Validation of Daily Increments Periodicity in Otoliths of Spotted Gar

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Abstract: Accurate age and growth information is essential in successful management of fish populations and for understanding early life history. We validated daily increment deposition, including the timing of first ring formation, for spotted gar (*Lepisosteus oculatus*) through 127 days post hatch. Fry were produced from hatchery-spawned specimens, and up to 10 individuals per week were sacrificed and their otoliths (sagitta, lapillus, and asteriscus) removed for daily age estimation. Daily age estimates for all three otolith pairs were significantly related to known age. The strongest relationships existed for measurements from the sagitta ($r^2 = 0.98$) and the lapillus ($r^2 = 0.99$) with asteriscus ($r^2 = 0.95$) the lowest. All age prediction models resulted in a slope near unity, indicating that ring deposition occurred approximately daily. Initiation of ring formation varied among otolith types, with deposition beginning 3, 7, and 9 days for the sagitta, lapillus, and asteriscus, respectively. Results of this study suggested that otoliths are useful to estimate daily age of spotted gar juveniles; these data may be used to back calculate hatch dates, estimate early growth rates, and correlate with environmental factor that influence spawning in wild populations. This early life history information will be valuable in better understanding the ecology of this species.

Key words: Lepisosteus oculatus, sagitta, lapillus, asteriscus, hatch date

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Gars (family: Lepisosteidae) are a basal lineage of fishes that are widespread in central and eastern North America and throughout Central America (Echelle and Grande 2014). Gars are native, large-bodied, top-level piscivores, and are important components of aquatic food webs (David et al. 2015). Nonetheless, these fishes have long been viewed as nuisance species, and as such, many aspects of their biology remain understudied (Scarnecchia 1992). Populations of several species within Lepisosteidae have declined as a result of habitat loss and removal efforts and now face conservation issues (Scarnecchia 1992, Alfaro et al. 2008, Staton et al. 2012, NatureServe 2016). Spotted gar (*Lepisosteus oculatus*), while globally secure, is a species of conservation concern at the northern edge of its range and is critically imperiled in Canada (Glass et al. 2011, Staton et al. 2012, David et al. 2015, NatureServe 2016, Ontario Ministry of Natural Resources and Forestry 2016) and Kansas, Ohio, and Pennsylvania and is thought to be extirpated in New Mexico (NatureServe 2016).

Research efforts on conservation for the spotted gar and other species of Lepisosteidae have mostly been directed toward understanding population dynamics. To date, most studies have focused on adults (Love 2004, Glass et al. 2011, Staton et al. 2012, David et al. 2015), but little is known about the early life history of gars. The recovery strategy for spotted gar in Canada emphasized the importance of early life history on population growth rates (Staton et al. 2012, Ontario Ministry of Natural Resources and Forestry 2016). Thus, these early life-history stages warrant further study to determine best practices to manage spotted gar populations.

Estimating daily age of young-of-year fish has been successfully used for many species to better understand early-life history ecology and the linkages to environmental factors. For example, daily increment analyses were used to determine that kelp bass (*Paralabrax clathratus*) spawned on a lunar cycle from June through August (Cordes and Allen 1997). Otoliths from juvenile European eel (*Anguilla anguilla*) provided a reliable record of larval life-history transition from marine to freshwater environments (Lecomte-Finiger 1992). Further, back-calculation of spawning dates from daily age estimates provided evidence for how water-level fluctuations affected spawning by alligator gar (*Atractosteus spatula*) in Lake Texoma, Oklahoma (Snow and Long 2015).

However, before daily ages can be successfully used for population management (e.g., estimation of spawning dates and calculating growth rates; Campana and Neilson 1985), validating their periodical formation is critical. Validation studies have been conducted for numerous freshwater and saltwater fish species (e.g. channel catfish [*Ictalurus punctatus*], Sakaris and Irwin 2008; gizzard shad [*Dorosoma cepedianum*], Davis et al. 1985; kelp bass, Cordes and Allen 1997; spotted seatrout [*Cynoscion nebulosus*], Powell et al. 2000; and white crappie [*Pomoxis annularis*], Sweatman and Kohler 1991). Although daily age validation studies exist for alligator gar (Sakaris et al. 2014, Long and Snow 2016), there are no studies currently available regarding daily age validation for *Lepisosteus* species. Additionally, validating daily increments of all three otolith pairs allows for a holistic assessment to determine the most suitable structure (Long and Snow 2016).

The purpose of this study is to determine the utility of each of three otolith pairs (sagitta, lapillus, and asteriscus) to estimate daily age of spotted gar. We validated daily increments and the timing of first ring formation in all three otolith pairs of known-age young-of-year (YOY) spotted gars. We also compared the precision among and between readers and accuracy of ring counts among the otolith pairs of known-age, hatchery-reared spotted gar.

Methods

In late April 2014, we collected mature spotted gar from Lake Thunderbird, Oklahoma, using boat electrofishing. After capture, fish were transported to the University of Oklahoma's Aquatic Research Facility (ARF), and housed in two 1600-L aerated holding tanks. Once water temperatures reached 20–22 °C (Frenette and Snow 2016) on 5 May 2014, an intramuscular injection of Luteinizing Hormone-Releasing Hormone Analogue at a concentration of 1 mg kg^{-1} of body weight was used to induce spawning. Fish were returned to the holding tanks, and branches from white willow (*Salix alba*) were added to act as spawning substrate (Frenette and Snow 2016). Spotted gar spawned within 24 to 36 h after injection. Peak hatch occurred on 11 May 2014 with lecithoextrophic (swim-up) beginning 7 days later. After swim-up, 16,452 juvenile spotted gar were transferred into four 0.04-ha grow-out ponds and held for 127 days. Gar were first fed pellets, krill, and wild-caught zooplankton until satiation within 30 min twice daily, transitioning to a weekly diet of 1,360 g (900–1050 fish) of live fathead minnows (*Pimephales promelas*). Up to 10 gar were sampled weekly from the ponds using dip nets, placed in labeled plastic bags, and frozen.

In the laboratory, otoliths were removed from each fish by positioning the specimen dorsal side down under a dissecting scope and removing the head with a transverse incision anterior to the pectoral girdle (Long and Snow 2016). The bottom jaw and gill structures were removed with forceps, exposing the ventral side of the braincase. The parasphenoid was then detached to expose the inner ear structures, located just under the large bulbous portion of the parasphenoid. After removing the parasphenoid, the saccule and lagena structures were revealed, allowing the sagittae and asterisci otoliths to be removed. The lapilli otoliths were then removed after removing brain matter from around the utricle structures (Mathiesen and Popper 1987, Long and Snow 2016).

Otoliths were browned for 2–5 min depending on size at 104 °C on a hot plate to increase contrast between accretion and discontinuous zones (Secor et al. 1992, Long and Snow 2016). After browning, otoliths were embedded in Loctite 349 (Mauck and Boxrucker 2004) and sectioned with a low speed IsoMet saw using a (127- × 0.4-mm) wafering blade. Sagittae were sectioned in a transverse plane near the anterior portion of the otolith (Sakaris et al. 2014, Long and Snow 2016) whereas asterisci and lapilli were sectioned near the center in a transverse and frontal plane, respectively (Long and Snow 2016; Figure 1) to remove excess material. After sectioning, otoliths were mounted to glass microscope slides with thermoplastic cement. Otoliths were polished wet using a 600-grit sand paper and routinely viewed under a compound microscope until daily increments were visible on the margin of the otoliths. Then the otoliths were flipped and polished until the core was visible (<0.5 mm thick depending on otolith size), then inverted again to estimate daily increments.

To estimate daily ages, otoliths were examined independently by two readers (Hoff et al. 1997) using a high resolution monitor connected to an optic-mount digital camera attached to an Olympus BH-2 compound microscope under 100×–400× objectives (Olympus Corporation, Lake Success, New York). Otoliths were selected at random with the readers having no reference to fish size or known age in order to reduce bias. Growth increments were first counted from the outer edge to the nucleus margin, and then a second count from the nucleus margin to the outer edge was conducted to verify the first count. If necessary, otoliths were polished multiple times to reveal growth increments near the nucleus (Roberts et al. 2004).

Linear regression and parameter estimates were calculated in Ex-

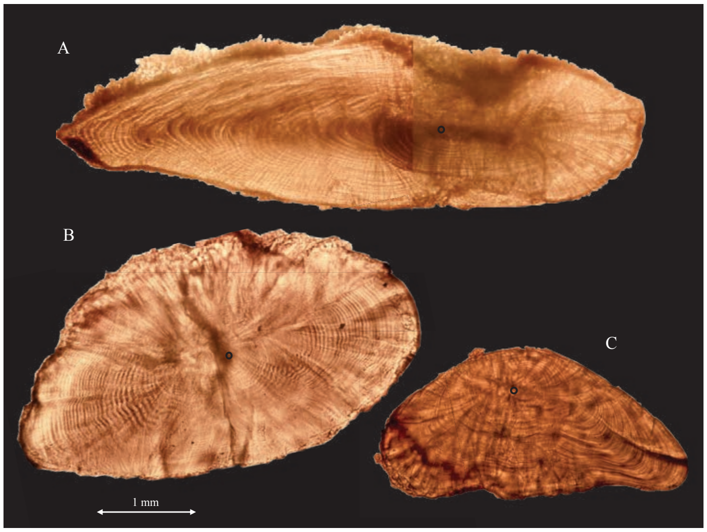

Figure 1. Photograph depicting daily increments in (A) sagitta, (B) lapillus, and (C) asteriscus otoliths from a 134-mm TL spotted gar that was 52 days old posthatch. The first ring of each otolith is identified with a circle.

cel to determine the relationship between mean assigned age and known age from hatch for each otolith type and test the hypotheses that slope = 1 (i.e., increment deposition occurred daily) and intercept = 0 (i.e., first increment formed at hatch) (Sakaris et al. 2014, Long and Snow 2016). Between-reader precision across otolith types was evaluated by the Coefficient of Variation (CV; Sakaris et al. 2014), which was tested by ANOVA (arc-sin square root transformation) using SAS software (SAS Institute 2012; Proc GLM) with post-hoc analyses for differences among otolith types with a Tukey HDS test (LSMEANS statement). Age-bias plots (mean age estimated by Reader 2 for each age estimated by Reader 1) were also constructed for each otolith type to assess reader bias (Campana et al. 1995). All statistical tests were evaluated for significance at $P \leq 0.05$.

Results

Eighty spotted gar were collected over 14 weeks, ranging in age from 12 to 127 days post-hatch (Table 1). Accuracy of age estimates was greatest for the sagitta and lapillus, and least for the asteriscus (Figure 2). Timing of ring formation varied among otolith types; approximately 3 days posthatch for sagitta, 7 days after hatch for lapillus, and 9 days after hatch for asteriscus (Table 2, Figure 2). There was high concordance between estimated and known age for all otoliths types ($r^2 > 0.95$). Ring formation occurred approximately daily, although slope estimates were statistically less than 1 for counts from sagitta and asterisci; the 95% CI for slope estimates included 1 only for ring counts based on lapilli (Table 2).

Table 1. Mean total length (TL) of known-age young-of-the-year spotted gar used for validating daily deposition of growth increments in otoliths.

Sample week (days from hatch)	*n*	Mean TL (mm)	SD
1 (12)	5	15.8	1.3
2 (16)	6	16.0	1.4
3 (22)	10	22.7	2.4
4 (29)	9	46.0	4.3
5 (38)	9	77.1	6.2
6 (47)	5	92.6	18.2
7 (52)	4	105.5	24.3
8 (65)	6	133.2	23.2
9 (75)	6	130.2	13.7
10 (85)	3	136.3	5.0
11 (100)	5	155.8	15.9
12 (108)	3	171.3	6.5
13 (117)	5	162.4	18.6
14 (127)	4	185.5	11.2

Table 2. Linear regression results (y-intercept and slope estimates including 95% confidence intervals) among otoliths for estimated ring counts from known age (from hatch) of spotted gar through 127 days. The *P*-value indicates whether the intercept or slope value significantly differs from the null hypothesis (0 for y-intercept and 1 for slope).

		95% CI		*P*-value		95% CI		*P*-value
Otolith	y-intercept	Lower	Upper	($H_0 = 0$)	Slope	Lower	Upper	($H_0 = 1$)
Sagittae	−3.17	−4.24	−2.10	0.01	0.96	0.94	0.97	0.01
Lapilli	−6.33	−7.23	−5.43	0.01	0.99	0.97	1.01	0.06
Asterisci	−8.77	−10.85	−6.67	0.01	0.87	0.84	0.90	0.01

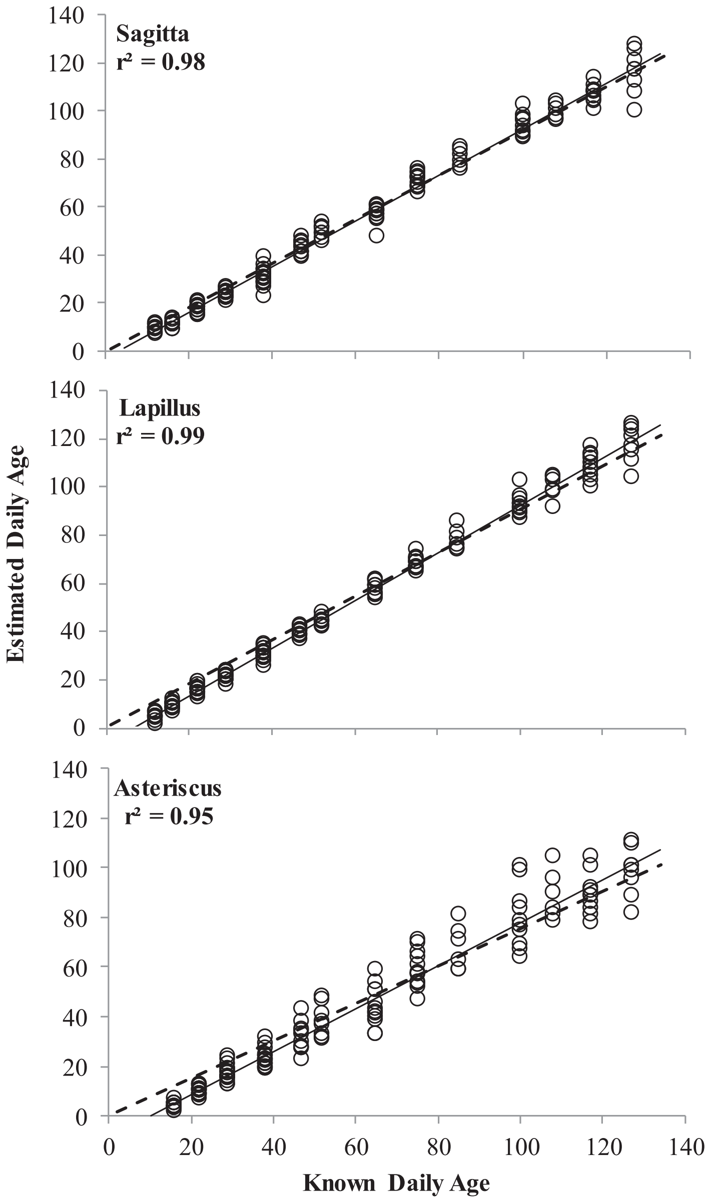

Figure 2. Linear regression between estimated daily increments among readers from otoliths of known-age spotted gar from hatch. The solid line represents the regression line and the dashed line represents a 1:1 relationship between estimated age and known age.

Precision of age estimates differed among otolith types ($F = 13.8$, df = 38, $P = 0.01$), with estimates from sagitta (CV = 7.5) and lapillus (CV = 7.4) being more precise (lowest CV estimates) than from the astersicus (CV = 15.6). No consistent bias was evident between

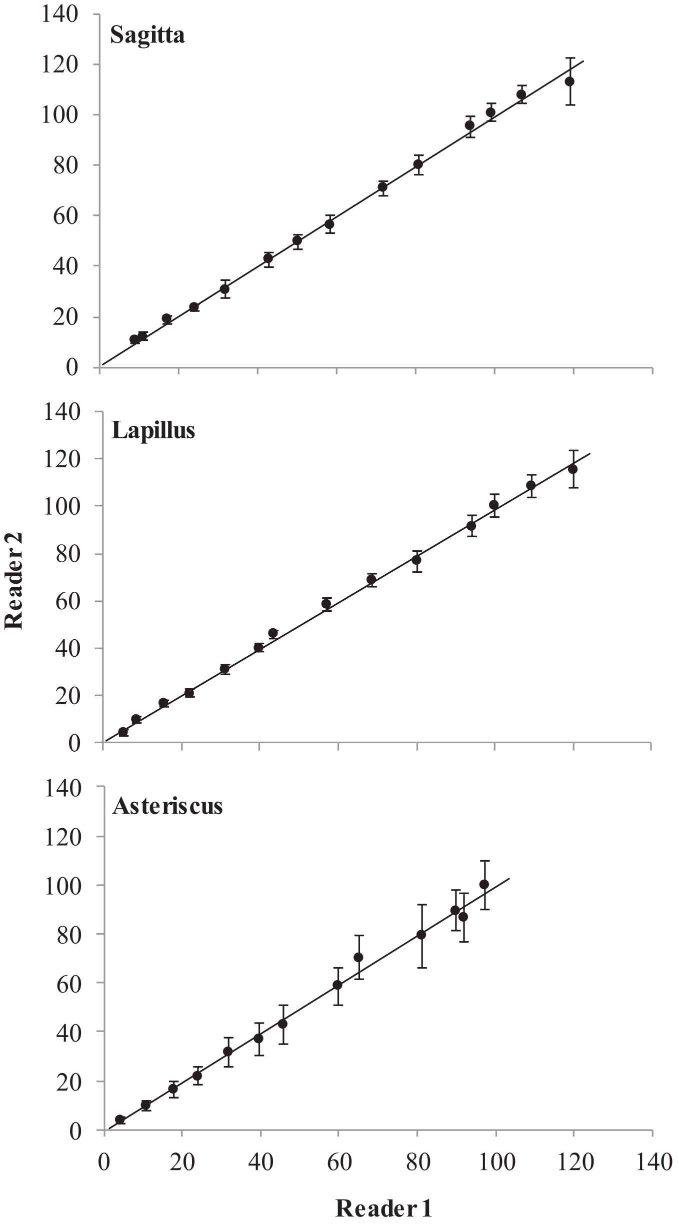

Figure 3. Age bias plots between mean age (±1 SD) estimated by Reader 2 and ages estimated by Reader 1 among otolith types. The solid line represents a 1:1 relationship between readers.

readers for any otoliths type, although errors bars around mean age estimates by Reader 2 increased with age estimated by Reader 1, especially for the asteriscus (Figure 3).

Discussion

We verified that increments were deposited approximately daily among otoliths of young-of-year spotted gar, at least through the first 127 days after hatching, and that the timing of first ring formation varied among otoliths. Daily age estimates from the lapillus and sagitta were the most useful when estimating daily age in spotted gar, but differences with regards to difficulty of preparation and examination were noted. Readers agreed that the morphology of the lapillus made it easier to prepare and view under the microscope. Further, daily increments in the lapillus were continuous when viewed on a single plane, in contrast to those from the sagittae, similar to what was observed in juvenile alligator gar (Long and Snow 2016). Thus, the nucleus margin of the sagitta often required multiple sanding efforts to reveal increment near the nucleus, which resulted in loss of outer rings. Therefore, accurate aging, especially for fish older than 85 days, was only possible when readers agreed on land marks in the otolith image and marked them on the monitor. Then, they removed the microscope slide, further sanded and polished the otolith to reveal the inner rings, and placed the slide back under the microscope, using the land marks for reference to continue counting. This likely explained the increase in error daily age estimates from sagittae of older, juvenile alligator gar (Sakaris et al. 2014, Long and Snow 2016). Although sagittae and lapilli provided similar results, sagittae were harder to process, but they were also much larger to manipulate and therefore easier to find and extract from the head. Lapilli, conversely, seemed easier to prepare and view, but required more effort to find. These are trade-offs that should be considered when determining which otolith to use.

Determining the maximum age utility for otoliths in gar is still in need of research. Additional polishing was required for older spotted gar otoliths, regardless of otolith type. This was especially necessary for sagittae, as also has been described for alligator gar (Sakaris et al. 2014, Long and Snow 2016). Also, other studies using otoliths for daily aging have reported increased error after approximately 100 days of age (Miller and Stork 1984, Sweatman and Kohler 1991, Sakaris and Irwin 2008). Whether daily ages can be estimated accurately beyond the 127 days found in this study for spotted gar is unknown and warrants further study. Sakaris et al. (2014) found that alligator gar can have reliable age estimates through 62 days post-hatch and cohorts could be assigned an age up to 104 days post-hatch based on sagittae. Similar results were found by Long and Snow (2016), with sagittae being accurate through 65 days post-hatch, but they also found that lapilli produced accurate age estimates for a longer period, up to 91 days post-hatch. In all these studies, maximum age estimates were limited because of the high degree of cannibalism that gar exhibit in hatchery systems. Whether refinements in hatchery maintenance or use of wild fish can be obtained for future studies is unknown but could be useful toward addressing maximum age utility.

Many previous studies of gar have relied on estimates of growth from laboratory or hatchery studies to back-calculate spawning dates (May and Echelle 1968, Echelle and Riggs 1972), which assumes similar growth rates between wild and captive fish. Because variables such as temperature and feeding regime can greatly affect growth rates (Johnson et al. 2002, Fey 2006), the assumption that captive fish can be surrogates for growth of wild fish is not necessarily valid. Back-calculating spawning dates from age estimates of wild-caught juvenile fish alleviates this concern, and our results provide evidence that counts of rings in otoliths (sagittae and lapilli) are accurate indicators of age for spotted gar to be used for this purpose. Results from this research can aid in conservation of gar species by providing a tool to elucidate early-life population dynamics which are often important for recruitment. For example, alligator gar hatch-date distribution in Lake Texoma, Oklahoma, estimated with otoliths, coincided with an increase in pool elevation from two pulses of water from the Red River which inundated herbaceous vegetation which, in turn, facilitated gar access to spawning and nursery habitats (Snow and Long 2015). In Ontario, Canada, where spotted gar is imperiled, this present validation study can enable additional research on factors affecting early life history which have been identified as a limiting factor for management toward recovery (Staton et al. 2012). Accurately back-calculating spawning dates can be correlated with environmental factors that affect spawning success and recruitment, to provide a better understanding of ecology to enable management for this species.

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