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ORIGINAL PAPER

Aspects of gonadal morphology in the South Georgian plunderfish *Artedidraco mirus* (Perciformes: Artedidraconidae)

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Abstract Research on reproduction in Antarctic fishes has been primarily focused on evaluation of macroscopic features while microscopic studies have been limited to very few species. In particular no data are available on plunderfishes of the family Artedidraconidae. In order to provide a preliminary description of gonads in the South Georgian plunderfish *Artedidraco mirus* morphological observations on ovary and testis were carried out using both macroscopic and histological approaches. All fishes were collected during the ICEFISH cruise, at South Georgia Island in June 2004. The ovary was a typical hollow structure: from the ovarian wall connective tissue projected into the ovarian cavity forming ovigerous folds where oocytes were located. The testis was of the lobular-type with lobules defined by connective tissue septa within the testicular wall. The presence of specimens showing post-spawning features suggested that the spawning process was being concluded during the sampling period.

The ICEFISH Cruise (International Collaborative Expedition to collect and study Fish Indigenous to Sub-antarctic Habitats) was conducted on board the RVIB Nathaniel B. Palmer in May to July 2004. For further information, please visit www.icefish.neu.edu.

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Introduction

Research on Antarctic fish reproduction has been primarily focused on macroscopic evaluation of maturity, fecundity, egg size and first maturity length (Everson 1984; North and White 1987; Kock and Kellermann 1991; Duhamel et al. 1993; Christiansen et al. 1998) while microscopic studies have been limited to a few species (Everson et al. 1991; Shandikov and Faleeva 1992; Macchi and Barrera Oro 1995; Fusco et al. 1997; Calvo et al. 1999; Eastman and De Vries 2000; Russo et al. 2000; La Mesa et al. 2003, 2006; Van der Molen and Matallanas 2003, 2004; Motta et al. 2005).

The family Artedidraconidae includes four genera (*Artedidraco*, *Dolloidraco*, *Histiodraco* and *Pogonophryne*) and 25 species (Eastman and Eakin 2000). Artedidraconids are an important component of the bottom fauna on the Antarctic continental shelf and upper slope. For example, in the Weddell and Ross seas, they account for 20–23% of species diversity (Hubold 1992; Eastman and Hubold 1999). They have been the subjects of taxonomic studies (Eakin 1977, 1981; Eakin and Kock 1984; Balushkin and Eakin 1998; Eakin and Eastman 1998, 2001; Eastman and Eakin 1999, 2000; Eakin et al. 2001) but their life cycles and reproduction are in general poorly known (Kock and Kellermann 1991; Duhamel et al. 1993; North 2001).

Table 1 Size and morphological data for six males and five females of *Artedidraco mirus*

Fish I.D.	Sex	TL (mm)	SL (mm)	TW (g)	GW (g)	I_G (%)
45/BT24/6	M	64.0	52.5	2.22	0.24	0.72
45/BT24/2	M	76.0	62.5	4.97	0.02	0.40
45/BT24/7	M	77.0	63.4	4.63	0.02	9.50
45/BT24/11	M	81.2	66.6	5.48	0.02	0.36
45/BT24/3	M	88.5	73.0	7.51	0.09	27.52
45/BT24/5	M	93.6	76.0	9.51	0.15	1.58
45/BT24/14	F	68.1	55.0	2.67	0.02	0.94
45/BT24/10	F	69.5	54.7	4.00	0.33	0.83
45/BT24/9	F	74.5	60.6	3.76	0.03	0.80
45/BT24/1	F	82.4	69.8	6.30	0.16	2.54
41/BT22/1	F	97.5	78.5	11.92	1.16	9.73

TL total length; *SL* standard length; *TW* total weight; *GW* gonadal weight; I_G gonadosomatoc index

Our focus here is *Artedidraco mirus* Lönnberg 1905, a species endemic to South Georgia. This small plunderfish has a nearshore and continental shelf distribution (Eakin 1990) and it spawns between May and June (Lönnberg 1905; North 2001). The International Collaborative Expedition to Collect and Study Fish Indigenous to Sub-Antarctic Habitats cruise (ICEFISH) of 2004 (17 May–17 July), designed to improve knowledge of the Sub-Antarctic fish fauna, presented an opportunity to extend our understanding of the reproductive biology of this species using both macroscopic and histological approaches.

Materials and methods

Collection of specimens

We collected 48 specimens of *A. mirus* at five stations near South Georgia (approximately 54°15′S, 36°45′W). These were taken during bottom trawling on the ICEFISH cruise (NBP 04-04) of the RVIB *Nathaniel B. Palmer* from 5 to 12 June 2004. We used a 1.5-m Blake trawl and a 7.6-m otter trawl and towed at a speed of 2.5 knots for 30 min. Bottom depth at these stations was 98–231 m and sea surface temperature was 0.9–1.9°C. After fixation in 10% formalin the specimens were stored by immersion in 70% ethanol.

Macroscopic methods

After fixation each specimen was measured for total length (L_T) and standard length (L_S) to the nearest millimeter and we weighed specimens for the total weight (W_T) and somatic weight (W_S) to accuracy of ± 0.01 g. Specimens were sexed by inspection of the gonads under magnification and macroscopic maturity was assessed according to the Kock and Kellermann scale (1991). We removed gonads from a sub-sample of 11 specimens (6 males and 5 females of different sizes), weighed them (W_G) to an accuracy of ± 0.01 g and the gonadosomatic index ($I_G = 100\ W_G\ W_T^{-1}$) was calculated. Absolute fecundity of the only mature female was determined by hand counting and then relative fecundity (N eggs/W_T) was calculated. The studied specimens are stored in the Italian National Antarctic Museum—Section of Biology in the University of Genoa.

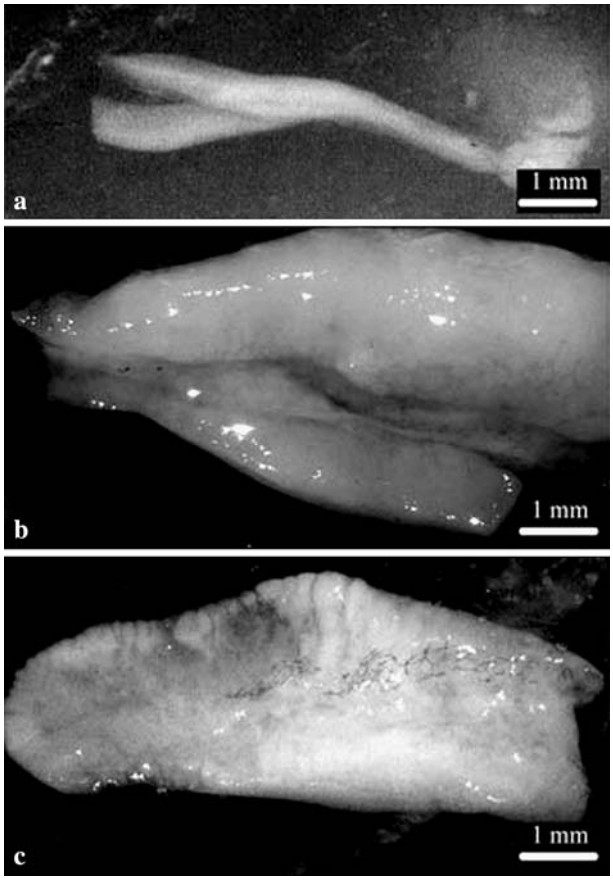

Fig. 1 Macroscopic view of *Artedidraco mirus* testes. **a** Immature testes in 64 mm L_T specimen. **b**, **c** Developed testes in 88.5 mm L_T and 76 mm L_T males

Histological techniques

Mid-portions of the gonads were removed from the selected 11 specimens, embedded in paraffin according to standard procedures and then 7 μm sections were stained with hematoxylin–eosin (HE). Sections were examined by an Olympus BX 60 and images were acquired by an Olympus digital CCD Color ViewII Camera with the analySIS software (Soft Imaging System GmbH, Germany).

Results

Macroscopic data

Size, gonad weight data and the values of I_G for our sample of 11 specimens are summarized in Table 1. Size range for male and female was 64–93.6 mm L_T and 68.1–97.5 mm L_T, respectively. Macroscopic features of gonadal maturation are reported in Table 2. Except for the smallest specimen (L_T = 64 mm), all the

Table 2 Comparison between gonadal macroscopic appearance and histological features of testes and ovaries in *Artedidraco mirus*

Fish I.D.	Sex	Macroscopic stage (according to Kock and Kellermann 1991)	Histological features
45/BT24/6	M	Immature	Immature testes
45/BT24/2	M	Developed	Testes in post-spawning condition
45/BT24/7	M	Developed	Fluent testes with also post-spawning lobules
45/BT24/11	M	Developed	Testes in post-spawning condition
45/BT24/3	M	Developed	Fluent testes
45/BT24/5	M	Developed	Fluent testes
45/BT24/14	F	Maturing virgin	Immature ovaries: previtellogenic oocytes (PVG) and others in lipid vesicle stage (LV)
45/BT24/10	F	Maturing virgin	Maturing ovaries: early vitellogenic oocytes with presence of lipid vesicles (LV) and vitellogenic oocytes with yolk granules (YG)
45/BT24/9	F	Developing	Maturing ovaries: early vitellogenic oocytes with presence of lipid vesicles (LV) and vitellogenic oocytes with yolk granules (YG)
45/BT24/1	F	Developing	Post-spawning ovaries: oocytes in lipid vesicles stage and also in vitellogenic stage. Presence of Post Ovulatory Follicles (POFs)
41/BT22/1	F	Ripe	Mature ovaries

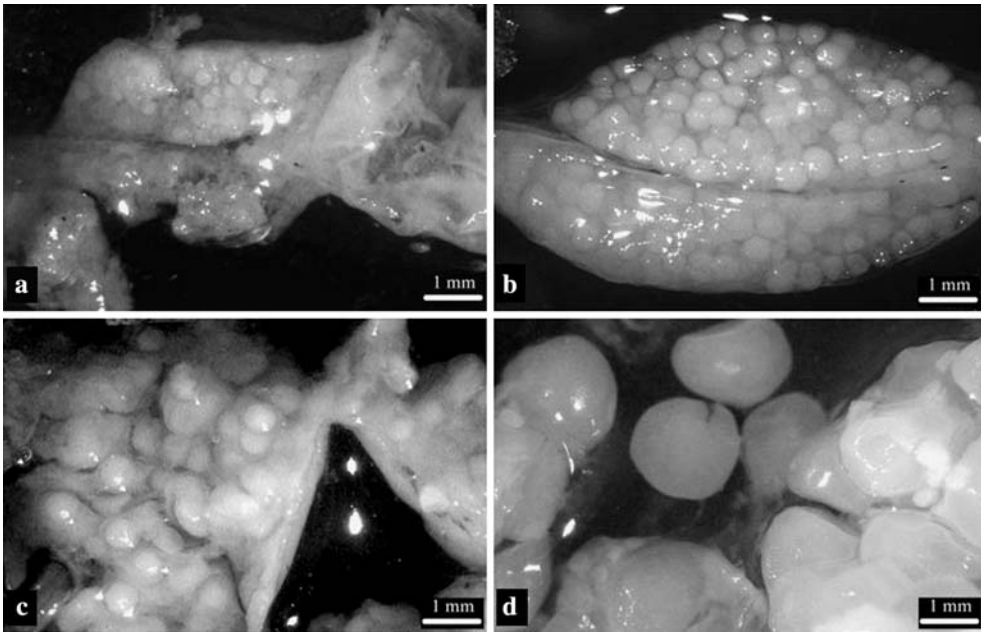

Fig. 2 Different macroscopic maturity stages of ovaries in *Artedidraco mirus*. **a** Maturing ovaries in 68.1 mm L_T specimen. **b**, **c** Developing ovaries in 74.5 mm L_T and 82.4 mm L_T females. **d** Ripe ovaries in 97.5 mm L_T female

males had testes showing similar macroscopic patterns of maturity (Fig. 1). The largest female specimen had a ripe ovary containing large eggs while smaller fishes exhibited gonads in different macroscopic maturity stages (Fig. 2). The number of oocytes in the ripe female was 193, the relative fecundity was 16 eggs/g and the maximum size of oocytes was 2 mm.

Gonadal histology

The comparison between gonadal macroscopic appearance and histological features in the males and females is presented in Table 2. Overall we observed different maturity features for both sexes.

Males

The testes were of the lobular-type with lobules defined by connective tissue septa within the testicular wall. Our analysis revealed three different histological stages in the specimens: immature, fluent gonads and post-spawning testes. The gonads of the only immature male showed a typical lobular organization where the lobules were lined by spermatogonia and did not contain spermatids or spermatozoa (Fig. 3a). Two specimens had fluent testes in which most cysts were broken and greatly enlarged lobules contained free spermatozoa (Fig. 3b). All other males were in post-spawning condition with gonads where most of the lobules were devoid of spermatozoa and only occasional cysts were present (Fig. 3c).

Females

The ovaries had the typical hollow structure: from the ovarian wall connective tissue septa projected into the ovarian cavity forming ovigerous folds containing oocytes. We observed four different histological maturity stages: immature, maturing, mature and post-spawning. The immature gonads contained oocytes with a large nucleus and a narrow area of cytoplasm that was strongly basophilic; some oocytes showed small lipid vesicles containing unstained lipid at the periphery (Fig. 4a). In the maturing ovaries we observed oocytes in early vitellogenesis characterized by the presence of lipid vesicles (LV) at the periphery and vitellogenic oocytes with their typical yolk granules (YG) (Fig. 4b). Only one female was mature: here oocytes had an irregular shape and the diameter reached approximately 2 mm; LV and YG coalesced to form slightly acidophilic material (Fig. 4c). In the post-spawning ovaries oocytes in lipid vesicles and vitellogenic stages could be observed together with Post Ovulatory Follicles (POFs); they were organized in convoluted strings formed by the follicular epithelium.

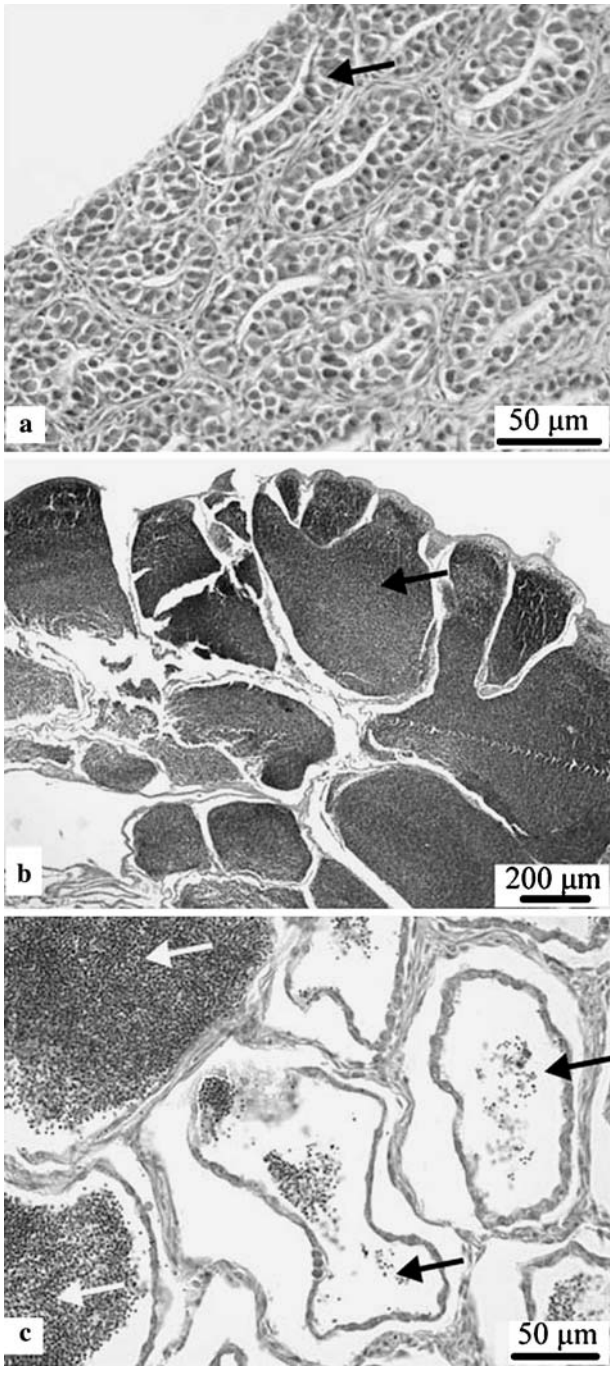

Fig. 3 Histology of *Artedidraco mirus* testes. **a** Immature testes of 64 mm L_T specimen showing lobules lined by spermatogonia (*arrow*) and without spermatids or spermatozoa. **b** Fluent testes in active male of 88.5 mm L_T with most cysts broken and greatly enlarged lobules containing free spermatozoa (*arrow*). **c** Testis in post-spawning condition of 76 mm L_T specimen showing most of lobules empty of spermatozoa (*black arrows*) and only rare cysts (*white arrows*)

They will gradually lose their lumina and will be invaded by the follicular cells (Fig. 4d).

Discussion

Most of species living in the Seasonal Pack-ice Zone (sensu Hempel 1985) and around the islands of the northern Southern Ocean are autumn or winter spawners (from May to August) (Kock and Kellermann 1991). The artedidraconid *A. mirus* Lönnberg, 1905 is a demersal fish endemic to South Georgia, where it is distributed mainly in the nearshore waters and on the continental shelf (Eakin 1990). In spite of the accessibility of this species there are no studies focused on its reproductive biology. Some authors indicate that the spawning period of *A. mirus* is between May and June (Lönnberg 1905; Kock and Kellermann 1991; North 2001). Our results are in concordance with these predictions in that the concurrence of mature active specimens with others showing post-spawning features suggest that the current season's spawn was in progress during specimen sampling, but may have been near conclusion. In addition, the presence of oocytes in vitellogenesis in the post-spawning females, potentially destined to spawn the next year, suggests that vitellogenesis is a protracted two year long process, as has been observed in other Antarctic fishes (Duhamel et al. 1993; Everson, 1994; Macchi and Barrera Oro 1995; Everson et al., 1996). Available data on artedidraconid fecundity are very limited but it is worth noting that absolute and relative fecundities of the only ripe female in our *A. mirus* sample are similar to those observed in *A. shackletoni* (Ekau 1991) indicating that the South Georgian plunderfish could also produce only a few hundred large eggs as observed in other Antarctic fishes (Ekau 1991; Kock and Kellermann 1991; North 2001).

Our study revealed discrepancies between the macroscopic and histological features of the gonads. This worth emphasizes once again the importance of the histological approach in research on Antarctic fish reproduction in order to verify the macroscopic observations, a point also noted by other authors (Macchi and Barrera Oro 1995; La Mesa et al. 2003,

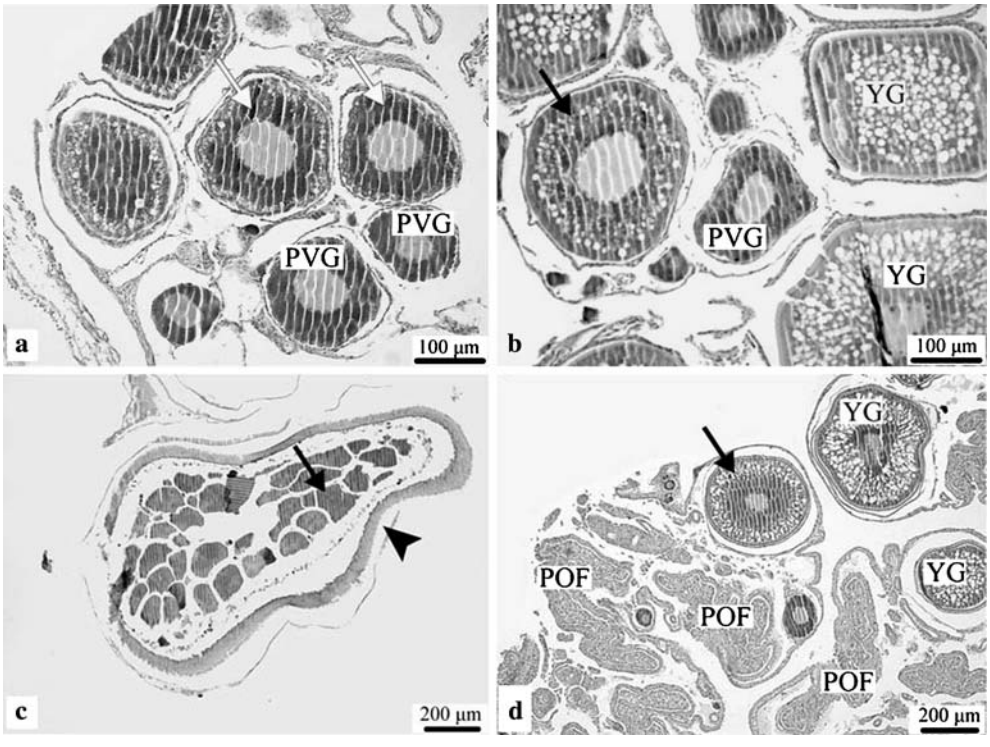

Fig. 4 Histology of *Artedidraco mirus* ovaries. **a** Immature ovary with oocytes in different early developmental stages of 68.1 mm L_T specimen: in particular pre-vitellogenic oocytes (*PVG*) and oocytes showing lipid vesicles (*LV*) containing unsaturated lipid at the periphery (*white arrows*). **b** Maturing ovary with vitellogenic oocytes showing many yolk granules (*YG*) of 74.5 mm L_T specimen: early vitellogenic oocytes (*arrow*) and pre-vitellogenic oocytes (*PVG*). **c** Mature ovary showing irregular shape (*arrowhead*) with coalesced to form slightly acidophilic material (*arrow*) in female of 97.5 mm L_T. **d** Post-spawning ovary with oocytes in yolk granules (*YG*) and vitellogenic stages (*arrow*) together with post ovulatory follicles (*POFs*) in female of 82.4 mm L_T

2006; Van der Molen and Matallanas 2003; Motta et al. 2005).

Our results, although derived from few specimens in only one season, provide the first substantial information on *A. mirus* reproduction and the first histological data on gonads in the plunderfishes of the family Artedidraconidae. A more detailed picture of the reproduction of this species will be facilitated by sampling in other seasons and by further analyses based on both macroscopic and histological approaches.

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