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[Management Brief]

A Comparison of Techniques Using Dorsal Spines to Estimate Sauger Age

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Abstract.—Fish age is useful to fishery managers when evaluating growth rates, mortality rates, and reproduction. Our objective was to determine whether two methods for estimating the age of saugers *Sander canadensis* using dorsal spines—side illumination and spine sectioning—resulted in similar age estimates at the same efficiency and cost. To do this, we determined whether the coefficients of variation (CV = 100 • SD/mean) differed between age estimates of fish using the two techniques in the Missouri River and a portion of the Kansas River. We found that the CVs did not differ significantly between techniques. While reader agreement did not differ significantly, side illumination took one-half as much time as sectioning. In addition, side illumination displayed outer annuli on older fish better than sectioning and did not require an expensive low-speed saw, microscope slides, or a mounting medium for processing. We conclude that side illumination is the most time- and cost-efficient method for estimating sauger ages from spines.

Fish age is useful to fishery managers when evaluating growth rates, mortality rates, and reproduction (Van Den Avyle and Hayward 1999). Dorsal spines are a nonlethal alternative to otoliths for estimating fish age (Campbell and Babaluk 1979; Erickson 1983; Borkholder and Edwards 2001). In general, age estimates from walleye *Sander vitreus* dorsal spines are more precise than those from scales, another nonlethal age-estimation structure (Borkholder and Edwards 2001; Bruesewitz et al. 2002; Isermann et al. 2003). Among eight different structures, dorsal spine cross-sectioning is preferred for estimating the age of walleyes (Campbell and Babaluk 1979). However, preparation time and the need for specialized equipment limit the use of spines. These drawbacks were mitigated in the case of whole channel catfish *Ictalurus punctatus* pectoral spines by cutting them and sanding them on the proximal end and then viewing them under a microscope using side illumination (Buckmeier et al. 2002). The same method is effective for estimating walleye age (Logsdon 2007). Faint or merged rings, partial rings, double rings, crowding of distal rings, false annuli, and difficulty distinguishing the first annulus frequently cause problems in age estimation from dorsal spines (Kocovsky and Carline 2000; Bruesewitz et al. 2002; Isermann et al. 2003; Logsdon 2007). To reduce some of these problems, side illumination of unsectioned dorsal spines was tried; this approach (95% reader agreement) replicated ages estimated from otoliths (98% reader agreement) in Red Lake, Minnesota, walleye while decreasing preparation time (Logsdon 2007).

Our goal was to find the most precise age estimation method for Missouri and Kansas River saugers *Sander canadensis*. Researchers at the Missouri Department of Conservation's Missouri River Field Station have estimated the age of saugers from dorsal spine sections (Beamish 1981) since 2007. Our objectives were to determine (1) whether the relative precision of age estimates differed between dorsal spines prepared by thin sectioning and by side illumination and (2) whether the two processing techniques differed in terms of their cost-benefit ratios. To our knowledge, this is the first comparison of the two techniques for this species.

Methods

The Missouri River was divided into an upper and lower sampling reach for the U.S. Army Corps of Engineers' Pallid Sturgeon Population Assessment Program (Drobish 2008a) based on longitudinal difference (e.g., tributary influence, geology, turbidity, degrading or aggrading stream bed, etc.) and length of growing season. The upper sampling reach includes the 327.5 river kilometers from Fort Peck Dam, Montana, downstream to the headwaters of Lake Sakakawea, North Dakota, and is the northernmost of the two reaches. The upper sampling reach is a meandering, often braided channel that lacks navigation structures and is influenced by the reservoir. The lower sampling reach includes (1) the 88.5 river kilometers (rkm) from Fort Randall Dam, South Dakota, downstream to the headwaters of Lewis and Clark Lake, Nebraska–South Dakota, (2) the 98.2 rkm from Gavins Point Dam downstream to Lower Ponca Bend (the only segment below Gavins Point Dam that is not channelized), and (3) the 1,207.0 rkm from Lower Ponca Bend to the confluence with the Mississippi River, which includes

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the entire channelized portion of the Missouri River. The lower sampling reach also includes the Kansas River from above the Johnson County Weir (Kansas) to the mouth (32.2 rkm). The lower sampling reach is highly engineered compared with its original state.

Sampling was conducted in the reaches just described from 23 October 2006 to 31 October 2007 and from 1 November 2007 to 23 October 2008 in accordance with guidelines established by the pallid sturgeon population assessment team as outlined in the *Missouri River Standard Operating Procedures for Sampling and Data Collection* (Drobish 2008b) and *Pallid Sturgeon Population Assessment Program* (Drobish 2008a). Gear types used to collect saugers included gill nets, trammel nets, otter trawls, hoop nets, mini-fyke nets, push trawls, beam trawls, and bag seines. The first three anterior dorsal spines were removed from each sauger sampled and stored for up to 23 months before processing and analysis.

Dorsal spines were soaked in isopropyl alcohol for 15 min, and then microforceps were used to separate the spines and remove all skin in preparation for side illumination (Buckmeier et al. 2002; Logsdon 2007). The proximal end of the second dorsal spine was then sanded with 600-grit sandpaper until the end was smooth. Modeling clay was used to hold the distal end of the spine for viewing, and mineral oil was applied to the sanded surface to reduce glare and sanding scars. Spines were illuminated from the side using a hand-held fiber optic light to count annuli. Images of prepared structures were viewed digitally using a Paxcam 3 digital microscope camera mounted on an Olympus SZ61TR stereo microscope using Paxcam 3 software. Structures were recorded at the highest magnification possible (up to 106.2×). Spines were stored for sectioning after two readers estimated age.

Dorsal spines were prepared for sectioning (Beamish and Chilton 1977) by placing one spine in a 4-cm section of a small tubular mold (soda straw) filled with a clear two-part epoxy resin. Spines encased in hardened resin were removed from the mold and secured in the saw chuck of a Beuhler low-speed Isomet saw. A 0.0305-mm wafering blade at a 90° angle cut spines into three 0.6350-mm cross sections from the proximal end. The three cross sections were affixed to a glass slide using Cytoseal 280 mounting medium and labeled with a unique code that identified where and when the fish was collected.

Age was assigned to each fish based on the number of annuli observed in the spine. With the sectioning technique, annuli appeared as light-colored rings against a dark background in transmitted light (Kocovsky and Carline 2000). With the side illumination technique, annuli appeared as dark-colored rings against a light background in direct light.

Two readers (always the same two individuals) independently analyzed each spine using both techniques without knowledge of the fish's sex or length. Age was recorded for each technique. The precision of the age estimates was determined by calculating percent reader agreement and mean CV between paired assignments of age (Logsdon 2007). The CV for each fish was calculated from the following formula:

$$\mathrm{CV}_j = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j},$$

where R is the number of times the age of each fish was estimated; X_j is the mean age estimated for the jth fish, and X_{ij} is the ith age estimate for the jth fish (Chang 1982). A mean CV was then calculated for each technique as a measure of the relative precision between techniques (Chang 1982). The mean CV among fish was compared between techniques using a paired t-test ($\alpha < 0.05$)

We randomly selected 10 spine samples and measured the time (min) required to process each one from the time when the structure envelope was opened to the time when an age estimate was obtained. This was done six times for each technique, for a total of 60 spines per technique. We then calculated the mean processing time for each technique among the 10 samples.

Results

Ages were estimated for 851 saugers, including 378 collected during 23 October 2006–31 October 2007 and 473 during 1 November 2007–23 October 2008. Total lengths ranged from 40 to 607 mm (mean = 345 mm, SE = 3.03).

The side illumination technique yielded age estimates ranging from 0 to 15 years, whereas the sectioning technique yielded age estimates ranging from 0 to 12 years. Age estimates from the two readers differed by as much as 5 years with the side illumination technique and 6 years with the sectioning technique. Further, the sectioning technique tended to yield older age estimates than the side illumination technique. Most fish (71.7%) were estimated to be 2–5 years old (Figure 1). Age estimates were the same for both readers for 55.2% (470/851) of the fish using either sectioning or side illumination and for 33.2% (283/851) of the fish using both techniques (Figure 1). Mean CV did not differ significantly between the side illumination and sectioned age estimates ($t = -0.50$, df = 1,699, P = 0.31). Readers agreed on their age

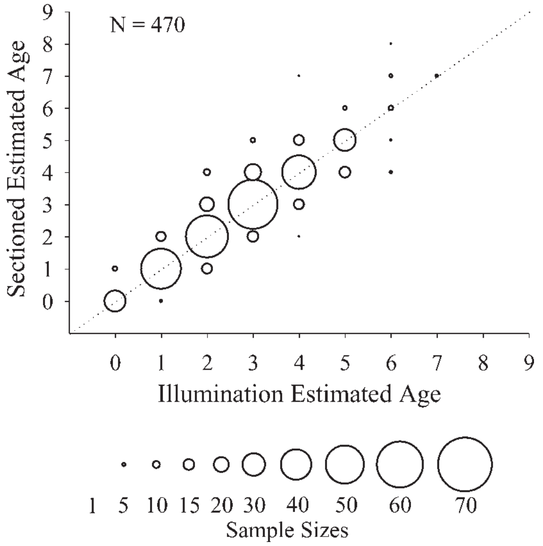

FIGURE 1.—Comparison of sauger ages (years) as determined from two methods of examining dorsal spines. The diagonal line represents 1:1 agreement between techniques.

estimates for 73.1% of all samples for the side illumination method (CV = 7.3%) and 70.9% of all samples for the sectioning method (CV = 7.6%).

Total processing time was less for the side illumination method than for the sectioning method. The side illumination method required an average of 5.84 min per sample and the sectioning method an average of 13.27 min per sample (Table 1). However, the mean viewing time of the sectioning technique was significantly shorter (0.91 min per sample) than that of the side illumination technique (2.36 min per sample) (t = 5.94, df = 8, P = 0.0002).

Discussion

Percent reader agreement for the sectioning technique (70.9%) was generally higher than that found in studies with walleye dorsal spines. For example, in Isermann et al. (2003) reader agreement for the sectioning method was 55.0%. Similarly, in Kocovsky and Carline (2000) reader agreement was 63.0% for males and 62.0% for females. And in Erickson (1983) it was relatively low in two Manitoba lakes—55.4% in Eardley Lake and 59.4% in Obukowin Lake—but 80.0% in a third, Lake Winnipeg. The dorsal spines of walleyes in Lake Winnipeg were easier to read because high exploitation in that lake resulted in younger, faster-growing fish with well-defined and well-spaced annuli (Erickson 1983). In contrast, the walleyes in the other two lakes experienced little exploitation, so they were generally older and more slowly growing and thus had annuli that were more difficult to read.

TABLE 1.—Mean processing times (min) per fish using the side illumination and sectioning techniques to estimate sauger ages from dorsal spines, by activity stage. Values with different letters are significantly different (t-test; $\alpha = 0.05$); na = not applicable.

Stage	Side illumination	Sectioning
Cleaning	3.48	na
Setting spines in mold	na	3.02
Sectioning and mounting	na	9.34
Viewing	2.36 z	0.91 y
Total processing	5.84	13.27

The percent reader agreement that we found for the side illumination method (73.1%, CV = 7.3%) was similar to that in another study of walleyes, namely, Logsdon (2007), in which the agreement between readers was 70.0% for walleyes from Mille Lacs Lake, Minnesota. However, the same study found 95.0% reader agreement for walleyes from Red Lake, Minnesota. Age structure was the likely reason for the differences in reader agreement between the two lakes because fish were younger in Red Lake than in Mille Lacs Lake (Logsdon 2007). However, in contrast to our study, precision was higher in both Red Lake (CV = 1.0%) and Mille Lacs Lake (CV = 2.7%; Logsdon 2007).

We found that side illumination displayed outer annuli on older fish better than sectioning, probably because of the ability to move the light to highlight annuli crowded near the margins, as suggested by Logsdon (2007). Logsdon (2007) also suggested that side illumination enhances the appearance of annuli that may appear split or discrete if the spine is viewed as a thin section. In contrast, we found that the outer annuli were difficult to distinguish because they were crowded together. This is similar to problems that have been experienced by others (Welch et al. 1993).

We found that with the side illumination technique preparation and age estimation required approximately 5.8 min per fish, whereas the sectioning technique required about 13.3 min per fish. In contrast, mean viewing time for the sectioning technique was 0.9 min per fish whereas that for the side illumination technique was 2.4 min per fish. Our processing times with the side illumination technique exceeded those of another study by nearly threefold (<2 min/fish; Logsdon 2007), perhaps because we were relatively inexperienced with a new method. Others may prepare and view spines faster or slower, depending on the experience of the individual(s). A reduction in the number of sections cut in the sectioning technique would also reduce preparation time, though we recommend cutting two sections because the first cut may miss the spine within the epoxy or may be unreadable and cutting more sections makes more sections available.

We recommend further experimentation with the intensity of the light used to illuminate the spine in the side illumination technique, especially with larger or older fish. Developing a holder for the fiber optic light instead of holding it by hand when viewing spines may reduce reading time. For example, a small block of wood with a clip to hold the light could speed up the process and make the image viewable for longer periods of time on the screen.

We found no significant differences in reader agreement between the two methods, but the processing time of the side illumination method was only half that of the sectioning method. Further, the side illumination method did not require an expensive low-speed saw, microscope slides, or a mounting medium for processing. Lastly, the side illumination method displayed outer annuli on older fish more clearly than the sectioning method. Therefore, we conclude that the side illumination method is the most time- and cost-efficient method for estimating sauger ages from dorsal spines.

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