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Application of Stable Isotope Techniques to Trophic Studies of Age-0 Smallmouth Bass

M. JAKE VANDER ZANDEN* AND MENNO HULSHOF

Department of Biology, McGill University
1205 Avenue Docteur Penfield, Montreal, Quebec H3A 1B1, Canada

MARK S. RIDGWAY

Harkness Laboratory of Fisheries Research, Ontario Ministry of Natural Resources
Aquatic Ecosystems Science Section
Third Floor North, 300 Water Street, Peterborough, Ontario K9J 8M5, Canada

JOSEPH B. RASMUSSEN

Department of Biology, McGill University

Abstract.—Naturally occurring stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be used to differentiate pelagic and benthic prey items and to characterize the trophic position of aquatic organisms. The isotopic signatures of age-0 smallmouth bass *Micropterus dolomieu* from six broods in Lake Opeongo, Ontario, were tracked between June 18 and July 24, 1995. Posthatch embryos (4–5 mm in total length) had elevated $\delta^{15}N$ values (9‰) that were attributed to the parental origin of their nitrogen pool. The $\delta^{15}N$ decreased rapidly, approaching 2‰ for 15-mm smallmouth bass; this complete dilution of the parentally derived $\delta^{15}N$ pool corresponded with metamorphosis from larvae into juveniles. The dramatic decline in $\delta^{15}N$ provided an opportunity to model the relative importance of somatic growth and tissue turnover in isotopic shifts; tissue accumulation (from exogenous feeding) accounted for 86% of the observed decline in $\delta^{15}N$. Nitrogen isotopes indicated a dietary shift and an increase in trophic position between 17 and 46 mm. By the final sampling date (July 24), body size of age-0 fish ranged from 38 to 46 mm; a positive relationship between $\delta^{15}N$ and body size suggested that intrapopulation trophic differences may be responsible for the observed variation in body size. The $\delta^{13}C$ values of premetamorphosis ($<$15-mm) smallmouth bass (-23.2‰ to -26.1‰) were generally higher than adult $\delta^{13}C$ values (-25.0‰ to -28.4‰). The $\delta^{13}C$ of juvenile smallmouth bass increased with body size (from -24‰ to -21‰), indicating a dietary shift from a mix of benthic and pelagic prey towards reliance on benthic food items.

Studies of trophic relationships are complicated by high levels of spatiotemporal (Paine 1988; Polis and Winemiller 1996; Wainright et al. 1996), intrapopulation (Gu et al. 1997), interpopulation (Vander Zanden and Rasmussen 1996), and ontogenic (Werner and Gilliam 1984) variation. Many of these problems are particularly serious for studies of age-0 fish because direct gut content analysis is logistically difficult, particularly when prey items smaller than 60 μm must be identified (McCarter and James 1993). Yet larval and juvenile life stages appear to influence recruitment and year-class strength because the largest age-0 fish generally have improved overwinter survival and condition (Shuter et al. 1980; Cargnelli and Gross 1996, 1997). This central role of body size (Miller et al. 1988) underscores the importance of understanding how variation in feeding of age-0 fish (at multiple levels and multiple scales) influences the body size attained by fish during these critical life stages.

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are increasingly used to provide time-integrated information about feeding relationships in aquatic food webs (Kling et al. 1992; Cabana and Rasmussen 1994; Hobson and Welch 1995). Laboratory and field studies report a consistent stepwise increase in the heavy nitrogen isotope (^{15}N) of 3–4‰ per trophic level increment (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987; Michener and Schell 1994). Thus, the $\delta^{15}N$ of an organism can be used to indicate trophic position of consumers, based on the pathways of energy flow through the food web (providing a continuous measure rather than discrete trophic levels; Vander Zanden and Rasmussen 1996; Cabana and Rasmussen 1996; Vander Zanden et al. 1997).

Although $\delta^{13}C$ signatures of consumers are sim-

* Corresponding author: jzanden@bio1.lan.mcgill.ca

ilar to those of their food (they generally differ by less than 1‰: DeNiro and Epstein 1978; Fry and Sherr 1984; France 1996a), pelagic and benthic algae in freshwater lakes often have distinct $\delta^{13}C$ signatures because benthic algae generally exhibit less ^{13}C fractionation during carbon fixation than their pelagic counterparts (France 1995; Hecky and Hesslein 1995). For systems in which benthic and pelagic primary producers have distinct $\delta^{13}C$ values, the $\delta^{13}C$ of consumers can be used to differentiate between consumption of pelagic and benthic prey (France 1995; Hecky and Hesslein 1995).

Gut content studies afford a high degree of taxonomic precision but provide only a snapshot in time of consumer diets. Stable isotopes, on the other hand, do not provide direct dietary information per se but serve to indicate long-term average feeding; the time period that is integrated depends on the metabolic rate of the tissue examined and the growth rate of the organism (Tieszen et al. 1983; Hobson and Clark 1992; Hesslein et al. 1993). Furthermore, stable isotopes reflect the materials actually *assimilated* by the consumer and can detect certain feeding interactions that are difficult to observe (Kling et al. 1992), thus making conventional gut content and isotopic lines of evidence highly complimentary sources of information.

In light of the potential importance of dietary differences to age-0 fish, the high degree of variation in feeding, and the limitations of conventional gut content analyses, this study uses the stable isotope approach to examine the trophic ecology of age-0 smallmouth bass *Micropterus dolomieu*. Smallmouth bass is an important species in fisheries management, and it is a particularly suitable species for such a study because nest fidelity allows isotopic tracking of individual broods through time. The early life history of northern smallmouth bass populations is well known (Doan 1940; Ridgway 1988; Ridgway and Friesen 1992); typical ontogenic development of Lake Opeongo smallmouth bass is summarized in Figure 1. This study explores potential applications of stable isotopes to the ecology of age-0 fish: (1) detection of the onset of exogenous feeding, (2) detection of ontogenic trophic shifts, and (3) determination of the relative importance of growth and nitrogen turnover in the observed isotopic shifts in age-0 smallmouth bass.

Methods

Age-0 smallmouth bass were collected from nests along a 2-km stretch of shoreline in central Jones Bay of Lake Opeongo (45°42′N, 78°22′W), a 5,860-ha oligotrophic lake in Algonquin Park, central Ontario. Samples were hand collected by snorkelers using an aquarium-sized net (20 cm × 15 cm) on days of the year 170, 173, 176, 179 (June 19, 22, 25, 28) in 1995, and days 183, 188, 191, and 205 (July 3, 7, 10, and 24), in 1995 from each of six individual nests. Adult smallmouth bass used for isotopic analysis were collected from Jones Bay on July 24 with rod and reel; the individual adult smallmouth bass that were defending the study nests were not sampled as this would have compromised the survival of the age-0 fish from the study nests. Total lengths (TL, mm) were recorded for all age-0 and adult smallmouth bass collected.

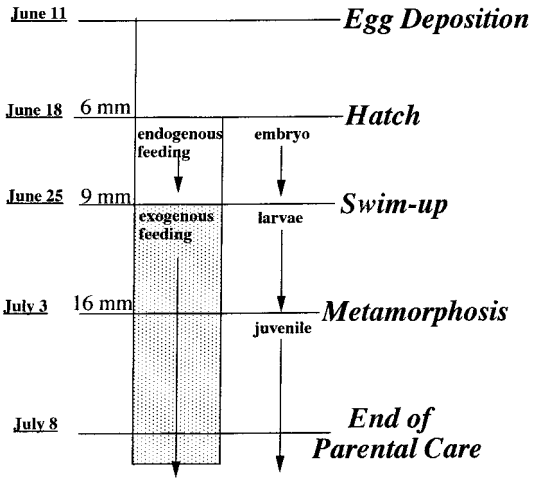

FIGURE 1.—Development during the early life stages of age-0 smallmouth bass from Lake Opeongo, Ontario. Dates are averages based on Ridgway and Friesen (1992).

Problems encountered interpreting isotopic data for primary producers include high levels of temporal variation in isotopic signatures (Cabana and Rasmussen 1996) and uncertainty as to the trophic importance of the particular plant materials analyzed (Vander Zanden and Rasmussen, in press). These problems are partially circumvented by considering the isotopic signatures of characteristic primary consumer organisms that undergo far less temporal isotopic variation (Toda and Wada 1990; Cabana and Rasmussen 1996) and that, by definition, reflect the integrated isotopic signatures of the energetically important primary producers. Crayfish were chosen as a time-integrated indicator of benthic $\delta^{13}C$ because they consume benthic algae, invertebrates, and detritus and exhibit less negative $\delta^{13}C$ values than pelagic consumers

TABLE 1.—Mean total lengths and $\delta^{13}C$ and $\delta^{15}N$ values (ranges in parentheses) for invertebrates and age-0 and adult smallmouth bass from central Jones Bay, Lake Opeongo, Ontario; *N* is the number of samples analyzed.

Taxon	*N*	Length (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Invertebrate				
Crayfish	5[a]	48	−20.8	3.8
Zooplankton (250–500 μm)	1		−28.5	−0.8
Zooplankton (>500 μm)	1		−27.6	0.6
Unionid mussels	3	6.3	−26.4	1.2
			(−26.8 to −26.0)	(1.0–1.5)
Smallmouth bass				
Embryos	10	6.3	−24.8	6.3
		(4–9)	(−26.1 to −23.2)	(4.2–10.1)
Larvae	8	12.8	−24.7	3.6
		(10–15)	(−25.9 to −23.7)	(2.3–5.3)
Juveniles	10	23.9	−23.9	2.7
		(17–30)	(−24.5 to −23.4)	(2.4–2.9)
Juveniles	5	41.2	−20.7	3.2
		(30–46)	(−22.1 to −19.4)	(2.9–3.8)
Adults	5	225	−26.0	7.0
		(165–281)	(−28.4 to −25)	(6.5–7.7)

[a] Represents a composite sample of abdominal muscle tissue from five individual crayfish.

(Hecky and Hesslein 1995; France 1996b; Cabana 1997). Unionid mussels and zooplankton were chosen as indicators of the $\delta^{13}C$ of pelagic primary production. Zooplankton provided a short-term indicator (specific to the study period), and unionids indicated long-term average signatures. Unionid mussels, zooplankton, and crayfish were collected from the littoral zone in the direct vicinity of the study nests on June 28, 1995.

All samples were frozen, dried at 75°C for 36 h, and ground into a fine powder with mortar and pestle. Age-0 smallmouth bass specimens collected on June 19, 22, 25, and 28 were analyzed as pooled samples of up to 10 individuals. After June 28, individual fish were analyzed and the mean $\delta^{15}N$ and $\delta^{13}C$ of the five measurements per nest date were used for subsequent analysis. Nest determination was not possible for fish collected on July 24 due to dispersal from their original nest area by this time. Abdominal muscle tissues from the five crayfish collected at the sample site were pooled for analysis. Dry sample material was packed into 4 × 6-mm tin capsules for subsequent isotopic analysis. Stable carbon and nitrogen isotope analysis was performed with a continuous-flow VG Micromass 903E isotope-ratio mass spectrometer at the Environmental Isotope Laboratory (Department of Earth Sciences, University of Waterloo, Waterloo, Ontario). Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰ or ''per mil'') deviation from a standard material: $\delta^{13}C$ (or $\delta^{15}N$) = $([R_{sample}/R_{standard}] - 1) \times 1{,}000$; $R = {}^{13}C/{}^{12}C$ (or ${}^{15}N/{}^{14}N$). A more positive (or less negative for carbon) isotopic value is said to be isotopically enriched, meaning that the sample contains proportionally more of the heavy stable isotope (^{13}C or ^{15}N). The standard materials are Pee Dee belemnite limestone for $\delta^{13}C$ (Craig 1957) and atmospheric nitrogen for $\delta^{15}N$ (both standards have a ‰ value set to 0). The working standard was DORM-1 powdered dogfish standard ($\delta^{15}N = 10.3$, $\delta^{13}C = -19.5$) provided by the National Research Council of Canada, Institute for Environmental Chemistry, Ottawa. We analyzed 15% of the samples in duplicate; the standard errors for replicates were 0.13‰ for $\delta^{13}C$ and 0.15‰ for $\delta^{15}N$.

Results

Patterns in Nitrogen Isotope Values

Stable nitrogen isotope ratios characterizing invertebrates (crayfish, unionid mussels, zooplankton) and smallmouth bass (adults and four classes of age-0 fish) are presented in Table 1. Zooplankton and unionid mussels, both considered to be primary consumers (trophic position = 2.0) had mean $\delta^{15}N$ values of −0.1‰ and 1.2‰, respectively. The $\delta^{15}N$ of crayfish abdominal muscle tissue was 3.8‰, indicative of predatory feeding habits. The average $\delta^{15}N$ of five adult smallmouth bass was 7.0‰; considerably lower than the $\delta^{15}N$ of embryos immediately after hatching (9–10‰). From this elevated signature, $\delta^{15}N$ of age-0 bass declined rapidly, approaching 2.0‰ for smallmouth bass 15 mm in total length (Figure 2). Length was a highly significant predictor of the $\delta^{15}N$ of smallmouth bass between 4 and 15 mm:

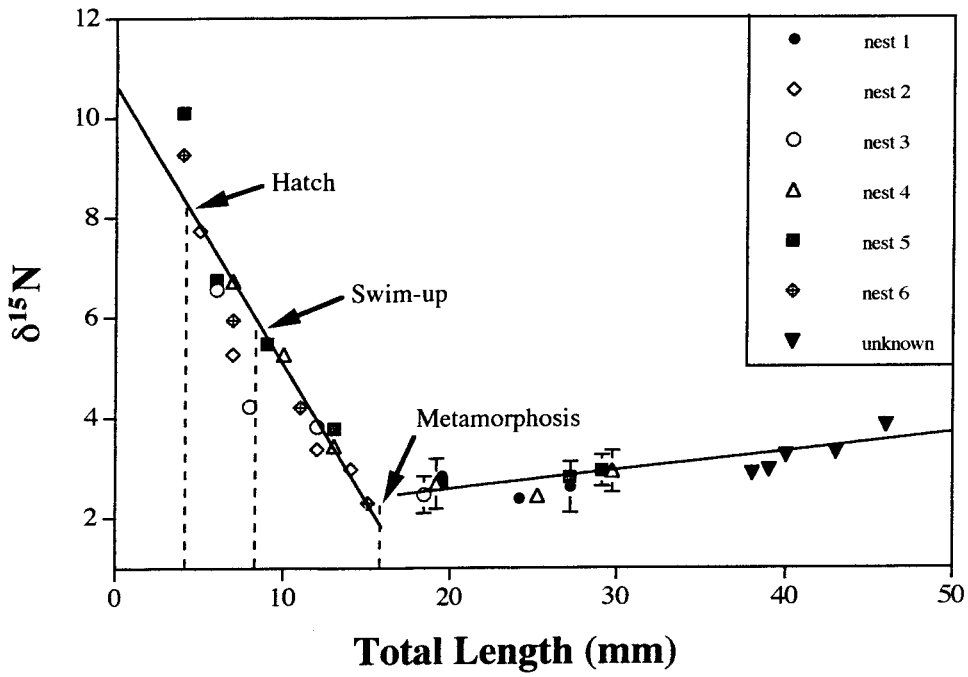

FIGURE 2.—Nest-specific mean $\delta^{15}N$ plotted against total length for age-0 smallmouth bass from Lake Opeongo between June 19 and July 24, 1995. Data points for lengths less than 15 mm represent pooled samples of up to 10 individual smallmouth bass, those between 17 and 35 mm represent nest-specific averages of 5 individually analyzed smallmouth bass (error bars are ±SD), and points over 35 mm represent individual bass for which nest identity could not be determined due to dispersal of fish from their original nest area.

$$\delta^{15}N = -0.566 \cdot \text{length (mm)} + 10.53$$

($N = 18$; $r^2 = 0.86$; $P < 0.001$; SE = 0.83; $F = 98.79$; df = 1, 16). Between 17 mm and 46 mm, there was an increase in $\delta^{15}N$ from 2.3‰ to 3.8‰, and total length was a highly significant predictor of smallmouth bass $\delta^{15}N$:

$$\delta^{15}N = 0.032 \cdot \text{length (mm)} + 1.90$$

($N = 14$; $r^2 = 0.63$; $P = 0.001$; SE = 0.24; $F = 20.11$; df = 1, 12). Finally, among the five juveniles collected on the final sampling date (July 24), total length ranged from 38 to 46 mm, and $\delta^{15}N$ and total length were positively related:

$$\delta^{15}N = 0.11 \cdot \text{length (mm)} - 1.37$$

($N = 5$; $r^2 = 0.92$; $P = 0.009$; SE = 0.12; $F = 36.46$; df = 1, 3). Thus, the within-population variation in body size of age-0 smallmouth bass that emerged by late July was related to individual-level (intrapopulation) differences in $\delta^{15}N$ and inferred trophic position.

Analysis of covariance (ANCOVA) was used to search for nest-specific differences in the observed $\delta^{15}N$ shift among smallmouth bass 4–15 mm in length. When total length was used as a measure of development, no significant nest effects were observed (Figure 2; $N = 18$, $r^2 = 0.91$, df = 12, 1, 4, $P_{length} < 0.001$, $F_{length} = 102.68$, $P_{nest} = 0.29$, $F_{nest} = 1.41$). Alternatively, replacing length with sample date (day of year) resulted in significant nest-specific effects in the observed $\delta^{15}N$ shift (Figure 3; $N = 18$, $r^2 = 0.93$, df = 12, 1, 4, $P_{date} < 0.001$, $F_{date} = 153.83$, $P_{nest} = 0.002$, $F_{nest} = 7.92$). The nest-specific differences in isotopic shift when sample date is used as a measure of development is probably due to the fact that intraannual spawning and hatching times of smallmouth bass vary considerably (Ridgway and Friesen 1992).

Patterns in Carbon Isotope Values

The mean values of $\delta^{13}C$ for crayfish, zooplankton, unionid mussels, adult smallmouth bass, and four size-classes of age-0 smallmouth are presented in Table 1. Crayfish muscle tissue (an indicator of the $\delta^{13}C$ of benthic production; see Methods) had a $\delta^{13}C$ of −20.8‰. Zooplankton

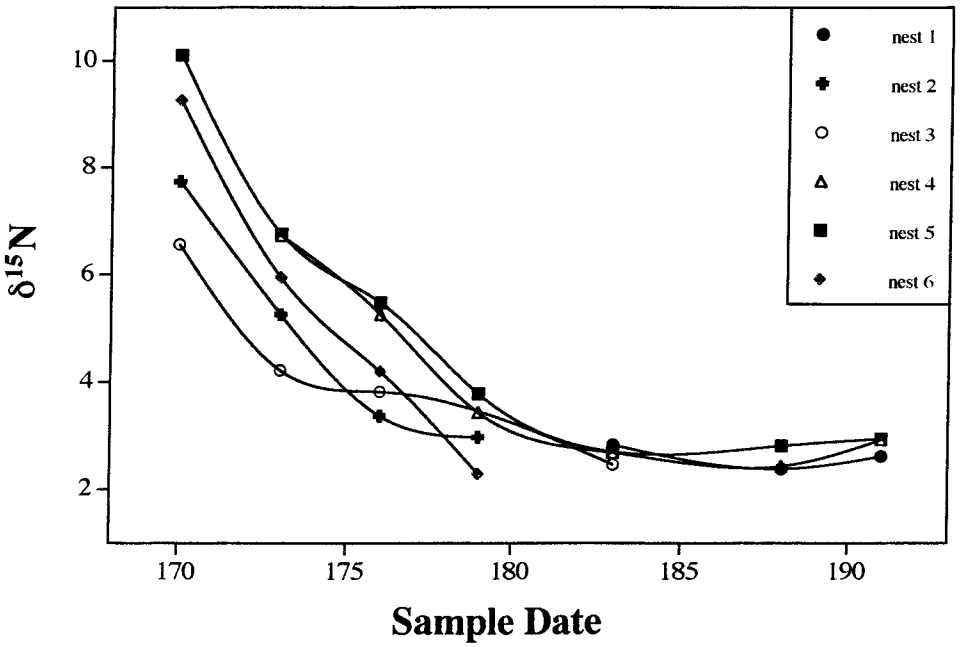

FIGURE 3.—Nest-specific mean $\delta^{15}N$ values plotted against sample date (day of year) for age-0 smallmouth bass collected from Lake Opeongo between June 19 (day 170) and July 10 (day 191), 1995. Smallmouth bass collected on July 24 were excluded because they could not be identified by nest. Lines connect the observations from each nest to emphasize nest-specific trends.

(250–500-μm and >500-μm size fractions) and unionid mussels (indicators of the $\delta^{13}C$ of pelagic production) had mean $\delta^{13}C$ values of −28‰ and −26.4‰, respectively.

The average $\delta^{13}C$ of the five adult smallmouth bass was −26‰. Values for adults were more negative than those for age-0 bass, which ranged in $\delta^{13}C$ from −19.4‰ to −26.1‰ (Figure 4). Overall, $\delta^{13}C$ of age-0 smallmouth bass increased significantly as a function of total length:

$$\delta^{13}C = 0.106 \cdot \text{length (mm)} - 25.87$$

($N = 33$; $r^2 = 0.67$; $P < 0.001$; SE = 0.95; $F = 61.69$; df = 1, 31). Within the 4–30 mm size range, the $\delta^{13}C$: body size relationship was weaker and exhibited a shallower slope:

$$\delta^{13}C = 0.052 \cdot \text{length (mm)} - 25.22$$

($N = 28$; $r^2 = 0.26$; $P = 0.003$; SE = 0.70; $F = 10.42$; df = 1, 27). Thus, much of the increase in $\delta^{13}C$ with total length is attributed to differences between bass longer than 30 mm and shorter than 30 mm (Student's *t*-test, df = 34, $P < 0.001$).

An ANCOVA was used to test for nest-specific differences in the $\delta^{13}C$ to total length relationship. There were significant ($P < 0.05$) effects of total length and nest, as well as a significant nest to length interaction, which indicated significant differences in the nest-specific slopes of the $\delta^{13}C$ to length relationships (Figure 5; $N = 29$; $r^2 = 0.79$; df = 1, 5, 5, 17; $P_{\text{length}} = 0.034$; $F_{\text{length}} = 5.30$; $P_{\text{nest}} = 0.003$; $F_{\text{nest}} = 5.56$; $P_{\text{length} \cdot \text{nest}} = 0.048$; $F_{\text{length} \cdot \text{nest}} = 2.84$). The overall nest-specific trend was that smallmouth bass from different nests began with distinct $\delta^{13}C$ signatures, but values converged by the time the fish metamorphosed (at 15 mm).

Discussion

Insights from $\delta^{15}N$ into the Feeding of Age-0 Fish

The elevated $\delta^{15}N$ signatures of posthatch smallmouth bass embryos (9–10‰ versus 7‰ for adults) was not surprising because the nitrogen pool of embryonic fish is inherited from their parents. Bass embryos subsist off the yolk sac and are thought to feed exogenously only during the later stages of the yolk sac period (referred to as the mixed-feeding period). Yet the decline in the $\delta^{15}N$ of smallmouth bass commenced almost immediately after hatching. This rapid posthatch decline in $\delta^{15}N$ can be attributed to one of two factors: (1) the trophic effect of embryos beginning

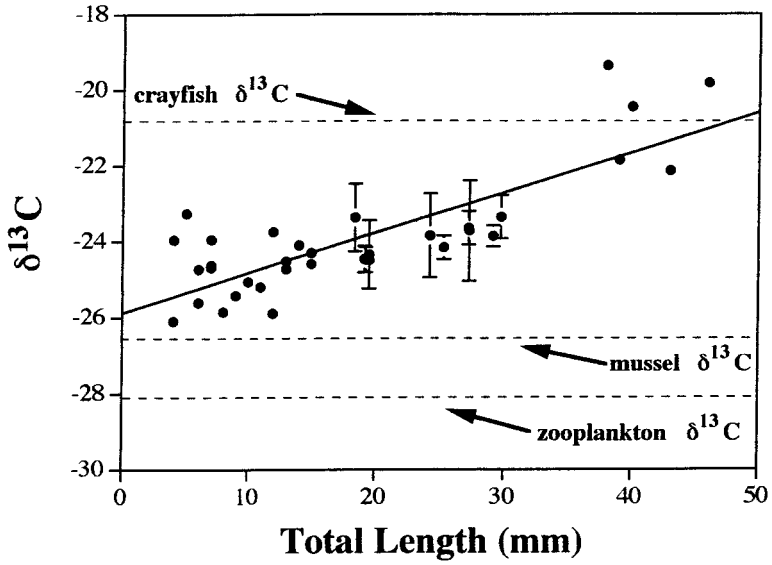

FIGURE 4.—The relationship between $\delta^{13}C$ and total length of age-0 smallmouth bass from Lake Opeongo. Each data point for lengths less than 15 mm represents a pooled sample of up to 10 individuals, those between 17 and 35 mm represent nest-specific averages of 5 individually analyzed age-0 smallmouth bass (error bars are ±SD), and points over 35 mm represent individual fry for which nest identity could not be determined. The $\delta^{13}C$ values characterizing crayfish, unionid mussels, and zooplankton are indicated by the dashed horizontal lines.

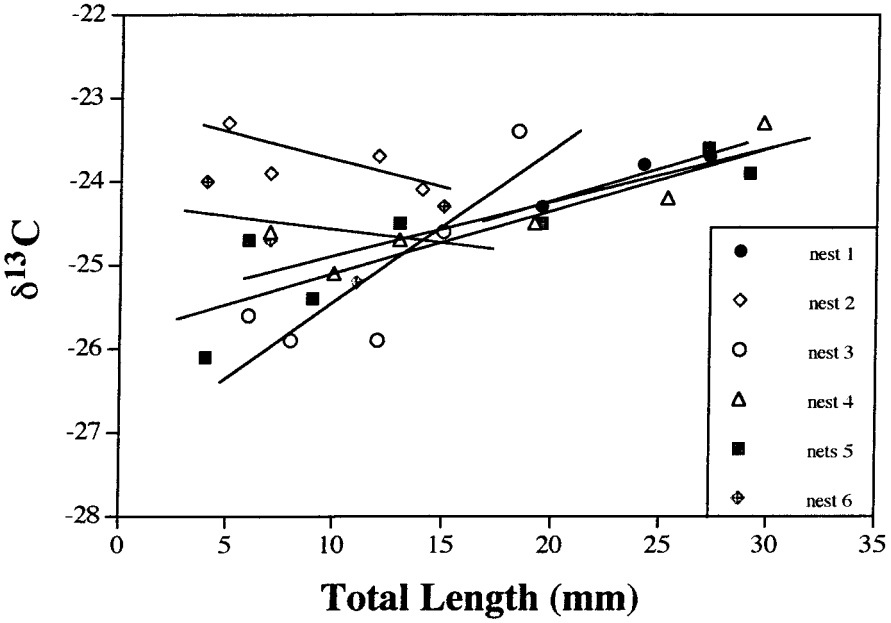

FIGURE 5.—Nest-specific differences in the relationship between total length and $\delta^{13}C$ for age-0 smallmouth bass from Lake Opeongo. Smallmouth bass collected on July 24 were excluded because their nest identity could not be determined.

to feed soon after hatching, which is earlier than previously believed or (2) the metabolic effect of embryos between 4 and 9 mm selectively using light nitrogen (^{14}N) over heavy nitrogen (^{15}N) from their available yolk sac nitrogen pool (isotopic fractionation) and excreting ^{15}N-rich waste products. Although no observational studies have investigated the timing of exogenous feeding for smallmouth bass, Kawamura and Washiyama (1989) documented behavioral changes in age-0 largemouth bass *M. salmoides* in the laboratory. They reported that exogenous feeding began 5 d after hatching and that the yolk sac was lost 9 d posthatch, indicating a 4–5 day mixed (exogenous–endogenous) feeding period. Additionally, it appears to be the act of exogenous feeding itself that stimulates the excretion of nitrogen by age-0 fish (Rice and Stokes 1973; Klumpp and von Westernhagen 1986).

Although swim-up marks the loss of the yolk sac and the transition from embryonic to larval life stage, $\delta^{15}N$ failed to detect a trophic shift corresponding with this event. The dramatic decrease in $\delta^{15}N$ that began at 4 mm was complete by 15 mm, by which time the endogenous $\delta^{15}N$ pool had been diluted by exogenous (dietary) nitrogen. Interestingly, the timing of full dilution of the endogenous $\delta^{15}N$ corresponded closely with metamorphosis (the dramatic morphological shift from larval to juvenile developmental stages) at 16 mm (Figure 2), potentially serving to define an ecological–life history boundary between larval and juvenile fish.

The dramatic decline in $\delta^{15}N$ between 4 and 15 mm provides a natural diet-changing experiment that allows comparison of the relative roles of growth and tissue turnover as the $\delta^{15}N$ pool of a consumer responds to changes in dietary $\delta^{15}N$. Because mass was not recorded when specimens were collected, lengths were converted to mass by using published length–weight relationships for age-0 smallmouth bass (George and Hadley 1979). A power curve was fit to the *observed* decline in $\delta^{15}N$ as a function of body mass (M):

$$\delta^{15}N = 0.20 \cdot M^{-0.89}.$$

Next, the shift in $\delta^{15}N$ that would be *expected* from considering only growth was modeled with an isotopic dilution equation (Fry and Arnold 1982):

$$\delta^{15}N_{gro} = \delta^{15}N_{asy} + (\delta^{15}N_{init} - \delta^{15}N_{asy})\,(M_{init}/M_x),$$

where, $\delta^{15}N_{gro}$ = modeled bass $\delta^{15}N$ due to growth at mass x; $\delta^{15}N_{asy}$ = asymptotic $\delta^{15}N$ value approached after extended growth; $\delta^{15}N_{init}$ = initial $\delta^{15}N$ value; M_{init} = initial mass of bass; M_x = mass of bass attained during growth. The highest $\delta^{15}N$ value predicted from the fitted power curve (8.7‰) was used as the initial $\delta^{15}N$ value, and the lowest $\delta^{15}N$ observed among smallmouth bass (2.3‰) as the asymptotic $\delta^{15}N$ value (Figure 6). A power curve was fit to the modeled shift in $\delta^{15}N$ attributed to growth alone:

$$\delta^{15}N = 0.70 \cdot M^{-0.59}.$$

These results indicate that the rate of change in the $\delta^{15}N$ of age-0 fish body tissue following changes in dietary $\delta^{15}N$ (and hence, the time interval integrated by stable isotope information) appears to be related to growth rate because 86% of the observed change in $\delta^{15}N$ was explained by the accretion of new tissue.

A relatively small proportion of the observed isotopic shift was unexplained by growth (14%) and is probably a result of the metabolic turnover of nitrogen. Because whole age-0 fish were analyzed, the observed nitrogen turnover can be attributed to the presence of tissues with elevated metabolic rates. Overall, the results of our model indicate that age-0 smallmouth bass tend to conserve nitrogen and that the time period integrated by stable isotopes for age-0 fish is a function of growth rate. Although a number of other diet-switching studies that used stable isotope have been published (Fry and Arnold 1982; Tieszen et al. 1983; Hobson and Clark 1992; Hobson 1995), the only other study to investigate isotopic shifts of fish in response to diet shifts was Hesslein et al. (1993). That study also reported that tissue accumulation explained the majority of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ shifts in muscle tissue of broad whitefish *Coregonus nasus* following an isotopic change in diet (although the $\delta^{15}N$ of experimental and control foods differed by only 1.9‰).

The $\delta^{15}N$ of juvenile smallmouth bass increased linearly with body size—from 2.3‰ for 15-mm fish to 3.8‰ for 46-mm fish. Although this isotopic shift may very well be indicative of an ontogenic increase in trophic position, part of this shift could alternatively be due to temporal changes in baseline $\delta^{15}N$ because the $\delta^{15}N$ of zooplankton undergoes seasonal and temporal shifts (Toda and Wada 1990; Gu et al. 1994; Kiriluk et al. 1995). Additionally, benthic primary consumers generally have lower $\delta^{15}N$ values than their pelagic counterparts (Vander Zanden and Rasmussen, in press). The relatively small size and high specific growth

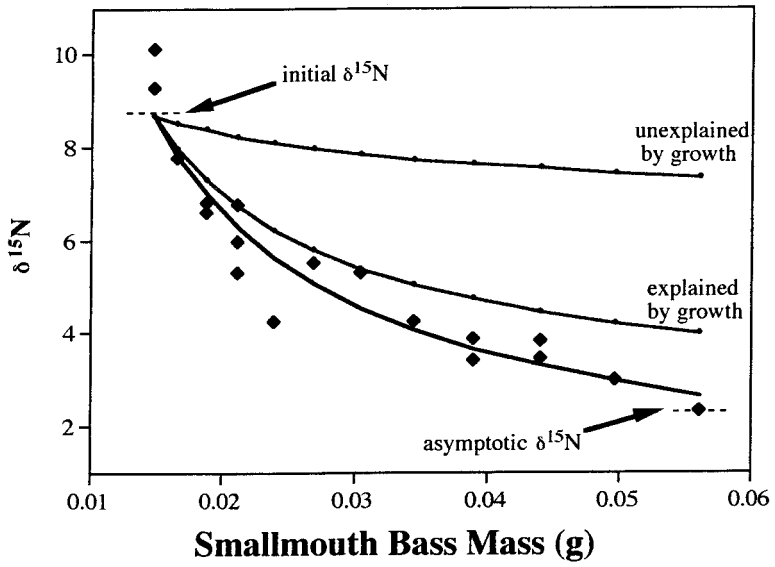

FIGURE 6.—A model of the relative contributions of growth and tissue turnover to the observed decline in $\delta^{15}N$ of age-0 smallmouth bass with size. Black diamonds are the observed shift from an endogenous to an exogenous $\delta^{15}N$ signature with size; the lower dashed line is the simulated shift in $\delta^{15}N$ attributed to tissue accumulation, which explains 86% of the observed $\delta^{15}N$ shift; the upper dashed line represents the portion of the observed shift in $\delta^{15}N$ unexplained by growth (14%), which is probably attributed to tissue turnover.

rates of juvenile smallmouth bass (compared with adults) means that their $\delta^{15}N$ values are likely to respond to shifts in the $\delta^{15}N$ of their prey. This sensitivity underscores the importance of accounting for both spatial and temporal variation in $\delta^{15}N$ signatures that characterize the base of the food web when making inferences concerning the trophic position of consumers. In this situation, this would require more frequent sampling of invertebrates during the study period.

Juvenile smallmouth bass collected on the final sampling date (July 24) ranged in length from 38 to 46 mm, and $\delta^{15}N$ was positively correlated with total length, indicating that the variation in body size established by midsummer was related to intrapopulation trophic differences. Perhaps these larger, high-$\delta^{15}N$ juvenile smallmouth bass also had an earlier birth date (Cargnelli and Gross 1996), thus providing these individuals with a longer growing season. Even so, the importance of this body size : $\delta^{15}N$ relationship is apparent in light of studies in northern temperate lakes that demonstrate that juvenile fish that attain the largest body size by winter experienced higher survivorship and were better able to survive winter starvation periods (Oliver et al. 1979; Shuter et al. 1980; Cargnelli and Gross 1996, 1997).

The elevated $\delta^{15}N$ of embryonic smallmouth bass (9–10‰) contrasts with the $\delta^{15}N$ of 2–4‰ characterizing typical secondary consumers from Lake Opeongo. These two distinct isotopic endpoints may provide a unique opportunity to trace predation upon embryonic–larval fish, as well as fish eggs. Evidence for predation on larval fish would not be evident in the muscle tissue of predators as the nitrogen pool probably turns over on the order of months to years (Hesslein et al. 1993). Short-term $\delta^{15}N$ shifts could be detected in predator tissues with high metabolic rates, such as liver tissue (Tieszen et al. 1983; Hobson and Clark 1992; but see Hesslein et al. 1993). This approach could be used as an indicator of cannibalism and predation upon larval fish and eggs, as long as the target potential prey item has a distinctive chemical signature in relation to other potential prey.

Insights from $\delta^{13}C$ into the Feeding of Age-0 Fish

Interpretation of smallmouth bass $\delta^{13}C$ was facilitated by considering their signatures in relation to pelagic and benthic $\delta^{13}C$ indicator taxa (Cabana 1997). Zooplankton and unionid mussels had similar carbon signatures (-26.4‰ to -28.5‰), which was expected as they are both pelagic primary consumers. Yet these two consumers differ in their utility as indicator taxa because unionids are orders of magnitude larger than zooplankton

and, thus, provide a baseline indicator that integrates over a much longer time period (Cabana and Rasmussen 1996). Consequently, zooplankton should provide a more appropriate indicator when considering age-0 smallmouth bass, and unionids should be a more suitable indicator for adult fish. The $\delta^{13}C$ value of crayfish tissue (−21‰) clearly indicates consumption of benthic prey, particularly in light of the literature synthesis by France (1995) showing that particulate organic matter and pelagic consumers seldom have such high $\delta^{13}C$ values.

The mean $\delta^{13}C$ of adult smallmouth bass from Lake Opeongo was −26‰, which is indicative of a pelagic prey source. Although the carbon pool of embryonic smallmouth bass is parentally derived, embryos were 1.2‰ more *enriched* on average relative to the carbon signature of adults. This was surprising, as lipid content of fish eggs and embryos is generally high relative to other life stages (Tocher and Sargent 1984; Tocher et al. 1985; Fraser et al. 1988) and lipids are *depleted* in ^{13}C relative to whole tissue (Peterson and Fry 1987; Kling et al. 1992; Hobson 1995). Consequently, eggs and embryos would be expected to have slightly lower (more negative) $\delta^{13}C$ than the parents based on this lipid effect. It should also be noted that the adult smallmouth bass sampled were not the same individual females who produced the eggs of our study nests. Future isotopic studies of age-0 fish should consider diet–egg fractionation and should include analysis of the actual parents (if a sample can be taken nonlethally).

Although isotopic analysis was performed on whole tissue samples, lipids are depleted in $\delta^{13}C$ relative to whole tissue (Peterson and Fry 1987; Kling et al. 1992). Because lipid content should decrease during the early development of age-0 smallmouth bass (Tocher and Sargent 1984; Tocher et al. 1985; Fraser et al. 1988; Miranda and Hubbard 1994) due to the loss of parentally derived (egg) lipids, the lipid-extracted $\delta^{13}C$ of embryonic and larval fish would be slightly higher than the values presented herein.

The primary shift in $\delta^{13}C$ is a 3–4‰ increase between smallmouth bass over 30 mm and smallmouth bass under 30 mm, which is indicative of a shift from a mixed pelagic–benthic diet to a primarily benthic diet. Bass over 30 mm are inferred to have a benthic diet, as their $\delta^{13}C$ (−21‰) is similar to crayfish from the study site (−20.8‰), as well as $\delta^{13}C$ values for benthic algae and benthic consumers from other studies (Hecky and Hesslein 1995; France 1995; Vander Zanden and Rasmussen, in press). This inference is corroborated by dietary studies reporting that juvenile smallmouth bass tend to shift from zooplankton to benthic invertebrates as they increase in size (Tester 1932; George and Hadley 1979). Benthic primary production appears to be an underappreciated source of production to food webs in general (Hecky and Hesslein 1995); stable isotope evidence established the importance of benthic primary production to certain size-classes of juvenile smallmouth bass.

The overall nest-specific trend in $\delta^{13}C$ was that smallmouth bass from different nests began with distinct signatures and that $\delta^{13}C$ values converge by the time the fish reach 15 mm (which is the size at which smallmouth bass approach their asymptotic $\delta^{15}N$; Figure 6). Thus, nest-specific differences were only apparent among embryos and larvae, whose $\delta^{13}C$ values are influenced by endogenous carbon. A slight decline in $\delta^{13}C$ was observed in all nests at approximately 10 mm (Figure 5), which, interestingly, corresponds with swim-up, which is when larvae lose their yolk sac and ascend into the water column to feed on zooplankton (which correspondingly, are depleted in ^{13}C).

Conclusion

Age-0 smallmouth bass undergo dramatic shifts in feeding strategy as they progress from embryonic, to larval, to juvenile life history stages. In this exploratory study, stable isotopes of carbon and nitrogen were useful in elucidating these trophic shifts. The utility of stable isotopes in trophic studies may be particularly fruitful considering the difficulties of gut content analysis for age-0 fish (McCarter and James 1993) and the high levels of spatial and temporal variation in feeding (Polis and Winemiller 1996). By providing time-integrated trophic information based on assimilated prey, stable isotopes can circumvent the problems of conventional dietary methods. In light of the importance of body size attained during early life stages to the survival and recruitment of fish populations (Miller et al. 1988; Cargnelli and Gross 1996), stable isotopes should be useful in determining the extent to which trophic differences are responsible for the observed variation in body size. Future studies of age-0 fish will benefit from combining isotopic and conventional approaches, whereby stable isotopes can provide a time-integrated depiction of feeding relationships, and dietary data can validate isotope-based inferences and provide a much finer taxonomic resolution.

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