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Stable isotopes reveal live prey support growth of juvenile channel catfish reared under intensive feeding regimens in ponds

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ABSTRACT

Juvenile channel catfish in nursery ponds are typically supplied large daily additions of manufactured feeds to increase growth and survival. However, mounting evidence suggests that naturally occurring zooplankton and insects support early fish growth. In this study, we leveraged the large natural differences in $\delta^{13}C$ and $\delta^{15}N$ contents between live prey in ponds and added manufactured feeds to quantify their relative contributions to fish growth. We conducted a tank experiment to quantify the isotopic transition patterns in muscle tissue of fish subjected to constant and abruptly changing diets between foods with different $\delta^{13}C$ and $\delta^{15}N$ values. We then applied these results to resolve temporal isotopic patterns in muscle tissue of fish reared in ponds under treatments of (1) no manufactured feed addition, (2) a low feeding regimen (1% body-weight/day, BW/d), or (3) a high feeding regimen (3% BW/d). We found that live prey entirely supported fish growth during the first 3 weeks in ponds, regardless of feeding regimen. Thereafter, about 50% of fish growth was supported by feed in the 1% and 3% BW/d ponds, which increased fish growth rates as compared to fish reared with no feed addition. Thus, live prey organisms are important in nursery ponds to support growth throughout the first growing season, and feed supplements growth only several weeks after stocking fish in ponds. Accordingly, we recommend manufactured feeds be withheld from nursery ponds until several weeks after fish stocking.

1. Introduction

Aquaculture ponds are commonly supplied manufactured feeds to enhance fish growth and survival above levels supported by ambient live prey (Lovell, 1989). The first foods ingested by larval fish in freshwater ponds are typically rotifers, copepod nauplii, and small cladocerans, followed by insect larvae when mouth gape size and digestive development allow (Qin et al., 1995). Fish can usually be weaned to partial or complete diets of dry feed during the juvenile life stage, although some species can be wholly weaned as larvae in tank systems with no alternate prey (Kolkovski, 2001; Lazo et al., 2011). To avoid wasteful feed use in ponds with dynamic live prey communities, managers must identify the timing of voluntary feed inclusion in fish diets and weigh the positive effects of feeding on fish growth and survival against its negative effects on water quality (Filbrun et al., 2013).

Stable isotopes provide a powerful tool for quantifying dietary support of fish tissue growth among foods with distinct isotope contents. Manufactured feeds provided to fish in aquaculture settings are typically different in $\delta^{13}C$, $\delta^{15}N$, and/or $\delta^{34}S$ from alternative foods used to support growth in tanks or ponds (e.g., *Artemia*, zooplankton; Hesslein et al., 1993; Schroeder, 1983; Su et al., 2008). Thus, the contribution of feeds to fish tissue growth can be easily quantified relative to alternate foods using mass-balance mixing models. For example, Gamboa-Delgado et al. (2008) and Jomori et al. (2008) leveraged natural isotopic differences between live *Artemia* nauplii and dry feeds to quantify the relative contributions of these two foods in combination to growth of larval Senegalese sole (*Solea senegalensis*) and pacu (*Piaractus mesopotamicus*), respectively. In both studies, *Artemia* contributed more to fish growth than feed, and growth was slower when fish were provided only the dry feed rather than a mixture of the two foods. Both studies also showed that fastest growth and highest survival were achieved by providing *Artemia* only or a mixture of both foods.

Channel catfish (*Ictalurus punctatus*) has been the most important commercially cultured fish in the United States by yield and value for over 50 years (FAO, 2011), and most states stock juveniles into ponds, lakes, and reservoirs to create or enhance recreational fisheries (Michaletz and Dillard, 1999). Channel catfish aquaculture has been widely successful because this species is exceptionally tolerant of poor water quality, and advanced juveniles readily accept dry feeds added to ponds (Hargreaves, 2002). However, early juvenile rearing in nursery ponds remains the most variable stage in commercial production, likely because managers ignore natural live prey support of early growth in favor of the use of manufactured feeds (Mischke et al., 2011, 2013). Despite growing evidence that swim-up and early juvenile catfish readily consume zooplankton and insects in ponds (Bonneau et al., 1972;

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Mischke et al., 2003), and have faster growth on diets that include zooplankton as compared to dry feed only (Mischke et al., 2009), large amounts of dry feed are added to nursery ponds, sometimes even before the fish are stocked (Tucker et al., 2004).

Mischke et al. (2013) recently used a delayed feeding experiment to test whether natural live prey can entirely support juvenile channel catfish growth in nursery ponds. They established "industry standard" and "delayed" feeding treatments by adding manufactured feed to the ponds immediately at the time of stocking, and 6 weeks after stocking, respectively. The delayed feeding treatment did not reduce fish growth or survival, suggesting that fish in the industry standard treatment either did not consume the feed for the first 6 weeks, or did not receive nutritional benefits by including it in their diets. However, fish diets were not quantified in their study, so the relative contributions of live prey versus feed to fish growth in ponds remains unclear.

In this study, we quantified the onset of feed assimilation by channel catfish using stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). In a recent pond experiment at the Hebron State Fish Hatchery, Ohio, USA, Filbrun et al. (2013) showed that the provided dry feeds were enriched relative to naturally occurring zooplankton and insects by about 4–6‰ in $\delta^{13}C$ and 4–7‰ in $\delta^{15}N$. Here, we combine a feeding trial in tanks and high-frequency fish collections from the same pond experiment described in Filbrun et al. (2013) to quantify live prey and feed contributions to fish growth through time. To our knowledge, this study is among the first to capture detailed isotopic dynamics of freely foraging juvenile fish with access to live prey and feed in a relatively complex pond ecosystem. Our findings have profound implications for improving channel catfish fingerling production by relying more heavily on live prey items to support fish growth in the early weeks after stocking fish in ponds.

2. Materials and methods

2.1. Study design

Concurrent tank and pond experiments were performed with juvenile channel catfish to resolve temporal patterns in the stable isotope values of fish with forced and natural diet shifts. The laboratory tank experiment was designed to quantify the isotopic transition patterns in muscle tissue of fish subjected to constant and abruptly changing diets between foods with known, distinct $\delta^{13}C$ and $\delta^{15}N$ contents. These results were then applied to interpret the temporal isotopic patterns in muscle tissue of fish reared in the pond experiment with access to dynamic live prey communities and manufactured feed. Channel catfish used in both experiments were hatched from the same brood stock at the Senecaville State Fish Hatchery, Ohio, USA. Eggs were collected from spawning cans placed in ponds, hatched in jars, and then larvae were reared for about two weeks in flow-through troughs with multiple daily feedings of a powdered manufactured feed.

2.2. Tank experiment

A 10-week laboratory feeding experiment was performed in 35 L glass tanks at The Ohio State University Aquatic Ecology Laboratory during July–September 2010. Tanks were filled with dechlorinated tap water and equipped with radiant heaters to maintain temperatures between 25 and 32 °C, similar to summer temperatures in Ohio ponds. The static tank systems were aerated vigorously throughout the experiment to maintain oxygen saturation and were subjected to a constant 12 hour photoperiod. All tanks had low NH_3 concentrations (<0.05 mg N/L) during the experiment.

Thirty two-week-old fish (mean wet weight = 0.05 g, mean total length = 19 mm) were stocked into each tank on 7 July 2010. The three feeding treatments consisted of supplying fish with constant diets of (1) freeze-dried chironomid larvae only (packaged as blood worms, Hikari Sales USA, Inc.), (2) Silver Cup No. 1 sinking feed granules (hereafter "feed"; 52–54% crude protein, 12–16% fat, Nelson and Sons, Inc.), or (3) an abrupt diet switch from chironomid larvae to feed after 5 weeks. (Additional feeding treatments of freeze-dried zooplankton [packaged as *Daphnia*, Hikari Sales USA, Inc.] were originally included in the experiment, but excluded from this study because the types of zooplankton and their $\delta^{13}C$ and $\delta^{15}N$ values varied tremendously among packages). Each treatment was replicated in 4 tanks each by random assignment. Fish were fed once daily, 7 days per week, after which wastes and any unconsumed food were siphoned from tanks during 20% water changes. Although food consumption was not quantified, food amounts were increased gradually during the experiment to ensure that slight amounts of excess food were present in tanks at 15 min after feedings. One to three fish were collected from each tank on a weekly basis and measured and weighed while fresh. The experimental foods were sampled from containers about every two weeks. Fish and food samples were fresh frozen (−20 °C) for later $\delta^{13}C$ and $\delta^{15}N$ measurements.

2.3. Pond experiment

The pond experiment was performed at the Hebron State Fish Hatchery during July–September 2010, as described by Filbrun et al. (2013). Briefly, nine earthen ponds were filled 7 days prior to fish stocking using water from a eutrophic reservoir, Buckeye Lake. The 0.4 ha ponds were not fertilized before or during the experiment. Two-week-old fish (mean wet weight = 0.05 g, mean total length = 19 mm) were stocked into ponds on 7 or 9 July 2010 at 13 fish/m^3 (40,000 fish/pond; 100,000 fish/ha). Three ponds were randomly assigned to treatments of (1) no manufactured feed addition, (2) a low feeding regimen (1% body-weight/day, BW/d), or (3) a high feeding regimen (3% BW/d). Feeds with similar nutritional content and $\delta^{13}C$ and $\delta^{15}N$ values (Silver Cup Starter, No. 1, and No. 2) were hand-broadcast to ponds near the draining structure between 1200 h and 1400 h, 5 days per week. Ten fish were collected from each pond weekly, measured and weighed while fresh, and then fresh frozen (−20 °C) for later $\delta^{13}C$ and $\delta^{15}N$ measurements. Zooplankton and insects were collected from ponds weekly, of which $\delta^{13}C$ and $\delta^{15}N$ contents were measured and reported by Filbrun et al. (2013).

2.4. Measuring $\delta^{13}C$ and $\delta^{15}N$ values of fish and diets

During the first two weeks of both experiments, fish were too small to measure stable isotope contents of individual fish, so the entire caudal peduncles of up to three fish from the same pond or tank were combined to achieve adequate dry mass for $\delta^{13}C$ and $\delta^{15}N$ measurements (Vander Zanden et al., 1998). During all subsequent weeks, measurements of muscle tissue in individual fish were performed. To account for anticipated size-dependent differences in fish diets within ponds, we selected the smallest, median, and largest fish (by weight) from each pond's weekly collection for stable isotope measurements (N = 3 fish/pond).

Fish muscle tissue samples were dried at 60 °C for 24 h, ground into fine powders using an Agate (SiO_2) mortar and pestle, and packed into tin capsules for solid samples. Stable C and N isotope ratios were measured at The Ohio State University Stable Isotope Biogeochemistry Laboratory using an elemental analyzer (Costech Analytical Technologies, Inc.) coupled to a Finnigan Delta IV Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc.) under continuous gas flow using a CONFLO III interface (Thermo Fisher Scientific, Inc.). Samples were measured relative to C and N reference materials and 10% of the samples were measured in duplicate. Stable isotope measurements are expressed using the standard delta (δ) notation, defined as parts per thousand (‰) deviation in isotope ratios from the Vienna Pee Dee Belemnite limestone (C) and air (N) international standards. The average standard deviations for repeated measurements of the USGS24, IAEA-N1 and IAEA-N2 standards were 0.05‰ for $\delta^{13}C$ and 0.14‰ for $\delta^{15}N$. The average standard deviations for duplicate fish tissue measurements were 0.04‰ for $\delta^{13}C$ and 0.09‰ for $\delta^{15}N$.