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Reproductive biology of female Antarctic spiny plunderfish *Harpagifer spinosus* (Notothenioidei: Harpagiferidae), from Îles Crozet

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Abstract: On the basis of histological examination, we present the first description of oogenesis in *H. spinosus*. Eight stages of oocyte development were identified using morphological and histochemical criteria. The development of the oocytes is synchronous, and two clutches grow simultaneously in the ovary. Spawning takes place during the end of summer and the beginning of autumn. Absolute fecundity is low, ranging from 787 to 1504 oocytes. The low fecundity is probably related to parental behaviour. Our data indicate that the features of the reproductive biology of *H. spinosus*, in spite of its unusual environment, are similar to those observed in other teleost fish.

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Introduction

The spiny plunderfishes are small, bottom dwellers in shallow waters along the Antarctic Peninsula, sub-Antarctic and South Atlantic islands (DeWitt 1971). As adults, harpagiferids inhabit shallow inshore waters and tide pools, sometimes living under rocks (Daniels 1979), and are also occasionally found among kelp (Hureau 1990). They show slow growth and attain sexually maturity between three and five years old (North & White 1987). The eggs are demersal and the larvae have a large pelagic phase (Kock & Kellermann 1991). Furthermore, it has been well demonstrated that some harpagiferids, for example *Harpagifer bispinis* Schneider, 1801, exhibit nesting behaviour (Daniels 1978, 1979).

The Harpagiferidae has traditionally included the artedidraconids, but Andriashev (1965) and later Eakin (1981) separated them into two families. Following the revision of Hureau (1990) the family Harpagiferidae contains only one genus, *Harpagifer*, with six species. However, recently three new species of this genus have been described (Prirodina 2000, 2002).

The main features of the Southern Ocean are its near-freezing temperatures and extreme seasonality in ice cover and light, leading to a markedly seasonal pattern of primary production. The breeding cycles of these fish are thought to be linked to the production cycle (Clarke 1988).

In notothenioids, some common features characterize the reproduction: prolonged gametogenesis, low fecundity and relatively large yolky eggs (Andriashev 1965). Little is known about the biology of *Harpagifer spinosus* Hureau, Louis, Tomo & Ozouf, 1980. This species is found in waters between 80 and 180 m deep around Îles Crozet and Kerguelen (Hureau 1990) and is also present in the South Orkney Islands, where is found at depths of 118 m (Matallanas 1997). The colonization of Îles Crozet and Kerguelen was probably facilitated by adults and juveniles transported on floating kelp, via the west wind drift from the South Orkney Islands (Matallanas 1997).

In the present study, sampling of the ovaries was conducted to investigate the characteristics of the reproductive cycle of *H. spinosus*. We describe the changes in the morphology of oocytes during development, estimate absolute fecundity, and propose an ovarian maturity scale.

Materials and methods

Fifteen female *H. spinosus* from the Paris Natural History Museum Antarctic fish collection were studied. The fish analysed were collected in the Îles Crozet region (46°27'S 52–59°W) during February and April in different expeditions between 1976 and 1982.

After a macroscopic examination of the paired ovary, ovaries were weighed and separated. The right one was used for fecundity calculations and the left one was processed for histological examination.

In many teleost fishes, significant differences in the average size of oocytes have been found along the length of the ovary (West 1990). Thus, for the histological and the fecundity study, subsamples were taken as complete cross-sections of the ovary, from the posterior, anterior and central parts of the gonad.

For the histological analysis of the ovaries, gonad samples were dehydrated through an increasing ethanol concentration series. Dehydrated samples were cleared and embedded in paraffin following a standardized procedure (Martoja & Martoja 1970). Sections 5–12 μm thick were

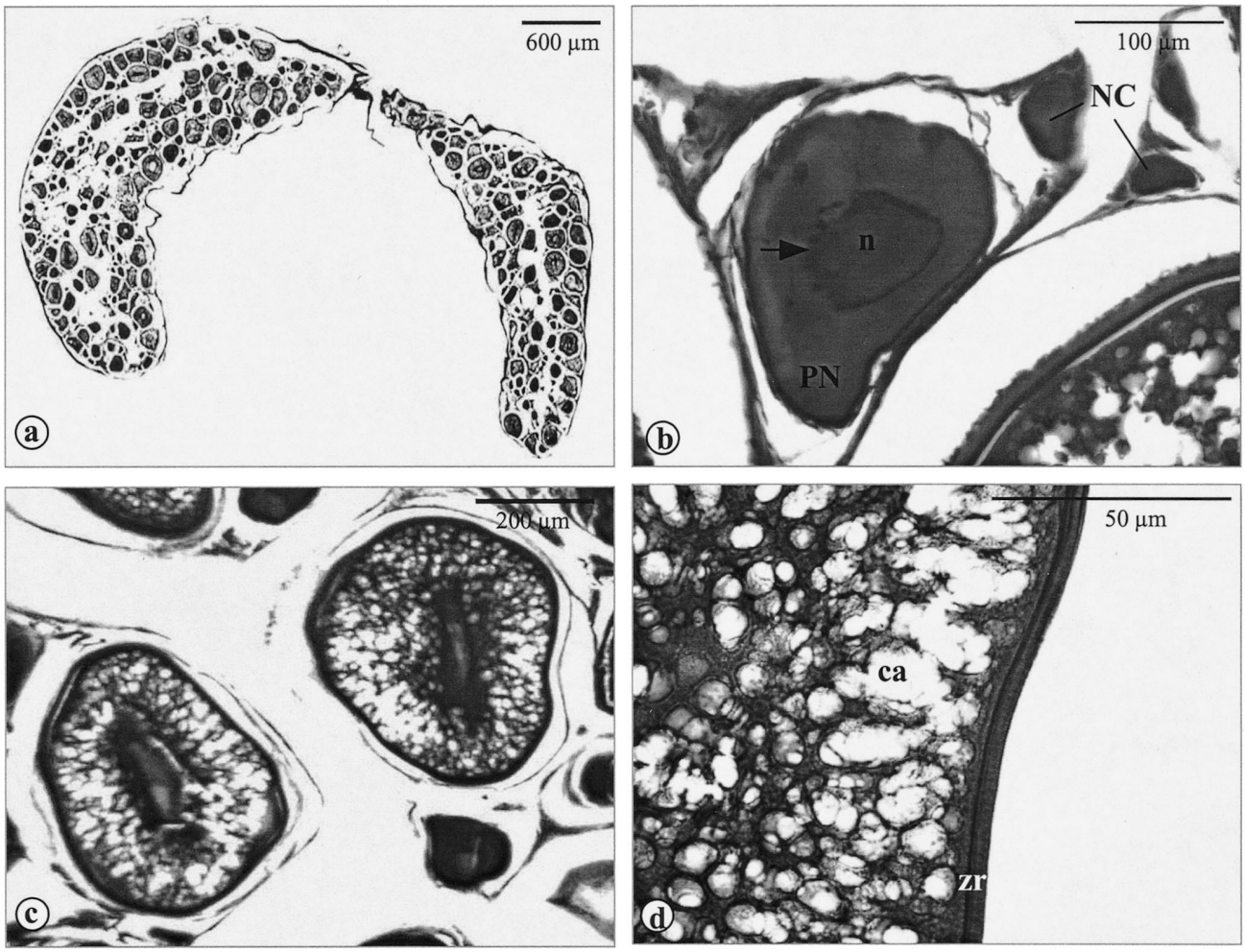

Fig. 1. Transverse sections of the entire ovary showing oocytes in different developmental stages. **a.** Complete section of both ovaries. **b.** Chromatin-nucleolar and perinuclear oocytes. **c.** Cortical alveolus II oocytes. **d.** Zona radiata detail in a cortical alveolus II oocyte. n = nucleus, arrow = nucleoli, NC = chromatin-nucleolar oocyte, PN = perinuclear oocyte, ca = cortical alveoli, zr = zona radiata.

cut, mounted on slides and stained with the standard staining methods. Haematoxylin-eosin (HE) was used as general stain, Mallory's trichromic for connective tissue, and the PAS (periodic acid-Schiff) reaction for neutral mucopolysaccharides (Hinton 1990).

Each section was examined using an optical microscope at magnifications ranging from x40 to x1000. Cells were measured using the Leica Q500 Mc (v.01.02-1995) Windows software. All oocytes on the slide were measured and assigned to a stage; cell and nuclear diameters were calculated as an average of two measurements, perpendicular to one another.

Because of the wide range of cell diameters, overlapping sizes among oocyte stages and shrinkage due to fixation, criteria for staging oocytes was based on histological appearances and cell structure (Bowers 1992). The nucleocytoplasmic ratio (NPR) was calculated, for each stage of oocyte development, as: NPR = Vn $(Vc–Vn)^{-1}$ where Vn is the nuclear volume and Vc is the cytoplasmic volume.

Patterns of oocyte development can be detected by plotting the frequency of oocytes in an ovarian sample as a function of oocyte diameter (Forberg 1982, De Vlaming 1983, West 1990) or oocyte developmental stage. An analysis of the stage/size-frequency distribution was

Table I. Mean ± SE of cellular diameters and nucleocytoplasmic ratio (NPR) for the different stages of oogenesis in *H. spinosus*.

Stage	Diameter (µm ± s.e.)	NPR
chromatin-nucleolar	45.64 ± 17.41	1.44
perinuclear	91.65 ± 30.65	1.01
cortical alveoli I	144.47 ± 31.56	0.71
cortical alveoli II	238.64 ± 49.02	0.39
yolk I	298.10 ± 53.94	0.28
yolk II	348.08 ± 68.72	0.26
yolk III	576.76 ± 157.15	0.14
mature	656.44 ± 125.80	-

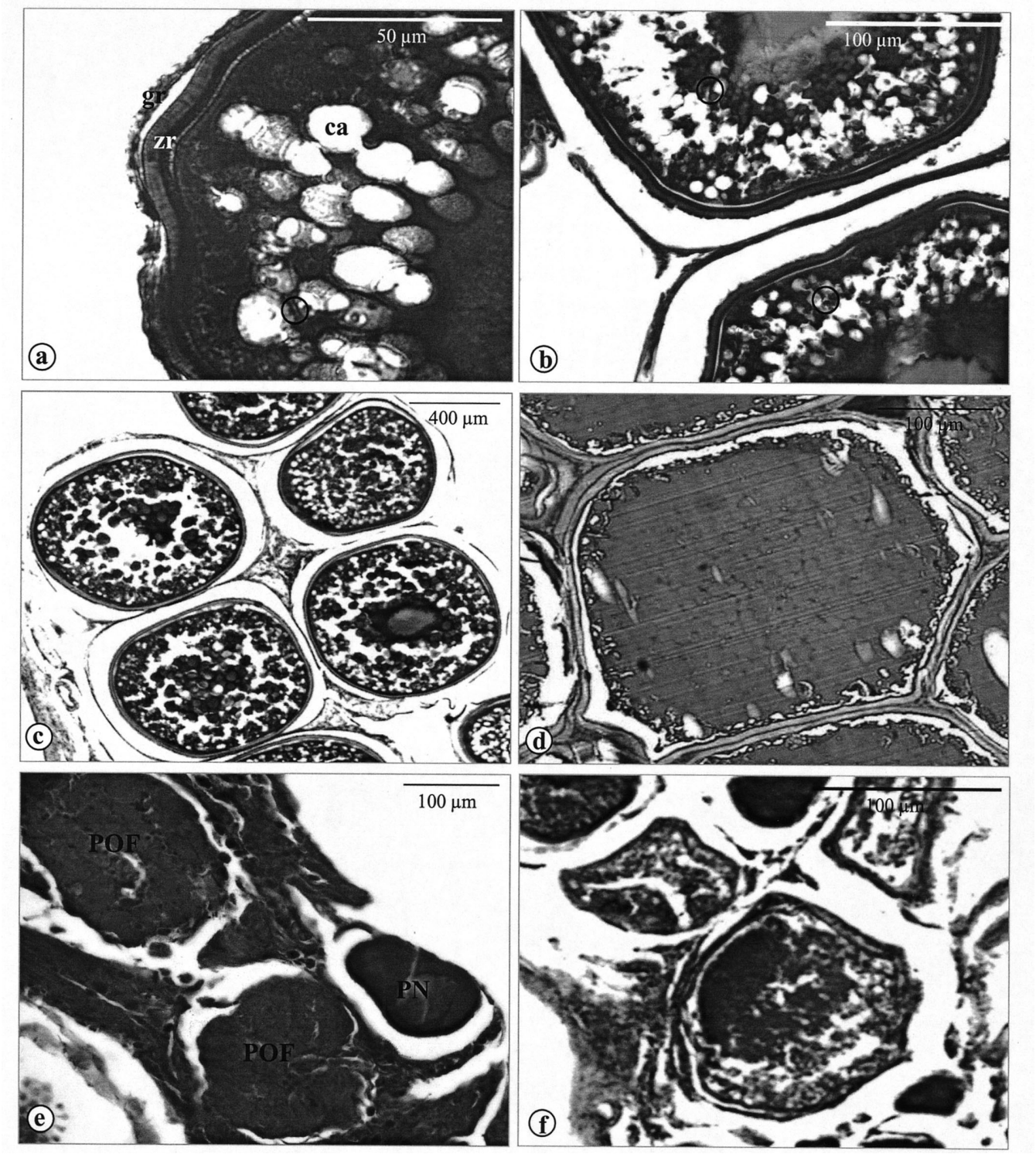

Fig. 2. a. Yolk I oocyte, showing the yolk granules at its periphery. **b.** Yolk II oocytes, showing the yolk granules and the zona radiata. **c.** Yolk III oocytes. **d.** Mature oocyte: coalescence of yolk granules, the nucleus is completely disintegrated. **e.** Post-ovulatory follicles and perinucleolar oocyte. **f.** Atretic oocytes. PN = perinuclear oocyte, ca = cortical alveoli, zr = zona radiata, gr = granulosa layer, circle = yolk granules, POF = post-ovulatory follicles.

performed following this approach.

The maturation stages of the ovary were determined after the macroscopic examination of the ovaries together with the analysis of the frequency distribution of the oocytes in the different stages. A five-stage ovarian maturity scale was utilized, following the classification proposed by Everson

(1977) and Kock (1989).

Absolute fecundity is defined as the number of ripening eggs found in the female prior to the next spawning period (Bagenal 1978). The absolute fecundity of *H. spinosus* was estimated using the gravimetric method. That is, weighing the ovary, counting a known weight of eggs (subsample) and estimating total fecundity by proportion.

Three subsamples of the right ovary were weighed to the nearest 0.001 g and then preserved, in separate jars, in Gilson's fluid. Jars were shaken periodically to facilitate the disaggregation and the hardening of the eggs and to break down the ovary tissue. Before counting, oocytes were washed with water in a consecutive series of sieves. Reserve oocytes (diameter estimated from histological preparations) and remaining small tissue particles were removed from the samples; thus, only oocytes from advanced stages of development onwards were counted. The total number of oocytes in the ovary, that is the absolute fecundity (AF), was estimated from the following equation:

$$AF = W \cdot D$$

where W is the ovary mass in grams and D is the mean oocyte density (average number of advanced oocytes per gram of ovarian tissue).

Even when some allowance is made for the mass of ovarian tissue and reserve oocytes (Bagenal 1978), in our data this was negligible when compared to the mass of the advanced oocytes.

Results

The paired ovary of *H. spinosus* is located in the dorsomedial region of the abdominal cavity. In the case of mature ovaries, the stomach adjusts to fit between both ovaries. Each ovary has a sac-like shape when immature (Fig. 1a) and, as they grow, they become more rounded.

Both ovaries join together at their posterior ends giving rise to a single oviduct, and the ovarian lamellae completely fill the central lumen. These ovaries belong to the cystovarian type, according to the classification of Hoar (1969) and Connaughton & Katsumi (1998). Two layers of smooth muscle make up the ovarian wall: the inner being formed by circular fibres and the outer by longitudinal ones; these layers contain numerous blood vessels.

Oogenesis

Oocyte development was divided into eight stages, plus oogonia cells. The nucleocytoplasmic ratio (NPR) and the mean oocyte diameter for each developmental stage are given in Table I. Note that as the oocyte grows the NPR decreases. Each of the eight stages of oocyte development, as well as the oogonia cells are described below.

Oogonia (OG): oogonia are small rounded cells grouped in nests of 8 to 11 cells, placed between the more advanced oocytes, in the germinal ridges. The oogonia are characterized by a clear cytoplasm and a large nucleus occupying most of the cellular volume.

Chromatin-nucleolar stage (CN): oocytes in this stage show a rounded shape. The cytoplasm is lightly basophilic, and a large and more basophilic nucleus occupies most of the cell. Some nucleoli, highly basophilic, are randomly distributed in the nucleus (Fig. 1b).

Perinucleolar stage (PN): oocytes in this stage keep their rounded shape. Most of the nucleus has an ovoid shape with numerous spherical nucleoli at their periphery (Figs 1b & 2e). A single layer of elongated cells makes up the oocyte envelope.

Cortical alveolus stage I (CAI): in this stage the cytoplasm is seen as a narrow, dense, deeply stained zone around the nucleus. The nucleoplasm is more or less rough and presents an average of 28 nucleoli at the periphery. Three to four layers of small PAS-positive vesicles appear in the middle cortical zone of the cytoplasm. These vesicles, the cortical alveoli, gradually increase in size and number and move to the centre of the cell (Fig. 1c). Below the flattened layer of granulosa cells a striated zone, weakly stained with HE, can be distinguished.

Cortical alveolus stage II (CAII): the oocytes in this stage undergo an increase in size, and the cortical alveoli occupy the entire cell (Fig. 1c). Larger vesicles are located in the central zone whereas the smaller ones can be seen in the periphery. These spherical structures appear to be empty vacuoles. Some of these vacuoles have inclusions inside, which stain light blue with Mallory's trichromic (Fig. 1d). Lipid droplets are absent. The weakly stained cytoplasm is reduced to the perinuclear zone and to a very narrow band just below the cellular membrane. The outer zona radiata can be distinguished from the inner zona radiata; both are clearly striated (average thickness 4.7 μm) (Fig. 1d).

Yolk stage I (YI): the true vitellogenesis begins in this stage, when small rounded yolk granules appear, among the cortical alveoli, in the outer cortex of the oocyte; they stain deep orange with Mallory's trichromic (Fig. 2a). The yolk granules are larger toward the centre of the cell and smaller at the periphery. The narrow band of cytoplasm persists around the nucleus. The nuclear membrane is folded and the nucleoli are adjacent to it. The zona radiata is markedly striated: the inner zona radiata is thinner (average thickness 2.5 μm) than the outer zona radiata (average thickness 3.1 μm) (Fig. 2a).

Yolk stage II (YII): the increase in the cell volume and in the size of the yolk granules is noticeable in this stage. Larger

Table II. Ovarian maturity scale for *H. spinosus*.

Stage	Macroscopic appearance	Histological features
I . Immature	Ovaries are small. No visible oocytes to the naked eye.	
II. Developing	Ovaries are still small in size, but show a granular aspect.	Previtellogenic oocytes of different sizes as well as oocytes in cortical alveolus stage. In some specimens atretic oocytes are present.
III. Maturing	Ovaries are medium in size and oocytes are visible to the naked eye. Ovarian wall becoming thinner.	Oocytes in all the developmental stages, including in some cases, post-ovulatory follicles.
IV. Ripe	Ovaries reach their maximum size. Large oocytes are clearly visible through the transparent and thin ovarian wall.	Oocytes of two developmental stages and sizes are dominant: oocytes in the last vitellogenic stage and mature ones, and another small clutch of previtellogenic oocytes.
V. Spawned	Ovaries are flaccid and reduced in size; the ovarian wall looks thicker.	Post-ovulatory follicles and oocytes in the first developmental stages (chromatin- nucleolar and perinuclear stages).

yolk granules are found near the nucleus. The cortical alveoli are reduced in number and size, and some of them are very small and accumulate in the outer cortex (Fig. 2b). The nucleus maintains its central position and the nucleoli are adjacent to the membrane. Both the inner and outer zona radiata have almost the same thickness (average thickness 4.0 and 4.1 μm, respectively). Over the zona radiata, a flat layer of granulosa cells with a conspicuous nucleus can be observed.

Yolk stage III (YIII): the yolk granules from the inner zone fuse to form plates, whereas in the cortical zone the small and rounded granules alternate with a few cortical alveoli (Fig. 2c). The cytoplasm of the oocyte in this stage is very scarce and is reduced to a perinuclear zone and to a thin cortical layer. The nucleus becomes irregularly shaped and is displaced from the centre of the cell; the nucleoli still appear at its periphery. The inner zona radiata stains deep red with Mallory's trichromic and is wider (average thickness 9.6 μm) than the outer zona radiata (average thickness 2.6 μm) that stains blue with the same stain.

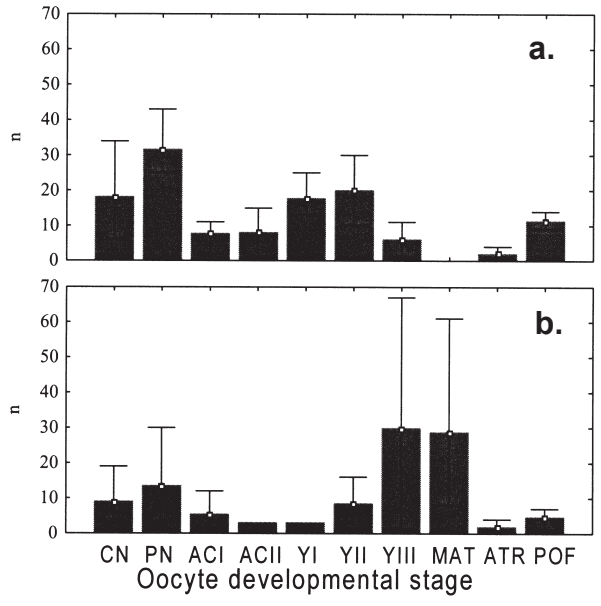

Fig. 3. Frequency distribution of oocytes in different developmental stages. **a.** Ovarian maturity stage III (five females), **b.** Ovarian Maturity stage IV (eight females). Oocyte developmental stages: CN = chromatin-nucleolar, PN = perinucleolar, CAI & CAII = cortical alveolus I and II respectively, YI, YII & YIII = yolk I, II and III respectively, MAT = mature, ATR = atretic, POF = post-ovulatory follicles. Middle point: mean; whisker value: min–max.

Mature (MAT): at this stage, the nucleus has disintegrated and the yolk granules are totally fused forming plates (Fig. 2d). The zona radiata is very prominent. The inner zona radiata is much thicker (average thickness 9.8 μm) than the outer (average thickness 2.0 μm).

Post-ovulatory follicles (POF): the follicle presents a much folded shape and granular material is present in its lumen (Fig. 2e). Granulosa cells are wider and the nucleus is very prominent and exocentric.

Atretic oocytes (ATR): the oocyte acquires an irregular shape, the zona radiata becomes fragmented at several points and the granulosa cells invade the cell. In the interior of the cell, different kind of unknown material can be observed; these present diverse affinities and in some of them, yolk granules can also be observed (Fig. 2f).

Ovarian maturity scale

Histological observation of the ovaries and the frequency distribution of the oocyte developmental stages leads to an accurate demarcation of the various stages. Five maturity stages of the ovary were identified, as it is shown in Table II. From the fifteen females analysed, most were in stages III (five specimens) and IV (eight specimens) of gonad maturity. One female was in stage II and another one was in post-spawning condition (stage V). The mean oocyte frequency distributions for ovaries in stages III and IV are shown in Fig. 3. Segregation between the most advanced

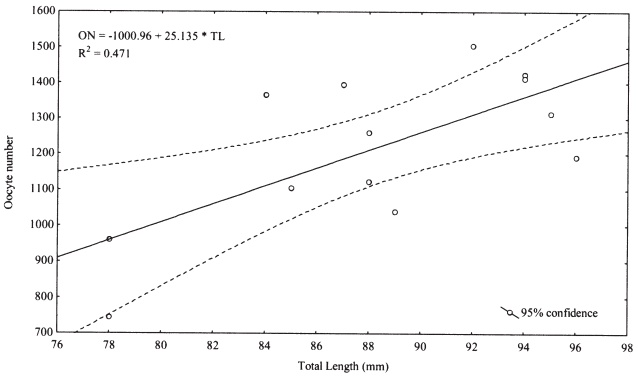

Fig. 4. Regression of Total Length against Absolute Fecundity. ON = oocyte number.

oocytes and those in the first stages of development can be distinguished.

Fecundity

Absolute fecundity was estimated for thirteen females in stages III and IV of ovarian maturity. The total length of the specimens studied for the fecundity analysis ranged from 78 to 96 mm. The absolute fecundity was between 787 and 1504 eggs per female, with a mean of 1227. Regression of the oocyte number on TL showed that a linear model would account for almost 47% of the fecundity variation (Fig. 4).

Discussion

Both ovaries of *H. spinosus* are functional, and they are of cystovarian type. This is the most common type of ovary in teleosts (Hoar 1969, Connaughton & Katsumi 1998).

Despite the numerous adaptations shown by the Antarctic ichthyofauna (see Eastman 1993, 2000, and Clarke & Johnston 1996, for a review) such as the antifreeze proteins or the reduction of blood haemoglobin, no distinctive characteristics have been observed in oocyte development. Two clutches of oocytes can be distinguished in the maturing ovaries; one more advanced (vitellogenic oocytes) which are to be ovulated in the current spawning season, and another group of less developed oocytes. The second clutch, composed of oocytes in the chromatin-nucleolar, perinucleolar and cortical alveoli stages, is the reserve stock for the next spawning season. This kind of oocyte development is similar to that reported for many Antarctic fish (Kock & Kellermann 1991). Both clutches have a synchronous development.

Production of demersal eggs is a common feature in Antarctic species (Kock 1985). Considering the absence of lipid droplets and the thickness of the chorion, the eggs of *H. spinosus* are probably demersal. According to Potts (1984) the production of demersal eggs is a fundamental step in the evolution of parental care in fish.

Absolute fecundity of *H. spinosus* is positively correlated with total length (Fig. 4). Correlation of absolute fecundity with fish length and weight is generally accepted, and according to Kock & Kellermann (1991) Antarctic fish are no exceptions to this rule. For example Calvo *et al.* (1999) found a positive correlation between absolute fecundity and fish total length in *Champsocephalus esox* Günther, 1861. Examination of mature ovaries show that absolute fecundity varied between 743 and 1504 eggs. Prirodina (2002) counted 1500 eggs from an *H. spinosus* female in stage III–V, and in *H. antarcticus* Nybelin, 1947 the absolute fecundity ranged from 1113 to 1522 (White & Burren 1992). It is possible that low fecundity of harpagiferids is related to parental care and nesting behaviours. This kind of behaviour has selective advantages among species that spawn a small number of eggs because of the protection offered from predators, dispersal to unsuitable habitats and infection (White & Burren 1992).

Nesting has been described in other members of the genus, for example in *H. bispinis* from Arthur Harbour in the Antarctic Peninsula (Daniels 1978) and in *H. antarcticus* from South Orkney Islands (White & Burren 1992). These species lay their eggs in nests, generally built in the ocean floor or in rocky caves, and both the male and the female care for the eggs. Considering the low absolute fecundity and the behaviour showed by these closely related species, it is likely that *H. spinosus* adopt the same reproductive strategy.

In their study of the reproduction and larval growth, White & Burren (1992) found that female *H. antarcticus* produced a single clutch of eggs. It is usually assumed that Antarctic notothenioids spawn once a year and lay eggs in a single batch (Everson 1984, Kock 1985). To date there are no records of Antarctic fish with intermittent or fractional spawning. However, we found that *H. spinosus* may display a different strategy. It is interesting to note that in the stage III ovaries of *H. spinosus* studied here, the presence of post-ovulatory follicles was frequent, indicating recent ovulation. According to De Vlaming (1983) separate ovulatory events may result in accumulation of a stock of eggs that could be spawned as a single batch or in fractions. The presence of post-ovulatory follicles in the maturing ovaries of *H. spinosus* may reflect this phenomenon. Fractional spawners spawn a part of an ovulated clutch at intervals over a relatively short period (De Vlaming 1983). Thus there is the possibility that this species is a fractional spawner. A more exhaustive study is needed to explain the presence of post-ovulatory follicles, because both hypotheses - separate ovulatory events or fractional spawning - could explain the presence of these structures in maturing ovaries.

According to Everson (1984), the spawning season in Notothenioidei is generally of limited duration. However in harpagiferids a 2–3 month period is typical (Daniels 1978, White & Burren 1992). Spawning of *H. antarcticus* occurs

from May to July in the South Orkney Islands (White & Burren 1992), and in the Antarctic Peninsula spawning of *H. bispinis* occurs slightly later, between June and August (Daniels 1978). In agreement with these reports we found gravid females ready to spawn from February to April, indicating a protracted spawning season of at least three months. At Îles Crozet the spawning season is earlier than those found for the other species of *Harpagifer*. Nevertheless differences in the time of spawning between localities are common phenomena in the Antarctic ichthyofauna (Kock & Kellermann 1991).

In the other species of this genus hatching occurs 3–4 months after spawning (Daniels 1978, White & Burren 1992), and the pelagic larvae are ready to feed when the phyto- and zooplankton are most abundant. This supports the idea of a close link between the seasonal production and the breeding cycles of the Antarctic ichthyofauna. Further studies on the hatch and larval growth are needed for more complete understanding of the reproductive cycle of *H. spinosus*.

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References

ANDRIASHEV, A.P. 1965. A general review of the Antarctic fish fauna. *In* VAN OYE, P. & VAN MIEGHEM, J., *eds. Biogeography and ecology of Antarctica.* The Hague: Junk, 491–550.

BAGENAL, T.B. 1978. Aspects of fish fecundity. *In* GERKING, S.D., *ed. Ecology of freshwater fish production*. Oxford: Blackwell Scientific Publications, 75–101.

BOWERS, M.J. 1992. Annual reproductive cycle of oocytes and embryos of yellowtail rockfish *Sebastes flavidus* (Family Scorpaenidae). *Fishery Bulletin*, **90**, 231–242.

CALVO, J., MORRICONI, E. & RAE, G.A. 1999. Reproductive biology of the icefish *Champsocephalus esox* (Günther, 1861) (Channichthyidae). *Antarctic Science*, **11**, 140–149.

CLARKE, A. 1988. Seasonality in the Antarctic marine environment. *Comparative Biochemistry and Physiology*, **90B**, 461–473.

CLARKE, A. & JOHNSTON, I.A. 1996. Evolution and adaptative radiation of Antarctic fishes. *Trends in Ecology and Evolution*, **11**, 212–218.

CONNAUGHTON, M.A. & KATSUMI, A. 1998. Female reproductive system, fish. *In* KNOBIL, E. & NEILL, J.D., *eds. Encyclopedia of reproduction*. London: Academic Press, 193–204.

DANIELS, R.A. 1978. Nesting behaviour of *Harpagifer bispinis* in Arthur Harbour, Antarctic Peninsula. *Journal of Fish Biology*, **12**, 465–474.

DANIELS, R.A. 1979. Nest guard replacement in the Antarctic fish *Harpagifer bispinis*: possible altruistic behaviour. *Science*, **205**, 831–833.

DE VLAMING, V. 1983. Oocyte development patterns and hormonal involvement among Teleosts. *In* RANKIN, J.C., PITCHER, J.J. & DUGGAN, R., *eds. Control processes in fish physiology*. London: Croom Helm, 176–199.

DEWITT, H.H. 1971. Coastal and deep-water benthic fishes of the Antarctic. *Antarctic Map Folio Series*, **15**, 1–10.

EAKIN, R.R. 1981. Osteology and relationships of the fishes of the Antarctic family Harpagiferidae (Pisces, Notothenioidei). *Antarctic Research Series*, **31**, 81–147.

EASTMAN, J.T. 1993. *Antarctic fish biology: evolution in a unique environment*. San Diego: Academic Press, 1–322.

EASTMAN, J.T. 2000. Antarctic notothenioid fishes as subjects for research in evolutionary biology. *Antarctic Science*, **12**, 276–287.

EVERSON, I. 1977. *The living resources of the Southern Ocean*. Rome: FAO/UN Development Programme, 156 pp.

EVERSON, I. 1984. Fish biology. *In* LAWS, R.M., *ed*. *Antarctic ecology*, vol. 2. London: Academic Press, 491–532.

FORBERG, K.G. 1982. A histological study of development of oocytes in capelin, *Mallotus villosus villosus* (Müller). *Journal of Fish Biology*, **20**, 143–154.

HINTON, D.E. 1990. Histological techniques. *In* SCHRECK, C.B. & MOYLE, P.B., *eds*. *Methods for fish biology*. Bethesda, MD: American Fisheries Society, 191–211.

HOAR, W.S. 1969. Reproduction. *In* HOAR, W.S. & RANDALL, D.J., *eds*. *Fish physiology*, vol. III. London: Academic Press, 1–72.

HUREAU, J.-C. 1990. Harpagiferidae. *In* GON, O. & HEEMSTRA, P.C., *eds*. *Fishes of the Southern Ocean*. Grahamstown, South Africa: JLB Smith Institute of Ichthyology, 350–363.

KOCK, K.-H. 1985. Marine habitats: Antarctic fish. *In* BONNER, W.N. & WALTON, D.H.W., *eds*. *Key environments: Antarctica*. Oxford: Pergamon Press, 173–192.

KOCK, K.-H. 1989. Reproduction in fish around Elephant Island. *Archiv für Fischereiwissenschaft*, **39**, 171–210.

KOCK, K.-H. & KELLERMANN, A. 1991. Review: reproduction in Antarctic notothenioid fish. *Antarctic Science*, **3**, 125–150.

MARTOJA, R. & MARTOJA, P.M. 1970. *Técnicas de histología animal*. Barcelona: Ediciones Toray, 1–355.

MATALLANAS, J. 1997. Sobre algunos peces con interés biogeográfico de las Islas Orcadas del Sur. *Boletín de la Real Sociedad Española de Historia Natural (Sección Biología)*, **93**, 87–92.

NORTH, A.W. & WHITE, M.G. 1987. Reproductive strategies of Antarctic fish. *In* KULLANDER, S.O. & FERNHOLM, B., *eds. Proceedings of the Vth Congress of European Ichthyologists, Stockholm 1985*. Stockholm: Swedish Museum of Natural History, 381–391.

POTTS, G.W. 1984. Parental behaviour in temperate marine teleost with special reference to the development of nest structures. *In* POTTS, G.W. & WOOTON, R.J., *eds*. *Fish reproduction: strategies and tactics*. London: Academic Press, 223–244.

PRIRODINA, V.P. 2000. On the systematic position of littoral and deep-water species of the genus *Harpagifer* (Harpagiferidae, Notothenioidei) from Macquarie Island with a description of two new species. *Journal of Ichthyology*, **40**, 488–494.

PRIRODINA, V.P. 2002. Redescription of littoral and deep-sea species of the genus *Harpagifer* (Harpagiferidae, Notothenioidei) off islands of the Indian Ocean sector of the Southern Ocean with the description of a new species. *Journal of Ichthyology*, **42**, 701–712.

WEST, G. 1990. Methods for assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwater Research*, **41**, 199–222.

WHITE, M.G. & BURREN, P.J. 1992. Reproduction and larval growth of *Harpagifer antarcticus* Nybelin (Pisces, Notothenioidei). *Antarctic Science*, **4**, 421–430.