REPRODUCTION IN *Notothenia neglecta* Nybelin

By INIGO EVERSON

ABSTRACT. Monthly gonad samples from *Notothenia neglecta* caught at Signy Island have been examined macroscopically and microscopically. Development of the ova takes 2 years, probably because of the low sea temperature and large egg size. Considerable variation around the Antarctic continent has been found in both spawning time and the size of eggs laid.

THE breeding season and size of ova contained or spawned by *Notothenia neglecta* have been described by several workers. Marshall (1953) described ova of *Notothenia coriiceps* (= *N. neglecta*) caught in August at Hope Bay that were 2·5–3·0 mm. in diameter and in a later publication (Marshall, 1964) he predicted the breeding season as late spring or early summer. Hureau (1962) found that *N. neglecta* spawned ova of only 1·5 mm. diameter in December at Terre Adélie and Nybelin (1951) suggested that spawning occurred in either late March or April at Deception Island. These results indicated a great variation in the reproductive season of *N. neglecta* and therefore warranted a thorough investigation of the breeding biology of the species.

The investigation was undertaken in two parts. At Signy Island, samples of gonads were taken for histological examination leading to the description of a standard gonad cycle. For the second part, specimens from different places were examined in the same manner as the Signy Island material so that variations in the breeding season will be seen.

MATERIALS AND METHODS

Freshly caught fish were weighed and measured (Everson, 1969). The roe was then removed, weighed and a part of it was fixed in either Smith's formol-bichromate (Pantin, 1948) in the case of ripening ovaries or Bouin's fluid for all others. Gonads fixed in Smith's solution were washed for 24 hr. in running water (fresh water if available, otherwise sea-water) and stored in neutral 4 per cent formol saline. Gonads fixed in Bouin's fluid were stored in 70 per cent alcohol. A small collection of whole ovaries was stored in neutral 4 per cent formol saline for fecundity determinations.

For histological examination, gonad samples were embedded in celloidin and wax by Peterfi's double-embedding method (Pantin, 1948) and sectioned at 10 μm. Ovaries fixed in Smith's solution were generally embedded in 45°C melting-point wax and sectioned at 6 μm. Sections were stained retrogressively with Erlich's haematoxylin and aqueous eosin.

For the fecundity study, ripe ova were washed out of the ovary with running tap water into a nest of sieves, having successive aperture sizes of 4·0, 2·0 and 0·50 mm. Ripening ova from gonads taken during March, April and May were retained by the 2 mm. mesh and these were either all counted or a measured volume of the total counted and multiplied up.

MACROSCOPIC EXAMINATION

The development of the ovary of many fish species may often be described by a series of arbitrary stages based on appearance (size and colour) and egg size. Dearborn (1965) described five stages in the ovarian development of *Trematomus bernacchii* based on subjective macroscopic examination and the diameter of ova.

A similar system was unsuitable for describing the maturation of *Notothenia neglecta* ovaries because only four categories were demonstrable: immature, ripening, spawning and spent.

An alternative method, used by Hureau (1964) to describe the gonad cycle of *T. bernacchii*, was used. A factor, the relative gonad size,* was determined for each fish and regression lines were fitted to the results of R.G.S. of mature fish plotted against standard length for each month, and the calculated mean R.G.S. was determined for the mean standard length of all mature fish. (This reduced variations due to a predominance of larger fish in certain months.) For both sexes the mean R.G.S. reaches a maximum during April and May (Fig. 1). The

* Relative gonad size (R.G.S.) $= \frac{\text{gonad weight}}{\text{total weight}} \times 100$.

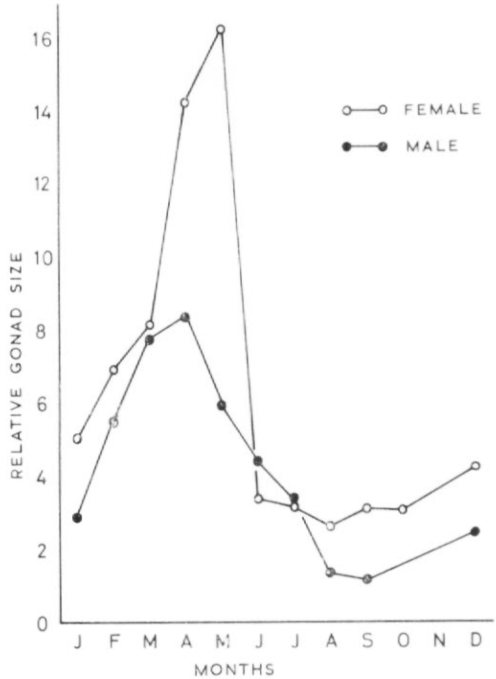


Fig. 1. Monthly mean relative gonad size for mature *Notothenia neglecta* at Signy Island.

testis shows a steady reduction in size during June, July and August caused by the release of sperm followed by a slow increase in size during the spring and a rapid increase in the summer, leading up to the peak prior to spawning.

The ovary starts to increase in size from November after being more or less constant at about 3 per cent of the total weight for the preceding 6 months. The increase is gradual until March when there is a rapid increase so that the gonad is nearly twice its March size when spawning occurs in May. The reduction in the gonad size at spawning does not leave the ovary as devoid of ova as either Hureau (1964) or Dearborn (1965) have described for the closely related *Trematomus bernacchii*. The spent ovary, although containing no large ova, contains many small ova (0·8 mm. diameter) which will presumably form the next year's spawn. The presence of these small ova, when spawning occurs, suggests that the development of the eggs takes more than 1 year.

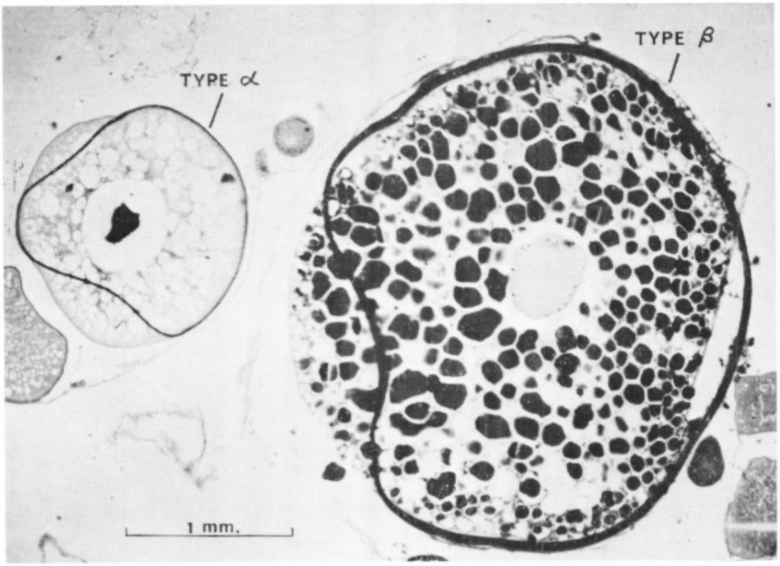
Microscopic Examination of the Ovary

Characteristically, mature ovaries contain oocytes in four developmental stages:

i. Oogonia; small cells recently differentiated from the germinal epithelium.
ii. Primary oocytes which have undergone a period of minor growth (0·08–0·13 mm. diameter).
iii. Circum-nuclear ring-phase oocytes; rounded cells with a central, well-defined reticular nucleus around which is a ring of deeply staining granulations (0·12–0·33 mm. diameter).
iv. Oocytes containing yolk droplets (0·27–3·0 mm.* diameter).

In addition, the oocytes containing yolk may be divided into two types mainly by their size (Fig. 2). The smaller yolky oocytes, which will be termed type a oocytes, are formed directly from the circum-nuclear ring-phase oocytes and have an initial diameter of about 0·3 mm. At this stage the nucleus is quite distinct with many nucleoli at the periphery and the cell wall

* Due to fixational shrinkage, the diameter of ripe ova as measured on histological sections is little more than 2·5 mm. Ripe ova from fresh ovaries were 3·0 mm. in diameter.



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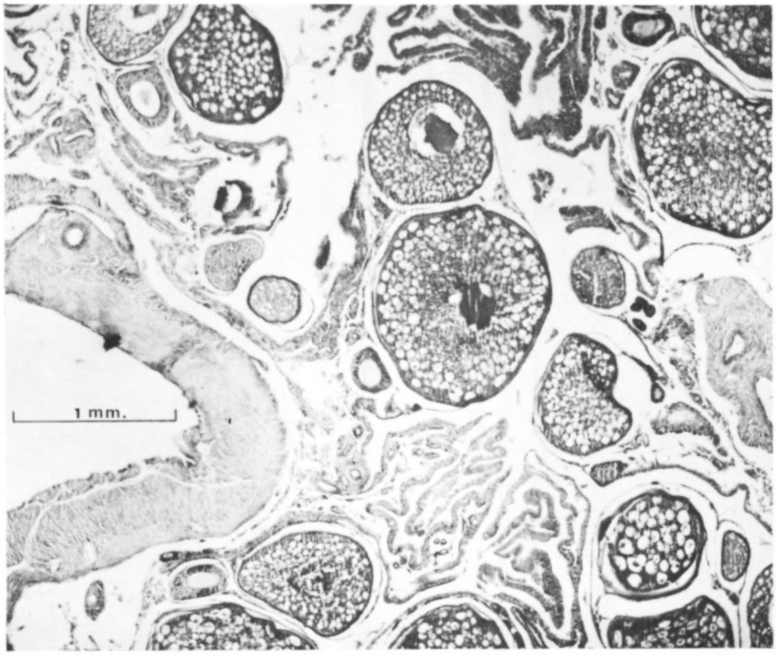

b

Fig. 2. Photomicrographs of mature *N. neglecta* ovaries.
a. Ripening ovary showing both a type α and a type β oocyte.
b. Spent ovary showing the ruptured follicles of the spawned oocytes and also many yolky oocytes remaining.

is thin and does not stain deeply. Larger yolky oocytes (type β oocytes) are formed by gradual changes from the type α oocytes. Ultimately they are large (3 mm. diameter) and contain much yolk. The nucleus has not increased in size but it has lost its initial spherical shape probably because of deformation by the yolk globules. The nucleoli are scattered throughout the nucleus. The cell wall becomes very much thicker (0·04 mm.) and takes up stain. It is important to note that there is no clear demarcation between the two types but rather a slow transformation from one to the other.

During the early summer months atretic yolky oocytes occasionally occur, and the few ripe oocytes that are not spawned undergo atresia during the winter.

Seasonal Cycle

The formation of ripe oocytes to be spawned is best described by consideration of the immature ovary and its development to a first breeding season. When the fish is 20 cm. long the ovaries are quite distinct firm sacs in the abdominal cavity. Development to the circum-nuclear ring phase has occurred in the majority of oocytes and some may have started yolk deposition. As the fish increases in size the number of oocytes with yolk increases and, although the process occurs for several years, no ova are spawned, since no ruptured follicles are present. The build-up is steady and slow until the fish is about 30 cm. long, by which time most of the volume of the ovary is made up of type α oocytes. The type α oocytes increase in size and gradually become transformed to type β oocytes while new type α oocytes are formed from the circum-nuclear ring-phase oocytes. Ova that are spawned are all type β oocytes. The post-spawning ovary contains type α oocytes and the ruptured follicles of the type β oocytes. This is shown in Fig. 3 and its effect is that at spawning the ovary size does not become reduced in size to a level comparable with fish in which all the oocytes with yolk are spawned (Fig. 1).

During the months following spawning the only difference between ovaries of spent fish and those preparing to spawn for the first time is the presence of ruptured follicles in the former which persist until about December. After this time no distinction could be made between fish which had spawned and fish preparing to spawn for the first time.

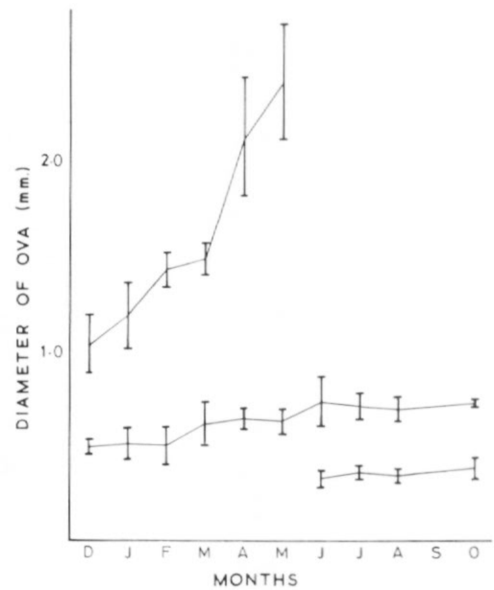

Fig. 3. The diameter of ova with yolk in each month of the year. The results plotted are the mean plus or minus one standard deviation.

Microscopic Examination of the Testis

Only three clearly defined stages can be described from a macroscopic examination of the testis: an immature stage where the gonad is very small and convoluted, an adult stage where the testis is large and creamy in colour, and a ripe stage where it is very large and opalescent white. From microscopic examination, the following stages of spermatogenesis and final ripening may be seen:

Stage 1. Primary germ cells; large cells with a central darkly staining nucleus and a distinct cell wall.

Stage 2. Spermatogonia; cells with dense tangled nuclear material and a relatively indistinct cell wall.

Stage 3. Primary spermatocytes; small cells with chromatin threads in a more or less tangled mass giving the nucleus a knobbly appearance.

Stage 4. Secondary spermatocytes; cells about half the size of the above having evenly dispersed chromatin.

Stage 5. Spermatids; smaller still, having an elliptically shaped nucleus and a small cytoplasm rim. They are slightly darker staining than the preceding stages and give sections a mottled appearance.

Stage 6. Spermatozoa develop directly from the spermatids and are about the same size. They are first seen aggregated in bunches with tails to the centre and latterly (*Stage 6a*) lie free in the testis.

A points system was developed for analysis of the sections based on the following criteria: six points for the dominant stage (if two stages were equally dominant a score of three points each was made, etc.); one point for each other stage that was present.

Three sections for each fish were examined, the points totalled and the mean total for all fish caught in each month determined. These results are shown in Fig. 4.

The maturation cycle is simple and except for timing is the same as that described for other teleosts (Turner, 1919; Woodhead and Woodhead, 1964). Primary germ cells and spermatogonia are present throughout most of the year, the latter reaching a peak during late spring. Primary spermatocytes, dominant during February, develop into secondary spermatocytes,

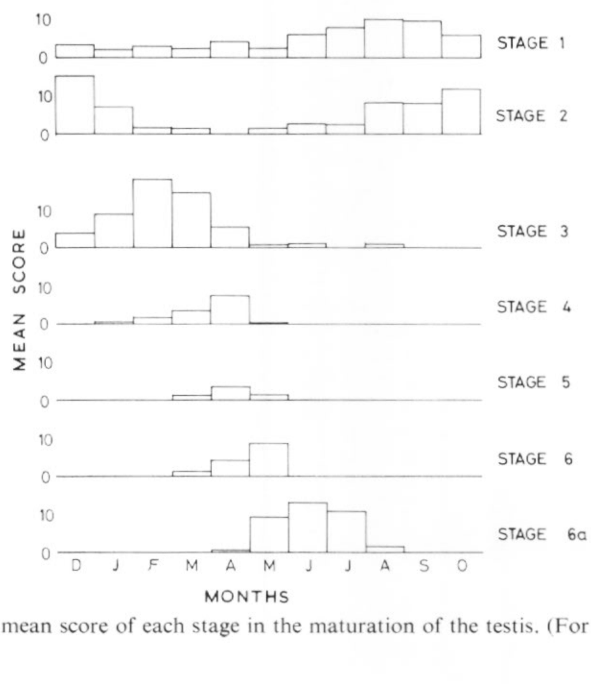

Fig. 4. The monthly mean score of each stage in the maturation of the testis. (For explanation see text.)

spermatids and finally spermatozoa during April and May. Sperm are found free in the testis for several months after spawning, during which time they are gradually resorbed and simultaneously new primary germ cells are formed from the germinal epithelium.

Age at Sexual Maturity

Since spent female fish retain burst follicles in the ovary for nearly 6 months following spawning, it follows that fish caught during this period, and which will spawn for the first time in the following May, will have both type α and β oocytes but no burst follicles. Fish in the latter category have been grouped by age (determined from otoliths (Everson, 1969)) and standard length in Figs. 4 and 5, respectively.

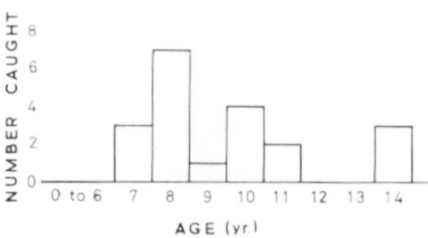

Fig. 5. Numbers of female *N. neglecta* of each age class preparing to spawn for the first time in the current year.

Although the numbers involved are small, it is clear from the irregularity in the histogram that age has only a limited influence on the onset of sexual maturity (Fig. 5), whereas size (Fig. 6), because of the more regular frequency distribution, appears to have a greater governing effect. Although no definite spawning zones (Rollefsen, 1933) are present on the otoliths to suggest annual reproduction, this may be inferred by the presence of type α and β oocytes in the ovaries of fish which are near to spawning.

Fig. 6. Numbers of female *N. neglecta* of each centimetre length group preparing to spawn for the first time in the current year.

A clear distinction is not possible between male fish that have spawned before and those spawning for the first time. From microscopic and macroscopic examination of the testis and ages determined from otoliths, the percentage of fish caught from each age and size group that are mature has been calculated (Figs. 7 and 8).

A few fish, developing precociously, had mature testes at 4 or 5 years of age, but the majority did not reach maturity until they were 8 to 11 years old (30 cm. standard length). All male fish matured before they were 13 years old.

Fecundity

The ovaries of *Notothenia neglecta* just prior to spawning are filled with ova in various stages of maturation. Most of the volume is occupied by large yolky oocytes which will form the current season's spawn and it is only with these mature eggs that any considerations of fecundity are concerned. Fig. 9 shows the number of eggs constituting a year's spawn plotted against the weight of the fish with a regression line fitted by the method of least squares.

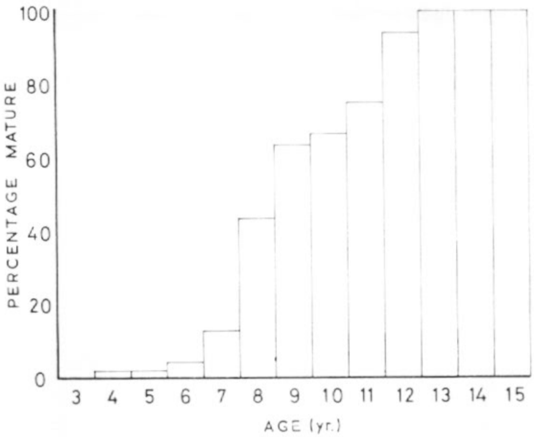

Fig. 7. The percentage of all male fish caught of each age class that are mature.

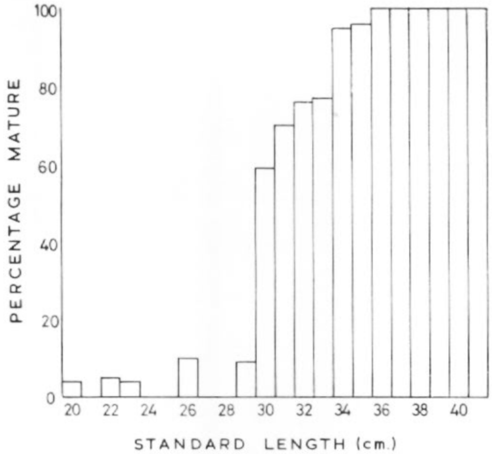

Fig. 8. The percentage of all male fish caught of each centimetre length group that are mature.

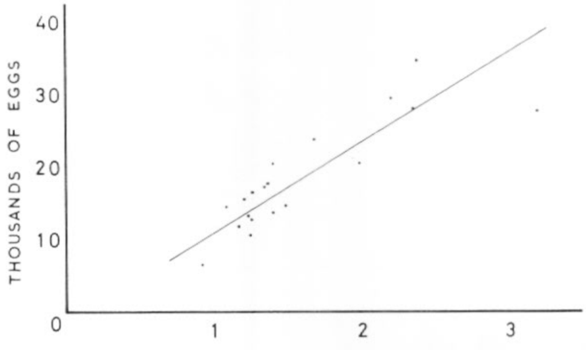

Fig. 9. Total number of eggs (in thousands) removed from ripe ovaries plotted against the total weight (kg.) of the fish. The line, calculated by the method of least squares, is $F = 18 \cdot 29 + 13 \cdot 12(\text{weight} - 1 \cdot 578)$.

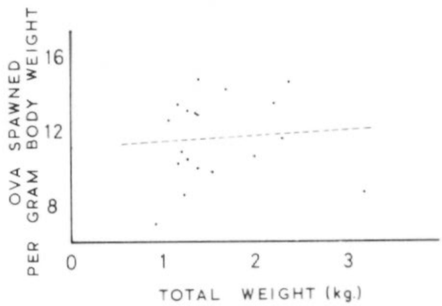

Fig. 10. The number of eggs spawned per gram body weight plotted against the total weight. The broken line, calculated by the method of least squares, has a slope not significantly different from zero, indicating that the number of eggs laid is directly proportional to the fish total weight.

In Fig. 10 the number of eggs per gram of total fish weight is plotted against the length of the fish; it can be seen that this remains fairly constant as the fish increases in size and has a mean of 11,600/kg.

Atretic ova, occasionally found in mature ovaries, never occurred in large enough numbers to determine fecundity and it is more likely that the process is controlled during the transformation of circum-nuclear ring-phase oocytes to yolky oocytes throughout the year and type α oocytes to type β oocytes after spawning.

Seasonal Gonad Cycle in Other Antarctic Localities

Material used for this part of the study was fixed and examined in the same way as that from Signy Island except that all ovaries were fixed in Bouin's solution.

The results for female fish are shown in Fig. 11. Female fish were caught in two other localities and in both cases they were all obviously immature. The result for South Georgia,

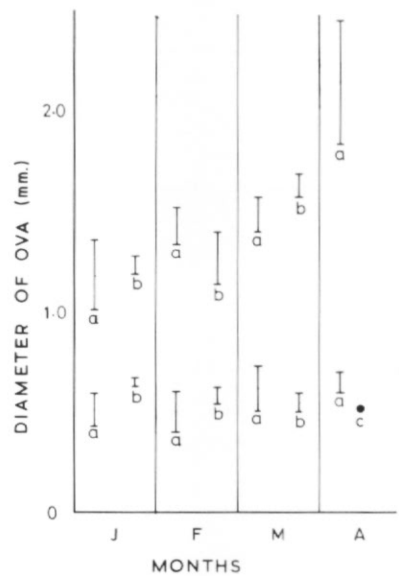

Fig. 11. A comparison of the results of egg diameters of *N. neglecta* caught in different localities.
a. Fish caught at Signy Island.
b. Fish caught at Deception Island.
c. One fish caught at South Georgia.

in which there were no type β oocytes present, is almost certainly due to the fish being immature. Although 33·8 cm. in standard length, and therefore large enough to have bred, it had obviously not spawned recently as there were no ruptured follicles in the ovary. Ova dissected from two mature fish caught at South Georgia in April were approximately 3 mm. in diameter, about the same size as ripening ova in the same month at Signy Island, if allowance is made for the shrinking effects of different fixatives.

Mature testis samples were obtained from three localities other than Signy Island and the results from their analyses are shown in Fig. 12. The shapes of the histograms produced for each locality are very nearly the same as those for the same months at Signy Island. It is clear, therefore, that the breeding season for *N. neglecta* is not more than 1 month different at any of the four localities.

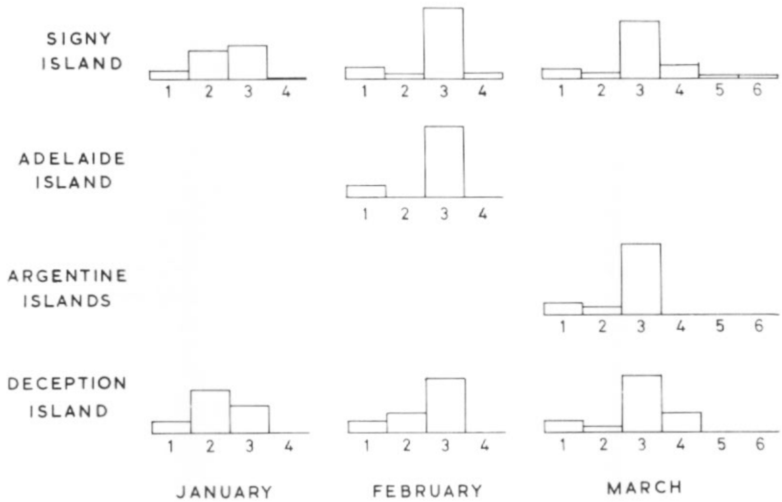

Fig. 12. The monthly mean score of each stage at three localities compared with the results for Signy Island.

Discussion

A characteristic of Antarctic fish is the production of large yolky eggs and in this respect *N. neglecta* conforms to the general pattern. The advantages in producing large yolky eggs are that the larger the individual, the more advanced it will be on hatching, the smaller its relative food requirements and the faster it will be capable of swimming in search of food (Marshall, 1953). The advantages may, however, be offset to a certain extent by the amount of organic matter that is required for ovogenesis. The development of mature ova takes 2 years in *N. neglecta* and, although a biennial process of yolk deposition has not been described for other fish, it is probable that it also occurs in *Trematomus bernacchii*. Hureau (1964) published results of egg-diameter measurements on *T. bernacchii* caught at Terre Adélie, and it is of interest to note that he found the spent ovaries contained eggs of about 1 mm. diameter. This is a very close parallel with the results for *N. neglecta* (Fig. 13) presented here, if allowance is made for fixational shrinkage in the *N. neglecta* material. It is therefore quite likely that yolk deposition takes about 2 years for *T. bernacchii* as well as *N. neglecta*.

The advantage to the fish in having a prolonged yolk-deposition cycle is not clear, as in any one year the weight of ova produced must be at least the sum of all type α and β oocytes present immediately prior to spawning. During the 3 months before spawning, when growth of ova is accelerated, the final yolk-deposition process may be governed by the availability of food, either as organisms or as an internal store. Variations in the relative liver size* have already been described for *N. neglecta* (Everson, 1969), and it is likely that the build-up in the

* Relative liver size $= \frac{\text{liver weight}}{\text{fish weight}} \times 100$.

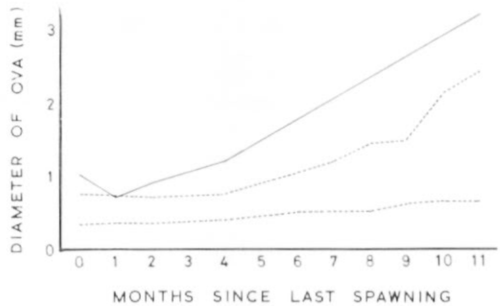

Fig. 13. Mean monthly results of egg-diameter measurements for *N. neglecta* at Signy Island (broken line), compared with the results of Hureau (1964) for *Trematomus bernacchii* (solid line). Since the two species do not spawn during the same month, the results are presented in months since last spawning rather than actual months of the year.

liver size (Fig. 14) and sudden decline following spawning are linked to the process of final maturation of the eggs. Continuous formation of type α oocytes from the circum-nuclear ring-phase oocytes results in the production of yolky oocytes of about 0·8 mm. diameter 1 year prior to their being spawned and represents a background activity of the ovary throughout the year. Fecundity is probably controlled during the process of transformation from circum-nuclear ring-phase to yolky oocytes and from type α to type β oocytes by limiting the numbers that make the final maturation; the type α oocytes remaining probably undergo atresia, although it is possible that they could remain and develop fully in the following year. The advantage of the 2 year yolk-deposition process is probably that the first year's background growth produces ova of such a size that a physiological boost to produce the ripe ova is of shorter duration and of lower organic production.

Variability in breeding season in a species at different geographical localities in the Antarctic has already been described by Dearborn (1965), who found that *T. bernacchii* spawned at McMurdo Sound 2 months after the same species at Terre Adélie.

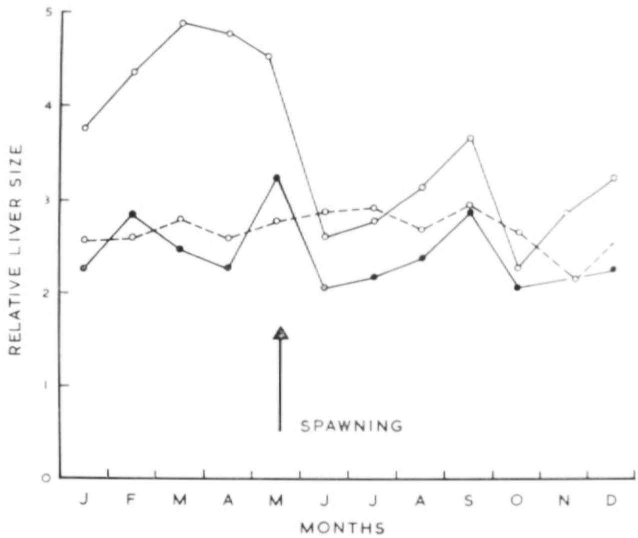

Fig. 14. The mean relative liver size ($\frac{\text{liver weight}}{\text{total weight}} \times 100$) in each month of the year: ○—○ females, ●—● males, ○ - - - ○ immature fish. Spawning occurs in late May.

However, the seasonal disparity in *N. neglecta* is very much greater, being 6 months between the two extremes (Fig. 15). Considering results along the Scotia arc, it is clear that apart from Marshall's (1953, 1964) predictions at Hope Bay breeding definitely occurs in May at all the other Antarctic Peninsula localities investigated and almost certainly, in addition, at South Georgia. Allowing for a relatively slow rate of development in Antarctic waters, large eggs spawned in May would result in post-yolk-sac stage larvae in the spring, coincident with the phytoplankton bloom and, as a result, maximum zooplankton growth. Phytoplankton production varies from a minimum at the southern end to a maximum in the north of the Scotia arc (Hart, 1942) and the distribution of macroplankton (Mackintosh, 1934) is very similar. Neither of these two important biological factors appear to affect the breeding season of *N. neglecta*.

Both the egg size and spawning period of *N. neglecta* at Terre Adélie are of singular interest. The eggs laid are the smallest of any Antarctic species and only about half the diameter of those laid by the same species (*N. neglecta*) elsewhere. Although the phenomenon is not un-

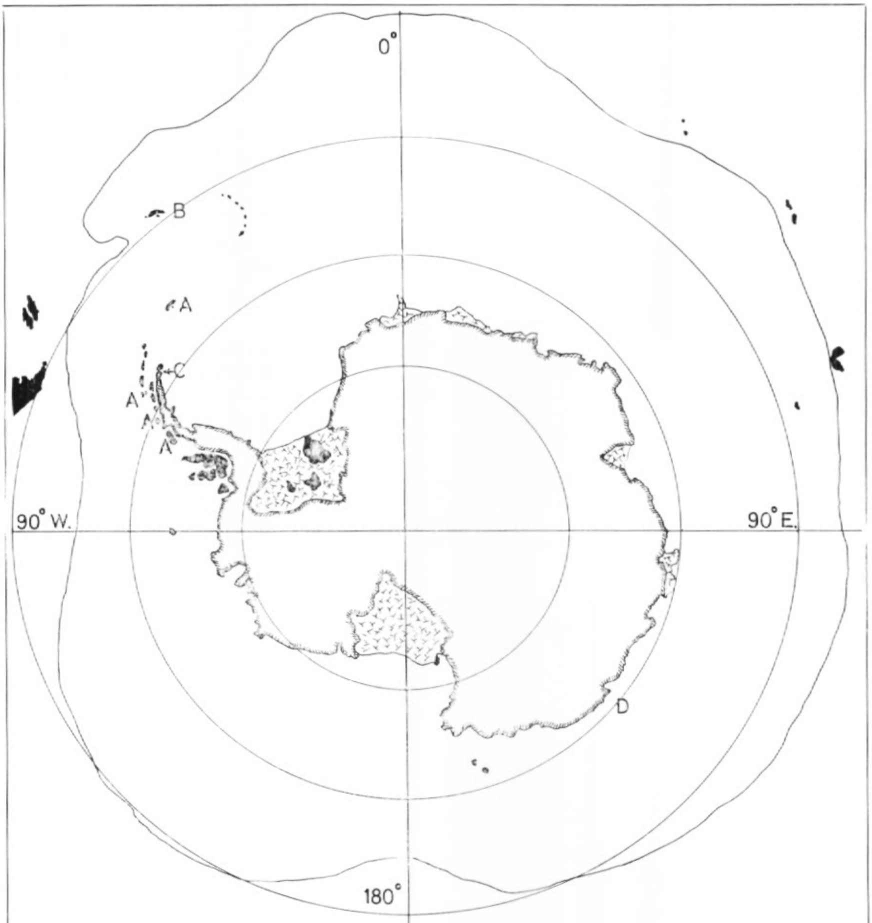

Fig. 15. Localities around the Antarctic continent for which a breeding season has been described.
A. Breeding season definitely May.
B. Breeding season probably May.
C. Breeding season predicted in spring (Marshall, 1953).
D. Breeding season described as early summer (Hureau, 1964).

known, Hoar (1957) described different populations of sockeye salmon (*Oncorhynchus nerka*) which lay different size eggs and occur in the same river system; the adaptive significance of this is not obvious. It is, however, possible that the spawning of small eggs in the summer may result in the production of larvae which are able to utilize the summer's growth of phytoplankton. In contrast, Antarctic fish generally lay large demersal eggs which develop into large larvae that feed immediately on the zooplankton or bottom-dwelling forms, e.g. copepods, etc., rather than spending an intermediate period as phytoplankton grazers (Marshall, 1953). At Terre Adélie it is possible that *N. neglecta* larvae spend an increased period in the plankton, although the adaptive significance of such a monospecific variation is not at all clear.

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