

RESEARCH ARTICLE

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Methods**

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Estimating Largemouth Bass Age: Precision and Comparisons among Scales, Pectoral Fin Rays, and Dorsal Fin Spines as Nonlethal Methods

Reid L Morehouse^{1*}, Steven B Donabauer², Angela C Grier^{2#}

¹Department of Zoology, Oklahoma State University, 501 Life Sciences West, Stillwater, OK 74078, USA.

²Northeast Regional Office, 1353 South Governors Drive, Columbia City, IN 46725, USA.

[#]Formerly at Northeast Regional Office as mentioned above.

*Correspondence: reid.morehouse@okstate.edu, reid_morehouse@hotmail.com

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Abstract

Scales are commonly used to age largemouth bass since the samples are easy to extract and process. Yet, scale annuli are often difficult to interpret among the largest (and presumably oldest) individuals. The goal of this study was to determine whether a more precise nonlethal structure could be used to age largemouth bass. We examined scales, pectoral fin rays, and dorsal fin spines from largemouth bass collected from six glacial lakes in northern Indiana. Collectively, 861 structures were aged by three analysts and post-concert reads concluded with 85%, 94%, and 99% agreement for scales, pectoral fin rays, and dorsal fin spines, respectively. Pre-concert exact (± 0 years) percent agreement among age analysts was highest for dorsal fin spines (27%) followed by pectoral fin rays (23%) and scales (2%). Mean coefficient of variation was lowest among dorsal fin spines (16%) followed by pectoral fin rays (22%) and scales (37%). Scales exhibited the most variability and bias, while dorsal fin spines were the least variable and most unbiased structure. We encourage researchers to use scales for younger fish and dorsal fin spines for larger fish when both: (1) nonlethal methods and (2) a higher standard of precision are sought to evaluate specific management objectives.

Keywords: Aging; dorsal spines; pectoral fin rays; precision; scales.

1. Introduction

Largemouth bass *Micropterus salmoides* are among the most sought after sport fish in the United States. The United States Fish and Wildlife Service [1] conducted a nationwide survey that revealed 40% of all freshwater anglers specifically target largemouth bass. In a 2005 angler survey, respondents ranked largemouth bass as the second most important species to manage in Indiana only behind bluegill *Lepomis macrochirus* [2]. Largemouth bass are an economically important species generating approximately \$136 million annually in Indiana [1]. Thus, fisheries managers must have a clear understanding of the structure and dynamics of these populations to ensure sustainability.

Accurate and consistent age determination is a key aspect of managing sport fish populations [3]. Scales are the most commonly used calcified structure for determining the age of black bass [4, 5] since they are easy to extract and process. However, scale annuli are often difficult to interpret amongst the largest (and presumably oldest) individuals. Evidence suggests that scales provide untrustworthy age estimates, as 73% of surveyed biologists felt that scales inaccurately aged fish to the maximum age [5]. Furthermore, researchers have demonstrated that otoliths are the most precise and unbiased structure to age largemouth bass [6-8]. However, the primary disadvantage of using otoliths is that the fish must be euthanized, and in some situations (i.e., mark-recapture studies) sacrificing the fish is not permissible. As a result, alternative nonlethal aging methods are required.

Alternative calcified structures can be extracted for age determination [9]. Fin spines and rays have been used to age other species (walleye, *Sander vitreus* [10]; yellow perch, *Perca flavescens* [11]; striped bass, *Morone saxatilis* [9]; river carp suckers, *Carpionodes carpio* [12]; black crappie, *Pomoxis nigromaculatus* [13]) with variable results. For example, Erickson [10] found that percent agreement between readers was higher for walleye dorsal fin spines than for scales. Conversely, Kocovsky and Carline [14] reported opposite results and suggested between reader agreements were higher for scales than for dorsal fin spines. Furthermore, when compared to scales, Welch

et al. [9] suggested that dorsal fin spines more accurately estimated age for striped bass. The disparity between results warrants further investigations comparing nonlethal aging methods within and among species.

Alternative nonlethal aging methods are gaining in popularity among fishery biologists and managers that do not want to or cannot sacrifice fish species for various reasons (i.e., research or public relation issues). While multiple studies have examined scales and otoliths for aging largemouth bass [7, 8, 15, 16], only one study has evaluated alternative nonlethal aging methods [15]. For example, Maraldo and MacCrimmon [15] found that dorsal fin spine and pectoral fin ray cross sections were unreliable for aging largemouth bass from northern waters. They reported that correlation coefficients were lower and significant biases towards inaccurate aging were greater in dorsal fin spines and pectoral fin rays relative to other aging structures used. Additionally, Isermann *et al.* [13] examined dorsal spines and scales as nonlethal aging methods relative to otoliths in black crappie and concluded that dorsal spines were not an adequate nonlethal alternative and scales are still recommended when otoliths cannot be used.

The Indiana Department of Natural Resources solely utilizes scales for age determination in largemouth bass populations around the state. As shown above, scales routinely result in under/over aging in multiple species. As many agencies cannot or do not sacrifice largemouth bass for public relations, research, or other reasons, alternative nonlethal methods aging methods need to be evaluated. The goal of this study was to evaluate the use of scales, pectoral fin rays, and dorsal fin spines to estimate the age the largemouth bass. The main objective was to determine the precision of ages estimated among calcified structures as assigned by three independent readers.

2. Methods

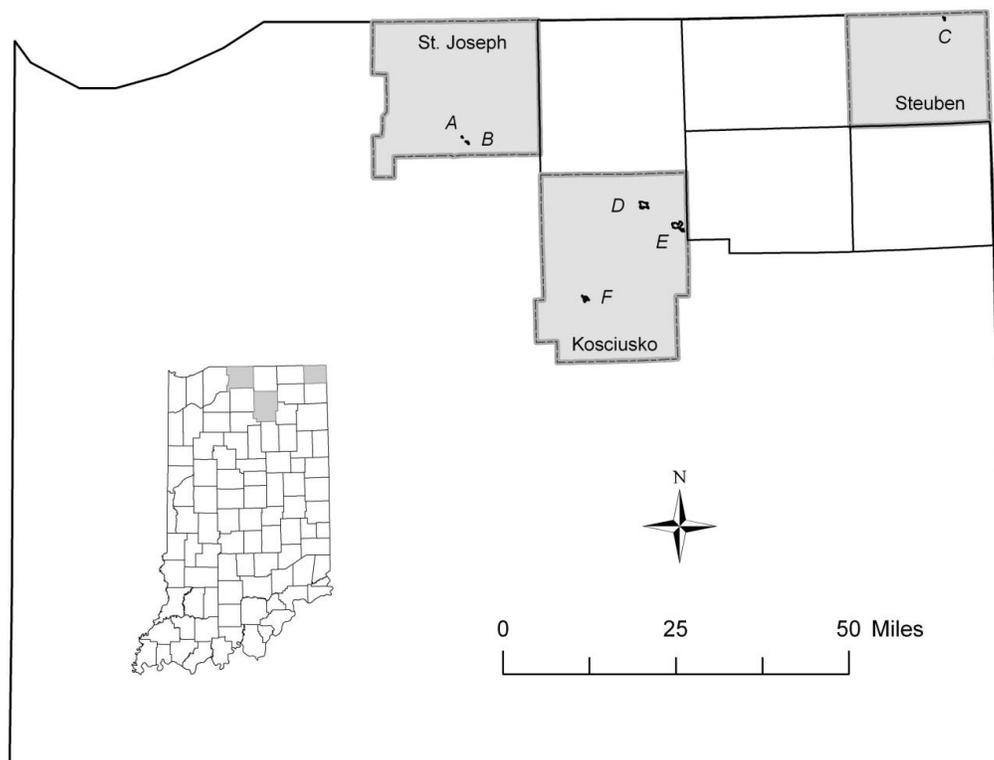
Six natural lakes in northern Indiana were sampled for largemouth bass from April to May 2006. Biologists collected largemouth bass from Pleasant and Riddles Lakes in southern St. Joseph County, Fish Lake in northern Steuben County, Dewart and Webster Lakes in northeastern Kosciusko County, and Palestine Lake in southwestern Kosciusko County (Figure 1). Largemouth bass were sampled by night electrofishing with boom-mounted pulsed DC boats equipped with Smith-Root Type VI-A electrofisher (60 PPS; 530-707V; 4-6 Amps). Calcified structures (scales, pectoral fin rays, and dorsal fin spines) were collected from five largemouth bass per 12.7 mm group in each of the six lakes. Scales were taken from below the lateral line midway between the pectoral and pelvic fins. The left pectoral fin ray and the first three anterior dorsal fin spines were clipped as close to the body as possible. All structures were stored in coin envelopes and left to air dry. Before largemouths were released, they were measured for total length (TL; ± 2.5 mm).

Approximately six largemouth bass scales per fish sample were heat pressed on acetate slides. To ensure that independent analysts read the same scale sample, the most readable impression of the series was projected with a microfiche reader and a high resolution image was captured with a 3.2 mega pixel digital camera (Fuji S3000, Valhalla, New York) mounted on a tripod. Pectoral fin rays and the second anterior dorsal fin spine (third anterior dorsal fin spine used only if the second dorsal fin spine was not suitable for sectioning) were embedded in a two-part epoxy resin (System Three, Auburn, Washington, D.C.) inside a polyester mold (Ted Pella, Inc., Redding, California). After the resin solidified, the samples were sectioned (70 μ m) with a modified lapidary saw (1725 rpm; 1/3 hp motor; 23 μ m) equipped with a diamond impregnated Blue Blazer blade (Raytech Industries, Middletown, Connecticut). The cross-sections were mounted with epoxy resin between two glass slides and viewed (60X) with reflected light under a stereo microscope (Olympus, Center Valley, Pennsylvania). Digital images of the pectoral fin ray and dorsal fin spine cross sections were captured with a digital camera (Model: Olympus Optical DP70) mounted on a trinocular compound microscope and Image Plus Pro 5.0 software (Media Cybernetics Inc., Silver Spring, Maryland). Images of all calcified samples were uploaded into PowerPoint® (Microsoft Corporation, Redmond, Washington) and given a unique identification number, which provided age analysts the ability to independently mark annuli without knowledge of fish length or age (i.e., pre-concert read). After marking annuli, independent analysts collectively resolved age discrepancies (i.e., post-concert consensus) and ages not agreed upon were excluded from further analysis. All age analysts had prior experience aging calcified structures.

Ideally, a sample of known-age fish would be used to test for age assignment bias between readers and among calcified structures [17]. However, known-age fish rarely exist [7] and were not available for this study. Thus, alternative methods were used to assess age assignment bias. Age frequency tables were constructed to illustrate how analysts' independent age assignments among structures compared to post-concert agreement. Ages above (under-aged) or below (over-aged) the 1:1 consensus produced measures of variability to identify bias

among age analysts. In addition, age-bias plots were used to illustrate differences of post-concert age assignments among calcified structures (i.e., within the same fish).

Figure 1: Northern Indiana lakes sampled for largemouth bass in 2006; Pleasant (A), Riddles (B), Fish (C), Dewart (D), Webster (E), and Palestine (F).



Precision (pre-concert age assignment) was estimated by: (1) percent agreement tables; and (2) calculation of the coefficient of variation among pairwise comparisons of independently assigned ages. The coefficient of variation (*CV*) was calculated by:

$$CV_j = \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{n-1}}}{X_j} \cdot 100$$

where X_{ij} is the i th age estimate for the j th fish; X_j is the mean age estimate of the j th fish; and n is the number of times the fish was independently aged. The coefficient of variation was averaged across all fish to obtain mean *CV* values for each calcified structure among all lakes.

3. Results and Discussion

Collectively, ages were assigned to 861 calcified structures among largemouth bass that ranged 66 to 569 mm TL. The maximum consensus age estimates were 11, 12, and 13 years for scales, pectoral fin rays, and dorsal fin spines respectively. Post-concert reads concluded with 85%, 94%, and 99% agreement for scales, pectoral fin rays, and

dorsal fin spines, respectively. Exact percent agreement (± 0 years) among analysts was lowest for scales (2%; Table 1) followed by pectoral fin rays (23%; Table 2) and dorsal fin spines (27%; Table 3). Analysts were able to independently reproduce dorsal fin spine age assignments more effectively than by using either pectoral fin rays or scales as supported by mean CV values (16%, 22%, 37% respectively). Thus, dorsal fin spines were more precise while scales were shown to have more age variability among independent analysts.

Table 1: Mean coefficient of variation (CV) and percent frequencies of agreement for largemouth bass ages determined by three independent analysts using scales.

	N	Agreement (%)					Discarded	CV
		Exact	± 1 yr	± 2 yr	± 3 yr	>4 yr		
Dewart	49	0	6	16	15	34	29	45.7
Fish	38	5	16	40	15	13	11	31.8
Palestine	74	1	15	19	18	38	9	43.9
Pleasant	33	3	42	37	6	0	12	31.1
Riddles	41	5	26	38	12	2	17	29.0
Webster	53	0	24	29	29	7	11	41.8
Mean	288 ^a	2	22	30	16	15	15	37.2

^aTotal largemouth bass samples aged from all six study lakes.

Table 2: Mean coefficient of variation (CV) and percent frequencies of agreement for largemouth bass ages determined by three independent analysts using pectoral fin rays.

	N	Agreement (%)					Discarded	CV
		Exact	± 1 yr	± 2 yr	± 3 yr	>4 yr		
Dewart	49	14	37	22	13	8	6	32.6
Fish	38	16	42	31	6	0	5	26.1
Palestine	73	15	33	29	3	8	12	26.2
Pleasant	33	27	49	15	6	0	3	18.2
Riddles	41	36	40	10	12	0	2	15.6
Webster	53	31	40	14	6	2	7	11.7
Mean	287 ^a	23	40	20	8	3	6	21.7

^aTotal largemouth bass samples aged from all six study lakes.

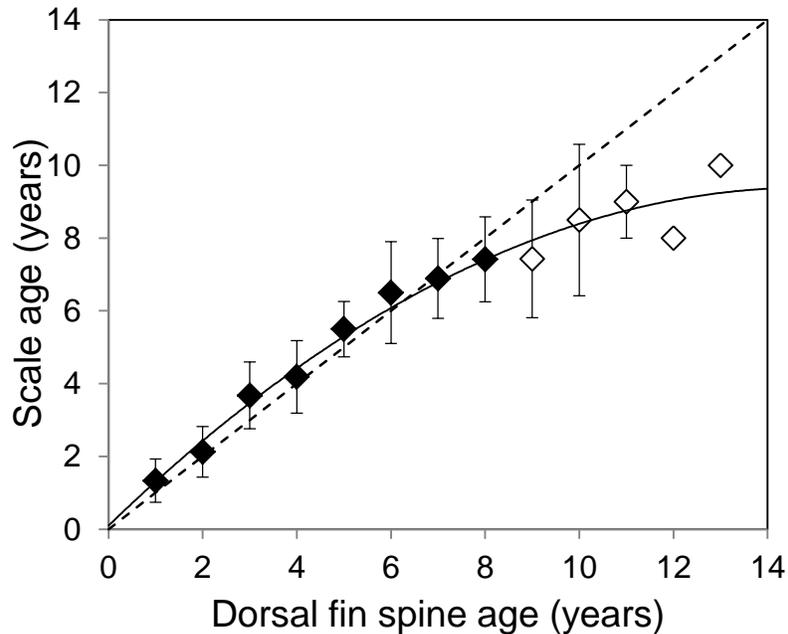
Table 3: Mean coefficient of variation (CV) and percent frequencies of agreement for largemouth bass ages determined by three independent analysts using dorsal fin spines.

	N	Agreement (%)					Discarded	CV
		Exact	± 1 yr	± 2 yr	± 3 yr	>4 yr		
Dewart	49	31	38	23	4	4	0	17.7
Fish	38	18	40	21	18	3	0	21.7
Palestine	73	27	52	10	6	2	3	12.9
Pleasant	33	33	43	3	18	3	0	15.5
Riddles	42	19	57	24	0	0	0	14.8
Webster	52	33	36	24	0	0	7	11.9
Mean	286 ^a	27	44	18	8	2	1	15.8

^aTotal largemouth bass samples aged from all six study lakes.

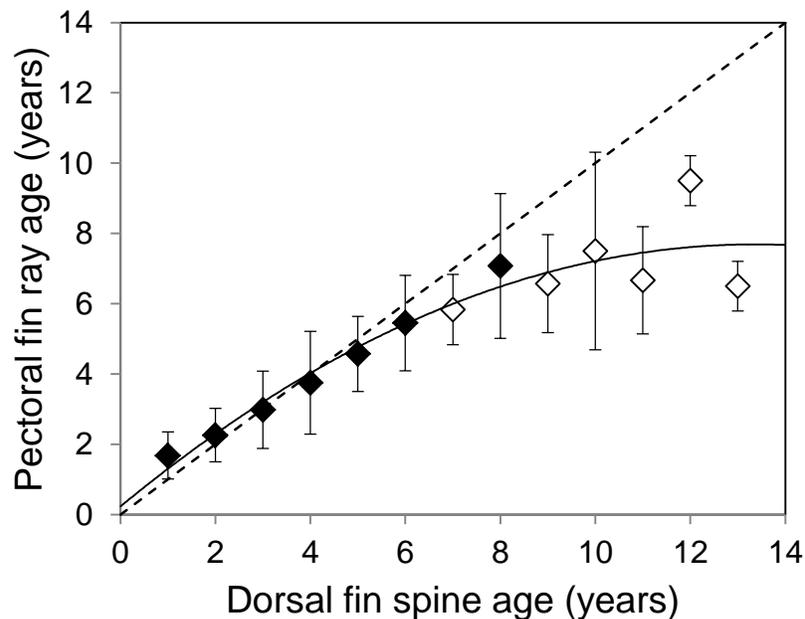
Post-concert agreement of calcified structures was compared with age-bias plots. When directly compared to dorsal fin spines, scales were generally unbiased up to age 8, but increasingly under-aged largemouth bass age greater than 8 (Figure 2).

Figure 2: Age-bias plots illustrating post-concert mean (\pm SD) age cross comparisons between scales (y-axis) and dorsal fin spines (x-axis) for largemouth bass. Solid data points represent unbiased mean values that are $< \pm 1$ year from theoretical equivalence (dashed line). Hollow data points represent biased mean values that are $\geq \pm 1$ year (solid line) from theoretical equivalence.



Similarly, when compared to dorsal fin spines, pectoral fin rays were generally unbiased up to age 6 but increasingly under-aged largemouth bass age greater than 6 (Figure 3).

Figure 3: Age-bias plots illustrating post-concert mean (\pm SD) age cross comparisons between pectoral fin rays (y-axis) and dorsal fin spines (x-axis) for largemouth bass. Solid data points represent unbiased mean values that are $< \pm 1$ year from theoretical equivalence (dashed line). Hollow data points represent biased mean values that are $\geq \pm 1$ year (solid line) from theoretical equivalence.



We found that dorsal fin spines yielded more precise age estimates for largemouth bass when compared to scales and pectoral fin rays. Scales were reliable to age-8 when compared to dorsal fin spines, however, they likely underestimated the ages of larger and presumably older fish. The annuli observed among scales indicate that they tend to crowd near the outer margin and become increasingly difficult to discern. This phenomenon has been observed in walleye scales by other researchers [10, 14, 18]. Given that scale growth is proportional to body growth [19], we can assume that older fish will exhibit crowded annuli that may cause underestimation of age. Our results suggest that annuli crowding at the edges among dorsal fin spines are less pronounced. Dorsal fin spines may be recommended for estimating age in fish species when sacrificing is not an option [18, 20]. The phenomena of annuli crowding the edges of scales was evident in our study as estimated ages for larger fish were generally below that of dorsal fin spine estimations. Post-concert percent agreement of age was 85% for scales and 99% for dorsal fin spines, indicating that annuli on dorsal fin spines were not as crowded at the edges and easier to read than scales.

Furthermore, partial rings, merged rings, crowding of distal rings, and difficulty distinguishing the first annuli (due to the lumen of the spine) frequently cause problems in estimating age from dorsal fin spines [13, 14, 21]. Furthermore, Isermann *et al.* [13] examined the use of dorsal fin spines and scales of black crappie as nonlethal alternatives to otoliths and found that dorsal fin spines resulted in higher among reader precision and reader agreements when compared to scales. Although they reported higher precision and reader agreements associated with dorsal fin spines, they did not recommend dorsal fin spines over scales due to the conspicuous lumen located at the center of each dorsal fin spine. The lumen, over time, can enlarge and partially or fully obscure annuli causing inaccurate and imprecise age estimates [22]. According to Isermann *et al.* [13], when compared to annuli from otoliths, results suggested that not all annuli were clearly visible on dorsal fin spines rendering them unreliable for accurate and precise age estimation. The lumen was clearly evident in our study, and our results support the findings presented by Isermann *et al.* [13]. It is unknown how many annuli may be obscured by the lumen of the dorsal spine in largemouth bass, since otoliths were not available for this study.

4. Conclusion

In a recent survey conducted by Maceina *et al.* [5], they detected a problem fishery managers face when a decision is made whether or not to sacrifice a fish for calcified aging structures. Accepting to not sacrifice the fish ultimately leads to a lower accuracy and precision of age estimates. Thus, it is important to thoroughly examine multiple nonlethal aging methods and validate these methods when possible. Furthermore, the preferred structure for estimating ages of fish will vary with species, locality, and size, so recommending one structure or method over another may not work for all situations [16]. This is the first study, to our knowledge, that has employed current methodology, examined, and compared multiple nonlethal methods of aging (e.g., scales, pectoral fin rays, and dorsal fin spines) for largemouth bass. Until further comparisons of alternative nonlethal methods can be evaluated, it is recommended that fishery managers still use scales for smaller individuals, due to time and resource need for analyzing dorsal fin spines. If more precise ages are required for large largemouth bass, managers should analyze dorsal fin spines. Further comparisons need to be conducted (with known aged fish) to fully examine the precision and accuracy of these structures as alternative aging methods.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

RLM and ACG participated in the study design. ACG collected all of the aging structures from the fish. RLM sectioned, mounted, and took pictures of all of the structures. RLM, SBD, and ACG participated in aging each structure independently and in concert. SBD carried out the statistical analyses. RLM and SBD wrote the manuscript.

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