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[Management Brief]

Use of Unsectioned Dorsal Spines for Estimating Walleye Ages

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Abstract.—Dorsal spines may be a suitable structure for aging walleyes *Sander vitreus* when the otoliths are unavailable due to live release of the catch, but the specialized equipment and additional time necessary to remove and mount a readable cross section can discourage their use. I evaluated a simple method of obtaining age estimates from unsectioned dorsal spines. The basal end of each dorsal spine was sanded smooth and viewed under a dissecting microscope with side illumination. For Red Lake, Minnesota, walleyes, where consensus otolith ages by two experienced readers indicated that most fish were younger than age 7, reader agreement rates (95%) and precision (mean coefficient of variation [CV] = 0.95%) of dorsal spine estimates were relatively high. Age estimates by individual readers inspecting spines generally agreed with consensus otolith ages (≥95%). Reader agreement rates (70%) and precision (mean CV = 2.71%) were much lower for a sample of walleyes collected from Mille Lacs Lake, Minnesota, where 94% of consensus otolith ages were greater than age 6. Furthermore, spine-based age estimates for Mille Lacs Lake walleyes exhibited relatively poor agreement with consensus otolith ages (≤40%); however, most of the discrepancies were noted for individuals of consensus otolith age 7 or more, and consistent underestimation of ages did not occur until age 13. Based on these findings, unsectioned dorsal spines offered a reasonably precise, nonlethal, and simple approach for replicating consensus otolith ages for walleyes younger than age 7. Although otoliths may still be necessary to estimate the ages of older individuals within a sample, use of unsectioned dorsal spines could allow biologists to sacrifice fewer walleyes while closely replicating otolith-based estimates of population age structure.

The use of scales is the most common approach for estimating ages of freshwater fish because of the ease and nonlethality associated with their collection. False annuli caused by resorption of calcium (Simkiss 1974; Devries and Frie 1996) and crowding of annuli in older or slow-growing fish, however, can cause ages estimated from scales to be less accurate than ages estimated from other calcified structures (Beamish and McFarlane 1987; Heidinger and Clodfelter 1987; Ross et al. 2005). Otoliths, on the other hand, provide an accurate method for estimating the ages of many fish species (Taubert and Tranquilli 1982; Heidinger and Clodfelter 1987; Buckmeier and Howells 2003) because annuli in otoliths are produced regardless of somatic growth (Simkiss 1974; Beamish and McFarlane 1987). Otolith extraction requires fish sacrifice and can be problematic in situations when live release of sampled fish is desired.

Dorsal spines are easily removed calcified structures that have previously been evaluated as a nonlethal alternative to otoliths for estimating the ages of walleyes *Sander vitreus* (Campbell and Babaluk 1979; Erickson 1983; Borkholder and Edwards 2001). Unfortunately, the specialized equipment and additional processing times necessary to prepare spines for age determination may discourage their use. In the past, methods for preparing spines required that the spines be cast individually in an epoxy or polyester resin and sectioned before inspection (Campbell and Babaluk 1979; Frie et al. 1989; Kocovsky and Carline 2000). If a low-speed bone saw (e.g., Buehler Isomet) is used, then the sections can be cut thin enough so that the samples can immediately be inspected with a microscope or microfiche reader (Frie et al. 1989; Borkholder and Edwards 2001; Bruesewitz et al. 2002). Use of less-precise cutting instruments, such as a jeweler's saw or Dremel tool, requires that the sections be ground thinner with fine-grit sandpaper to produce a sample suitable for inspection (Olson 1980; Marwitz and Hubert 1995; Kocovsky and Carline 2000). Both methods are time consuming and tedious.

Buckmeier et al. (2002) recently described a method of preparing spines of channel catfish *Ictalurus punctatus* that negates the need for mounting and sectioning the spines. Their method consists of making a single cut through the dorsal and anterior processes of the spine, then inspecting the cut with the side illumination technique described by Heidinger and Clodfelter (1987) to inspect broken otoliths. The objective of this study was to modify the technique of Buckmeier et al. (2002) for use on walleye spines and to compare the ages obtained by this technique with those obtained from inspecting otoliths.

Methods

Walleyes from two lakes were used to evaluate the use of unsectioned dorsal spines as age-estimating structures. During September 2002, a sample of walleyes was collected from Red Lake, a 111,000-ha natural lake located in northwest Minnesota. The

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population had suffered a severe decline due to overexploitation; at the time of fish collection, it was being rehabilitated through stocking and a harvest ban. Hence, the sample was expected to contain mostly young fish (age $\leq$ 6 years). To ensure that the use of spines was evaluated for older individuals, a sample of walleyes larger than 500 mm total length (TL) was obtained in October 2004 from Mille Lacs Lake, another large (54,000 ha), natural lake located in north-central Minnesota.

Sagittal otoliths and dorsal spines were removed from captured walleyes. The first three dorsal spines were cut flush at the point of attachment with a pair of side cutters and then were torn free of the membrane that attached them to adjacent spines (Kocovsky and Carline 2000; Borkholder and Edwards 2001). Detached spines were stored for up to 4 months in coin envelopes before inspection; spines were not cleaned prior to storage. Ages were estimated from the second anterior dorsal spine (Campbell and Babaluk 1979; Marwitz and Hubert 1995; Borkholder and Edwards 2001). The spine to be inspected was first separated from the other spines. The basal end of the spine was dipped into a 70% ethanol solution to soften the flesh, which was then pulled back from the end of the spine with a pair of forceps. The cut end of the spine was sanded flat by wet sanding with 1,000-grit sandpaper. Sanding was best accomplished by securing the spine with forceps and moving the cut end of the spine in a circular pattern against a sheet of sandpaper placed on top of a smooth table or thick sheet of glass. The spine was then broken into two pieces so that approximately 2 cm remained on the basal section; the broken end of the basal section was embedded in a block of clay. The sanded end of the spine was coated with mineral oil to reduce the glare from the sanding marks and then was examined under a binocular dissecting scope with side illumination (Buckmeier et al. 2002). Side illumination was provided by a 150-W quartz halogen illuminator and was directed across the sanded surface of the spine with a 0.9-mm, handheld flexible fiber light guide. Otolith ages were estimated using the methods of Heidinger and Clodfelter (1987). The otoliths were broken in half perpendicular to the longest axis; the freshly broken surface was then coated with mineral oil and examined under a binocular dissecting scope with side illumination.

Two readers independently estimated ages from otoliths and spines. Spines were read by both an experienced spine reader and an inexperienced spine reader who was provided brief training before reading the spines. The inexperienced spine reader was proficient at estimating ages from walleye scales but had never determined ages from spines. Including an inexperienced spine reader provided some insight into the level of precision that could be expected by fisheries professionals new to the procedure. To ensure the best possible agreement among the otoliths and the highest standard for comparison, only experienced readers estimated ages from otoliths.

Precision of age estimates from spines was determined by calculating percent agreement and mean coefficient of variation (CV; Chang 1982) of pairwise comparisons of age assignments among readers of the same spine. The CV was calculated for each fish by the following formula:

$$\mathrm{CV}j = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(Xij - Xj)^2}{R-1}}}{Xj},$$

where R = number of times the age of each fish was estimated; X_j = mean age estimated for the jth fish; and X_{ij} = ith age estimation for the jth fish. The CV was then averaged across fish to produce a mean CV. Percent agreement and mean CV were also calculated between otolith readers to serve as a standard for comparison of the spine results.

Percent agreement between spine and otolith ages was calculated as a measure of the ability of unsectioned spines to replicate otolith-based age estimates. To assure the best otolith-based ages for comparisons, only walleyes with consensus otolith ages (i.e., the same age was assigned by both experienced readers independently) were used.

Results

Ages were estimated from both otoliths and unsectioned spines of 126 walleyes captured from Red Lake and 69 walleyes captured from Mille Lacs Lake. The combined sample included individuals from 226 to 733 mm TL (Figure 1). Ages assigned to walleyes inspected during this study ranged from 1 to 12 years for Red Lake fish and from 5 to 18 years for Mille Lacs Lake fish. Lengths at age derived from otoliths indicated that growth was slightly faster in Mille Lacs Lake than in Red Lake (Table 1).

Annuli of the unsectioned spines appeared as dark, hyaline bands against a lighter opaque background (Figure 2). Annuli were generally discernable under 25× magnification after sanding of the spine's basal end, application of mineral oil, and illumination of the sanded surface. Occasionally, the innermost annuli appeared wispy and split, thus requiring additional sanding to increase their visibility. Often, annuli of older walleyes appeared to be crowded near the outer edge of a spine. These older fish were more difficult to age and required repositioning of the fiber light to bring

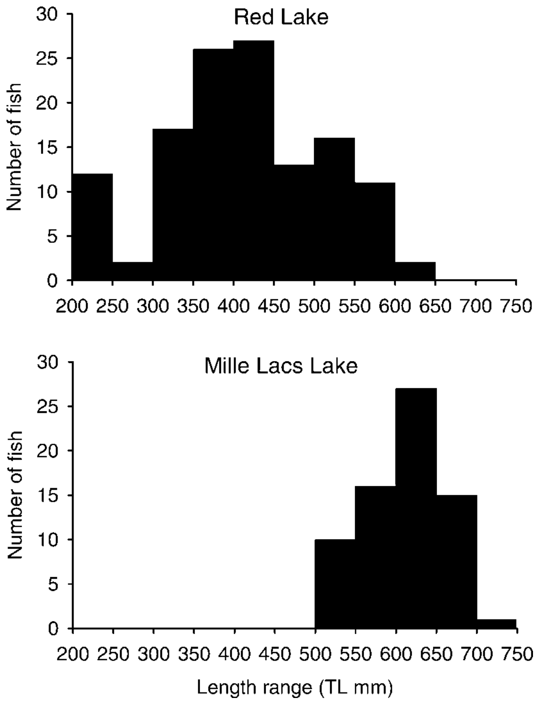

FIGURE 1.—Length frequency distribution (TL, mm) of walleyes collected from Red Lake, Minnesota, during September 2002 and Mille Lacs Lake, Minnesota, during October 2004.

TABLE 1.—Mean TL at age as estimated from otoliths of walleyes (n = number examined) collected from two Minnesota lakes: Red Lake (September 2002) and Mille Lacs Lake (October 2004). Only data from fish that were independently assigned the same age by both otolith readers (consensus otolith age) are presented.

	Red Lake Mean			Mille Lacs Lake Mean		
Otolith age	*n*	TL (mm)	SE	*n*	TL (mm)	SE
1	13	244.3	3.4			
2						
3	46	362.6	3.9			
4	1	419.0				
5	39	464.9	8.5	1	534.0	
6	16	531.8	12.0	3	533.3	14.8
7	1	520.0		4	564.5	9.5
8	1	495.0		8	563.4	16.1
9	3	539.7	12.8	9	592.6	8.2
10				5	622.0	10.2
11	1	521.0		3	571.7	33.9
12	1	553.0		5	632.8	9.9
13				10	649.8	11.9
14				2	672.5	27.5
15				2	609.0	66.0
16				11	640.8	14.1
17						
18				1	700.0	

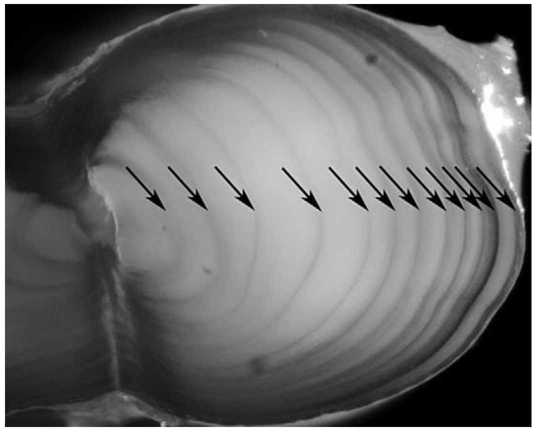

FIGURE 2.—Annuli visible on the basal end of a walleye spine collected from Mille Lacs Lake, Minnesota, in October 2004. The spine end was polished smooth, coated with mineral oil, illuminated from the side by directing a 150-W fiber light across the cut surface, and viewed under a binocular dissecting microscope at 25× magnification.

greater highlight to the individual annulus being viewed. Preparation time also was greatest for spines of the oldest walleyes because their larger size required removal of more material during the polishing stage, and a smoother surface was required to discern crowded annuli near the edge. Total preparation and inspection time, even of these older fish, seldom exceeded 2 min/spine.

Reader agreement rate for unsectioned dorsal spines was 95% for Red Lake walleyes. Reader age assignments for the remaining 5% of the sample differed by only 1 year (Table 2). The reader agreement rate for spines (95%) was similar to that achieved by two experienced readers using otoliths (98%; Table 2). Average precision associated with spine-based age estimates for Red Lake walleyes was relatively high (mean CV = 0.95%) but was lower than that observed for experienced readers using otoliths (mean CV = 0.29%; Table 2). Spine-based age estimates by both readers agreed with consensus otolith ages in 95% or more cases (Table 3). All discrepancies with the consensus otolith age were 2 years or less (Table 3) and typically occurred for individuals with consensus otolith ages of 7 years or more (Figure 3).

Reader agreement rate for unsectioned dorsal spines was only 70% for walleyes collected from Mille Lacs Lake (Table 2). Although 90% of the discrepancies were only 1 year, differences in age assignments of up to 3 years were observed (Table 2). The reader agreement rate associated with the two experienced readers using otoliths was higher (93%) than that observed for spines (70%) and approached rates

TABLE 2.—Mean CV and percent agreement between two readers using unsectioned dorsal spines and otoliths to estimate ages of walleyes (n = number examined) collected from two Minnesota lakes: Red Lake (September 2002) and Mille Lacs Lake (October 2004).

Structure	n	Age range	Percent agreement among readers				CV (%)
			Exact	1 year±	2 years±	3 years±	
Red Lake							
Spine	126	1–12	95	100	100	100	0.95
Otolith	125	1–12	98	100	100	100	0.29
Mille Lacs Lake							
Spine	69	5–18	70	90	99	100	2.71
Otolith	69	5–18	93	97	100	100	0.53

associated with otoliths and spines for walleyes collected from Red Lake (Table 2). Average precision of spine-based age estimates (mean CV = 2.71%) was substantially lower than that observed for the two experienced readers using otoliths (mean CV = 0.53%; Table 2). Furthermore, spine-based age estimates (both readers) and consensus otolith ages agreed for not more than 40% (Table 3) of the fish from the Mille Lacs Lake sample. Although most of the observed discrepancies were of 1 year or less, differences of up to 4 years were observed (Table 3). Most of the discrepancies occurred for individuals with consensus otolith ages of 7 years or more, and a bias toward spine underestimation of the consensus otolith age became apparent for age-10 and older walleyes (Figure 3).

Discussion

The study results indicated that unsectioned dorsal spines could successfully be used to replicate ages obtained from otoliths for many of the walleyes collected from Red and Mille Lacs lakes. Based on the consensus ages estimated from otoliths, unsectioned spines were most effective at replicating otolith ages of individuals younger than age 7 from both lakes. Much of the difference in spine-to-otolith agreement that did occur between Red and Mille Lacs lakes was probably due to differences in the age structure of the samples. The Mille Lacs Lake sample consisted wholly of individuals that were over 500 mm TL and that ranged in age from 5 to 18 years. The Red Lake sample included ages 1–12, but individuals younger than age 6 composed 79% of the total sample. The oldest fish in both samples showed the highest differences in spine-to-otolith agreement rates. Other researchers have reported similar results. Erickson (1983) reported increasing differences in age of walleyes between spine cross sections and otoliths beginning at age 6 in Lake Winnipeg, Manitoba, and at age 5 in lakes Eardley and Obukowin. Kocovsky and Carline (2000) reported 44–65% agreement between spine cross sections and otoliths for walleyes younger than age 4 in Pymatuning Reservoir, Pennsylvania, and 20–30% agreement for walleyes older than age 4.

Although spine-to-otolith agreement rates serve as a measure of the ability of one age-estimating method to replicate the results of another method (Welch et al. 1993), reader agreement rate serves as an assessment of the method for relative ease of annuli identification (Campana et al. 1995). During this study, reader agreement rates associated with unsectioned dorsal spines were not as high as those observed with otoliths. Reduced reader agreement rates may have been due in part to differences in reader experience levels; nevertheless, rates were consistent with the results of

TABLE 3.—Percent agreement between age estimates from unsectioned dorsal spines and otoliths in 122 walleyes collected from Red Lake (September 2002) and 64 fish from Mille Lacs Lake (October 2004). Only data from fish that were independently assigned the same age by both otolith readers (consensus otolith age) are presented.

Reader number	Age range	Percent agreement with consensus otolith age				
		Exact	1 year±	2 years±	3 years±	4 years±
Red Lake						
1	1–12	98	99	100	100	100
2	1–12	95	99	100	100	100
Mille Lacs Lake						
1	5–18	36	78	84	100	100
2	5–18	40	78	89	98	100

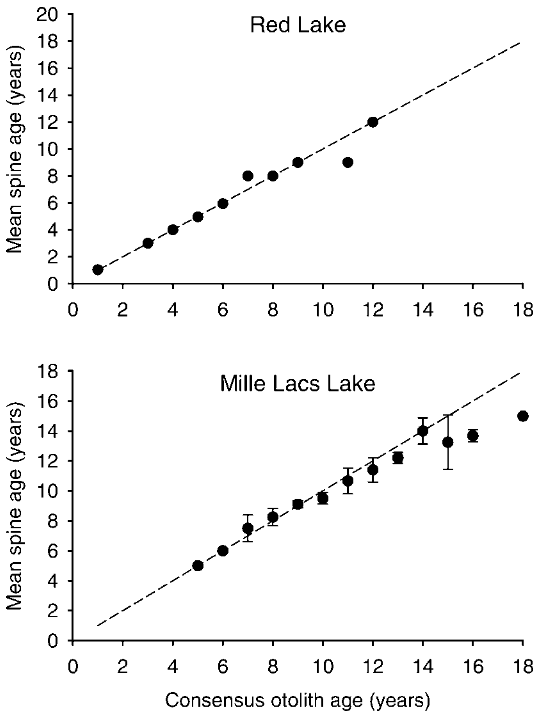

FIGURE 3.—Age bias plots comparing mean walleye ages estimated from unsectioned dorsal spines and otoliths. Fish were collected from Red Lake, Minnesota, during September 2002 and Mille Lacs Lake, Minnesota, during October 2004. Only consensus otolith ages (i.e., same age was independently assigned by both otolith readers) are presented. Error bars represent 95% confidence limits; the dashed line represents 1:1 agreement between structures.

previous studies that found greater between-reader agreement in walleye age for otoliths than for other structures (Erickson 1983; Kocovsky and Carline 2000; Isermann et al. 2003). The agreement rates I observed between readers of unsectioned spines did, however, exceed most spine-based agreement rates in other studies that used sectioning techniques: 47% reported by Olson (1980), 55% and 59% reported by Erickson (1983), and 55% reported by Isermann et al. (2003). Only reader agreement rates for sectioned walleye spines from Lake Wapun (81%; Campbell and Babaluk 1979) and Lake Winnipeg (80%; Erickson 1983), Manitoba, exceeded the reader agreement rates of unsectioned dorsal spines in this study. The high reader agreement rates in lakes Winnipeg and Wapun were probably influenced by the age structure of the populations. Campbell and Babaluk (1979) reported that although the overall reader agreement rate was 81%, the rate for fish older than age 8 was only 53%. Erickson (1983) estimated that all of the walleyes in his sample were less than age 10.

It is possible that the use of side illumination to highlight annuli on unsectioned spines reduces some of the error observed when estimating walleye ages from sectioned spines. Kocovsky and Carline (2000) reported problems with faint or merged rings, partial rings, double rings, and crowding of distal rings on sectioned spines. Bruesewitz et al. (2002) reported apparent false annuli on several spine cross sections from Ann Lake, Minnesota. Isermann et al. (2003) reported that much of the lack of agreement among readers was associated with identification of the first annulus on spine sections. Using a dissecting microscope with side illumination to view the polished basal end of an unsectioned spine can enhance the appearance of the annuli by providing depth and convergence to annuli that otherwise may appear split or discrete when the spine is viewed as a thin section. The additional depth of calcified material below the focal plane contributes to the contrast in appearance between the opaque and hyaline zones when illuminated from the side with a high-intensity fiber light. This contrast is further enhanced by use of a dissecting microscope, which provides a greater depth of focal field than that of a compound microscope. Occasionally, the innermost annulus of an unsectioned spine appeared to be wispy or split when its basal end was viewed under a dissecting microscope with side illumination. Removal of additional material by sanding of the basal end increased its visibility as a single annulus and thus indicated that the split appearance was caused by viewing the spine too close to the base. The occurrence of inner annuli that appear wispy or split could be exacerbated by the use of sectioned spines. Unlike channel catfish spines (Sneed 1951; Buckmeier et al. 2002), walleye spines lack good reference points that identify where to remove sections for age estimation. Consequently, it is unknown whether a section was taken from the correct location until the time necessary to cast, cut, and mount the section has been invested.

In addition, to improve identification of inner annuli, the flexibility in lighting conditions afforded by the high-intensity light with a flexible light guide may provide better resolution of the crowded annuli near the edges. The fiber light guide could be held against the side of the spine to emit transmitted light, as is traditionally used to illuminate sectioned spines, or could be shone across the cut and sanded surface. When the fiber light guide was held against the spine's side, the light was redirected along the axis of the spine and the cut surface gave the appearance of illumination from below. This made the entire spine glow, and the annuli appeared as light, hyaline bands against an opaque brown background. Annuli were easily discerned in spines of younger fish, but it was often difficult to examine crowded annuli near the spine edges in older fish. When the fiber light was shone across the cut and sanded surface of the spine, annuli appeared as dark, hyaline bands against a lighter opaque background (Figure 2) and the crowded annuli were much more visible because the light could be directed to bring greater highlight to the individual annuli being viewed.

The greatest advantages of the use of unsectioned spines over the traditional use of sectioned spines are simplicity and speed of preparation. By eliminating the need to section spines, the entire preparation process is simplified. Instead of casting the spine in resin, sectioning the spine, and mounting the section to a microscope slide, the spines are prepared simply by polishing the cut end smooth with sandpaper. The cost of preparation is reduced by eliminating the need for expensive sectioning equipment and consumable materials, such as resin and microscope slides. By reducing steps in the preparation process, total preparation time is also reduced. Kocovsky and Carline (2000) reported that the time required to section, mount, and polish walleye spines was longer than the preparation techniques for either scales or otoliths. Welch et al. (1993) also reported longer preparation time for spines over scales for estimating ages of striped bass *Morone saxatilis*. Isermann et al. (2003) compared times required to remove, process, and view walleye scales, dorsal spine cross sections, whole otoliths, and sectioned otoliths. Dorsal spines were the quickest to remove, but slower than all but sectioned otoliths in the length of time required to process and view. The mean time reported by Isermann et al. (2003) to section, mount, and view a walleye spine was 2.4 min. The time taken to process and view unsectioned spines during this study seldom exceeded 2 min/spine. Much of the processing time for preparing unsectioned spines was spent sanding the cut end of the spine perpendicular to its length. Thus, assuring that spines are cut perpendicular to their shafts during removal from the fish can minimize processing time.

This study indicates that unsectioned dorsal spines offer a simple, fast, inexpensive, and reasonably precise approach for replicating consensus otolith ages of younger walleyes. Although otoliths may still be necessary to estimate the ages of older fish within a sample, the use of unsectioned dorsal spines can allow biologists to reduce the number of walleyes sacrificed and closely approximate otolith-based estimates of population age structure. Based on mean lengths at age in Red and Mille Lacs lakes, 530 mm TL would be an appropriate threshold to begin taking otoliths for walleye age estimation. Alternatively, an age error matrix could be developed for a walleye population through comparison of spine and otolith ages, and error-corrected spine ages could be used for routine age assignment. Periodic updating of the error matrix could be accomplished through inspection of a smaller sample of otoliths.

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