Evaluation of Supplemental Pellet Feeding and Threadfin Shad Addition on Stable Isotope Signature and Potential Influence on Fish Growth in Recreational Fishing Ponds

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Abstract: Pond enhancements such as adding pelleted feed or stocking threadfin shad (*Dorosoma petenense*) are sometimes used in the management of pond fisheries, but their relative impacts on growth and reproduction at multiple levels of the food web are not often fully evaluated. We used stable isotope analysis to indicate the contribution of pelleted feed to bluegill (*Lepomis macrochirus*) reproduction and growth, and ultimately to largemouth bass (*Micropterus salmoides*) growth in the presence and absence of threadfin shad via two different approaches: a pond experiment and sampling of established ponds. Bluegill growth and reproductive metrics increased with increased rates of pelleted feed provided. Bluegill nitrogen signature decreased with pelleted feed in the small pond experiment, suggesting feeding at a lower trophic level with increased feed. Largemouth bass nitrogen signature results showed similar trends to that of bluegill, although not statistically significant. In established ponds, pelleted feed appeared to alter the carbon isotopic signatures of both bluegill and largemouth bass independent of threadfin shad presence. These results suggest that adding pelleted feed to recreational largemouth bass-bluegill ponds can contribute to carbon flow, possibly through multiple trophic levels. The apparent lack of effect of pelleted feed on largemouth bass growth in established ponds is likely due to low and variable feeding rates and other management limitations found in more typical non-controlled field settings.

Key words: largemouth bass, bluegill, threadfin shad, stable isotopes, pelleted feed

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Small impoundments (hereafter "ponds") are abundant throughout the United States (Smith et al. 2002), and have many uses including recreation, irrigation, aesthetics, and livestock watering. The most common use of ponds is recreational fishing (USDA-SCS 1982, Dauwalter and Jackson 2005, Haley 2009). For ponds managed for recreational fishing, goals of small pond owners range from a consistent supply of fish for harvest (fish for consumption) or high catch rates to a focus on trophy fish production with little interest in fish harvest.

Largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) are the most common combination of predator and prey fish species stocked into ponds in North America (Wright and Kraft 2012). These two species can provide a balanced predator-prey combination (i.e., provide sustainable long-term harvest; Swingle 1950) under commonly-applied management techniques such as fertilization and selective harvest. More intensive management enhancements can lead to increased growth and perhaps density of preferred size fish (Haley et al. 2012). While there are many types of enhancements that can influence the production and catchability of largemouth bass and bluegill in ponds, stocking additional forage species (Wright and Kraft 2012) and providing pelleted feeds (Stone et al. 2012) are among the most common and direct approaches used by pond owners and managers.

Threadfin shad (*Dorosoma petenense*) stocking is one of the most common forage enhancements used in the southeastern United States to increase largemouth bass growth rate (Noble 1981, DeVries and Stein 1990, Wright and Kraft 2012). The addition of threadfin shad may increase bass production but can sometimes have a negative effect on bluegill production via competition for a common food source (DeVries and Stein 1990, DeVries and Stein 1992). In situations where such competition may occur, other management strategies may be used to mitigate the effects of competition, such as providing pelleted feed to enhance bluegill growth and/or abundance (Berger 1982, Murnyak et al. 1984, Porath and

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Hurley 2005). Pelleted feed can improve condition of stunted fish that may be limited by food availability (Schalles and Wissing 1976, Berger 1982, Murnyak et al. 1984, Porath and Hurley 2005) and can also attract and concentrate bluegill making them more vulnerable to angling (Berger 1982). While the addition of pelleted feeds can enhance the growth and catchability of adult bluegill, it is not clear that these effects can be transmitted up the food web to influence the growth of largemouth bass. Bluegill that are fed pelleted feed may have increased reproduction thereby producing more forage for largemouth bass or pellet-fed bluegill may have higher caloric density (Woodard et al. 2013) making them a more profitable prey resource.

Stable isotope analysis has been used to examine the sources and flow of materials and therefore energy through ecosystems (Peterson and Fry 1987, Jardine et al. 2003). The ratio of ^{12}C to ^{13}C in the tissues of consumers is a direct reflection of that ratio in its food. The ratio of ^{14}N to ^{15}N reflects the trophic position of an organism because ^{15}N is selectively retained with each trophic transfer up the foodweb (DeNiro and Epstein 1978, Jardine et al. 2003).

A controlled small pond experiment combined with sampling of established ponds with and without pelleted feed additions and threadfin shad allowed us to determine if the addition of pelleted feed could cause shifts in stable isotope signatures of bluegill and largemouth bass in a relatively controlled environment, as well as to explore whether similar patterns existed under realistic field conditions. Our objectives were to 1) quantify the potential effect of pelleted feed on bluegill growth and reproduction and largemouth bass growth, and 2) use stable isotope analysis to estimate the contribution of carbon and nitrogen isotopes from pelleted feed to bluegill and ultimately to largemouth bass when threadfin shad are present or absent.

Methods

Controlled Small Pond Experiment

Study Site and Design—We conducted an experiment in 10 0.1-ha earthen ponds (max depth = 1.5 m) at the E. W. Shell Fisheries Center, Auburn, Alabama, to determine the relationship between rate of feed addition and ratios of stable isotopes of carbon and nitrogen over a 5-month period. In February 2012, ponds were drained and refilled to ensure they did not contain fish before stocking. Ponds were refilled through 300-µm mesh filters to prevent larval fish from entering each pond and fertilized with 10-34-0 (N-P-K) liquid fertilizer after pond temperatures stabilized above 15.6° C. This stimulated a phytoplankton bloom that was maintained with fertilization at a targeted Secchi depth of 45–60 cm (Swingle and Smith 1939, Wright and Masser 2004). We stocked 250 bluegill (50–100 mm total length, TL) into each pond in early March 2012, and 25 largemouth bass (150–250 mm TL) were stocked in early April 2012. One of five feed rates (0, 1.3, 1.9, 3.2, and 4.4 kg ha^{-1} d^{-1}) of 3-mm diameter, 36% protein floating catfish feed was randomly assigned to each of the 10 ponds (2 ponds per feeding rate), and feeding began in mid-March. While there are feeds specifically designed for bluegill and largemouth bass, floating catfish food is the feed type commonly used in ponds and is readily consumed by bluegill (Woodard et al. 2013). Automatic fish feeders dispersed the given ration into each pond at 0800 h every day. Throughout the experiment, herbicide (diquat dibromide) was applied as needed to help prevent and treat excess aquatic vegetation growth. During times of application, dissolved oxygen (DO: mg L^{-1}) levels were monitored to assure that if DO did not fall below 5.0 mg L^{-1} which is the level considered stressful for long-term exposure for warmwater fishes (Boyd 1990). While herbicide applications can result in changes in water quality including declines in dissolved oxygen in ponds (Boyd 1990), dense growth of aquatic plants can also negatively affect water quality (Boyd 1990) and alter the predator-prey relationship between largemouth bass and bluegill (Cooper and Crowder 1979, Mittelbach and Chesson 1987). After careful consideration, we elected to treat the plants with herbicide rather than risk altering predator-prey dynamics among ponds.

Sampling Methods—Temperature and DO were measured at the surface in the deep end of each pond once per month from April to July using a Yellow Springs Instrument Model 51 B dissolved oxygen meter. Secchi depth was also measured (nearest cm) and surface water samples were collected for chlorophyll-*a* measurements. Two replicate zooplankton samples were collected once per month from each pond using a vertical tow of a zooplankton net (30-cm diameter; 50-µm mesh) from 1-m to the surface at the deepest point of each pond. Two replicate larval fish samples were collected from each pond using a larval fish net (0.5-m diameter; 500-µm mesh) pulled by hand by two individuals on either side of the pond through the length of each pond (tow speed approximately 1.0 m sec^{-1}) once a month from April to July.

Fish from the originally stocked individuals were collected in August when ponds were drained. We measured and weighed all bluegill >80 mm TL and all largemouth bass that were collected. Bluegill >80 mm TL were assumed to represent the size where they would be able to consume pelleted feed. Muscle tissue was collected from all collected largemouth bass and 20 bluegill at the end of the experiment for stable isotope analysis. Adult bluegill gonads of the subsample of fish sacrificed were weighed (nearest 0.0001g) and 5 ovaries from fish from each pond were frozen for stable isotope analysis. Ovaries were analyzed for stable isotopes to determine if pelleted feed contributed to egg development.

In August, just prior to draining the ponds, potential prey organisms were collected for stable isotope analysis using a sweep net for macroinvertebrates in the vegetation, and an Eckman dredge to collect benthic macroinvertebrates both near feeders and away from feeders if present. These collections were not quantitative assessments of macroinvertebrate density but were meant only to collect sufficient organisms for analysis. The macroinvertebrates were primarily odonate larvae and chironomids. Two zooplankton samples were collected from each pond, following the same protocol for the monthly sampling. Immediately after collection the macroinvertebrate (i.e., pooled benthic and water column samples) and zooplankton samples were frozen for stable isotope analysis.

Stable Isotope Analysis—Frozen fish samples were dried and homogenized according to standard methods for stable isotope mass spectroscopy (Jardine et al. 2003). A 5-g sample of dorsal muscle tissue was used for large fish, and whole fish were used as samples when 5 g of dorsal muscle tissue could not be extracted. Whole specimens were used as samples for prey items other than fish. Samples were dried to a constant weight at 60° C, homogenized using a mortar and pestle, and dried again at 60° C to a constant weight. Dried samples (0.4 mg) were packed into tin capsules and sent to Southern Illinois University, Center for Fisheries, Aquaculture, and Aquatic Sciences for stable isotope analysis. Stable isotope data were reported as δ values defined as parts per thousand (‰) differences of either ^{13}C or ^{15}N from a standard material, and calculated according to the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R\text{sample}/R\text{standard}) - 1] \times 1000$$

where $R_{sample} = {}^{13}C / {}^{12}C$ or $^{15}N / {}^{14}N$ of the sample, and $R_{standard} = {}^{13}C/{}^{12}C$ of PDB (Pee Dee Belemnite) and for carbon and $^{15}N / {}^{14}N$ of atmospheric nitrogen for nitrogen standards (Walsworth 2011).

Statistical Analysis—All data were tested for normality and homogeneity of variance prior to inferential statistical analysis. For those variables not meeting those assumptions the data were $\log_{10}$ transformed which corrected any non-normality and non-homogeneity of variance. For all inferential statistical tests an α of 0.05 was chosen to indicate a significant difference. Single factor ANOVA was used to test for differences in mean TL ($\log_{10}$ transformed) and mean body weight ($\log_{10}$ transformed) of stocked largemouth bass and bluegill (i.e., ≥75 mm TL) across feed delivery rates at the beginning of the experiment. We used linear regression models to test for relationships between feed rate and 1) mean body weight of bluegill and largemouth bass, and 2) female bluegill ovary weight and gonadosomatic index (GSI = ovary weight/body weight). Ovary weight is a correlate of egg production, whereas GSI indicates a correlate of condition and energy allocation to reproduction (Wooton 1979). Linear regression was used to test for relationships between feed rate and $\delta^{15}N$ and $\delta^{13}C$ for bluegill, largemouth bass, and bluegill ovaries. Normality and homogeneity of variance were assessed to ensure that the assumptions of linear regression were met. We tested for relationships between feed rate and dissolved oxygen, Secchi depth ($\log_{10}$ transformed), chlorophyll-*a* concentration ($\log_{10}$ transformed), zooplankton density ($\log_{10}$ transformed), and larval fish density over the duration of the experiment using separate linear mixed effect models with feed rate and month treated as a fixed effects and individual ponds as random effects. Statistical Analysis System ver. 9.1.3 (SAS Institute 2008) was used for all mixed model analyses. All other statistical analyses used R ver. 2.15.2 software (R Development Core Team 2012).

Established Ponds

Pond Descriptions—We sampled 30 fertilized ponds (0.8–23.3 ha) in southern Alabama and western Georgia with established fish populations during April through August of 2012 ($n = 9$) and 2013 ($n = 21$). We selected ponds that had no threadfin shad and did not receive any pelleted feed ("control"; $n = 3$ in 2012, $n = 7$ in 2013), ponds that received pelleted feed but had no threadfin shad ("feed-only"; $n = 3$ in 2012, $n = 7$ in 2013), and ponds that both contained threadfin shad and received pelleted feed ("feed-shad"; $n = 3$ in 2012, $n = 7$ in 2013). While including ponds with just threadfin shad and no pelleted feed would have completed a balanced design, ponds with this combination were typically much larger and rarer, thus they were not included in our study.

Sampling Methods—Boat-mounted pulsed-DC electrofishing (5.0 GPP, Smith-Root, Inc., Vancouver, Washington, operated to produce 4–6 A) was used to collect largemouth bass and bluegill once in each established pond. Two 10-min electrofishing transects were conducted in each pond, one centered around the feeder and the other beginning at least 25 m away from the end of the feeder-centered transect. A subsample of both largemouth bass ($n = 10$ for each transect) and bluegill ($n = 10$ for each transect) was euthanized according to Auburn University Institutional Animal Care and Use Committee protocol 2011–1944 and transported on ice to the lab for further analysis. The subsample included individuals from across the size range collected. All largemouth bass and bluegill >80 mm TL that were not sacrificed were measured (nearest mm TL) and weighed (nearest g).

To assure that selected ponds had similar productivity, surface water samples were collected for chlorophyll-*a* analysis. Temperature, DO, and Secchi depth were measured in each pond as described above. Two replicate zooplankton samples were collected from each pond using a vertical tow of a zooplankton net (30-cm

diameter, 50-µm mesh) from 1 m to the surface at the deepest point of each pond.

Laboratory Processing—Adult fish were weighed (g), measured (mm, TL), and sexed, and adult bluegill gonads were weighed (nearest 0.0001g). Sagittal otoliths to be used for age estimation were removed from largemouth bass and bluegill and stored dry. Ages of fishes were estimated using otoliths in whole mount except those with annuli that were difficult to read. The problematic otoliths were set in an epoxy base, cross-sectioned using a diamond blade saw, and viewed under oil immersion with a compound microscope (400X magnification). All otoliths were aged independently by two readers. When the two independent reads differed, a third independent read was made. For the stable isotope analyses, all samples were prepared and analyzed following the protocols described above.

Statistical Analysis—Single factor ANOVA was used to test for differences in female bluegill and largemouth bass GSI across treatments in the established ponds. When significant treatment effects were detected, Tukey post-hoc analysis was used to determine significant pair-wise comparisons. Female bluegill and largemouth bass GSI values were $\log_{10}$ transformed. We used back-calculation from otolith radius measurements to determine length-at-age for both largemouth bass and bluegill in the established ponds using the direct proportion method (Le Cren 1947). Differences in back-calculated TL at age 2 for bluegill and largemouth bass ($\log_{10}$ transformed) across treatments were tested using one-way ANOVA. Relative weight (W_r) was quantified to estimate bluegill and largemouth bass condition using the published standard weight equation for each species (Hillman 1982, Henson 1991, Neumann et al. 2012). Differences in relative weight across treatments were tested using one-way ANOVA for each species.

We tested for differences in $\delta^{15}N$ and $\delta^{13}C$ among treatments using analysis of covariance with treatment (control, feed only, feed and shad) as a categorical independent variable and mean fish length as a continuous predictor variable. Mean fish length was included in the analysis to account for potential differences in $\delta^{15}N$ and $\delta^{13}C$ attributable to differences in fish size. We ran the analysis on pooled transect data (near vs. away from feeder) from each pond after paired *t*-tests revealed that $\delta^{15}N$, $\delta^{13}C$, and mean length did not differ between transects for either species (all $P < 0.05$). When significant treatment effects were detected, Tukey post-hoc tests were used to determine which pair-wise comparisons were significant.

One-way ANOVA was used to test for differences in surface temperature, dissolved oxygen, Secchi depth, chlorophyll *a* ($\log_{10}$ transformed), zooplankton density ($\log_{10}$ transformed), and pond size ($\log_{10}$ transformed) across treatments. Statistical Analysis System ver. 9.1.3 (SAS Institute 2008) was used for all mixed model analyses. All other statistical analyses used R ver. 2.15.2 software (R Development Core Team 2012).

Results

Controlled Small Pond Experiment

We found no statistically significant differences in chlorophyll *a* concentration, Secchi depth, DO, zooplankton density, or larval bluegill density among treatments in the small pond experiment (Table 1).

At the start of the experiment, there were no differences in mean TL or weight of stocked bluegill assigned across feed treatments (Table 2). Bluegill mean body weight was positively related to feed rate at the end of the experiment (Table 2; Figure 1A). Both bluegill ovary weight and GSI were positively related to feed rate at the end of the experiment (Table 2; Figure 1B). Bluegill $\delta^{15}N$ values were negatively associated with feed rate (Table 2; Figure 1C) but $\delta^{13}C$ values did not differ across feed rates (Table 2). At the end of the small pond experiment, bluegill ovary $\delta^{15}N$ and $\delta^{13}C$ values did not differ across feed rates.

At the start of the experiment, there were no differences in mean total length or weight of stocked largemouth bass across feed treatments (Table 2). The average weight of largemouth bass was not significantly related to the feed delivery at the end of the experiment (Table 2). Adult largemouth bass $\delta^{15}N$ and $\delta^{13}C$ values were also not related to feeding rate (Table 2; Figure 1D).

Pelleted feed had the lowest mean $\delta^{15}N$ of all potential food items,

Table 1. Abiotic, plankton, and larval bluegill model results for separate linear mixed effect models from the pond experiment. Statistical significance at $P < 0.05$ is indicated by bold text. For graphical analyses and time series see Henderson (2014).

Variable	df	*F* value	*P* value
Treatment effects			
Chlorophyll *a* (µg L^{-1})	1,8	0.11	0.75
Secchi depth (cm)	1,8	< 0.01	0.96
Dissolved oxygen (mg L^{-1})	1,8	0.07	0.80
Larval bluegill (No. m^{-3})	1,8	0.44	0.52
Zooplankton (No. L^{-1})	1,8	0.39	0.55
Time effects			
Chlorophyll *a* (µg L^{-1})	**4,32**	**6.82**	**0.01**
Secchi Depth (cm)	4,31	0.76	0.56
vDissolved Oxygen (mg L^{-1})	4,32	0.99	0.43
Larval Fish (No. m^{-3})	**4,32**	**5.19**	**0.01**
Zooplankton (No. L^{-1})	**4,31**	**8.58**	**0.01**
Interaction Effects (Time*Treatment)			
Chlorophyll *a* (µg L^{-1})	4,32	0.31	0.87
Secchi depth (cm)	4,31	0.80	0.53
Dissolved oxygen (mg L^{-1})	4,32	1.10	0.37
Zooplankton (No. L^{-1})	4,31	0.13	0.97
Larval fish (No. m^{-3})	4,32	0.11	0.98

Table 2. Statistical results for comparisons of invertebrates, bluegill and largemouth bass characteristics among feed rates in the controlled pond experiment. Statistical significance at $P \leq 0.05$ is indicated by bold text. For graphical analyses and time series see Henderson (2014).

Variable	df	*F* value	*P* value	R^2
Invertebrates				
Zooplankton $\delta^{15}N$ at end	1,8	0.51	0.49	0.40
Zooplankton $\delta^{13}C$ at end	1,8	1.52	0.25	0.25
Macroinvertebrates $\delta^{15}N$ at end	1,8	0.01	0.97	0.01
Macroinvertebrates $\delta^{13}C$ at end	1,8	0.08	0.79	0.10
Bluegill:				
TL at stocking	4,5	0.08	0.99	
Weight at stocking	4,5	0.08	0.99	
Weight at end	**1,8**	**8.34**	**0.02**	**0.45; Figure 1A**
Female GSI at end	**1,8**	**8.64**	**0.02**	**0.46**
$\delta^{15}N$ at end	**1,8**	**10.78**	**0.01**	**0.52; Figure 1C**
Ovary $\delta^{15}N$ at end	1,8	1.48	0.21	0.09
$\delta^{13}C$ at end	1,8	0.22	0.65	0.09
Ovary $\delta^{13}C$ at end	1,8	0.34	0.57	0.08
Largemouth bass:				
TL at stocking	4,5	0.09	0.98	—
Weight at stocking	4,5	0.03	0.99	—
Weight at end	1,8	0.59	0.47	0.05
$\delta^{15}N$ at end	1,8	2.71	0.12	0.16; Figure 1D
$\delta^{13}C$ at end	1,8	0.62	0.46	0.04

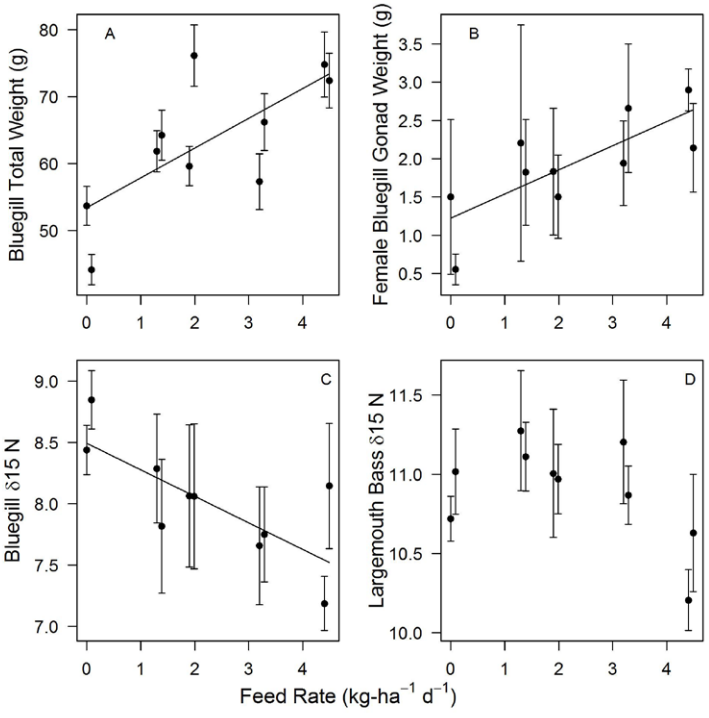

Figure 1. Mean (± 95% CI) bluegill total weight (g; Panel A), female bluegill gonad weight (g; Panel B), bluegill $\delta^{15}N$ (Panel C), and largemouth bass $\delta^{15}N$ (Panel D) as a function of feed rate at the end of the small pond experiment. The lines represent the predictions from statistically significant regression models.

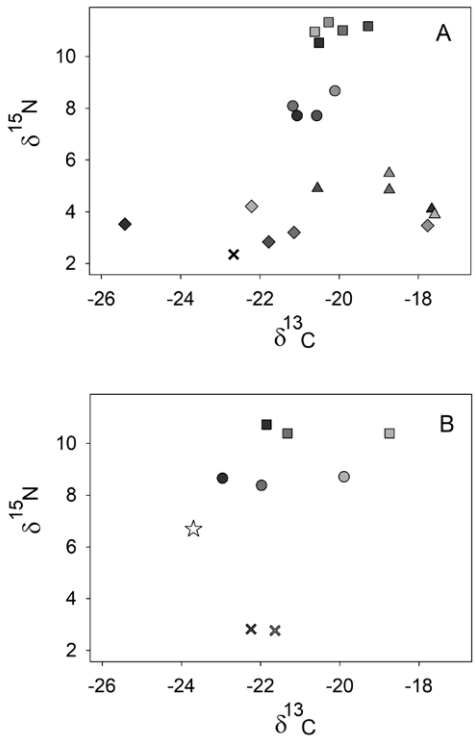

Figure 2. Panel A: Small pond experiment mean $\delta^{15}N$ and $\delta^{13}C$ for largemouth bass (squares), bluegill (filled circles), benthic macroinvertebrates (triangles), zooplankton (diamonds), and feed (X); darker shading indicates higher feed rates. Panel B: Established ponds mean $\delta^{15}N$ and $\delta^{13}C$ for largemouth bass (squares), bluegill (filled circles), threadfin shad (open star), and feed (X); shading indicates different feed and threadfin shad treatments: control (outlined light gray), feed-only (dark gray) and feed-shad (black).

but overlapped with zooplankton in terms of $\delta^{13}C$ (Figure 2A). Zooplankton $\delta^{13}C$ values were highly variable ranging from –25.4 to –17.7 (Figure 2A). Macroinvertebrate $\delta^{13}C$ values were generally higher than bluegill and zooplankton values, although some overlap existed (Figure 2A). Feed rate was not related to $\delta^{15}N$ and $\delta^{13}C$ values of zooplankton and macroinvertebrates, or potential bluegill prey (Table 2; Figure 2A).

Established Ponds

Control ponds generally had higher chlorophyll-*a* concentration and lower Secchi depth than the feed-only and feed-shad ponds (Table 3). There were no differences in zooplankton density, surface DO, DO at 1.0 m, or surface temperature across treatments, although temperature at 1 m was significantly less in the control treatment than in the feed-only and feed-shad treatments (Table 3).

There were no differences in bluegill length at age 2 or relative weight across established pond treatments, but female bluegill GSI was significantly lower in feed-only ponds versus control and feed-shad ponds (Table 3). Bluegill $\delta^{15}N$ values did not differ

Table 3. Statistical results for comparisons of characteristics of the ponds, adult bluegill, and adult largemouth bass among pond type treatments for the established pond portion of the study. Statistical significance at $P \leq 0.05$ is indicated by bold text, and treatment comparisons are indicated for significant test results (C = control; FO = Feeder only; FS = feeder and threadfin shad). For graphical analyses and time series see Henderson (2014).

Variable	df	*F*-value	*P*-value
Chlorophyll-a concentration	**2,27**	**11.58**	**<0.01**
Secchi depth	**2,27**	**7.05**	**<0.01**
DO-surface/@ 1 m	2,23/2,23	1.34/0.51	0.28/0.61
Temperature-surface/@ 1 m	2,25/2,25	2.98/3.54	0.07/0.04
Zooplankton density	2,26	2.93	0.07
Surface area	**2,27**	**4.99**	**0.01**
Bluegill			
Length at age-2	2,27	1.31	0.29
Wr	2,27	0.95	0.40
Female GSI	**2,27**	**4.66**	**0.02**
$\delta^{15}N$ values			
Treatment effect	2,27	0.57	0.57
Length effect	1,26	1.21	0.28
Treatment x length	2,24	1.22	0.31
$\delta^{13}C$ values			
Treatment effect	**2,27**	**6.14**	**<0.01**
Length effect	1,26	1.38	0.25
Treatment x length	2,24	0.07	0.93
Largemouth bass			
length at age-2	**2,27**	**3.97**	**0.03**
Wr	2,27	0.37	0.69
Female GSI	2,27	1.15	0.33
$\delta^{15}N$ values			
Treatment effect	2,27	0.85	0.44
Length effect	1,26	0.06	0.81
Treatment x length	2,24	0.01	0.99
$\delta^{13}C$ values			
Treatment effect	**2,27**	**5.78**	**<0.01**
length effect	1,26	0.20	0.66
Treatment x length	2,24	0.64	0.53

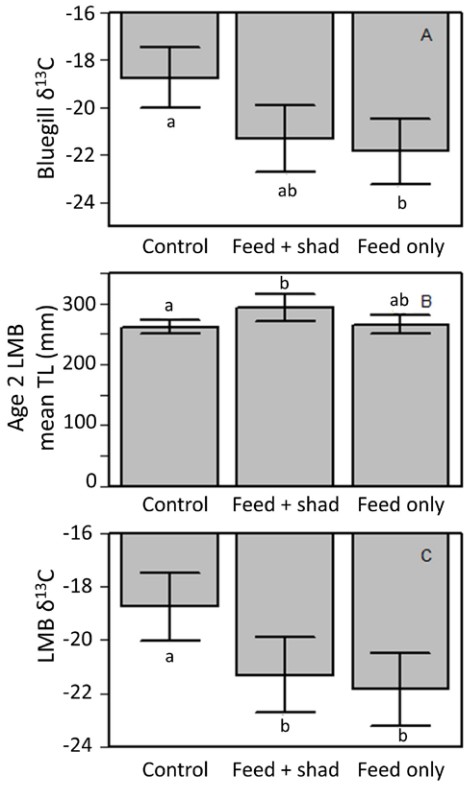

Figure 3. Panel A: bluegill mean (± 95% CI) $\delta^{13}C$ in established control, feed-shad, and feed-only ponds; Panel B: largemouth bass mean (± 95% CI) length at age 2; Panel C mean $\delta^{13}C$ (Panel B) in established control, feed-shad, and feed-only ponds. Different letters indicate treatments that differed significantly within panels.

across treatments and were not associated with bluegill mean TL (Table 3). Bluegill $\delta^{13}C$ values were significantly more negative in the feed-only treatment than the control treatment, but were not associated with mean bluegill TL (Table 3; Figures 2B, 3). These lower bluegill $\delta^{13}C$ values were similar to $\delta^{13}C$ values of the pelleted feed at these ponds (Figure 2B).

Adult largemouth bass length at age 2 was greater in the feed-shad treatment than in the controls, and the feed-only treatment did not differ from either one (Table 3; Figure 3). However, relative weight and female GSI did not differ statistically among treatments (Table 3).

Largemouth bass $\delta^{15}N$ values did not differ statistically among treatments (Table 3; Figure 2) and were not related to largemouth bass mean TL (Table 3). Largemouth bass $\delta^{13}C$ values were significantly more negative in the feed-only and feed-shad treatments versus the control (Table 3; Figure 3), but were not related to mean TL (Table 3). Largemouth bass $\delta^{13}C$ values were similar to pelleted feed but higher than threadfin shad $\delta^{13}C$, a potential prey item (Figure 2).

Discussion

The isotopic signature of pelleted feed was evident in bluegill tissues but responses differed between $\delta^{15}N$ and $\delta^{13}C$, as well as between experimental and established ponds. The $\delta^{15}N$ values of animals are usually more positive than those of their diets (DeNiro and Epstein 1981), which was also true for bluegill and their natural prey. However, $\delta^{15}N$ values of pelleted feed were lower than that of natural prey items. Thus, the inverse relationship between $\delta^{15}N$ values in bluegill tissues and rates of pelleted food application in experimental ponds is consistent with the hypothesis that increased rates of pelleted feed application led to higher levels of assimilation of energy and nutrients from feed into tissues. In contrast, we found no difference in $\delta^{15}N$ among treatments in established ponds. Low feed-delivery rates in established ponds likely prevented significant shifts in bluegill $\delta^{15}N$ ratios in these ponds.

Informal interviews with pond owners suggested that feed rates in established ponds averaged approximately 1.0 kg ha^{-1} d^{-1}, which is at the low end of recommended rates (Woodard et al. 2013) and less than our lowest experimental pond feed rate (1.3 kg ha^{-1} d^{-1}).

In contrast to $\delta^{15}N$ results, adult bluegill $\delta^{13}C$ ratios were unrelated to feed rate in the pond experiment. Zooplankton and macroinvertebrate $\delta^{13}C$ ratios were highly variable and overlapped with the feed signature in the experimental ponds, which may have masked increased reliance on pelleted feed by bluegill under higher feed rates. Stable isotopes likely will not perform well at tracking trophic flow of pelleted feed through the food web if feed is isotopically similar to other available prey items. Similarly, Duffy et al. (2011) found that isotopic signatures of crayfish in natural ponds did not clearly match pelleted feed signatures due to substantial consumption of natural prey items by crayfish and overlap in the signatures of feed and natural prey.

While pelleted feed effects were less evident overall in $\delta^{15}N$ and $\delta^{13}C$ ratios of largemouth bass than bluegill, largemouth bass $\delta^{13}C$ values were significantly lower in the established ponds containing pelleted feed when compared to the control ponds. This finding suggests that pelleted feed may have influenced largemouth bass $\delta^{13}C$ values in the established ponds, presumably via lower $\delta^{13}C$ values of the bluegill prey; had the largemouth bass fed directly on pelleted feed, their $\delta^{15}N$ would have decreased, which was not observed. This result was somewhat unexpected given that we found no clear pelleted feed signal in largemouth bass stable isotopes in controlled pond experiment despite higher pelleted feeding rates there versus in the established ponds. The long term nature of feeding over multiple years may account for this effect in the established ponds.

Although stable isotopes did not consistently indicate a strong signal of pelleted feed in bluegill, our findings do build on a growing body of evidence that supports the management approach of providing pelleted feed to enhance bluegill growth and reproductive potential in small impoundments. We found increased bluegill weight and GSI with increasing feed rate in the pond experiment, although not in established ponds. Food availability and quality are important in determining fish size differences among populations (Hewett and Kraft 1993). Pelleted feed has a higher caloric density and is likely easier to capture than natural foods (Schalles and Wissing 1976, Porath and Hurley 2005) which likely translates into greater allocation of energy to somatic and gonadal growth when rations are sufficient. Presumably, the lack of effect of pellet feeding on bluegill growth and reproduction observed in the established ponds was due to low feeding rate.

Even though bluegill gonad weight and GSI increased with increasing feed rate in the pond experiment, this did not translate to higher larval bluegill density across treatments. Our monthly sampling for larval bluegill may have not been frequent enough to detect differences in larval bluegill density among feeding rate treatments. Woodard et al. (2013) found similar results during one year of a controlled experiment. Specifically, they found increased bluegill GSI with pelleted feed, yet no difference in age-0 bluegill abundance were observed in the fall despite observing increased larval bluegill density during the summer, which they attributed to density dependence.

Largemouth bass growth (length at age 2, mean weight), relative weight, and reproductive investment (gonad weight, GSI) were not related to pelleted feeding in either experimental or established ponds, but length at age 2 was greater in established ponds with threadfin shad. These findings agree with Woodard et al. (2013) who reported no relationship between feed rate and largemouth bass total length in a controlled small pond experiment. Longer term experiments with consistent and higher rates of pelleted feed addition may be necessary to generate significant effects through the food web to growth and reproductive investment by largemouth bass. The positive effect of threadfin shad on largemouth bass size at age is likely a direct effect of consumption. Largemouth bass may shift their diet to feed on threadfin shad when present (Davies et al. 1979, Noble 1981) and threadfin shad has been shown to enhance largemouth bass growth (Tharratt 1966, Miller 1971, von Geldern and Mitchell 1975, Haley et. al 2012).

Given the increase in largemouth bass length at age 2 in the established ponds with threadfin shad and their documented influence on largemouth bass growth when present (Davies et al. 1979, Noble 1981, Wydoski and Bennett 1981), we expected largemouth bass $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures to differ in the presence versus absence of threadfin shad. In contrast, we found no relationship between largemouth bass $\delta^{13}C$ and $\delta^{15}N$ isotopic values and presence of threadfin shad. Largemouth bass $\delta^{13}C$ isotopic signatures were more similar to bluegill $\delta^{13}C$ in the established ponds regardless of treatment suggesting that largemouth bass primarily consumed bluegill. Previous studies suggest that even in the presence of *Dorsoma* spp., centrarchids sometimes remain the primary prey for largemouth bass (Timmons et al. 1980, Jackson et al. 1992, Bettoli et al. 1992, Irwin et al. 2003, Haley et al. 2012). In the established ponds, largemouth bass may have been primarily consuming bluegill (as suggested by stable isotope analysis) but supplementing their diets (and growth) with threadfin shad. To separate the effects of feeding pellets from that of threadfin shad, it would be necessary to include ponds with shad but no pellet feeding in sampling. Adding a stable isotope unique to pelleted feed as a tracer may have also provided a more definitive analysis of feed contribution through multiple levels of the food web.

Previous research suggests a potential for competition between threadfin shad and sunfishes (Davies et. al. 1979, Noble 1981, DeVries et al. 1991); however, a literature review of field manipulations of gizzard shad (*Dorosoma cepedianum*) and threadfin shad found mixed results for effects of threadfin shad on bluegill (DeVries and Stein 1990). We expected bluegill $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures to differ in the feed-only and feed-shad treatments because of a heavy reliance upon pelleted feed when threadfin shad were present due to likely competition (Davies et. al. 1979) and a possible reduction in zooplankton densities (Ziebell et. al. 1986, Prophet 1988, DeVries et. al. 1991, Garvey and Stein 1998, Haley et. al. 2012); however, there were no differences in zooplankton densities nor bluegill isotopic signatures between treatments. This suggests that either bluegill relied on pelleted feed in the feed-only treatment at the same rate as in the feed-shad treatment, or food was not limited for bluegill in the presence of threadfin shad in the established ponds.

Management Implications

Pelleted feed has the potential to enhance bluegill growth and reproductive allocation. Our findings also suggest that threadfin shad can increase largemouth bass growth in small impoundments as has been observed in other studies. Our stable isotope analyses in established ponds suggest that isotopic signatures of pelleted feed may still be evident in bluegill and largemouth bass even at feeding rates below levels that produced significant enhancement of growth in controlled experiments. However, the presence of the feed isotopic signature did not coincide with increased growth and condition of these populations, a primary concern of pond owners. Thus pond owners should not expect positive effects of pelleted feed on growth of bluegill or on growth and condition of largemouth bass when feeding below recommended rate of 2.7–11 kg ha^{-1} day^{-1} (Wright and Masser 2004, Woodard et al. 2013).

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