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A Comparison of Methods for Estimating Ages of Unexploited Walleyes

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Abstract.—Age estimation is an important diagnostic tool for fisheries managers. Previous studies on comparison of age estimation methods of walleyes *Stizostedion vitreum* have shown otoliths are the best method overall and that dorsal spine cross sections are a suitable alternative to scales, the most commonly used method. We compared ages estimated from scales, dorsal-spine cross sections, and otoliths for unexploited walleyes from Pymatuning Sanctuary, Pennsylvania. We collected these structures from fish sampled in trap nets during the spawning run in March and April 1997. Two readers independently examined these structures without knowledge of fish length or gender. Otoliths provided the best agreement between readers for both genders (94% for females, 86% for males) and dorsal-spine cross sections provided the poorest agreement (63% of females, 62% of males). Younger ages were estimated from scales than from spines, and this difference was greater for males than for females. Age estimates from both scales and dorsal spines agreed poorly with age estimates from otoliths for both sexes. Ages estimated from scales were consistently lower than ages estimated from otoliths, especially after age 4; differences between ages estimated from otoliths versus spines were inconsistent. We concluded that otoliths are the best method of age estimation for this population and that scales are the best alternative for fish less than 500 mm long. This research underscores the need to validate age estimates with fish of known age and to test multiple age estimation methods when beginning research on previously unstudied populations.

Fisheries managers and researchers require reliable methods of age estimation for studies of age structure and dynamics of fish populations. Age-frequency data can be used to estimate growth and mortality, monitor effects of changes in regulations, and diagnose environmental changes (Hammers and Miranda 1991). Scales have been the most widely used method of age estimation among managers because they can be collected without killing fish and are the least time consuming and least expensive to prepare. However, studies have shown that scales underestimate ages compared with other calcified structures (e.g., fin rays, spines, and otoliths), especially among older or slowly growing fish (Beamish and Harvey 1969; Mills and Beamish 1980; Belanger and Hogler 1982; Erickson 1983; Boxrucker 1986; Welch et al. 1993). Otoliths are considered to be the most accurate method of age estimation for most species, in part because otolith growth is not directly linked to somatic growth (Simkiss 1974), but otoliths are not routinely used because fish must be killed to extract otoliths.

Few published studies compare age estimation methods for walleyes *Stizostedion vitreum.* Belanger and Hogler (1982) compared fin rays, spines, scales, and otoliths for walleyes from a heavily exploited lake in Michigan. Erickson (1983) compared age estimation for scales, dorsal spine cross sections, and otoliths for walleyes from several Manitoba lakes with different levels of exploitation. In both studies, as estimated fish age increased, younger ages were estimated from scales than from spines, and from spines versus otoliths. Otoliths were considered the most precise method in both studies. Erickson (1983) also found that otoliths were more accurate than scales or spines. Campbell and Babaluk (1979) concluded dorsal spine cross sections were the preferred method of age estimation for an unexploited walleye population in a eutrophic lake in Manitoba.

This study was conducted in conjunction with research into the dynamics of the unexploited walleye population of Pymatuning Sanctuary, Pennsylvania. Because of the disadvantage of having to kill fish to extract otoliths, and the bias that killing fish from an otherwise unexploited population would introduce into a study of population dynamics, we sought to determine whether a non-

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TABLE 1.—Percentage agreement of walleye age estimates between readers using scales, dorsal-spine cross sections, and otoliths. The last three columns show the percentage of the time that readers' age estimates were within 1, 2, and 3 years of each other for the same fish (e.g., if one reader estimates a fish's age as 7 years and another estimates it as 9 years, the percentage agreement falls in the category).

Sex	Structure	*N*	Percentage exact agreement	Percentage agreement by number of years		
				± 1 year	± 2 years	± 3 years
Female	Otolith	31	94	97	100	
	Scale	129	86	98	100	
	Dorsal spine	130	63	83	92	99
Male	Otolith	29	86	100		
	Scale	147	84	99	100	
	Dorsal spine	147	62	86	93	96

lethal method of age estimation would be sufficient. Our objective was to determine the relative precision of age estimates from scales, dorsal spine cross sections, and otoliths.

Methods

We collected walleyes during the spring spawning run in Pymatuning Sanctuary, a 530-ha eutrophic impoundment on the upper Shenango River in Crawford County, Pennsylvania. The sanctuary flows into Pymatuning Reservoir over a 1-m high concrete spillway that prevents immigration (emigration is possible). The sanctuary has been closed to angling since its creation in 1933.

We collected scales, dorsal spines (hereafter spines), and otoliths from mature walleyes sampled with trap nets between 19 March and 11 April 1997. We collected both scales and spines from 130 females and 147 males. Scales were taken inferior to the lateral line and posterior to the point of insertion of the pectoral fin. We removed the second or third dorsal spine by cutting as close to the base as possible. We extracted both saggital otoliths from 31 females and 29 males from which we took scales and spines.

Using a microfiche reader, we viewed acetate impressions of scales at 24× magnification. Scale annuli were identified using methods described by Carlander (1961). We prepared spine cross sections by first casting them in an epoxy resin, allowing them to set at least 24 h before sectioning, and then cutting three or four cross sections approximately 1–3 mm thick from the base of the spine. We polished both sides of each cross section with 400-grit sandpaper to improve clarity. Spine cross sections were viewed under a compound microscope at 40× magnification. Marks on spine cross sections appear as light rings on a dark matrix. Only complete rings were counted as annuli. We prepared otoliths by breaking them through the nucleus and mounting them on a piece of acetate slide; we then ground each half of each otolith to approximately 1 mm thick. We viewed otolith cross sections under low magnification using a dissecting microscope. Immersion oil was used for spine and otolith cross sections to improve image clarity.

Two readers independently estimated ages from each calcified structure without knowledge of the length or gender of the fish from which it was taken. We simultaneously reread all structures for which readers disagreed on estimated age. If disagreement persisted, the structure was eliminated from subsequent analyses. We calculated percent agreement between readers for each structure to determine which structures yielded the most consistent (precise) age estimates. We generated age-bias plots similar to those used by Mills and Beamish (1980) and Campana et al. (1995) for pairs of structures to visually assess whether there were consistent differences in age estimates between structures. Females and males were analyzed separately and by estimated age-group to determine if differences were related to gender or estimated age.

Results

Agreement between readers was best for otoliths and worst for dorsal spine cross sections for both genders (Table 1). Readers agreed on ages estimated by otoliths for 94% of the females and 86% of the males. Agreement between readers for dorsal spine cross sections was noticeably poorer but consistent between genders (63% of females, 62% of males). Ages estimated by scales agreed 86% of the time for females and 84% for males.

Agreement of age estimates from different structures was poor. Ages estimated from scales were generally lower than ages estimated from spine cross sections for both genders (Figure 1). The

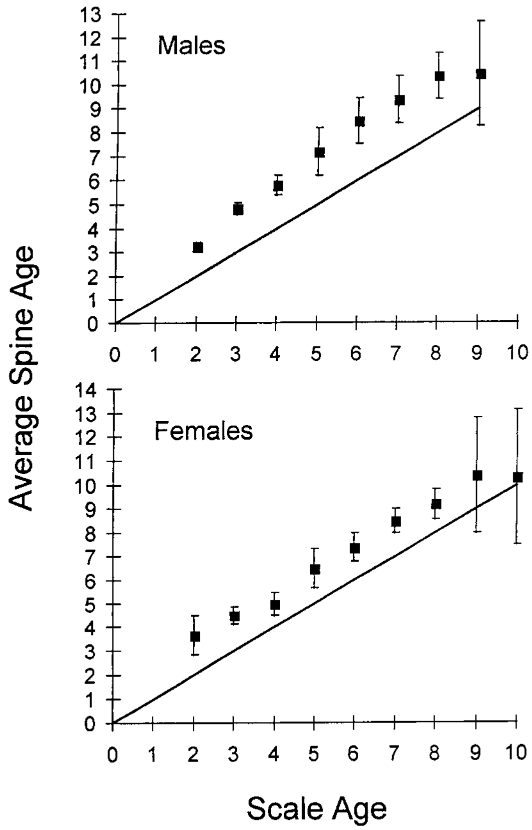

FIGURE 1.—Age bias graphs for male and female walleyes from Pymatuning Sanctuary, Pennsylvania, comparing average ages estimated from spines with ages estimated from scales. Vertical bars are approximate 95% confidence limits (very narrow for scale estimates of age-2 males). The diagonal line represents agreement between age estimates.

difference between ages estimated by scales versus spines was more pronounced for males age 3 and older (confidence bars are provided only as measure of variability and cannot be used to determine statistical significance; see Campana et al. 1995). Otoliths generally provided the highest estimated age. Agreement between either scales or spines and otoliths was also poor but better than agreement between scales and spines (Figure 2). Agreement was best for dorsal spine cross sections versus otoliths. Older ages were estimated from spines than from otoliths for younger fish but younger ages were estimated from otoliths for older fish.

Differences in percentage agreement between structures varied by gender and age. For both genders, percent agreement between structures was much higher for fish less than age 4 than for older fish (Table 2). Agreement between scales and otoliths decreased from 90% to 20% for females and from 58% to 0% for males older than age 4. The same pattern of distinct decrease in agreement for older fish was evident for spines versus otoliths; percentage agreement decreased from 65% to 30% for females and from 44% to 20% for males older than age 4.

Discussion

For walleyes in Pymatuning Sanctuary, otoliths provided the most precise age estimate overall, followed by scales and spines. This conflicts with Belanger and Hogler (1982), who found that spines and scales yielded equally precise age estimates, and Erickson (1983), who found that spines yielded more precise estimates than scales. Our results also conflict with those of Campbell and Babaluk (1979), who determined dorsal spine cross sections provided the most precise age estimates. We had difficulty reading spines because of faint or merged rings, partial rings, and crowding of distal rings, especially for males. Scales were also difficult to read for fish older than age 6 because of crowding or lack of annulus formation. We attribute difficulties in reading scales and spines primarily to poor readability (spines) and slow growth (scales and spines), especially following sexual maturity.

Relative inexperience of readers was not a factor in precision of age estimates. Before initiating this research, we received instruction on preparation and reading of spine cross sections from a biologist who uses walleye spines for most age estimation work (R. King, Wisconsin Department of Natural Resources [WDNR]). We also had a subsample of our spine cross sections read by an experienced fisheries technician (T. Brecka, WDNR) as a means of testing our ability relative to that of someone with more experience. Our age estimates were consistent with those of the reader with more experience, indicating no effect of relative inexperience on age estimates. We also used otoliths when available to help us identify annuli on spine cross sections, based on the assumption that otoliths are the most accurate age estimation method (Erickson 1983; Boxrucker 1986; Welch et al. 1993). Compared with the more experienced reader, our age estimates from spine cross sections were equally precise and accurate.

Differences in relative precision of age estimates between males and females beyond age 4 are caused by gender-related differences in growth and age at sexual maturity. All female walleyes in Pymatuning Sanctuary are mature by age 4 and all

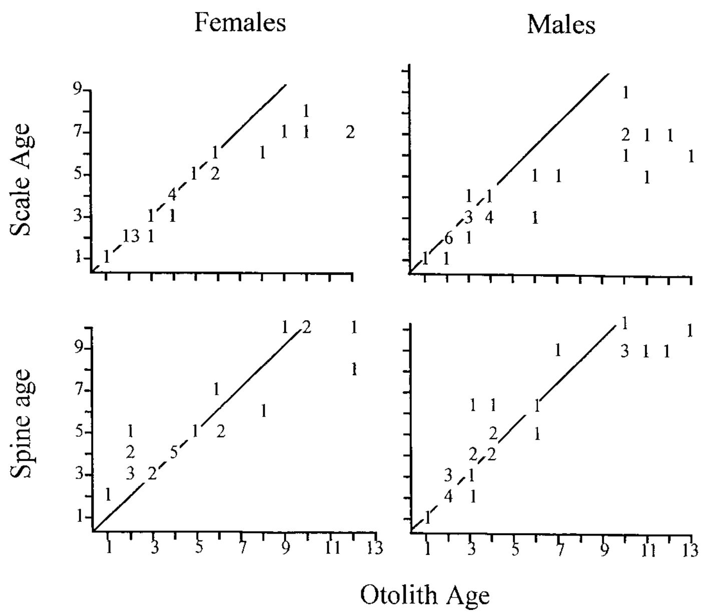

FIGURE 2.—Age bias graphs for male and female walleyes from Pymatuning Sanctuary, Pennsylvania, comparing ages estimated from scales and spines to ages estimated from otoliths. Numerals indicate numbers of fish at a data point. The diagonal line represents agreement between age estimates.

males by age 3 (Kocovsky 1999). After sexual maturity, growth slows substantially for both genders, but the decrease is more distinct for males. For otolith estimates, length-at-age for males age 10 and older averaged 85 mm (total length) shorter than for females, compared with only 13–25 mm at age 5 (Kocovsky 1999). Slower somatic growth for males probably results in crowding of annuli near the edges of scales and spines and lack of annulus formation.

Poor readability of spine cross sections is also probably due to poor adult walleye growth. Summer water temperatures in the sanctuary in 1997 exceeded the optimum temperature for walleye growth throughout the summer months (Kocovsky 1999). Because the sanctuary population is unexploited, adult biomass is high. The combination of an unfavorable thermal regimen and high walleye biomass resulted in slow adult growth (Kocovsky 1999), which probably affected annulus formation on scales and spines, hence their readability.

For a few of our fish, ages estimated from spine cross sections were twice those estimated from otoliths for both reader's age estimates—as well as those of the more experienced reader who validated our age estimates. Double rings interpreted as separate annuli on some spine cross sections could only be identified when otoliths were available for age verification. It is likely that ages estimated from spine cross sections overestimated fish ages for several fish for which we did not have otoliths, including fish as young as age 2. Because we did not have otoliths for all fish, we lack confidence in the validity of ages estimated from spine cross sections. Anomalous ring formation was more important than reader experience in identification of annuli on spine cross sections.

TABLE 2.—Percentage agreement of walleye age estimates between either scales or spine cross sections and otoliths by gender and age group.

Comparison	Otolith age	Females		Males	
		Exact	± 1 year	Exact	± 1 year
Scale–otolith	≤ 4 years	90	100	58	95
	> 4 years	20	40	0	18
Spine–otolith	≤ 4 years	65	85	44	89
	> 4 years	30	70	20	60

Using ages estimated from otoliths as a basis for comparison, age estimates from scales were better for younger walleyes, whereas age estimates from dorsal spine cross sections were better for older walleyes from Pymatuning Sanctuary. Poor agreement between readers resulted in a high level of uncertainty for age estimates from spine cross sections compared with scales and otoliths. Additionally, the finding of older spine ages than otolith ages for young fish and younger otolith ages than spine ages for older fish led to further uncertainty of spine age estimates.

In our experience with this population, preparation of spine cross sections was much more time consuming and expensive than preparing either otolith cross sections or scales. We experienced considerable difficulty in preparing cross sections that were interpretable. Sectioning spines took relatively little time (comparable to cleaning and pressing scales and breaking, mounting, and grinding otoliths) but polishing spine cross sections to improve readability took extensive effort and often failed to improve image clarity.

The results of this research suggest that otoliths are the best of the three age estimation methods for a detailed study of the dynamics of the unexploited walleye population of Pymatuning Sanctuary. Based on the objectives of our population dynamics study and uncertainty regarding readability of spines, we determined scales were the best alternative to otoliths for fish less than 500 mm long because they are easily and inexpensively collected, prepared, and read; can be collected without killing fish; and provide precise and relatively accurate (compared with otoliths) age estimates. We chose 500 mm as the length beyond which we would use otoliths because the average age of 500-mm walleyes in Pymatuning Sanctuary is 5–6 years (Kocovsky 1999) and because ages estimated from scales are noticeably lower than ages estimated from otoliths for older fish (Figure 2). Although we were unable to do so, this research underscores the importance of validating age estimation methods by comparing estimates from hard parts to known-age fish. This research also underscores the importance of testing multiple methods of age estimation when beginning research on a previously unstudied population.

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