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Validation of age estimates obtained from juvenile pallid sturgeon *Scaphirhynchus albus* pectoral fin spines

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Summary

Pallid sturgeon *Scaphirhynchus albus* are a federally-listed endangered species, yet little is known about their population dynamics, including age and growth. Age validation is needed before the accuracy of age estimates and ensuing analyses is known. Therefore, we evaluated the accuracy and precision of age estimates obtained from 36 pallid sturgeon recaptured from the lower Missouri River. Accuracy of age estimates varied among three readers from 28 to 41%. Most deviations from the actual age were within 1 year, and most fin spine sections were overaged due to an apparent false annulus near the focus of the section. Between-reader agreement varied by reader combination but was generally around 30%. Our findings suggest that careful attention be paid to age and growth information obtained from hatchery-reared pallid sturgeon fin spines and further methods of accurately estimating age of pallid sturgeon need to be developed.

Introduction

Many hard structures produce periodic marks used for age estimation of fishes (e.g. scales, otoliths, vertebrae, fin spines, cleithra, opercula), but before age estimates are used, their accuracy needs to be evaluated, including the methodology and the interpretation of growth marks (Beamish and McFarlane, 1983; Campana, 2001). Inaccurate age estimations can have dramatic affects on age-dependent population metrics (e.g. growth, recruitment, mortality), and can result in the mismanagement of fisheries (Campana, 2001). As such, standard practices recommend that whenever hard structures are used to estimate age of fishes, the method of age determination must be both verified and validated (Beamish and McFarlane, 1983; DeVries and Frie, 1996). Verification refers to the assessment of precision of age estimates which is usually evaluated by two or more readers independently counting annuli on the same hard structure (DeVries and Frie, 1996). These verification assessments might be precise among readers, but they may not necessarily be accurate (Maceina et al., 2007). Validation refers to the accuracy of age estimates and is generally accomplished by assigning age estimates to known-age fish (DeVries and Frie, 1996).

The pallid sturgeon *Scaphirhynchus albus* is native to large rivers in the Mississippi River drainage, has experienced substantial population declines, and has been listed as a federally-endangered species since 1990 (Pflieger, 1997). Little is known about age and growth characteristics of existing pallid sturgeon populations, primarily due to their lack of scales and other calcified structures used for age determination (U. S. Fish and Wildlife Service, 2000; Quist et al., 2004). The most widely-used structure for estimating ages of sturgeons (family Acipenseridae) are sectioned pectoral fin spines (Helms, 1974; Rossiter et al., 1995; Hurley et al., 2004; Whiteman et al., 2004; Killgore et al., 2007; Bruch et al., 2009); however, validation of fin spine age estimates has been attempted for few sturgeon species. Rossiter et al. (1995) validated age estimates of lake sturgeon *Acipenser fulvescens* by collecting and marking individuals and recapturing a subset of the marked individuals in subsequent years. Rien and Beamesderfer (1994) used a similar study design for validating ages of white sturgeon *A. transmontanus*. Bruch et al. (2009) utilized bomb radiocarbon assays to assess the validity of lake sturgeon ages obtained from pectoral fin spines and otoliths.

Only three attempts have been made to validate age estimates of river sturgeons (genus *Scaphirhynchus*). Helms (1974) attempted to validate shovelnose sturgeon *S. platorynchus* age estimates using length-frequency histograms, while Whiteman et al. (2004) used marginal increment analysis to validate periodicity of annulus formation in shovelnose sturgeon pectoral fin spines. Hurley et al. (2004) conducted the only age validation study for pallid sturgeon by examining fin spine samples obtained from 16 age-6 fish that were reared and held in captivity until the time of fin spine removal. Although Hurley et al. (2004) reported that pallid sturgeon age estimates exhibited low accuracy and precision, captivity may have affected the growth of individuals and the resulting annulus formation in calcified structures (Campana, 2001). Therefore, the objective of this study was to assess the accuracy and precision of age estimates obtained from multiple age-classes of known-age, juvenile hatchery-reared pallid sturgeon that were stocked in the Missouri River and recaptured at a later date.

Materials and methods

Pallid sturgeon were collected from the Missouri River, Nebraska (river kilometers 811–1086) during 2007 and 2008 using multifilament gill nets and trot lines baited with nightcrawlers *Lumbricus terrestris*. All pallid sturgeon were measured to the nearest millimeter (fork length) and the left marginal pectoral fin spine was removed using methods described by Koch et al. (2008). Fin spines were only removed from fish with a unique tag or combination of tags (e.g. PIT

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tag, elastomer, scute removal) that identified the year-class of the individual. Fin spines were placed in individually-numbered coin envelopes and allowed to air-dry for at least 2 weeks prior to processing. Fin spines were cleaned of residual tissue and mounted in epoxy prior to sectioning. Encapsulated fin spines were sectioned with a Buehler Isomet low-speed saw (Buehler, Lake Bluff, IL). Four 0.6-mm thick cross-sections were removed from the region immediately distal to the conspicuous curve near the articulating process of the fin spine (Koch et al., 2008). Fin spine sections were photographed with a digital camera linked to a light microscope and examined on a computer monitor. Fin spine sections were independently aged by three readers. Readers 1 and 2 had substantial experience (i.e. > 5 years) aging fishes, including shovelnose sturgeon. Reader 3 possessed less experience aging fish (i.e. approximately 2 years), but had previous experience aging shovelnose sturgeon. During initial aging, all readers noted the position of presumptive annuli on an individual copy of the fin spine section image. Upon completion of initial aging, the three readers met in concert, discussed differences in opinion regarding presumptive annuli and agreed on consensus annuli and ages.

Due to difficulties associated with determining which mark was the first annulus on fin spine sections, annuli were measured from all fin spines, and mean back-calculated lengths (MBCL) at age 1 were estimated using the Dahl-Lea method (DeVries and Frie, 1996):

$$L_i = (L_c/R_c) * R_i$$

where L_i, length at annulus i, L_c, length at capture, R_c, fin spine radius at capture, and R_i, fin spine radius at annulus i. In an attempt to validate which mark on the fin spine was the actual first annulus, these back-calculation estimates were compared to empirical length measurements taken at the time of stocking (i.e. approximately 1 year).

Because pallid sturgeon were collected in both spring and fall, we assumed annulus formation to be complete in the fall, as marginal increment analyses indicated that annuli were formed during the summer in shovelnose sturgeon collected from the Missouri River (Whiteman et al., 2004). Therefore, only annuli presumed to be complete were counted towards the age of the specimen. Annuli were considered to be the combination of a continuous light band immediately outside of a dark band, as described by Whiteman et al. (2004). Each section was aged without knowledge of the fish's length or weight. We also assessed between-reader agreement for exact age and age within 1 year, while precision of age estimates was evaluated using the coefficient of variation (CV; Campana et al., 1995).

Results

Pectoral fin spines were removed from 36 pallid sturgeon that varied in length from 347 to 764 mm. Six year-classes from 2001 to 2007 (i.e. ages 1–7) were represented in the sample set (Table 1). Exact between-reader agreement (i.e. precision) varied from 30 to 36% and coefficients of variation for between-reader comparisons varied from 11.2 to 16.0% (Table 2). Agreement within 1 year varied by reader comparison and was between 58.4 and 75.1%, while agreement within 2 years was above 83% for all three reader comparisons.

Accuracy of age estimates varied by reader from 27.8 to 41.7% (Fig. 1). Readers 1 and 2 generally over-estimated ages of pallid sturgeon, especially young fish. All six fin spine sections from pallid sturgeon that were 2 years of age or less were over-aged by Readers 1 and 2. Age estimates of Reader 3 were usually lower than those of the other readers, and as such, had a higher rate of accuracy. Consensus ages were accurate 27.8% of the time and were within 1 year of the actual age 77.8% of the time. Consensus age estimates were generally higher than actual ages. Of all age consensus estimates that were inaccurate, 20 of 26 (76.9%) ages were overestimated.

Mean back-calculated lengths at age 1 were compared to length measurements taken at the time of stocking for the 25 individuals with available stocking data (Fig. 2). All lengths at stocking were greater than mean back-calculated lengths at the first mark (i.e. when counting the inner mark as an annulus). When the inner mark was not counted as an annulus, mean back-calculated length at age 1 was within the range of lengths at stocking of the fish, indicating that the inner mark on hatchery-reared pallid sturgeon is not the actual first annulus (Fig. 3).

Discussion

The precision of age estimates obtained from the hard structures of sturgeon species is generally low. In studies examining white sturgeon pectoral fin spines, exact between-reader agreement varied from 17 to 37% (Kohlhorst et al., 1980; Brennan and Cailliet, 1989; Rien and Beamesderfer, 1994). Studies that have examined shovelnose sturgeon fin spines reported similar levels of precision; Whiteman et al. (2004) reported exact between-reader agreement of 18%, while Morrow et al. (1998) had 32% agreement. The precision of our age estimates obtained from pallid sturgeon fin spines was lower than values reported by Hurley et al. (2004) who

Table 1
Year class, number, mean length at stocking, and mean length at capture from pallid sturgeon sampled from the middle Missouri River, 2007–2008

Year class	N	Mean length (mm) at stocking	Mean length (mm) at capture
2001	4	230 (20.0)	547 (37.6)
2002	10	303 (5.8)	588 (32.9)
2003	8	232 (14.2)	498 (24.6)
2004	4	253 (0.0)	407 (22.7)
2005	7	322 (13.2)	433 (13.6)
2007	3	292 (29.3)	353 (4.5)

Standard errors are reported in parentheses.

Table 2
Precision of age estimates between readers for sectioned pectoral fin spines from pallid sturgeon sampled from the middle Missouri River, 2007–2008. Precision between readers is expressed as percent exact agreement, percent agreement within 1 year, and percent agreement within 2 years

Comparison	Exact agreement	Within 1 year	Within 2 years
Reader 1 vs 2	30.6 (11.2)	72.3	86.1
Reader 1 vs 3	30.6 (12.6)	75.1	94.5
Reader 2 vs 3	36.1 (16.0)	58.4	83.4

The coefficient of variation (CV, %) for percent exact agreement is shown in parentheses.

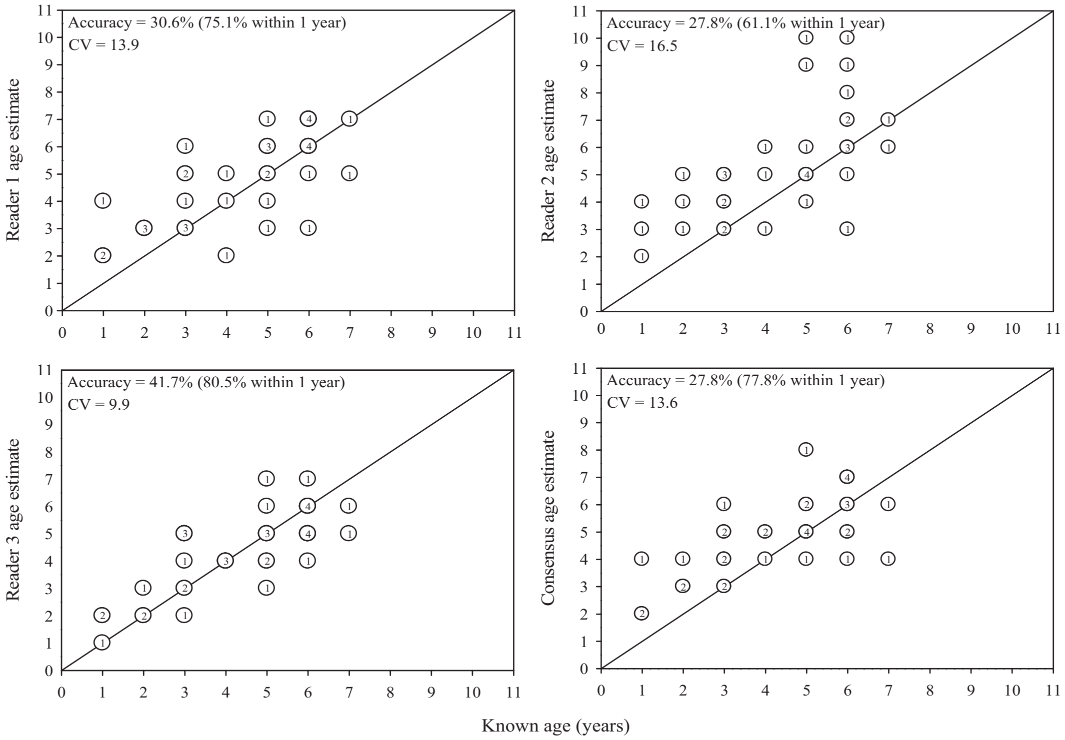

Fig. 1. Age-bias plots for individual reader and consensus ages of sectioned fin spines from pallid sturgeon. Accuracy, mean coefficient of variation (CV), and accuracy within 1 year (value inside parentheses), are shown. Numbers in circles are the number of pallid sturgeon at each age. The diagonal line (1 : 1 line) illustrates departure from exact accuracy

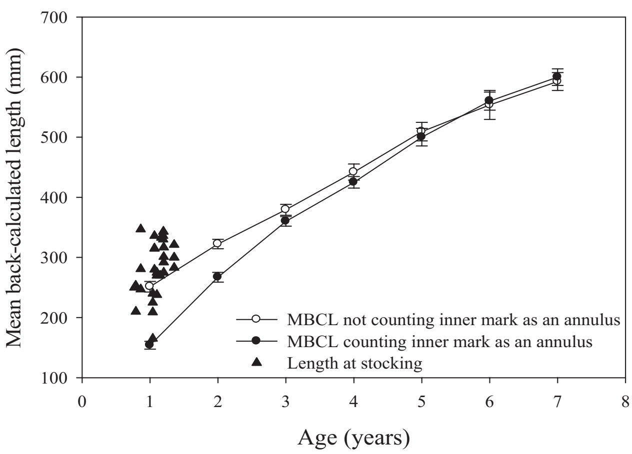

Fig. 2. Mean back-calculated length at age (MBCL) of hatchery-reared pallid sturgeon used in the study which were sampled from the middle Missouri River, 2007–2008. Mean back-calculated lengths were estimated using measurements acquired when inner mark on fin spine section was counted as an annulus (●) and also when not counting the inner mark as an annulus (○). The lengths (mm) of at time of stocking for the individual hatchery-reared pallid sturgeon are indicated by ▲. Error bars represent one standard error

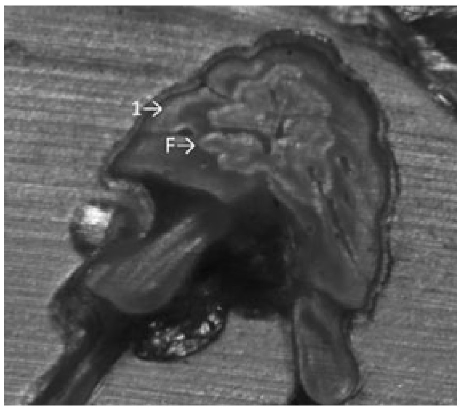

Fig. 3. Hypothesized false (F) and first (1) annuli in fin spine section of an age-1 hatchery-reared pallid sturgeon sampled from the middle Missouri River, 2007–2008. This specimen was spawned in 27 June 2007 and recaptured in 17 November 2008

reported an exact between-reader agreement of 47% and an agreement within 1 year of 94%. Coefficients of variation in our study were higher than those reported for white sturgeon (e.g. 7.8%; Rien and Beamesderfer, 1994) and juvenile lake sturgeon (0%; Bruch et al., 2009). Additionally, our levels of precision were more variable than those recommended as a minimum target level in the literature (i.e. 5% CV; Campana, 2001).

The fin spines we examined were taken from pallid sturgeon stocked and re-collected from the wild, yet our accuracy was similar to that reported by Hurley et al. (2004) for pallid sturgeon held in hatchery facilities. All readers in our study agreed that poor readability (e.g. false and unclear annuli) affected their ability to assign ages to the fin spine sections. Additionally, readers concurred that annuli on shovelnose sturgeon fin spines collected concurrently with fish from this study were clearer and more readable than those from the hatchery-reared pallid sturgeon fin spines. Poor readability of pallid sturgeon fin spines may be attributed to the influence of stress associated with captive rearing and stocking. These influences of a captive environment could affect annulus deposition (Schramm, 1989) and create false annuli in the rays that can be confused with true annuli. It is also possible that fin spines removed from wild pallid sturgeon would not have these false annuli and be more readable and useful in age and growth analyses.

Most age estimate errors were within 1 year and the result of overestimation, especially in young specimens. This is contrary to research suggesting that age estimates from fin spines underestimate true ages of white sturgeon (Paragamian and Beamesderfer, 2003) and lake sturgeon (Bruch et al., 2009); although those results were for fish substantially older than the pallid sturgeon used in our study. The disparity between empirical measurements at the time of stocking and estimates of back-calculated lengths at age 1 suggest the first mark on the inner portion of the fin spine section is not an annulus. As such, many of the fin spine sections were likely over-aged by 1 year. The apparent inner, false annulus on the fin spines may be a result of stress associated with captive rearing (Campana, 2001) such as a transition in diet, drastic temperature changes, low oxygen levels, or disease contracted in hatchery facilities. Regardless, the possibility of a false annulus should be noted when estimating ages of hatchery-reared pallid sturgeon. Although the sample size for our study was relatively small, it should be noted that pallid sturgeon are an extremely rare species and it may take several years to collect enough hatchery-reared pallid sturgeon to determine the specific causes and frequency of these incidences. As more pallid sturgeon are stocked and subsequently collected, further investigations into this phenomenon are warranted.

Sectioned pectoral fin spines will likely remain the preferred aging structure for pallid sturgeon because of the conservation status of the species and the non-lethal nature of fin spine removal. Most studies validating methods of aging sturgeon have concluded that age and growth information obtained from sectioned pectoral fin spines should be viewed with caution (Rien and Beamesderfer, 1994; Rossiter et al., 1995; Hurley et al., 2004; Whiteman et al., 2004). Although our study supports these findings and further emphasizes the need for researchers to approach age- and growth-related population parameters for pallid sturgeon with caution, promising age-determination methods for lake sturgeon (e.g. using otoliths and bomb radiocarbon aging; Bruch et al., 2009) have recently been developed. Although bomb radiocarbon aging would only be useful for older or archived fish, further research of juvenile (i.e. to 15 years of age) and adult (i.e. to 60 years of age) pallid sturgeon age and growth utilizing new methods is warranted as it may provide means to acquire more accurate information that is critical to the recovery of the species.

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