Comparison of the Precision of Age Estimates Generated from Fin Rays, Scales, and Otoliths of Blue Sucker

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Abstract - Evaluating the precision of age estimates generated by different readers and different calcified structures is an important part of generating reliable estimations of growth, recruitment, and mortality for fish populations. Understanding the potential loss of precision associated with using structures harvested without sacrificing individuals, such as scales or fin rays, is particularly important when working with imperiled species, such as *Cycleptus elongatus* (Blue Sucker). We collected otoliths (lapilli), scales, and the first fin rays of the dorsal, anal, pelvic, and pectoral fins of 9 Blue Suckers. We generated age estimates from each structure by both experienced (n = 5) and novice (n = 4) readers. We found that, independent of the structure used to generate the age estimates, the mean coefficient of variation (CV) of experienced readers was approximately 29% lower than that of novice readers. Further, the mean CV of age estimates generated from pectoral-fin rays, pelvic-fin rays, and scales were statistically indistinguishable and less than those of dorsal-fin rays, anal-fin rays, and otoliths. Anal-, dorsal-, and pelvic-fin rays and scales underestimated age compared to otoliths, but age estimates from pectoral-fin rays were comparable to those from otoliths. Skill level, structure, and fish total-length influenced reader precision between subsequent reads of the same aging structure from a particular fish. Using structures that can be harvested non-lethally to estimate the age of Blue Sucker can provide reliable and reproducible results, similar to those that would be expected from using otoliths. Therefore, we recommend the use of pectoral-fin rays as a non-lethal method to obtain age estimates for Blue Suckers.

Introduction

Accurate and precise age determinations are vital for generating reliable estimates of demographic parameters, such as recruitment, mortality, and growth, that are necessary for the effective management and conservation of fishes (Campana 2001). There are a number of calcified structures, including various bones, fin rays, and scales, that can be used for age determination, but otoliths have generally been found to produce the most accurate and precise age estimates for most species (Buckmeier et al. 2002, Erickson 1983, Hining et al. 2000, Maceina and Sammons 2006, Secor et al. 1995). However, the process of otolith removal results in mortality to the fish. Given that age and growth studies can require significant sample sizes

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to generate reliable estimates (Campana 2001, Quist et al. 2012) , researchers working with imperiled species are often left seeking non-lethal alternative structures to make age determinations. For example, scales can produce reliable estimates of age (Devries and Frie 1996), but they can also severely underestimate the age of individuals of long-lived species, such as catostomids (Rupprecht and Jahn 1980, Spiegel at al. 2010, Sylvester and Berry 2006). The use of fin rays and spines for age estimation can be difficult due to variation in shape, deterioration of early annuli in older fish, and lack of a well-defined centrum (Rupprecht and Jahn 1980, Weber and Brown 2011). Therefore, a full evaluation of the precision and accuracy of age estimates generated from calcified structures, particularly those that can be harvested non-lethally, is important to determine their utility for age estimation in imperiled species.

Cycleptus elongatus Lesueur (Blue Sucker) is a large-bodied, long-lived riverine catostomid that is increasingly becoming of conservation concern throughout its range. The species is found throughout the Mississippi River drainage and Gulf Slope drainages in Louisiana and Texas (Gilbert 1980, Harris et al. 2014). Even though it is widely distributed, Blue Sucker is listed variously as a species of greatest conservation need, threatened, presumed extirpated, or endangered by 19 of the 23 states it inhabits (Steffensen et al. 2015). However, the status of most populations is uncertain because relatively little information exists regarding growth, recruitment, and mortality (Bacula et al. 2009). Although scales have been used for Blue Sucker age determination (Beal 1967, Labay et al. 2011, Morey and Berry 2003, Rupprecht and Jahn 1980), they have been found to underestimate the age of older individuals in most populations (Labay et al. 2011, Rupprecht and Jahn 1980). The first pectoral-fin ray has also been used to determine the age of Blue Sucker (Bacula et al. 2009, Labay et al. 2011, Rupprecht and Jahn 1980) and seems to yield more-accurate estimates than those from scales (Labay et al. 2011). However, there has been no report of a comprehensive comparison of the precision and accuracy of age estimates from calcified structures that can be non-lethally harvested from Blue Sucker relative to those from structures, such as otoliths, that typically yield more-accurate age estimates in other species and that require sacrifice of individuals. Therefore, our objective was to compare precision of age estimates generated from several calcified structures by readers with different skill levels, and their accuracy relative to those from lapilli of Blue Sucker.

Study Site and Methods

We captured adult Blue Suckers from the Colorado River in Texas (n = 170) and Sabine River on the Texas and Louisiana border using a boat-mounted electrofisher. Blue Sucker is a protected species in Texas; thus, our samples were limited to individuals that did not recover after capture (n = 5, all from the Colorado River) or that were sacrificed for other purposes (n = 4, all from the Sabine). We euthanized Blue Suckers that were still exhibiting opercular movement but no other sign of recovery 15–20 min post-capture through immersion in a >400-mg/L aqueous solution of clove oil (eugenol; Leary et al. 2013) and stored them on ice for processing in the lab. The Blue Suckers used in this study had a mean (± SD) total length (TL) of 606 ± 86 mm (range = 507–720 mm TL). Lapilli, hereafter referred to as otoliths, were removed and stored dry. We used lapilli because the sagittae of ostariophysan fishes, such as catostomids, are highly modified and difficult to use for age estimation (Secor et al. 1992). We harvested the first ray of the dorsal, anal, pectoral, and pelvic fins, removed tissue from the fins, and stored the samples dry (Bacula et al. 2009, Labay et al. 2011). We also collected and stored dry a sample of 3–5 scales from between the lateral line and dorsal fin just posterior to the origin of the pectoral fin as described by Labay et al. (2011).

We embedded the otoliths in epoxy and used a low-speed isomet saw to cross-section them along the transverse plane through the nucleus (South Bay Technologies, San Clemente, CA) as described by Quist et al. (2012). We also embedded the fin rays in epoxy and made 0.8-mm sections from the proximal end of the ray. Multiple sections were taken from each fin ray starting at approximately 1.0–1.5 cm from the articulation point of the ray in order to produce sections that would be comparable to those taken from fish that were released alive (Bacula et al. 2009, Labay et al. 2011). We cleaned and placed scales between 2 glass slides to hold them flat. We took digital photographs of each calcified-structure section at 3.0–11.0x magnification using an Olympus SZX16 stereo microscope (Olympus Corporation, Tokyo, Japan) equipped with an Infinity 1-5C 5.0-MP digital camera (Lumenera Corporation, Ottawa, ON, Canada).

We used ImageJ v. 1.48 image-analysis software (Abramoff et al. 2004) to enhance the visibility and clarity of the annuli. We assigned images a random identifier that allowed images of different sections of the same fin ray or scales from the same fish to be grouped together but prevented readers from identifying which structures came from the same fish. Readers were not provided any information regarding the individual fish. In addition, we inserted duplicate images from 3 fish into the dataset to assess the precision within an individual reader. We instructed readers to identify annuli following published criteria for otoliths, scales, and fin rays (Casselman 1983, DeVries and Frie 1996, Quist et al. 2012). Each of the 9 readers was assigned to a skill level based on whether they had participated as a reader in a previous age and growth study (experienced; $n = 5$) or not (novice; $n = 4$).

We tested hypotheses related to the variability of age estimates among readers, age estimates generated from otoliths and other structures, and precision of individual readers. We calculated the variability of age estimates among readers as the coefficient of variation (CV), i.e., the standard deviation of the observations divided by the mean multiplied by 100. We calculated a separate CV for the novice readers and the experienced readers for each structure from each individual Blue Sucker. We conducted analysis of covariance (ANCOVA) to test the null hypothesis that aging structure (fixed effect), reader skill level (fixed effect), and the TL of the fish (covariate) did not influence the CV of age estimates. The difference between the age estimate generated for a particular fish from its otolith compared to the other aging structures of that fish was calculated for each reader. We employed a mixed-model ANCOVA to test the null hypothesis that age estimates from other structures did not differ from those from otoliths. Aging structure, reader skill level, and TL of the fish were used as fixed effects in the model, individual readers were used as a random effect, and individual fish were treated as a subject effect. We calculated the precision of individual readers as the difference in the age estimates by a given reader between repeated reads of the same structure from the same fish. We ran a mixed-model ANCOVA to assess the effects of aging structure, reader skill level, and TL of the fish (fixed effects) on reader precision. Individual readers were treated as a random effect and individual fish as a subject effect. We tested all interactions, but removed them from the final models if they were not statistically significant. We assessed parametric assumptions of independence, normality, and equality of variance and set $\alpha = 0.05$ for all tests of statistical significance. We employed Tukey's HSD post hoc test to compare means among structures and 2-tailed *t*-tests to test the null hypothesis that mean differences between estimates did not differ from zero. Analyses were performed in SAS 9.4 (SAS Institute, Inc., Cary, NC).

Results

The CV of age estimates varied by aging structure and skill level of the reader (Table 1; Fig. 1), but no interaction effect was evident, nor did TL influence the

Table 1. Results from analysis of covariance assessing the influence of TL, structure used for age estimation (anal, dorsal, pectoral, and pelvic fin rays, lapillar otolith, and scales), and reader skill level (novice vs. experienced) on the coefficient of variation of age estimates of Blue Sucker ($n = 9$) collected from the Colorado River, TX, and the Sabine River, TX–LA, during 2015.

Fixed effect	df	*F*-value	*P*-value
TL	1134	0.42	0.52
Structure	5134	5.44	<0.01
Skill	1134	36.52	<0.01

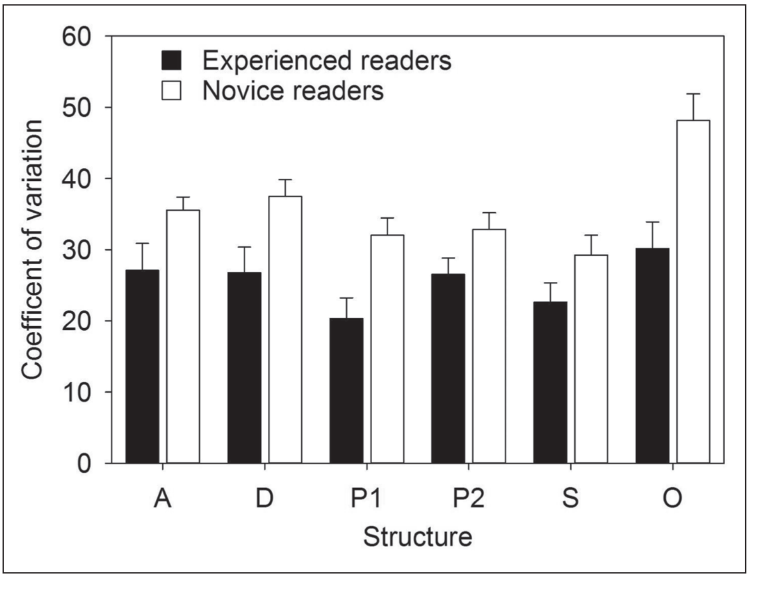

Figure 1. Mean coefficient of variation for age estimates derived by experienced ($n = 5$) and novice ($n = 4$) readers from the first ray of the (A) anal fin, (D) dorsal fin, (P1) pectoral fin, and (P2) pelvic fin, (S) scales, and (O) lapillar otoliths of Blue Sucker ($n = 9$) captured from the Colorado River in TX, and the Sabine River in TX–LA. Error bars represent standard error.

CV of the age estimates. Independent of the structure used to generate the age estimates, the mean CV of experienced readers was ~29% lower than that of novice readers. The mean CVs of age estimates from otoliths were greater than those from pectoral fin rays, pelvic fins rays, and scales ($P < 0.02$). The mean CVs of age estimates from all structures other than otoliths were statistically indistinguishable from each other ($P > 0.17$).

The difference between the age estimated for the same individual from its otolith and that from other aging structures was influenced by the structure used to generate the age estimate (Table 2, Fig. 2) and tended to increase with TL. The skill level of the reader did not have a detectable effect. Anal, dorsal, and pelvic fin rays underestimated the age compared to otoliths (Table 3; the least-square mean differences were all less than zero ($t_{441} \leq -2.37$, $P \leq 0.02$; Fig. 2). However, age estimates from pectoral fin rays ($t_{441} = -1.62$, $P = 0.11$) and scales ($t_{441} = -0.95$, $P = 0.75$) did not exhibit any bias relative to otoliths.

Skill level, structure, and TL influenced the readers' precision between subsequent estimates of the same structure of a particular fish (Table 4, Fig. 3). Precision

Table 2. Tests of fixed effects from a mixed-model analysis of covariance assessing the influence of TL, structure used for age estimation (anal, dorsal, pectoral, and pelvic fin rays, and scales), and reader skill level (novice vs. experienced) on the difference between the age estimated from that structure and the age estimated from the lapillar otolith of Blue Suckers (n = 9) collected from the Colorado River, TX, and the Sabine River, TX–LA, during 2015. Individual readers were treated as a random effect and individual Blue Suckers as a subject effect in this model.

Fixed effect	df	F-value	P-value
TL	1441	19.42	<0.01
Structure	4441	2.44	0.05
Skill	1441	0.16	0.69
TL*Structure	4441	3.78	0.01

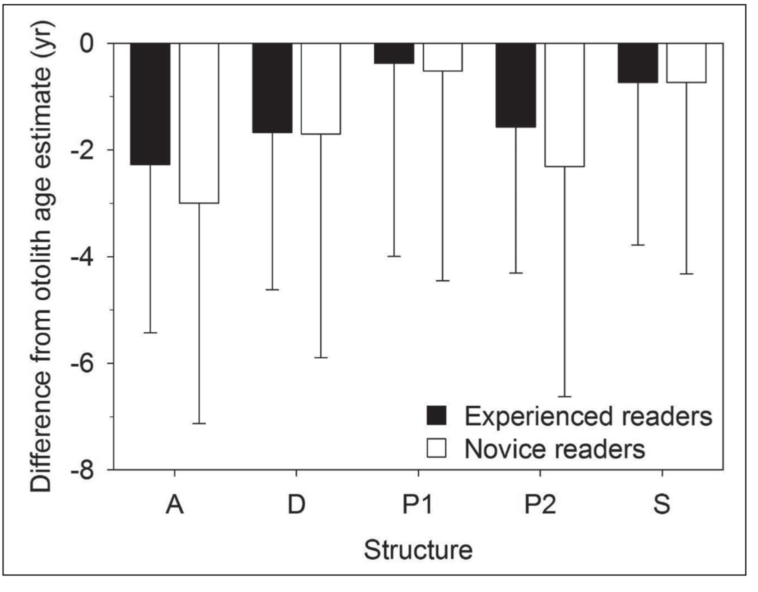

Figure 2. Mean difference between age estimates generated from anal (A) fin, (D) dorsal fin, (P1) pectoral fin, and (P2) pelvic fin rays, and (S) scales of Blue Sucker, and the age estimated from the lapillar otolith of that individual by experienced (n = 5) and novice (n = 4) readers. Error bars represent one standard deviation.

Table 3. Total length (TL) and mean (± SE) age estimates generated by 9 independent readers using the anal, dorsal, pectoral, and pelvic fin rays, lapillar otoliths, and scales of Blue Sucker (n = 9) collected from the Colorado River, TX, and the Sabine River, TX–LA, during 2015.

River	TL (mm)	Anal fin rays	Dorsal fin rays	Otoliths	Pectoral fin rays	Pelvic fin rays	Scales
Colorado	596	5.9 ± 0.8	5.4 ± 0.5	9.0 ± 1.0	7.1 ± 0.9	7.3 ± 0.7	6.3 ± 0.7
	642	4.4 ± 0.3	7.8 ± 0.7	9.2 ± 1.3	8.8 ± 0.6	6.0 ± 0.6	7.9 ± 0.8
	674	7.0 ± 0.8	5.6 ± 0.6	11.2 ± 1.0	7.2 ± 0.7	6.8 ± 0.6	9.0 ± 1.4
	691	3.7 ± 0.5	6.6 ± 1.0	10.8 ± 1.5	6.7 ± 0.6	4.1 ± 0.3	8.6 ± 1.1
	720	6.7 ± 0.5	6.5 ± 0.5	8.3 ± 0.8	9.2 ± 0.3	6.3 ± 0.5	7.1 ± 0.4
Sabine	507	4.4 ± 0.4	4.2 ± 0.5	5.4 ± 0.5	4.8 ± 0.5	4.2 ± 0.4	5.9 ± 0.3
	513	4.1 ± 0.6	6.2 ± 0.7	5.4 ± 1.1	9.0 ± 0.8	3.3 ± 0.4	5.7 ± 0.5
	516	4.6 ± 0.6	4.8 ± 0.5	6.2 ± 1.2	5.7 ± 0.7	4.8 ± 0.4	5.8 ± 0.6
	538	3.9 ± 0.4	6.3 ± 1.2	5.0 ± 0.7	5.6 ± 0.6	6.3 ± 0.7	6.1 ± 0.6

Table 4. Tests of fixed effects from a mixed-model analysis of covariance assessing the influence of TL, structure used for age estimation (anal, dorsal, pectoral, and pelvic fin rays, and scales), and reader skill level (novice vs. experienced) on the within-reader precision of age estimates of Blue Suckers (n = 9) collected from the Colorado River, TX, and the Sabine River, TX–LA, during 2015. Individual readers were treated as a random effect and individual Blue Suckers as a subject effect in this model.

Fixed effect	df	F-value	P-value
TL	1120	3.63	0.06
Structure	5120	1.27	0.28
Skill	1120	0.24	0.62
TL* Structure*Skill	11,120	2.16	0.02

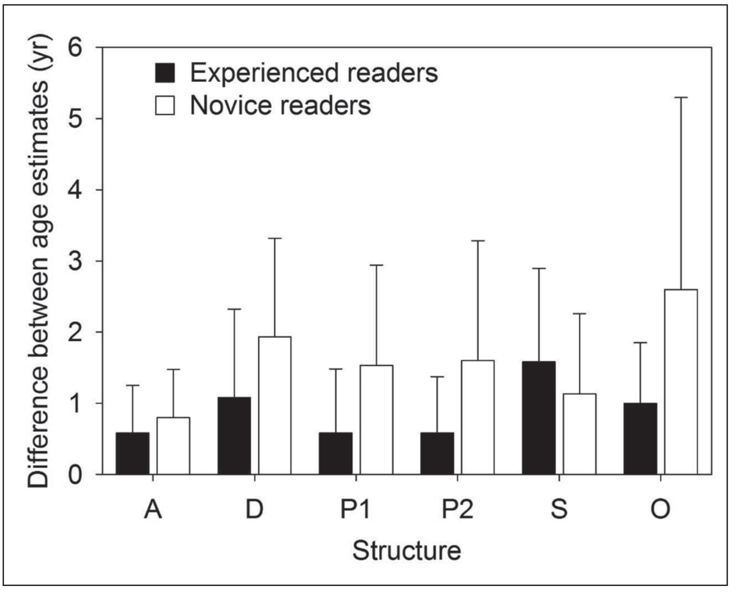

Figure 3. Mean difference between age estimates generated by experienced (n = 5) and novice (n = 4) readers during their first and second readings of (A) anal fin, (D) dorsal fin, (P1) pectoral fin, and (P2) pelvic fin rays, (S) scales, and (O) lapillar otoliths of Blue Sucker collected from the Colorado River in TX, and the Sabine River in TX–LA. Error bars represent one standard deviation.

tended to decrease with increasing TL and subsequent estimates from the same structure by experienced readers were overall about 77% more precise than those of novices, though aging of scales was a notable exception where novices' age estimates were closer to the actual ages than were those of experienced readers (Fig. 3). Structure was not identified as an important factor influencing precision by itself because only the precision of estimates from anal fin rays and otoliths exhibited an appreciable difference (Fig. 3).

Discussion

Although age estimates from pectoral fin rays for Blue Sucker previously have been shown to be more precise than those from scales (Labay et al. 2011), our results represent the first assessment of the accuracy of age estimates generated from fin rays and scales relative to otoliths. Previous research with Blue Sucker and other catostomids has shown that age estimates generated from scales tend to underestimate the age of the fish relative to otoliths (Quist et al. 2007, Sylvester and Berry 2006). However, age estimates for pectoral fin rays and otoliths have been shown to be similar in species such as *Catostomus discobolus* Cope (Bluehead Sucker) and *Catostomus latipinnis* S.F. Baird & Girard (Flannelmouth Sucker) (Quist et al. 2007). Age estimates from the pectoral fin rays of *Catostomus commersonii* Lacépède (White Sucker) generally were in agreement with those from otoliths, but discrepancies tended to occur in older individuals, particularly after age 7 (Sylvester and Berry 2006). Our results indicated that not only were the age estimates of Blue Sucker generated from pectoral fin rays comparable to those produced from otoliths, but the estimates from pectoral fin rays also had a lower CV and greater reproducibility than those from otoliths. Furthermore, the age estimates generated from Blue Sucker scales did not show the expected significant negative bias relative to those from otoliths. Finally, our results indicate that rays from the pelvic, dorsal, and anal fins of Blue Sucker do not produce reliable and reproducible age estimates.

The high degree of precision and reproducibility of age estimates generated from pectoral fin rays and scales relative to those from otoliths was unexpected. Otoliths, particularly the sagittal otoliths, are the preferred structure for age determination for most fish taxa (Campana 2001, Maceina et al. 2007, Secor et al. 1995), specifically because they have a higher degree of accuracy and precision than other structures (Beckman 2002, Boxrucker 1986, Buckmeier et al. 2002). However, in ostariophysan fishes, such as Blue Sucker, the size and morphology of the sagittal otoliths render them unusable for age estimation (Secor et al. 1992, Sylvester and Berry 2006). The lapillar otolith is used instead for estimating the age of ostariophysan fishes, but the ease of interpreting annuli can vary among species (Campana 2001, Phelps et al. 2007). Experienced and novice readers both generally regarded the annuli from Blue Sucker otoliths examined for this study as difficult to identify and count. The potential difficulties interpreting the otoliths could account for the relatively low reproducibility and high CV of age estimates from otoliths, particularly those made by the novice readers. In contrast, the annuli on the pectoral fin rays and scales were relatively clear and distinct, which would reduce the degree of subjectivity involved in identifying annuli and lead to higher precision regardless of reader-experience level. The unexpectedly high levels of reproducibility and agreement with otolith age estimates obtained from the scales also was likely due in part to the relatively young fish (Table 3) used in our study compared with those from other studies (Bacula et al. 2009, Labay et al. 2011, Morey and Berry 2003, Rupprecht and Jahn 1980).

In summary, we demonstrate that age estimation using scales and pectoral fin rays is a viable alternative to otoliths for generating information on age structure of Blue Sucker populations. It is also important to note that although we used the age estimates generated from otoliths as the "true age" for analysis purposes, our results suggest lapilli in this species may not very useful. Validating the formation of annual increments on the calcified structures of Blue Sucker using known-age or marked individuals is therefore an important next step to identifying the structures and procedures capable of producing accurate age estimates for this species.

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