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ORIGINAL PAPER

Using scales to clarify the transition from blue-phase to brown-phase fingerling in *Notothenia rossii* from the South Shetland Islands

Esteban Barrera-Oro · Nadia Alescio · Eugenia Moreira · Enrique Marschoff

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Abstract Scales and whole otoliths were read for age determination in early stages of *Notothenia rossii* caught in Potter Cove, South Shetland Islands, in summer seasons 2003–2006 and 2008. The sample comprised blue-phase pelagic fingerlings of 7.0–7.6 cm (TL) of age group 0 year and demersal brown-phase fingerlings/juveniles of 8.5–20.9 cm and predominant age groups 1–2 years. Counting of sclerites facilitated the interpretation of the rings, particularly in the central scale. To clarify two previous issues of controversy, we deduce that the duration of the offshore pelagic blue-phase fingerling stage is less than one year before migration to the nearshore demersal habitat. Furthermore, the first well-defined ring in scales corresponded to the first annulus, while a contiguous ring was a secondary ring sometimes deposited after the first winter during the second year of life, attributable to a shift of habitat from pelagic to demersal. A von Bertalanffy growth curve was computed by combining age/length data of the juvenile phase of *N. rossii* from this and a previous study at Potter Cove with literature data from the offshore adult population, resulting in the following equation: $L_t = 86.9\left(1 - e^{-0.091(t-0.668)}\right)$.

E. Barrera-Oro (✉) · E. Marschoff
Instituto Antártico Argentino, Cerrito 1248,
1010AAZ Buenos Aires, Argentina
e-mail: ebarreraoro@dna.gov.ar

E. Barrera-Oro · N. Alescio · E. Moreira
CONICET, Buenos Aires, Argentina

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

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Introduction

The nototheniid *Notothenia rossii* (Richardson 1844) is a circum-Antarctic coastal fish widely distributed in waters of the Scotia Arc (South Georgia, South Sandwich, South Orkney and South Shetland Islands), around the Kerguelen, Crozet, Marion, Macquarie, Prínce Edward, Heard and Macdonald islands, Ob and Lena Banks (Gon and Heemstra 1990). It was the first Antarctic fish species depleted by the commercial fishery, which started activities around South Georgia in the late 1960s (Kock 1992). Since the establishment of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) in 1982, a series of conservation measures have been adopted in an attempt to promote recovery of this and other overexploited fish species in many Antarctic regions.

The life cycle of *N. rossii* has been well described for the population at South Georgia by Olsen (1954) and Burchett (1983a) and for the population at Kerguelen Islands by Duhamel (1982). *Notothenia rossii* hatches between September and November in offshore waters, where larval and young pelagic fingerling stages inhabit the upper layer zone. After about 5–6 months (according to Burchett 1983a, b, 1984), the pelagic blue-phase fingerlings enter the fjords, change morphologically and become demersal in habitat. In nearshore waters the species turns to the brown-phase fingerling and, after 15 months, it enters the juvenile phase of its life cycle. At about 5–7 years of age and 41–45 cm TL, *N. rossii* reaches sexual maturity and migrates offshore joining the adult population. It spawns between April and June on the bottom at 120–350 m depth and spends the mature phase in continental shelf areas. These offshore–inshore phases in the life cycle of *N. rossii* are assumed to be similar in other geographical areas (Kock et al. 1985), such as off South Shetlands (Barrera-Oro 2002).

Studies on age/growth of *N. rossii* have been conducted using scales and otoliths of specimens mainly from South Georgia (Olsen 1954; Crisp and Carrick 1975; Shcherbich 1975, 1976; Linkowski and Rembiszewski 1978; Shust and Pinskaya 1978; Freytag 1980a, b; Burchett 1983a, b, 1984; Kock 1986; North 1988; Radtke 1990) and to a lesser extent from Kerguelen (Hureau 1970; Tankevich 1990) and the South Shetland Islands (Freytag 1980a, b; Linkowski and Zukowski 1980; Kock 1988; Barrera-Oro and Casaux 1992). Information on age determination for the species has increased progressively, but the identification of the first annulus in scales and otoliths, and also of a secondary ring whose deposition has been linked to a change from pelagic to demersal-benthic habitat, remains unclear (Shcherbich 1975; Freytag 1980b). Misinterpretation of these two rings may lead to inaccuracy in age estimates.

Most of the von Bertalanffy growth curves of *N. rossii* were calculated for the population around South Georgia (Olsen 1954; Freytag 1980b; Burchett 1983b). The VB growth curve estimated from fish sampled off Elephant Island included a small number of juveniles combined with adult specimens (Freytag 1980b). However, it is known that fish from this area have slower growth than those from South Georgia (Kock et al. 1985): therefore, age and growth data from both areas are not comparable.

Although in the South Shetlands some studies dealt with juveniles caught nearshore, they were generally relatively large-sized fish (being more than 18 cm TL), so that age and growth rate of early stages of the juvenile phase are still unknown. Linkowski and Zukowski (1980) and Barrera-Oro and Casaux (1992) analysed the age of intermediate and late brown-phase juvenile *N. rossii* of the inshore King George/25 de Mayo Island population of ages 3–7 years, but there is still uncertainty on the occurrence and the age determination of the age groups 0–2. The study by Barrera-Oro and Casaux (1992) was carried out in Potter Cove, where extensive ichthyological research has been conducted using trammel nets with mesh sizes that allowed the capture of specimens usually over 15 cm TL (reviewed in Barrera-Oro and Casaux 2008). After 2003, the scope of this research was expanded using a trawl net of smaller mesh within the cove, able to sample small sizes of fish down to approximately 4 cm TL (Barrera-Oro and Winter 2008).

Based on specimens collected with this new sampling methodology, this paper reports on the occurrence and age determination of blue-phase and brown-phase fingerlings/juveniles of *N. rossii* in Potter Cove and completes the information on intermediate and late juvenile stages from the same site given in Barrera-Oro and Casaux (1992). An attempt to clarify the controversy over the location of the first annulus and the secondary ring possibly linked to settlement is also provided. Likewise, a von Bertalanffy growth curve was obtained including age/length data estimated from the juvenile phase of *N. rossii* at Potter Cove with literature data from the offshore adult population (Freytag 1980b).

Materials and methods

A total of 243 *N. rossii* were collected at Potter Cove, King George/25 de Mayo Island, close to the scientific station Jubany (62°14′S and 58°40′W) in the summer seasons 2003–2006 and 2008 (Fig. 1). Detailed information of the biotic and abiotic features of this site is given in Casaux et al. (1990). Two different gear were used from rubber boats at different sites in the cove, preferably where the seabed was a uniform rocky bottom covered mainly with red and brown macroalgae (Fig. 1b). A bottom trawl net (mouth 1 m^2, length 2 m and mesh 4 mm) was towed at daylight for 15–30 min at depths of 6–30 m (average, 12 m). Trammel nets (length 15 m, height 1.5 m, inner mesh 2.5 cm, outer mesh 12 cm) were fixed to rocks and set on the bottom at depths of 4–43 m (average, 14 m) for 16–24 h. In order to obtain data complementary to a previous study at the same site (Barrera-Oro and Casaux 1992), only fish <21 cm TL were included in this study. Further data on sampling stations and fish examined are presented in Table 1.

Soon after capture, scales below and above the lateral line in the pectoral fin area and sagittal otoliths were extracted, cleaned and stored until processing. Each fish was measured as total length (TL) and standard length (SL) to the nearest 0.1 cm below, weighed (g) and sexed. As all the specimens were sexually immature (Kock and Kellermann 1991), data from both sexes were pooled.

Age determination was based on scales and whole otolith readings. The procedure of cleaning and preparing scales and otoliths, as well as the optical equipment, reading method and terminology used for ageing is described in Barrera-Oro and Casaux (1992). Unlike the previous study, otoliths of early stages of *N. rossii* were not sectioned, being too small and fragile. Overall, scales and otoliths removed from 8 and 25 individuals, respectively, were considered not suitable for ageing and therefore they were discarded (Table 1).

A growth curve was computed by combining size at age data of juvenile inshore fish obtained in this (0–3 years) and in a previous study (3–6 years, Barrera-Oro and Casaux 1992) at Potter Cove.

Because Freytag's (1980b) age determinations of the offshore population are not consistent with the method used here (see "Discussion"), it was not feasible to

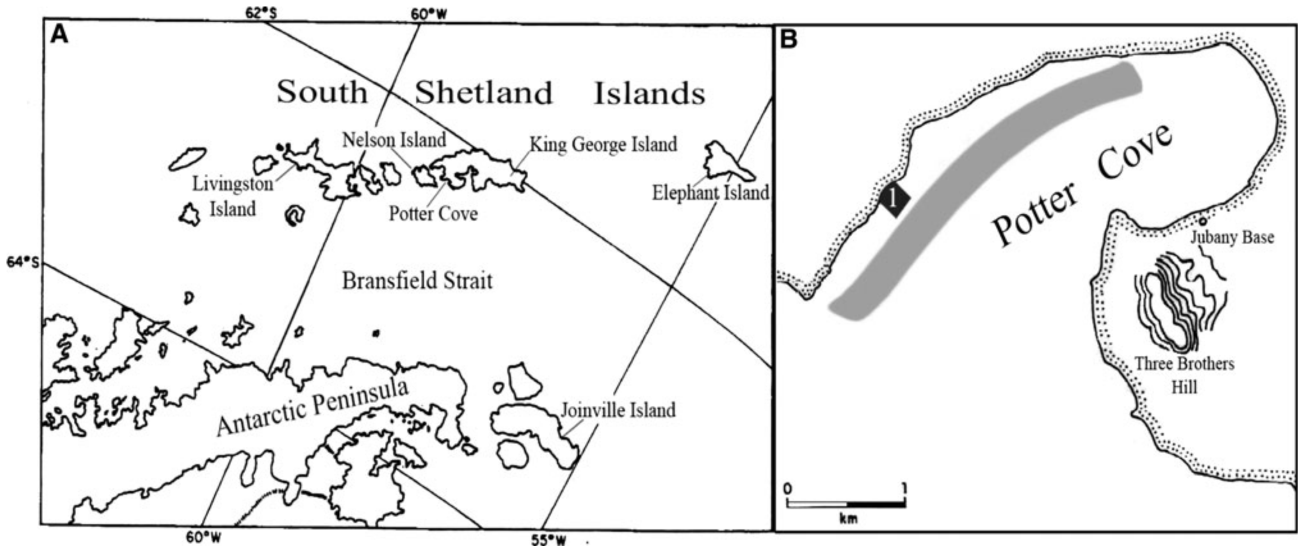

Fig. 1 **a** The South Shetland Islands. **b** Enlargement of Potter Cove, showing the sampling areas for *Notothenia rossii* with pelagic net (shadow) and trammel nets (1)

Table 1 Details of sampling stations and fish examined

Date	No. of fish caught		Length range TL (cm)	No. of fish aged	
	Trawl net	Trammel net		Scales	Otoliths
February 2003	3	–	11.2–11.6	3	2
December 2004–January 2005	10	–	9.0–13.3	10	6
December 2005–March 2006	89	56	7.6–20.9	143	134
Jan-Feb 2008	67	18	7.0–19.2	79	76
Total	169	74	7.0–20.9	235	218

combine both data sets. On the other hand, because our sampling was limited in season and in size, it is likely that only the youngest individuals of 0-age-class are represented in our data set. Therefore, we fitted the von Bertalanffy equation setting the L_∞ to 86.9 cm as estimated by Freytag (1980b) and minimizing the sum of squares of differences between observed and estimated values at Potter Cove. The L_∞ from Freytag's data provides a better estimate of L_∞ than that can be obtained from the younger stages. This is because L_∞ is better estimated from the older ages, where the effect of the added year becomes relatively small.

Results

Fish sample

The fish sample was composed of five pelagic blue-phase fingerlings (7.0–7.6 cm) and 238 demersal brown-phase fingerlings/juveniles (8.5–20.9 cm), the latter with well defined colour and meristic characters. The smaller fish caught by trawl and trammel nets were 7.0 and 15.5 cm TL, respectively. The length frequency distribution of the fish aged ($N = 236$) was bimodal, with peaks at 9.0–10.9 and 16.0–17.9 cm (Fig. 2). The fish weighed 3–112 g. In 11.5% of the specimens the sex could not be determined with naked eye. In the remaining fish, the ratio between males and females was 1.29**:**1. They were all sexually immature, at stages I and II of development.

Age interpretation

Scales

We counted the winter rings (checks) along the axis from the focus to the anterior margin (Fig. 3). A check is defined as a zone of narrowly spaced sclerites that corresponds to winter growth. The zone of widely spaced sclerites corresponds to summer growth. To confirm the position of the

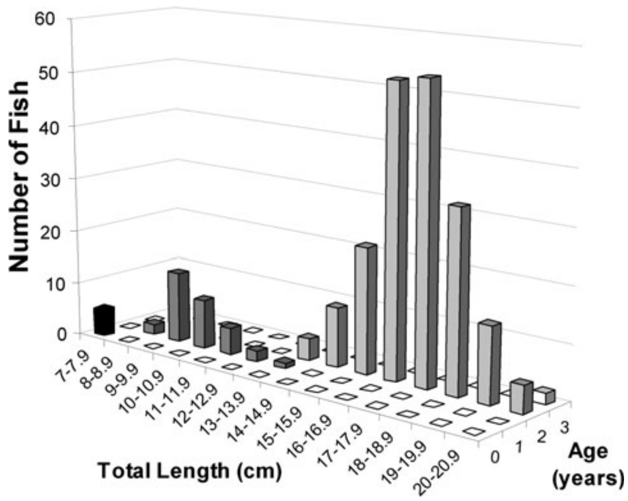

Fig. 2 Length–frequency distribution of early juvenile *Notothenia rossii* caught at Potter Cove

checks, the number of sclerites was counted in scales of all specimens (Table 2). When the sclerite follows a continuous line around the focus, the term “circuli” can be used as synonymous. Different scales from a single fish showed similar sclerite patterns. The five blue-phase fingerlings had scales with 10–12 circuli, without any checks, indicating that they belonged to age group 0 (Fig. 3a).

The procedure of counting sclerites facilitated interpretation of the scale area adjacent to the first check, as it was often not easily distinguishable. The first check appeared at a mean of about ten sclerites from the focus (the lower mean for age group 3 can be considered not representative because $N = 2$) (Table 2). The area contiguous to the beginning of the first winter ring showed the following patterns:

1. 1–3 narrow (winter) spaced sclerites followed by a widely spaced sclerites zone (summer) up to a distant second check (36% of the specimens) (Fig. 3b).
2. A not clearly discernible band of 5–9 narrow-intermediate spaced sclerites followed by a summer sclerite zone up to the second check (35% of the specimens) (Fig. 3c).
3. 2–3 winter sclerites followed by 2–3 more spaced sclerites and then by 1–2 narrow spaced sclerites. These last narrow-spaced circuli constituted a rather well-defined ring which was separated from the beginning of the first check by 7–9 sclerites. The formation of this secondary ring, in the present study called “X”, is presumably linked to a change in fish habitat (discussed below). Ring X was followed by the summer sclerite zone up to the second check (29% of the specimens) (Fig. 3d).

The second check or second winter ring was always formed by 2–3 narrow-spaced sclerites, although its distance (i.e. number of sclerites) from the focus varied significantly (Table 2).

The proportion of fish with a discernible ring X on scales is summarized in Table 3. Fish of age group 0 (i.e. blue phase fingerlings) are excluded from the analysis because ring X was deposited after the first annulus. Of the

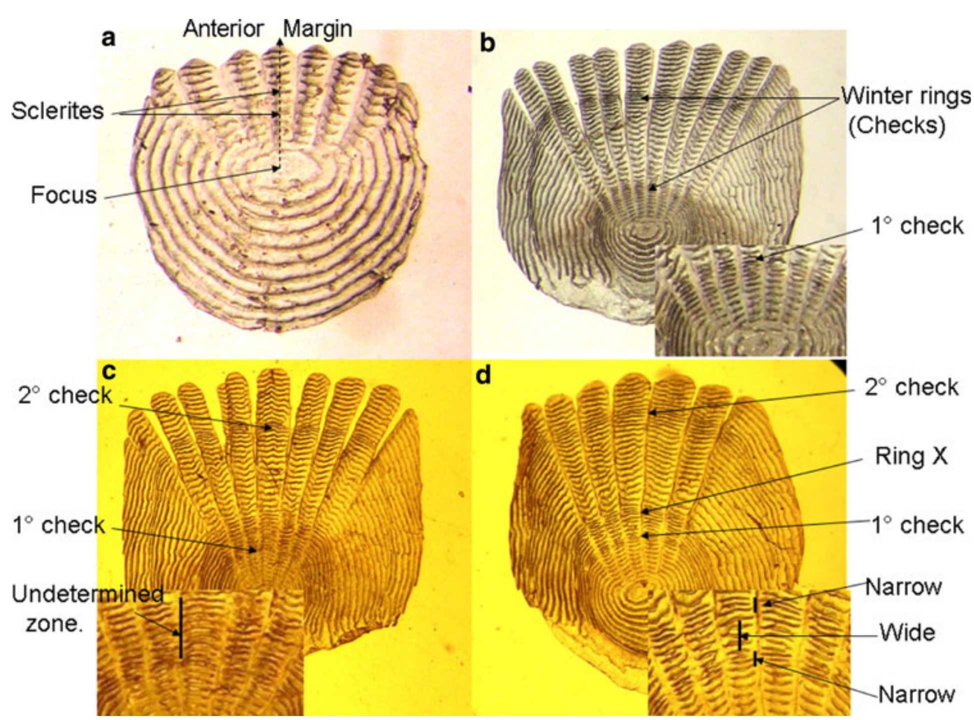

Fig. 3 Scales of early juvenile *Notothenia rossii*: blue-phase fingerling, age group 0, 7.5 cm TL (**a**); juvenile brown phase, age group 2, 17.1 cm TL, pattern 1 (**b**), 19.5 cm TL, pattern 2 (**c**), 17.5 cm TL, pattern 3 (**d**)

Table 2 Number of sclerites counted to the start of winter rings (checks) and secondary ring X and to the edge in scales of early juvenile *Notothenia rossii*

Age (years)	0			1			2			3		
	Range	Mean	*N*	Range	Mean	*N*	Range	Mean	*N*	Range	Mean	*N*
Sclerites												
1st check	–	–	0	7–11	9.9	31	7–12	9.7	172	8–9	8.5	2
Ring X	–	–	0	17–18	17.7	4	15–20	17.2	55	–	–	0
2nd check	–	–	0	–	–	0	24–40	32.4	171	27–29	28.0	2
3rd check	–	–	0	–	–	0	–	–	0	43–46	44.5	2
To edge	10–12	10.8	5	16–28	21.8	32	22–52	45.2	172	50–51	50.5	2

Table 3 Proportion of fish with a clearly discernible ring X in scales

Age (years)	No. of fish examined	TL (cm)	Identification of ring "X"	
			N	%
0	5	7.0–7.6	0	0
1	30	8.5–13.3	4	13.3
2	195	13.0–20.5	55	28.2
3	2	20.3–20.9	0	0

32 specimens of age group 1 available, only those that were sufficiently old for the deposition of ring X in their scales were considered in the calculation. Accordingly, the analysis of this age group was conducted on 30 fish with a total number of sclerites $\geq$18 in their scales, which is the mean value for ring X deposition (Table 2).

Conversely, two of the smaller brown-phase fingerlings (TL = 8.9 and 9.0 cm) of age group 1 presumably did not reach the chronological time for the deposition of ring X in their scales, showing a total number of 16 sclerites to the edge. These fish were caught in late December 2005, and they exhibited scales with only one well-defined ring. This fact is particularly important when discussing which of the two rings, first annulus or X, is deposited first in the life cycle of the species.

The identification of ring X increased from 13 to 28% in scales of age groups 1 and 2, respectively. The small number of specimens of age group 3 prevented any additional analysis.

Otoliths

On the distal side of the otolith, we counted the annuli in the dorsal area. On the medial side, we used the posterior and dorsal areas. Under reflected light, the nucleus was opaque, with a thin hyaline border. One opaque and one hyaline ring were taken as representing an annual ring (annulus).

In general, age readings from otoliths were not as comprehensible as those from scales. This is because the area surrounding the nucleus was sometimes dark/translucent, and the annuli were not clearly discernible. In this case the age was not determined, so that 25 otoliths were excluded from the further analysis (Table 1).

Age/length observed values

The comparison between age estimates obtained from scale and otolith readings gave a full agreement in 99.5% of fish aged. Likewise, the agreement between different repeated readings from either scales or otoliths was over 90%.

Length at age values is summarized in Table 4. Mean values obtained from the analysis of scales and otoliths were comparable each other. Fish sample consisted of 0–3 year-old-individuals, although most of them were aged 1 or 2 years. The 3-year-old-fish (two individuals) were aged only by scale reading, as they were released at sea after removing scales.

Since our sampling was biased by size and season selection it is likely that only the earliest hatching, fastest growing and more precocious members of 0 age-class were caught. Therefore, our estimate of *N. rossii* early growth rate (age 0) may be an overestimate.

Age validation

The identification of peaks in length frequency analyses (Petersen method) is one of the traditional age validation methods. In the length frequency distribution according to age, the first two peaks are normally distributed and agree with the mean length values of age groups 1 and 2, respectively (Fig. 2; Table 4). Data for age groups 0 and 3 are not included in the analysis, as they were based on few specimens.

Growth curve

The combined age/length data set obtained in this and in the previous study (Barrera-Oro and Casaux 1992) at Potter Cove is presented in Table 4. The length interval between

Table 4 Age-length observed values of early juvenile (this study) and intermediate-late juvenile (Barrera-Oro and Casaux 1992) *Notothenia rossii* at Potter Cove

Age (years)	Barrera-Oro and Casaux (1992)						This study					
	Scales			Otoliths			Scales			Otoliths		
	N	TL (cm)	Mean	*N*	TL (cm)	Mean	*N*	TL (cm)	Mean	*N*	TL (cm)	Mean
0	–	–	–	–	–	–	5	7.0–7.6	7.2	4	7.0–7.6	7.1
1	–	–	–	–	–	–	33	8.5–13.3	10.2	26	8.9–12.7	10.1
2	–	–	–	–	–	–	195	13.0–20.5	17.0	188	13.0–20.5	17.0
3	108	18.0–31.0	24.5	95	20.9–31.0	24.6	2	20.3–20.9	20.6	–	–	–
4	77	24.3–35.1	30.1	78	24.3–35.1	30.4	–	–	–	–	–	–
5	54	29.0–40.0	35.4	44	29.0–38.1	35.0	–	–	–	–	–	–
6	45	33.0–44.3	38.8	45	33.0–42.2	39.3	–	–	–	–	–	–
7	2	42.0–43.0	42.5	3	42.0–44.3	43.1	–	–	–	–	–	–

the means of age groups 1 and 2 fits well in the decreasing series of length intervals between age groups 3–7.

The growth parameters were obtained by fitting the von Bertalanffy growth equation to the mean sizes at age estimated from Potter Cove, constraining L_∞ to the value obtained from offshore data, namely 86.9 cm (Freytag 1980b) (Fig. 4).

Age group 7 was not included in the estimate of growth parameters because at this age only the smallest individuals remain into the cove.

Discussion

It is known that the inshore phase of the life cycle of *N. rossii* includes only immature specimens (Olsen 1954; Duhamel 1982; Burchett 1983a). The size, age, weight and gonad maturity of the specimens caught in Potter Cove indicate that they were all juveniles, including blue-phase and brown-phase fingerlings, as well as early juveniles. Size and age ranges are complementary to those of the intermediate and advanced juvenile fish (22.3–44.0 cm TL, 3–7 years) caught exclusively with trammel nets in the same site (Casaux et al. 1990).

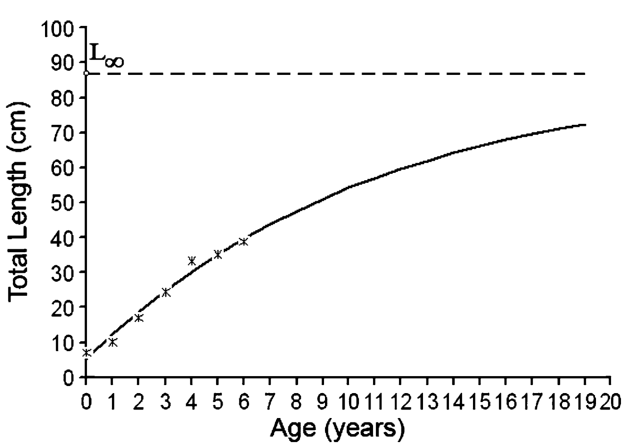

Fig. 4 Von Bertalanffy growth curve for *Notothenia rossii* in the South Shetlands

Ageing of early juvenile *N. rossii* can be interpreted clearly from scales. Unlike the report in Barrera-Oro and Casaux (1992) on the feasibility of age estimation in age groups 3–7 from whole otoliths, in this study we found that otoliths were not as useful as scales. This was particularly true for age group 1 specimens, where a dark/translucent unclear zone surrounding the nucleus was regularly observed. Therefore, we used selected otoliths mainly to confirm the age determination from scales. The good agreement between age readings from these structures confirms reports on the formation of scales in early stages of *N. rossii* (Shcherbich 1975; Freytag 1980b; Burchett 1984).

The duration of the pelagic fingerling stage at sea has been long controversial, lasting from 5–6 months (Burchett 1983b) to 2 years (Shcherbich 1975; Freytag 1980b). In our study, the five blue-phase fingerlings were caught in the nearshore waters of Potter Cove in middle and late February and had the typical morphological characteristics of the pelagic phase, including silverly colour and forked caudal fin. As reported by Freytag (1980b) for South Georgia fingerlings, the border of the fins was yellowish, showing signals of the beginning of the process of migration to the bottom. In agreement with the description of Burchett (1984) for fingerlings arriving nearshore in late March at South Georgia, blue-phase fingerlings of 7.0–7.6 cm TL found in Potter Cove had scales with a mean of 10.8 circuli at the edge, in concomitance with the starting of formation of the first check (Table 2; Fig. 3a). Likewise, the examination of the corresponding otoliths in both studies did not show the presence of a complete annulus.

Burchett (1984) reported growth in length and scale size (as number of circuli) of fingerlings sampled offshore and nearshore from summer to winter in South Georgia: young fingerlings in February, 38.5 mm, 0–1 circulus; blue-phase fingerlings in March, 61 mm, 7–11 circuli; demersal brown-phase fingerlings in June, length not provided, 12–19 circuli. Larval hatching took place in September at 15.1 mm and fingerlings grew up to 10.9 cm in first year of life, indicating that the pelagic blue-phase fingerlings grow to a length of about 6–7 cm during 6–7 months before entering the fjords (Burchett 1983b). Size and the elapsed time from hatching of fingerlings caught in Potter Cove are in agreement with aforementioned results from South Georgia. Therefore, on the basis of our material, we agree with Burchett (1984) that there is no evidence to support the hypothesis that some fingerlings may overwinter epi-pelagically at sea before migrating inshore.

According to Burchett (1983b), demersal brown-phase fingerlings appear inshore South Georgia from about April on, but we could not make an analogous observation at Potter Cove because our sampling was conducted only during summer months.

The analysis of the proportion of scales where ring X was clearly identified (Table 3) supported the assumption that the first ring corresponded to the first annulus. The following considerations can be made:

– While the first defined ring was identified in all individuals (except in the blue phase fingerlings) in the same location (Table 2), the presence of a second, less pronounced contiguous ring (X) was clearly identified in only scales of 13 and 28% of age groups 1 and 2, respectively.
– All age group 1 specimens were already in the brown-phase stage, i.e. transitioned to demersal life. It is impossible that these brown-phase specimens, which were caught in December–February, moved from the pelagic to the demersal phase and grew to 8.5–13.3 cm TL in only a few months from hatching in spring. Hence, they likely overwintered in the inshore waters of the cove, with the evidence that in most of them (87%) the single defined ring identified in their scales and the preceding summer band is the first annulus.
– Two small brown-phase specimens, also of this cohort (TL = 8.9 and 9.0 cm), which were too young for the deposition of ring X in their scales, had scales with only one well-defined ring, which corresponded unequivocally to the first annulus.

Our interpretation of the sclerite sequence from the first winter ring to ring X (structure 3) is as follows: after the conventional winter ring pattern, the 2–3 widely spaced sclerites corresponded to a short period of growth during spring-summer interrupted by migration to the bottom. The deposition of the contiguous 1–2 narrowly spaced sclerites (ring X) are explained by the morphological and ecological changes experienced during change of habitat (Fig. 3d). Although in structure (2) the sclerite pattern could not be accurately distinguished (Fig. 3c), it was likely that ring X was masked in it. In such a case, the proportion of fish with scales where the secondary ring X is present is higher, in this work 64% [35% of structure (2) + 29% of structure (3)]. The reason for the absence of ring X in structure (1) is unknown. It is possible that in some fish (36%) the change from pelagic to demersal habitat does not necessarily result in a deposition of a narrowly spaced sclerite band.

Ageing criteria starting from fingerling stages adopted in scale reading were reported by Shcerbich (1975) and Freytag (1980b). Among Freytag's results for *N. rossii* caught along the Scotia Arc islands, those for fish sampled off Elephant Island (age groups 5–12 years), in the South Shetland Islands, are comparable to our study. Freytag (1980b, Fig. 8) described two main sclerites patterns, namely (a) the first two rings wide apart (18% of individuals) and (b) the first two rings very close together and difficult to distinguish (60% of individuals), concluding that the interpretation of the first annulus was still an open question. The pattern (a) is identical to our structure (1), where the first two rings corresponded unequivocally to the first two annuli. Conversely, the pattern (b) is a combination of our structures (2) and (3), which together constituted 64% of cases. In conclusion, the differences between the results of both studies are (1) Freytag did not identify ring X as included in structure (b); (2) for age determination, she considered the two closely located rings in structure (b) as two separate annuli.

We are in partial agreement with the general observation of Shcherbich (1975) that the first annulus appears during the transition from pelagic to demersal life, with our original observation that the deposition of the secondary ring X occurs in the second year, after the first winter when the fish have already spent some time in the demersal stratum during the juvenile brown phase.

Based on the differences between Freytag's (1980b) and our results, in the first study the age determinations in 60% of the specimens might include fish 1 year younger in every age class, producing a shift on the age axis which becomes proportionally less important in older specimens. Since these older specimens have the greatest influence in the estimation of the L_∞ parameter, setting Freytag's value of L_∞ to the older specimens is expected to produce the best available estimate of von Bertalanffy's parameters. However, many caveats should be taken into account: L_∞ was obtained some 30 years before the actual data, the k parameter might overestimate the actual value since only young specimens are considered and sex was not taken into account neither in Freytag's (1980b) nor in our fit.

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