterygidae	5	0.61	3	2.4
Plecoptera				
Capniidae	15	1.83	4	3.2
Trichoptera				
Hydropsychidae	5	0.61	3	2.4

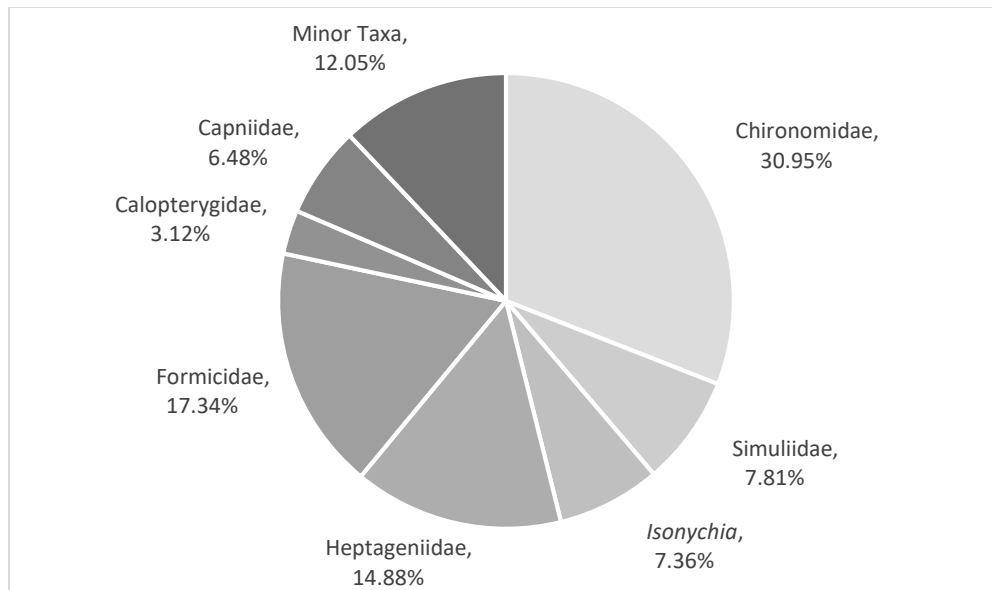| Philopotamidae | 6 | 0.73 | 5 | 4 |
| unidentified larvae | 5 | 0.61 | 2 | 1.6 |
| Total | 821 | | | |

Figure 19. A pie chart showing the composition of diet by biomass. Minor taxa include Dytiscidae, Elmidae, Ceratopogonidae, Chaoboridae, Culicidae, Ichneumonidae, Sialidae, Hydropsychidae, and Philopotamidae.

<div align="center">**Discussion**</div>

Age and growth

The population of *N. boops* in Triplett Creek in 2017 predominantly contained age class 1 individuals, at 72.7%. Age class 2+ individuals made up the second largest group at 25.5% of the population, and young of year (YOY) (age class 0) made up the smallest group at 1.8%. A previous study in Briar Creek, Oklahoma found that YOY made up the largest proportion of sampled populations (Lehtinen and Echelle 1979), possibly indicating poor recruitment in Triplett Creek in 2017. Triplett Creek has historically had a healthy population of *N. boops* (pers. comm., David Eisenhour). The rarity of YOY in 2017 is puzzling, and there are several possible explanations.

First, it could be possible that these fish move upstream during the spring and summer months to spawn, resulting in YOY occurring outside of the study area. I am involved in an ongoing mark-and-recapture study of this species, which indicates fairly high mobility in this species, although spawning locations have not been identified. Previous studies have heavily sampled the tributaries of Triplett Creek during the summer and fall months and found no *N. boops* (McCafferty 2000). Given the above information this hypothesis does not seem to be a valid explanation for the rarity of YOY captured in the fall of 2017.

The second explanation is that the multiple flooding events that occurred in early summer of 2017 could have disrupted spawning or recruitment of young. In the Morehead State University Fish Museum there are catalogued lots with numerous YOY from fall samples. This indicates that when present they are susceptible to standard capture methods. During the follow up sampling events for the mark-and-recapture study I have seen decreased numbers of age class

1 individuals in 2018, indicating poor recruitment in 2017. There are multiple ways that flood

pulses could have disrupted *N. boops* reproduction leading to poor recruitment in 2017. It is

possible the high waters washed away the eggs or larvae during one of the flood pulses. Eggs or

larvae may have been buried in the excess of sediments introduced into the stream during the

flood pulses. Also, altered water temperature or stream chemistry could have been unfavorable

for early development. Triplett Creek reached flood stages several times over this study period

reaching a discharge of around 3.800 ft^3/sec in June, and 2,600 ft^3/sec in July (Figure 20), going

well beyond the "two standard deviation rule" which indicates if a flood event might negatively

impact fish populations (Matthews 1998). This is double what the highest discharges were in

2016 (median discharge from May-July is typically 10-100 ft^3/sec) (USGS 2018). These flood

pulses greatly restructured the habitat at both sites multiple times. Flood pulses raised, or moved

gravel bars, and changed the substrate composition in riffle complexes. These events also

reduced available cover at the upstream site: *Justicia* beds were washed out, and fallen trees were

washed downstream out of the sampling reaches.

 Notropis boops are most active during their spawning season where I saw the highest

catch per unit effort during the months of May – July 2017. Catch per unit effort drastically

dropped off by November when these fish, presumably, had entered overwintering habitats. Most

N. boops captured in November were found in dense leaf packs at the bottom of large pools.

 There are limited data published on the growth of *N. boops*. The maximum reported total

length is 91 mm (Etnier and Starnes 1993). The largest specimen captured during this study was

63 mm SL. The length frequency distributions in Triplett Creek populations were similar to those

reported by Lehtenin and Echelle (1979) for the Oklahoma population. The few YOY do not

provide much information about growth during their first summer. However, looking at the age

class 1 individuals captured in February and March it is evident that *N. boops* reaches a SL of about 30 mm by the spring following their first winter. The computed growth curve shows that by their second winter *N. boops* reach about 45 mm SL, 59 mm SL by the third winter, and 65 mm SL by the fourth winter (Figure 10).

Notropis boops' life span in Triplett Creek seems to be 2 to 3 years, similar to the estimate of the populations in Brier Creek, Oklahoma (Lehtinen and Echelle 1979), and is comparable to other cyprinids of this size in stream environments (Holder and Powers 2010). Age class 1 individuals made up the largest portion of the population for most fishes. Because of small sample size and variations in growth rates it is difficult to determine distinct age class 2 and 3 fish from length frequency data, although there are likely a few fish older than 2 years included in age class 2+, based on the generated growth curves (Figure 10), and scale annuli counts. This age class included individuals with SL ranging from 46-62 mm in our population. Determination of age by length-frequency analysis for the largest individuals is difficult due to growth greatly slowing. During scale annuli counting there were no fish with four annuli, indicating that no fish live past three years, as reported by Lehtinen and Echelle (1979).
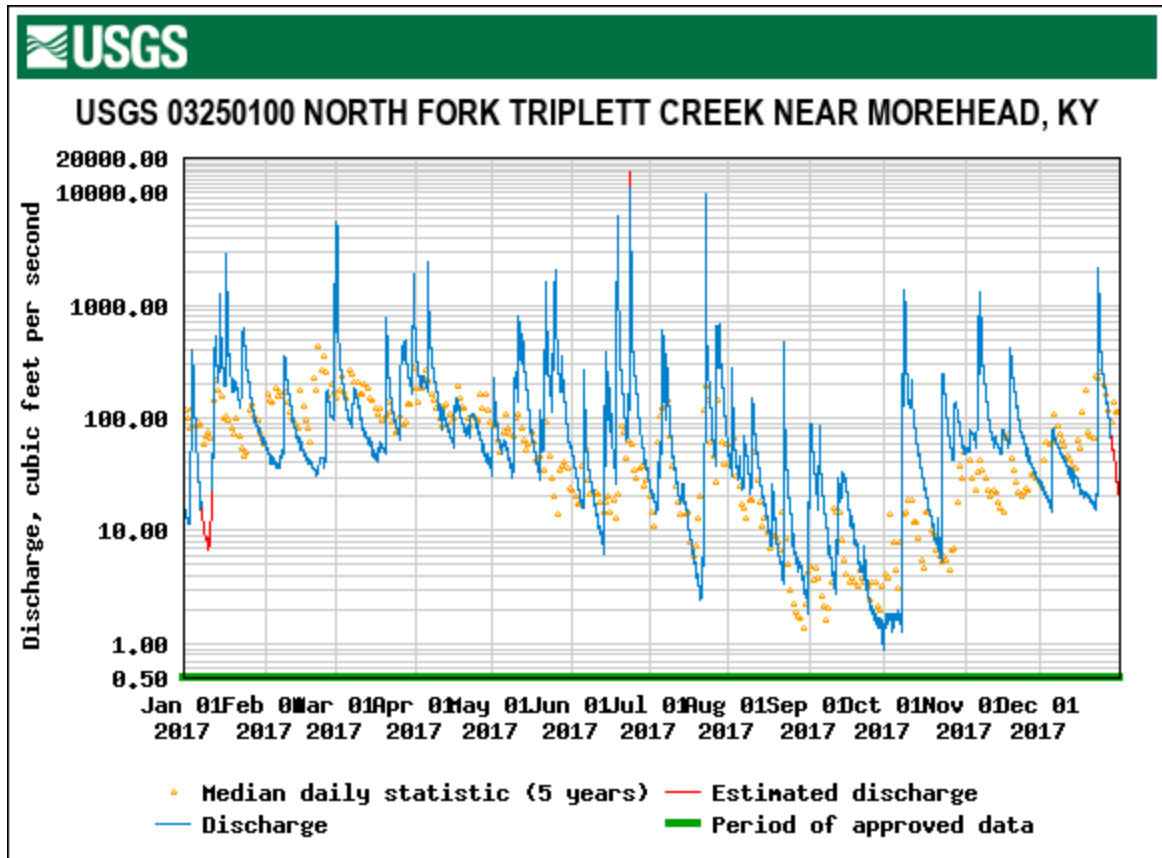
Figure 20. Daily discharge in 2017 from an USGS gauge in North Fork Triplett Creek, a large tributary of Triplett Creek. Courtesy of USGS.

Reproductive biology

Testes of male *Notropis boops* were similar in appearance and structure to published descriptions of other cyprinid testes (Núñez and Duponchelle, 2009, Hodgskins et al. 2016). GSI data and the seasonal pattern of tuberculation showed that *N. boops* spawn May through early July in this study population. These data are consistent with accounts in various state fish books, in which spawning season was estimated by the presence of tuberculate males (Trautman 1981). However, this is a shortened spawning season compared to the only other study to examine GSI for *N. boops* (Lehtenin and Echelle 1979). Lehtenin and Echelle reported an extended spawning season that they attributed to higher than normal temperatures, and the southern latitude of their study populations. Another study looking at *N. boops'* sister species *N. xaenocephalus* in Moore Creek, Georgia reported a three-month spawning period April – June (Jolly and Powers 2008). This spawning season is about the same length as the one I saw in my population of *N. boops*; however, that of *N. xaenocephalus* is shifted one month earlier, most likely due to the higher temperatures earlier in the year at this southern latitude. My study population showed similar age at sexual maturity to that reported in Brier Creek, Oklahoma. Both our study populations occurred in similar climates, and the majority of fish not reaching sexual maturity until their second summer (Lehtinen and Echelle 1979). During visual examination of ovaries, I saw that mature oocytes were surrounded by many immature oocytes. Multiple stages of oocyte development is consistent with an iteroparous species as suggested by published data (Lehtinen and Echelle 1979).

Habitat utilization

During this survey *N. boops* was found in permanent pools, occupying locations in the pools immediately adjacent to faster moving riffles or runs. These locations usually contained some form of cover in the form of emergent vegetation (i.e., *Justicia americana*) or coarse woody debris. These locations had silt-free heterogeneous substrates, with cobble and sand predominating.

The results from the Kruskal-Wallis rank test, and the PCA are consistent with anecdotal reports on *N. boops* habitat use (Trautman 1981). *Notropis boops* are considered intolerant of heavy siltation, and pools are habitats that are disproportionally affected by siltation events (Rice et al. 1998). A substrate high in cobble and gravel indicates lower levels of smaller sediments. The PCA did show an association between % sand and presence of *N. boops*, although this relationship was not shown to be significant in the univariate tests. A sand association would not be unexpected as *N. boops* is reported to inhabit stream with clean sand substrates (Clay 1975, Ross 2001).

It was consistently seen that dense populations of age class 1 and 2+ individuals used the same type of microhabitat throughout the year, although they might move to different locations in the stream as changing water levels created or eliminated these microhabitats. Thus, I saw no evidence that indicated different ages used different microhabitats. I saw the most fish during the late spring through summer when these fish are most active, and breeding is occurring. The visual surveys showed that *N. boops* were occurring in the same habitats throughout the year as they were during the spawning season may possibly mean that they are spawning in these habitats. The few YOY encountered during this survey provided only limited information about

their habitat usage. However, most were found at the margins of the stream in beds of detritus or *Justicia*, suggesting the importance of cover for YOY.

Diet

Notropis boops is classified as a bentho-pelagic, sight-feeding invertivore that uses its large eyes to feed on insects drifting in the water column, plucking them from the benthos, or the surface, even reportedly jumping out of the water to capture terrestrial insects (Trautman 1981, Etnier and Starnes 1993). Analysis of gut contents showed that they were eating insects that occur in the substrate such as capniid and heptageniid nymphs. It is difficult to say if this is enough to determine whether *N. boops* was plucking insects from the benthos, because these prey items could have been suspended in the water column due to a drift event. However, during the visual snorkel survey *N. boops* was observed most often in the lower third of the water column, occasionally making jabs into the substrate, presumably to pick prey items from the substrate. *Notropis boops* was not observed doing this as often as *Pimephales notatus*, but apparently were foraging from the benthos. A considerable portion of their diet consisted of insects that occur at the surface, such as culicid pupae, and terrestrial insects such as Formicidae, Ichneumonidae, and adult chironimids, simuliids, and ceratopogonids. During examination of gut contents, no non-insect invertebrates were identified. This may mean that *N. boops* do not consume them, or that they were small, rapidly digested and not apparent during examination. This might also reflect limited numbers of non-insects in the environment. These results suggest that *N. boops* are generalists that move throughout the water column foraging for a diverse range of invertebrates. The large eyes suggest that they are primarily sight-feeders and assist in this behavior. The microhabitat location, the visual survey, and my analysis of the diet suggests that

N. boops exploits multiple feeding modes, including foraging on drifting insects, plucking insects from the substrate, and possibly foraging in beds of emergent vegetation.

Microhabitat location influences what foraging opportunities are available for a species. *Notropis boops* occupies a microhabitat that is on the edge of multiple microhabitats, this provides several unique foraging opportunities. For example, the proximity to faster flowing water allows *N. boops* to capitalize on insects drifting in the faster moving waters without having to use the large amounts of energy required to maintain position in a riffle or run. The variability of substrate is indicative of the proximity to many varied flows, and the trend towards substrates consisting of particles larger than silt is in line with the published reports of these fish being sensitive to siltation (Rice et al. 1998). Silt free substrates provide greater interstitial space which many prey items of *N. boops* occupy, providing another foraging opportunity in this microhabitat.

Implications for conservation

Notropis boops are reported to be sensitive to heavy siltation (Rice et al. 1998). The mechanisms of this sensitivity are unclear and indicate a topic for further research. In general, heavy siltation can inhibit the ability of sight-feeders to forage, limit available microhabitat, reduce the volume of interstitial space, clog gill arches, and may alter water chemistry (Hollis et al. 1964, Ryan 1991). The microhabitat *N. boops* occupies is especially vulnerable to siltation events, due to the low rate of flow. Siltation comes from many sources as water moves into, and within waterways. Natural sources are generally water eroding loose dirt from the banks or carried into waterways. Anthropogenic factors, such as agricultural, construction, and impervious

surface runoff can increase the level of siltation in surrounding waterways. Heavy siltation also occurs during flood events due to the excessive amount of runoff. Heavy siltation in *N. boops* microhabitat could lower the available interstitial space within the substrate burying prey items. This situation could also limit *N. boops* ability to forage since the depend on their large eyes. With these factors combined this could greatly reduce the ability of *N. boops* to capture prey. Another concern is that prolonged droughts can extirpate populations when permeant pools where *N. boops* take refuge during dry spells become isolated. An isolated pool has the potential to become too hot or hypoxic, these conditions are unfavorable for *N. boops* survival (Etnier and Starnes 1993, Rice et al. 1998, Page and Burr 2011).

Notropis boops microhabitat location requires a heterogeneous mix of habitats in a stream. Pools with adjacent riffles or runs require meandering flow and natural barriers (e.g., gravel or sand bars). Emergent vegetation has to establish in areas with slower flow requiring the same stream conditions as stated above. Channelization straightens waterways and homogenizes the benthos. Other anthropogenic modifications (e.g., dam, bridge, etc.) can have similar detrimental effects. Under different flow conditions their preferred microhabitat may be in different areas of the stream. These areas may be removed or inaccessible after channelization or major anthropogenic modification. These situations could lead to a local extirpation event, as well as not being conducive for reestablishment of *N. boops* in an area of local extirpation.

Notropis boops is also a short-lived fish with no individuals living past three years, and only a small fraction of age class 1 individuals reaching spawning condition by the spawning season of their first year. Erratic weather patterns or anthropogenic disturbances in two successive years could very well disrupt recruitment enough to extirpate a population. Reestablishment in such an area may not be possible if other populations are isolated by barriers.

If no barrier exists and there is a contiguous metapopulation, reestablishment in an area of local

extirpation could readily occur.

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