

Gametogenesis in the dragonfishes *Akarotaxis nudiceps* and *Bathydraco marri* (Pisces, Notothenioidi: Bathydraconidae) from the Ross Sea

MARIO LA MESA¹*, VINCENZO CAPUTO² and JOSEPH T. EASTMAN³

¹ISMAR-CNR, Istituto di Scienze Marine, Sede di Ancona, Largo Fiera della Pesca, 60125 Ancona, Italy

²Istituto di Biologia e Genetica, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

³Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, Athens, OH 45701-2979, USA

*m.lamesa@ismar.cnr.it

Abstract: We analysed histological characteristics of gonads and reproductive effort of the small deep-living dragonfishes *Akarotaxis nudiceps* (Waite) and *Bathydraco marri* Norman collected in the south-western Ross Sea. From a macroscopic point of view, most specimens of *B. marri* were juveniles in early stages of gonad maturity, except for a maturing female. Conversely, the sample of *A. nudiceps* was composed of both immature and adult fish in different stages of maturity. A single *A. nudiceps* female was mature with a gonadosomatic index of 9.8%. Its absolute and relative fecundity was 260 oocytes and 31.5 oocytes g⁻¹ TW, respectively, with a mean size of ripe oocytes of 1.9 mm. Gametogenesis in both species closely resembled that observed in other notothenioids, with females possessing two well-defined groups of oocytes. One group consisted of previtellogenic oocytes as a reserve stock while the other group was maturing oocytes to be ovulated in the current spawning season. A distinctive feature of oogenesis in recovering and maturing females of *A. nudiceps* was the presence of both postovulatory follicles in different stages of reabsorption and atretic oocytes. Based on low absolute fecundity, it is possible that *A. nudiceps* provides parental care and egg guarding.

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Introduction

The Bathydraconidae, commonly known as dragonfishes, are a group of small benthic notothenioid fishes endemic to the Southern Ocean. This family is composed of 11 genera, mostly monospecific, and 16 species (Eastman & Eakin 2000). Most species are found over the Antarctic continental shelf and slope, but some are known from Antarctic and sub-Antarctic islands as well. They are mostly bottom dwellers distributed over a wide depth range, from shallow, inshore waters to about 3000 m (Gon 1990, Kock 1992). Although dragonfishes currently have no commercial value, they play an important role in the Antarctic ecosystem, representing the intermediate level in the local trophic web (Van der Molen & Matallanas 2003).

Dragonfishes are generally most common and attain their greatest diversity in the coldest and deepest shelf waters of Antarctic high latitudes (Schwarzbach 1988, Kock 1992, Eastman 1993). Indeed, more than 70% of known dragonfishes species inhabit the cold waters of the Ross Sea continental shelf, where they account for about 10–20% of the catch by bottom trawls (Eastman & Hubold 1999, Donnelly *et al.* 2004). However, despite the importance and wide distribution of dragonfishes, many aspects of their biology are still poorly known (Gon 1990).

The genus *Bathydraco* is composed of five species of

small fishes inhabiting relatively deep water from 500 to over 2500 m. *Bathydraco macrolepis* Boulenger, *B. marri* and *B. scotiae* Dollo are found in East Antarctica, and particularly on the continental shelf of the Ross Sea, except for *B. scotiae*, which has been collected only in deep waters of continental slope (> 2000 m). A similar pattern of distribution is also shown by *Akarotaxis nudiceps*, previously assigned to the genus *Bathydraco* (as *B. nudiceps* and *B. wohlshlagi*) due to their morphological similarity (Waite 1916, DeWitt & Tyler 1960).

Compared to most of the dragonfishes, the biology of species belonging to the genera *Bathydraco* and *Akarotaxis* is almost completely unknown (Gon 1990), probably as a consequence of their relatively low abundance and deep habitat. To date, the available literature on these species has focused on feeding habits and reproductive biology, although sample sizes were small and drawn exclusively from the Weddell Sea (Schwarzbach 1988, Kock & Kellermann 1991, Hubold 1992). In addition, studies dealing with reproduction were based exclusively on the macroscopic morphology of gonads during or in close proximity to the spawning season of each species.

A recent trawl survey in the south-western Ross Sea yielded a considerable number of specimens of *B. marri* and *A. nudiceps*, which accounted for 10.4% and 3.2% of total

catch, respectively (Eastman & Hubold 1999). Conducting a histological analysis on the gonads of this relatively large sample, we report data on the reproductive biology of these poorly known species, providing further insight into the reproductive strategies of endemic Antarctic dragonfishes.

Materials and methods

The samples of *A. nudiceps* and *B. marri* analysed in the present study were collected during cruises 96-6 and 97-9 of the RV *Nathaniel B. Palmer*, in the south-western Ross Sea between 11 December 1996–8 January 1997 and 20 December 1997–10 January 1998, respectively. Fishes were caught using both an otter trawl and a smaller Blake trawl. Further details on sampling data for the two species are reported in Eastman & Hubold (1999).

After sorting, dragonfishes were identified according to Gon (1990) and preserved in formalin. In the laboratory, total length (TL) and standard length (SL) were measured to the nearest millimetre below and total weight (TW) recorded (g) for each specimen. After dissection, specimens were sexed and their gonads were weighed to 0.1 g. The maturity stage of gonads was preliminarily assessed according to a five-point scale for Antarctic fish (Everson 1977, Kock & Kellermann 1991). When possible, the gonadosomatic index (GSI) was calculated as the percentage of gonad weight to total weight of the fish. In gravid females (i.e. stage 4 of Everson's scale), absolute fecundity, defined as total number of ripe eggs found in gonads prior to the next spawning period, was calculated by counting all eggs in the ovaries, and relative fecundity (eggs g^{-1} of total weight) subsequently derived (Kartas & Quignard 1984). Finally, the mean size of ripe eggs was determined by measuring the maximum diameter (0.1 mm) of 20 oocytes representing as much as possible of the size range of eggs observed in the ovary (West 1990).

Gonad samples for histological studies were fixed in Bouin's solution for 12 h, dehydrated through increasing concentrations of ethanol and embedded in paraffin. Histological sections (7 μ m thick) were cut, mounted on slides and stained with Mayer's haematoxylin-eosin and Galgano's trichrome (Beccari & Mazzi 1972). Sections were examined with a Nikon Eclipse 800 optical microscope at magnifications of 40–400x. On the basis of histological appearance and cell structure, ovarian follicles were classified in the following development stages: I. chromatin nucleolar (immature), II. perinucleolar (immature), III. yolk vesicle or cortical alveoli formation (early maturation), IV. vitellogenic (late maturation), V. mature, VI. postovulatory follicle (post-reproductive) (Wallace & Selman 1990, West 1990). To determine the oocyte developmental pattern throughout the ovary (De Vlaming 1983), an analysis of the stage/size frequency distribution was performed by counting oocytes at different stages of maturity in five sections, taken at 1 mm intervals,

for each ovary. As notothenioids generally exhibit "group synchronous ovaries" (Wallace & Selman 1981, Kock & Kellermann 1991), each specimen was staged on the basis of the most advanced histological stage of development observed in the ovary sections. For each stage of development, cellular and nuclear diameters (μ m) were measured on approximately 20 oocytes under a light microscope coupled to a CCD video camera using image analysis software (Nikon Lucia 4.51). Finally, the nucleocytoplasmic index (NP) was calculated as follows: $NP = V_n (V_c - V_n)^{-1}$, where V_n is the nuclear volume and V_c is the cellular volume.

The spermatogenic activity of males was assessed by the evaluation of different types of gametocytes (i.e., from spermatogonia to spermatozoa) in the seminiferous lobules of each testis. The presence of spermatogonial mitoses was also recorded. The maturity of testes was estimated according to a five-point scale (Billard 1986): I. immature stage (presence of spermatogonia and spermatogonial mitoses), II. early development stage (first meiotic division), III. advanced development stage (second meiotic division), IV. mature stage (presence of spermatozoa cysts), V. post-reproductive stage (presence of collapsed lobules and residual spermatozoa).

Results

Fish data

We examined gonadal histology in 41 specimens of *B. marri* and 25 specimens of *A. nudiceps*. The sample of *B. marri* consisted of 24 females between 100–166 mm TL and between 2.1–12.4 g TW and 17 males between 91–143 mm TL and between 1.7–7.9 g TW. The sex ratio was not significantly different from unity ($\chi^2 = 1.19$, $df = 1$, $P > 0.1$). The sample of *A. nudiceps* included 14 females between 96–133 mm TL and between 3.4–8.2 g TW and 11 males between 95–123 mm TL and between 2.9–6.6 g TW. The sex ratio was not significantly different from unity ($\chi^2 = 0.36$, $df = 1$, $P > 0.5$).

Reproductive effort and macroscopic appearance of gonads

As a measure of the reproductive effort of each species, we calculated the GSI in both sexes as well as fecundity and size of ripe eggs in gravid females. Unfortunately, the sample of *B. marri* consisted almost exclusively of juveniles, whose gonads were too small to be reliably weighed. Macroscopically, males and females were all staged as immature (stage 1, Everson 1977). Considering the small size, only one female of *B. marri* was probably a maturing virgin (stage 2), attaining a GSI of about 0.5%.

As for *A. nudiceps*, three males (95–108 mm TL) were immature (stage 1), and their gonads too small to provide a reliable weight. All other males (8 specimens) were in resting stage (stage 2), with a GSI between 0.2–0.5% (mean

0.4%). Most females (8) were in recovering stage (stage 2), with a GSI range of 0.3–0.7% (mean 0.5%). Two females were small (96–101 mm TL) and immature (stage 1), with very small ovaries (weight not recorded). Three other specimens were in maturing stage (stage 3) and their GSI ranged between 0.6–0.7% (mean 0.7%). A single large female (133 mm TL) was gravid (stage 4), attaining a GSI of 9.8%. Its absolute and relative fecundity were 260 oocytes and 31.5 oocytes g^{-1} TW, respectively, with a mean size of mature oocytes of 1.9 mm (range 1.6–2.0 mm).

Spermatogenesis and testicular structure

As observed in other notothenioids (Rae & Calvo 1996, Calvo *et al.* 1999, Russo *et al.* 2000, La Mesa *et al.* 2003, 2006), testes of both species had a cystic lobular pattern, with long finger-like lobules. The paired testes, dorsally situated near the kidneys, had a thick wall composed of connective tissue fibres and muscle tissue (i.e. the *tunica albuginea*). The inner structure of testes in *A. nudiceps* closely resembled that of most Perciformes, described as “unrestricted spermatogonial type” (Grier *et al.* 1980), and consisted of spermatogonia randomly distributed along the entire length of lobules.

The histological analysis of testes of both species allowed us to describe only the early stages of spermatogenesis, as most specimens were small juveniles. The stages of maturity were:

I immature stage - testes very small and uniformly consisting of spermatogonial cysts densely filled with spermatogonia, characterized by weakly-stained cytoplasm and a large spherical nucleus (Fig. 1a). Evidence of some spermatogonia in mitosis. Small and fusiform Leydig cells irregularly located around the spermatogonial cysts. Inner lumina of lobules fully occupied by cysts of spermatogonia.

II early development stage - testes of small size, with a less dense inner structure than in the previous stage. Lobules consisting of dividing spermatogonia cysts, as well as of several cysts of primary spermatocytes in first meiotic division (pachytene stage) (Fig. 1b).

Oogenesis and ovarian structure

In both dragonfishes, ovaries were paired subcylindrical structures oriented cranio-caudally between the kidneys and distal intestine. According to Hoar (1969), the ovaries are cystovaries, a classification characterizing most teleosts, with the ovarian lumen in continuity with the oviduct. The ovarian wall was formed by a peritoneal epithelium and two layers of smooth muscle tissue.

On the basis of the histological appearance of gonads, we recognized these stages of oogenesis:

I chromatin nucleolar stage - oocytes in this stage

showed deeply stained cytoplasm and a central rounded nucleus occupying most of cell (Fig. 2a & b). Their size range was 29.6–56.2 μm in *B. marri* and 19.3–52.8 μm in *A. nudiceps*.

II perinucleolar stage - at this stage, oocytes were increasing in size and had a lightly basophilic cytoplasm. The nucleus was still spherical and characterized by a large number of nucleoli located at its periphery (Fig 2a & b). The size range of these oocytes was 115.3–193.1 μm in *B. marri* and 59.6–139.6 μm in *A. nudiceps*.

III cortical alveoli formation - this stage was characterized by oocytes starting primary or endogenous vitellogenesis. With respect to the previous stages, oocytes showed a steady increase in size, reaching 168.6–222.4 μm in *B. marri* and 155.4–459.5 μm in *A. nudiceps*. Their slightly stained cytoplasm contained a series of rows of vesicles in the region

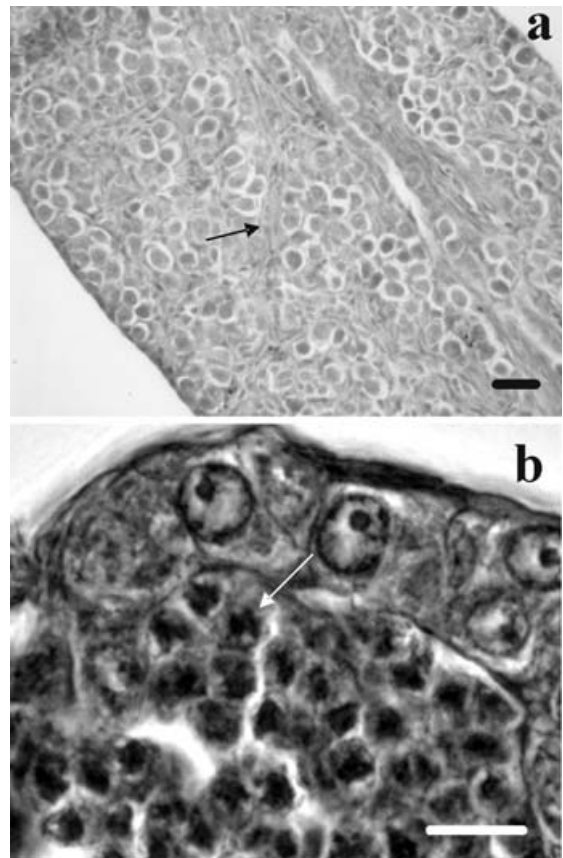


Fig. 1. Early stages of spermatogenesis in dragonfishes from the south-western Ross Sea. **a.** *Bathydraco marri*, immature stage (I), showing lobules filled with spermatogonia and fusiform Leydig cells irregularly located around the spermatogonial cysts (arrow); scale bar = 15 μm . **b.** *Akarotaxis nudiceps*, early development stage (II), showing lobules with spermatogonia and several cysts of primary spermatocytes in first meiotic division (stage of pachytene) (arrow); scale bar = 10 μm .

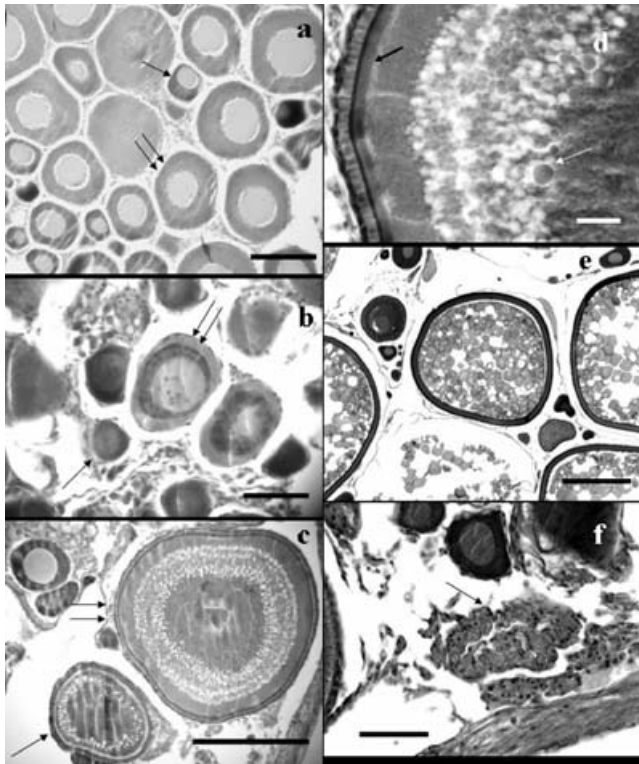


Fig. 2. Oogenesis of dragonfishes sampled in the south-western Ross Sea. **a.** *Bathhydraco marri*, chromatin nucleolar stage (I), showing oocytes with a deeply stained cytoplasm (arrow) and perinucleolar stage (II), showing oocytes with a lightly basophilic cytoplasm and a spherical nucleus with several nucleoli located at its periphery (double arrows); scale bar = 45 μ m. **b.** *Akarotaxis nudiceps*, as above; scale bar = 30 μ m. **c.** *A. nudiceps*, cortical alveoli stage (III), showing an oocyte in endogenous vitellogenesis, characterized by cytoplasm filled with vesicles (i.e. cortical alveoli) close to the plasma membrane (arrow) and exogenous vitellogenic stage (IV), showing an oocyte starting vitellogenesis (double arrows); scale bar = 350 μ m. **d.** *A. nudiceps*, detail of a vitellogenic oocyte, showing the formation of yolk granules in the cytoplasm (arrow) and the zona radiata (black arrow); scale bar = 30 μ m. **e.** *A. nudiceps*, mature stage, showing ripe oocytes in which the coalescence of yolk granules gives rise to yolk plates; scale bar = 600 μ m. **f.** *A. nudiceps*, postovulatory follicle stage (VI), showing a follicle with a reduced lumen (arrow) found in a maturing female; scale bar = 100 μ m

adjacent to the plasma membrane. These vesicles, the cortical alveoli, gradually increased in size and number and tended to migrate towards the nucleus so as to occupy the entire cytoplasm (Fig. 2c). At the same time, a thin zona radiata (4 μ m) began to develop in the periphery of these oocytes.

IV vitellogenic stage - zona radiata 10 μ m, yolk deposition begins from the periphery of the oocyte in the form of yolk granules of different size (5–12 μ m) (Fig. 2c & d); the nucleus is still in a central position within the oocyte.

Table I. Morphological characteristics of oocytes at different stages of development in *Akarotaxis nudiceps* (above) and *Bathhydraco marri* (below) from the south-western Ross Sea. mean value \pm standard error; NP = nucleo-plasmic index; I = chromatin nucleolar, II = perinucleolar, III = yolk vesicle, IV = vitellogenic, V = mature.

Stage	Cellular diameter (μ m)	Nuclear diameter (μ m)	NP
I	40.3 \pm 2.1	27.8 \pm 1.5	0.49
II	90.2 \pm 4.1	51.9 \pm 1.9	0.23
III	281.6 \pm 17.1	116.1 \pm 3.4	0.07
IV	523.3 \pm 11.0	137.5 \pm 5.4	0.02
V	1542.7 \pm 124.8	-	-
I	48.9 \pm 2.4	24.7 \pm 1.9	0.15
II	152.8 \pm 7.5	81.8 \pm 4.3	0.18
III	187.3 \pm 5.0	85.3 \pm 5.5	0.10

V mature - at this stage, the nucleus was completely dissolved and the coalescence of yolk granules gives rise to yolk plates completely filling the oocytes (Fig. 2e). The thickness of the zona radiata increases to an average of 50 μ m. An inner and outer zona radiata are easily distinguished. In *A. nudiceps*, the size of mature oocytes was 1463–1941 μ m.

VI postovulatory follicle - the follicle was convoluted and folded, some with a reduced lumen and in different states of reabsorption (Fig. 2f).

For each stage of oocyte development, the mean values of cellular and nuclear diameters, as well as the nucleoplasmic index (NP), are summarized in Table I.

Maturity scales

Analysing the histological sections of ovaries, we obtained

Table II. Maturity scale for *Akarotaxis nudiceps* collected in the south-western Ross Sea.

Macroscopic stage of maturity	Histological features
Females	
1. Immature	clusters of oogonia, chromatin nucleolar and perinucleolar oocytes (stages I and II)
2. Recovering	some oogonia, few basophilic oocytes at stage I and II and dominance of cortical alveolus oocytes (stage III); some postovulatory follicles with lumina and atretic oocytes
3. Maturing	oocytes in all developmental stages, i.e. previtellogenic (stages I and II), endogenous (stage III) and exogenous (stage IV) vitellogenic oocytes; in some cases, postovulatory follicles in reabsorption and atretic oocytes
4. Mature	a few previtellogenic oocytes, mature oocytes free in the ovarian lumen (stage V) filling the ovaries
Males	
1. Immature	cysts of spermatogonia filling the entire lobule (stage I); some spermatogonia in mitotic activity
2. Developing	seminiferous lobules with cysts of spermatogonia and spermatocytes I (stage II)

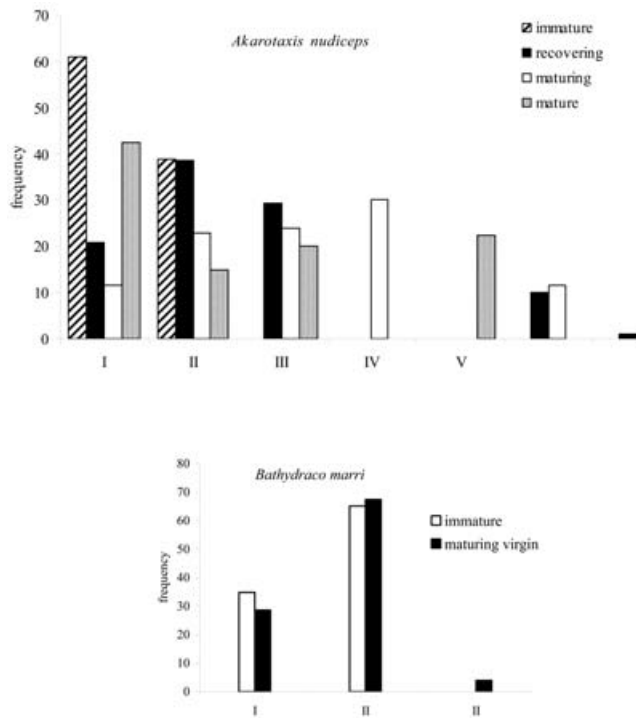


Fig. 3. Frequency distribution of oocytes at different stages of development in gonads of dragonfishes sampled in south-western Ross Sea. Stage of development: I = chromatin-nucleolar, II = perinucleolar, III = cortical alveoli formation, IV = vitellogenic, V = mature, VI = postovulatory follicle, at = atretic oocytes.

the frequency distribution of oocytes at different stage of development, respectively, in gonads of immature, developing, maturing and mature females (Fig. 3). In addition, based on histological observations and macroscopic appearance of gonads, we provide a maturity scale for females and males (partially) of *A. nudiceps* (Table II) and *B. marri* (Table III).

Discussion

Most data concerning the reproductive characteristics of dragonfishes are derived from specimens collected off South Georgia and in the Weddell Sea (Kock & Kellermann 1991). Despite the variability observed among the species investigated some common macroscopic reproductive

Table III. Maturity scale for *Bathhydraco marri* collected in the south-western Ross Sea.

Macroscopic stage of maturity	Histological features
Females	
1. Immature	cysts of oogonia, chromatin nucleolar and perinucleolar oocytes (stages I and II)
2. Maturing virgin	some oogonia, dominance of basophilic oocytes at stage I and II, few oocytes at cortical alveolus stage (stage III)
Males	
1. Immature	cysts of spermatogonia filling the entire lobule (stage I); some spermatogonia in mitotic activity

features of dragonfishes can be seen: high reproductive effort of females (mean GSI between 10% and 30%), from medium to large yolky eggs (1.5–3.5 mm) and relatively low absolute fecundity (200–10000 eggs). On the other hand, histological studies on gametogenesis of dragonfishes are scarce and only include a few species, such as *Gymnodraco acuticeps* and *Gerlachea australis* (Van der Molen & Matallanas 2003, Motta *et al.* 2005).

Unfortunately, except for a maturing virgin female, all specimens of *B. marri* sampled in the south-western Ross Sea were juveniles of small size (< 15 cm TL), not approaching sexual maturity. Only a single maturing female of 16.6 cm TL (14.6 cm SL) had attained sexual maturity, with oocytes of about 200 µm at the beginning of endogenous vitellogenesis. However, this specimen would probably spawn in the next season at a larger size, considering the current low GSI (about 0.5%). This is in good agreement with literature data, indicating that *B. marri* approaches a length at sexual maturity of 18–19 cm TL (see Table IV). Interestingly, taking into account the maximum length of *B. marri* reported to date (23 cm SL, Gon 1990, Miller 1993), we can deduce that this species attains sexual maturity at approximately 70–75% of its maximum size.

Fortunately, the sample of *A. nudiceps* collected in the south-western Ross Sea allowed us to determine most of their gametogenetic cycle, at least for females. From a histological perspective, oogenesis and ovarian maturation observed in *A. nudiceps* closely resembled that of other notothenioids and more generally of other teleosts, although the timing of events is different. Similarly, the structure of

Table IV. Reproductive characteristics of *Akarotaxis nudiceps* and *Bathhydraco marri* mature females from the High-Antarctic Zone.

Species	Size range TL (cm)	GSI	Egg size (mm)	Spawning period	Absolute fecundity	Relative fecundity	Site	Source
<i>Akarotaxis nudiceps</i>	13.3	9.8	1.6–2.0	summer	260	31.5	Ross Sea	1
	-	9.8–12.2	1.9–2.5	summer	200	16.2	Weddell Sea	2
	14.4	-	2.6	autumn	-	-	Weddell Sea	3
<i>Bathhydraco marri</i>	21–24	4.4–8.9*	1.4–1.6	early winter	1549–2208	34.0–46.6	Weddell Sea	4

* the values are referred to females at maturity stage 3. Data source: 1 = present data, 2 = Ekau 1991, 3 = Lisovenko 1987, 4 = Duhamel *et al.* 1993.

the paired ovary of both investigated dragonfishes, defined as cystovarian type (Hoar 1969), is the most widely represented type in teleosts. As a common feature reported in several species of notothenioids (Rae & Calvo 1996, Calvo *et al.* 1999, Van der Molen & Matallanas 2003, 2004, La Mesa *et al.* 2003, 2006), *A. nudiceps* also showed asynchronous oogenesis which is typical of those fish spawning once in each reproductive season. Indeed, considering the size and frequency distribution pattern of oocytes observed in gonad sections of maturing females (see Fig. 3), two distinct groups of oocytes can be distinguished: one group of previtellogenic oocytes composed of cells in the chromatin nucleolar, perinucleolar and cortical alveoli stages, constituting the reserve stock for the next spawning season, and a second group of oocytes which undergo vitellogenesis and will be ovulated in the current spawning season.

Some histological features characterizing the oogenesis of *A. nudiceps*, are also shared with other notothenioids. The presence of postovulatory follicles in different states of reabsorption in recovering and maturing females of *A. nudiceps* have two possible explanations. Postovulatory follicles with an evident lumen found in recovering females (stage 2) suggest a recent spawning of these specimens, which could be assigned to a transition stage between post spawning and recovering. Alternatively, as suggested in some species of *Trematomus* (Butskaya & Faleeva 1987), postovulatory follicular reabsorption in *A. nudiceps* is a slow process lasting several months. The presence of postovulatory follicles in maturing females has also been reported in *Harpagifer spinosus* (Van der Molen 2003, Van der Molen & Matallanas 2004), which was tentatively described as a fractional spawner.

Evidence of atresia or oocyte reabsorption was found in both recovering and maturing females of *A. nudiceps*. This phenomenon is also quite common in Antarctic notothenioids (Calvo *et al.* 1999, La Mesa *et al.* 2003, Van der Molen & Matallanas 2003, 2004, Vanella *et al.* 2005), probably playing an important role in the size-segregation of oocyte batches at early and advanced maturation stages through the selective reabsorption of oocytes of intermediate size (Rae & Calvo 1996). Another interesting feature observed in *A. nudiceps* concerns an apparent delay of vitelline homogenisation in mature oocytes compared to several species of channichthyids, in which this process starts long before oocyte growth is completed (Shandikov & Faleeva 1992).

As far as reproductive effort and spawning season of *A. nudiceps* are concerned, present data are in good agreement with those obtained from the Weddell Sea (Table IV). Compared to other bathydraconids (Kock & Kellermann 1991), and in particular to *B. marri* (Table IV), *A. nudiceps* has the lowest absolute fecundity with only two thousand eggs per female. Thus the existence of parental care and egg guarding is possible in this species, a

reproductive behaviour recently described for the first time in this family in *Gymnodraco acuticeps* (Evans *et al.* 2005). Other than in reproductive effort, *A. nudiceps* and *B. marri* differ also in the spawning season (see table IV), further evidence of relatively different reproductive strategies adopted by each species.

In conclusion, although the general histological and macroscopic patterns of gonad maturation in bathydraconids resemble those observed in other notothenioids, some features such as spawning time and reproductive effort suggest a different life strategy in the two species studied. The possibility of investigating the reproductive biology of other dragonfishes species would provide a more comprehensive picture of reproductive strategies within this poorly known family of Antarctic fish.

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