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Effects of Vegetation Density on the Ontogeny to Piscivory of Juvenile Largemouth Bass

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Abstract

First-year overwinter survival is a frequent bottleneck to the recruitment of Largemouth Bass *Micropterus salmoides*. Early ontogeny to piscivory provides increased overwinter survival through increased growth and the accumulation of lipids. This ontogeny is thought to be slowed by dense and complex vegetative habitats, but this hypothesis has not been directly tested. To address this question, we conducted enclosure experiments for 5 weeks during the typical time that juvenile Largemouth Bass would transition to piscivory (i.e., midsummer). Thirty-two enclosures were constructed across two 0.10-ha ponds and given one of four stem densities of simulated vegetation (0, 50, 250, and 500 stems/m²). Three juvenile Largemouth Bass and 30 juvenile Bluegills *Lepomis macrochirus* were added to each enclosure. All fish were sampled twice per week. Largemouth Bass growth was measured on all sample dates, and stomach samples were collected to determine diets on one date each week. Bluegill sizes and densities were manipulated to maintain the number of fish at 30 fish that were 25–35% of Largemouth Bass TL after each sampling event. Largemouth Bass stomach contents (percent by weight) were initially dominated by insects, and the bass transitioned to mostly fish prey by the end of the experiment. The use of fish prey, as measured by the presence of surviving Bluegills in the enclosures, significantly increased at the beginning of the second week at all stem densities, but fewer fish prey were eaten by bass in the 250-stems/m² treatment than in all other treatments throughout the experiment. Largemouth Bass also grew less in the 250-stems/m² treatment. We concluded that vegetation density does affect the foraging rate of piscivorous juvenile Largemouth Bass, but not necessarily the timing of the ontogeny to piscivory. However, differing results between this experiment and other previously published studies suggests vegetation may have an interactive effect with available prey types.

Largemouth Bass *Micropterus salmoides* undergo an ontogenetic shift in feeding habits from invertebrates to piscine prey during their first year of life (Werner and Gilliam 1984; Olson 1996; Ludsin and DeVries 1997; Mittelbach and Persson 1998). The switch to piscivory facilitates increased growth, as fish prey are a greater source of energy than invertebrate prey (Keast and Eadie 1985; Olson 1996; Mittelbach and Persson 1998). With this increased growth comes an ability to eat larger prey, thus available prey is increased, which further enhances growth (Mittelbach and Persson 1998). First-year growth is important as it facilitates an individual's ability to avoid

predation (Hambright 1991) and increases overwinter survival (Ludsin and DeVries 1997; Mittelbach and Persson 1998), both of which are considered major bottlenecks in Largemouth Bass recruitment (Houde 1987; Galarowicz and Wahl 2005; Parkos and Wahl 2010). Therefore, understanding the factors influencing the timing of ontogeny to piscivory is important for understanding recruitment dynamics of Largemouth Bass.

Availability of fish prey that are small enough for juvenile Largemouth Bass to consume (approximately $\leq 50\%$ of predator TL: Johnson and Post 1996) affects the timing of ontogeny to piscivory for this species (Mittelbach and

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Persson 1998). Prey availability can be affected by prey community structure, relative hatch timing of both Largemouth Bass and prey species, and prepiscivorous growth of Largemouth Bass (Johnson and Post 1996; Olson 1996; Garvey and Stein 1998). These factors contribute to the wide range of sizes reported for the onset of piscivory in Largemouth Bass (30–140 mm TL, but typically 40–60 mm TL: Bettoli et al. 1992; Johnson and Post 1996; Olson 1996; Mittelbach and Persson 1998; Brown et al. 2001; Huskey and Turingan 2001; Post 2003). Although ontogeny to piscivory may begin at these lengths, a steady supply of appropriately sized fish is required to maintain piscivory (Garvey and Stein 1998). Piscine prey community composition affects prey availability as some prey species may quickly outgrow gape limitations (e.g., shad *Dorosoma* spp.: Hambright 1991) of juvenile Largemouth Bass, whereas others spawn throughout the summer (e.g., Bluegill *Lepomis macrochirus*: Cargnelli and Gross 1996; Garvey et al. 2002; Santucci and Wahl 2003) and provide a consistent supply of appropriately sized prey fish.

Habitat complexity may also influence the timing of ontogeny to piscivory for juvenile piscivores through reduced foraging efficiency, but this has not been adequately studied. Optimal foraging theory predicts that organisms will forage in a way that maximizes their net energy gain (MacArthur and Pianka 1966). Habitat complexity affects foraging rates and growth in many species of fish (Bettoli et al. 1992; Dibble and Harrel 1997; Miranda and Pugh 1997; Olson et al. 1998; Reid et al. 1999; Shoup et al. 2012; Stahr and Shoup 2015, 2016) and may also lead to changes in diet if certain prey types require more energy to capture in certain environments (Mittelbach 1981), ultimately resulting in habitat-specific differences in diet (Schramm and Zale 1985; Dibble and Harrel 1997). Thus, prey selection in a given type of habitat will likely be a function of the way habitat complexity alters predator and prey behavior.

Complex habitats, such as vegetation, may delay the ontogeny to piscivory in Largemouth Bass because highly mobile fish prey may be more difficult to capture in these complex habitats. Juvenile Largemouth Bass initially prey on zooplankton and soon transition to macroinvertebrates and eventually fish (Galarowicz and Wahl 2005; Graeb et al. 2006). Zooplankton are often the most abundant and easily captured prey type (Mittelbach 1981; Graeb et al. 2005), but their small size requires the consumption of large quantities to meet predator energetic needs (Cummins and Wuycheck 1971). Macroinvertebrate prey items are less abundant and more mobile than zooplankton, making them somewhat harder to consume (Mittelbach 1981; Graeb et al. 2005). However, macroinvertebrates are larger and therefore provide better energy returns than do zooplankton, which may help offset the additional effort required to capture them (Cummins and Wuycheck 1971). Piscine prey

are the least abundant and the most difficult prey type to capture (Graeb et al. 2005), but are also the most energetically valuable (Cummins and Wuycheck 1971; Miranda and Muncy 1989). Dense vegetation affects the ability of Largemouth Bass to find and capture prey primarily by obstructing vision (Breck 1993) and hampering maneuverability (Savino and Stein 1982). Therefore, it is logical that increased habitat complexity would have a disproportionately large effect on the ability of juvenile Largemouth Bass to forage when they feed on fast-moving or highly maneuverable fish prey relative to when they feed on slower-moving and less-maneuverable zooplankton or macroinvertebrates.

Of all the factors influencing the timing of ontogeny to piscivory in Largemouth Bass, vegetation density is the least studied and yet is one of the factors most commonly manipulated by fisheries managers in their attempts to improve Largemouth Bass recruitment. Earlier ontogeny to piscivory in Largemouth Bass has been correlated with the removal of complex vegetation (Bettoli et al. 1992); however, vegetation removal can affect the fish community by altering the abundance of prey and competitors, both of which can also affect the ontogeny to piscivory (Bettoli et al. 1992; Garvey and Stein 1998; Brown et al. 2001; Huskey and Turingan 2001). In laboratory studies, adult Largemouth Bass have reduced foraging return when they feed on fish prey in vegetated habitats (250–1,000 stems/m²: Savino and Stein 1982; Gotceitas and Colgan 1989; Stahr and Shoup 2015). However, juvenile Largemouth Bass foraging rates are unaffected by vegetation at stem densities up to 1,000 stems/m² when they feed on nonmobile invertebrate prey (e.g., chironomid larvae: Stahr and Shoup 2016) and increase at moderate vegetation densities (Largemouth Bass tested at 125 stems/m² had a higher foraging rate than those at 0, 250, or 1,000 stems/m²) when they forage on piscine prey (Broderius 2015). Despite the large amount of research directed at the effects of vegetation on adult Largemouth Bass foraging, it is unclear whether vegetation is likely to affect the timing of the ontogeny of piscivory in juvenile Largemouth Bass. The purpose of this experiment was to determine how vegetation density affects the timing and extent of piscivory by juvenile Largemouth Bass and ultimately how it affects growth. We used enclosures that combined the control of a laboratory experiment with the applicability of a field study by controlling vegetation densities and piscine prey densities for juvenile Largemouth Bass during summer months, when they would naturally transition to piscivory.

METHODS

Experimental design.—To determine the effect of vegetation density on the timing of ontogeny to piscivory in juvenile Largemouth Bass, juvenile Largemouth Bass diets

and growth were monitored over a 5-week experiment during a time frame when they would typically switch to piscivory (midsummer). Square enclosures (1×1 m) with vegetation densities of 0, 50, 250, or 500 stems/m² (hereafter, referred to as control, low, medium, and high) were constructed as experimental replicates within 0.10-ha research ponds at the Oklahoma State University Aquatic Ecology Research Ponds. Stem densities were selected such that they ranged from ineffective (0 and 50 stems/m²) to highly effective (250 and 500 stems/m²) at providing predation refuge for Bluegills under the threat of Largemouth Bass predation (Savino and Stein 1982; Gotceitas and Colgan 1987; Gotceitas 1990). Enclosures were constructed from plastic diamond-shaped mesh (3.3-mm bar mesh, approximately 7 mm diameter across the longest axis of openings) attached to a U-post frame with the mesh buried approximately 15 cm in the pond bottom and tops were left uncovered. Approximately 20 cm of plastic mesh extended above water level when ponds were filled. Enclosures were set up in four rows of four enclosures within each of the two ponds (i.e., 16 enclosures per pond), arranged with one of each of the four vegetation densities per row, in a randomized order. Row number and pond were treated as random blocking variables in the analysis. Enclosures were spaced approximately 2 m apart along the deepest portion of the pond, were placed 3 m from shore to ensure all enclosures were located on level substrate at similar depth (± 0.05 m) and were situated far enough apart so that they were unlikely to affect adjacent enclosures.

Vegetation was simulated in the enclosures by affixing 3-mm-diameter yellow twisted polypropylene rope to 1-m² mats of galvanized steel, hexagonal, poultry netting and then securing the poultry netting to the substrate with garden staples. For continuity, control treatments also received poultry netting mats even though no simulated vegetation was attached. Once the enclosures were built and vegetation mats installed, the ponds were filled to a depth of 0.6 m (± 0.05 m) with water from Lake Carl Blackwell (filtered through an 800- μ m-mesh sock). This left approximately 20 cm of the plastic mesh sides of each enclosure extending above the water surface. The ponds held water (but no fish) prior to enclosure construction and were only partially drained during enclosure construction such that invertebrates were present at the time of refilling and rapidly colonized the enclosures (observed inside the enclosures before the 1-d filling procedure was complete). We gave invertebrates in the pond 1 week after refilling to adjust to the change in water level and further disperse into enclosures. After 1 week, three juvenile Largemouth Bass (mean = 40 mm TL, SD = 1.9) were stocked in each enclosure at a density of 3 fish/m². This fish density is lower than the density reported for complex vegetated habitats (i.e., up to 15 fish/m²; Hayse and Wissing 1996),

but higher than typical lake-wide density estimates (0.02–0.53 fish/m²; Bettoli et al. 1992, 1993; Maceina et al. 1993; Valley and Bremigan 2002; Strakosh et al. 2009) and likely reflects a density that would be common for the middle vegetation densities used in our experiment (i.e., 50 or 250 stems/m²). Each Largemouth Bass was given a different fin clip so the three fish in the same enclosure could be individually identified. Enclosures were also stocked with juvenile Bluegills (30 fish/m²) as a source of fish prey that were approximately 30% (range, 25–35%) of the length of juvenile Largemouth Bass. Juvenile Largemouth Bass can eat prey as large as 50% their length (Johnson and Post 1996), but availability of fish 30% of predator length is required to sustain piscivory (Post 2003). All prey fish were sufficiently large to be retained by the 3.3-mm-bar-mesh enclosure material. A density of 30 Bluegills/m² was chosen to provide adequate numbers of prey (10 prey fish per predator) while providing a realistic density for vegetated habitats (0.2 to 108 age-0 Bluegills/m²; Hayse and Wissing 1996). Both juvenile Largemouth Bass and Bluegills were obtained from adjacent ponds stocked with adult fish earlier in the spring.

All enclosures were sampled twice per week from a boat (to minimize sediment disturbance and maintain similar turbidity levels throughout the experiment) within a 3-h period after sunrise using a frame net. Enclosures in each pond were sampled on different days (i.e., one pond was sampled on Mondays and Thursdays, the other on Tuesdays and Fridays). The frame net was built to specifically fit the 1-m² enclosures to maximize sampling efficiency and was constructed from 3.8-cm-diameter PVC pipe that formed a 1-m² square frame with 1-m long handles. A 1-m² net with 1.6-mm bar mesh was attached to the frame. To sample, the leading edge of the frame was submerged to the bottom along one enclosure side and was then run across the bottom of the enclosure to the opposite side where it was raised to the surface while keeping the net bottom in contact with the far side. The frame net was highly effective, as most fish were captured on the first sampling event, but to ensure thoroughness, frame-net samples were repeatedly taken until three consecutive samples came back empty. All sampled Largemouth Bass were measured (mm TL) during every sample (i.e., twice per week), and their stomach contents were collected via gastric lavage (Culp et al. 1988) once per week on every other sample time to avoid undue handling stress. Gastric lavage was performed with a 3-mL veterinary-grade syringe with a dulled tip filled with water that was placed in the stomach via the esophagus. The plunger was depressed, and stomach contents were flushed onto a 300- μ m-mesh screen and preserved in vials with 70% ethanol to be analyzed later. Preliminary data indicated the method was 100% effective at removing all prey items and resulted in minimal mortality (<2% at 24 h postlavage).

To maintain consistent levels of prey fish, sampled Bluegills were counted and evaluated for appropriate size (25–35% of Largemouth Bass size), and fish were replaced or added to each enclosure after sampling (i.e., twice per week) to ensure the density was maintained at 30 appropriately sized fish/m². Zooplankton and macroinvertebrate densities were not adjusted because the openings in the enclosure (7 mm in diameter) allowed recolonization from the pond (invertebrates were observed in the enclosures throughout the experiment). Any dead Largemouth Bass that were observed were replaced with similar-sized fish from an adjacent pond, where bass spawned earlier in the spring (18% of Largemouth Bass died during the experiment; all but one died during the first 2 weeks of the experiment when the fish were small and still primarily insectivorous). Trials were conducted for 5 weeks, at which time Largemouth Bass in all treatments had become primarily piscivorous.

Data analysis.—Stomach contents from each Largemouth Bass were identified to the lowest possible taxonomic level. Each diet item was measured using the imaging software, ImageJ. The length (mm) of each diet item was converted to a predigested mass (mg) using organism-specific length–mass relationships published by previous studies (Smock 1980; Culver et al. 1985; Benke et al. 1999). After conversion to mass, diet items were classified into four broad taxonomic groups: fish, insects, zooplankton, and other prey types. The “other” group included rare diet items such as an amphipod, a bivalve, and a spider. Unidentifiable items were excluded from predigested mass analysis (<1% of items could not be assigned to a prey category).

The onset and degree of piscivory of sampled Largemouth Bass was identified using three metrics: (1) the mean mass of fish prey found in diets on each date, (2) the number of surviving Bluegills per Largemouth Bass sampled in enclosures on each date (i.e., dead Largemouth Bass were not counted), and (3) the number of fish from each enclosure with piscine prey in their stomach. Because the number of surviving Bluegills were sampled twice per week, the duration between samples alternated between 3 d and 4 d. Initial attempts to standardize by day made it obvious there was no good way to account for the difference in duration (all methods appeared to overly penalize shorter or longer samples once the correction for sample duration was applied). As such, only the 4-d sampling intervals were used in the analysis to ensure a consistent and unbiased sample duration (i.e., number of surviving Bluegills was only analyzed once per week even though Bluegills were counted and densities adjusted twice per week). These three metrics of piscivory provided different balances between direct measurements (i.e., actual stomach contents confirming piscivory) that suffer from temporal bias (only able to detect predation events

occurring within a few hours of the sample) with less direct, but less temporally biased metrics (e.g., number of surviving Bluegills). Largemouth Bass growth was analyzed as cumulative absolute growth (mm TL) from the start of the experiment. Growth was summed across individuals in cases where replacement fish were used because similar-sized replacements were always selected.

Vegetation density, date, and their interaction were tested as fixed effects within generalized linear statistical models to predict piscivory (mass of prey types in diets, ratio of surviving Bluegills to Largemouth, and frequency of piscivorous diets) and Largemouth Bass growth (cumulative absolute growth rate). The model testing the predigested mass of fish prey in the diets was analyzed with multivariate ANOVA in SAS proc Mixed (Khattree and Naik 1999; SAS Institute 2014) so that the mass of all prey types (fish, insect, zooplankton, and other) could be included in the model to document changes in prey use over time. The models testing the ratio of surviving Bluegills to Largemouth Bass and Largemouth Bass growth were tested with SAS Proc Mixed (SAS Institute 2014). The model testing the frequency of piscivorous diets was tested with SAS Proc Glimmix (SAS Institute 2014) specifying a binomial data distribution. For all models, pond and rows within pond were used as random blocking variables in the analysis. For response variables taken at the enclosure level (i.e., number of Bluegills per predator remaining, frequency of piscivorous diets), enclosures were specified as subjects on which repeated measurements were taken. For metrics measured on individual fish (i.e., mass of prey types consumed and growth), Largemouth Bass (identified by fin clip) nested within enclosures were specified as subjects. Remaining density of Largemouth Bass was used as a random blocking variable in growth and piscivory models but not in the surviving number of Bluegills per predator model, as remaining Largemouth Bass was already accounted for in the number-of-Bluegills-per-predator metric. Response variables for the models testing mean predigested mass of prey items, number of remaining Bluegills per Largemouth Bass, and growth were $\ln(x + 1)$ transformed to normalize their residuals. When significant differences were detected, the Tukey–Kramer multiple comparison procedure was used to test for pairwise differences.

RESULTS

Total mass of the four different prey types consumed had a significant date \times taxa interaction ($F_{12, 921} = 4.64$, $P < 0.01$; Figure 1) indicating that different amounts of some taxa were eaten on different dates, but these differences in prey biomass did not differ between vegetation treatments (vegetation density was not significant, and there were no significant interactions involving vegetation

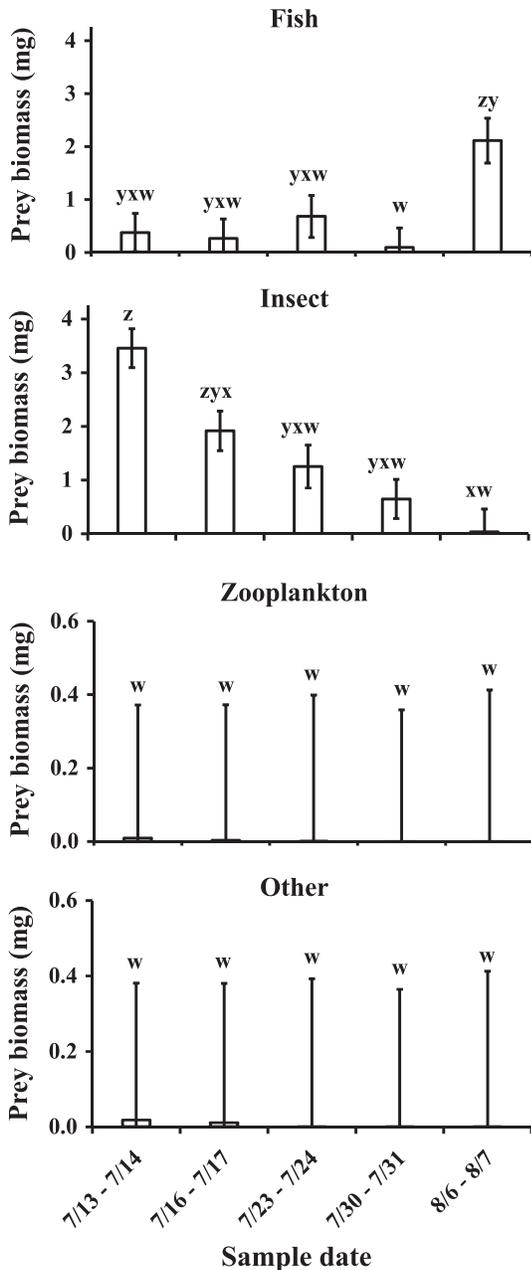


FIGURE 1. Mean predigested mass (mg; calculated from length-weight regression equations) of four taxonomic groups consumed by Largemouth Bass held in 1-m² enclosures with one of four different artificial vegetation densities. Diets were collected by gastric lavage once per week during the 5-week experiment. Letters represent Tukey-Kramer groupings across all four prey groups; letters not in common represent significant differences ($P < 0.05$); error bars represent ± 1 SE. Sample dates are given as month/day.

density). Largemouth Bass ate more insects than any other prey type on the first sampling date. The mass of insects consumed declined throughout the experiment such that it was significantly lower by the third week. By the final week of the experiment, significantly more fish prey were

consumed than any other prey type. The amount of zooplankton and the other prey consumed was consistently low throughout the experiment.

The number of surviving Bluegills per Largemouth Bass declined throughout the experiment across all treatments (date effect: $F_{4, 120} = 17.68, P < 0.01$), but there were still significant differences among vegetation densities (vegetation effect: $F_{3, 120} = 4.59, P < 0.01$; Figure 2; no date \times vegetation interaction: $F_{12, 120} = 0.94, P = 0.51$). Most of the available Bluegills survived each week in the medium stem density treatment (250 stems/m²) throughout the entire experiment, whereas in the other stem densities, including the control without vegetation, fewer than half the replenished Bluegills were observed on all sampling dates starting the second week of the experiment. The proportion of Largemouth Bass with Bluegills in their stomachs was highly variable (mean = 0.065, SD = 0.210) and did not differ among any of the vegetation-density treatments ($F_{3, 173} = 0.30, P = 0.83$) or sample dates ($F_{1, 173} = 0.04, P = 0.84$), and there was no treatment \times date interaction ($F_{3, 384} = 0.12, P = 0.95$).

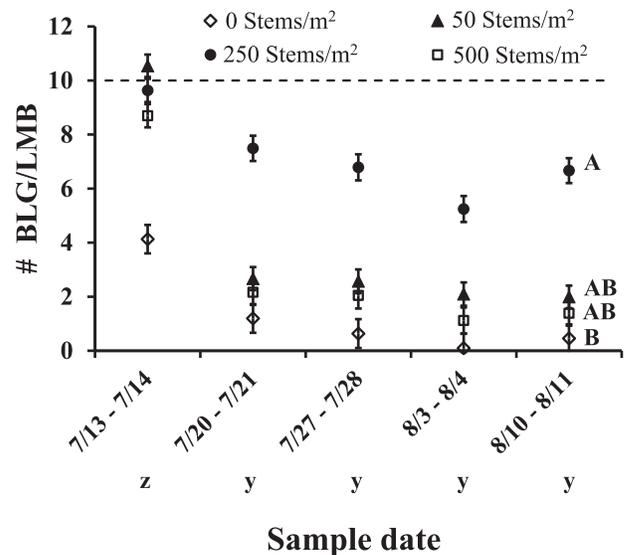


FIGURE 2. Mean number of surviving Bluegills (BLG) per Largemouth Bass (LMB) from nine sampling dates during an experiment testing the effects of vegetation stem density on piscivory by juvenile Largemouth Bass in 1-m² enclosures. The horizontal dashed line indicates expected number of Bluegills per Largemouth Bass if no Bluegills were consumed. Fish were sampled once during the week the experiment began and twice per week during the remaining weeks of the 5-week experiment, and Bluegills were added to bring total prey abundance to 30 at each sampling event. Lowercase letters below dates represent Tukey-Kramer groupings for significant date effects, and uppercase letters to the right of data series represent Tukey-Kramer groupings for significant stem-density effects; letters not in common represent significant differences ($P < 0.05$); error bars represent ± 1 SE. There was no significant date \times stem density interaction. Sample dates are given as month/day.

Cumulative absolute growth of Largemouth Bass was greater in the control, low vegetation, and high vegetation treatments than in the medium treatment (vegetation effect: $F_{3, 382} = 17.40$, $P < 0.01$; all Tukey–Kramer P -values ≤ 0.01 ; Figure 3). Growth was also higher in the control and high vegetation treatments than in the low vegetation treatment. Largemouth Bass growth increased during the experiment in all treatments ($F_{8, 382} = 44.04$, $P < 0.01$), and there was no vegetation density \times date interaction, indicating the observed vegetation-density effects on growth were consistent throughout the experiment (vegetation \times date: $F_{3, 382} = 0.61$, $P = 0.93$).

DISCUSSION

Managers often attempt to manipulate vegetation coverage to improve Largemouth Bass recruitment (Strakosh et al. 2005, 2009), but potential tradeoffs between predation refuge (Stahr and Shoup 2015) and growth (dense vegetation is hypothesized to delay ontogeny to piscivory in Largemouth Bass: Bettoli et al. 1992; Miranda and Pugh 1997; and reduces growth of Bluegills: Gotceitas 1990; Theel and Dibble 2008) that occur when vegetation is manipulated have been poorly studied in this species. We found the ontogeny to piscivory for juvenile Largemouth Bass happened at approximately the same time when stem densities ranged from 0 to 500 stems/m² (i.e., mass of Bluegills in stomach contents increased and the surviving number of Bluegills per predator decreased around the same sample dates in all vegetation-density treatments). Largemouth Bass in the medium stem density treatment

(250 stems/m²) did not significantly differ with respect to the temporal pattern for increased mass of Bluegills in the diet, but they ate considerably fewer of the available Bluegill prey (i.e., surviving number of Bluegills per Largemouth Bass) throughout the experiment, which suggests a less complete switch to piscivory occurred in that treatment. We also found Largemouth Bass grew slowest at this medium vegetation level. In natural systems with a wider range of piscine prey sizes, the difference in piscivory between the medium vegetation density and other densities could be even more pronounced due to the observed growth differences (i.e., as faster-growing predators gain a size advantage over a greater proportion of the prey community, even more piscine prey, which slower-growing predators may not be able to use, would be available to them; Phillips et al. 1995; Post 2003). Ultimately, this more complete shift to piscivory and the related increase in growth that occurred at 0, 50, and 500 stems/m² should allow juvenile Largemouth Bass to accumulate the amount of lipids necessary for overwinter survival (Ludsin and DeVries 1997). Therefore, medium vegetation densities might lead to reduced recruitment of Largemouth Bass, but this would require further study.

Of the three metrics of piscivory used in our study, only one (surviving Bluegills per predator) differed between vegetation densities. Both the mass of Bluegills found in stomachs and the number of Largemouth Bass with Bluegills in their diet are dependent upon sampling Largemouth Bass shortly after they ate. Less than 50% of ingested fish prey remains in the stomach of juvenile Largemouth Bass after 2 h of digestion at 26°C (Hayward and Bushmann 1994). The number of surviving Bluegills per predator is a less temporally sensitive metric that we used to measure any piscivorous feeding that occurred over the 4-d sampling interval, but it is a less direct metric of piscivory (i.e., other factors could lead to Bluegill mortality besides Largemouth Bass predation). The indirect nature of the number-of-Bluegills-per-predator metric would only be a problem if other sources of Bluegill mortality varied with treatment, which would not be expected. It is possible the more direct piscivory metrics would have had similar treatment effects as did the Bluegills per predator metric if diet sampling could have been conducted more than once per week, but this would have been stressful for the Largemouth Bass. Given the infrequent sampling, it is not surprising that piscivorous events were only rarely observed. Further, the percentage of empty stomachs increased more than sixfold (from <5% in the first week to over 30% in the final 2 weeks) during the experiment. The frequency of empty stomachs increases with the extent of piscivory for most fish (Arrington et al. 2002; Wallus and Simon 2008; Vinson and Angradi 2011), which not only further validates the patterns observed for the number of Bluegills per predator metric but also

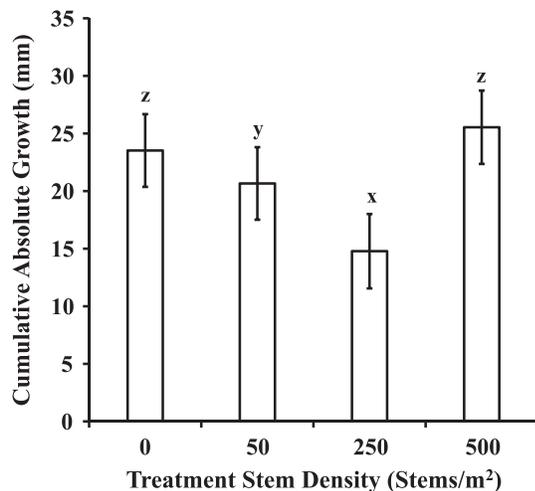


FIGURE 3. Mean cumulative absolute growth of individual Largemouth Bass during a 5-week experiment conducted in 1-m² enclosures with four vegetation stem density treatments. Letters represent Tukey–Kramer groupings; letters not in common represent significant differences ($P < 0.05$); error bars represent ± 1 SE.

explains the lack of statistical power in the metrics that relied on stomach contents. The increased frequency of empty stomachs and the tight coupling of patterns between the number of surviving Bluegills per predator and weight gain by Largemouth Bass across treatments (greater growth is indicative of the ontogeny to a piscivorous diet: Keast and Eadie 1985; Olson 1996; Ludsin and DeVries 1997) suggests that the number-of-surviving-Bluegills-per-predator metric was the most sensitive measure of piscivory.

We found no effect of dense vegetation (i.e., 500 stems/m²) on the timing or extent of piscivory relative to the vegetation-free treatment, which is in conflict with field studies that suggest dense vegetation slows the ontogeny to piscivory in Largemouth Bass (Bettoli et al. 1992; Miranda and Pugh 1997). Several factors could have caused these differences between field studies and the current study. First, the field studies did not include control over the prey fish community, which changed over the course of the study (Bettoli et al. 1993). Thus, changes detected in the field studies could be driven by changes in the piscine prey community (i.e., to less desirable or more difficult-to-capture fish prey types, or simply to lower piscine prey densities overall) rather than by vegetation density directly. Second, reductions in vegetation typically result in lower invertebrate abundance (Wiley et al. 1984; Cyr and Downing 1988; Rennie and Jackson 2005), so field studies with vegetation reductions may have had lower abundance of alternate (invertebrate) prey, forcing Largemouth Bass to switch to piscivory due to lack of available invertebrates. Third, the use of a single fish species as the prey (Bluegill) in the current study may have driven the patterns we observed. Those patterns may not have been observed in previous field research where diverse prey assemblages existed. If multiple fish prey species had been available in our study, the lower degree of piscivory and corresponding reduced growth in Largemouth Bass observed in the medium vegetation density may not have been as profound because different piscine prey types may be easier to capture at different vegetation densities, potentially providing Largemouth Bass alternatives when Bluegills become difficult to capture (e.g., as has been observed for adult Largemouth Bass: Savino and Stein 1989). Lastly, although enclosures provided control over some of the environmental conditions that would otherwise confound the experimental design, this control also led to some unnatural conditions that could have caused differences between our enclosure results and what happens in the field. For example, Bluegills were confined within the enclosure such that they could not change habitats to avoid predation. Similarly, juvenile Largemouth Bass could not select habitats outside of the enclosure, so they were forced to forage in what may have been a sub-optimal vegetation density, whereas in field studies they

are free to choose areas of lower or higher vegetation density, if such patches existed in their environment.

One possible explanation for the high degree of piscivory in the control, low, and high vegetation treatments but not the medium (250 stems/m²) vegetation treatment is that juvenile Largemouth Bass may use different predation techniques in different densities of vegetation, a phenomenon that has been described for adult Largemouth Bass (Savino and Stein 1982, 1989). Adult Largemouth Bass employ a searching technique at low densities of vegetation (<250 stems/m²). However, when their foraging rate is diminished by their inability to maneuver at higher vegetation densities (>250 stems/m²), they adopt a lie-in-wait strategy (Savino and Stein 1982). It is possible that there is a transitional phase for juvenile Largemouth Bass when vegetation is too dense to employ a searching technique yet not dense enough to provide enough concealment to effectively employ a lie-in-wait technique. A threshold of 516 stems/m² is needed before Bluegills specifically select a patch of vegetation as a predation refuge (Gotceitas and Colgan 1989). Therefore, it is possible that the searching ability of juvenile Largemouth Bass was slowed in the medium treatment (250 stems/m², the density above which adults adopt an ambush strategy: Savino and Stein 1982), whereas juvenile Bluegills were still predator-cautious at this density (likely hiding rather than searching for food as they might do at stem densities > 516 stems/m²: Gotceitas and Colgan 1989), making an ambush strategy less effective at medium vegetation density than at higher vegetation densities. In high vegetation densities (500 stems/m²), Bluegills may perceive less predation risk and resume their own foraging behaviors, which would increase their encounter rates with juvenile Largemouth Bass that use a lie-in-wait strategy within the dense vegetation. Our experimental design precluded the possibility of collecting behavioral data that would be able to address that hypothesis, so further research is needed to determine the mechanisms underlying this pattern of low piscivory at 250 stems/m².

Different results might have been found if different fish prey were used in this experiment because different prey can exhibit different antipredatory behaviors (Schramm and Zale 1985). Juvenile piscivorous Largemouth Bass foraged more efficiently on Fathead Minnows *Pimephales promelas* at moderate (125 stems/m²) vegetation densities than in open water (Broderius 2015). The different pattern of consumption between the current study and previous laboratory work suggests that prey community and habitat complexity interact to effect ontogeny to piscivory in juvenile Largemouth Bass. Previous experiments with adult Largemouth Bass found the effects of vegetation density were different when they fed on Fathead Minnows than when they fed on Bluegills (Savino and Stein 1989), so the same might be true of juvenile Largemouth Bass.

Our results in combination with previous lab (Broderius 2015) and field (Bettoli et al. 1992; Miranda and Pugh 1997) studies indicate that vegetation density is an important factor determining the ontogeny to piscivory in juvenile Largemouth Bass, but predation and the transition to piscivory in particular are complex processes. In fact, the ontogeny to piscivory should be viewed as a part of a continuum of prey selection whereby the majority of prey selected is initially invertebrates and subsequently becomes dominated by piscine prey. Neither the preontogeny nor postontogeny period are typically characterized by fish eating exclusively one or the other prey type. Individual fish may consume both prey types throughout a large portion of their life. Further, a host of environmental conditions besides vegetation density and complexity likely influence predator-prey interactions and ultimately prey selection. For example, the piscine prey community assemblage may be an important factor that interacts with vegetation density (Savino and Stein 1989; results of the current study versus Broderius 2015) and alters the ontogeny to piscivory. Further research is needed to determine how these other factors interact with vegetation density to affect the ontogeny to piscivory in juvenile Largemouth Bass. However, it is clear that structural complexity can affect the extent of piscivory in young of year Largemouth Bass, so changes in vegetation levels in lakes and reservoirs may affect Largemouth Bass year-class strength. Therefore, managers attempting to improve Largemouth Bass recruitment should consider the role vegetation coverage plays in this process. Our results indicate that moderate vegetation densities (250 stems/m²) may not be optimal for Largemouth Bass recruitment, but we did not find any evidence that dense vegetation delays the ontogeny of piscivory for this species.

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REFERENCES

- Arrington, D. A., K. O. Winemiller, W. F. Loftus, and S. Akin. 2002. How often do fishes "run on empty"? *Ecology* 83:2145–2151.

- Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* 18:308–343.
- Bettoli, P. W., M. J. Maceina, R. L. Noble, and R. K. Betsill. 1992. Piscivory in Largemouth Bass as a function of aquatic vegetation abundance. *North American Journal of Fisheries Management* 12:509–516.
- Bettoli, P. W., M. J. Maceina, R. L. Noble, and R. K. Betsill. 1993. Response of a reservoir fish community to aquatic vegetation removal. *North American Journal of Fisheries Management* 13:110–124.
- Breck, J. E. 1993. Foraging theory and piscivorous fish: are forage fish just big zooplankton? *Transactions of the American Fisheries Society* 122:902–911.
- Broderius, C. R. 2015. The effect of vegetation on the ontogeny to piscivory in juvenile Largemouth Bass. Master's thesis. Oklahoma State University, Stillwater.
- Brown, G. E., V. J. LeBlanc, and L. E. Porter. 2001. Ontogenetic changes in the response of Largemouth Bass (*Micropterus salmoides*, Centrarchidae, Perciformes) to heterospecific alarm pheromones. *Ethology* 107:401–414.
- Cargnelli, L. M., and M. R. Gross. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (Bluegill: *Lepomis macrochirus*). *Canadian Journal of Fisheries and Aquatic Sciences* 53:360–367.
- Culp, J. M., I. Boyd, and N. E. Glozier. 1988. An improved method for obtaining gut contents from small, live fishes by anal and stomach flushing. *Copeia* 1988:1079–1082.
- Culver, D. A., M. M. Boucherle, D. J. Bean, and J. W. Fletcher. 1985. Biomass of freshwater crustacean zooplankton from length-weight regressions. *Canadian Journal of Fisheries and Aquatic Sciences* 42:1380–1390.
- Cummins, K. W., and J. C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. *Mitteilung Internationale Vereinigung fuer Theoretische und Angewandte Limnologie* 18:1–158.
- Cyr, H., and J. A. Downing. 1988. Empirical relationships of phytomacrofaunal abundance to plant biomass and macrophyte bed characteristics. *Canadian Journal of Fisheries and Aquatic Sciences* 45:976–984.
- Dibble, E. D., and S. L. Harrel. 1997. Largemouth Bass diets in two aquatic plant communities. *Journal of Aquatic Plant Management* 35:74–78.
- Galarowicz, T. L., and D. H. Wahl. 2005. Foraging by a young-of-the-year piscivore: the role of predator size, prey type, and density. *Canadian Journal of Fisheries and Aquatic Sciences* 62:2330–2342.
- Garvey, J. E., T. P. Herra, and W. C. Leggett. 2002. Protracted reproduction in sunfish: the temporal dimension in fish recruitment revisited. *Ecological Applications* 12:194–205.
- Garvey, J. E., and R. A. Stein. 1998. Linking Bluegill and Gizzard Shad prey assemblages to growth of age-0 Largemouth Bass in reservoirs. *Transactions of the American Fisheries Society* 127:70–83.
- Gotceitas, V. 1990. Variation in plant stem density and its effects on foraging success of juvenile Bluegill sunfish. *Environmental Biology of Fishes* 27:63–70.
- Gotceitas, V., and P. Colgan. 1987. Selection between densities of artificial vegetation by young Bluegills avoiding predation. *Transactions of the American Fisheries Society* 116:40–49.
- Gotceitas, V., and P. Colgan. 1989. Predator foraging success and habitat complexity: quantitative test of the threshold hypothesis. *Oecologia* 80:158–166.
- Graeb, B. D., M. T. Mangan, J. C. Jolley, D. H. Wahl, and J. M. Dettmers. 2006. Ontogenetic changes in prey preference and foraging ability of Yellow Perch: insights based on relative energetic return of prey. *Transactions of the American Fisheries Society* 135:1493–1498.

- Graeb, B. D. S., T. Galarowicz, D. H. Wahl, J. M. Dettmers, and M. J. Simpson. 2005. Foraging behavior, morphology, and life history variation determine the ontogeny of piscivory in two closely related predators. *Canadian Journal of Fisheries and Aquatic Sciences* 62:2010–2020.
- Hambright, K. D. 1991. Experimental analysis of prey selection by Largemouth Bass: role of predator mouth width and prey body depth. *Transactions of the American Fisheries Society* 120:500–508.
- Hayse, J. W., and T. E. Wissing. 1996. Effects of stem density of artificial vegetation on abundance and growth of age-0 Bluegills and predation by Largemouth Bass. *Transactions of the American Fisheries Society* 125:422–433.
- Hayward, R. S., and M. E. Bushmann. 1994. Gastric evacuation rates for juvenile Largemouth Bass. *Transactions of the American Fisheries Society* 123:88–93.
- Houde, E. D. 1987. Fish early life dynamics and recruitment variability. Pages 17–29 in R. D. Hoyt, editor. 10th annual larval fish conference. American Fisheries Society, Symposium 2, Bethesda, Maryland.
- Huskey, S., and R. Turingan. 2001. Variation in prey-resource utilization and oral jaw gape between two populations of Largemouth Bass, *Micropterus salmoides*. *Environmental Biology of Fishes* 61:185–194.
- Johnson, J. M., and D. M. Post. 1996. Morphological constraints on intracohort cannibalism in age-0 Largemouth Bass. *Transactions of the American Fisheries Society* 125:809–812.
- Keast, A., and J. M. Eadie. 1985. Growth depensation in year-0 Largemouth Bass: the influence of diet. *Transactions of the American Fisheries Society* 114:204–213.
- Khattree, R., and D. Naik. 1999. Multivariate data reduction and discrimination with SAS software. SAS Institute, Cary, North Carolina.
- Ludsin, S. A., and D. R. DeVries. 1997. First-year recruitment of Largemouth Bass: the interdependency of early life stages. *Ecological Applications* 7:1024–1038.
- MacArthur, R. H., and E. R. Pianka. 1966. On optimal use of a patchy environment. *American Naturalist* 100:603–609.
- Maceina, M. J., S. J. Rider, and D. R. Lowery. 1993. Use of a catch-depletion method to estimate population density of age-0 Largemouth Bass in submersed vegetation. *North American Journal of Fisheries Management* 13:847–851.
- Miranda, L., and R. Muncy. 1989. Bioenergetic values of shads and sunfishes as prey for Largemouth Bass. *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies* 43:153–163.
- Miranda, L. E., and L. L. Pugh. 1997. Relationship between vegetation coverage and abundance, size, and diet of juvenile Largemouth Bass during winter. *North American Journal of Fisheries Management* 17:601–610.
- Mittelbach, G. G. 1981. Patterns of invertebrate size and abundance in aquatic habitats. *Canadian Journal of Fisheries and Aquatic Sciences* 38:896–904.
- Mittelbach, G. G., and L. Persson. 1998. The ontogeny of piscivory and its ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1454–1465.
- Olson, M. H. 1996. Ontogenetic niche shifts in Largemouth Bass: variability and consequences for first-year growth. *Ecology* 77:179–190.
- Olson, M. H., S. R. Carpenter, P. Cunningham, S. Gafny, B. R. Herwig, N. P. Nibbelink, T. Pellett, C. Storlie, A. S. Trebitz, and K. A. Wilson. 1998. Managing macrophytes to improve fish growth: a multi-lake experiment. *Fisheries* 23(2):6–12.
- Parkos, J. J., and D. H. Wahl. 2010. Influence of body size and prey type on the willingness of age-0 fish to forage under predation risk. *Transactions of the American Fisheries Society* 139:969–975.
- Phillips, J. M., J. R. Jackson, and R. L. Noble. 1995. Hatching date influence on age-specific diet and growth of age-0 Largemouth Bass. *Transactions of the American Fisheries Society* 124:370–379.
- Post, D. M. 2003. Individual variation in the timing of ontogenetic niche shifts in Largemouth Bass. *Ecology* 84:1298–1310.
- Reid, S. M., M. G. Fox, and T. H. Whillans. 1999. Influence of turbidity on piscivory in Largemouth Bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Sciences* 56:1362–1369.
- Rennie, M. D., and L. J. Jackson. 2005. The influence of habitat complexity on littoral invertebrate distributions: patterns differ in shallow prairie lakes with and without fish. *Canadian Journal of Fisheries and Aquatic Sciences* 62:2088–2099.
- Santucci, V. J., and D. H. Wahl. 2003. The effects of growth, predation, and first-winter mortality on recruitment of Bluegill cohorts. *Transactions of the American Fisheries Society* 132:346–360.
- SAS Institute. 2014. Base SAS 9.4 procedures guide: statistical procedures. SAS Institute, Cary, North Carolina.
- Savino, J. F., and R. A. Stein. 1982. Predator-prey interaction between Largemouth Bass and Bluegills as influenced by simulated, submersed vegetation. *Transactions of the American Fisheries Society* 111:255–266.
- Savino, J. F., and R. A. Stein. 1989. Behavioural interactions between fish predators and their prey: effects of plant density. *Animal Behaviour* 37:311–321.
- Schramm, H. L., and A. V. Zale. 1985. Effects of cover and prey size on preferences of juvenile Largemouth Bass for Blue Tilapia and Bluegills in tanks. *Transactions of the American Fisheries Society* 11:725–731.
- Shoup, D. E., M. A. Nannini, and D. H. Wahl. 2012. The effect of vegetation density on juvenile Bluegill diet and growth. *Journal of Freshwater Ecology* 27:199–209.
- Smock, L. A. 1980. Relationships between body size and biomass of aquatic insects. *Freshwater Biology* 10:375–383.
- Stahr, K. J., and D. E. Shoup. 2015. American water willow mediates survival and antipredator behavior of juvenile Largemouth Bass. *Transactions of the American Fisheries Society* 144:903–910.
- Stahr, K. J., and D. E. Shoup. 2016. The effects of macrophyte stem density and structural complexity on foraging return of invertivorous juvenile Largemouth Bass. *North American Journal of Fisheries Management* 36:788–792.
- Strakosh, T. R., J. L. Eitzmann, K. B. Gido, and C. S. Guy. 2005. The response of water willow *Justicia americana* to different water inundation and desiccation regimes. *North American Journal of Fisheries Management* 25:1476–1485.
- Strakosh, T. R., K. B. Gido, and C. S. Guy. 2009. Effects of American water willow establishment on density, growth, diet, and condition of age-0 Largemouth Bass in Kansas reservoirs. *Transactions of the American Fisheries Society* 138:269–279.
- Theel, H. J., and E. D. Dibble. 2008. An experimental simulation of an exotic macrophyte invasion and its influence on foraging behavior of Bluegill. *Journal of Freshwater Ecology* 23:79–89.
- Valley, R. D., and M. Bremigan. 2002. Effects of selective removal of Eurasian watermilfoil on age-0 Largemouth Bass piscivory and growth in southern Michigan Lakes. *Journal of Aquatic Plant Management* 40:79–87.
- Vinson, M. R., and T. R. Angradi. 2011. Stomach emptiness in fishes: sources of variation and study design implications. *Reviews in Fisheries Science* 19:63–73.
- Wallus, R., and T. P. Simon. 2008. Reproductive biology and early life history of fishes in the Ohio River drainage: Elasmobranchia and Centrarchidae, volume 6. CRC Press, Boca Raton, Florida.
- Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics* 15:393–425.
- Wiley, M. J., R. W. Gorden, S. W. Waite, and T. Powless. 1984. The relationship between aquatic macrophytes and sport fish production in Illinois ponds: a simple model. *North American Journal of Fisheries Management* 4:111–119.