Comparison of Scales, Pectoral Fin Rays and Opercles for Age Estimation of Ontario Redhorse, *Moxostoma,* Species

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Reid, Scott M. 2007. Comparison of scales, pectoral fin rays and opercles for age estimation of Ontario Redhorse, *Moxostoma,* species. Canadian Field-Naturalist 121(1): 29–34.

Opercle based age estimates of four redhorse species (*Moxostoma anisurum, M. carinatum, M. macrolepidotum* and *M. valenciennesi*) were compared with those from two structures that can be obtained non-lethally (scales and pectoral fin rays). For all species, age estimates from scales were significantly lower than those obtained from fin rays and opercles. After ages 4 to 5, age estimates from scales were consistently lower than those from the opercle. Overall differences between age estimates from fin rays and opercles were only detected for *M. anisurum*. For all species, age estimates from fin rays were consistently lower than opercles after ages 12 to 15. Closer agreement among different structures was identified for populations of *M. macrolepidotum* comprised of younger individuals. Maximum ages reported for Ontario populations were higher than those previously reported across the species' ranges, reflecting either geographic differences in growth and longevity, or the historical use of scales for age estimation.

Key Words: redhorse, *Moxostoma*, age interpretation, calcified structures, Ontario.

Redhorses (*Moxostoma* spp.) are relatively large, laterally compressed and superficially similar suckers (Scott and Crossman 1979). In southern Ontario rivers, redhorse comprise a large proportion of fish biomass (Cooke and Bunt 1999). However, like many non-sport fish species in Canada, little is known about redhorse life history (Cooke et al. 2005). Of the six redhorse species in Ontario, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) designated the Black Redhorse (*M. duquesnei*) as nationally threatened and the River Redhorse (*M. carinatum*) as a species of special concern (COSEWIC 2005*). These two species and the Greater Redhorse (*M. valenciennesi*) are also imperiled in several neighbouring Great Lakes states (NatureServe 2005*). Recovery efforts for fish species at risk in Canada are limited by a lack of knowledge regarding their ecology, population size and factors that affect distribution and abundance.

Population age information is necessary for determining growth and mortality rates, age at maturity, and understanding the effects of environmental changes. Most redhorse age interpretations have used scales (Meyer 1962; Carlander 1969; Bowman 1970; Hackney et al. 1970; Curry and Spacie 1984; McAllister et al. 1985; Sule and Skelly 1985; Kwak and Skelly 1992; Cooke and Bunt 1999). Compared to fin rays or the opercle, scales typically underestimate age of older catostomids (Chalanchuk 1984). In older fish, annuli may be crowded at the distal edge due to minimal or non-existent scale growth and/or scale edges may be reabsorbed and annuli lost. Annulus formation in Shorthead Redhorse (*M. macrolepidotum*) pectoral fin rays was validated by Harbicht (1990). However, while less likely to underestimate age than scales, fin ray based age interpretations for older redhorse can be less accurate than those from either the opercle or otolith (Huston 1999). Re-absorption of the central part of the fin rays can obscure the first annulus and crowding of annuli in distal portions may occur in older fish, leading to potential errors in age determination (Beamish and Chilton 1977; Quinn and Ross 1982). If the fin ray is cut too far from the body, the core may not be seen or the annulus may be missing (Howland et al. 2004). The primary disadvantage of age estimation based on the opercle or otolith is that the fish must be killed in order to obtain the structure. As well, burnt or cracked otoliths have poor archival properties compared to fin rays (Howland et al. 2004).

Population monitoring of fish species at risk may require non-lethal sampling methods. Method comparisons assist in evaluating which is most efficient, reliable and appropriate for a given population study (Beckman 2002). If similar ages are observed using different structures then the most accessible and easily interpreted should be used (Howland et al. 2004). The objective of this study was to compare opercle based age estimates of four redhorse species (Greater Redhorse, River Redhorse, Shorthead Redhorse and Silver Redhorse (*M. anisurum*)) with age estimates from two structures that can be obtained non-lethally (scales and pectoral fin rays). The opercle was used, instead of the otolith, because it requires less preparation time and provides age estimates that are in close agreement with the otolith (Howlett 1999; Huston 1999).

Methods and Materials

Between 2000 and 2004, redhorse were collected from multiple sites across three southern Ontario drainages: Bay of Quinte (44°6'N, 77°28'W), Grand River (43°6'N, 80°19'W), and Ottawa River (45°39'N,

TABLE 1. Number and size range (total length: TL) of redhorse sampled from the Ottawa River, Bay of Quinte and Grand River drainages in Ontario.

	Greater Redhorse		River Redhorse		Shorthead Redhorse		Silver Redhorse	
Drainage	Number	TL (cm)	Number	TL (cm)	Number	TL (cm)	Number	TL (cm)
Ottawa River	7	51.0–63.5	8	40.0–65.0	51	18.8–55.4	87	15.2–66.0
Bay of Quinte	17	39.2–59.8	18	47.3–73.2	26	38.4–55.6	67	33.8–63.3
Grand River	11	40.3–63.9	1	64.7	37	24.5–53.7	–	–
All	35	39.2–63.9	27	40.0–73.2	114	18.8–55.6	154	15.2–66.0

76°42'W) (Table 1). Sampling occurred either as part of targeted redhorse inventories or Ontario Ministry of Natural Resources (OMNR) fish community sampling programs. Redhorse were collected using gill-nets, hoop-nets or a boat electro-fishing unit. Scales were collected below the dorsal fin above the lateral line on the left side of the fish. The most anterior pectoral fin ray on the left side of the body was removed using wire cutters as close to the body as possible. The opercle, a thin flat bone that covers the gills, was also removed. All structures were placed in a scale envelope to dry.

Two to five scales were pressed onto acetate slides using a roller press and read on a microfiche projector. Pectoral fin rays were embedded in epoxy, sectioned with a low-speed Isomet saw (section width 600 µm) mounted on a slide and polished with 320 and 600 grit wet sandpaper. Opercles were placed in hot water for several minutes to facilitate tissue removal with a toothbrush and then air dried. Fin ray sections and opercles were interpreted using a dissecting microscope and transmitted light. Identification of scale, fin ray and opercle annuli was based on illustrations provided by Chalanchuk (1984), Harbicht (1990) and Marcogliese (1996). Age assignment was based on annulus counts and edge condition data (Casselman 1987). Annuli number and edge condition were assigned without knowledge of fish size or date of collection. Before structures were interpreted, a subsample (~10) of each structure was interpreted with a second experienced reader to improve confidence in annuli identification.

For each species, statistical differences between age estimates based on scale, pectoral fin ray and opercle were tested using Friedman's test and a non-parametric multiple comparisons procedure for ranked data (Zar 1999). Age bias plots (Campana et al. 1995) were constructed to assess age bias from scales and pectoral fin rays compared to opercle bones. Repeatability of age estimates was evaluated by re-interpreting a subset (30 to 50) of each structure within one year of first interpretation. Percent agreement (absolute and ± 1 year) of first and second interpretations was calculated.

Results

For all species, age estimates from scales, fin rays and opercles were not equal (Friedman's test statistic: 31.8 to 134.7; $P < 0.001$). Age estimates from scales were significantly lower than those obtained from either fin rays or opercles (Table 2). After ages 4 to 5, age estimates from scales were consistently lower than those

TABLE 2. Comparisons of redhorse age estimates and repeatability for each aging structure. Statistically different age estimates are indicated by different superscripts (A, B or C).

Structure	Greater Redhorse	River Redhorse	Shorthead Redhorse	Silver Redhorse
Mean age[1]				
Scale	5.9 (2-9)^{A}	7.4 (4-13)^{A}	5.1 (1-10)^{A}	7 (1-11)^{A}
Fin ray	8.7 (4-15)^{B}	11.3 (7-19)^{B}	8.5 (2-18)^{B}	11.4 (2-23)^{B}
Opercle	9.4 (4-20)^{B}	11.6 (6-27)^{B}	8.7 (1-20)^{B}	12.9 (1-27)^{C}
Mean pairwise difference in age estimates[1]				
Scale vs. Fin ray	3.0 (0-8)	3.9 (1-11)	3.7 (0-10)	4.4 (0-15)
Scale vs. Opercle	3.5 (0-11)	4.3 (0-16)	3.9 (0-12)	6.3 (0-22)
Fin ray vs. Opercle	1.4 (0-6)	2.3 (0-9)	1.5 (0-9)	2.4 (0-11)
Percent agreement between structures[2]				
Scale vs. Fin ray	3.4 (13.8)	0 (8.7)	4.3 (17.0)	1.9 (18.1)
Scale vs. Opercle	3.2 (22.6)	8.3 (37.5)	9.4 (27.1)	5.7 (19.0)
Fin ray vs. Opercle	27.3 (63.6)	29.6 (48.1)	30.6 (63.1)	28.7 (54.3)
Percent agreement between first and second readings[2]				
Scale	74 (100)	72 (89)	60 (74)	48 (68)
Fin ray	85 (100)	80 (94)	87 (98)	64 (93)
Opercle bone	64 (91)	96 (100)	54 (88)	70 (94)

[1]Age range in parentheses. [2]Percent agreement ± one year in parentheses.

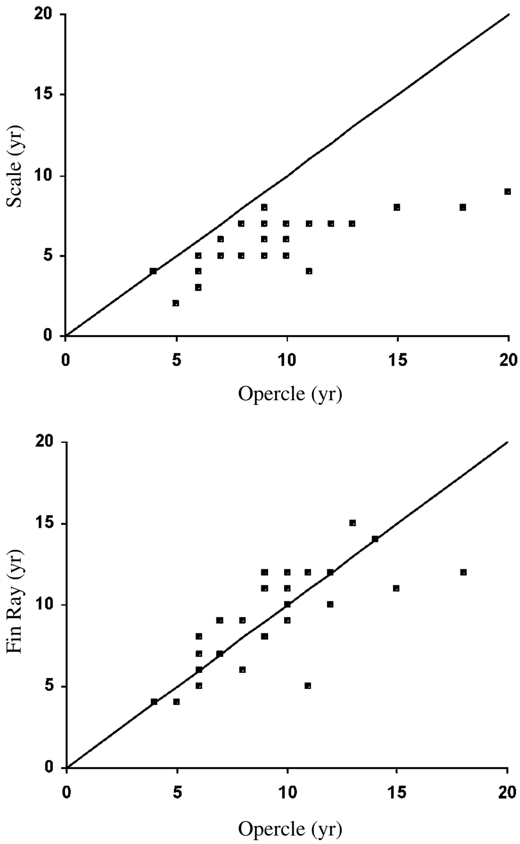

FIGURE 1. Comparison of Greater Redhorse opercle-based age estimates with those from scales (top) and pectoral fin rays (bottom). Opercle vs. scale CV (mean ± SE): 20.8 ± 2.3. Opercle vs. pectoral fin ray CV: 10.6 ± 3.6.

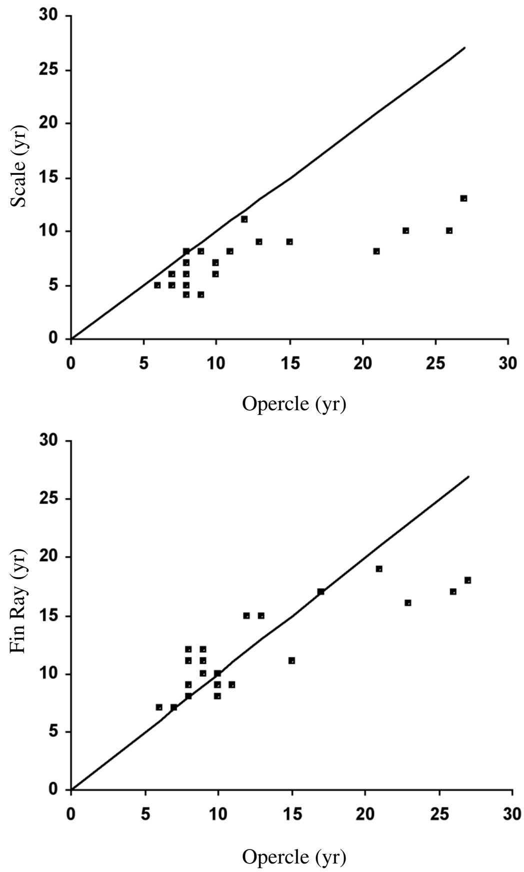

FIGURE 2. Comparison of River Redhorse opercle-based age estimates with those from scales (top) and pectoral fin rays (bottom). Opercle vs. scale CV (mean ± SE): 28.6 ± 5.8. Opercle vs. pectoral fin ray CV: 8.4 ± 1.4.

from the opercle (Figures 1 to 4). Mean pairwise differences between scale-based age estimates and those from fin rays and opercles were at least double that between fin rays and opercles (Table 2). Regenerated scales were common.

Agreement between age estimates from fin rays and opercles was greater than that between scales and the other two structures (Table 2). For all species except Silver Redhorse, there were no significant differences between age estimates from fin rays and opercles. However, the maximum difference between age estimates from these two structures was as large as 6 to 11 years. Fin ray based maximum age estimates were also 2 to 4 years less than those from opercles (Table 2). More frequent underestimation of age using fin rays began between ages 12 and 15 (Figures 1-4). For all species except Greater Redhorse, agreement between first and second interpretations of each structure was greater for pectoral fin rays and opercles than for scales (Table 2).

For Shorthead Redhorse and Silver Redhorse, sample numbers permitted an evaluation of whether differences in age estimates among structures were consistent across drainages. For Shorthead Redhorse, there was a greater level of agreement among Grand River structures (mean pairwise differences: 1.2 to 2.9 years) than among those from the Bay of Quinte (mean pairwise differences: 1.6 to 4.4 years) and Ottawa River (mean pairwise differences: 2.0 to 4.8 years) populations. The pattern reflects among drainage differences in the age of redhorse sampled, as Grand River Shorthead Redhorse were younger (mean age: 7.3) than those from Bay of Quinte (mean age: 9.2) and Ottawa River (mean age: 10.7). The level of agreement among structures from Silver Redhorse was equivalent for the similarly aged Bay of Quinte (mean pairwise differences: 2.4 to 5.9 years) and Ottawa River (mean pairwise differences: 2.4 to 6.5 years) Silver Redhorse populations. The mean age of both populations was 13 years.

TABLE 3. Comparison of maximum size (TL in cm) and maximum age for redhorse reported in past studies. For maximum age, superscript indicates structure used in the study: S:scale; F:fin ray: and O:opercle.

Location	Maximum Size	Maximum Age	Reference
	Greater Redhorse		
Grand River, Ontario	–	13^{S}	Cooke and Bunt (1999)
Grand River, Ontario	66.7	20^{O}	This study
Trent River, Ontario	68.8	15^{O}	This study
Ottawa River and tributaries, Ontario	63.5	10^{O}	This study
St. Lawrence River and tributaries, Quebec	59.4	15^{F}	Mongeau et al. (1992)
	River Redhorse		
Cahaba River, Alabama	59.7	5^{S}	Tatum and Hackney (1970)
Meramec River, Missouri	59.7	12^{S}	Carlander (1969)
James River, Missouri	67.5[1]	15^{O}	Huston (1999)
Grand River, Ontario	77.8	23^{F}	This study
Trent River, Ontario	78.7	27^{O}	This study
Ottawa River and tributaries, Ontario	46.1[2]	14^{S}	McAllister et al. (1985)
Ottawa River and tributaries, Ontario	79.8	28^{O}	Campbell 2001
St. Lawrence River and tributaries, Quebec	66.9	20^{F}	Mongeau et al. (1992)
	Shorthead Redhorse		
Des Moines River, Iowa	65.5[3]	8^{S}	Meyer (1962)
Kankakee River, Illinois	40.4	8^{S}	Sule and Skelly (1985)
Grand River, Ontario	53.7	17^{O}	This study
Trent River, Ontario	61.5	19^{O}	This study
Ottawa River and tributaries, Ontario	55.4	20^{O}	This study
St. Lawrence River and tributaries, Quebec	41.6	11^{F}	Mongeau et al. (1992)
Saskatchewan River, Saskatchewan	41.9[4]	12^{S}	Scott and Crossman (1979)
Dauphin Lake, Saskatchewan	50.5	18^{F}	Harbicht (1990)
	Silver Redhorse		
Cahaba River, Alabama	62.0	10^{S}	Hackney et al. (1970)
Des Moines River, Iowa	51.8	9^{S}	Meyer (1962)
Trent River, Ontario	67.5	23^{O}	This study
Ottawa River and tributaries, Ontario	66.0	25^{O}	This study
St. Lawrence River and tributaries, Quebec	57.8	21^{F}	Mongeau et al. (1992)

[1]Based on age-length figure; [2]Standard length; [3]Jenkins and Burkhead (1993) suggest specimen was either Greater Redhorse or River Redhorse; and, [4]Fork length.

Discussion

This study indicates that pectoral fin rays are a more precise and accurate non-lethal age interpretation structure for redhorse than scales. However, underestimation of age can occur for fish older than 12 to 15 years. Observed overestimation of age at younger ages indicates the presence of false annuli or checks. Errors in the age interpretation of long-lived fishes can lead to substantial errors in estimates of growth, reproductive lifespan, age at maturity and survivorship. For northern redhorse populations where there is a larger representation of old individuals, pectoral fin ray based ages could overestimate growth and mortality rates and underestimate age at maturity. Determination of whether re-absorption of initial annuli occurs (Penha et al. 2004) would assist in further assessments of error associated with pectoral fin rays. If re-absorption is occurring in redhorse, age interpretations of older individuals could be improved by estimating the location of the first and second annuli using their average diameter calculated from juvenile fish, or those with clearly visible annuli (McFarlene and King 2001).

Maximum ages for Greater Redhorse (20 years), Shorthead Redhorse (20 years) and Silver Redhorse (27 years) are the oldest reported for these species (Table 3). The maximum for River Redhorse (27 years) was similar to that reported by Campbell (2001) for the Mississippi River, Ontario (28 years). The substantially higher maximum ages for Ontario populations could be the result of either the geographic differences among the populations compared or that most past studies have used scales to estimate age. Many of the redhorse species found in Ontario are near the northern limit of their range (Scott and Crossman 1979). For freshwater species with wide geographic distributions, northern populations tend to exhibit lower growth rates, later maturity and greater longevity than southern populations (Colby and Nepszy 1981; Mills 1988). For example, Huston (1999) reported the maximum age (interpreted from opercles) of more southerly River Redhorse populations in Missouri to be 15 years. Alternatively, the low maximum age of 5 years reported by Tatum and Hackney (1970) for river redhorse in the Cahaba River, Alabama, was likely an underestimation of age as a result of using scales (Jenkins and Burkhead 1993). For similarly sized (~60 cm TL) River Redhorse, scale-based age estimates from Huston (1999), Campbell (2001) and this study were up to 15 years less than

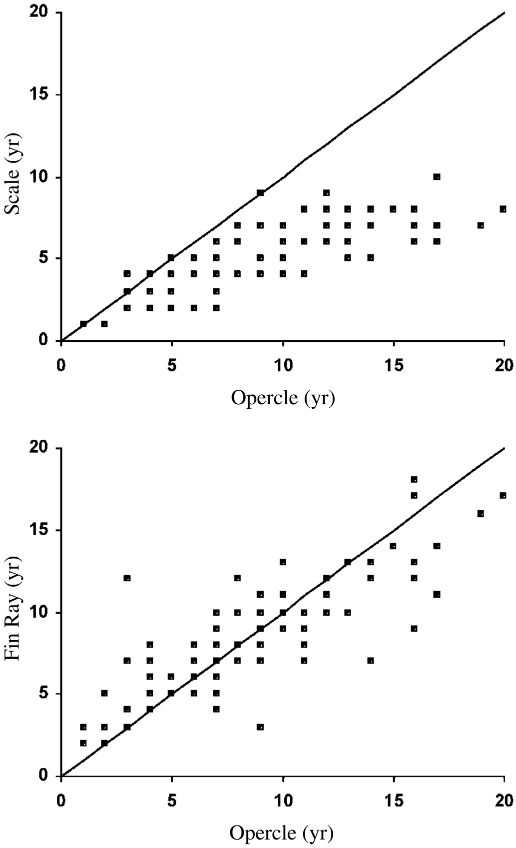

FIGURE 3. Comparison of Shorthead Redhorse opercle-based age estimates with those from scales (top) and pectoral fin rays (bottom). Opercle vs. scale CV (mean ± SE): 35.5 ± 2.8. Opercle vs. pectoral fin ray CV: 9.4 ± 1.0.

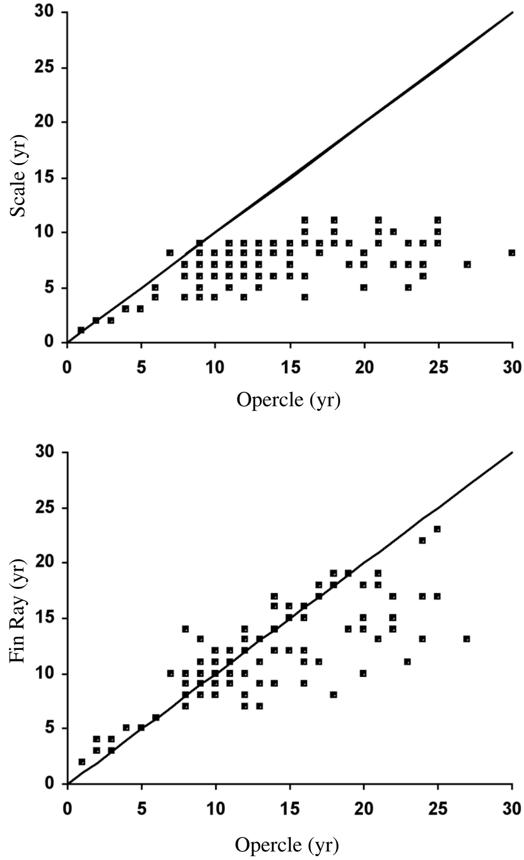

FIGURE 4. Comparison of Silver Redhorse opercle based age estimates with those from scales (top) and pectoral fin rays (bottom). Opercle vs. scale CV (mean ± SE): 31.8 ± 2.0. Opercle vs. pectoral fin ray CV: 25.3 ± 3.2.

opercle-based estimates. Underestimation of age resulting from the use of scales may also apply to maximum ages reported for Silver Redhorse in Alabama and Iowa (Meyer 1962; Hackney et al. 1970). For similarly sized Silver Redhorse in Ontario, scale-based age estimates were as much as 18 years lower than those from opercles.

Monitoring the status of imperiled redhorse species in Ontario will require non-lethal sampling methods. Results from this study indicate that pectoral fin rays are suitable for monitoring recruitment and detecting changes in population age structure. However, estimates of growth, mortality and maximum age are expected to be inaccurate. If more accurate estimates are necessary to meet monitoring objectives, opercles could be removed from a subset of the individuals sampled and used to: (1) back-calculate growth rates from measurements of annuli radii; and (2) adjust pectoral fin ray based age estimates of large (and presumably older) individuals.

Acknowledgments

The study was supported by the Ontario Ministry of Natural Resources and Industrial NSERC and Ontario Graduate scholarships awarded to S. Reid. Pectoral fin ray sections were prepared by Ontario Federation of Anglers and Hunters (Peterborough, Ontario). C. Robinson assisted with age interpretations. Some of the aging structures used in this study were provided by Fisheries and Oceans Canada. The manuscript was improved by comments provided by L. Carl, A. Dextrase, S. Stoklosar and two anonymous reviewers.

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Received 20 January 2006
Accepted 29 January 2007