Precision and comparability of Black Redhorse (*Moxostoma duquesnei*) age estimates using scales, pectoral fin rays, and opercle bones

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ABSTRACT

Determination of age and related demographic parameters has an important role in the assessment and recovery of fishes at risk. In this study, we compared opercle-based age estimates for Black Redhorse (*Moxostoma duquesnei*), a nationally Threatened riverine fish, with two structures that can be obtained non-lethally (scales and pectoral fin rays). Age estimates from each structure were significantly different. Compared to the opercle, age estimates were lower using scales after age-5, and using fin rays after age-8. Agreement between-readers and among-replicate age interpretations were greater for fin rays than scales. We recommend the use of pectoral fin rays for monitoring recruitment and population age structure. To improve the accuracy of some parameter estimates (*e.g.* growth rate and maximum age), the opercle could be removed from a subsample of individuals.

RÉSUMÉ

La détermination de l'âge et des paramètres démographiques connexes est importante pour l'évaluation et le rétablissement des espèces de poissons en péril. Dans cette étude sur le chevalier noir (*Moxostoma duquesnei*), un poisson fluvial considéré comme menacé à l'échelle nationale, nous avons comparé les estimations de l'âge établies à partir des opercules, et à l'aide de deux structures qui peuvent être obtenues sans tuer le poisson (les écailles et les rayons des nageoires pectorales). Les résultats obtenus à partir de chaque structure différaient considérablement. Par rapport aux estimations de l'âge établies à partir des opercules, les estimations étaient inférieures après l'âge-5 lorsqu'elles reposaient sur l'analyse des écailles et après l'âge-8 dans le cas des rayons des nageoires pectorales. Les résultats étaient plus uniformes entre les lecteurs et entre les interprétations de l'âge répétées pour les rayons des nageoires que pour les écailles. Nous recommandons d'utiliser les rayons des nageoires pectorales pour surveiller le recrutement et la structure par âge des populations. Pour améliorer la précision des estimations de certains paramètres (p. ex., le taux de croissance et l'âge maximal), nous avons retiré l'opercule chez un sous-ensemble des individus.

1.0 INTRODUCTION

Determination of age has an important role in the conservation and management of fishes (Beckmand and Hutson 2012). For Canadian fishes at risk, life history and demographic information are necessary for assessing population status, population viability modelling, and identifying critical habitat (Rosenfeld and Hatfield 2006; Vélez-Espino and Koops 2009). Age-related information is also important for interpreting the influence of habitat degradation or other environmental changes on population characteristics (Drake et al. 2008; Haxton and Findlay 2008). The interpretation of fish age commonly involves analysis of calcified structures such as scales, fin rays, or internal bones such as the otolith (DeVries and Frie 1996; Maceina et al. 2007). However, accuracy and precision varies among structures influencing estimates of population metrics such as growth and annual mortality rates (Sylvester and Berry 2006). Accurate (and regionally representative) age estimates are essential for modelling minimum viable population sizes of Ontario fishes at risk and the amount of habitat required to recover populations. Variation in interpreted ages between investigators or between studied populations has been found to dramatically influence estimates of viable population sizes (Bouvier et al. 2013; Young and Koops 2013).

Black Redhorse (*Moxostoma duquesnei*) is at the northern edge of its North American range in Canada, where it is found in a small number of southwestern Ontario rivers. It is has been assessed as both provincially and nationally Threatened (COSEWIC 2007). Other imperiled redhorse species in Canada are the Endangered Copper Redhorse (*M. hubbsi*) and Special Concern River Redhorse (*M. carinatum*). Studies of redhorse (*Moxostoma* spp.) populations have often used scales for aging specimens (see Reid 2007). Scales are non-lethal to collect and easy to prepare for interpretation, but annuli may be difficult to discern for older individuals (Reid 2007). Although less likely to underestimate age than scales, agreement between interpretations of pectoral fin rays and opercle or otoliths is less consistent for older catostomids (suckers) (Quinn and Ross 1982; Huston 1999; Reid 2007). Errors in age determination can arise from the re-absorption of the central part of the fin ray, crowding of annuli in distal portions of the fin ray, and the cutting of fin ray too far from the body (Quinn and Ross 1982; Howland et al. 2004; Zymon and McMahon 2008). Preparation of fin ray sections is also time and labour intensive (Walsh et al. 2008). While likely providing more accurate age estimates, the use of internal bones requires lethal sampling, which is less desirable for species at risk.

Method comparisons assist in evaluating which is the most efficient, reliable, and appropriate for a given population study (Beckman 2002). If similar ages are observed using different structures, then the most accessible, easily interpretable, and reproducible should be used (Howland et al. 2004). In this study, we compared the precision and bias of Black Redhorse age estimates associated with two structures that can be obtained non-lethally (scales and pectoral fin rays) with the opercular bone (opercle).

2.0 METHODS

Black Redhorse (n = 57) were collected during the fall of 2007 and 2008 from two reaches of the Grand River in southwestern Ontario (43°26'N, 80°30'W). Reaches were located near Kitchener-Waterloo and Inverhaugh. Sampling was undertaken with a backpack electrofisher, or a 5-KW pulsed DC boat-mounted electrofisher. Total lengths (TL) of individuals sampled ranged from 150 to 472 mm. Scales were collected below the dorsal fin above the lateral line. The most anterior pectoral fin ray was removed using wire cutters as close to the body as possible. The opercle, a thin flat bone that forms part of the gill cover, was also removed. All structures were collected from the left side of the body, and placed in scale envelopes to dry.

Two to five scales were pressed between two microscope slides (1 mm thickness). Fin rays were embedded in a two-part epoxy resin using micro-centrifuge tubes as a mould (Koch and Quist 2007). Rays were sectioned as close to the base as possible using BuehlerIsomet low-speed saw to a width of 500 µm. Sections were mounted on a microscope slide using Crystalbond 509 (Electron Microscopy Sciences, Hatfield PA). Opercles were placed in hot water for several minutes to facilitate tissue removal with a toothbrush, and then air dried. Annuli were identified under magnification with transmitted light, using illustrations provided in Chalanchuk (1984), Harbicht (1990), and Marcogliese (1996) (see also: den Haas et al. 2013). Age assignment was based on annulus counts and undertaken without knowledge of fish length. Scale regeneration was evident for 83% of individuals.

To compare precision among structures and readers, ages from each structure were interpreted once by two readers (Readers 1 and 2) independently at different locations, and in triplicate by the first reader. Repeat readings (Reader 1) were separated by at least one week. For all three structures, Reader 1 had previously interpreted the ages of several thousand individuals representing five redhorse species (including Black Redhorse). Reader 2 had prior experience using pectoral fin rays and otoliths to interpret the ages of Spotted Gar (*Lepisosteus oculatus*), and Bluegill (*Lepomis macrochirus*).

Age-bias plots, percent agreement, and coefficient of variation were used to assess the precision for each structure, as well as, the agreement among matched pairs of scales, fin rays and opercles (Chang 1982; Campana et al. 1995). Overall differences between age-estimates among structures were tested with repeated-measures Analysis of Variance (ANOVA) (Reader 1 data only). Differences between pairs of structures were tested with the paired T-test. Significance values were adjusted with the Bonferonni correction for multiple tests.

3.0 RESULTS AND DISCUSSION

Mean age estimates (Reader 1) from opercles (5.8 years) were greater than fin rays (5.3 years) (ANOVA: $F = 22.0$, $p < 0.001$; paired t-test: $T = 4.6$, $p < 0.001$) and scales (4.9 years) (paired t-test: $T = 5.4$, $p < 0.001$). Estimates from scales and fin-rays were also significantly different (paired t-test: $T = 2.2$, $p = 0.016$). Ages interpreted using opercles and fin rays generated a wider range of age classes (1 to 10 years) than scales (1 to 8 years). Compared to the opercle, age estimates were lower using scales after age-5, and using fin rays after age-8 (Figure 1). This corresponded to individuals larger than 390 (scale) and 420 (fin ray) mmTL. Reid (2007) and den Haas et al. (2013) found similar differences among these structures when interpreting the age of other redhorse species found in Ontario rivers. After age-5, den Haas et al.

(2013) reported that annuli of scales collected from Shorthead Redhorse (*M. macrolepidotum*), collected in the Grand River, were too crowded to accurately interpret. For Ontario populations of Greater Redhorse (*M. valenciennesi*), River Redhorse, Shorthead Redhorse, and Silver Redhorse (*M. anisurum*), consistent disagreement with opercle bones began at ages 4 to 5 for scales and ages 12 to 15 for pectoral fin rays (Reid 2007).

As reported in Reid (2009), our sample of Black Redhorse from the Grand River included a larger proportion of older individuals than more southern populations (*e.g.* Missouri Ozarks region: Beckman and Howlett 2013). However, the maximum age identified is substantially lower than previously reported for the Grand River (17 years: Reid 2009). This likely reflects the much smaller sample size in our study (57 individuals) than Reid (2009) (522 individuals). Black Redhorse were also younger than another redhorse species that has been studied in the Grand River, Shorthead Redhorse (Reid 2007; Reid 2009; den Haas et al. 2013).

Agreement within 1 year between readers was generally high for all three structures (Table 1). Compared to fin rays, exact agreement between readers was lower for scales and opercles. A consistent difference in opercle-based age estimates between readers was evident for Black Redhorse older than age-6 (Figure 2). The largest difference was associated with scale interpretation, where the age assigned to a single individual differed by 3 years. Compared to scales and the opercle, greater between-reader agreement using pectoral fin rays has also been reported for several other North American riverine catostomids (Quist et al. 2007; Spiegel et al. 2010). Percent agreement between two repeat interpretations by Reader 1 was lowest for scales, and similarly greater for fin ray and opercle (Table 1). Reid (2007) also found repeat interpretations of redhorse age using fin rays to be more consistent than scales. CV calculated using triplicate age interpretations found within-reader precision to be highest for the opercle (mean CV = 4.9), intermediate for fin ray (mean CV = 9.0), and highest for scales (mean CV = 11.8).

In this study, we did not estimate Black Redhorse age using the otolith (another lethally obtained, but widely used structure). Exact agreement between opercle and otolith age estimates has been reported for a Missouri population of Black Redhorse (Beckman and Howlett 2013). Also, for River Redhorse, Beckman and Hutson (2012) found 94% of age estimates to be in exact agreement. In another study of catostomid age interpretation using multiple structures, exact agreement between age estimates for Bluehead Sucker (*Catostomus discobolus*), Flannelmouth Sucker (*C. latipinnis*), and White Sucker (*C. commersonii*) was generally high; ranging from 82 to 94% (Quist et al. 2007). In contrast, den Haas et al. (2013) reported poor agreement (31%) between ages determined by opercle and otoliths taken from Shorthead Redhorse. After age-15, the mean difference between opercles and otoliths was 2.5 years; with higher age-estimates associated with otoliths. After 15 years, the authors indicated that the opercle bone was too thick to reliably identify the first one or two annuli.

Validation of age estimates for different structures has often been neglected by researchers (Beamish And McFarlene 1983; Campana 2001), and estimates of precision (i.e. reproducibility) cannot be substituted for measures of accuracy (Maceina et al. 2007). We did not evaluate annuli formation for any of the structures.

Using mark-recapture or marginal incremental analysis methods, validation studies have been undertaken for the following redhorse and sucker species: Black Redhorse and Golden Redhorse (*M. erythrurum*) (otolith: Beckman and Howlett 2013), Brassy Jumprock (*Moxostoma* spp.) and Notchlip Redhorse (*M. collapsum*) (otolith: Bettinger and Crane 2011), River Redhorse (scale, opercle: Huston 1999), Shorthead Redhorse (pectoral fin ray: Harbicht 1990), and White Sucker (scale and pectoral fin ray: Beamish and Harvey 1969; Quinn and Ross 1982). For these species, annuli formation each year was found to be generally reliable for opercles, otoliths, and pectoral fin rays, but not scales.

Given that large sample sizes are required to accurately and precisely estimate demographic parameters such as growth and mortality (Coggins et al. 2013), the use of lethally obtained structures to study endangered fish populations is not feasible. Our study indicates that, compared to scales, Black Redhorse age-estimates from fin ray sections will be more precise and closer to age estimates obtained from the lethally obtained opercle. Therefore, we recommend the use of pectoral fin rays for monitoring recruitment and population age structure. However, to improve the accuracy of parameter estimates, the opercle (or otolith) could be removed from a subset of sampled individuals to: (1) back-calculate growth rates from measurements of annuli radii; and, (2) adjust estimates based on pectoral fin rays for larger (and presumably older) individuals.

4.0 ACKNOWLEDGEMENTS

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Table 1 Percent agreement between two readers and two replicate (Reader 1) interpretations for three hard structures collected from Black Redhorse in the Grand River, Ontario.

	Opercle	Fin Ray	Scale
Between-reader difference (yr)			
0	35	68	58
±1	91	98	91
±2	100	100	98
Between-reading difference (yr)			
0	71	67	53
±1	98	97	86
±2	100	100	98

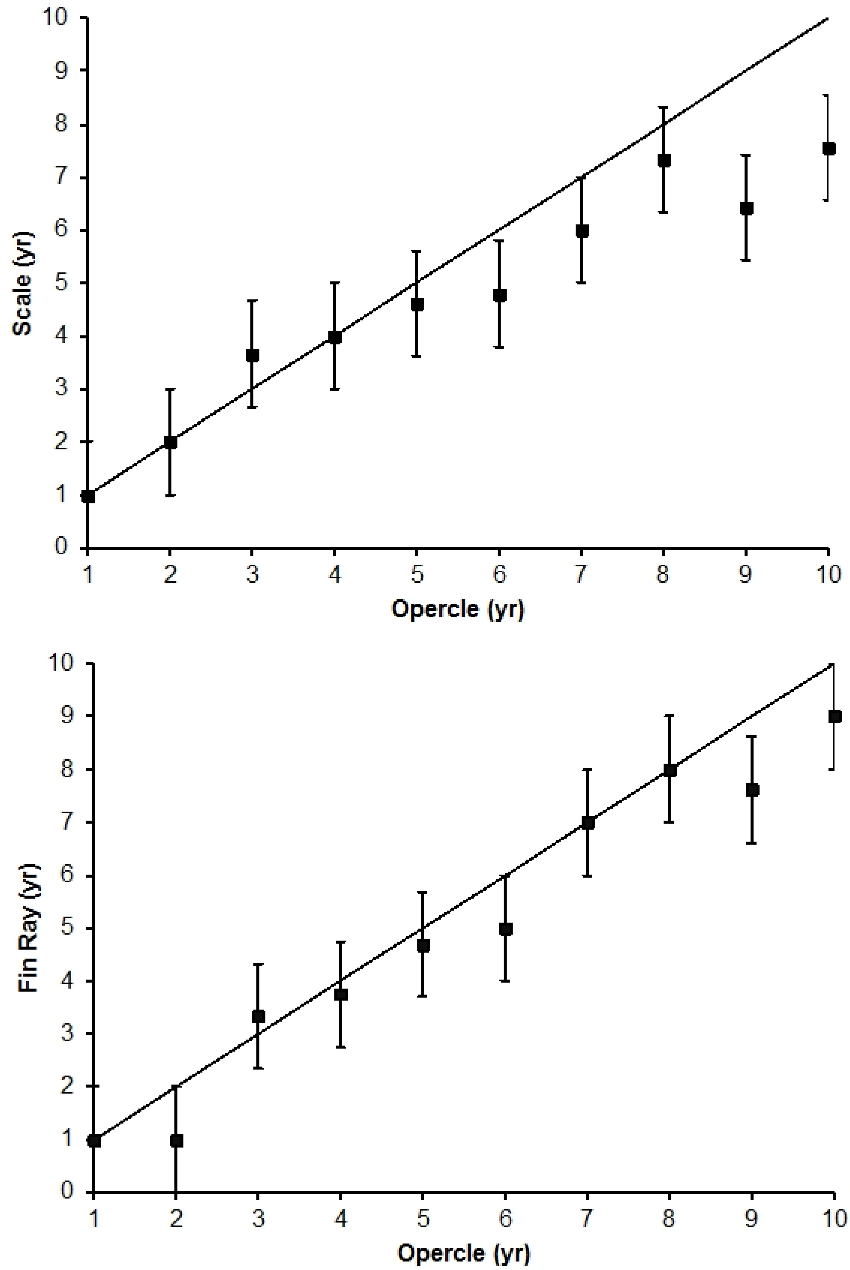

Figure 1 Age-bias plots comparing age-estimates (Reader 1 only) from scales and opercle bones (mean CV ± SD: 10.4 ± 11.5); and fin rays and opercle bones (mean CV ± SD: 9.0 ± 10.6). Error bars indicate Standard Deviation (SD) about the mean. The solid line represents 1:1 equivalence between structures.

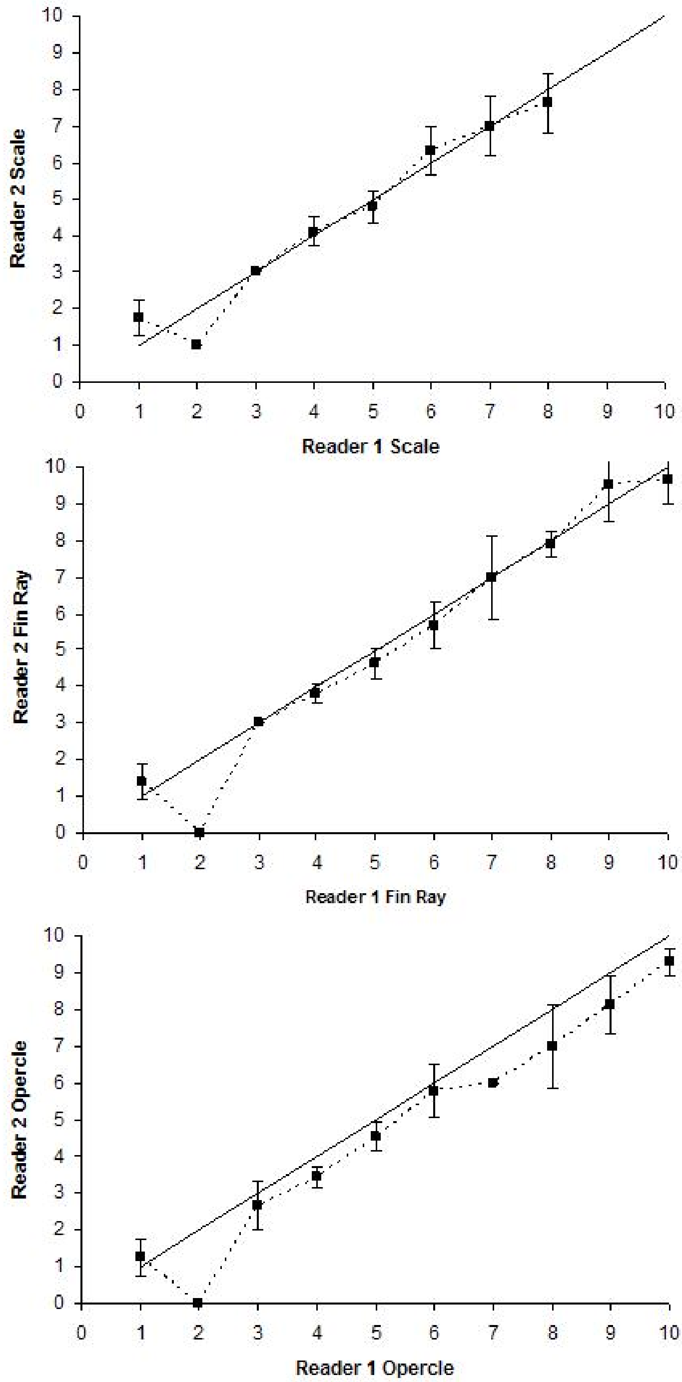

Figure 2 Age-bias plots comparing age-estimates from two readers using scales (mean CV ± SD: 9.6±14.2), fin rays (mean CV ± SD: 5.6±10.8), and opercle bones (mean CV ± SD: 12.3±19.8). Error bars indicate SD about the mean. The solid line represents 1:1 equivalence between readers.