Otolith Daily Increment Deposition in Age-0 Smallmouth Bass Reared in Constant and Fluctuating Water Temperatures

Angela A. Hill & Kevin R. Bestgen

Larval Fish Laboratory, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, Colorado 80523, USA

Published online: 07 Aug 2014.

To cite this article: Angela A. Hill & Kevin R. Bestgen (2014) Otolith Daily Increment Deposition in Age-0 Smallmouth Bass Reared in Constant and Fluctuating Water Temperatures, North American Journal of Fisheries Management, 34:4, 774-779, DOI: 10.1080/02755947.2014.910575

To link to this article: http://dx.doi.org/10.1080/02755947.2014.910575
Otolith Daily Increment Deposition in Age-0 Smallmouth Bass Reared in Constant and Fluctuating Water Temperatures

Angela A. Hill* and Kevin R. Bestgen
Larval Fish Laboratory, Department of Fish, Wildlife, and Conservation Biology, Colorado State University, 1474 Campus Delivery, Fort Collins, Colorado 80523, USA

Abstract
We reared embryos and larvae of Smallmouth Bass Micropterus dolomieu in constant and diel fluctuating water temperatures (mean of 20°C) to clarify when otolith first daily increments were deposited and the periodicity of increment formation. Unlike the results of previously published studies, we found that first-increment formation began at hatching rather than 7–11 d later at swim-up. We confirmed that increment deposition was daily and extended the daily increment validation period from 21 d to 30 d posthatch. Accurate and precise age estimation was possible for Smallmouth Bass reared in both constant and fluctuating temperature environments, as age estimates rarely varied more than 1 d from true age. We also found consistent estimated ages using either left or right sagittae and showed relationships for Smallmouth Bass total length as a function of age, and otolith diameter as a function of total length. Otolith daily increment counts allow accurate and precise estimates of Smallmouth Bass age, which enables determination of hatch date, timing of spawning, and growth rate. These findings may assist with the management of this species, as well as provide information that can be used to disadvantage reproductive success of invasive Smallmouth Bass.

The number of daily increments in fish otoliths, patterns of increment deposition, and otolith growth may record life history events such as hatching, growth, periods of physiological stress, movements, and changes in water temperature or food abundance (Pannella 1971; Campana and Neilson 1985; Bestgen et al. 2006; Falke et al. 2010). Such information is particularly useful for fishes that are captured for commercial or recreational use, or for those that are rare and endangered, because understanding life history and ecological processes can contribute to their improved management and conservation.

Validation studies are required to enhance the accuracy and precision of information obtained from otoliths, particularly the timing of deposition of the first increment and the frequency (daily or otherwise) of increment formation (Beamish and McFarlane 1983; Campana 2001). Accurate predictions of the hatch time of young fish that are derived from otolith microincrement analyses may be especially important when managers attempt to influence year-class strength of a species. For example, understanding the reproductive timing of invasive populations of Smallmouth Bass Micropterus dolomieu in the Green River, Colorado and Utah, would allow for appropriately timed disruption of spawning via nest destruction, mechanical removal of adults, or by-flow manipulations that might reduce the survival of embryos and young (Winemiller and Taylor 1982; Ridgway and Friesen 1992; Jager et al. 1993; Knoteck and Orth 1998). Using embryos reared in the laboratory at 20–22°C and larvae reared at 17–23°C, but presumably not under a regular daily temperature fluctuation, Graham and Orth (1987) reported that first-increment deposition occurred at swim-up (7 d posthatch) and that increment deposition in larvae was daily for 14 d after swim-up. However, their age estimates for Smallmouth Bass larvae that were sacrificed on the same day and that were presumably the same age, varied widely. For example, estimates of age for Smallmouth Bass larvae that were 10 d after swim-up (e.g., should have had 10 daily age increments) ranged from 7 to 14 d, variation which they attributed...
to difficulty in distinguishing between daily and subdaily rings (increments). Graham and Orth (1987) also reported that the clarity of otolith increments of laboratory-reared Smallmouth Bass was lower than that of wild individuals, which may have contributed to variable age estimates.

While aging wild Smallmouth Bass from the Yampa River, Colorado, for another project, we found an inconsistency in previously reported aging techniques. We suspected that either the daily increment formation rate for Smallmouth Bass reported in Graham and Orth (1987) was different than one per day or that deposition of the first otolith increment occurred prior to swim-up. First-increment formation at hatch with a daily deposition pattern is the norm in many fishes, including Largemouth Bass *M. salmoides* (Campana and Neilson 1985; Isely and Noble 1987 [but see Miller and Storck 1982]; Bestgen and Bundy 1998). Therefore, we undertook our validation study to (1) document the timing of first-increment deposition in Smallmouth Bass, (2) determine if increment deposition rate in Smallmouth Bass sagittae was one per day, (3) understand the effects of temperature on initial and daily increment formation and otolith growth, (4) determine if left and right sagittae differed in rate of increment deposition, and (5) expand the validation period for otolith age estimation to 30 d.

**METHODS**

We collected fertilized Smallmouth Bass eggs from a pond at the Colorado Parks and Wildlife Hatchery in Wray, Colorado, on June 10, 2010. Hatchery-cultured broodfish were placed in ponds earlier in the spring and allowed to spawn naturally over existing pond substrate or gravel-filled containers. After Smallmouth Bass broodfish deposited eggs, the broodfish were removed from the ponds and the larvae were left to hatch and grow. We suctioned 670 eggs from a single nest with a baster and transported them in aerated coolers to the Aquatic Research Laboratory at Colorado State University, Fort Collins, Colorado. Taking all eggs from a single nest increased the likelihood that they were in a similar developmental state and that hatching would occur in a relatively short time period. Knowing that larvae hatched in a relatively short time frame was essential to interpreting when otolith formation and first-increment deposition occurred in Smallmouth Bass larvae. Observations showed eggs were in a similar and early stage of development (no somites or other readily identifiable early life stage features present), perhaps just fertilized and within 12 h old. The temperature of the pond when eggs were collected was 24°C.

Eggs were sorted from debris and divided in approximately equal numbers into four 2-L flow-through tanks. Two randomly chosen tanks were maintained at a constant 20°C water temperature, and two tanks were subjected to a fluctuating temperature treatment with a mean daily temperature of 20°C and diel fluctuation of ±2°C (see Bestgen and Bundy [1998] for more details on experimental setup); measured minima and maxima were 18°C and 23°C. In fluctuating tanks, temperature change was gradual over several hours and the diel change minimally approximated that for the Yampa River, Colorado, in early summer when Smallmouth Bass were spawning (U.S. Geological Survey Gauge 09251000). The diel light cycle was 14 h light : 10 h dark. The drip water supply to flow-through tanks was sufficient to change water volume about every 90 min. Live nauplii of brine shrimp *Artemia* spp. were hatched daily, and Smallmouth Bass larvae fed to satiation twice per day after jaw formation was noted.

We observed development daily and removed embryos that had fungus or were dead. Most hatching occurred on June 13 for both treatments and the 13 eggs that did not hatch that day were moved to a separate constant temperature tank, where they then hatched 1 d later on June 14. The separation of fish with different hatching dates was important to make inferences to timing of otolith formation and first-increment deposition. Most larvae (about 90%) in fluctuating tanks died on June 13, and we supplemented those with larvae from the constant temperature tanks. Thus, valid comparisons between temperature treatments could be achieved because larvae were subjected to their correct treatments since the day of hatch.

Five live eggs or larvae from each temperature treatment were preserved daily in late morning from June 11 until June 30 in 100% ethanol. Only healthy embryos or larvae were selected. After June 30, preservation continued every third day until no fish remained. The 13 embryos that did not hatch until June 14 were preserved last, and their later hatch date was adjusted in analyses. Preserved samples were labeled with date, time, and treatment type. After experiments were completed, preserved samples were reassigned random numbers so that fish sample date was unknown when the reader was counting otolith increments. We treated individual fish as independent experimental replicates (e.g., Bestgen and Bundy 1998). We randomly selected three fish from each sample and used samples collected the first 5 d posthatch (June 13–18), samples collected every other day from June 19 to 30, and samples from every third day after that. We measured each fish with electronic calipers to the nearest 0.01 mm TL. Both left and right sagittal otoliths were extracted and mounted on separate microscope slides (Stevenson and Campana 1992). Otoliths were fixed to the slide with cyanoacrylate glue that bonded to glass and were then polished using lapping film (0.3–12.0-micron grit size). A compound microscope fitted with a calibrated ocular micrometer was used to measure the maximum diameter of each sagittae, typically from the tip of rostrum to the postrostrum (Stevenson and Campana 1992). Core diameter was measured at 320 × magnification and was the maximum diameter of the first distinct and dark band surrounding the primordia. Counts of otolith microincrements were at 320 × magnification; immersion oil placed on the otolith increased increment clarity. Increments were counted in the sagittal plane of each otolith.
and one increment consisted of one light band and one dark band (the L-zone and D-zone, after Kalish et al. 1995).

Reader One (first author) counted increments in all left and right sagittae one time to gain familiarity with otolith growth and aging. Reader Two (second author) reread several of those otoliths to confirm age estimates. All counts by each reader were performed blind to the true age or any knowledge of the specimen, measured otolith size, or preservation date. The first reader then conducted a second series of three consecutive increment counts for each otolith, again in a blind fashion; those counts were averaged to determine the final increment count for each otolith. The mean difference between the first and second otolith readings, calculated only after all readings were completed, was 0.03 increments with a range of −1 to +2 increments. The maximum difference between Reader One and Reader Two was ±1 increment, demonstrating the validity of having a single reader conduct all increment counts.

We used analysis of covariance (ANCOVA) to compare slope and intercept of age estimates as a function of known age, using water temperature regime (fluctuating and constant) as the covariate. We then used regression to estimate the effects of constant and fluctuating temperature regimes on relationships of increment count as a function of age in days posthatch (true age). We also used regression to evaluate if increment counts were different for the left and right sagittae of individual fish. All statistical analyses were conducted using SAS statistical software (SAS Institute 2012; version 9.3).

**RESULTS**

Observations showed that embryo development proceeded at similar rates for fish in constant and fluctuating temperature treatments. Movement of embryos in each treatment was first detected on June 12, and most hatching began and was completed on June 13. Thus, hatching occurred about 4 d after embryo collection and likely within 5 d of fertilization; mean TL of 1-d-old larvae was 5.6 mm. Both sagittae and lapilli were easily observed in just-hatched live fish under a dissecting microscope at 10× magnification. The eyes of larvae were pigmented by June 16, 3 d posthatch, and a few fish were attempting to swim. Pectoral fins were noted on June 17, followed by the formation of jaws and a functional mouth on June 18. All larvae were buoyant (gas bladder inflated), swimming in the water column, and actively feeding on June 22, 9 d posthatch, when mean TL was 8.5 mm.

Constant and fluctuating thermal regimes had no effect on otolith microstructure or fish length but had a small effect on otoliths from fish in the fluctuating temperature treatment were 0.03 increments with a range of −1 to +2 increments. The maximum difference between Reader One and Reader Two was ±1 increment, demonstrating the validity of having a single reader conduct all increment counts.

We used analysis of covariance (ANCOVA) to compare slope and intercept of age estimates as a function of known age, using water temperature regime (fluctuating and constant) as the covariate. We then used regression to estimate the effects of constant and fluctuating temperature regimes on relationships of increment count as a function of age in days posthatch (true age). We also used regression to evaluate if increment counts were different for the left and right sagittae of individual fish. All statistical analyses were conducted using SAS statistical software (SAS Institute 2012; version 9.3).

**TABLE 1.** Numerator and denominator degrees of freedom, F-values, and P-values for ANCOVA relationships of the following: (1) estimated age (increment counts) as a function of true age (days posthatch) for Smallmouth Bass from constant or fluctuating temperature treatments, (2) estimated age as a function of true age when compared for left and right sagittae, (3) TL of Smallmouth Bass larvae as a function of true age for fish from constant or fluctuating temperature treatments, and (4) sagittae diameter as a function of TL for fish from constant or fluctuating temperature treatments.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1.81</td>
<td>0.30</td>
<td>1</td>
<td>0.59</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.149</td>
<td>0.005</td>
<td>1</td>
<td>0.07</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1.82</td>
<td>1.23</td>
<td>1</td>
<td>2.34</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1.82</td>
<td>3.81</td>
<td>1</td>
<td>0.71</td>
<td>0.40</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Constant and fluctuating thermal regimes had no effect on otolith microstructure or fish length but had a small effect on the diameter of the otoliths. The ANCOVA showed that the timing of first-increment deposition and increment deposition rates were not different in otoliths of fish from constant or fluctuating temperature treatments for the relationship of estimated age (increment counts) as a function of true age (days posthatch; Table 1). Consequently, increment counts for fish in both treatments were combined for further analyses. Additionally, slopes and intercepts for relationships of estimated age as a function of true age were similar when compared for left and right sagittae. Thus, the median increment count of the right and left sagittae (three readings each) was used as the estimated age for each fish in subsequent analyses. Likewise, TL of Smallmouth Bass larvae as a function of true age in the two temperature treatments was similar. Finally, the ANCOVA showed that sagittae in fish from the fluctuating temperature treatment grew only slightly faster than in fish in the constant temperature treatment, and otoliths from fish in the fluctuating temperature treatment were only about 8% larger.

The least-squares regression relationship, estimated using combined data from constant and fluctuating temperature treatments and the median values from left and right sagittae, supported the idea that increment deposition began at hatching and continued at a rate of one per day through 30 d posthatch (Table 2). This was true because the slope and intercept parameter values were close to 1 and 0, respectively (P = 0.606 and P = 0.656), and estimated age from increment counts showed little variation over the range of true ages (CV [100 · SD/mean] = 4.5%; Figure 1). Observations of otolith margins of fish preserved 1 d posthatch (June 14) showed a small but distinct light–dark band, which also supported the notion that first-increment deposition occurred the day of hatching (Figure 2).

**DISCUSSION**

Validation studies continue to be a necessary precursor for obtaining reliable information from fish otoliths, particularly for investigations that use daily increments (Beamish and McFarlane 1983; Campagna and Neilson 1985; Campagna 2001). We verified earlier findings of the validation study by Graham and Orth (1987), who suggested otolith microincrement deposition in Smallmouth Bass was daily, and we were able to obtain both accurate and precise estimates of age (Rice 1987; Campagna 2001). Our findings also extended the period of validation, based on known-age specimens, from 21 to 30 d posthatch.
TABLE 2. Least-squares statistics for regression relationships of the following: (1) Smallmouth Bass total length (TL; mm) as a function of days since hatching (true age) with fish from constant and fluctuating temperature regimes combined, (2) sagittae diameter (µm; mean of left and right sagittae) as a function of true age for fish from constant and fluctuating temperature regimes, and (3) otolith increment counts (estimated age in days) as a function of true age with fish from constant and fluctuating temperature regimes combined (P-value for significance test of slope not significantly different from 1). True ages were the median age from left and right sagittae (three counts each) in all cases.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Intercept (SE, P)</th>
<th>Slope (SE, P)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) TL–true age</td>
<td>5.56 (0.084, &lt;0.0001)</td>
<td>0.304 (0.006, &lt;0.001)</td>
<td>0.971</td>
</tr>
<tr>
<td>(2) Sagittae diameter–TL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant temperature</td>
<td>−270 (14, &lt;0.0001)</td>
<td>47 (1.4, &lt;0.001)</td>
<td>0.971</td>
</tr>
<tr>
<td>Fluctuating temperature</td>
<td>−280 (15, &lt;0.0001)</td>
<td>48 (1.6, &lt;0.001)</td>
<td>0.963</td>
</tr>
<tr>
<td>(3) Estimated age–true age</td>
<td>−0.043 (0.095, 0.656)</td>
<td>0.997 (0.007, 0.606)</td>
<td>0.996</td>
</tr>
</tbody>
</table>

Daily increment deposition is routinely observed in the otoliths of many fishes (Campana and Neilson 1985; Campana 2001), and we found increments in Smallmouth Bass otoliths from both temperature treatments to be clear and easy to read. While even a 30 d validation period is relatively short, the finding that wild Smallmouth Bass deposit clear and easily identifiable otolith increments suggests that reliable aging is possible as long as fish are actively growing, typically when water temperatures are > 10°C (Graham and Orth 1987). Smallmouth Bass otoliths will be useful to estimate recruitment, growth, and survival of larvae relative to biotic and abiotic stressors (Crecco and Savoy 1985; Bunnell et al. 2003; Bestgen et al. 2006).

A major departure of our findings from those of Graham and Orth (1987) was that increment deposition began at hatching rather than at swim-up. Timing of first-increment deposition (daily or annual) is an important aspect of any aging or validation study because, as Campana (2001) noted, a correctly defined starting point is needed or age determinations will be wrong by a constant amount. We first suspected discrepancies in the timing of first-increment deposition based on the presence of increments positioned close to the nucleus in otoliths from wild Smallmouth Bass in the Yampa River, Colorado. Those included just-hatched, preswim-up fish, which, according to Graham and Orth (1987), should not have readily identifiable daily increments. We also suspected that the wide variation in age estimates of laboratory-reared Smallmouth Bass in Graham and Orth (1987) derived from confusion regarding the identification of daily or subdaily increments. Similarly, Miller and Storck (1982) suggested that “prolarval rings” (a minimum of seven increments are visible in their Figure 1) in Largemouth Bass otoliths were present at hatching but were difficult to see when fish were older because otoliths became increasingly opaque.
and the rings would not be visible in later life. Isely and Noble (1987) later confirmed the deposition of first otolith increments at hatching in Largemouth Bass. Specimens used by Graham and Ort\(\text{h} \) (1987) were from an irregularly fluctuating environment (17–23°C) over 14 d after swim-up that might have caused deposition of irregular increments of varying clarity and thus yielded results different from ours.

The potential bias caused by the assumption that first-increment deposition in Smallmouth Bass begins at swim-up rather than hatching depends, in part, on the specific use of otolith information. The range of time from hatch to swim-up was reported as 7–11 d (Graham and Orth 1987; Ridgway and Friesen 1992); time to swim-up in our study was 9 d at 20°C. Thus, estimation of hatch dates based on subtracting increment-based fish ages from fish capture date could be biased by as much as 7–11 d and affect comparisons of hatch timing in disparate geographic areas, such as Virginia, South Dakota, and Ontario (e.g., Ridgway and Friesen 1992; Sabo and Orth 1995; Phelps et al. 2008). Further, estimation of growth rates based on the size of fish at capture could be biased low, again if changes in fish length posthatching (about 5.5 mm TL) are divided by the number of daily increments counted and inflated by adding an additional 7 (or up to 11) d. The relative bias would be greatest for relatively young fish and decrease for older fish since the proportion of days before swim-up to total age would decrease over time. The bias induced by this method would be increased if the length at swim-up (8.5 mm TL), rather than the length at hatching (5.5 mm TL), was used to determine the change in length to capture, as was done by Phelps et al. (2008), because the divisor for the daily growth calculation was the number of days between hatching and capture, not swim-up and capture. Regardless, investigators may wish to assess if potential aging bias for young Smallmouth Bass affected the conclusions of previous studies.

Our specific purpose for estimating Smallmouth Bass age using otoliths was to predict periods of reproduction relative to environmental cues so that spawning and hatching periods can be predicted. This would allow for appropriately timed disturbances in places like the dam-regulated Green River, where flow spikes could reduce reproductive success and disadvantage invasive Smallmouth Bass populations. Accurate and precise age estimation will allow managers to correctly time disturbances that target specific portions of the reproductive effort and aid in reducing the negative effects of Smallmouth Bass on native fishes in the upper Colorado River basin.

ACKNOWLEDGMENTS

We thank J. McKissick and B. Egloff of Colorado Parks and Wildlife, Warmwater Fish Hatchery in Wray, Colorado, for assistance in obtaining Smallmouth Bass embryos. We also thank J. Fowler and J. Charles for assistance with embryo collection and laboratory activities, D. Propst, and anonymous reviewers for helpful comments on the manuscript. This is Larval Fish Laboratory Contribution 176.

REFERENCES


