Polar Biol (2017) 40:199–211
DOI 10.1007/s00300-016-1945-7

ORIGINAL PAPER

Reproductive features of the Antarctic silverfish (*Pleuragramma antarctica*) from the western Ross Sea

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Received: 14 April 2015 / Revised: 1 February 2016 / Accepted: 12 April 2016 / Published online: 21 April 2016

Abstract The Antarctic silverfish *Pleuragramma antarctica* is the most abundant pelagic fish inhabiting the Ross Sea. Given its ecological relevance in the local food web, it is considered a keystone species in the Antarctic coastal ecosystems. Many aspects of its biology have been elucidated, but knowledge of important parts of its life cycle, including reproduction, is still poor. Here we use macroscopic and histological approaches to describe the reproductive features of the Antarctic silverfish based on fish sampled in late summer in the Ross Sea. Both males and females were at an early developmental stage, consistent with what has been reported for the same species from other Antarctic sectors and with spawning occurring in late winter. Widespread follicular atresia has been detected in the fish examined. The analysis of its intensity and prevalence suggests that skipped spawning (not all adults spawn every year) is likely to be a reproductive strategy of the Antarctic silverfish.

Keywords Antarctic silverfish · Ross Sea · Gonadal histology · Atresia · Skipped spawning

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Introduction

The Antarctic silverfish (*Pleuragramma antarctica*) is the most abundant pelagic fish inhabiting high-Antarctic waters (DeWitt et al. 1990). In the Ross Sea this species accounts for more than 90 % by number and biomass of midwater fishes (DeWitt 1970), and over 97 % of the total ichthyoplankton (Guglielmo et al. 1998; Vacchi et al. 1999; Granata et al. 2000, 2002). As key species in the Antarctic food web, the Antarctic silverfish occurs in the stomach of top predators ranging from large fish such as Antarctic toothfish to penguins, marine flying birds and seals [reviewed in Hubold (1985)]. It is predominantly a zooplankton feeder, but its diet ranges from phytoplankton and meso-zooplankton to copepods and euphausiids, and to other fish depending on size, location and season (Giraldo et al. 2011; Mayzaud et al. 2011; Pinkerton et al. 2013).

Given its abundance and ecologic role, the Antarctic silverfish has been extensively studied and many aspects of its biology have been elucidated [reviewed in La Mesa and Eastman (2012)]. Nevertheless, knowledge on important steps of its life cycle, including reproduction, is still poor and patchy.

The first research on the spawning period of the Antarctic silverfish (Andriashev 1965; Hubold 1984) leads to hypothesize that spawning occurred during winter and hatching in spring. A more extensive study in east Antarctica (Faleeva and Gerasimchuk 1990; Gerasimchuk 1992) allowed the determination of the length of sexual maturity for both sexes, confirmed winter or spring spawning, and provided the first details on gametogenesis including the description of two oocyte cohorts (pre-vitellogenic and yolked) in maturing ovaries. A recent study from the Antarctic Peninsula geographically extended the knowledge on the reproductive biology of this species, confirmed the occurrence of two oocyte cohorts, and that

vitellogenesis occurred in the austral autumn (late March to early May; La Mesa et al. 2015).

Faleeva and Gerasimchuk (1990) reported the presence of follicles undergoing resorption (atretic) in the ovaries of all observed females. The presence of atretic follicles in two females was also reported for specimens from the Antarctic Peninsula (La Mesa et al. 2015). Ovarian follicular atresia has already been described for other notothenioid species (e.g., Everson et al. 1991; Calvo et al. 1999; Vanella et al. 2005).

In fish, the presence of many atretic follicles is considered a failure to achieve final maturation of oocytes, usually caused by poor nutritional condition (Hunter and Macewicz 1985). Regardless of the reasons for mass ovarian atresia, this phenomenon is suggested to be one of the features underlying "skip spawning" (the fact that adults may not spawn every year), described in detail for teleosts by Rideout et al. (2005). The increasing number of species recognized as skip spawners supports the acknowledgment that this is a widespread phenomenon in fish (Rideout and Tomkiewicz 2011). Among Antarctic teleosts, skip spawning has been reported for the demersal Patagonian toothfish *Dissostichus eleginoides* (Arana et al. 2009), Antarctic toothfish *D. mawsoni* (Parker and Grimes 2010), blackfin icefish *Chaenocephalus aceratus* (Vanella et al. 2005), mackerel icefish *Champsocephalus gunnari* (Kock and Kellermann 1991) and pike icefish *C. esox* (Calvo et al. 1999). In most of those cases, skipped spawning was deduced from high intensity of pre-vitellogenic follicular atresia, indicated by Rideout et al. (2005) as an accurate criterion to identify non-reproductive individuals.

Data on the reproduction of the Antarctic silverfish are fragmentary, and in particular, information from the Ross Sea is lacking. The fact that the only known nursery ground for this species is located in Terra Nova Bay in the Ross Sea (Vacchi et al. 2012) emphasizes the importance of this area for developing an understanding of the Antarctic silverfish reproductive cycle and life history.

To obtain information on the reproductive traits of Antarctic silverfish from the Ross Sea, we analyzed specimens from various locations on the continental shelf. Macroscopic and histological approaches were integrated allowing an accurate description of the gonadal structure and the assessment of reliable maturity stages of the specimens. Particular attention was devoted to the analysis of follicular atresia, considering the current interest in this process.

Materials and methods

Sampling and data collection

Sampling was carried out during the New Zealand research voyage in the western Ross Sea from the RV *Tangaroa* in February 2008 (Hanchet et al. 2008). The voyage carried out a number of demersal trawls using a rough bottom trawl with a mouth width of 25 m and cod-end mesh of 40 mm, as well as a fine-mesh midwater trawl with a mouth opening of 12 m diameter and a cod-end mesh of 10 mm. The locations of all bottom trawl stations and three midwater stations were randomly selected, while the remaining two midwater stations (49 and 103) were non-random, targeted trawls on midwater layers (see also O'Driscoll et al. 2011). Antarctic silverfish were sampled from all eight bottom trawl and midwater trawl stations where adult fish were caught (Table 1). Five to ten specimens were randomly subsampled from each station and promptly fixed in 10 % formalin for further analyses, except for the trawling station 79 where only three specimens were sampled. A total of 58 fish were sampled in this manner in depths ranging from 272 to 752 m. Further details of the survey area, sampling design and sampling methods can be found in O'Driscoll et al. (2011) and Hanchet et al. (2008).

For each fish, the following variables were determined: standard length (SL) to the nearest millimeter below; total body weight (TW) to the nearest centigram below. After dissection, sex and stage of maturity were assessed on the basis of macroscopic appearance of gonads according to the five-point scale of maturity for notothenioid fish (Everson 1977; Kock and Kellermann 1991). Gonads and livers were removed, weighed with an accuracy of 0.01 g and stored in 70 % alcohol for further analyses. Somatic weight (SW) was determined to the nearest centigram below.

Indexes calculation

The gonadosomatic index (GSI) was calculated as the percentage of gonad weight (GW) per somatic body weight (Crim and Glebe 1990) in order to avoid biases due to the stomach fullness.

Absolute and relative fecundity were estimated in females at gonadal macroscopic stage III and IV using the gravimetric method (Murua et al. 2003) that relate the number of most advanced oocytes in the weighed subsample to the GW. A piece of ovarian tissue was taken from the median portion of a randomly selected ovarian lobe from each specimen. The subsample of each ovary, representing 1.5–8.2 % of the GW, was weighed and immersed in ethanol 70 % in a Petri dish. Oocytes were spaced and photographed by a Cell Pad E "all-in-one" microscope camera (TUCSEN). To exclude pre-vitellogenic oocytes a threshold size of 400 μm diameter was set. Oocytes larger than the threshold size were counted through the Adobe Photoshop Software.

Table 1 Summary of sampling information

Station #	Sampling method	Latitude	Longitude	Gear bottom depth (m) (start–end)	Vessel bottom depth (m) (start–end)	Specimens
49	FMMT	74.92967	168.07383	395–378	402–430	4 m, 6 f
54	FMMT	75.63100	169.76500	478–50	525–502	7 m, 3 f
70	BT	76.77500	167.83600	724–752	738–765	2 m, 1 f, 2 u
77	BT	76.83283	180.04967	664–653	663–648	1 m, 9 f
79	FMMT	76.61883	176.69350	320–50	358–358	3 f
81	BT	76.59367	176.82750	369–371	365–366	1 m, 2 f, 2 u
92	FMMT	76.18017	176.36350	442–50	452–452	1 m, 7 f, 2 u
103	FMMT	74.52117	177.53367	201–236	276–287	2 m, 3 f

Sampling method: *FMMT* fine-mesh midwater trawl, *BT* bottom trawl. Sample: f, female; m, male; u, macroscopically unsexed. Latitude is expressed as decimal degrees south. Longitude is expressed as decimal degrees east. Gear bottom depth and vessel bottom depth are expressed in meters

Absolute fecundity (F_{abs}) was estimated as:

$$F_{abs} = n \times GW/w$$

where n = number of oocytes larger than 400 µm in the subsample and w = weight of the subsample (g). Relative fecundity (F_{rel}) was calculated as the number of oocytes per gram of fish body weight (TW). The hepato-somatic index (HSI) was obtained as the percentage of liver weight (LW) per TW.

Gonad histology

Histology was performed on the gonads of all 58 specimens. Fixed ovaries and testes were cut in half along the medial–lateral axis before embedding, and a subsample was taken from the middle to minimize potential variation due to section location. The subsamples were then dehydrated, cleared with xylene, embedded in Paraplast (McCormick Scientific, USA) and sectioned at 5-µm-thick sections. Hematoxylin–eosin staining (Bio-Optica, Milano Italy) was performed. Sections were examined using Leica DMRB light microscope, and images were acquired with a Leica CCD camera DFC420C (Leica, Switzerland).

Histological classification of the maturity stage for male is based on the progression of spermatogenesis from spermatogonia (Sg) to spermatozoa (Sz) and on the changes in the germinal epithelium. Testes were therefore staged based on morphological and histological criteria according to Brown-Peterson et al. (2011).

The ovarian follicles were classified as: primary growth oocytes (PG) including pre-follicular and early growth follicles, initial alveolus stage oocytes (IA) with at least one visible row of peripherally situated vacuoles, cortical alveolus stage oocytes (CA) characterized by multiple rows of membrane limited cytoplasmatic vesicles, vitellogenic oocytes (Vtg) with accumulation of yolk granules in the ooplasm. In addition, atretic oocytes (A) and post-ovulatory follicles (POF) were detected and counted. Each gonad was classified based on the most advanced follicle stage observed in the histological sections according to the five-point maturity scale of Brown-Peterson et al. (2011).

Correspondence between the macroscopic gonad maturity stages described by Kock and Kellermann (1991) and the histological stages described in Brown-Peterson et al. (2011) are summarized in Table 2.

Analysis of follicular atresia

Follicular atresia is a degenerative process by which ovarian follicles lose their integrity and are reabsorbed prior to ovulation (Santos et al. 2008). Here we refer to the alpha-stage of the atretic process as detectable in the histological sections, characterized by breaks in the chorion, hypertrophy of the granulosa cells and disorganization of the oocyte structure (Üçüncü and Çakici 2009).

Following Kjesbu et al. (2010) the relative intensity of atresia (A_{RI}) was expressed as:

$$(\text{Nr of atretic oocytes})/(\text{Nr of normal and atretic oocytes}) \times 100$$

In mature females, where two cohorts of oocytes were clearly distinguishable, intensity of atresia pertaining to each oocyte population was calculated as the percentage of atretic oocytes in a given cohort over the total oocytes (normal and atretic) in that cohort.

Statistical analyses

Statistical analyses were performed using R 3.2.2 (R Development Core Team 2015). When dealing with indices and ratios, data were *arcsin* $\sqrt{\frac{p}{100}}$ transformed. One-way ANOVA and post hoc Tukey HSD test were applied after

Table 2 Maturity scales

C1	C2	C3
Females		
(I) Immature	(I) Immature	EF, PG
(III) Developing	(II) Developing	PG, CA, early Vtg
		Some A may be present
(IV) gravid	(III) Spawning capable	Late Vtg, POF, A
		Hydrated oocyte may be present
(V) Spent	(IV) Regressing	Mass atresia, POFs
		Sporadic CA and Vtg may be present
(II) Maturing virgin or resting	(IV) Regenerating	EF, PG
		A and old POFs may be present
Males		
(I) Immature	(I) Immature	Small testes
		No lumen in lobules
		Sg1
(III) Developed	(II) Developing	Small testes
		Sg2, Sc1, Sc2
		St and Sz may be present in spermatocysts
(IV) Ripe	(III) Spawning capable	Large testes
		Sg1, Sg2, Sc1, Sc2, St, Sz
		Sz in the lumen of lobules or ducts
(V) Spent	(IV) Regressing	Small flaccid testes
		Sporadic Sz residual in the lumen of lobules and sperm ducts
		Little or no spermatogenesis
(II) Developing or resting	(V) Regenerating	Small testes, small spermatocysts
		Proliferating Sg, Sz occasionally present in the lumen of lobules and sperm duct

Maturity scales used for the gonad staging of Antarctic silverfish. C1: five-point scale from Kock and Kellermann (1991) used for the macroscopic assessment of the gonads; C2: maturity scale by Brown-Peterson et al. (2011) used in the microscopic staging of gonads; C3 main features of each stage. Corresponding stages in the two scales are placed in the same row. EF = early follicles; PG = primary growth oocytes; CA = cortical alveolus stage oocytes; Vtg = vitellogenic oocytes; A = atretic follicles; POF = post-ovulatory follicles; Sg1 = primary spermatogonia; Sg2 = secondary spermatogonia; Sc1 = primary spermatocytes; Sc2 = secondary spermatocytes; St = spermatid; Sz = spermatozoa

having verified normality and homogeneity of variances through Shapiro–Wilk and Levene tests. Kruskal–Wallis nonparametric test and post hoc Wilcoxon test were used when data did not meet the normality and homogeneity of variances assumptions for ANOVA. Significance for all statistical analyses was determined at $\alpha = 0.05$.

The effect of size on the gonadosomatic index (GSI) was investigated in both sexes separately (18 males and 34 females). The subsamples were grouped by length (small adults 152–178 mm, large adults 179–236 mm) based on the criteria of Pinkerton et al. (2013).

Variation of GSI values in response to ovarian maturation stage was tested on the 32 developing females (the two immature females were excluded). Oocyte maturation was classed in three levels according to the maturation stage of the most advanced follicle: initial alveolus (IA), cortical alveolus (CA) and vitellogenic (Vtg).

The Wilcoxon test was applied to test for differences in the incidence of atresia between the two oocyte cohorts (primary and secondary growth cohorts).

As potential factors influencing the intensity of atresia, oocyte maturation stage and condition level were taken into consideration. In order to gain a finer resolution, the analyses were performed on the two oocyte cohorts separately. Oocyte maturation was classed in the three previously described levels (IA, CA and Vtg). Specimens condition, evaluated through the HSI, was classed in three levels ($HSI < 1.20$, $1.20 \leq HIS < 1.70$, $HSI \geq 1.70$).

To test the effect of geographic origin on the incidence of atresia, stations from which at least six specimens

(replicates) were sampled were taken into consideration (stations 49, 77 and 92, see Table 1).

Results

The sample of Antarctic silverfish included 18 males, 34 females and six specimens of undetermined sex, ranging in size from 13 to 19 cm SL (Fig. 1). Males were homogeneous in size ranging between 16 and 18 cm SL, with average size 16.9 cm ± 0.69 SD. The size distribution of females ranged from 13 to 19 cm SL, but the average size (17.1 cm ± 1.28 SD) was comparable to the males. The specimens of undetermined sex were among the smallest, ranging between 13 and 16 cm SL.

Macroscopic staging, gonadosomatic index and fecundity estimate

Based on macroscopic examination of the gonads, most males (67 %) were developed (maturity stage III) with the remainder developing (stage II) or ripe (stage IV; Table 3). Male GSI ranged from 1.29 to 3.04 and the mean GSI increased progressively from stage II, III and IV (Fig. 2a). The effect of size on GSI was not able to be evaluated given the homogeneity in the size of males (all small adults, according to Pinkerton et al. 2013).

Most females (62 %) were developing (macroscopic maturity stage III), and of the remainder two were immature (stage I), seven were either maturing virgin or resting (stage II) and four were gravid (stage IV; Table 3). The mean GSI increased progressively from maturity stage I to maturity stages II, III and IV (Fig. 2a), and was positively correlated with SL ($r = 0.6$). According to Kruskal–Wallis test, GSI values were not significantly influenced by size ($\chi^2 = 2.3339$, $p > 0.1$).

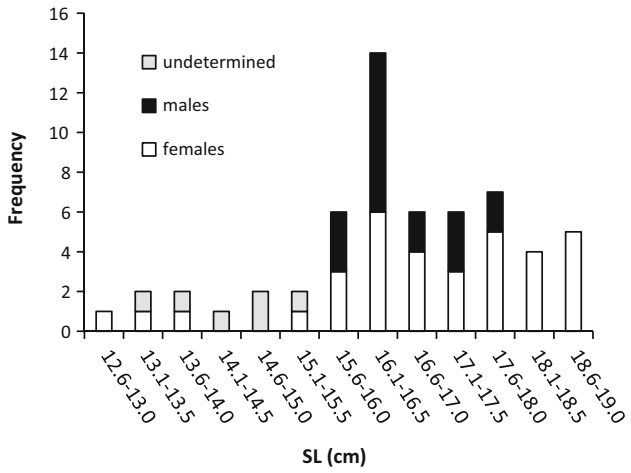

Fig. 1 Length frequency distribution of the *Pleuragramma antarctica* sample by sex

Table 3 Numbers of fish in each gonad maturity stage based on macroscopic features (following Kock and Kellermann 1991) and corresponding gonadosomatic index (GSI) range, mean and standard deviation

Maturity stage	Sex	*n*	GSI range	GSI mean ± SD
I	F	2	0.27–0.73	0.50 ± 0.33
II	F	7	0.50–1.71	0.91 ± 0.54
II	M	2	1.55–1.65	1.60 ± 0.07
III	F	21	0.97–2.71	2.07 ± 0.43
III	M	12	1.29–3.04	2.16 ± 0.85
IV	F	4	2.51–3.43	2.88 ± 0.39
IV	M	4	2.08–3.04	2.45 ± 0.42

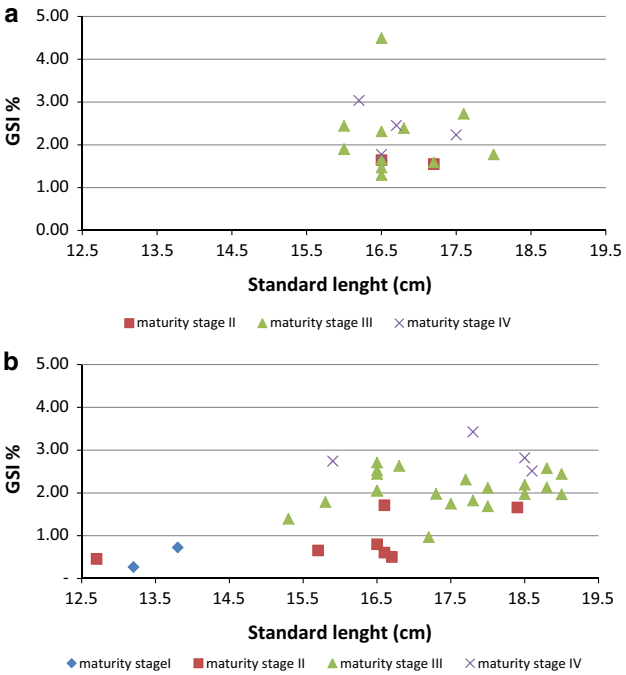

Fig. 2 Plot of gonadosomatic index (%GSI) versus standard length (SL). Individuals are labeled according to the macroscopic stage of gonad maturity in **a** males, **b** females

Fecundity was estimated for 25 females at macroscopic stages III and IV (size range 15.8–19 cm SL). The largest oocytes (diameter larger than 400 µm) were not homogeneous, among them two groups of oocytes were clearly distinguishable based on size: a first group including oocytes of approximately 460 µm diameter and a second one including oocytes of approximately 600 µm diameter. Absolute and relative fecundity was calculated based on the sum of oocytes from the two groups. F_{abs} was found to range between 2360 and 6700 eggs per female with a mean

of 4939 ± 1289 SD. The relative fecundity ranged between 65.8 and 188.4 eggs per gram of body weight, with a mean F_{rel} of 111.3 eggs/g of body weight ±27.8 SD.

Histological characterization

Histological examination of testes revealed homogeneity at the level of microscopic organization. The tissues in the cross sections were organized in cysts homogeneously composed by primary spermatocytes (Fig. 3). All the males were classified as developing (stage II) despite different size and GSI (Fig. 4a).

Histological examination of ovaries allowed the detection of two different gonad states. The ovaries of the two smallest females were exclusively composed of primary growth oocytes (PG), were homogeneous in size and were characterized by a large nucleus that occupied most of the cell surrounded by a thin strongly basophilic layer of cytoplasm (Fig. 5a). The gonads of all the remaining females were classified as developing (stage II). Based on size and cytological characteristics, two cohorts of oocytes were clearly distinguishable in the ovaries. The first cohort (primary growth cohort) was composed of pre-vitellogenic oocytes (PG), sometimes heterogeneous in size (Fig. 5a–c), and generally smaller than 100 µm diameter. The other cohort (secondary growth cohort) included maturing oocytes at a homogeneous developmental stage within each ovary. As a general rule, the secondary growth oocytes were larger in size than the primary growth cohort, and generally larger than 400 µm diameter. Based on the morphological properties, three distinct stages of vitellogenic cells were recognizable: initial alveolus (IA), cortical alveolus (CA), vitellogenic (Vtg). The cells were round with a thin acidophilic layer at the periphery of the cytoplasm (the future zona radiata) beneath the follicle. The nucleus was spherical in the IA cells (Fig. 5b) and

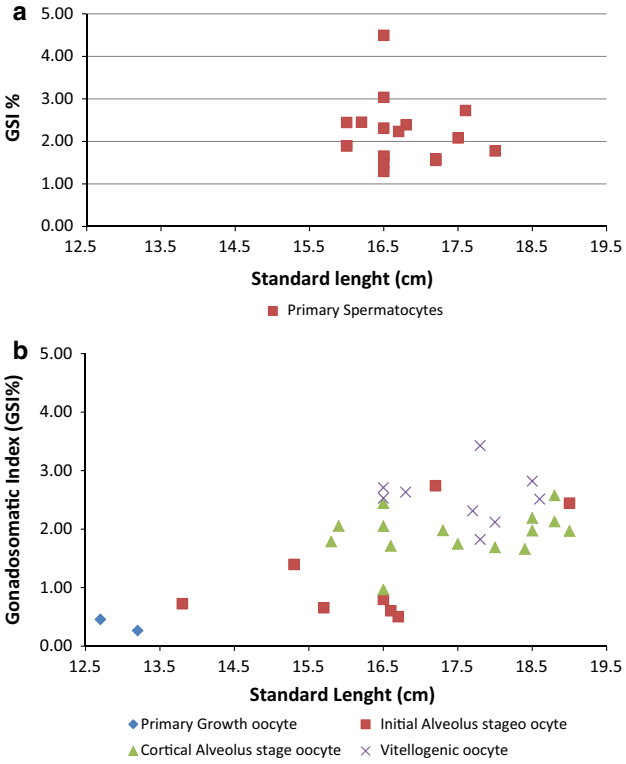

Fig. 4 Plot of gonadosomatic index (%GSI) versus standard length (SL). Individuals are labeled according to the most advanced stage of spermatocyte/oocyte maturity found from histological analysis in **a** males, **b** females

became irregularly shaped, with niches along its wall, in the CA (Fig. 5c) and Vtg (Fig. 5d) cells. A large number of basophilic nucleoli were accumulated at the periphery of the nucleus; small grainy basophilic, not marginated, nucleoli were sometimes interspersed in the nucleoplasm or circularly displaced around the central area of the nucleus. Vesicles started to be accumulated at the

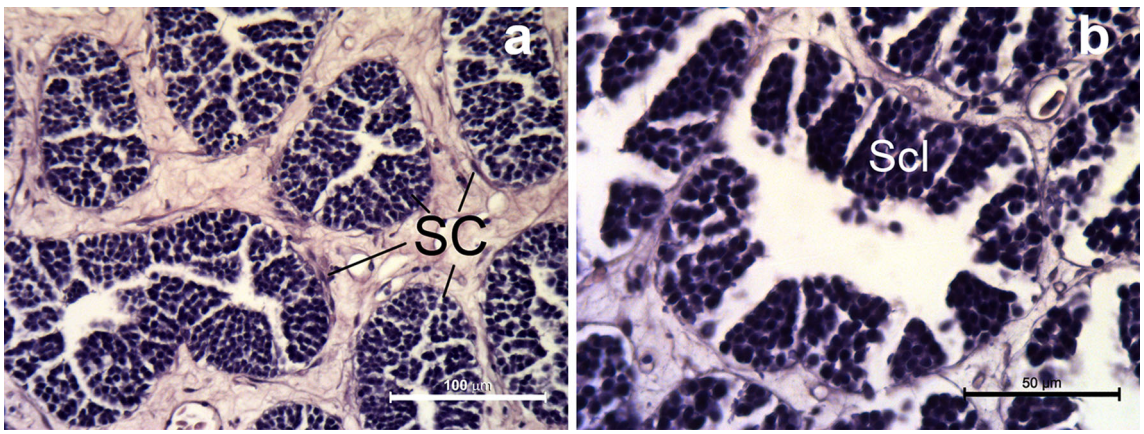

Fig. 3 Cross sections of testes of *P. antarcticum* (16.2 cm SL, IV macroscopic testis maturity stage). Male gonad is composed of cysts **a** each one composed only by primary spermatocytes (**b** detail of cyst). SC = spermatocyst; ScI = primary spermatocyte. Hematoxylin and eosin stain

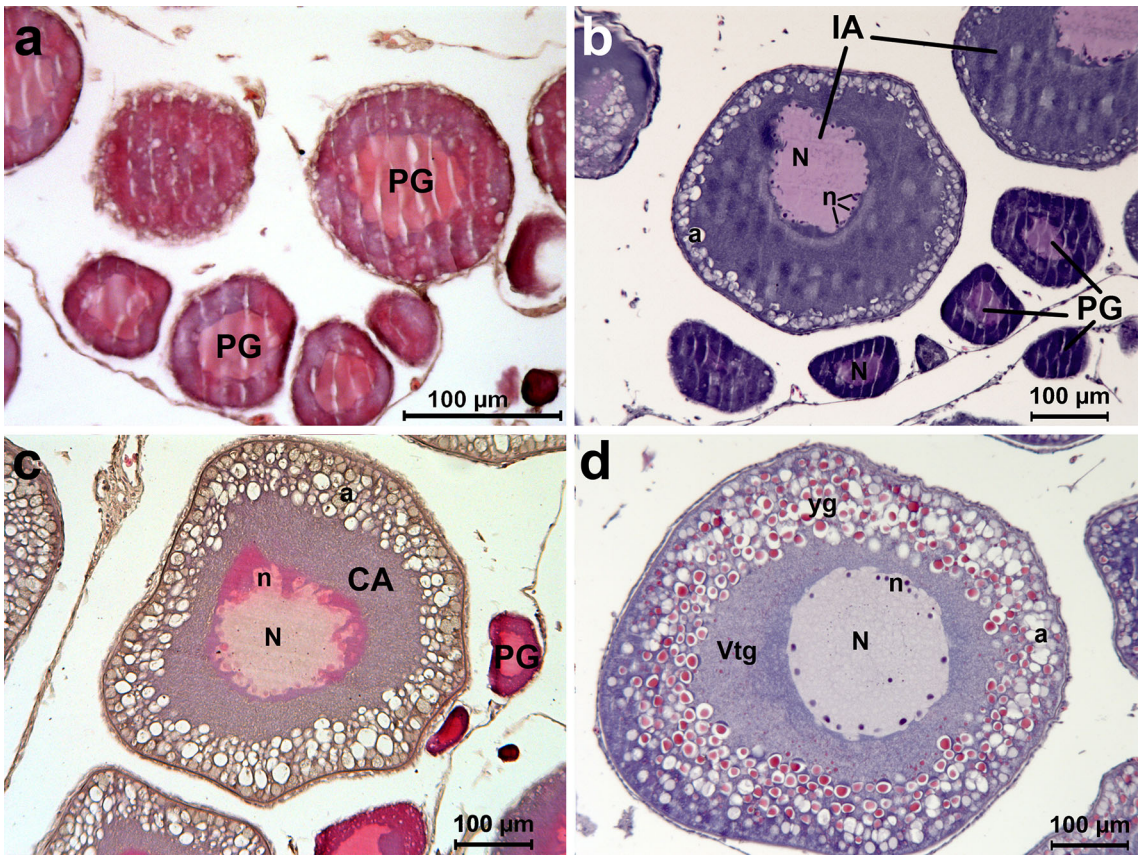

Fig. 5 Cross section of ovaries of *P. antarcticum* at different maturity stages: pre-vitellogenic (**a**), initial alveolus (**b**), cortical alveolus (**c**) and vitellogenic (**d**). PG = primary growth oocyte; IA = initial alveolus stage oocyte; CA = cortical alveolus stage oocyte; Vtg = vitellogenic oocyte; N = nucleus; n = nucleoli; a = alveoli; yg = yolk granules. Hematoxylin and eosin stain

periphery of the cytoplasm, in a few rows (IA), many rows (CA) or multiple rows occupying almost the entire cytoplasm (Vtg). In the vitellogenic oocytes, yolk granules started to be deposited from the periphery of the oocyte toward the centrally located nucleus.

Based on the most advanced oocyte developmental stage, and according to the Brown-Peterson et al. (2011) five-point scale, the females were classified as: immature (2 specimens) and developing (32 specimens). This latter group included females with different maturation stages of the most advanced follicles, eight were at the IA stage, 15 were at the CA stage and nine were at Vtg stage (Fig. 4b). A significant effect of ovarian maturation stage on the GSI for IA, CA and Vtg ovaries was detected through one-way ANOVA ($F = 12.54$, $p < 0.01$). However, post hoc Tukey HSD test revealed that for IA stage the value of GSI was significantly lower than that for the other stages.

Follicular atresia

Atretic follicles were present in all sampling groups. In most cases, the atretic process was in its initial phase (alpha atresia) characterized by oocyte shrinkage, distortion of the follicle resulting in irregular oocyte shape, disorganization of the cytoplasm in the oocyte, fragmentation of the chorion leading to cytoplasm flow out of the cell, deformation and sometimes dissolution of the nucleus (Fig. 6a–c).

The number of atretic follicles was counted in each female specimen with a minimum of 74 and a maximum of 846 oocytes. The two immature females showed 90 and 27 % of the oocytes undergoing resorption.

In developing females, the intensity of atresia was separately calculated in the primary growth oocytes (thereafter referred to as cohort 1) and in the secondary growth oocytes (thereafter referred to as cohort 2). The incidence of atresia was significantly different in the two cohorts (Wilcoxon test $V = 257$, $p = 0.01143$), and differed according to the ovarian maturation stage of the individuals (Fig. 7). Specimens at the earliest stages of maturation had higher intensity of atresia in cohort 2 relative to cohort 1; conversely, specimens at the later vitellogenic stages showed higher atresia in the oocytes of cohort 1. The Kruskal–Wallis test showed no effect of ovarian stage on

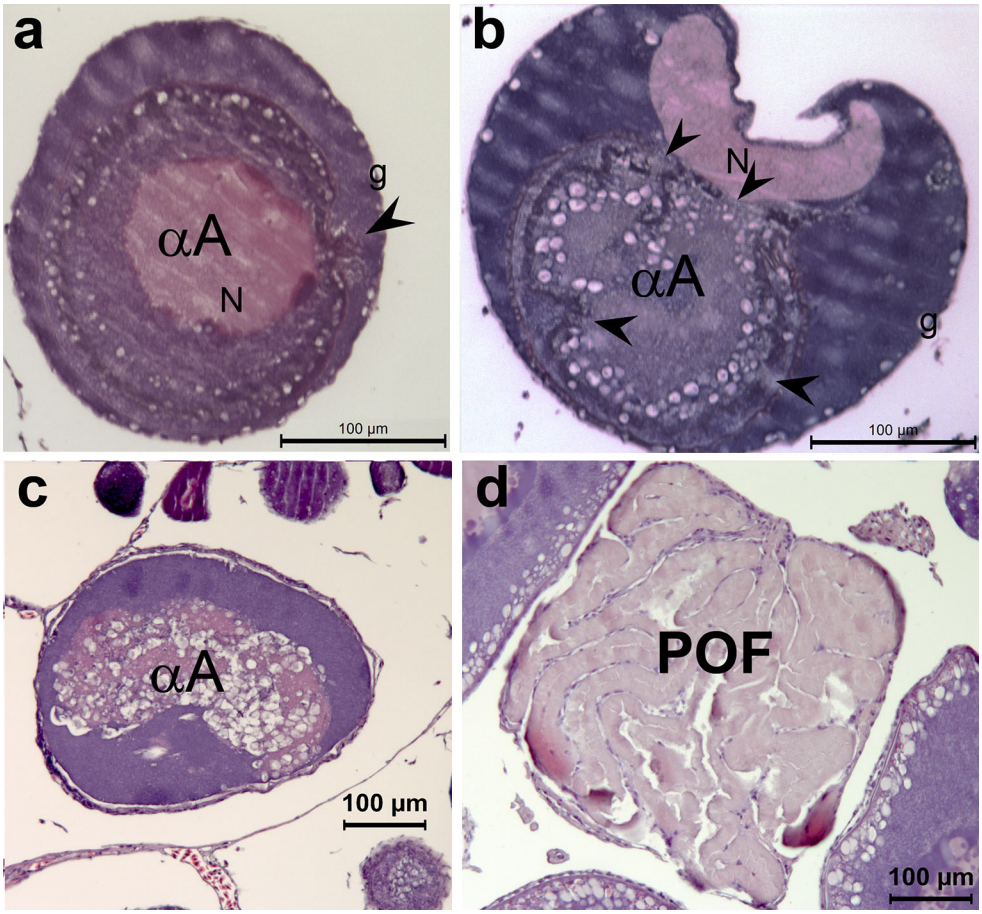

Fig. 6 Alpha atretic follicles in cross sections of *P. antarcticum* ovaries. **a** Initial alpha atretic oocyte, granulosa cells (g) start to look hypertrophic, clearly show a break in the chorion and nuclear envelope (*arrowhead*). **b** Alpha atretic oocyte characterized by hypertrophy of the granulosa cells, several breaks in the chorion (*arrowheads*) and disorganization of the oocyte structure emphasized by the outflow of the nucleoplasm (N) from the oocyte wall. **c** Terminal alpha atretic follicle, the de-structured oocyte collapsed in the middle of the follicle, granulose cells are phagocyting the oocyte remnants. **d** Post-ovulatory follicle characterized by convoluted shape next to initial alveolus stage oocytes. αA = alpha atretic oocyte; N = nucleus (or nucleoplasm where the nuclear envelope is disrupted); g = follicular granulosa cells; POF = post-ovulatory follicle. Hematoxylin and eosin stain

the intensity of atresia in cohort 1 ($\chi^2 = 3.305$, $p > 0.10$). Conversely, the incidence of atresia in cohort 2 was significantly different in ovaries at different maturity stages ($\chi^2 = 9.6862$, $p < 0.01$). Wilcoxon post hoc test highlighted that the incidence of atresia was higher in the IA stage compared to the others.

One-way ANOVAs showed a significant effect of fish condition (HSI) on intensity of atresia in the oocytes of cohort 2 ($F = 4.609$, $p < 0.05$) and no effect in the oocytes of cohort 1 ($F = 0.54$, $p > 0.10$). Post hoc Tukey HSD test for cohort 2 showed a decreasing incidence of atresia with better fish condition.

One-way ANOVAs applied to investigate the influence of the geographic origin of the specimens on the intensity of follicular atresia revealed a highly significant effect of the variable in cohort 1 ($F = 22.83$, $p < 0.01$) and no significant effect on cohort 2 ($F = 1.303$, $p > 0.10$). Post hoc Tukey HSD test highlighted significant differences among all stations, with the highest incidence of atresia at station 77 and the lowest incidence of atresia at station 92 (Fig. 8).

Post-ovulatory follicles (POFs) were occasionally found in three fish. In all cases, the POFs were convoluted and folded with reduced lumen (Fig. 6d). In one of the specimens, POFs were still recognizable but almost completely reabsorbed.

Discussion

The reproductive traits of Antarctic silverfish inferred from our study are consistent with those reported from other geographic areas (Faleeva and Gerasimchuk 1990; Duhamel et al. 1993; La Mesa et al. 2015).

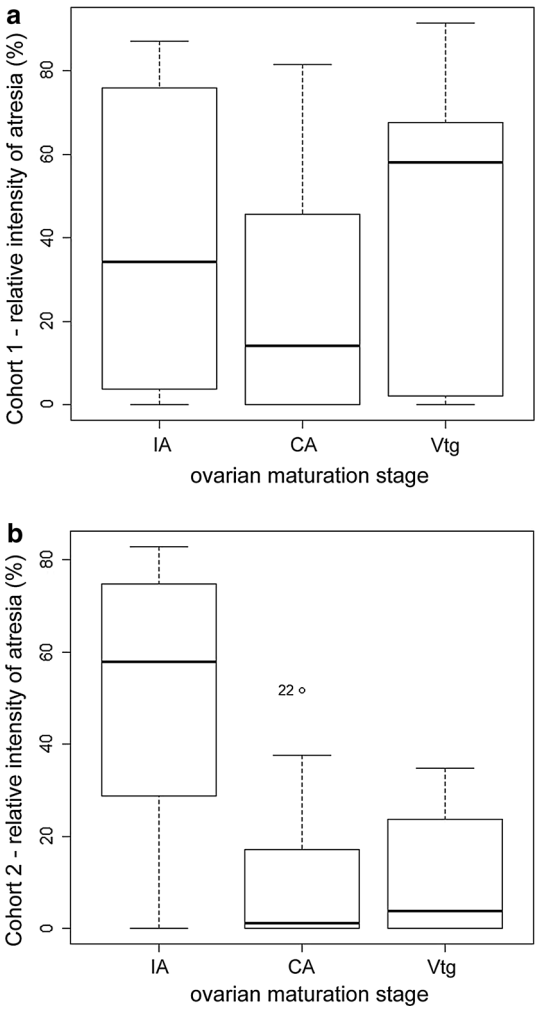

Fig. 7 Relative intensity of atresia in cohort 1 (**a**) and cohort 2 (**b**) in ovaries at different microscopic maturation stages. IA = initial alveolus stage oocyte; CA = cortical alveolus stage oocyte; Vtg = vitellogenic oocyte

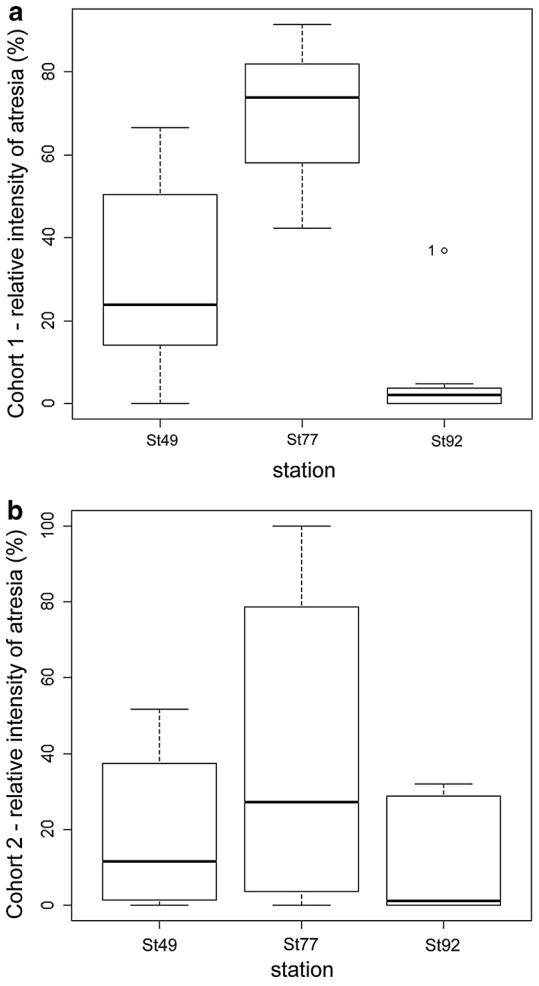

Fig. 8 Relative intensity of atresia in cohort 1 (**a**) and cohort 2 (**b**) in ovaries of specimens caught at three different stations

The average GSI values of maturing specimens were quite low (Table 3) and similar to that found at the same time in the Weddell Sea (Duhamel et al. 1993), slightly lower that those found in March–April from the Antarctic Peninsula (La Mesa et al. 2015), and lower than those recorded in April from the Cosmonaut, Commonwealth and Mawson Seas (Faleeva and Gerasimchuk 1990). The histological data presented showed that the testes were at a very initial developing state with only primary spermatocytes occurring in the cysts, while females showed early-vitellogenesis oocytes as the most advanced stage. Based on these results spawning in the Ross Sea is likely to occur in the late austral winter/early austral spring and at a similar time to that hypothesized for Antarctic silverfish in east Antarctica (Faleeva and Gerasimchuk 1990; Duhamel et al. 1993).

According to our analysis, the Antarctic silverfish from the Ross Sea have absolute fecundity values lower that those reported elsewhere for the species. Gerasimchuk (1987) reported absolute fecundity values ranging from 4315 to 17774 (average of 7499 ± 524) for Antarctic silverfish individuals collected at the end of March beginning of April in the Mawson Sea. In the same period of the year at the Antarctic Peninsula La Mesa et al. (2015) found values ranging from 3953 to 11613. Our results for the Ross Sea are from mid-February, where we recorded values ranging between 2360 and 6700 eggs per female. This might be an underestimate of the absolute fecundity because samples were collected early in the maturation period, and therefore, early vitellogenic oocytes might still be under the threshold size for fecundity count. However, we cannot exclude the possibility that low fecundity values might be typical of Antarctic silverfish in the Ross Sea. Samples of fish collected from later in the season in the Ross Sea would be required to resolve those alternatives.

From a histological perspective, the main traits of gametogenesis for specimens collected in the Ross Sea are consistent with those described for fish caught in other Antarctic areas and similar to those of other notothenioid species (e.g., Shandikov and Faleeva 1992; Calvo et al. 1999; Molens and Matallanas 2004; La Mesa et al. 2008). Females show group synchronous ovarian development, with two cohorts of oocytes: an advanced batch of large vitellogenic oocytes that will be ovulated in the current spawning season, and a set of smaller pre-vitellogenic oocytes, which form the reserve stock for the following spawning seasons. Follicles at advanced stages of reabsorption, residual from the previous spawning event, were detected in three out of the 34 females. Given that residual oocytes are thought to disappear completely within 6–8 months in other Antarctic notothenioid species (Butskaya and Faleeva 1987), the scarcity of POFs found in February, and their advanced state of phagocytosis, is consistent with the occurrence of spawning in late winter (July/August).

Is the Antarctic silverfish a skipped spawner?

The occurrence of atretic follicles in Antarctic silverfish has been reported from east Antarctica (Faleeva and Gerasimchuk 1990) and from the western Antarctic Peninsula (La Mesa et al. 2015). Although the capability of the Antarctic silverfish to skip spawn has already been suggested (Faleeva and Gerasimchuk 1990), analyses of the intensity and prevalence of follicular atresia, necessary to assess actual non-reproductive condition, were not performed prior to the present study.

We detected follicular atresia in all the examined ovaries. The determination of the intensity rate of atretic process individually, as well as the relative prevalence of atresia in groups of specimens, has provided clues to better understand the extent of the phenomenon.

The finding of atresia in immature females is not surprising since this process is normally occurring in ovaries as a means to regulate the physiological development of gonads. However, its role in mature specimens is less clear. A limited proportion of atresia is generally included among the mechanisms to down-regulate the ovarian maturation in early gametogenesis and to ensure vitellogenesis only occurs for the number of eggs that could be supported by the individual. By contrast, massive ovarian atresia should be interpreted as an anomalous status, possibly induced by external factors such as stress, fasting, biocidal agents, light, temperature, confinement and inadequate hormone levels (Miranda et al. 1999).

In the present study, there was a significant inverse relationship between the condition of the fish and the incidence of follicular atresia. Thus, condition (as a proxy of the physiological state of an individual) was a key factor influencing the intensity of reabsorption in the secondary growth oocytes.

The feeding opportunities during vitellogenesis are likely to play a critical role for the spawning success, and nutritional condition has proved to be an important measurement to estimate fecundity in fishes (Witthames et al. 2010). It is therefore reasonable to hypothesize that females would first try to maximize their spawning ability by producing as many pre-vitellogenic oocytes as possible (limited only by the size of the ovary), but subsequently they would be forced to adjust the threshold for vitellogenic eggs to the number of oocytes that they can effectively take to maturation. Condition is likely to play a key role in this second part of the gametogenesis acting as a fine-tuning factor.

If down-regulation is taken to its extreme in an individual, it would result in extensive oocyte reabsorption and, therefore, in diminished reproductive potential of that fish, whose contribution to the spawning of the following season would then be limited or nil (skip spawning).

This behavior is advantageous in the short term because it avoids the energy waste for specimens in poor nutritional status that therefore would be unable to sustain the reproductive effort, but it also represents an investment in the future. Indeed, the energy of a fish can be allocated to survival, growth or reproduction depending on the individual's characteristics (age, size, stored energy, etc.) as well as on the state of the environment (food availability, pollution, etc.) (Jørgensen and Fiksen 2006). Therefore, by foregoing spawning an individual can incur survival and growth benefits. In this respect, skipping a spawning season represents a trade-off between current and future reproduction, and survival (Rideout et al. 2005). Such a strategy has already been reported for several Antarctic fish species including *D. eleginoides* (Arana et al. 2009), *D. mawsoni* (Parker and Grimes 2010), *C. aceratus* (Vanella et al. 2005), *C. gunnari* (Kock and Kellermann 1991) and *C. esox* (Calvo et al. 1999). It also seems plausible for the Antarctic silverfish in the Ross Sea given the dramatic seasonal and annual fluctuations in food availability, related to changes in the physical environmental factors (Smith et al. 2007).

On an individual basis, and on a short timescale, skip spawning following decrease in nutritional status would simply represent the biological response to physiological and ecological unfavorable conditions.

However, on an evolutionary timescale, such individual phenotypic plasticity of life history strategies could also represent an adaptation that guarantees a higher fitness to the species. If this holds true, skipped spawning might not be just an abnormal occurrence brought by exceptional environmental and physiological conditions, but rather an

adaptive trait leading to increased lifetime reproductive output.

In this broader context, and to the best of our knowledge about its life history, the Antarctic silverfish provides some interesting clues that might support such a hypothesis.

Indeed, the Antarctic silverfish is specialized for pelagic life (Eastman 1997) and known to undertake vertical as well as horizontal migrations during its lifetime (La Mesa and Eastman 2012). Of particular interest is its supposed homing behavior (Koubbi et al. 2011) that would lead the fish to undertake breeding migrations (even long ones) moving from open waters to the coastal sites where they were born (natal homing, Papi 1992) or where environmental conditions are similar to those experienced at the larval stage (environmental homing, Cury 1994). Such a breeding strategy might be energetically costly. Therefore, taking into consideration that variability in the energetic state is usually present within populations, and that according to body size and condition some specimens could not be in the optimal shape to undertake the migration, skipped spawning on an individual basis would represent an advantage not only for the single specimen, but for the whole population.

Partial breeding migration, where some individuals migrate and reproduce while others in the same population do not migrate and forgo reproduction, has been reported for several pelagic and bathypelagic fish species (Shaw and Levin 2011; Chapman et al. 2012). It has also been inferred indirectly by the occurrence of ovarian massive atresia (Burton 1999; Rideout et al. 2005; Loher and Seitz 2008; van Damme et al. 2009; Skjæraasena et al. 2012). In the case of the Antarctic silverfish in the Ross Sea, the migration from open waters to the coastal spawning ground, potentially happening during the early winter, poses further challenges. The only known nursery ground for this species in the Ross Sea is located in a coastal embayment covered by sea ice for most of the year, thus implying that during migration the silverfish would encounter progressively harsher environmental conditions including ice crystals, besides exposure to a wider variety of coastal predators. In such an environmental scenario, the partial breeding migration strategy, sustained by the capability to down-regulate the oocytes development through follicular atresia, would acquire adaptive advantage.

To what extent our observations on specimens from the Ross Sea would allow generalization is an issue that deserves further investigations. However, the systematic analysis of ovarian atresia reported here lays the necessary basis to undertake those in-depth analyses aimed at clarifying various aspects of the Antarctic silverfish reproduction including the possible occurrence of skip spawning as a widespread adaptive breeding strategy for the species.

Acknowledgments This work was carried out within the project RAISE (PdR 2013/AZ1.18) supported by the Italian National Programme for Antarctic Research. We thank Michela Angiolillo for her contribution to part of the laboratory activity. The officers and crew of RV Tangaroa, and the scientific staff during the survey, are gratefully acknowledged.

References

Andriashev AP (1965) A general review of the Antarctic fish fauna. In: van Mieghem J, van Oye P (eds) Biogeography and ecology in Antarctica. Springer, Netherlands, pp 491–550. doi:10.1007/978-94-015-7204-0_15

Brown-Peterson NJ, Wyanski DM, Saborido-Rey F, Macewicz BJ, Lowerre-Barbieri SK (2011) A standardized terminology for describing reproductive development in fishes. Mar Coast Fish 3:52–70. doi:10.1080/19425120.2011.555724

Burton MPM (1999) Notes on potential errors in estimating spawning stock biomass: determining the effects of non- participatory adults for some groundfish species. J North-west Atl Fish Sci 25:1–9

Butskaya NA, Faleeva TI (1987) Seasonal changes in gonads and fecundity of Antarctic fishes *Trematomus bernacchii* Boulenger, *Trematomus hansoni* Boulenger and *Pagothenia borchgrevinki* (Boulenger). Voprosy Ikhtiologii 27:27–36

Calvo J, Morriconi E, Rae GA (1999) Reproductive biology of the icefish *Champsocephalus esox* (Günther, 1861) (Channichthyidae). Antarctic Sci 11:140–149. doi:10.1017/S0954102099000206

Chapman BB, Skov C, Hulthén K, Brodersen J, Nilsson PA, Hansson LA, Brönmark C (2012) Partial migration in fishes: definitions, methodologies and taxonomic distribution. J Fish Biol 81:479–499. doi:10.1111/j.1095-8649.2012.03349.x

Crim LW, Glebe BD (1990) Reproduction. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 529–553

Cury P (1994) Obstinate nature: an ecology of individuals. Thoughts on reproductive behaviour and biodiversity. Can J Fish Aquat Sci 51:1664–1673. doi:10.1139/f94-167

DeWitt HH (1970) The character of the midwater fish fauna of the Ross Sea, Antarctica. In: Holdgate MW (ed) Antarctic ecology. Academic Press, London, pp 305–314

DeWitt HH, Heemstra PC, Gon O (1990) Nototheniidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern Ocean. Smith Institute of Ichthyology, Grahamstown, pp 279–331

Duhamel G, Kock KH, Balguerias E, Hureau JC (1993) Reproduction in fish of the Weddell Sea. Polar Biol 13:193–200. doi:10.1007/BF00238929

Eastman JT (1997) Phyletic divergence and specialization for pelagic life in the antarctic nothotenioid fish *Pleuragramma antarcticum*. Comp Biochem Physiol 118A:1095–1101

Everson I (1977) The living resources of the Southern Ocean. FAO Report, GLO/S0177/1, Rome, pp 1–156

Everson I, Kock KH, Campbell S, Parkes G, Cielniaszek Z, Szlakowski J (1991) Reproduction in the mackerel icefish, *Champsocephalus gunnari*, at South Georgia. Working Document CCAMLR WG-FSA 91/7, pp 1–12

Faleeva TI, Gerasimchuk VV (1990) Reproduction biology peculiarities of Antarctic silverfish, *Pleuragramma antarcticum* (Nototheniidae). Quest Ichthyol 30:67–79 **(in Russian)**

Gerasimchuk VV (1987) On the fecundity of Antarctic sidestripe, *Pleuragramma antarcticum*. J Ichthyol 28:98–100

Gerasimchuk VV (1992) A brief outline of the biology of the Antarctic silverfish, *Pleuragramma antarcticum* Boulenger,

1902 (Nototheniidae) from the Antarctic Indian Ocean. Working Document CCAMLR WG-FSA-92/11, 47

Giraldo C, Cherel Y, Vallet C, Mayzaud P, Tavernier E, Moteki M, Hosie G, Koubbi P (2011) Ontogenic changes in the feeding ecology of the early life stages of the Antarctic silverfish (*Pleuragramma antarcticum*) documented by stable isotopes and diet analysis in the Dumont d'Urville Sea (East Antarctica). Polar Sci 5:252–263. doi:10.1016/j.polar.2011.04.004

Granata A, Guglielmo L, Greco S, Vacchi M, Sidoti O, Zagami G, La Mesa M (2000) Spatial distribution and feeding habits of larval and juvenile *Pleuragramma antarcticum* in the western Ross Sea (Antarctica). In: Faranda F, Guglielmo L, Ianora A (eds) Ross Sea ecology. Springer, Berlin, pp 369–393

Granata A, Cubeta A, Gugliemo L, Sidoti O, Greco S, Vacchi M, La Mesa M (2002) Ichthyoplankton abundance and distribution in the Ross Sea during 1987–1996. Polar Biol 25:187–202. doi:10.1007/s00300-001-0326-y

Guglielmo L, Granata A, Greco S (1998) Distribution and abundance of postlarval and juvenile *Pleuragramma antarcticum* (Pisces, Nototheniidae) off Terra Nova Bay (Ross Sea, Antarctica). Polar Biol 19:37–51. doi:10.1007/s003000050214

Hanchet SM, Mitchell J, Bowden D, Clark M, Hall J, O'Driscoll R (2008) Ocean survey 20/20: New Zealand IPY-CAML Final Voyage Report. NIWA Client Report: WLG2008-74, October 2008. (Unpublished report held in NIWA library, Wellington)

Hubold G (1984) Spatial distribution of *Pleuragramma antarcticum* (Pisces: Nototheniidae) near the Filchner and Larsen Ice Shelves (Weddell Sea, Antarctica). Polar Biol 3:231–236. doi:10.1007/BF00292628

Hubold G (1985) The early life history of the high Antarctic Silverfish, *Pleuragramma antarcticum*. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Heidelberg, pp 445–451

Hunter JR, Macewicz BJ (1985) Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fish Bull 83:119–136

Jørgensen C, Fiksen Ø (2006) State-dependent energy allocation in cod (*Gadus morhua*). Can J Fish Aquat Sci 63:186–199. doi:10.1139/f05-209

Kjesbu OS, Fonn M, Gonzáles BD, Nilsen T (2010) Stereological calibration of the profile method to quickly estimate atresia levels in fish. Fish Res 104:8–18. doi:10.1016/j.fishres.2010.03.013

Kock KH, Kellermann A (1991) Reproduction in Antarctic notothenioid fish—a review. Antarct Sci 3:125–150. doi:10.1017/S0954102091000172

Koubbi P, O'Brien C, Loots C, Giraldo C, Smith M, Tavernier E, Vacchi M, Vallet C, Chevallier J, Moteki M (2011) Spatial distribution and inter-annual variations in the size frequency distribution and abundances of *Pleuragramma antarcticum* larvae in the Dumont d'Urville Sea from 2004 to 2010. Polar Sci 5:225–238

La Mesa M, Eastman JT (2012) Antarctic silverfish: life strategies of a key species in the high-Antarctic ecosystem. Fish Fish 13:241–266. doi:10.1111/j.1467-2979.2011.00427.x

La Mesa M, Caputo V, Eastman JT (2008) The reproductive biology of two epibenthic species of Antarctic notothenid fish of the genus *Trematomus*. Antarct Sci 20:355–364

La Mesa M, Riginella E, Mazzoldi C, Ashford J (2015) Reproductive resilience of ice-dependent Antarctic silverfish in a rapidly changing system along the Western Antarctic Peninsula. Mar Ecol 36:235–245. doi:10.1111/maec.12140

Loher T, Seitz AC (2008) Characterization of active spawning season and depth for eastern Pacific halibut (*Hippoglossus stenolepis*) and evidence of probable skipped spawning. J Northwest Atl Fish Sci 41:23–36

Mayzaud P, Chevalier J, Tavernier E, Moteki M, Koubbi P (2011) Lipid composition of the Antarctic fish *Pleuragramma antarcticum* influence of age class. Polar Sci 5:264–271. doi:10.1016/j.polar.2010.12.003

Miranda ACL, Bazzoli N, Rizzo E, Sato Y (1999) Ovarian follicular atresia in two teleost species: a histological and ultrastructural study. Tissue Cell 31:480–488

Molens S, Matallanas J (2004) Reproductive biology of female Antarctic spiny plunderfish Harpagifer spinosus (Notothenioidei: Harpagiferidae), from Îles Crozet. Antarct Sci 16:99–105. doi:10.1017/S0954102004001865

Murua H, Kraus G, Saborido-Rey F, Witthames PR, Thorsen A, Junquera S (2003) Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. J Northwest Atl Fish Sci 33:33–54

O'Driscoll RL, Macaulay GJ, Gauthier S, Pinkerton M, Hanchet S (2011) Distribution, abundance and acoustic properties of Antarctic silverfish (*Pleuragramma antarcticum*) in the Ross Sea. Deep Sea Res Part II: Top Stud Oceanogr 58:181–195. doi:10.1016/j.dsr2.2010.05.018

Papi F (1992) Animal homing. Chapman & Hall, London

Parker SJ, Grimes PJ (2010) Length and age at spawning of Antarctic toothfish (*Dissostichus mawsoni*) in the Ross Sea. CCAMLR Sci 17:53–73

Pinkerton MH, Forman J, Bury SJ, Brown J, Horn P, O'Driscoll RL (2013) Diet and trophic niche of Antarctic silverfish *Pleuragramma antarcticum* in the Ross Sea, Antarctica. J Fish Biol 82:141–164. doi:10.1111/j.1095-8649.2012.03476.x

R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for statistical computing. Vienna, Austria. www.R-project.org

Rideout RM, Tomkiewicz J (2011) Skipped spawning in fishes: more common than you might think. Mar Coast Fish 3:176–189. doi:10.1080/19425120.2011.556943

Rideout RM, Rose GA, Burton MPM (2005) Skipped spawning in female iteroparous fishes. Fish Fish 6:50–72. doi:10.1111/j.1467-2679.2005.00174.x

Santos HB, Thome RG, Arantes FP, Sato Y, Bazzoli N, Rizzo E (2008) Ovarian follicular atresia is mediated by heterophagy, autophagy, and apoptosis in *Prochilodus argenteus* and *Leporinus taeniatus* (Teleostei: Characiformes). Theriogenology 70:1449–1460. doi:10.1016/j.theriogenology.2008.06.091

Shandikov GA, Faleeva TI (1992) Features of gametogenesis and sexual cycles of six notothenioid fishes from East Antarctica. Polar Biol 11:615–621. doi:10.1007/BF00237956

Shaw AK, Levin SA (2011) To breed or not to breed: a model of partial migration. Oikos 120:1871–1879. doi:10.1111/j.1600-0706.2011.19443.x

Skjæraasena JE, Nashb RDM, Korsbrekkeb K et al (2012) Frequent skipped spawning in the world's largest cod population. Proc Natl Acad Sci USA 109:8995–8999. doi:10.1073/pnas.1200223109

Smith WO, Ainley DG, Cattaneo-Vietti R (2007) Trophic interactions within the Ross Sea continental shelf ecosystem. Philos Trans R Soc B 362:95–111. doi:10.1098/rstb.2006.1956

Üçüncü Sİ, Çakıcı Ö (2009) Atresia and apoptosis in preovulatory follicles in the ovary of *Danio rerio* (zebrafish). Turk J Fish Aquat Sci 9:215–221

Vacchi M, La Mesa M, Greco S (1999) Summer distribution and abundance of larval and juvenile fishes in the western Ross Sea. Antarct Sci 11:54–60. doi:10.1017/S0954102099000085

Vacchi M, DeVries AL, Evans CW, Bottaro M, Ghigliotti L, Cutroneo L, Pisano E (2012) A nursery area for the Antarctic silverfish *Pleuragramma antarcticum* at Terra Nova Bay (Ross Sea): first estimate of distribution and abundance of eggs and

larvae under the seasonal sea-ice. Polar Biol 35:1573–1585. doi:10.1007/s00300-012-1199-y

Van Damme CJG, Dickey-Collas M, Rijnsdorp AD, Kjesbu OS (2009) Fecundity, atresia, and spawning strategies of Atlantic herring (*Clupea harengus*). Can J Fish Aquat Sci 66:2130–2141. doi:10.1139/F09-153

Vanella FA, Calvo J, Morriconi ER, Aureliano (2005) Somatic energy content and histological analysis of the gonads in Antarctic fish from the Scotia Arc. Sci Mar 69:305–316

Witthames PR, Greenwood L, Lyons B (1999) Ovarian atresia in *Solea solea* (L). In: Norberg OS, Kjesbu GL, Taranger GL, Andersson E, Stefansson SO (eds) Proceedings of the 6th international symposium on the reproductive physiology of fish, Bergen, Norway, July 4–9, 1999. Jon Greg AS, Bergen, p 106

Witthames PR, Thorsen A, Kjesbu OS (2010) The fate of vitellogenic follicles in experimentally monitored Atlantic cod *Gadus morhua* (L.): application to stock assessment. Fish Res 104:27–37. doi:10.1016/j.fishres.2009.11.008