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Eugenia Moreira, Esteban Barrera-Oro & Mario La Mesa

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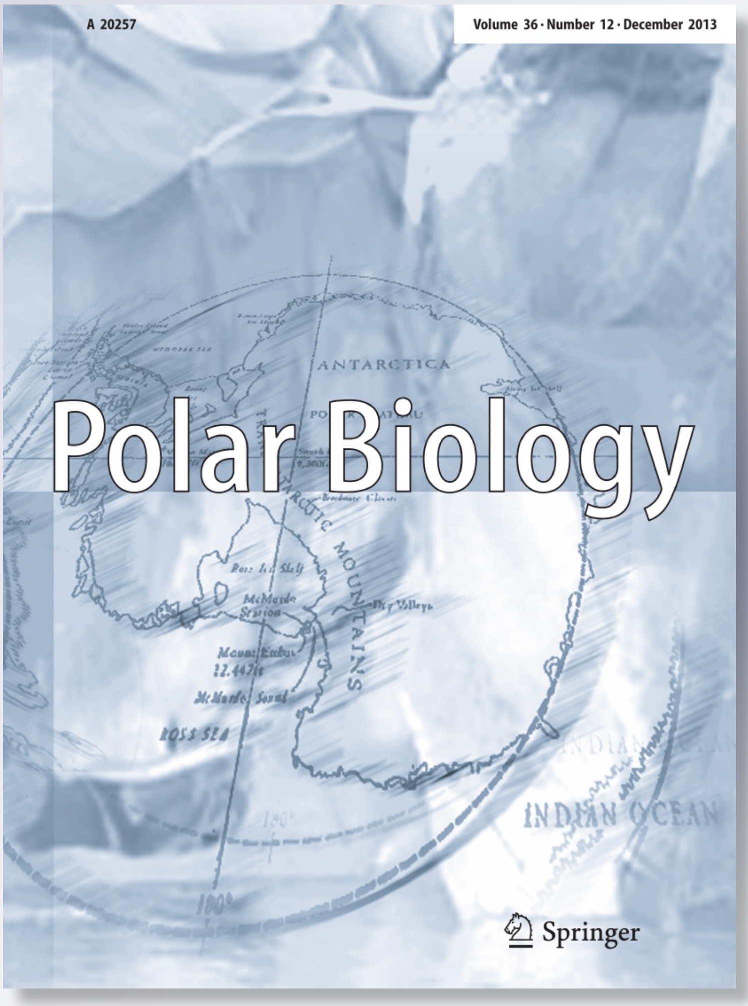

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SHORT NOTE

Age validation of juvenile *Notothenia rossii* at Potter Cove, South Shetland Islands, using mark-recapture data

Eugenia Moreira · Esteban Barrera-Oro · Mario La Mesa

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Abstract Among all validation methods of age determination in fish, release of known age and marked specimens gives the most reliable information. We carried out a tag-recapture experiment on *Notothenia rossii* at Potter Cove, to validate, for first time for this species using this method, the principle of annual deposition of an annulus in scales and otoliths. Of 132 juvenile specimens (TL = 22.1–38.1 cm) tagged and released in successive years from 2004 to 2010, 7 were recaptured at the same site after periods of 1–13 months. In scales of five specimens recovered after 10–13 months, one extra annulus was laid down, exhibiting an additional winter zone of closely spaced sclerites. Consistently, the same analysis in two individuals marked and recaptured during the same summer, after 1–3 months at liberty, did not show the deposition of an additional annulus. All the fish tagged or recaptured during the experiment period (December to March) showed in their scales an edge zone of widely spaced sclerites, in agreement with the known pattern of growth in summer. Likewise, an analysis in selected specimens showed good consistency between the numbers of sclerites deposited in scales and the time of fish release. The comparative analysis between scales taken at recapture and otoliths of the same individual allowed a simultaneous counting of the annuli with complete correspondence. The growth in length of fish ranged from 0.5 to 6.1 cm, depending on the time of release.

Keywords Antarctic coastal fish · Scales–otoliths · Notothenioidei

Introduction

The assessment of the dynamics of fish populations relies heavily on the accuracy of age determinations, basically used for the estimation of mortality and growth. Inaccurate estimates of these parameters can lead to the mismanagement of a fishery, and therefore, age readings must be supported by consistent validation methods. A range of techniques have been utilized in Antarctic fish to obtain age estimates from rhythmic patterns revealed in bony structures like scales and otoliths, but most ageing data have not been validated (North 1988; Kock 1990; White 1991; La Mesa and Vacchi 2001; Barrera Oro et al. 2010; among others).

The marbled notothenia, *Notothenia rossii* (Richardson 1844), is a circum-Antarctic species, widely distributed in coastal waters of the Scotia Arc, around the Kerguelen, Crozet, Marion, Prince Edward, Macquarie, Heard and Macdonald Islands, and Ob and Lena Banks (Gon and Heemstra 1990). It was the first Antarctic fish depleted by the industrial fisheries in the late 1970s (Kock 1992). Nevertheless, more than two decades since the prohibition of this fishery in the Southern Ocean in 1991, the stock condition of *N. rossii* around the South Shetland Islands is still uncertain (Barrera Oro and Marschoff 2007; Marschoff et al. 2012). An updated assessment of its population dynamics is essential for the appropriate monitoring of its recovery in the region.

E. Moreira (✉) · E. Barrera-Oro
Instituto Antártico Argentino and CONICET, Cerrito 1248, A1010AAZ Buenos Aires, Argentina
e-mail: eugeniamoreira@yahoo.com.ar; ebarreraoro@dna.gov.ar

E. Barrera-Oro
Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", A. Gallardo 470, CI405DJR Buenos Aires, Argentina

M. La Mesa
ISMAR-CNR, Istituto di Scienze Marine, Sede di Ancona, Largo Fiera della Pesca, 60125 Ancona, Italy

In juvenile and adult *N. rossii*, the annual deposition of presumed annuli in scales and otoliths has been validated by the following methods:

- Examination of the deposition of calcareous material at the edge of otolith and scales throughout the year to demonstrate that one opaque and one hyaline zone (otoliths) or one widely spaced and one closely spaced zone of sclerites (scales) are formed (Burchett 1983a; North 1988). A sclerite is a periodic marc, which has been formed on the scale proper to deposition process.
- Identification of peaks/modes in the length-frequency distribution (Petersen method) (Barrera Oro and Casaux 1992; Barrera Oro et al. 2010).
- Otolith microstructure analysis using daily ring counts (Barrera Oro and La Mesa 2012).

Recently, lead-radium dating has also been used successfully in both toothfish species *Dissostichus mawsoni* (Andrews et al. 2011) and *D. eleginoides* (Brooks et al. 2011) to test accuracy of age data. However, the technique is expensive, and Campana (2001) argued that among all the validation methods, release of known age and marked fish provides the most reliable information. Nevertheless, mark-recapture requires much work in the field and can be particularly problematic in the Antarctic where sampling may be difficult due to bad weather. For this reason, when applied for the first time in an Antarctic fish species in the 1990s, it was used in relatively protected coastal-littoral waters of the South Shetland Islands on the dominant nototheniid there, *Notothenia coriiceps*, to validate age based on conventional scale and otolith readings (Barrera Oro and Casaux 1996). Later, mark-recapture experiments were applied to validate age in *D. mawsoni* using otolith-oxytetracycline injection (Horn et al. 2003).

Since 2005, a long-term program using mark-recapture of *N. rossii* specimens has been carried out during the austral summer at King George/25 de Mayo Island, South Shetland Islands. The offshore–inshore phases in the life cycle of this species are well known from several localities within its geographical distribution (Olsen 1954; Burchett 1983b; Duhamel 1982). In the South Shetlands, the inshore period lasts up to 6–7 years of life (summarized in Barrera Oro et al. 2010). Consequently, we expected to obtain some recaptures in the following years. As a result, in this study, we used a tag-recapture validation study to test the annual deposition of annuli identified in previous studies (Barrera Oro and Casaux 1992; Barrera Oro et al. 2010) from scales and otoliths of *N. rossii* collected in inshore waters from the same site.

Materials and methods

Juvenile *Notothenia rossii* were caught at Potter Cove, close to the scientific station Carlini (62°14′S and 58°40′W) from December 2004 to February 2010 using similar trammel nets set on rocky, macroalgae beds at

Fig. 1 Recaptured *Notothenia rossii* specimen (TL: 33.9 cm) (**a**) and two types of plastic tags used in the experiment (**b** and **c**)

5–40 m depths for 16–24 h. A detailed description of the sampling procedures as well as the biotic and abiotic features of this site is given in Barrera Oro et al. (2010) and Casaux et al. (1990), respectively. Immediately after retrieval of the net, the fish that were healthy were measured for total length (TL) to the nearest 0.1 cm and marked with a numbered tag. Scales from the left side under the pectoral fin (below and above the lateral line) were extracted and stored in paper envelopes. A total of 132 individuals were liberated at the same site. The length range of the tagged fish was 22.1–38.1 cm.

Two different plastic tags were used in the experiment depending on fish size (Fig. 1). The fish recaptured were measured and weighed, and scales and sagittal otoliths were removed and stored. Sex was determined according to Everson (1977). In the laboratory, approximately 70 scales from both sides of fish body were placed in a detergent solution for 24–48 h in parallel with similar number of scales removed before release. Regenerated scales were discarded. Then, selected scales were cleaned mechanically, dried, and mounted between microscope slides. An average of 10 scales per specimen was used for sclerite counts. A binocular microscope with transmitted light was used, at magnifications from 12× to 25×.

The otoliths were placed in a detergent solution for 5–20 min to remove adhering organic tissue. These time periods are critical because longer time period result in the otoliths becoming irreversibly transparent. Otoliths were observed entire on both medial and distal sides. On some occasions, the center of the otolith was manually polished with an abrasive stone. The reading method was similar to that used by Barrera Oro and Casaux (1992). Whole otoliths were submerged in 96 % alcohol and then examined with the same optical equipment and magnifications as for the scales, but using reflected light, against a dark background. The annuli estimated from otoliths were compared with those obtained from scales.

All readings were done independently, without any ancillary data on tag and recapture dates or scale ages.

Results

Seven marked fish were recaptured (5 small and 2 large tags; 3 males and 4 females; recovery rate = 5.3 %) at the same release points, after periods of 1–13 months (Table 1). They were juvenile specimens from 25.2 to 38.1 cm TL. The specimens were in good condition indicating that the tagging wound had minimal effect (Fig. 1). The scales removed at the beginning of the experiment from the left flank had completely regenerated and were easily identifiable, showing the typical structure of a replacement scale (Barrera Oro and Casaux 1996).

The growth in length of fish ranged from 0.5 to 6.1 cm, depending on the time at liberty.

In scales, we counted the annuli as the narrowing area of the sclerites (winter rings, checks) along the axis from the focus to the anterior margin (Barrera Oro et al. 2010). An annulus is considered to be a zone of widely spaced sclerites that corresponds to summer growth, followed by a zone of closely spaced sclerites. When the sclerite follows a continuous line around the focus, the term "circuli" can be used as synonymous. A regular counting of the number of sclerites facilitated interpretation of the scale area adjacent to the first check, as it was in some cases not easily distinguishable (discussed below). Our results from this method are similar to those obtained with scales taken from a larger number of *N. rossii* specimens ($N = 243$) provided in detail in Barrera Oro et al. (2010), and so they are not presented in this study.

In entire otoliths, the annuli were counted mainly on the distal side in the dorsal area (Barrera Oro and Casaux 1992). The nucleus consisted of opaque material (light) surrounded by a thin hyaline (dark) edge (Fig. 2c).

Table 1 Comparative results of the annuli counting in scales and otoliths of *Notothenia rossii* obtained during the tag/recapture experiment at Potter Cove, South Shetland Islands

Tag	Sex	Tag date	Recapture date	Release period. (months. days)	Growth length (cm)	Number of annuli		Otoliths
						Scales at tagging	Scales at recapture	
WE	MII	27/12/2008	25/01/2010	12.29	32.0–38.1	5	6	6
ZF	FI	17/01/2009	19/12/2009	11.02	22.1–25.2	3	4	4
YH	MII	06/01/2009	23/12/2009	11.17	22.2–27.5	3	4	4
ADX	MII	11/02/2009	25/01/2010	11.14	22.5–27.3	3	4	4
AAI	FII	21/01/2009	12/12/2009	10.21	29.9–33.9	5	6	6
631*	F	17/12/2005	06/03/2006	2.17	29.9–30.8	4	4	4
667*	F	15/01/2006	13/02/2006	0.29	25.2–25.7	4	4	4

* = blue plastic tag. M = male, F = female

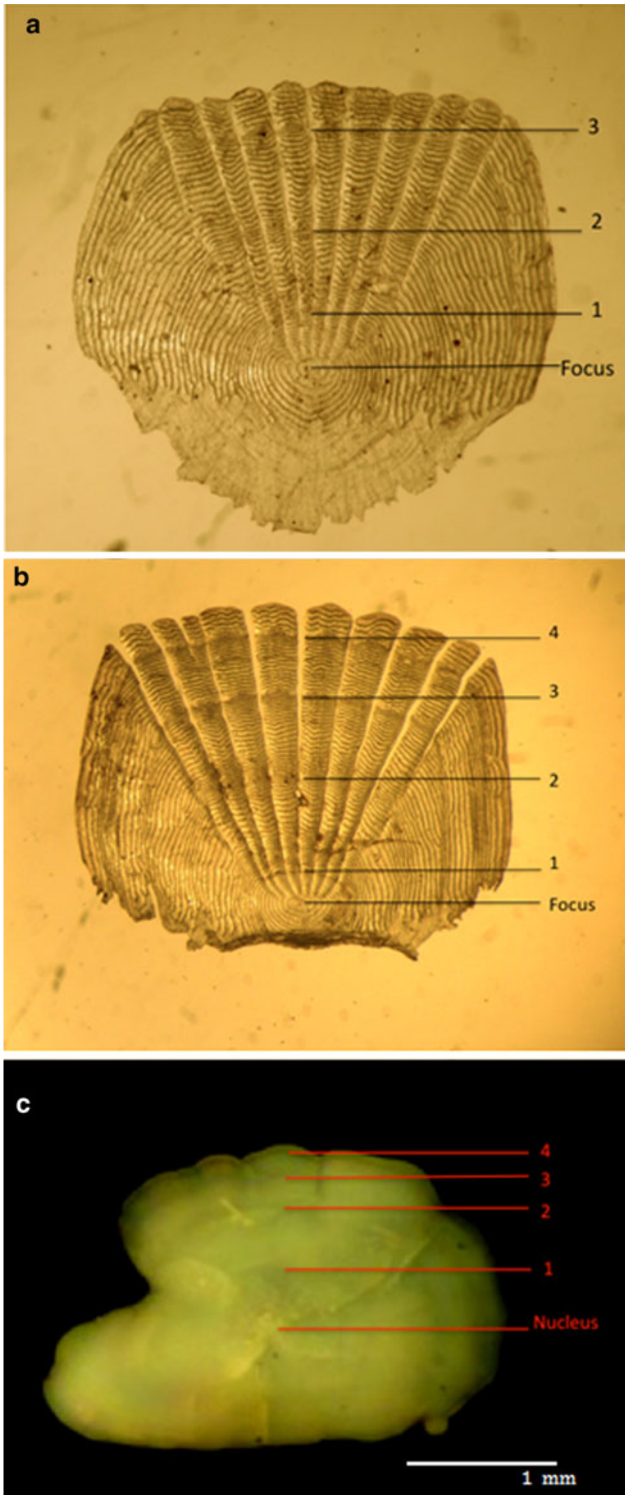

Fig. 2 Annuli counting on scales and otolith of a single *Notothenia rossii* specimen at different times of the experiment. Three annuli were observed in scales at tagging (**a**). After 10–13 months of release, 4 annuli were counted in scales (**b**) and otoliths (**c**)

In specimens WE, ZF, YH, ADX, and AAI, the comparison between the annuli counted in scales removed before the release and at recapture showed that one annulus was laid down during the elapsed 10–13 months (Table 1; Fig. 2a, b). Specimens # 631 and 667 were recaptured during the same summer, after about 3 and 1 months at liberty, respectively. Consistently, their scales did not exhibit an extra-closely spaced sclerites zone (Table 1). All the fish tagged or recaptured during the experiment time (December to March) showed in their scales an edge zone of widely spaced sclerites, in agreement with the known pattern of growth in summer (North 1988).

Likewise, a comparative analysis of the sclerites deposited in scales of the specimens of similar size ZF, YH, and ADX that remained at liberty for about 1 year showed a similar mean of 18–22 sclerites between the date of marking and recapture (Table 2). The same analysis carried out for specimens 631 and 667, which remained at liberty for 77 days the first and 29 days the latter, showed mean depositions of 6 and 2 sclerites, respectively.

The comparative analysis between scales at recapture and otoliths of the same individual allowed a simultaneous counting of the annuli with full agreement (Table 1; Fig. 2b, c).

Discussion

The data obtained by the recapture of marked *N. rossii* in Potter Cove have been utilized to validate the classical ageing technique based on the examination of conventional structures. The goal of the tag-recapture program developed in the late 1980s was also to obtain information on migration-site fidelity and age and growth of the fish species regularly caught in the area. Marking experiments on *N. rossii* have not been reported so far. The relatively low recapture number of individuals obtained in this study (5.3 %), as already reported for the ecologically similar *N. coriiceps* (2.2 %) collected at Potter Cove (Barrera Oro and Casaux 1996), would suggest a high tag loss or tag-induced mortality. Five out of seven *N. rossii* specimens recaptured have been tagged with smaller and lighter tags, suggesting that the larger and heavier blue tags might have a more detrimental effect on fish survival (Fig. 1). For comparison, the similar *N. coriiceps* had a significantly higher recapture rate (10 %) in inshore waters of Signy Island (South Orkney Islands) (Everson, 1970). As in the studies mentioned, we observed little damage in the tag insertion zone.

As in many Antarctic fish, scales of *N. rossii* can be interpreted properly in juvenile specimens, but in older fish, the outer annuli generally became difficult to differentiate (Linkowski and Zukowski 1980; Burchett 1984; North 1988; Barrera Oro and Casaux 1992).

The identification of the second check in scales is sometimes difficult, as it may be confounded with the presence of a fainter ring which is attributed to the shift from pelagic to demersal habitat (Freytag 1980; Barrera

Table 2 Number of sclerites counted to winter rings (checks) and to edge in scales of selected specimens of juvenile *Notothenia rossii* marked and recaptured at Potter Cove

Age	3 years at tagging						4 years at recapture						4 years at tagging and recapture				
Specimens	YH		ADX		ZF		YH		ADX		ZF		Specimens	631		667	
N° of sclerites to:	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Total no of sclerites	Range	Mean	Range	Mean
1st check	7–12	10.4	9–11	10.1	8–12	10.6	8–11	9.7	9–11	10.0	8–11	9.8	At tagging	67–71	69	62–70	66
2nd check	32–36	34.1	27–29	28.0	28–31	30.0	29–37	34.7	31–37	33.6	30–37	32.8	At recapture	69–81	75	62–74	68
3th check	44–50	47.3	44–48	46.0	41–47	43.9	42–51	48.1	50–58	54.3	43–54	48.9					
4th check							59–73	68.4	62–73	68.0	58–68	62.9					
To edge	52–58	54.6	54–58	55.6	50–57	53.6	66–78	75.2	74–83	77.6	66–77	71.6					

Oro and Casaux 1992). The regular counting of the number of sclerites proved to be a valid tool to confirm the position of the checks, a procedure that we followed but do not provide in this study, as it has been explained in detail in Barrera Oro et al. (2010).

The conventional procedure of scale analysis before release and after a time at liberty validated the annual deposition of an annulus for age range 3–6 years (Table 1). Five specimens (WE, ZF, YH, ADX, and AAI) showed in their scales an extra adjacent wide and closely spaced sclerite zone after 10–13 months from release (Fig. 2a, b). This actually corresponds to one winter season during which the fish remained in the cove between their tag and recapture dates. Consistently, the scales of the two specimens recaptured during summer after only 1–3 months at liberty (# 631 and 667) exhibited just a wider edge of widely spaced sclerites (Table 1). Likewise, in the selected specimens examined, there was good consistency between the numbers of sclerites deposited in scales and the time of fish release (Table 2). The pattern of sclerite deposition was similar to that analyzed for *N. rossii* in a previous study (Barrera Oro et al. 2010). The age range of specimens recaptured (3–6 years) is in agreement with the life history pattern reported for juvenile *N. rossii* in the South Shetland Islands, which indicates that they remain in inshore waters until 6–7 years of age before migrating offshore and joining the adult population (Freytag 1980; Linkowski et al. 1980; Barrera Oro and Casaux 1992).

The same conclusion is valid for otoliths, owing to the good agreement found between the age readings from whole otoliths and scales of recaptured specimens (Table 1; Fig. 2b, c). Sometimes, in otoliths, the zone closely surrounding the nucleus was not clearly visible, due to the thickening process occurring with increasing age of the fish (Barrera Oro and Casaux 1992). The polishing procedure of the otolith center helped to identify the ring pattern. Nevertheless, the counting of annuli on whole otoliths of *N. rossii* is feasible in juvenile specimens only, since for age determination in adults, an otolith sectioning technique should be used (Barrera Oro and Casaux 1992).

Therefore, in otoliths of *N. rossii*, only one opaque and one adjacent hyaline zone should be considered to represent an annual ring (i.e., an annulus). The agreement in number of annuli found in scales and otoliths within each individual supports the evidence that scales are formed in early stages of *N. rossii* (Barrera Oro and Casaux 1992).

The validation method of tag-recapture is in general not widely used due to difficulties associated with the migration/movements of fish species, and the effort required for recapture that often results in small sample sizes. In the Antarctic, such difficulties are magnified by extreme climate conditions and limited logistic facilities. Nevertheless, the data from the specimens recovered in this study were consistent with annual deposition of the annulus in scales and otoliths of *N. rossii*, and similar results were found in mark-recapture validations on other Antarctic fish that were also constrained by a limited number of recaptures, i.e., nine individuals of *Notothenia coriiceps* at Potter Cove (same methodology, Barrera Oro and Casaux 1996) and six individuals of *Dissostichus mawsoni* at Mc Murdo Sound (tetracycline injection, Horn et al. 2003). Despite the general drawbacks inherent in mark-recapture approaches, including potential biases where assumptions are not fulfilled, the information obtained for age validation can be highly reliable and easily amenable to laboratory determinations with the simple use of light microscopy. Validation studies using this methodology are an important option in the Antarctic, especially for nonmigratory demersal fish inhabiting inshore coastal waters, like juvenile *N.rossii* and *N.coriiceps* in Potter Cove.

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