Precision and Bias of Using Opercles as Compared to Otoliths, Dorsal Spines, and Scales to Estimate Ages of Largemouth and Smallmouth Bass

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Abstract - Several structures can be used to estimate ages of *Micropterus salmoides* (Largemouth Bass) and *Micropterus dolomieu* (Smallmouth Bass). Otoliths are often employed for these black bass age determinations, but processing otoliths can be time consuming and requires an investment in training and equipment. Scales and dorsal spines can also be analyzed to measure age, but precision and accuracy problems have been documented. Use of opercles to estimate age in Largmouth and Smallmouth Bass has not been previously examined. Utilization of both otoliths and opercles requires sacrificing the fish, but opercles are easier to remove and process than otoliths. In our study, four readers estimated the ages of the fish using each of the four structures. Opercles had the lowest coefficient of variation (CV) for both species (Largemouth Bass = 6.31, Smallmouth Bass = 5.23), but underestimated the ages of Largemouth Bass older than nine and Smallmouth Bass older than six, relative to otoliths. Opercles proved easier to prepare and read, and the results showed lower age-bias, higher precision, higher among-reader agreement, and less reader bias than scales and dorsal spines.

Introduction

Estimates of fish ages provide important demographic information that is a key component of fisheries research and management (Maceina and Sammons 2006). Determinations of fish longevity, growth rate, and age at maturity hinge on correct age information; inaccurate age estimations may hinder sound management of the fishery resource (Everhart and Youngs 1981). Scales are the most commonly used structure for estimating ages of many species, including *Micropterus salmoides* Lacépède (Largemouth Bass; LMB) and *Micropterus dolomieu* Lacépède (Smallmouth Bass; SMB) because they are easy to remove and sample collection is non-lethal (Besler 1999, Maceina et al. 2007). Other bony structures (e.g., otoliths, dorsal spines, and opercles) can be used to estimate ages of bass and other fishes, but the use of opercles is rare in North American studies (Besler 1999, Long and Fisher 2001, Maraldo and MacCrimmon 1979). Opercles have been successfully used to estimate ages of *Perca fluviatilis* L. (European Perch), *Morone saxatilis*

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Walbaum (Striped Bass), *Cyprinus carpio* L. (Common Carp), *Perca flavescens* Mitchill (Yellow Perch), *Xyrauchen texanus* Abbott (Razorback Sucker), *Salvelinus alpinus* L. (Arctic Char), and *Morone chrysops* Rafinesque (White Bass) (Baker and Timmons 1990, Bardach 1955, Kilambi and Prabhakaran 1987, Le Cren 1947, McCarthy and Minckley 1987, Phelps et al. 2007, Soupir et al. 1997).

Otoliths and scales have been verified to have annual ring deposition in LMB and SMB (DeVries and Frie 1996, Heidinger and Clodfelter 1987, Taubert and Tranquilli 1982); dorsal spines are also assumed to have annual marks, though their use for age estimation has not been validated (Hanchin 2011, Maraldo and MacCrimmon 1979). Age estimates using scales and dorsal spines were found to be biased and to have low precision (Long and Fisher 2001, Maraldo and MacCrimmon 1979). For this reason, otoliths are often the preferred structure to estimate ages of LMB and SMB, but otolith analysis requires sacrificing the fish and more preparation time than for scale analysis (Hoyer et al. 1985, Long and Fisher 2001).

A 2007 survey conducted by the American Fisheries Society found that, out of 45 American and Canadian management agencies, 75% used scales, 60% used otoliths, 5% used spines, 2% used fin rays, and 0% used opercles to estimate LMB and SMB age (Maceina et al. 2007). It is important to use a structure that can be analyzed quickly and provide precise results; however, scales do not yield precise age estimates for older fish (Besler 1999), and otoliths and spines are costly to prepare (e.g., time and supplies). Opercles were extracted from LMB and SMB to collect age data for a lake management plan (MDNR 2006, Winemiller and Taylor 1987), but the authors did not provide data regarding the presicion of their results, nor did they compare these results with those obtained from otoliths. Because opercles have been used for age estimation in other fish species, they warrant consideration for use in estimating the ages of LMB and SMB.

Methods

We collected LMB and SMB caught during fishing tournaments on Lake Champlain in Plattsburgh, NY June–September 2012. All fish collected from tournament anglers were longer than 300 mm. We obtained fish shorter than 300 mm via boat electrofishing in northern Lake Champlain, using a Smith-Root VVP-15B (Smith-Root, Inc., Vancouver, WA) boat-mounted electrofisher. We used opercles, sliced sagittal otoliths, dorsal spines, and scales to age a sample of bass (LMB: n = 63, SMB: n = 66) that ranged in length from 150 mm to 500 mm; the wide length range assured that different age classes were present in the sample.

We assigned each fish a unique identification code, then measured its total length and weight (Anderson and Neumann 1996). We collected scale samples from below the lateral line and behind the pectoral fin (Besler 1999, DeVries and Frie 1996). The first 3 dorsal spines were removed from fish using wire cutters. Scale and spine samples were stored dry in coin envelopes. We removed opercles by making two cuts—one above and one below the opercle bone—then pulling it off the fish (Le Cren 1947). Opercles were stored in 70% ethyl alcohol for several days until they were processed; post-processed opercles were stored dry. We removed otoliths from the fish following standard procedures and stored them dry in coin envelopes (Buckmeier and Howells 2003).

We cleaned and mounted scale samples onto a microscope slide with a cover slip and observed them at 25x magnification on a Zeiss Stemi 2000-C dissecting microscope with an overhead light. We set dorsal spines in epoxy and sectioned them using a Buehler Isomet® low speed saw 11-1280-160 (Buehler, Ltd., Lake Bluff, IL). We mounted spine sections on a microscope slide, added 3-In-One SAE 20 oil to make polishing smoother, and then polished them using 600-b-grit sandpaper. Spines were viewed with transmitted light at 200x magnification under a Leitz-Wetzler compound microscope. We sliced otoliths in half through the focus using the Buehler Isomet saw, lightly burned the otolith, mounted the samples in clay with the cut surface parallel to the clay base, submerged them in water, and viewed them under the dissecting microscope (Buckmeier and Howells 2003). We boiled opercles for ~30 seconds, scrubbed them briefly with a wire brush to remove excess flesh and scales, then viewed them with the naked eye to count annuli. We captured images of all structures using a Canon Powershot S51S digital camera mounted on a dissecting microscope.

Four readers estimated ages independently using digital images of otoliths, spines, and scales; opercles were aged with the naked eye. We calculated percent agreement among the four readers for each structure. We estimated precision of determinations for each structure by calculating the coefficient of variation (CV; Campana et al. 1995) as follows:

$$CV_j = 100 \text{ x } \left(\sqrt{\left[\sum_{i=1}^{R} ([X_{ij} - X_j]^2 / [R - 1])\right]} / X_j\right)$$

where R is the number of readers, Xj is the mean age estimated for the jth fish, and Xij is the ith age estimate for the jth fish (Chang 1982). To determine whether there were differences in estimated ages among structures, age-bias plots were constructed for each pair of structures using average age to test if the slope was equal to 1; t-tests were performed using GraphPad Prism (GraphPad 2012).

Results

Precision and agreement

Age estimation using opercles produced the most precise determinations for both species —LMB CV = 6.3, SMB CV = 5.2—compared with scales: CV = 14.6 and 12.1, spines: CV = 13.2 and 9.1, and otoliths: CV = 9.1 and 7.4 for LMB and SMB, respectively (Table 1). Age estimates from opercles also had the highest between-reader agreement compared to the other structures (Table 2).

Structure bias

When compared to the results from otoliths, age-bias plots for LMB opercles (P = 0.62, df = 15), spines (P = 0.98, df = 7), and scales (P = 0.30, df = 6), had a slope that did not significantly differ from 1, indicating there is very little bias between structures (Fig. 1). Opercles underestimated the ages of fish older than 9 compared to otoliths, and older than 6 when compared to spines; however, compared to scales, opercles underestimated ages of younger fish and overestimated ages of older fish.

Age-bias plots for SMB opercles showed a significant difference from a slope of 1 when compared to scales (P = 0.01, df = 7), which indicated that bias was present. However, when compared to spines (P = 0.09, df = 8) and otoliths (P = 0.17, df = 12), there was no significant difference from a slope of 1, indicating no bias (Fig. 2). Opercles underestimated ages when compared to otoliths and spines, and underestimated ages of younger fish when compared to scales.

Reader bias

Reader age-bias regressions for LMB indicated that determinations using otoliths had the least amount of bias (average slope of 0.91) between readers, compared

Table 1. Coefficient of variation (CV) among readers for four age-estimation structures in Largemouth and Smallmouth Bass.

	Reader				
Structure	1	2	3	4	Mean
Largemouth Bass					
Scales	15.3	11.5	14.9	16.5	14.6
Spines	11.1	15.6	12.6	13.6	13.2
Otoliths	7.2	9.8	9.8	9.6	9.1
Opercles	6.5	6.9	6.3	5.5	6.3
Smallmouth Bass					
Scales	11.9	10.1	11.8	14.5	12.1
Spines	7.7	9.7	10.9	8.1	9.1
Otoliths	5.8	8.5	7.7	7.6	7.4
Opercles	5.2	6.2	4.9	4.7	5.2

Table 2. Percent agreement between readers for each of the four age-estimation structures in Largemouth and Smallmouth Bass.

Between-reader difference	Scales	Spines	Otoliths	Opercles
Largemouth Bass				
0	20.2	30.8	31.7	46.3
± 1	53.4	66.1	75.7	84.7
± 2	74.2	84.7	91.8	96.6
± 3	87.9	92.8	96.2	98.4
± 4	93.8	97.5	100.0	99.7
> ± 4	100.0	100.0	-	100.0
Smallmouth Bass				
0	17.4	37.4	29.3	43.9
± 1	48.5	73.2	73.0	89.9
± 2	68.7	89.7	91.0	97.5
± 3	81.8	93.9	96.2	99.8
± 4	89.9	97.7	98.7	100.0
> ± 4	100.0	100.0	100.0	-

to an average slope of 0.44 for scales, 0.62 for spines, and 0.81 for opercles. Results for all structures except otoliths showed some between-reader bias; all 6 pairwise comparisons between readers using scales, 5 comparisons using opercles, and 4 comparisons using spines were significantly different from 1 ($P < 0.05$). No comparisons were significantly different for analyses of otoliths.

Reader age-bias regressions for SMB indicated that results for opercles (average slope of 0.84) and otoliths (average slope of 0.91) had the least bias between readers. Results for scales (slope = 0.36) and spines (slope = 0.59) showed more bias between readers. All 6 reader pairwise comparisons using scales, 5 comparisons using spines, and 2 comparisons using opercles significantly differed from a slope of 1 ($P < 0.05$), whereas no comparisons differed for otoliths.

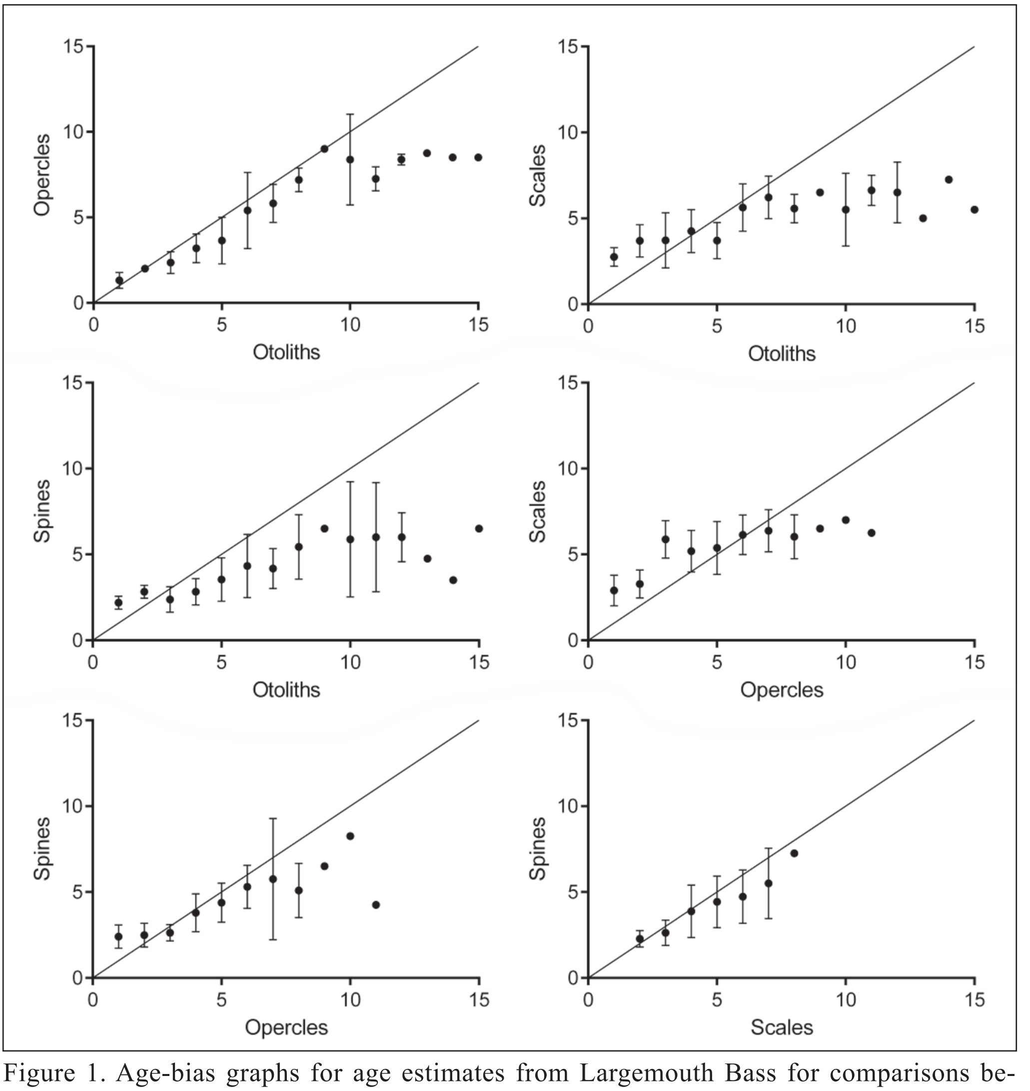

Figure 1. Age-bias graphs for age estimates from Largemouth Bass for comparisons between all structures. Solid line indicates a 1:1 agreement in age estimations, error bars represent one standard deviation around the mean.

Discussion

Analyses of opercles had higher percent agreement between readers, were found to be more precise, had higher among-reader agreement, and the structures were easier to remove and process than spines, scales, and otoliths in LMB and SMB. As Muir et al. (2008) pointed out, otoliths require removal via dissection, slicing, and mounting, which can be very costlyand time consuming. Dorsal spines also require slicing and mounting, but, as Koch and Quist (2007) suggested, there are cheaper alternatives. Ours is the first study to evaluate age determination using LMB and SMB opercles; opercles can simply be pulled off fish after two cuts and require only 30 seconds of cleaning to prepare them for age estimation (Le Cren 1947).

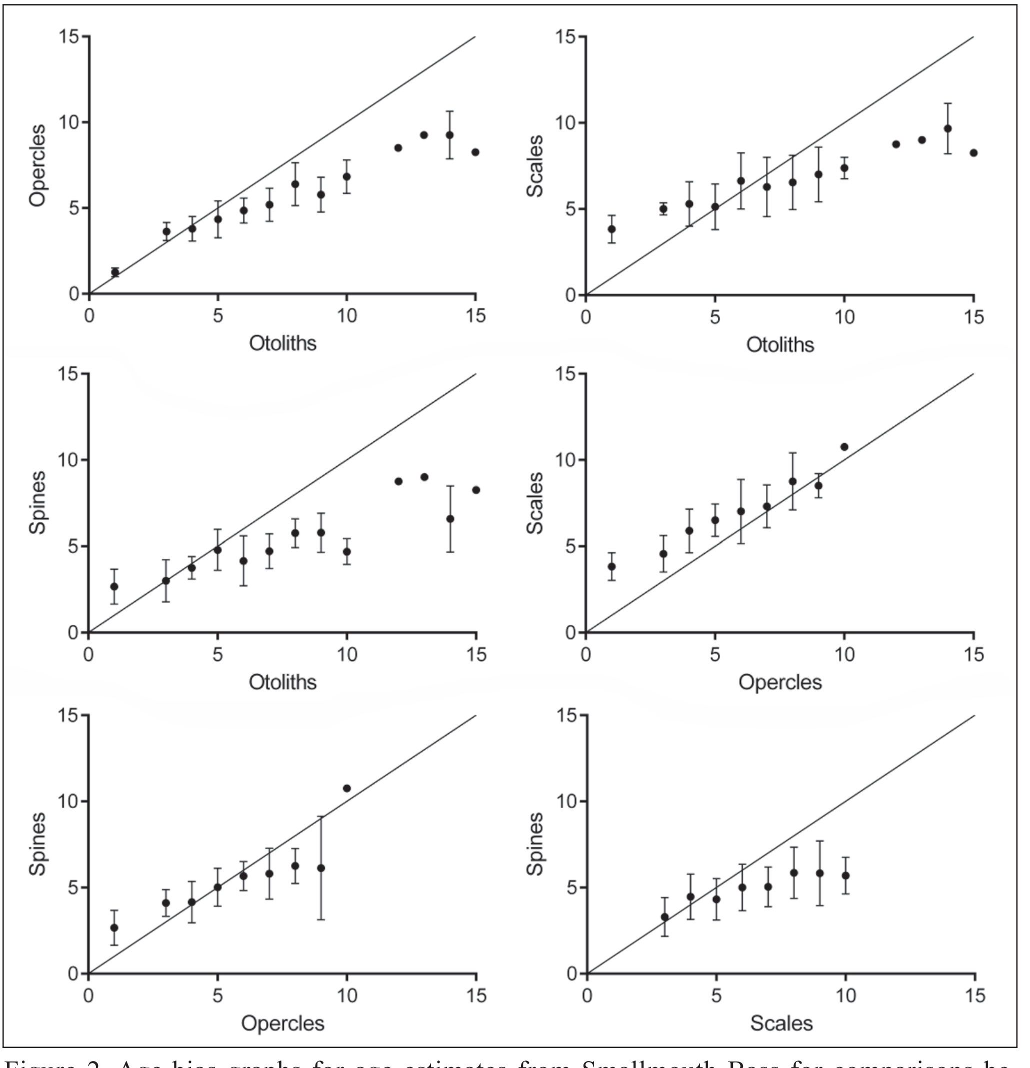

Figure 2. Age-bias graphs for age estimates from Smallmouth Bass for comparisons between all structures. Solid line indicates a 1:1 agreement in age estimations, error bars represent one standard deviation around the mean.

Use of otoliths to estimate LMB and SMB age has been validated (Buckmeier and Howells 2003, DeVries and Frie 1996), therefore, in order to determine if opercles are a viable age-estimation structure, age estimates must be comparable to those derived from otoliths. Ages estimated from opercles agreed with those estimated from LMB otoliths until age 9, and with SMB otoliths to age 6. Based on our results for reader agreement, precision, and ease of processing, opercles are a viable alternative to use of otoliths for age estimation, particularly for LMB. However, otoliths yield more accurate estimates for older fish than counts made from opercles.

High reader precision among age estimates from a given structure indicates the structure is generally easy to use for age estimation, and the age estimates can be easily reproduced due to the presence of distinct annuli. The high precision of opercles and otoliths among the four readers indicates that both structures are valuable for age estimation of LMB and SMB in Lake Champlain.

Between-reader agreement is the most common method to assess age estimation structures (Campana et al. 1995) because high agreement indicates higher reliability and reader confidence. Fisheries-management agencies often rely on inexperienced seasonal employees to conduct age estimations, so it is advantageous to use a method that requires little training and has high precision. We found that analysis of opercles had the highest between-reader agreement and precision, and therefore would be the most appropriate structure for use by inexperienced readers, but use of opercles needs further validation through analysis of opercles from known-age fish.

In conclusion, opercles are easier to remove, process, and observe for estimating SMB and LMB ages than otoliths, scales, and spines; opercles have also been verified as more accurate than scales in other species (Bardach 1955, Kilambi and Prabhakaran 1987, Le Cren 1947). Age estimation using scales and otoliths requires more experienced readers due to the inherent ambiguity in the clarity of the annuli, whereas opercles can be read by novice readers due to their well-defined annuli. A high percentage of between-reader agreement, high precision, and little bias among readers, along with ease of preparation, make opercles an ideal alternative for estimating the ages of LMB to age 9, and SMB to age 6; however, validation is required for older fish.

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