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Egg-shell morphology of the Antarctic fish, *Notothenia rossii* Richardson, and the distribution and abundance of pelagic eggs at South Georgia

MARTIN G. WHITE, RICHARD R. VEIT[1], ANTHONY W. NORTH and KEN ROBINSON

British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK
[1]Department of Zoology NJ-15, University of Washington, Seattle, WA 98195, USA

Abstract: During winter research cruises to South Georgia (1983 and 1993) fish eggs were abundant in the neuston. Examination of the external sculpturing and the micropyle morphology of the eggs collected during 1993 using scanning electron microscopy indicated that these were fertilized ova of *Notothenia rossii marmorata*. A comparison between the eggs of *N. rossii* from Iles Kerguelen and South Georgia showed these to have a similar surface morphology but a difference in the structure of the micropyle. In 1993, the abundance of fish eggs in the neuston varied from 0–116 eggs m^{-3} with an average of 4.5 eggs m^{-3}. During 1983, eggs were most abundant in the neuston but at lower average levels (0.04 eggs m^{-3}, range 0–1.17 eggs m^{-3}) and these were distributed from the surface to >380 m.

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Introduction

Many Antarctic species of fish spawn during the autumn–winter seasons, when sea-ice and poor weather conditions restrict access to the region. Fish eggs were among routine samples of zooplankton collected during the British Antarctic Survey (BAS) Offshore Biological Programme (OBP) Cruise to South Georgia during the winter of 1983 (White & North 1984, North 1988). However, these fish eggs were unable to be identified at the time. Subsequently a winter cruise to South Georgia was undertaken by the RV *Nathaniel B. Palmer* at South Georgia during June 1993 and fish eggs were also collected in surface zooplankton samples. These samples are described here and compared with earlier data from South Georgia and Iles Kerguelen.

The ova of teleosts are protected by a proteinaceous covering referred to as the zona radiata or cortex radiatus (Götting 1967). This covering has a surface morphology that is different among species and may be used as a means of identification (Guraya 1986, Lönning & Hagström 1975, Riehl 1980, Riehl & Schulte 1977). Riehl & Kock (1989) and Riehl & Ekau (1990) first demonstrated the possibility of using these structures to identify the ova of notothenioids collected from the eastern Weddell Sea, South Shetland Islands and the Iles Kerguelen. They described the surface sculpturing and micropyle structures of ova from seven species: *Notothenia rossii* Richardson, *N. neglecta* Nybelin (synonymized with *Notothenia coriiceps* Richardson by DeWitt *et al.* (1990)), *Lepidonotothen nudifrons* (Lönnberg), *L. larseni* (Lönnberg), *Trematomus eulepidotus* Regan, *Champsocephalus gunnari* Lönnberg and *Chaenocephalus aceratus* (Lönnberg). Ova from all of these had a species-specific morphology.

Material and methods

In June 1993, neuston samples were collected at 13 stations distributed among three N–S transect lines off the eastern end of South Georgia (Fig. 1). At each station a 1 m ring-net (505 mm mesh) was towed at the surface for 15 min *c.*1 knot. All three transects were sampled during 2–4 June, and the westernmost two were sampled again on 11–12 June and 19–20 June. Fish eggs were abundant in near-surface samples and a representative collection was made on 2 June 1993. The eggs were fixed in ethanol shortly after capture. In the laboratory the whole sample of eggs was examined and their diameter measured to the nearest 0.01 mm. A small sample of eggs were then prepared for viewing by scanning

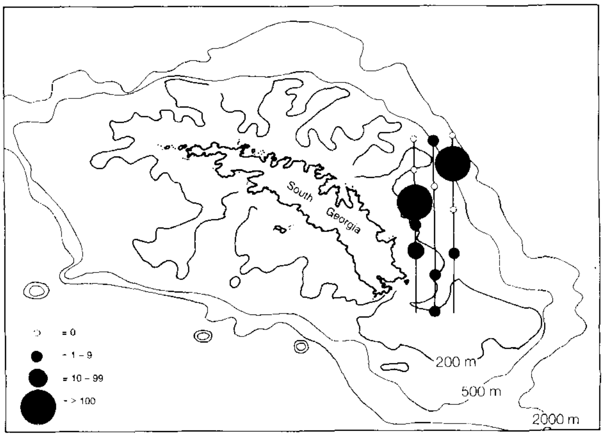

Fig. 1. Stations at which neuston samples were collected during winter cruise by RV *Nathaniel B. Palmer* to South Georgia in June 1993.

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electron microscopy (SEM). The surface of the eggs was examined to determine the location of the micropyle then the outer 'egg-shell' was separated from underlying structures and divided into approximate quarters by dissection with a micro-scalpel and fine needles. The egg-shells were then washed in distilled water and dehydrated in a series of water/acetone mixtures and then critical-point dried using liquid carbon dioxide. The resultant fragments were attached to SEM stubs and sputter coated with gold using a Biorad SC502 sputter coating unit — for examination using a Leica Cambridge 360 SEM. Subsequently focused beam milling was carried out using a FEI Europe instrument (FIB 200) (Young *et al.* 1993) to cut a vertical section through the egg-shell at the micropyle to confirm its structure.

At sea in 1993, eggs were measured as the displacement volume of eggs per sample. For the purposes of estimating the egg abundance, the number of eggs per sample was calculated by dividing the volume of eggs in each sample by the volume of the average egg (4.79 mm diameter). The number of eggs per unit volume in the neuston was calculated by multiplying the number of eggs per haul by the 1 m ring-net swept volume.

Samples of eggs were also collected during 1983 as part of the BAS Offshore Biological Programme at South Georgia (cf Bonner *et al.* 1978 for a description of this programme). The number and size of eggs were recorded from oblique hauls using an open/closing $8m^2$ rectangular midwater trawl (RMT8) and horizontal tows using a $1m^2$ frame net trawled at the surface (FNET). Samples were collected at stations arranged on a regular 30 x 30 n miles grid around South Georgia and at greater resolution in four selected study areas: A [Cumberland East Bay], B [eastern shelf], C [outer shelf] and D [inner shelf] (Fig. 2).

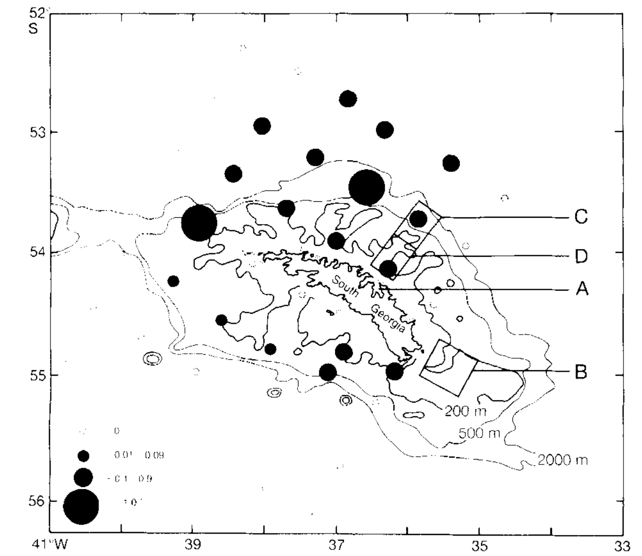

Fig. 2. Stations at which zooplankton samples were collected from RRS *John Biscoe* during July–September 1983. Circles = 30x30 n miles grid stations, A = Cumberland East Bay, B = eastern shelf, C = outer northern shelf, D = inner northern shelf.

Results

Distribution and abundance

The location of stations where the neuston was sampled and the abundance values for the first sample period in 1993 is given in Fig. 1. Mean egg diameter at South Georgia was 4.79 ± 0.17 mm (n = 50; range 4.41–5.12 mm). Most eggs were fertilized and were at an early stage of development. The distribution and abundance of the fish eggs showed no clear pattern temporally or spatially, other than a trend for a higher proportion of neritic stations to have positive results. The abundance of pelagic eggs collected during the three sample periods is given in Table I as the original values in displacement volume and these values transformed to number of eggs per unit volume sampled. The average abundance of *N. rossii* eggs within the neuston on the eastern continental-shelf at South Georgia during June 1993 was 4.7 eggs m^{-3} (range 0–116 eggs m^{-3}). High abundance levels occurred both over on the shelf and beyond the shelf-break. The highest single catch (2440 ml) and highest average catches (293.3 ml) occurred during the middle period (11–12 June) — and these were approximately one order of magnitude higher than the samples taken during the first and last transect series (Table I).

Samples of eggs collected in 1983 were not able to be identified at that time although the majority were recorded to be of the 'Notothenia rossii/coriiceps' type. The fish eggs comprized two size modes; the majority were 4.0–5.1 mm diameter while a small number were 2.5–2.7 mm across. The smaller size group were few in number, did not have the characteristics of the 'N. rossii/coriiceps' type and so were excluded from subsequent analyses. Eggs occurred in the water column from the surface to >380 m depth. Combining all nets the relative abundance was 0.01 eggs m^{-3} in the water-

Table I. Abundance of pelagic *Notothenia rossii* eggs collected in the neuston at South Georgia, June 1993. (Volume of eggs at each station [ml] and abundance standardized as eggs m^{-3}).

Date	2–4 June	11–12 June	19–20 June	Total
ΣNets*	18(7)	10(9)	10(9)	38(25)
Eggs (ml)				
Mean	23.3	293.3	42.7	99.5
SE	16.1	241.3	25.6	16.1
Range	0–2440	0–260	0–2440	0–280
Eggs m^{-3}				
Mean.	1.1	13.9	2.0	4.7
SE	0.8	11.5	1.2	3.1
Range	0–13	0–116	0–12	0–116

* numbers of stations in parenthesis is number at which fish eggs were collected.

Table II. Abundance of *Notothenia* spp. eggs (4.0–5.1 mm) collected at South Georgia during July–September 1983 in RMT8 and Foredeck net samples.

	Total	Grid	A	B	C	D
RMT samples						
Σ Nets*	220(154)	86(28)	65(63)	30(24)	13(13)	26(26)
Eggs/net	17.07	2.5	21.9	29.9	12.7	39.3
Eggs m^{-3}	0.001	0.0001	0.001	0.001	0.0006	0.002
SE	0.203	0.04	0.37	0.52	0.3	0.66
Range	0–0.028	0–0.002	0–0.019	0–0.009	0–0.004	0–0.013
FNET samples						
Σ Nets*	51(41)	2(2)	22(17)	8(6)	7(5)	12(11)
Eggs/net	674	925	34.4	28.0	8.7	45.3
Eggs m^{-3}	0.043	0.599	0.022	0.017	0.005	0.029
SE	0.022	0.571	0.007	0.007	0.002	0.007
Range	0–1.17	0.03–1.17	0–0.136	0–0.065	0–0.020	0–0.066
Combined nets						
Σ Nets*	271(196)	88(30)	87(80)	38(30)	20(18)	38(37)
Eggs/net	26.34	23.7	25.03	29.55	11.30	41.21
Eggs m^{-3}	0.009	0.014	0.006	0.005	0.002	0.010
SE	0.004	0.013	0.002	0.002	0.001	0.003
Range	0–1.17	0–1.17	0–0.136	0–0.065	0–0.020	0–0.066

* numbers of nets in parenthesis is number at which fish eggs were collected.

column and 0.04 eggs m^{-3} in the neuston. (Table II). In Cumberland Bay (East) the depth distribution was examined in more detail by sampling at five depth horizons (cf. North & Murray 1992) and these results clearly demonstrated that most eggs were to be found in the upper water column (Table III).

Figure 2 shows the occurrence of fish eggs captured on the sampling grid around South Georgia and the four areas where intensive sampling occurred. The majority of stations at which fish eggs were collected were from the northern side of the island, especially in coastal locations and over the continental shelf. Eggs were also found over deeper water to the north of the island.

Egg identification

All the eggs from the samples collected in June 1993 were from one species and these were identified as *Notothenia rossii* when compared with a former description of nototheniid eggs by Riehl & Kock (1989) from Iles Kerguelen.

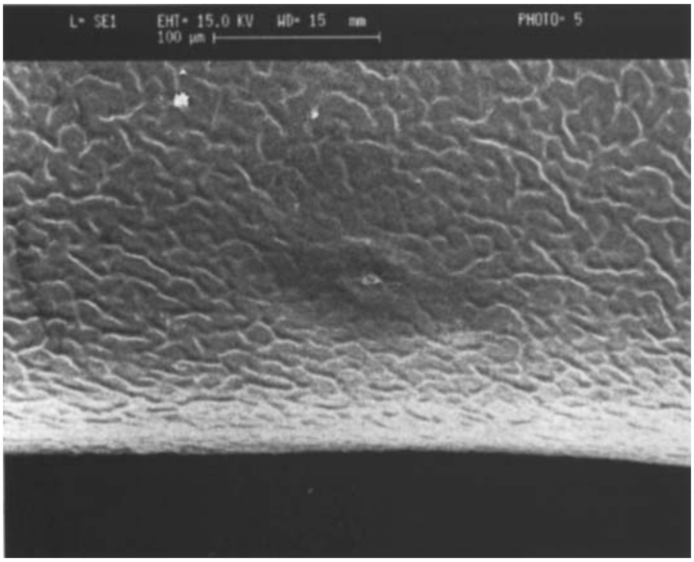

Fig. 3. Surface morphology of *Notothenia rossii* egg from South Georgia showing "wave like" reticulation and micropyle. Scale bar = 100 μm.

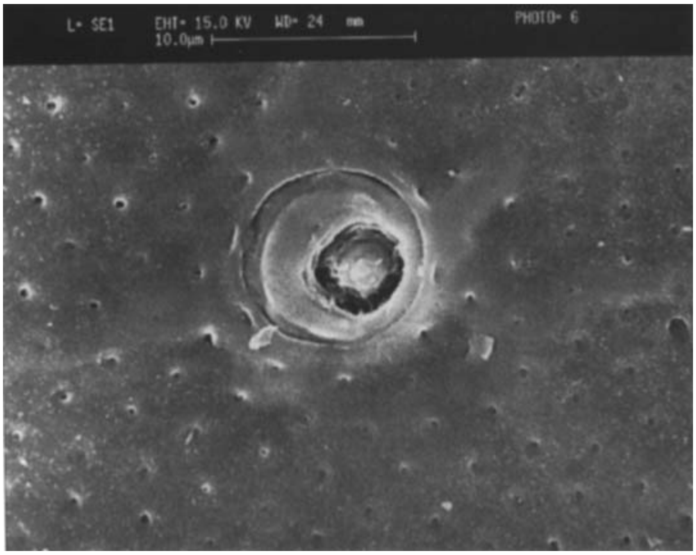

Fig. 4. Detail of *Notothenia rossii* egg micropyle showing micropores on micropyle pit, and canal with inner annulus and column. Scale bar = 5 μm.

Table III. Abundance of *Notothenia* spp. eggs in Cumberland East Bay, South Georgia, 20–26 September 1983. (0–2m layer sampled with Foredeck net, remaining layers using an RMT8 net).

Strata (m)	Total	0–2m*	0–75m*	75–150m	150–200m
eggs/1000m³ ± SE	5.33 ± 1.44	16.92 ± 4.75	2.66 ±0.58	0.29 ±0.05	0.803 ± 0.44
Σ Nets	88	23	21	22	22

*The Foredeck net is deployed over the ship's side clear of the wash whereas the RMT8 net is deployed over the stern and so the upper 5–10m are poorly sampled because of turbulence from the ship's wake.

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The external surface shows superficial sculpturing of the surface which appears as a wave-like reticulation covering the whole of the outer surface and measuring a maximum of 10–20 μm between ridges (Fig. 3). Small regularly spaced pores perforate the external surface, these micropores are 0.26 μm ± 0.035 μm in diameter and the inter-pore distance is 3.03 μm ± 0.27 μm.

The micropyle (Fig. 4) is surrounded by a circular pit 36–38 μm in diameter. The micropyle canal is 8.7–9.0 μm in diameter within which is an annular structure surrounding a central column 4.5–4.9 μm across with lateral pores 0.5–0.6 μm in diameter. A section of the egg-shell through the micropyle showed that these structures are relatively superficial being only about 2 μm deep but the lateral pores surrounding the central column and annulus penetrate the thickness of the egg-shell (20–25 μm).

Discussion

Aspects of the reproductive biology, such as fecundity, spawning period, size and age at sexual maturity and first spawning of most common Antarctic notothenioids are well known (cf. Everson 1984, Kock 1992). However, other key aspects such as the spawning grounds, site and duration of incubation, dispersal and mortality of eggs and early larval stages are poorly understood. Of species that spawn at South Georgia during the autumn–winter, *N. coriiceps* and *N. rossii* are known to produce pelagic eggs and other species such as *Chaenocephalus aceratus, Champsocephalus gunnari, Lepidonotothen larseni,* and *Dissostichus eleginoides* may do so (Kellermann 1991, Camus & Duhamel 1985). The diameter of the pelagic eggs collected during June 1993 at South Georgia was almost identical with the range reported for *N. rossii* (4.5–5.0 mm) (Kock & Kellermann 1991) although this is larger than the size reported for *N. coriiceps* (4.1 ±0.4 mm) by White *et al.* (1982) and Burchett *et al.* (1983). The surface sculpturing is unlike the distinctive 'elephant skin' covering described for the eggs of *N. coriiceps* but very similar to that for *N. rossii rossii* from the Iles Kerguelen described by Riehl & Kock (1989). The detailed micropyle morphology was found to be different between the Iles Kerguelen samples and those from South Georgia. In part, these differences may result from the samples of eggs from Iles Kerguelen being unfertilized while those from South Georgia were fertilized examples. The central column described here is a similar feature to a part of the 'fertilization cone' described by Iwamatsu *et al.* (1991).

The detailed structure of the micropyle has been shown to be important in orienting spermatozoa during the fertilization process and may be involved in avoiding interspecific cross fertilization among fish (Amanze & Iyengar 1990). The provenance, pelagic distribution, size and the surface morphology indicate that eggs collected from the neuston at South Georgia in June 1993 were of *N. rossii marmorata.* The differences observed in the micropyle morphology between the two subspecies of *N. rossii* may assist in maintaining genetic separation of these in the event of a chance displacement of eggs or fish between South Georgia and the Kerguelen region.

Eggs collected during July to September 1983 were not identified at the time but at least two species were represented because two size modes (2.5–2.7 mm and 4.0–5.0 mm diameter) were present. The latter mode spans the size range for *N. coriiceps* and *N. rossii*, the eggs of both of which can be expected to be present in the plankton at South Georgia

The spawning migrations and the location of spawning grounds are not confirmed for *N. rossii* at South Georgia. However, the likelihood of adults aggregating to spawn in localized areas of the continental shelf can be inferred from observations of the spawning behaviour of this species at Iles Kerguelen, where adults assemble to spawn during winter in submarine canyons on the south-east shelf of the main island (Duhamel 1987). In addition, informal reports from the Soviet fishing vessels working at South Georgia in the 1970s described the trench at the eastern end of the Island, between South Georgia and Clerke Rocks, as 'Marmorata trench' because large numbers of *N. rossii* could be caught at that locality, especially during the spawning season. Pelagic eggs dispersing from spawning in this area would be carried north and west on the prevailing currents. The transects sampled during June 1993 (Fig. 1) run north from this trench across the breadth of the north-eastern shelf and area D sampled in 1983 (Fig. 2) is also over this part of the shelf. The broad distribution pattern exhibited by stations at which fish eggs occurred conforms to a generalized hypothesis of the oceanic circulation patterns at South Georgia (cf. Hardy & Gunter 1935) whereby currents converge on the northern shelf and then may be deflected northwards away from the island. Gyres adjacent to the island shelf are a consistent feature of circulation patterns described by several authors (Hardy & Gunter 1935, Priddle *et al.* 1986, Latogursky *et al.* 1991). Eggs advected from the shelf are therefore lost or entrained in a gyre adjacent to the shelf. The eggs must remain over or near the shelf until hatching. This interval is protracted among nototheniids, for example, 90–120 days after spawning in *N. rossii* (Camus & Duhamel 1985). The successful retention of eggs over or near the shelf by a shelf-break frontal system or gyres will have implications for the survival of the larval stages and subsequent recruitment juvenile *N. rossii.* Latogursky *et al.* (1991) argued a similar mechanism for the development of aggregations of *Euphausia superba* at South Georgia and they asserted that the presence of large aggregations, suitable for commercial exploitation, depended upon the development and persistence of gyres near the island.

There are few other records of the abundance and distribution of *N. rossii* eggs in Antarctic waters. Joint French/Soviet ichthyological surveys during 1987 and 1988 around the Iles Kerguelen included plankton surveys which assessed the abundance and distribution of *Champsocephalus gunnari* and *N. rossii* eggs (Duhamel 1993). During these surveys *N. rossii* eggs were found over 'shelf' and 'slope' waters at low abundance, mostly <0.05 eggs m^{-3} although one station due east of Iles Kerguelen had >0.1 eggs m^{-3}. At South Georgia similar values for fish egg abundance were observed for fish eggs during 1983 and values two orders of magnitude higher in 1993 (neuston sample mean = 4.7 eggs m^{-3}). However, direct comparisons are not possible because of different nets and deployment techniques that were used, and the highly patchy nature of egg distributions.

The balance between loss and retention of shelf water and its associated zooplankton communities may have an important impact on the survival of the larval stages and recruitment of 'O' group fish stocks at the peri-Antarctic islands. Future studies on their early life histories at South Georgia should help to better understand variations in year-class strength of *N. rossii* and other species with epipelagic eggs and larvae.

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