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Early life history timings in marbled rockcod (*Notothenia rossii*) fingerlings from the South Shetland Islands as revealed by otolith microincrement

Esteban Barrera-Oro · Mario La Mesa · Eugenia Moreira

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Abstract Although it has been reported that *Notothenia* rossii elsewhere hatches in spring, our daily increment back-counting from the capture date in otoliths of fingerlings caught in Potter Cove, South Shetland Islands, in the 2000s, showed two main periods of larval hatching, one in summer (February-March) and another in winter (July). In concordance, the simultaneous presence of two cohorts born the same year was identified in the age/length frequency distribution of fish sampled in spring 2010, which belonged to biological ages 0+ and 1+ and hatched, respectively, in summer and winter-spring. Maximum and minimum ages of pelagic blue-phase and demersal brownphase fingerlings were, respectively, 227 and 240 days, indicating a demersal settlement after about 8 months from hatching. The estimated growth rate was 0.23-0.33 mm/ day, equivalent to that of South Georgia fingerlings and higher than those of other nototheniids of similar size range. Based on early life events associated with the hatching periods, two types of life cycles are hypothesised for South Shetland fingerlings. The pelagic blue-phase fingerlings (6.5–7.6 cm TL, age group 0+) hatched in July (winter cohort), entering in Potter Cove in February-March. The brown-phase fingerlings (6.3–10.6 cm, mostly of age group 0+) hatched in February-March (summer cohort) and were collected in the cove in spring (the smaller specimens) or in summer (the larger ones). Finally, early juveniles (10.7-15.5 cm, age group 1+) hatched in winter, mainly in July (winter cohort), entering in the cove the following year to spend the second winter inshore.

Keywords Daily rings · Notothenioidei · Juvenile stages · Hatching · Spawning time

Introduction

The marbled rockcod (Notothenia rossii, Richardson 1844) is a wide spread species present in waters of the Scotia Arc, around the Kerguelen, Crozet, Marion, Prince Edward, Macquarie, Heard and Macdonald Islands, and the Ob and Lena Banks (Gon and Heemstra 1990). It was the first Antarctic fish species depleted by the commercial fishery, which began around South Georgia in the late 1960s (Kock 1992). Upon the establishment of the Convention for the Conservation of Antarctic Marine Living Resources (CAMLR Convention) in 1982, a series of conservation measures were enacted in order to promote recovery of this and other overexploited fish species in many Antarctic zones. Although finfishing has been prohibited in the South Shetlands-Antarctic Peninsula area since 1991, the local population of N. rossii is still recovering more than three decades after the heavy fishing in the late 1970s (Barrera-Oro and Marschoff 2007; Marschoff et al. 2012).

In comparison to other notothenioids, the life cycle of *N. rossii* is rather complex and at South Georgia was well described by Olsen (1954) and Burchett (1983), as was the population at Kerguelen Islands by Duhamel (1982). Spawning takes place between April and June on the bottom of continental shelf areas at about 120–350 m depth, where ripe adults migrate during fall. The eggs hatch in spring between September and November in offshore waters, the newly hatched larvae being about 10–15 mm in

E. Barrera-Oro (⊠) · E. Moreira Instituto Antártico Argentino and CONICET, Cerrito 1248, 1010 Buenos Aires, Argentina e-mail: ebarreraoro@dna.gov.ar

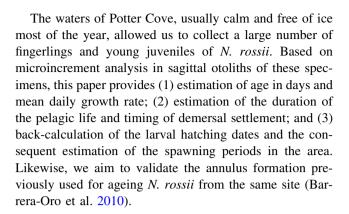
M. La Mesa ISMAR-CNR, Istituto di Scienze Marine, Sede di Ancona, Largo Fiera della Pesca, 60125 Ancona, Italy



length. Larvae and young offshore fingerlings (18–38 mm TL) are pelagic. After about 6–8 months, the pelagic bluephase fingerlings migrate inshore, change morphologically to the brown-phase fingerling stage and become demersal, settling in the algae beds. After a period of about 15 months, fingerlings acquire the typical mottled brownish colour of the juvenile stage. Juveniles live in association with algae beds for approximately 5–7 years and, at the onset sexual maturity, young adults become semipelagic and migrate to the offshore shelf feeding area to join the adult population. The aforementioned life cycle of *N. rossii* is assumed to be similar in other areas of its range (Kock et al. 1985), as off South Shetlands (Barrera-Oro 2002).

Despite the several studies on age and growth of N. rossii, the transition from pelagic blue-phase to demersal brown-phase fingerlings and the formation of the first annulus have long been a matter of controversy (Barrera-Oro et al. 2010 and references therein). Recently, interpretation of sclerites in scales of N. rossii fingerlings caught in Potter Cove, South Shetland Islands, helped to clarify these issues, as well as the deposition of a secondary ring after the first winter ring, linked to the shift from pelagic to demersal habitat during the second year of life (Barrera-Oro et al. 2010). In the referred study, the annual deposition of the annulus in scales and otoliths was validated by the traditional method of identification of peaks/ modes in the length frequency distribution (Petersen method). Nevertheless, to date ageing methodologies for estimating age and growth of this species have been based solely on otolith or scale macrostructure. As widely recognised for fish species elsewhere (Campana 2001) and also demonstrated in Antarctic nototheniids (Townsend 1980; La Mesa 2007; La Mesa and Ashford 2008), otolith microstructure analysis involving daily ring counting is useful in corroborating annulus formation and in providing more precise information on early life history timings such as the hatching period.

It has been suggested that *N. rossii* around Elephant and the lower South Shetland Islands spawns exclusively in May–June (Kock 1989) and, as described above, it is assumed that hatching occurs in spring. This information is based on samples collected offshore in February–March, where most fish were immature, at least 2 months from spawning (Kock 1989; Jones et al. 2003). Nevertheless, some specimens exhibited a post-spawning condition (Jones et al. 2003), presenting the possibility of two main spawning seasons, but no data are currently available on fish caught in early summer to testing this hypothesis. The back-counting of daily rings in otoliths of *N. rossii* from the date of capture is a useful method to determine the hatching period and hence might be an appropriate tool to clarify the spawning periodicity.



Materials and methods

A total of 364 N. rossii fingerlings and young brown-phase juveniles were collected at Potter Cove, King George/25 de Mayo Island, South Shetland Islands, close to the scientific station Carlini (formerly "Jubany") (62°14S; 58°40W), in summer 2003 (Feb), 2004-05 (Dec-Jan), 2005-06 (Dec-Feb), 2008 (Jan-Feb), 2008-09 (Dec-Mar), 2009-10 (Dec-Feb) and spring 2010 (Sept-Nov) (Fig. 1). Some samples were used in other ecological investigations, including a recent study on age and growth of early stages of the species (Barrera-Oro et al. 2010). The biotic components and abiotic features of this area are described in Casaux et al. (1990). The fish were caught by means of two different gears: a bottom trawl (mouth 1 m², length 2 m and mesh 4 mm) towed from a rubber boat on rocky bottoms covered with macroalgae during daylight for 15-30 min at depths of 6-40 m; trammel nets (length 15 m, height 1.5 m, inner mesh 2.5 cm, outer mesh 12 cm) set on the bottom in the same area at similar depth for 16-24 h. Some trawling was also conducted in the water column at various depths, but without fish captures. Shortly after capture, sagittal otoliths and scales were extracted, cleaned, dried and stored until processing. We recorded total length (TL) and standard length (SL) to the nearest 0.1 cm below, weight in g and sex of fish. Specimens <16 cm TL were included in this study. As all the fish were sexually immature (stage 1 or stage 2 virgin, Kock and Kellermann 1991), data from both sexes were combined. Further data on sampling stations and fish examined are presented in Table 1.

For each specimen, one sagittal otolith was randomly selected from each pair and attached, medial side down, to a glass slide with CrystalbondTM 509 adhesive. The length (OL, 0.01 mm) of the anterior–posterior growth axis through the otolith primordium was measured under a light microscope linked to a CCD videocamera. The relationship between OL and fish size (TL) was estimated using linear least-squares regression.



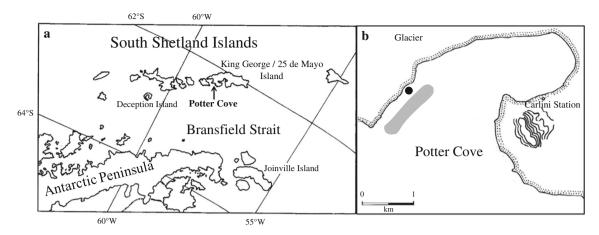


Fig. 1 a The South Shetland Islands and a detail of King George/25 de Mayo Island showing the location of Potter Cove. b Sampling sites for *Notothenia rossii* fingerlings with trawl net (shadow) and trammel net (dot)

Table 1 Details of sampling stations and fish examined. The number of hauls is indicated between parentheses

Date	N° of fish c	aught and hauls	Length range	N° of fish age	d
	Trawl net	Trammel net	TL (cm)	Otolith/scale annuli	Otolith microincrements
Feb 2003	3 (1)	_	11.2–11.6	3	_
Dec 2004-Jan 05	10 (6)	_	9.0-13.3	10	2
Dec 2005-Feb 06	38 (14)	1 (1)	7.6-15.9	39	5
Jan-Feb 2008	30 (7)	1 (1)	7.0-15.8	31	5
Dec 2008-March 09	109 (17)	_	6.5-15.7	109	10
Dec 2009-Feb 10	39 (12)	13 (7)	7.8-15.9	52	6
Sep-Nov 2010	119 (17)	1 (1)	6.3-15.2	120	18
Total	348 (74)	16 (10)	6.3–15.9	364	46

The sagittal plane of otoliths was chosen for counting, as microincrements were wide enough to be easily identified and counted. Each otolith was ground with abrasive paper (800 grit) and polished with alumina powder (0.05 μm) by a grinding wheel (REMET LS2) until the increment pattern was evident from the primordium to the otolith margin. In this way, thin sections of approximately 150 μm were obtained directly on slides. For microincrement counts, otolith sections were viewed under a light microscope at $400\times$ magnification. All microincrement counts generally took place along two main paths, i.e. from the core towards a posterior–dorsal or posterior–ventral axis. The sections were observed using green light and polarising filters to improve the readability of microincrements.

Typically, each increment revealed a bipartite structure consisting of a discontinuous zone (D-zone) and an incremental zone (L-zone), which appeared, respectively, dark and light under transmitted light (Secor et al. 1995). The total number of microincrements from the primordium to otolith edge was recorded twice by the same reader, and the mean value taken as a measure of fish age in days. Although we were unable to validate the daily deposition

of microincrements in *N. rossii*, they were assumed to be laid down daily showing similar width and deposition pattern observed in otoliths of other Antarctic fish that have been validated (Slosarczyk 1987; Kellermann et al. 2002; Morley et al. 2005).

To assess ageing precision between readings, we calculated the index of average per cent error (APE) (Beamish and Fournier 1981) and the mean coefficient of variation (CV) (Chang 1982). The relationship between fish size and age was estimated by fitting a linear equation in the following form:

$$TL = a + b age (days)$$

where TL is the total fish length (cm), a is the fish size at age 0 (i.e. larval size at hatching), and b is the instantaneous growth rate of *N. rossii* fingerlings.

Fitting the linear models to the relationships between OL/TL and age/TL, assumption of homogeneity of variance and normality of data was checked applying the common graphical methods (probability and residuals plots) and the Shapiro-Wilk test, respectively (Quinn and Keough 2002). As a result, all data were normally



distributed (OL, n = 46, W = 0.989, P > 0.1; TL, n = 46, W = 0.953, P > 0.05; OL, n = 46, W = 0.951, P > 0.05) and neither pattern in the residuals nor extreme values (outliers) was observed in both linear regressions.

The monthly distribution of hatching dates was backcalculated by subtracting age estimates (i.e. the mean number of microincrements) from the date of capture.

By means of the microincrement counting method, we assigned the age groups in years considering the real biological time elapsed from hatching to capture, using the nomenclature 0+ (<365 days) and 1+ (>365 days). The term "+" is useful to indicate the transition of months, meaningful especially for aged 0 fish (young of the year).

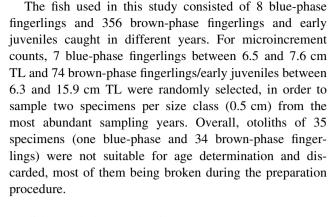
The age estimates obtained by microincrement counts were compared with those from the conventional method of annual ring (annuli) counting in scales and whole otoliths (see Barrera-Oro et al. 2010). The technique for preparing these structures, reading method and terminology is described in detail in Barrera-Oro and Casaux (1992), remarking that 1st of July is taken as the birth date of the species and the age group is assigned to an entire year as "0", "1", etc.

Results

Fish sample

The early stages of *N. rossii* were identified on the basis of their distinct body shape and colour. The blue-phase fingerlings (age group 0+) are more laterally compressed and silver-blue, whereas the brown-phase fingerlings and early juveniles (age groups 0+ and 1+) are more depressed and golden brownish (Fig. 2). They were all sexually immature, mostly at gonadal stage 1 of development.

Fig. 2 Young stages of Notothenia rossii caught at Potter Cove, South Shetland Islands: blue-phase fingerling, 6.4 cm TL (above); brownphase fingerling, 6.8 cm TL (below)



Otolith morphology and ratios

In *N. rossii* fingerlings, sagittal otoliths have an ovate shape, with straight ventral and rounded dorsal margins. With ontogeny, otoliths exhibit a more evident rostrum and the indented dorsal margin (Hecht 1987). The length (OL) of anterior–posterior growth axis ranged from 0.92 to 2.17 mm. The linear fit describing the relationship between fish length (TL, cm) and otolith length (OL, mm) explained 82 % of parameters variation (Fig. 3), as summarised in the following equation:

$$OL = 0.34 + 0.12 \text{ TL}$$
 $n = 46$ $r^2 = 0.82$

Under reflected light at low magnifications $(6-25\times)$ against a black background, the nucleus consisted of opaque material (light) surrounded by a thin hyaline (dark) edge. Based on the conventional annuli counting method, in fish of age group 0 (blue-phase fingerlings), the nucleus was followed by a continuous wide opaque band up to the otolith margin. In fish of age group 1 (brown-phase fingerlings), the first opaque band was followed by a thin





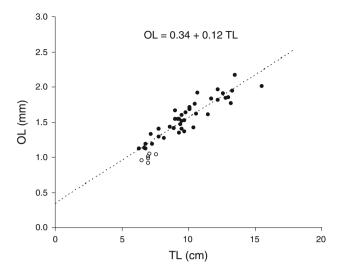


Fig. 3 Relationship between otolith size (OL) and fish size (TL) estimated for *Notothenia rossii* fingerlings, showing the linear fit to the experimental data. *Empty circle = blue phase*; *filled circle = brown phase*

hyaline ring thus constituting the first annulus and then by the onset of a second opaque band in the otolith margin (Table 2).

The otolith microstructure consisted of microincrements with alternating discontinuous (dark) and incremental (light) zones (Fig. 4). In the core area, the primordium was an optically dense structure composed of multiple nuclei. The primordium was generally encircled by two–three faint rings followed by more defined and evenly spaced microincrements. Off the core area, all otoliths showed an evident check located on average at about 13 microincrements (range 10–19, SD 2.76) of distance from the primordium (Fig. 4). Along the count path, microincrement width ranged approximately between 1 and 3.0 μ m, becoming progressively wider outside the check and narrower again at the otolith margin.

Age

Based on microincrement counts, six blue-phase fingerlings and 40 brown-phase fingerlings and early juveniles were successfully aged (Table 2). Age estimates ranged from 204 to 227 days and from 240 to 560 days for blue-phase and brown-phase fingerlings, respectively. Counting variability between readings was generally very low, with differences in less than 5 % of the relevant mean. Indices of ageing precision APE and mean CV were both very low too, respectively, 2.0 and 2.9 %, supporting the reliability of the ageing procedure and the consistency between readings.

Growth rate

The growth rate of *N. rossii* fingerlings was estimated by fitting a linear regression to the age-length data set (Fig. 5). Blue-phase and brown-phase fingerlings were pooled together due to the few individuals aged. The relationship between fish size (TL, cm) and age (days) was described by the following equation:

TL =
$$1.505 + 0.024$$
 age $n = 46$ $r^2 = 0.86$

The instantaneous growth rate was then 0.24 mm/day, at least in the size range investigated. A further estimate of growth rate has been calculated following the increase in the mean length of population over time recorded in summer (January–February 2009, summer hatchers) and spring (September–November 2010, winter hatchers) (Fig. 6). The slopes of the straight lines fitted to the data were 0.33 mm/day in summer and 0.23 mm/day in spring, being the last one very close to the value estimated by microincrement counts.

Duration of the pelagic blue-phase fingerling stage and timing of demersal settlement

In the present study, we were not able to clearly identify, on otolith sections, any settlement mark or secondary ring similar to that observed previously on scales (Shcherbich 1975; Barrera-Oro et al. 2010). Nevertheless, ageing data of blue-phase and brown-phase fingerlings of *N. rossii* may provide an indirect estimate of duration of the pelagic blue-phase fingerling stage and timing of demersal settlement. This period would be tentatively about eight months from hatching, considering that the maximum and minimum ages estimated for the blue-phase and brown-phase fingerlings were, respectively, of 227 and 240 days (Table 2, discussed below).

Hatching period

The hatch date distribution of *N. rossii*, back-calculated from the date of capture and pooled by year of sampling on a monthly basis, showed two main periods of larval hatching separated by 5–6 months, one in late summer between February and March and another in winter, mainly in July (Fig. 7; Table 2). The remaining specimens analysed hatched patchily in spring (7) and autumn (2).

In agreement with this finding, the age/length frequency distribution of all the fish sampled in spring 2010 showed the simultaneous presence of two different cohorts (or modes) belonging to age 0+ and 1+ that hatched predominantly on February–March and July, based on daily ring back-calculation (Fig. 8).



Table 2 Results of microincrement analysis/daily ring counting in otoliths of *Notothenia rossii* fingerlings from Potter Cove showing estimation of larval hatching dates and biological age, assignation of life cycle type and validation of annulus formation

assignation of the cycle type and varidation of annuals formation	וווכ כאכוכ	type and	, terrene																					
Capture date	TL (cm)	Annuli	Biological age	gical	Hatching date	Fingerling morph stage	Life cycle scheme	Mont	ths fro	om ha	tching	to ca	ıtch ac	cordi	ng to a	se es	Months from hatching to catch according to age estimate, with annuli period showed in italics	, with	annu	ıli peı	riod s	showe	in ba	italics
			days	years																				
16/02/2008	7	0	218	+0	13/07/2007	Blue phase	1	-	A	S	N O		ı.	ഥ										
25/02/2006	9.7	0	222	+0	18/07/2005	Blue phase	1	ſ	Ą	S	N 0	I L	ſ	Ħ										
18/02/2008	7	0	221	+0	12/07/2007	Blue phase		ſ	Ą	S	N 0	I I	ı .	Ħ										
18/02/2008	7	0	206	+0	27/07/2007	Blue phase	1	ſ	Ą	S	N 0	I L	ſ	Ħ										
04/03/2009	7.1	0	227	+0	20/07/2008	Blue phase	1	ſ	Ą	S	N 0	I L	ſ	Ħ	Σ									
20/02/2009	6.5	0	204	+0	31/07/2008	Blue phase	1	ſ	Ą	S	N 0	I L	ſ	Ħ										
22/12/2005	6.8	1	305	+0	20/02/2005	Brown phase	2	Г	M	A	M J	ſ	A	S	0	Z	О							
01/02/2008	9.4	1	326	+0	12/03/2007	Brown phase	2	\mathbf{Z}	Ą	M	l J	A	S	0	Z	О	ſ	Г						
29/12/2008	9.5	1	305	+0	28/02/2008	Brown phase	2	Г	M	A	M J	ſ	A	S	0	Z	О							
29/12/2008	6	1	303	+0	01/03/2008	Brown phase	2	Σ	Ą	M	l J	A	S	0	Z	О								
29/12/2008	9.5	1	314	+0	19/02/2008	Brown phase	2	ഥ	M	A	M J	ſ	A	S	0	Z	Ω							
05/01/2009	6	1	350	+0	21/01/2008	Brown phase	2	J	H	M	A	1 J	ſ	A	S	0	Z	О	_					
05/01/2009	13.5	2	489	+	04/09/2007	Brown phase	1	S	0	Z	D J	Щ	Σ.	A]	Σ	ſ	ſ	A	S	0	z	Q	_	
07/01/2009	10.1	1	413	+	21/11/2007	Brown phase	Atypical	z	D	J	F	1 A	M	<i>f</i>]	ſ	A	S	0	z	Ω	_			
06/02/2009	10.1	1	428	+	06/12/2007	Brown phase	Atypical	О	ı	F	M A	Σ	I J	ſ	A	S	0	z	О	ſ				
11/02/2009	13.2	2	502	+	28/09/2007	Brown phase	Atypical	S	0	z	D J	Щ	Σ.	[A	Σ	ſ	ſ	A	S	0	z	О	_	ъ
18/01/2010	9.8	1	245	+0	18/05/2009	Brown phase	Atypical	\mathbb{Z}	ſ	ſ	A S	0	Z	О	ſ									
12/02/2010	9.3	1	319	+0	30/03/2009	Brown phase	2	M	A	M	<i>l</i>	A	S	0	Z	О	ſ	Г						
19/02/2010	10.6	1	337	+0	19/03/2009	Brown phase	2	\mathbb{Z}	Ą	M	l J	A	S	0	Z	О	_	Г						
20/02/2010	10.1	1	379	+	06/02/2009	Brown phase	2	ഥ	M	A	M J	ſ	A	S	0	Z	О	ſ	ц					
20/02/2010	6.7	1	367	+	18/02/2009	Brown phase	2	ഥ	M	A	M J	ſ	A	S	0	Z	О	ſ	ц					
15/09/2010	12.2	2	457	+	15/06/2009	Brown phase	1	ſ	'n	δ 8	S O	Z	Q I	ſ	ц	Σ	Ą	Σ	ſ	ſ	А	S		
15/09/2010	12.2	2	439	+	03/07/2009	Brown phase	1	ſ	A	S	N 0	Q Z	<u> </u>	Г	Σ	A	Σ	ſ	ſ	А	S			
17/09/2010	10.7	1	370	+	12/09/2009	Brown phase	1	S	0	z	D J	Ц	Σ.	A]	Σ	r	^	Α	S					
24/09/2010	7.8	1	244	+0	23/01/2010	Brown phase	2	ſ	H	M	A M	1 J	ſ	A	S									
25/09/2010	7.2	1	255	+0	13/01/2010	Brown phase	2	ſ	ц	M	A	1 J	ſ	A	S									
20/10/2010	13.3	2	411	+	04/09/2009	Brown phase	1	S	0	z	D J	Щ	Σ.	l A	Σ	7	ſ	Α	S	0				
20/10/2010	11.5	1	373	+	12/10/2009	Brown phase	Atypical	0	z	D J	I F	Σ	1 A	M	7	7	Α	S	0					
09/11/2010	8.2	1	275	+0	07/02/2010	Brown phase	2	Г	Σ	A	M	,	Α	S	0	Z								
29/11/2010	6.7	1	405	+	23/10/2009	Brown phase	Atypical	0	z	D J	J F	Σ	1 A	Σ	'n	ſ	Α	S	0	z				
04/01/2006	13	2	525	+	28/07/2004	Brown phase	1	,	Ą	S	N 0	Q 7	<u> </u>	Ц	Σ	Ą	Σ	ſ	ſ	А	S	0	z	D J
08/01/2005	8.6	1	317	+0	26/02/2004	Brown phase	2	Ľ	Σ	A	M	ſ	A	S	0	Z	Ω	ſ						
24/01/2005	10.5	1	346	+0	13/02/2004	Brown phase	2	ഥ	\mathbf{Z}	A	N J	,	A	S	0	Z	Ω	_						



Capture date TL (cm) Annuli Biological	TL (cm)	Annuli	Biolog	gical	Hatching date	Fingerling morph stage	Life cycle Months from hatching to catch according to age estimate, with annuli period showed in italics scheme	Mont	hs fro	ım hatı	ching	to cal	ch ac	cordin	g to a	ge est	imate	, with	annı	uli pe	riod 8	showe	ui þa	italic	l re
			days	years																					
15/01/2006	15.5	2	999	1+	04/07/2004	Brown phase	1	J	A) S	0	Q I	ſ	F	M	A	M	J	ſ	A	S	0	N) J	
31/01/2006	10.4	1	335	+0	02/03/2005	Brown phase	2	\mathbb{Z}	A	M	,	, A	S	0	z	О	ī								
04/02/2008	9.2	1	315	+0	26/03/2007	Brown phase	2	Σ	A	M	ر ،	Α .	S	0	z	О	J	щ							
29/10/2010	8.9	1	264	+0	07/02/2010	Brown phase	2	щ	Σ	A N	M	ſ	A	S	0										
29/11/2010	7.3	1	283	+0	19/02/2010	Brown phase	2	ц	Σ	A	M	<i>f</i>	A	S	0	Z									
29/10/2010	6.7	1	240	+0	03/03/2010	Brown phase	2	Σ	A	N J	ر ،	Α .	S	0											
20/10/2010	6.5	1	255	+0	07/02/2010	Brown phase	2	щ	Σ	A	M	<i>f</i>	A	S	0										
20/10/2010	8.9	1	243	+0	19/02/2010	Brown phase	2	Н	Σ	A N	M	<i>f</i>	A	S	0										
15/09/2010	12.6	2	417	+	25/07/2009	Brown phase	1	_	A	S	(D	<u>-</u>	ц	Σ	A	Σ	ſ	ſ	A	S				
15/09/2010	8.9	1	274	+0	15/12/2009	Brown phase	Atypical	О	_	F	M	\ \ \	1	7	A	S									
19/10/2010	12.8	2	414	+	31/08/2009	Brown phase	1	A	S	0	ı z	J)	Ц	\boxtimes	A	\mathbf{Z}	ſ	ſ	А	S	0				
22/09/2010	6.3	1	280	+0	16/12/2009	Brown phase	Atypical	О	_	F	M	\ \ \	1	7	A	S									
28/12/2009	7.8	1	279	+0	24/03/2009	Brown phase	2	Σ	A	M	ر ،	Α .	S	0	z	D									

Based on the above information and on the age estimates (Table 2) and assuming pelagic phase duration of about 8 months, the events of the life cycle associated with the presence of the two main hatching periods can be summarised in the schemes shown in Fig. 9. The type of life cycle, 1 or 2, is assigned to most of the specimens included in Table 2, except for a few fish hatching in spring and middle autumn, defined arbitrarily as "atypical". The specimen that hatched at the end of autumn, the 15 of June 2009, was considered as belonging to the winter cohort (Table 2).

All the pelagic blue-phase fingerlings (age group 0+) correspond to the scheme 1, entering in Potter Cove in February–March (Table 2). The smaller brown-phase fingerlings measuring 6.3–8 cm TL (age group 0+) correspond to the scheme 2 and can be found in the cove already in spring, i.e. from September–October onwards (see in Table 2 the fish collected in spring 2010). The larger brown-phase fingerlings measuring 8–10.6 cm TL (mostly of age group 0+) correspond also to the scheme 2 and were collected in summer months, from December to February. The early juveniles measuring 10.7–15.5 cm (age group 1+) correspond to the scheme 1, hatching in winter and entering in the cove the following year to spend the second winter inshore.

Annulus formation

To validate the timing and position of the first annulus in sagittal otoliths of *N. rossii*, we drew the individual life cycle from hatching to date of capture based on microincrement counts (Table 2). Comparing the number of annuli (otoliths) or checks (scales) to the winter seasons experienced by each fish (as indicated in Table 2, excluding the winter hatch), they were almost all consistent with a single winter deposition. The discrepancy between the individuals aged 2 and the winter seasons experienced by each of them could be explained by linking the exceeding annulus or check to settlement, as previously reported (Barrera-Oro et al. 2010).

Discussion

Among notothenioids, the marbled rockcod *N. rossii* is by far the most affected species by anthropogenic factors, in particular the commercial fishery. As young specimens inhabit littoral waters, major changes in the offshore reproductive stock are reflected in the inshore juvenile populations through the recruitment processes. A sharp decline in abundance of juvenile *N. rossii* inshore at the South Shetland Islands was attributed to a reduction in



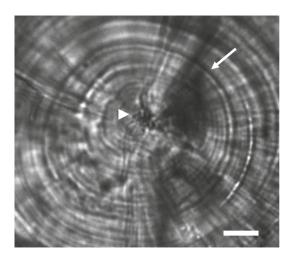


Fig. 4 Optical micrograph of sagittal otolith thin section of *Notothenia rossii* fingerlings, showing the core area with primordium containing multiple nuclei (arrow head) and the evident check (arrow), tentatively associated to the beginning of exogenous feeding. *Scale bar* 10 μ m

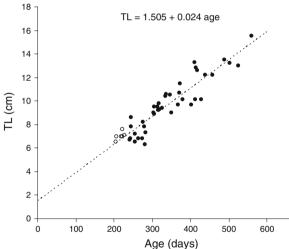
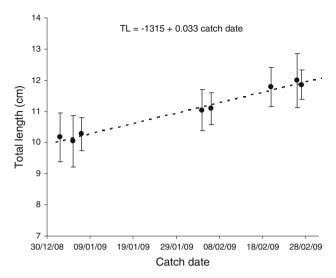


Fig. 5 Age-length relationship estimated for *Notothenia rossii* fingerlings collected in Potter Cove, showing the linear fit to the experimental data. *Empty circle = blue phase*; *filled circle = brown phase*

recruitment due to the decline of the offshore populations caused by industrial fishing in the area at the end of the 1970s (Barrera-Oro et al. 2000; Barrera-Oro and Marschoff 2007). Although some signs of recovery have been recently observed, the levels of abundance are far below those documented in the early 1980s (Marschoff et al. 2012). In addition, the detrimental effect of the climate change on Antarctic fish cannot be ignored since the Antarctic marine ecosystems are increasingly threatened by alterations of the abiotic and biotic environment (Mintenbeck et al. 2012 and references therein). Hence, the possibility that this



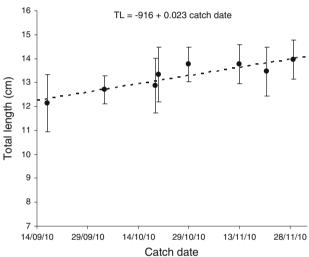


Fig. 6 Linear relationships fitted to mean length increase (\pm SD) in *Notothenia rossii* fingerlings collected on subsequent sampling dates in summer 2009 (N=67) and spring 2010 (N=39). The estimated growth rate (slope) was 0.33 mm/day in summer (*above*) and 0.23 mm/day in spring (*below*)

phenomenon is also partially responsible for a possible decrease in the survival of early stages and long recovery process of *N. rossii* populations in the area, should not be neglected. An extensive knowledge of the early events in the life cycle of this species is therefore essential for the appropriate monitoring of its recovery in the region, and the otolith microstructure analysis provided valuable information for this goal.

The timing of exogenous feeding observed in *N. rossii* (10–19 days) falls within the range reported for other nototheniids, being 9–10 days in *Dissostichus mawsoni* (La Mesa 2007), 18–24 days in *Lepidonotothen nudifrons* (previously referred to as *Nototheniops nudifrons*) (Hourigan and Radtke 1989) and 20 days in *Trematomus newnesi* (Radtke et al. 1989).



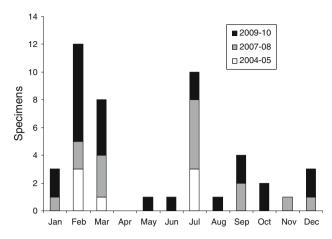


Fig. 7 Hatch date distribution of *Notothenia rossii* sampled at Potter Cove, estimated by otolith microincrement analysis

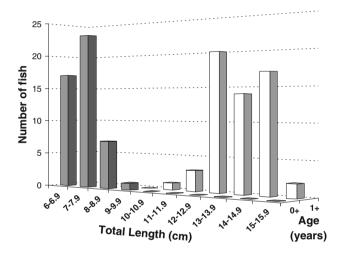


Fig. 8 Age/length frequency distribution of *Notothenia rossii* brownphase fingerlings and early juveniles caught in spring 2010 (September–November) with two main peaks, indicating the occurrence of two cohorts

The growth rate of *N. rossii* fingerlings from Potter Cove, estimated in this study by two different methodological approaches, was between 0.23 and 0.33 mm/day (Figs. 5 and 6). At South Georgia, *N. rossii* fingerlings hatch at 15.1 mm SL in September and attain 109 mm during the first year of life (Burchett 1984), which we estimated to be equivalent to a daily growth rate of 0.26 mm. Compared with other nototheniids within a similar size range, these growth rates are rather high. *Lepidonotothen larseni* (previously referred to as *Nototheniops larseni*) fingerlings, for example, grow at approximately 0.08 mm/day off South Georgia (Pakhomov and Pankratov 1994).

Taking into account the maximum age estimated for blue-phase fingerlings and the minimum age estimated for the brown-phase fingerlings, the duration of the pelagic phase and the timing of settlement in the demersal habitat would take place at about 8 months of life (namely between 227 and 240 days). Our estimation is in general agreement with data reported elsewhere (Duhamel 1982; Burchett 1983); nevertheless, its accuracy should be viewed with caution as fish were collected irregularly only from September to March, lacking data at Potter Cove between April and August.

Unfortunately, no data on age or size-selective mortality rates of *N. rossii* fingerlings are currently available. However, the cumulative mortality difference between youngest and oldest specimens should probably be reduced as fish were relatively old at the time of collection, providing a stable hatch date distribution of survivors without any correction for differential mortality rates (Campana and Jones 1992).

Although potentially biased for the population, the hatch date distributions for the survivors of the early mortality may be valuable in any case, as it often determines cohort strength.

The hatch date distribution of *N. rossii* (Fig. 7) and the significant difference in size between the fingerlings caught simultaneously in Potter Cove in spring 2010 (Fig. 8) would indicate the presence of two different cohorts: a summer cohort hatched mainly in February–March and a winter cohort hatched mainly in July (Table 2).

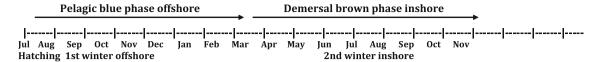
All the blue-phase fingerlings belonged to the winter cohort and to age group 0+ (about 7 months old fish), as evidenced by their hatching date in July estimated by back-counting of daily rings from the capture date. Accordingly, the conventional method of annual ring counting assigned 0 year to all of them, because the first annulus in otoliths and scales was still incomplete (Table 2). The brown-phase fingerlings aged 0+ belonged to the summer cohort, whereas most of those aged 1+ belonged to the winter cohort.

Based on the early life events associated with the two hatching periods in *N. rossii*, we hypothesise the existence of two main types of life cycles for South Shetland Islands fingerlings, which are illustrated in the schemes 1 and 2 and assigned to most of the specimens aged in Table 2. With respect to the schemes (Fig. 9), the following hypotheses can be formulated:

- The specimens of the summer cohort overwinter epipelagically before migrating inshore in September— October. This would be partially in agreement with the suggestion that the duration of the pelagic fingerling stage at sea may last up to 2 years (Shcherbich 1975; Freytag 1980).
- Alternatively, the pelagic blue-phase fingerlings of the summer cohort enter Potter Cove and settle to demersal



1) Winter cohort, hatched mainly in July:



2) Summer cohort, hatched mainly in February-March:

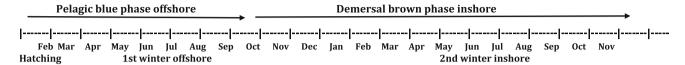


Fig. 9 Events of the life cycle associated with two main hatching periods in Notothenia rossii from the South Shetland Islands

life at smaller size than the winter cohort, already in July-August. Unfortunately, this hypothesis could not be tested because individuals of this stage were not caught due to the lack of sampling during winter months.

A remaining uncertainty is the approximate proportion of fish that do not belong either to the summer cohort or to the winter cohort and that hatches in other periods, e.g. the cases named arbitrarily as "atypical" (Table 2).

The individual life cycle derived from microincrement analysis in sagittal otoliths of *N. rossii* validated the timing and position of the first annulus (Table 2). The exceeding annulus or check observed in specimens aged 2 might be linked to the passage from pelagic to demersal habitat, as reported in the literature (Barrera-Oro et al. 2010).

As in other nototheniids, it has been assumed until now that this species spawns annually in late autumn/early winter and hatches in spring (Kock 1989). However, a proportion of post-spawners of both sexes (4 % in stage 5 of Kock and Kellermann's scale) was found in late summer north of Elephant Island and King George Island (Jones et al. 2003), suggesting an additional spawning period besides autumn. Taking into account the two hatching periods identified, two main spawning periods can be hypothesised. Based on the incubation period of eggs of *N. rossii* reported from Kerguelen (70–100 days, Camus and Duhamel 1985), spawning would occur in April–May (as commonly reported in the literature) and in November–December.

What could be the ecological advantage of the occurrence of two annual spawning periods in *N. rossii*? A possible interpretation would be the trophic benefit of having an "insurance" against larvae—plankton mismatch. And if so, should we expect such pattern in other species' stocks around Antarctica?

A fact is that in contrast with most notothenioids, to date only a few other species of nototheniids are reported to spawn twice a year. In particular, double annual spawning has been hypothesised in the sub-Antarctic *Patagonotothen tessellata* in the Beagle Channel (Rae and Calvo 1996), as well as in *Dissostichus eleginoides* at South Georgia and Shag Rocks (Agnew et al. 1999) and in *L. nudifrons* off the Antarctic Peninsula, although the latter was under laboratory conditions (Hourigan and Radtke 1989).

In summary, the technique of daily ring back-counting in sagittal otoliths of *N. rossii* from the capture date provided novel information on the hatching periods of the species in the South Shetland Islands and was useful to validate annulus formation. Nevertheless, the present study should be taken as preliminary, considering that more otolith microstructure analysis coupled with further research on the spawning stages of fish sampled offshore in early summer and on fingerling stages sampled inshore in winter months is needed to confirm our findings and to clarify other uncertainties relative to early life history timings of the species.

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