Analyzing the shift to piscivory in age-0 largemouth bass using stable isotopes

Research Thesis

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By

Melissa Bittner

The Ohio State University
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Project Advisor: Dr. Elizabeth Marschall, Department of Evolution, Ecology, and Organismal Biology
Introduction

Most organisms spend a significant portion of their lives searching for, handling, and consuming food. An individual must weigh, among other factors, predation risks and available energy when foraging. Young individuals often are vulnerable to predators because of their size. They are also likely to have low fat reserves, which makes consistent foraging a necessity to avoid starvation. As size increases, the risk of mortality from predation or starvation tends to decrease. Larger individuals decrease their predation risk by outgrowing the handling capability of the predator, or increasing their ability to evade the predator (Lundvall et al. 1999, Werner 1986, Dixon and Baker 1988). Large individuals are better able to outcompete conspecifics and other species for limited food resources (Graham et al. 2007, Olsen et al. 1995, Ware 1978). Large size also increases an individual's ability to capture, handle, and consume large food items (Einfalt et al. 2015, Scharf et al. 2000). Given all the advantages of large size, individuals should favor activities that increase their growth rates.

Diet has been shown to influence growth rates, especially during larval and juvenile life stages when fast growth yields the greatest benefit in decreasing the risk of predation and starvation (Buckley 1979, Edozien and Switzer 1978, Nielsen 1992). While overall food consumption is important for growth, not all food provides the same energetic benefit. As a predator moves up the food chain, food becomes more energy dense (Bowen et al. 1995). This energetic gain may be what drives ontogenetic diet shifts in some species.

Ontogenetic diet shifts have been well studied in fish. In many species, this is a shift from eating smaller prey items, such as zooplankton and macroinvertebrates, to
eating fish. Body size, prey availability, and environmental conditions interact to
determine the timing of this shift. A number of studies have shown that body length
constrains an individual’s ability to be piscivorous (Wu and Culver 1992, Mittelbach and
Persson 1998, Persson and Brönmark 2002). Both mouth gape and swimming ability
are positively correlated with body length and, consequently, have been shown to
positively correlate with the likelihood that an individual is piscivorous (Karpouzi and
Stergiou 2003). However, body size is not the single determinant of piscivory. In
largemouth bass in northern lakes, early hatch dates tend to correlate to early shifts to
piscivory (Post 2003). Both prey density (Wu and Culver 1992, Galarowicz and Wahl
2005) and water temperature (Olson 1996) have been shown to play a role in the shift
to piscivory. The relative effect of each of these factors on the shift to piscivory is highly
dependent on the study system. This makes generalizations on the timing of
ontogenetic diet shifts in fish difficult, and increases the need for system-specific
studies.

Our aim is to examine the ontogenetic shift to piscivory of juvenile largemouth
bass (Micropterus salmoides) in two Ohio reservoirs. Our study systems, Burr Oak Lake
and Deer Creek Lake, maintain viable populations of largemouth bass. Generally, adult
largemouth bass from Burr Oak Lake reach larger adult body lengths than their counter
parts in Deer Creek Lake (Ohio Division of Wildlife, unpublished data). However, a
study has shown that first-year growth rates in Deer Creek Lake largemouth bass
surpass those in Burr Oak Lake (E.A. Marschall, unpublished data). These differences
do not appear to be correlated with water temperature or zooplankton density (E.A.
Marschall, unpublished data). This study is focused on examining the incorporation of
zooplankton, macroinvertebrates, and larval fish into juvenile largemouth bass diets in each reservoir over time. In this way, we aim to determine if timing of first-year diet shifts are playing a role in the growth-rate differences between largemouth bass in Burr Oak Lake and Deer Creek Lake.

To estimate timing of ontogenetic diet shifts, we needed to distinguish long-term dietary changes from day-to-day fluctuations. Traditional gut content analyses provide only short-term information and depend on the ability to identify stomach contents. Stable isotopes have proven to be a reliable source of long-term dietary information across many taxa (Knoff et al. 2008, Hopkins 2012, Hobson and Clark 1992, Angela et al. 2016). Carbon and nitrogen are the two primary isotopes used for dietary stable isotope analyses. Carbon can be used to distinguish between primary producers, such as C3 and C4 plants (DeNiro and Epstein 1978). In aquatic systems, carbon has been shown to differentially label allochthonous from autochthonous inputs (France 1996, Peterson and Fry 1987). Nitrogen stable isotopes have been used by researchers to assign relative feeding position within a food chain (Vander Zanden et al. 1997, Post 2002). Lighter nitrogen isotopes are more readily metabolized, leading to an enrichment in heavier nitrogen isotopes in higher trophic levels (Gannes et al. 1997, Mill et al. 2007). Combining the information from carbon and nitrogen stable isotopes, we aim to track changes in diet of juvenile largemouth bass.

In this study, we will be using stable isotopes to estimate timing of the shift to piscivory in juvenile largemouth bass from Burr Oak Lake and Deer Creek Lake. To better estimate the timing of the shift to piscivory, we gathered baseline isotope measurements for the potential prey of juvenile largemouth bass: zooplankton, benthic
macroinvertebrates, and larval fish. We used these baseline measurements in conjunction with juvenile largemouth bass isotope measurements to determine changes in diet over time in both reservoirs. Diets heavier in fish will be categorized as having higher abundances of heavy nitrogen isotopes and having carbon isotope ratios that resemble the baseline larval fish measurements. In both reservoirs, we expect to see a positive correlation between nitrogen isotope ratios and length, since larger body size would allow for the capture and consumption of fish. We also expect to see an earlier shift to piscivory in the largemouth bass from Deer Creek Lake, correlating to the faster growth rates observed in this reservoir. Results of this study could have management implications. Understanding how, and if, the timing of the shift to piscivory plays a significant role in the growth of largemouth bass in these reservoirs could allow for managers to control system conditions to achieve desired results.

**Materials and Methods**

**Sample Collection**

To test our hypotheses, we collected zooplankton, macroinvertebrates, larval prey fish, and juvenile largemouth bass from Deer Creek Lake and Burr Oak Lake. These reservoirs were selected out of six reservoirs included in a previous pilot study because they displayed drastic differences in primary productivity, surrounding land-use, and patterns in juvenile largemouth bass growth rates (E.A. Marschall, unpublished data). Deer Creek Lake has a surface area of 526 ha, and is surrounded by agriculture,
while Burr Oak Lake has a forested watershed and encompasses 270 ha. Deer Creek Lake has much higher levels of primary productivity than Burr Oak Lake.

Sampling sites pre-determined from the Ohio Department of Natural Resources standard monitoring protocols were used for the collection of zooplankton, macroinvertebrates, larval prey fish, and juvenile largemouth bass from the littoral zone of the reservoirs. The reservoirs were split into “upper”, “middle”, and “lower” sections; at least one sampling site from each section was randomly chosen on each sampling date. Samples were collected weekly from May through August 2014 in the littoral zone. Age-0 juvenile largemouth bass were collected via electrofishing. Otoliths were extracted from juvenile largemouth bass for aging. Zooplankton were collected by vertical tow with a 0.5-m net with 64-um mesh. Macroinvertebrates were caught by a hand-held net. Larval prey fish species were collected with a 1x2-m neuston net with 500-um mesh. Samples were brought back to the lab for processing and stored immediately in a freezer at -80°C.

Sample Processing

A total of 182 juvenile largemouth bass, 24 zooplankton, 19 macroinvertebrate, and 14 larval fish samples were submitted for stable carbon and nitrogen isotope analyses. For each of the 9 sampling dates in Deer Creek Lake and the 10 sampling dates in Burr Oak Lake, we selected largemouth bass across the range of sizes from the total sample from that week. Muscle tissue samples were submitted for juvenile largemouth bass, and whole organism samples were submitted for zooplankton, macroinvertebrates, and larval prey fish. We aimed for a minimum wet sample weight of
200 mg to meet the 1.6-mg dry weight requirement. Zooplankton and macroinvertebrate samples were not separated by species; however, species found in each macroinvertebrate sample were identified and the data recorded.

**Stable Isotope Analysis**

Stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope ratios are determined by comparing sample ratios to a standard (equation 1) (Jardine et al. 2003),

$$
\delta_{\text{heavy}X} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
$$

where $\delta_{\text{heavy}X}$ is the amount of the heavier of the two isotopes of element $X$, $\delta_{\text{light}X}$ is the amount of the lighter of the two isotopes of element $X$, and $R = \frac{\text{heavy}X}{\text{light}X}$. The standards used were atmospheric nitrogen and Vienna Pee Dee Belemnite for carbon. All stable isotopes will be reported in this format.

$\delta^{15}\text{N}$ was used to estimate the trophic position of the bass. The trophic fractionation of nitrogen $^{15}\text{N}/^{14}\text{N}$ (i.e. the enrichment of $^{15}\text{N}$ between prey and predator) is assumed to be a 3-4‰ ($\pm$1‰) increase per trophic level (Post 2002, Vander Zanden and Rasmussen 2001). We used 3.4‰, as established by Vander Zanden and Rasmussen (2001), in their study of lake trout ($\text{Salvelinus namaycush}$):

$$\text{trophic position}_{\text{consumer}} = \frac{\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}}{3.4} + 3$$

Ideally, a baseline organism will incorporate temporal variation in system-wide $\delta^{15}\text{N}$ while remaining dependent upon either benthic or pelagic carbon. Because organisms that typically meet these requirements (i.e. long-lived, benthic, primary consumers) are
rare in our study systems, we were unable to use them for our baseline data. Instead, we used larval fish data, as they best incorporated temporal δ¹⁵N variation and reflected a single trophic level. We adjusted the equation, which was constructed to use a baseline primary consumer, to account for the higher trophic position (i.e. secondary consumer) of our baseline organism, larval fish.

Data Analysis

Results from a Bayesian isotope mixing model (IsotopeR; Hopkins and Ferguson 2012) were inconclusive; instead, we chose to use the results of our trophic position calculations to determine if there was a general trend towards piscivory over our sampling period in each reservoir. We used one-way ANOVAs to examine the correlation between collection date and trophic position. For this test, and all statistical analyses used, statistical significance was set at α = 0.05. To examine the temporal variation observed in the baseline isotope samples, we used a two-way ANOVA for carbon and nitrogen for each reservoir.

Results

Juvenile largemouth bass had δ¹⁵N values ranging from 7.54 to 19.62 and δ¹³C values from -30.41 to -24.54 (Table 1). Deer Creek Lake had consistently higher δ¹⁵N values than Burr Oak Lake. δ¹³C values were similar between reservoirs, with Burr Oak having only slightly more depleted values. δ¹³C values were found to be a poor indicator of primary carbon source due to exclusively littoral sampling.
In both reservoirs, stable isotope ratios of juvenile largemouth bass were influenced by collection date. In Burr Oak Lake, $\delta^{13}$C but not $\delta^{15}$N varied significantly between months (one-way ANOVA, $p < 0.001$), while in Deer Creek Lake both $\delta^{15}$N and $\delta^{13}$C varied significantly by month (one-way ANOVA, $p < 0.001$). In Deer Creek Lake we saw a positive correlation between both $\delta^{15}$N and $\delta^{13}$C, and collection month (Figure 1b).
To quantify a shift to piscivory, we calculated the trophic position for each individual largemouth bass from both reservoirs. Larval fish were used as a baseline, with an assumed trophic position of 3 correlating to their role as secondary consumers. Each whole-number increase in trophic position is equivalent to a single step within a food chain. Fractional trophic positions represent a diet that comprises more than one prey type of different trophic positions. In both reservoirs, we saw a weak positive correlation (Burr Oak Lake, $r^2 = 0.17$; Deer Creek Lake, $r^2 = 0.19$) between length and trophic position (Figure 2a,b), suggesting that length was not the sole determinant of diet.

We observed temporal variation in $\delta^{15}$N and $\delta^{13}$C for all prey types and in both systems. Direction of changes over the season differed between reservoirs and isotopes, but not among prey types (Figure 3a, b, c, d). Within some months, prey types within a reservoir had overlapping isotope ranges, making it impossible to use these isotopes to distinguish among prey types. Reservoir-specific $\delta^{15}$N data were examined for temporal patterns and differences among prey types using 2-way ANOVA. In Deer Creek Lake, $\delta^{15}$N varied by sample month (2-way ANOVA, $p = 0.013$), but not by prey type ($p = 0.220$) whereas in Burr Oak Lake, $\delta^{15}$N varied by both sample month (2-way ANOVA, $p < 0.001$) and prey type (2-way ANOVA, $p < 0.001$). Using the same analysis, $\delta^{13}$C was examined to identify temporal variation and differences between prey types. We found that $\delta^{13}$C varied only by sample month, and not prey type, in both Deer Creek Lake (2-way ANOVA, $p = 0.013$) and Burr Oak Lake (2-way ANOVA, $p = 0.018$).
Figure 2. Trophic positions of juvenile largemouth bass by length in (a) Burr Oak Lake and (b) Deer Creek Lake.

(a) Burr Oak

(b) Deer Creek
Figure 3. Isotope ratios of each of three prey types from samples in each of three months. (a) δ^{13}C in Burr Oak Lake, (b) δ^{13}C Deer Creek Lake, (c) δ^{15}N in Burr Oak Lake, and (d) δ^{15}N in Deer Creek Lake.

Discussion
Our trophic position results from both reservoirs supported our hypothesis that trophic position and body length would be positively correlated. However, we did not see a discrete shift from lower to higher trophic positions dependent on length, as has been observed in other studies of largemouth bass (Post 2003). Additionally, from our results, it appears that, in addition to length, there is another factor driving the shift to piscivory.

Collection date played a strong role in influencing $\delta^{15}N$ in Deer Creek Lake. The upward trend in the $\delta^{15}N$ of baseline prey measurements make it difficult to discern whether the significant increase in $\delta^{15}N$ observed in the juvenile largemouth bass is due to shifting baseline values, a shift in diet, or both.

In addition to the variation in $\delta^{15}N$ over time in Deer Creek Lake, there was also a discrete shift in carbon source from June to July and August. In June, the $\delta^{13}C$ for all prey types was low. Therefore, the depleted $\delta^{13}C$ values for largemouth bass in June could have been a reflection of consuming any of these prey types. However, in July and August, zooplankton $\delta^{13}C$ remains relatively depleted, while largemouth bass $\delta^{13}C$ ratios become enriched. This indicates that dependence on zooplankton as a source of food decreases over the summer for age-0 largemouth bass from Deer Creek.

It is clear that in both reservoirs, but Burr Oak Lake especially, fluxes in baseline stable isotope ratios occur throughout the summer. In order for future studies to accurately use stable isotopes to determine food web dynamics in these systems, a better understanding of baseline fluctuations is needed. The seasonality of stable isotope ratios within an aquatic system is not novel; zooplankton and macroinvertebrates were meant to serve as a measure of the flux in each system at the
level of a primary consumer. However, our results have shown that this is not a sufficient method for understanding and utilizing baseline isotope ratios in these systems. For this we need to better parse out the changes among ratios of primary producers and particulate organic matter (POM), and future studies should concentrate on obtaining these measurements.

The observed variation in nitrogen isotope ratios among prey types could be due to changes within the system, or experimental methodology. While both systems saw significant change in $\delta^{15}N$ over time, the direction of change was not uniform between them. The increase in $\delta^{15}N$ seen in Deer Creek Lake could be reflective of a constant enrichment in $^{15}N$ due to watershed inputs. Alternatively, it could also be due to an enrichment in $^{15}N$ due to preferential uptake of $^{14}N$ during denitrification, as suggested by Syvaranta and colleagues (2001). Over time, $^{15}N$ is more readily available and is incorporated by primary producers, and reflected in primary consumers. It is also possible that a combination of both forms of $^{15}N$ enrichment could be playing a role in seasonal increases in Deer Creek Lake. Conversely, the observed decrease in $\delta^{15}N$ in Burr Oak Lake has not been observed in other systems. It is possible that by varying sampling sites throughout the season we incorporated spatial variation without generating discrete endpoints, as previous studies have observed high spatial variation in littoral zones (Syvaranta 2001). However, more information on baseline nitrogen ratios in Burr Oak Lake is needed to draw further conclusions.

The increase in prey $\delta^{13}C$ over the summer months in both systems likely correlates to seasonal shifts in the dependence on allochthonous versus autochthonous carbon. Zooplankton have been shown to change their reliance on allochthonous
carbon over the winter to a reliance on autochthonous carbon throughout the summer (Grey and Jones 2001). The same would apply to non-carnivorous macroinvertebrates. The changing carbon isotope ratios in primary consumers would have a bottom up effect on the δ^{13}C of predatory species.

We are far from a complete understanding of food web dynamics of Burr Oak Lake and Deer Creek Lake. In order to utilize stable isotopes to their full potential, further studies should focus on parsing out the underlying isotopic variation within each system. Future experimental designs should focus on incorporating spatial variation while developing endpoints; this could be done by maintaining a set of sampling sites throughout all sampling dates. Additionally, collecting samples of particulate organic matter and phytoplankton could be useful in determining carbon source dependence in each system. Not every system is suitable for using stable isotope analyses; if future studies establish that variation within these reservoirs is too great to discern dietary relationships, other methods will have to be utilized.
Literature Cited:


