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Validation of Daily Ring Deposition in the Otoliths of Age-0 Blue Catfish and Flathead Catfish

Peter C. Sakaris*
Southern Polytechnic State University, Department of Biology and Chemistry
1100 South Marietta Parkway, Marietta, Georgia 30060, USA

Daniel J. Daugherty and David L. Buckmeier
Texas Parks and Wildlife Department, Heart of the Hills Fisheries Science Center
5103 Junction Highway, Mountain Home, Texas 78058, USA

Abstract.—We validated methods for estimating daily age of age-0 blue catfish *Ictalurus furcatus* and flathead catfish *Pylodictis olivaris*. Larvae of each species were reared in the laboratory and were sampled approximately every 10 d over a 4-month period. Five individuals of each species were randomly selected for daily age estimation from each of nine age-groups, ranging from 40 to 119 d posthatch for blue catfish and 20–121 d posthatch for flathead catfish. Mean daily ring count and known age were related for both species, indicating that daily ring deposition occurred in the otoliths of these fishes. Daily increment counts were accurate through 60 d posthatch for blue catfish and through 72 d posthatch for flathead catfish, with mean deviance of estimated age within 1 d of the known age. For both species, daily increments could be enumerated for older fish; however, accuracy decreased with age. We encourage researchers to utilize our aging technique to estimate hatch dates, the timing and frequency of hatching, and early growth rates of catfishes in wild populations. Such early life history information will be valuable in enhancing the management and conservation of important catfish populations.

Introduction

Fisheries biologists have used otoliths to age larval fish for a variety of purposes, including the estimation of early growth and mortality rates, determination of timing and frequency of spawning, and evaluation of hatching success. These early life history characteristics have been related to abiotic and biotic factors, such as hydrology, environmental conditions, water temperature, and zooplankton abundance (e.g., Crecco and Savoy 1987; Limburg et al. 1999; Sammons et al. 2001). By identifying factors that affect hatching success and early growth and mortality, we can better understand what influences recruitment and ultimately the dynamics of a fish population. With this information, managers can improve stock assessment models and make better management decisions.

Researchers have validated techniques for using larval otoliths to estimate the daily age of various fish species (Miller and Storck 1982; Davis et al. 1985; Graham and Orth 1987; Sweatman and Kohler 1991; Parrish et al. 1994; DiCenzo and Bettoli 1995). Studies verifying daily increment deposition in the otoliths of larval catfish have been limited to the channel catfish *Ictalurus punctatus*. Holland-Bartels and Duval (1988) observed daily increments deposited in the otoliths of larval channel catfish up to 18 d posthatch, but difficulties with ring resolution were apparent after 20 d. Sakaris and Irwin (2008) developed a technique using transverse sections of otoliths to estimate the daily age of larval channel catfish. All channel catfish were accurately aged through 60 d posthatch while faster growing fish were accurately aged up to 100 d posthatch (Sakaris and Irwin 2008). Faster growth rates appeared to be associated with wider daily increments near the edges of otolith sections, facilitating accurate estimates over a broad range of larval channel catfish ages (Sakaris and Irwin 2008).

In addition to channel catfish, blue catfish *I. furcatus* and flathead catfish *Pylodictis olivaris* support

* Corresponding author: psakaris@spsu.edu

important sport and commercial fisheries throughout much of the United States (Graham 1999; Hubert 1999; Jackson 1999). Blue catfish and flathead catfish are also ecologically important as apex predators in their native environments. Unfortunately, these highly piscivorous species have been introduced outside of their native ranges where they have negatively impacted fish assemblages through predation (Jackson 1999; Graham 1999). By validating and refining larval aging techniques for these species, we will be able to collect early life history information that would be valuable in managing important native fisheries and possibly controlling introduced populations. Therefore, the objectives of our study were to (1) determine if deposition of daily increments occurs in the otoliths of larval blue and flathead catfishes, and (2) evaluate how accurately we can estimate larval age of these species.

Methods

Known-age blue catfish fry ($N \approx 400$) were obtained from the Texas Parks and Wildlife Department (TPWD), Jasper State Fish Hatchery (Jasper, Texas) on April 16, 2008 and transferred to the TPWD, Heart of the Hills Fisheries Science Center (Mountain Home, Texas). Fish were reared in a 2,362-L circular, flow-through tank and fed a combination diet of granulated feed (Silver Cup Granulated Salmon/Trout #1; Nelson and Sons, Inc., Murray, Utah), brine shrimp Artemia spp., and bloodworms (Chironomidae) to satiation throughout the study period. Flathead catfish were produced on site following the procedures described by Daugherty et al. (2011, this volume). Fish were reared in a 218-L, flow-through raceway on the same diet as described for blue catfish. Water temperature was monitored (hourly) using an optic temperature logger (HOBO Pendant; Onset Computer Corporation, Bourne, Massachusetts). Water temperature during the study varied on a diel basis (±0.5–2.5°C/d) but did not vary greatly over time (range = 19.4–22.9°C).

Ten individuals of each species were randomly sampled from the pool of available fish approximately every 10 d throughout the 4-month study period. Age (d) was recorded, and fish were frozen whole for age analyses. Five individuals of each species were then randomly subsampled from each age-group for daily age estimation (blue catfish: 40, 50, 60, 70, 83, 90, 96, 111, and 119 d posthatch; flathead catfish: 20, 30, 40, 50, 62, 72, 85, 104, and 121 d posthatch). We encountered difficulties extracting otoliths from blue catfish younger than 40 d, primarily a result of their small body (≤25 mm total length [TL]) and otolith sizes. Therefore, blue catfish were aged beginning at 40 d posthatch. Fish were measured to the nearest 1 mm TL and weighed to the nearest 0.1 g. Lapillar otoliths were extracted using fine-tipped forceps with a dissecting microscope and stored dry prior to preparation.

Otolith Preparation and Aging

Methods for otolith processing and daily age estimation were followed as described for channel catfish by Sakaris and Irwin (2008) with some modification. Otoliths of fish larvae were embedded in a clear epoxy resin (WestSystem, 105 Epoxy Resin, 206 Slow Hardener, Bay City, Michigan). After the resin cured (~24 h), we used an Buehler Isomet high-precision sectioning saw (Buehler, Ltd., Lake Bluff, Illinois) to cut out a smaller section of the resin block that contained the otolith. This step was added to previously described procedures to minimize the amount of sanding and polishing time and to provide a clean, flat plane for further processing. Otoliths embedded in resin were mounted with thermoplastic cement to microscope slides using the approach described for adult channel catfish otoliths (Buckmeier et al. 2002), with the anterior-posterior axis of each otolith positioned perpendicular to the plane of the slide. Otoliths were incrementally ground wet with 600-grit sandpaper and routinely viewed with a compound microscope (under 100× and 400× magnification) until the core and daily increments were visible in a transverse plane. The otolith was then inverted and ground incrementally on the other side until a thin, section (<0.1 mm thick) was obtained. Otolith sections were polished several times with 1,500-grit sandpaper until otolith cores and rings were clearly visible for age determination.

Otoliths were read three times each in random order without reference to known ages, and no two counts of the same otolith were made consecutively (Miller and Storck 1982; DiCenzo and Bettoli 1995; Sakaris and Irwin 2008). All otolith sections were viewed using an image analysis system at 400× magnification. Otolith rings were counted from the outer edge to the inner core of each otolith section. The mean of the three counts for each otolith was used for analyses. Otolith radii (mm) were measured along a transect line from the center of the core to the outer edge of each otolith (Figure 1).

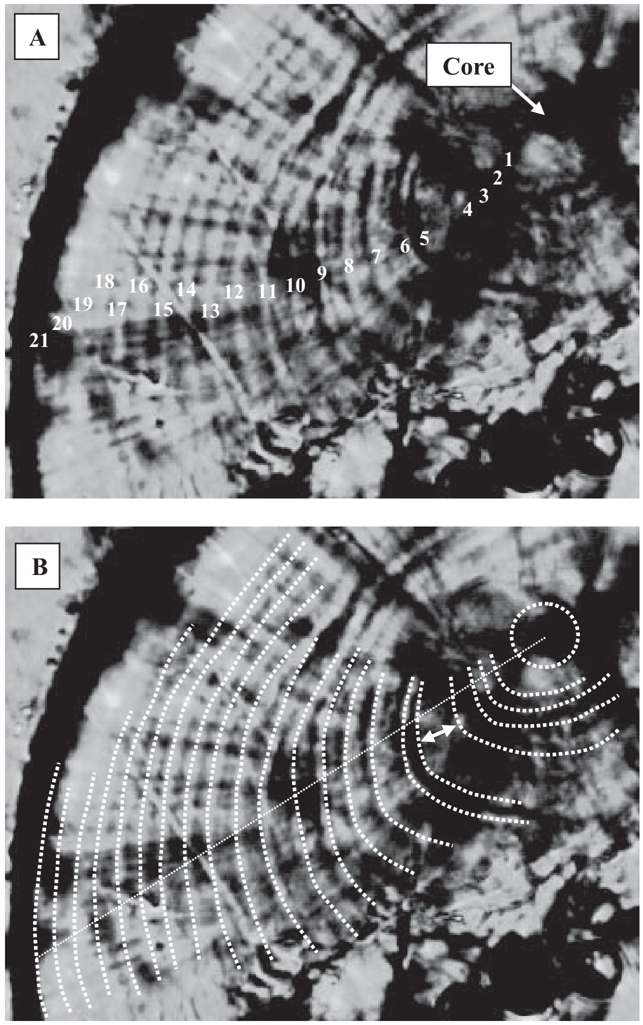

FIGURE 1. (A) Transverse section of an otolith extracted from a 20-d-old flathead catfish, revealing the otolith core and daily increments. (B) Same otolith with daily rings marked to illustrate how otoliths were read and interpreted. Rings near the core were typically tightly packed and required careful examination. Double arrow indicates the transition from endogenous to exogenous feeding. The age of this fish was overestimated by 1 d.

Statistical Analysis

Linear regression analysis was used to evaluate relations between the following variables: (1) mean ring count and known age, (2) fish total length and known age, and (3) fish total length and otolith radius. We tested null hypotheses that slopes and intercepts of regressions between mean ring count and known age equaled one (i.e., 1:1 ratio) and zero (i.e., intercept at the origin), respectively, using *t*-tests. Mean deviance was calculated for each age-group of each species as the mean difference between the estimated age and known age. Growth rates (mm/d) of blue catfish and flathead catfish were also compared using a *t*-test. Significance was set at $\alpha = 0.05$, and all statistics were conducted using SAS® software (SAS Institute 2003).

Results

We estimated the ages of 43 blue catfish (43 of 45; 96%) and 42 age-0 flathead catfish (42 of 45; 93%). Four blue catfish otoliths shifted in resin during preparation and were sectioned and read in an angled (near sagittal) plane. Five otolith sections were considered unreadable as a result of either errors during processing (i.e., missed cores) or an inability to resolve daily rings near otolith edges (i.e., two blue catfish and three flathead catfish otoliths; 5/90 or 6%). Total lengths and weights of blue catfish ranged from 26 to 63 mm and 0.1–1.7 g while lengths and weights of flathead catfish ranged from 16 to 61 mm and 0.1–2.6 g. Mean growth rates (mm/d) of blue catfish (0.57 ± 0.08 SD) and flathead catfish (0.55 ± 0.13 SD) were similar (t = 0.698, P = 0.49). Fish length was strongly related to known age for both species (blue catfish: r^2 = 0.923, P < 0.01; flathead catfish: r^2 = 0.922, P < 0.01) and positively related to otolith size (blue catfish: r^2 = 0.685, P < 0.01; flathead catfish: r^2 = 0.823, P < 0.01; Figures 2A–B).

Mean ring count was closely related to known age of blue catfish (r^2 = 0.976, P < 0.01; Figure 3A). However, the intercept of the regression was not equal to zero (df = 41, t = 3.02, P < 0.01), and the slope (0.897) was less than one for blue catfish (df = 41, t = 4.67, P < 0.01). Mean ring count was also closely related to known age of flathead catfish (r^2 > 0.962, P < 0.01; Figure 3B). The intercept was not equal to zero (df = 40, t = 3.74, P < 0.01), and the slope (0.833) was less than one for flathead catfish (df = 40, t = 6.34, P < 0.01).

Daily age estimates of blue catfish were reasonably accurate through 60 d posthatch, with mean devi-

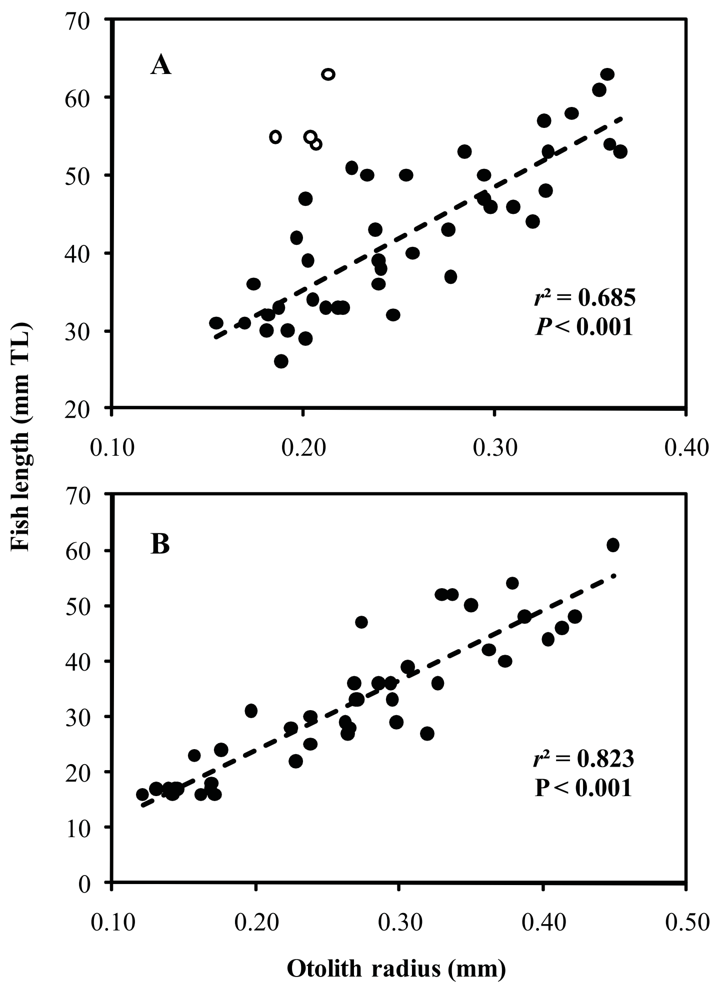

FIGURE 2. Relations between total length (TL; mm) and otolith radius (mm) for age-0 blue catfish (A) and flathead catfish (B). Open symbols (graph A) represent otoliths that shifted in resin during preparation and were sectioned in a sagittal plane. These data were not included in the regression analysis.

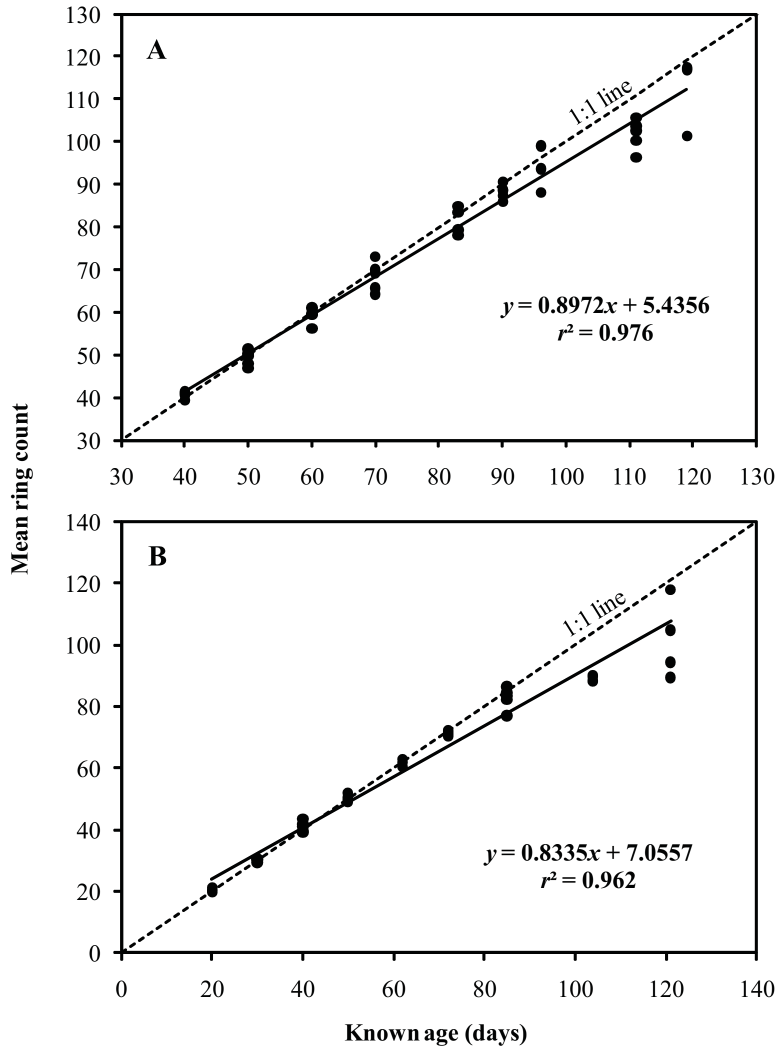

FIGURE 3. Relations between mean ring count and known age for age-0 blue catfish (A) and flathead catfish (B).

ance of estimated age within 1 d of the known age (Table 1). After 70 d posthatch, aging accuracy generally declined with age (mean deviance range: –1.5 to –9.3 d). Ages of blue catfish were underestimated by 2–18 d after 100 d posthatch (Table 1). Daily age estimates of flathead catfish were accurate through 72 d posthatch, with mean deviance of estimated age within 1 d of the known age (Table 2). After 72 d posthatch, accuracy of daily age estimation declined (mean deviance range: –2.4 to –16.1 d). Ages of flathead catfish were underestimated by 3–32 d after 100 d posthatch (Table 2). In general, ages of fish with the slowest growth rates were most severely underestimated (Figure 4).

Discussion

Our study has demonstrated that daily increments are deposited in the otoliths of larval blue catfish and flathead catfish. In addition, we determined that flathead catfish can be accurately aged through 72 d posthatch while blue catfish may be aged through 60 d under conditions similar to those in this study. Daily increments could be enumerated up to 80 d posthatch in both species; however, increments were more difficult to distinguish for older ages. Sakaris and Irwin (2008) reported similar results, with channel catfish accurately aged through 60 d posthatch.

TABLE 1. Aging statistics for the blue catfish, including coefficients of variation (CV), estimated age ranges, and mean deviance from known age for each age-group.

Known age (d)	*N*	CV (%)	Estimated age range (d)	Mean deviance (d)
40	5	2.0	39–41	0.7
50	5	3.6	47–51	–0.7
60	5	2.9	56–61	–0.8
70	5	5.1	64–73	–1.5
83	5	4.0	78–85	–2.3
90	5	2.0	86–91	–1.7
96	5	4.2	88–99	–2.4
111	5	3.5	96–106	–9.3
119	3[a]	8.2	101–117	–7.1

[a] Two of the five otolith sections from 119-d-old blue catfish were considered unreadable.

However, daily increments were enumerated up to 100 d posthatch in the otoliths of faster growing individuals (Sakaris and Irwin 2008).

We attribute decreased aging accuracy after 60 d posthatch to slow growth exhibited by blue and flathead catfish during the study. Slower growth rates result in narrower growth increments in the otoliths of fishes, resulting in the compression of daily rings near the edges of otolith sections. These closely spaced rings are more difficult to distinguish, which likely results in the underestimation of age. In this study, ages of older flathead catfish were more severely underestimated (up to 32 d) than ages of older blue catfish (up to 18 d), which was likely a result of slower growth rates among flathead catfish after 100 d posthatch. A post hoc growth comparison of older (>100 d posthatch) fish indicated that blue catfish grew at a significantly higher rate than flathead catfish ($t = 3.87$, $P < 0.01$). Similar results have been reported for other freshwater fishes (largemouth bass *Micropterus salmoides*, Miller and Storck 1982; spotted bass *M. punctulatus*, DiCenzo and Bettoli 1995). We suspect accuracy may be improved for older fish that are faster growing similar to the findings of Sakaris and Irwin (2008) for channel catfish.

Our experimental fish grew more slowly than age-0 catfishes from wild populations. Sakaris (2006) reported that early growth rates of age-0 channel catfish from the Tallapoosa River watershed (Alabama) ranged from 0.83 to 1.50 mm/d in the coastal plain and 0.60–1.07 mm/d in the piedmont region. Slow growth during our experiment was likely a result of cooler water temperatures (19.4–22.9°C). Catfishes typically begin spawning in late spring into early summer when water temperatures reach 21–29°C (Hubert 1999; Graham 1999). Thus, age-0 catfishes are usually growing during their first 100 d in warmer, midsummer water temperatures that would likely enhance fish growth. The relationship between growth

TABLE 2. Aging statistics for the flathead catfish, including coefficients of variation (CV), estimated age ranges, and mean deviance from known age for each age-group.

Known age (d)	*N*	CV (%)	Estimated age range (d)	Mean deviance (d)
20	5	2.5	20–21	0.2
30	5	2.0	29–31	–0.1
40	5	4.2	39–43	0.9
50	5	2.4	49–52	0.1
62	3[a]	1.9	60–63	–0.4
72	5	1.1	70–72	–0.7
85	5	4.2	77–86	–2.4
104	4[a]	1.1	88–90	–15.0
121	5	12.6	89–118	–16.1

[a] Three otolith sections were considered unreadable.

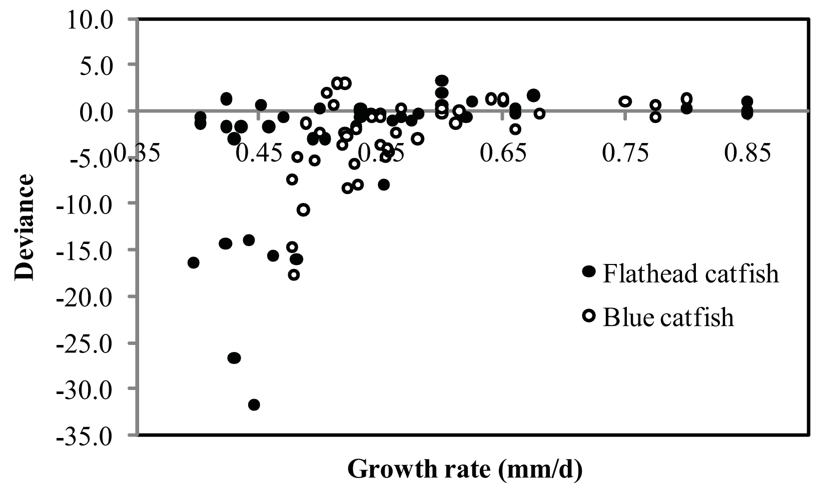

FIGURE 4. Deviance of estimated age from known age, as a function of fish growth. Ages of slow-growing fish (<0.55 mm/d) were often underestimated by up to 32 d.

of juvenile channel catfish and water temperature has been verified in several laboratory studies (Andrews and Stickney 1972; Fracalossi and Lovell 1995; Li et al. 2008). Andrews and Stickney (1972) reported that channel catfish fingerlings achieved the highest gains in total body lipid content and fatty acid composition and the best food conversion ratios at 30°C. Juvenile channel catfish exhibited the lowest growth rates at reduced temperatures (18°C and 22°C; Andrews and Stickney 1972). Li et al. (2008) observed a significant increase in growth of juvenile catfish with water temperature from 17°C to 27°C. We expect that similar effects of water temperature on growth would apply to blue catfish and flathead catfish.

Daily fluctuations in water temperature may also result in increased otolith clarity and overall reading accuracy of daily rings. Bestgen and Bundy (1998) reported that otolith increments were clearer and counts of increments more accurate for juvenile Colorado pikeminnow *Ptychocheilus lucius* reared at fluctuating temperatures than at constant temperatures. Water temperature was fluctuated ±2.5°C from 18, 22, or 26°C, and fluctuations mimicked the natural daily cycle (Bestgen and Bundy 1998). Diel water temperatures in our study also varied up to ±2.5°C; however, daily variation was not consistent throughout the study. This inconsistency, coupled with slower growth rates among older ages of fish, may explain the decreased aging accuracy we observed in older fish. However, future studies should specifically test the effects of water temperature regime on otolith clarity and reading accuracy.

Although we observed significant relations between fish length and otolith size, we did not confirm that otolith growth was directly proportional to fish growth. Other factors may influence the growth of otoliths in addition to somatic growth. Bestgen and Bundy (1998) reported that otoliths in larval Colorado pikeminnow continued to grow for up to 15 d after fish growth ceased, indicating that other factors contributed to otolith growth. Secor and Dean (1992) observed linear otolith growth in larval striped bass that were starved and exhibited negative somatic growth. In our study, approximately 18–32% of the variation in fish length could not be explained by otolith size. Therefore, back-calculation methods for determining growth histories should account for other factors (e.g., water and food quality) that predict length at age in addition to otolith size. Future studies should also examine how environmental factors affect otolith growth in catfishes.

We encourage fisheries scientists to utilize the daily aging technique to examine the early life history and determine biotic and abiotic factors that influence population dynamics and recruitment of catfishes. Age-0 blue catfish and flathead catfish can be accurately aged through 60 d posthatch. In addition, we postulate that faster growing individuals (>0.60 mm/d; Figure 4) may be aged up to 100 d posthatch, but further experiments are needed to test this hypothesis. Identifying factors that affect spawning timing and age-0 growth of these species will provide important information to maintain or enhance important catfish populations.

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