Identifying and Comparing Annuli across Calcified Structures in Shorthead Redhorse (*Moxostoma macrolepidotum*)

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Estimating the Age of Shorthead Redhorse (*Moxostoma macrolepidotum*) Using Various Calcified Structures

by

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ABSTRACT

Five types of calcified structures from 32 Shorthead Redhorse (*Moxostoma macrolepidotum*) were compared to determine their use for age estimation. The structures were prepared and growth marks counted based on criteria that were adapted from studies on White Sucker (*Catostomus commersonii*). Scales and pectoral fin rays were found unsuitable for age estimation of mature Shorthead Redhorse due to crowding of annuli. Pectoral fins can only be used in combination with scales for age estimation of immature fish due to the presence of apparent false annuli in the first year. Otoliths, vertebra and opercula yield comparable result in mature fish and appear to be the most reliable structures for age estimation of mature Shorthead Redhorse. Specifically, we recommend the following techniques for aging Shorthead Redhorse at various size-classes; use of scales for fish younger than five years [< 400 mm fork length (FL)], opercula for fish 5 to 15 years (400 – 450 mm FL), and otoliths for fish older than 15 years (> 450 mm FL).

RÉSUMÉ

On a comparé cinq types de structures calcifiées prélevées sur 32 chevaliers rouges (*Moxostoma macrolepidotum*) afin d'établir leur efficacité pour la détermination de l'âge. On a adapté les critères utilisés dans l'étude du meunier noir (*Catostomus commersonii*) pour préparer les structures et effectuer le dénombrement des marques de croissance. Les écailles et les rayons des nageoires pectorales se sont révélés inadéquats pour la détermination de l'âge du chevalier rouge adulte en raison du chevauchement des annuli. Étant donné la présence de faux annuli la première année, les nageoires pectorales doivent être utilisées uniquement en combinaison avec les écailles pour déterminer l'âge des juvéniles. Les otolithes, les vertèbres et les opercules ont donné des résultats similaires chez les adultes et semblent être les structures les plus fiables pour la détermination de l'âge du chevalier rouge. Plus précisément, nous recommandons les techniques suivantes pour déterminer l'âge du chevalier rouge selon diverses catégories de taille : l'utilisation des écailles pour les poissons âgés de moins de 5 ans (longueur à la fourche = < 400 mm); des opercules pour les poissons âgés de 5 à 15 ans (longueur à la fourche = < 400 à 450 mm); des otolithes pour les poissons âgés de plus de 15 ans (longueur à la fourche = > 450 mm).

1.0 INTRODUCTION

Estimating the age of fishes using their calcified structures is widely accepted and has been validated for many fish species. The use of calcified structures for age estimation has been studied and validated for River Redhorse (*Moxostoma carinatum*; Beckman and Hutson 2012), White Sucker (*Catostomus commersonii*; Sylvester and Berry 2006), and Notchlip Redhorse (*M. collapsum*; Bettinger and Crane 2011), three members of the sucker family (Catostomidae). Scales, pectoral fins and opercles were compared for four redhorse species (*M. anisurum, M. carinatum, M. macrolepidotum* and *M. valenciennesi*), and concluded that for all four species age estimates from scales were significantly lower than those obtained from fin rays and opercles (Reid 2007). The aim of this study was to provide comparative results and did not report on validation of age estimates. Sylvester and Berry (2006) concluded that the relative precision of age estimates from pectoral fin rays and otoliths was higher than that of scales and otoliths. Similarly, Beckman and Hutson (2012) concluded that opercles and otoliths showed the lowest variability and most precision in comparisons of ages when compared to counts obtained from annuli of scales. Using a catch-recapture method, Beamish and Harvey (1969) reported the pectoral fin rays are reliable for fish age estimation of White Sucker. However, Quinn and Ross (1982) found pectoral fin rays of mature White Sucker hard to read. Thompson and Beckman (1995) studied White Sucker in a coldwater reservoir in southwest Missouri and reported that the otoliths are a reliable estimator of age for White Sucker aged 2-18 years. Most authors agree that scales cannot be used for aging White Sucker after maturity (Beamish 1973; Quinn and Ross 1982; Thompson and Beckman 1995). The annuli on the scales of mature White Sucker are very close to each other because White Sucker attains 93% of their lifetime growth by age five (Quinn and Ross 1982). Few studies have examined the age and growth in redhorse species (*Moxostoma* sp.). The purpose of this study was to compare age estimates across five calcified structures for Shorthead Redhorse and to determine which structure is more appropriate to use for aging purposes at various life stages.

2.0 METHODS

Using an electrofishing boat, 32 Shorthead Redhorse were collected from the Grand River, near Paris, Ontario on December 3, 2003. At Paris, 80 km from its outlet into Lake Erie, The Grand River is a large warm-water river with a minimum average summer flow of 25 m^3/s and daily summer high water temperatures are around 25°C. Weight, fork, and total length of each captured specimen were recorded. In the lab, five types of aging structures (scales, fin rays, otoliths, vertebra, and opercula) were removed and the sex of the fish was recorded. Individual fish measurements are provided in Appendix 1.

2.1 SCALES

Three scales were collected from the right side of the fish above the lateral line between the origin and rear insertion of the dorsal fin and stored in a manila coin envelope. The scales were later cleaned with water and a small brush and were subsequently air dried. Cleaned scales were pressed between microscope slides and viewed under a dissecting microscope. The annulus on scales of Shorthead Redhorse is characterized by dense, often interrupted circuli (Figure 1).

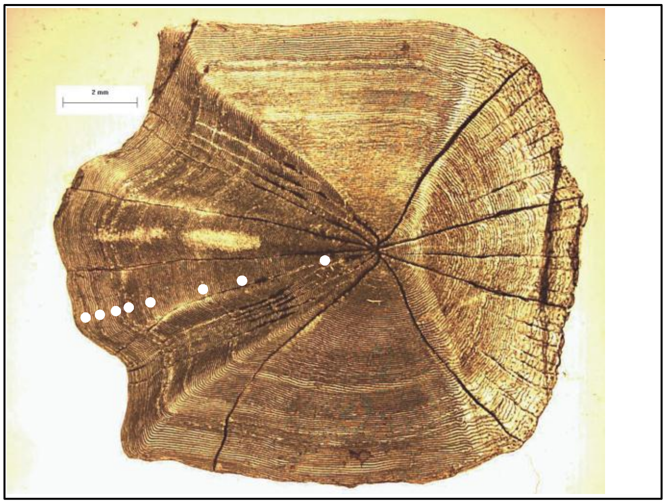

Figure 1. Scale from male Shorthead Redhorse, 420mm FL (SH_82) photographed with transmitted light. The annuli (indicated with white dots) correspond to areas where the circuli are closer, cross over, or interrupted.

2.2 PECTORAL FIN RAYS

To determine if the method of fin ray collection influences age estimates, left and right rays were collected using different physical means. The left pectoral fin ray was clipped with a pair of pliers as close to the base as possible. The right pectoral fin ray was sawed off with a bone cutter saw as close to the base of the ray as possible. The thickest part of each pectoral fin ray was embedded in epoxy resin and cut into 0.35 mm sections using a Buehler Isomet low speed saw with a 10 cm diamond edge blade. The sections were fixed between two microscope slides with resin. The annuli appear as light bands when the pectoral fin ray is observed through a compound microscope using transmitted light (Figure 2).

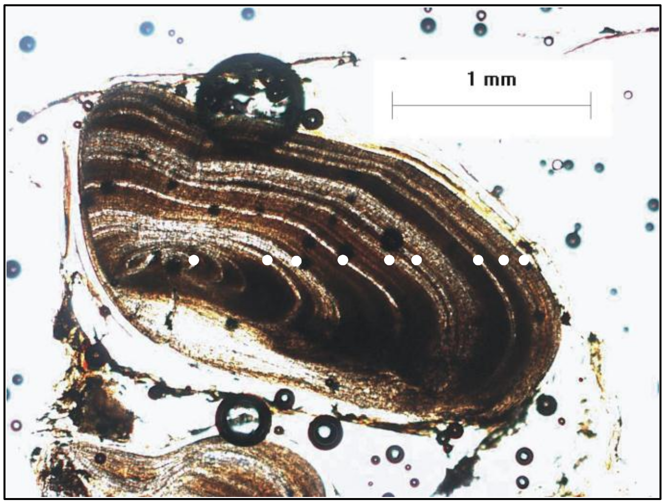

Figure 2. Section (0.35 mm) of the right pectoral fin ray of a male Shorthead Redhorse, 420 mm FL (SH_82) photographed with transmitted light. Annuli indicated with white dots.

2.3 OTOLITHS

Both sagittal otoliths were removed, rinsed with water, air dried, embedded in epoxy resin, and sectioned (0.35 mm) with the Buehler Isomet saw. Otoliths weighing less than 25 mg were fixed on a microscope slide and sanded until a thin transverse section through the nucleus remained. The annuli appear as light bands when the otolith is observed through a compound microscope using transmitted light (Figure 3).

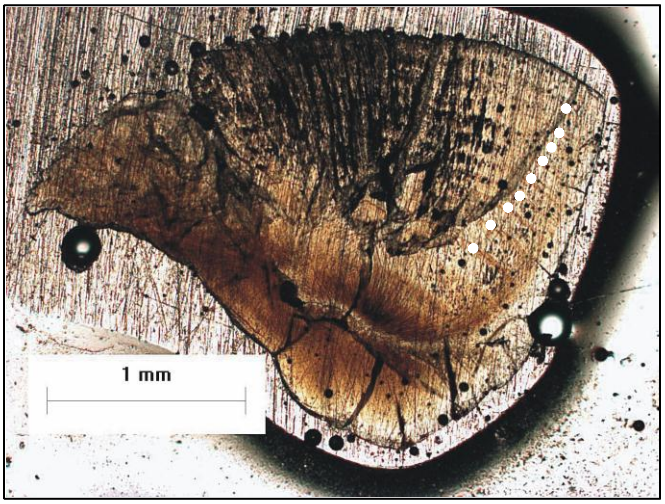

Figure 3. Section (0.35 mm) of the sagittal otolith of a male Shorthead Redhorse, 420 mm FL (SH_82) photographed with transmitted light. Annuli indicated with white dots.

2.4 VERTEBRA

The second vertebra was removed, boiled in water for three minutes to remove blood and tissue, air-dried, and embedded in epoxy resin. The vertebra in the epoxy resin was cut with the Buehler Isomet saw diagonally through the center into 0.5 mm sections (Figure 4). The annuli appear as light bands when the vertebra is observed through a compound microscope using transmitted light (Figure 5).

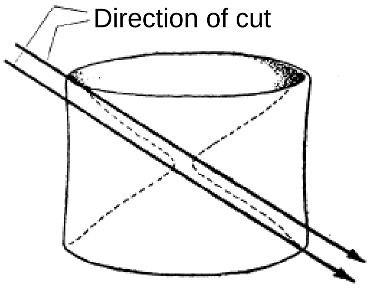

Figure 4. Direction of the cuts of the vertebra (redrawn from Ovchynnyk 1965).

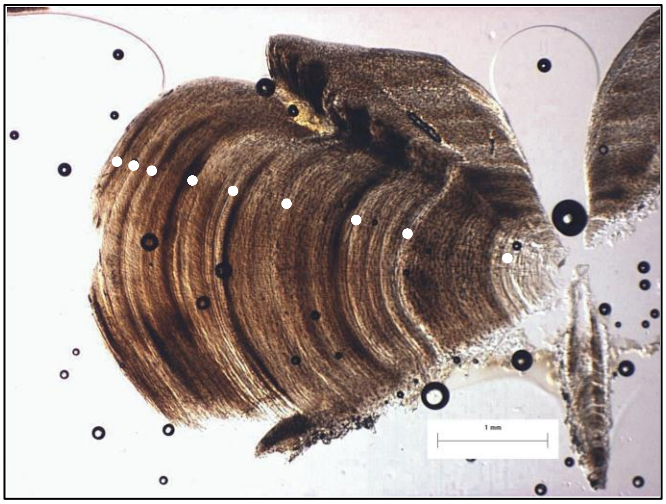

Figure 5. Section (0.50 mm) of the second vertebra of a male Shorthead Redhorse, 420 mm FL (SH_82) photographed with transmitted light. Annuli indicated with white dots.

2.5 OPERCULA

The opercula were removed, boiled in water for three minutes and air dried. The annuli on the opercula appear as thin light bands when observed through a stereo-microscope (Figure 6).

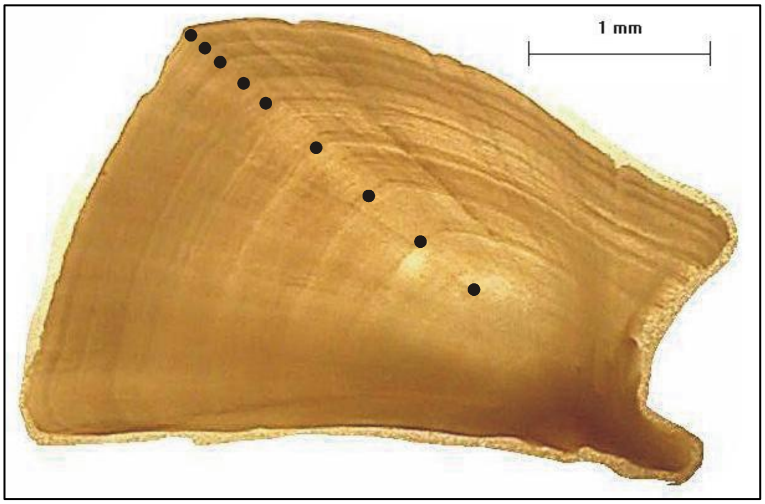

Figure 6. Left opercula of a male Shorthead Redhorse, 420 mm FL (SH_82) photographed with transmitted light. Annuli indicated with black dots.

2.6 AGE INTERPRETATION

All structures were analyzed without knowledge of the size and weight of the fish. To increase the accuracy of the readings, all structures were read on two separate occasions. If the two counts resulted in a different age interpretation, the structure was aged a third time (Appendix 1). Using the results from all structures, we estimated the age of each fish in the following manner: If the number of annuli on the scale was either one or two, the fish was estimated to be one or two years old. If the number of annuli on the scale was three to five, the age was estimated by taking the average number of annuli (rounded to the nearest whole number) from all five structures. If the number of annuli on the scale was more than five, the age was estimated by averaging the counts from otoliths, vertebra, and opercula, rounded to the nearest whole number. The method of using annuli to assign ages to calcified structures involves the general underlying assumption that annuli are created yearly in response to the coldwater growth period and, thus, represent discrete and identifiable age markers across time. The difference between the number of annuli counted on each structure and the estimated age of the fish was compared across all age classes by calculating the index of precision (Equation 1).

$$(1)\ \text{Index of Precision} = \frac{\text{Absolute [(\# annuli counted on the structure)} - \text{(age assigned to the fish)]}}{\text{age assigned to the fish}}$$

If there was no annuli count from one of the structures of a fish that was assigned an age, a standard value was entered for that structure as compensation. This standard value was chosen as 1 divided by the age class. For example, we estimated the age of Shorthead Redhorse #72 to be 5 years using all structures. Annuli count on the otoliths was 5 so the index of precision is $|5-5|/5=0$. Annuli count on the scales was 4 thus the index of precision = $|4-5|/5=0.2$. Index values of zero represent perfect agreement between the number of annuli counted on a single structure and the estimated age of the fish as described above. The average index of precision for each structure at different age fish is shown in Table 1.

Table 1. Index of precision for the age estimates of calcified structures of Shorthead Redhorse. Grey shaded cells indicate low precision of the structure (bottom 33% of the total precision of the structures). The compensation value is used to compensate for structures that were not counted and is calculated as one divided by the age class. (nv= No Value)

Age	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Scales	0	nv	nv	0.375	0.4	nv	0.43	0.36	0.33
Left Pectoral	0.5	nv	nv	0.125	0.2	nv	0.14	0.29	0.33
Right Pectoral	0.25	nv	nv	0	0.34	nv	0.29	0.36	0.33
Otolith	1.5	nv	nv	0	0.26	nv	0.143	0.125	0.33
Vertebra	0.25	nv	nv	0.125	0	nv	0.43	0	0.11
Opercula	0	nv	nv	0	0.2	nv	0	0.14	0.11
Compensation Value	1	0.5	0.333	0.25	0.2	0.167	0.143	0.125	0.111

Age	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Scales	0.4	0.45	0.5	nv	0.57	0.6	0.63	0.71
Left Pectoral	0.3	0.54	0.48	nv	0.71	0.57	0.56	0.71
Right Pectoral	0.1	0.36	0.33	nv	0.625	0.6	0.63	0.65
Otolith	0.27	0.21	nv	0.11	0.2	0.06	0	0
Vertebra	0.1	0.36	0.104	nv	0.07	0.13	0.06	0.12
Opercula	0.2	0.09	0.10	nv	0	0	0.06	0.06
Compensation Value	0.1	0.091	0.083	0.077	0.071	0.067	0.063	0.059

3.0 RESULTS

3.1 SCALES

Until maturity, between three years (Carlander 1969) and five years of age (Burr and Morris 1977; Curry and Spacie 1979, 1984; Sule and Skelly 1985), the scales of Shorthead Redhorse grow rapidly and annuli are clearly visible. The annuli on the scales of mature Shorthead Redhorse (i.e., individuals greater than five years of age) are too crowded to accurately estimate the age of the individual (Figure 7a).

3.2 PECTORAL FIN RAYS

Until maturity, the annuli on pectoral fin rays of Shorthead Redhorse are clear and easily distinguished. The growth of the pectoral fin rays slows down after maturity and annuli cannot be distinguished with certainty. The closer the section is taken to the base of the pectoral fin ray (i.e., by sawing rather than clipping), the further apart the annuli are which makes them easier to interpret (Figure 7b).

3.3 OTOLITHS

The otoliths of Shorthead Redhorse are relatively small and consist of very dense material. They are difficult to saw and sections transmit little light. In smaller otoliths, the nucleus is hard to locate precisely and several of the otoliths weighing 15 mg or less were not sectioned properly through the nucleus for this reason. Growth bands were visible when transmitted light was used on sections 35 mm thick. Yearly patterns of the growth bands could be distinguished in all age classes (Figure 7c). The highest annuli count among all structures was from an otolith (total annuli count = 19).

3.4 VERTEBRA

The vertebrae of Shorthead Redhorse are larger, and easier to collect and section than otoliths. The yearly growth patterns that form on vertebra were easily distinguished in all age classes (Figure 7d).

3.5 OPERCULA

Opercula require relatively little preparation and can be examined using low powered magnification. Opercula form clear growth bands until approximately 15 years (Figure 7e). Around this age, the opercula becomes too thick and the first one or two annuli become obscured.

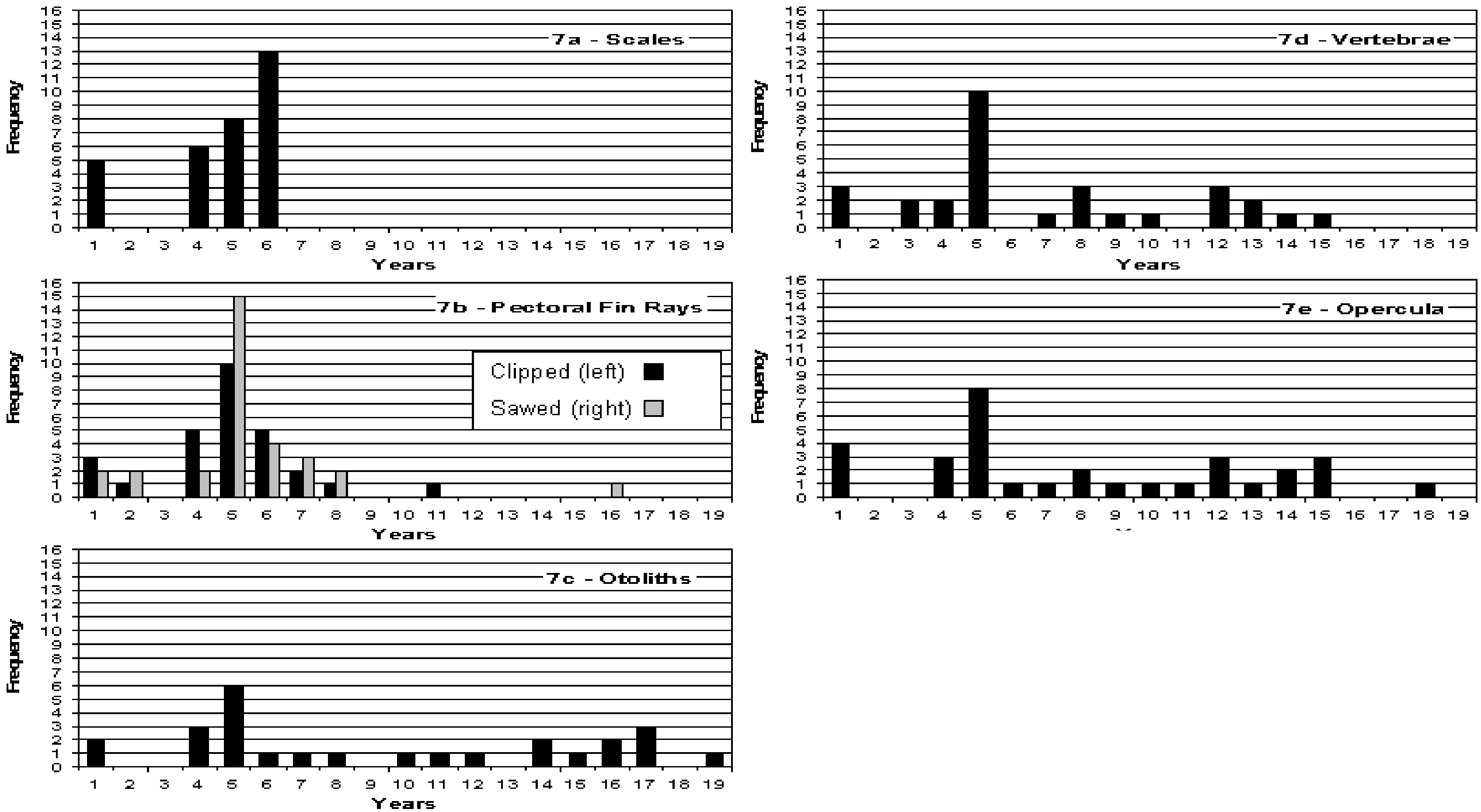

Figure 7. Age frequency diagram using annuli counts from (a) scales (n=32); (b) pectoral fin rays clipped (n=28), and sawed (n=31); (c) otoliths (n=26); (d) vertebrae (n=32); and (e) opercula (n=32) taken from Shorthead Redhorse.

4.0 DISCUSSION

Determining the most appropriate aging structure to be used for each life stage can be difficult as growth patterns may differ across species. In general, scales are convenient to collect and pose minimal injury to the fish during removal, especially compared with other structures, such as otoliths and opercula that require lethal sampling. Scales are also easy to prepare for age estimation, unlike other structures that require intricate preparation using specialized tools. Interpretation of the growth bands on scales resulted in precise age estimates until the fish reaches maturity [between 3 and 5 years, or 300 to 400 mm fork length (FL)]. Use of scales for age estimation of Shorthead Redhorse older than 5 years is not recommended as growth of the scale appears to slow down and annuli crowd the edge, making it difficult to distinguish annuli from one another. This recommendation is supported by Reid (2007) who, during his study comparing scales, pectoral fin rays, and opercles of Ontario redhorse species, concluded that age estimates from scales after ages 4 to 5 were consistently lower than those from opercles.

Pectoral fin rays did not yield precise age estimates in any age class and are not recommended for age estimation of Shorthead Redhorse. Like scales, growth of the pectoral fin rays slows down after five years and annuli crowd the edge. This phenomena was also reported by Reid (2007) who indicated that underestimation of age can occur for fish older than 12 to 15 years of age as annuli are very close and sometimes difficult to distinguish. When pectoral fin rays are used, sawing the pectoral fin rays, rather than clipping them with pliers, is recommended to preserve the part of the ray closest to the body.

The use of otoliths in age determination requires lethal sampling techniques and otoliths can be difficult to extract and prepare, especially for fish younger than five years. However, when successfully sectioned, otoliths give good age estimates across all ages.

The use of vertebrae also requires lethal sampling techniques but are more easily collected and prepared than otoliths. Vertebrae also resulted in age estimates similar to those from otoliths.

The annuli on opercula can be easily distinguished in all age classes except for the earliest annuli (i.e., those laid down during the first years of life) collected from old specimens. In these cases, the first one or two annuli are difficult to distinguish due to the thickness of the opercula.

To determine the most appropriate structure to be used for age determination, consideration must be given to the degree of sampling mortality due to structure collection. Certain structures, such as scales and pectoral fins, may be collected without lethal injury to the fish. Therefore, despite some shortcomings at certain ages, these structures may be particularly appropriate when studying rare species or species at risk, in which lethal methods may compromise the survival or recovery of the species. Since this is not a consideration for Shorthead Redhorse as it is currently abundant and widely distributed across central Canada (Reid 2009), we recommend the following techniques for aging at various size-classes; use of scales for fish younger than 5 years (< 400 mm FL), opercula for fish 5 to 15 years (400 to 450 mm FL), and otoliths for fish older than 15 years (> 450 mm FL).

5.0 ACKNOWLEDGMENTS

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Appendix 1. Weight, fork length, total length, sex, maturity, presence of tubercles, annuli count on otolith, scales, opercula, vertebra, right pectoral fin ray, and left pectoral fin ray, authors best estimate of the age of the fish.

Fish Number	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**	**81**	**82**	**83**	**85**	**86**	**87**	**88**
Fish weight (g)	1156	1498	1143	1337	357	1675	1266	1337	872	1150	786	774	1473	479	1213	321
Fish total length (mm)	449	499	464	476	312	523	472	469	416	451	420	397	511	357	464	317
Fish fork length (mm)	405	454	418	435	282	480	428	423	373	408	381	362	473	319	425	286
Sex	F	F	M	F	?	F	F	F	F	F	M	M	F	?	F	?
Mature	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	NO
Tubercles present	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO
Individual otolith weight (µg)	29.2	67.1	52.2		37.2	59.4	39.3	34.5	23.3	10.9	49	22.7		15.6	27.2	10.9
Otolith annuli count	5	14	12		5	10	7	5	4	1	8	4		4	6	1
Scale annuli count	4	6	6	5	1	6	5	5	5	4	6	4	6	1	4	1
Opercula annuli count	5	11	8	5	1	12	9	5	5	7	8	4	14	4	4	1
Vertebra annuli count	5	12	8	5	1	9	8	5	5	4	8	4	13	3	5	1
Pectoral fin (right) annuli count	5	16	5	6	1	7	8	5	5	6	6		5	4	4	1
Pectoral fin (left) annuli count		6	5	5	1	11	5	5	5	5	6	4	8	4	4	1
Assigned age	5	12	9	5	1	10	8	5	5	7	8	4	14	4	5	1

Fish Number	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**100**	**101**	**102**	**103**	**104**
Fish weight (g)	1342	198	1382	1087	1315	372	1341	1798	1766	1183	1297	1152	1144	775	1378	1020
Fish total length (mm)	495	269	504	453	496	316	489	526	537	460	482	455	455	400	504	430
Fish fork length (mm)	446	242	455	409	464	289	441	482	484	419	436	409	412	362	459	388
Sex	F	?	M	F	F	?	F	F	F	M	M	F	F	M	F	M
Mature	YES	NO	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES
Tubercles present	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	NO	YES
Individual otolith weight (µg)	73.9	9.3	54.6	25	73.5	12	61.2	70.3	66	56.8	70.8	32	30.8	25.2	74.4	33.6
Otolith annuli count	16		17	5	19		17	11	16	14	15	5	5		17	
Scale annuli count	6	1	6	4	6	1	6	6	6	6	6	4	5	5	5	5
Opercula annuli count	12	1	15	5	15	1	15	13	14	12	10	6	5	5	18	5
Vertebra annuli count	9		15	5	12	1	14	12	13	7	10	5	5	5	15	5
Pectoral fin (right) annuli count	5	2	7	5	5	2	8	7	6	5	5	5	5	5	5	5
Pectoral fin (left) annuli count		2	6		5	1	7		5	7	4	5	4	6	6	5
Assigned age	12	1	16	5	15	1	15	12	14	11	12	5	5	5	17	5