Invertebrate Reproduction & Development

ISSN: 0792-4259 (Print) 2157-0272 (Online) Journal homepage: https://www.tandfonline.com/loi/tinv20

On the reproduction of two deep-sea Arctic holothurians, *Elpidia heckeri* and *Kolga hyalina* (Holothuroidea:Elpidiidae)

Antonina Kremenetskaia, Olga Ezhova, Anatoliy L. Drozdov, Elena Rybakova & Andrey Gebruk

To cite this article: Antonina Kremenetskaia, Olga Ezhova, Anatoliy L. Drozdov, Elena Rybakova & Andrey Gebruk (2020) On the reproduction of two deep-sea Arctic holothurians, *Elpidia heckeri* and *Kolga hyalina* (Holothuroidea:Elpidiidae), Invertebrate Reproduction & Development, 64:1, 33-47, DOI: 10.1080/07924259.2019.1692915

To link to this article: https://doi.org/10.1080/07924259.2019.1692915

Published online: 28 Nov 2019.

INVERTEBRATE REPRODUCTION & DEVELOPMENT
2020, VOL. 64, NO. 1, 33–47
https://doi.org/10.1080/07924259.2019.1692915

Taylor & Francis
Taylor & Francis Group

On the reproduction of two deep-sea Arctic holothurians, *Elpidia heckeri* and *Kolga hyalina* (Holothuroidea:Elpidiidae)

Antonina Kremenetskaia[a], Olga Ezhova[b], Anatoliy L. Drozdov[c,d], Elena Rybakova[a] and Andrey Gebruk[a]

[a]Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, Russia; [b]Biological Faculty, Department of Invertebrate Zoology, Lomonosov Moscow State University, Moscow, Russia; [c]Zhirmunsky National Scientific Center of Marine Biology, FEB RAS, Vladivostok, Russia; [d]Department of Bioorganic Chemistry and Biotechnology, Far Eastern Federal University, Vladivostok, Russia

ABSTRACT

Internal gonad morphology was examined in two species of elpidiid holothurians, *Elpidia heckeri* and *Kolga hyalina*, commonly occurring in the Central Arctic Ocean. The holothurians were sampled in August – September 2012 and in August 2018, at several locations in the Nansen and Amundsen Basins, in areas under the constant ice cover and in areas free of ice. Both species were observed at different reproductive stages and both are presumed to reproduce periodically. In 2012, spawning occurred at the beginning of September in *Elpidia heckeri* and from late August to early September in *Kolga hyalina*. There are reasons to believe that gametogenesis in *K. hyalina* depends on the availability of freshly deposited phytodetritus. The maximum oocyte size in *Kolga hyalina* (240 µm) was smaller than in *Elpidia heckeri* (390 µm).

ARTICLE HISTORY
Received 8 August 2019
Accepted 11 November 2019

KEYWORDS
Abyssal Arctic; deep-sea species; reproduction biology; Elasipodida; Echinodermata; sea cucumbers

Introduction

The elpidiid holothurians (Elpidiidae) are the most diverse family within the Order Elasipodida. They are very common representatives of soft-bottom abyssal communities throughout the ocean. Many elpidiids form aggregations on the seafloor. In particular, high densities were reported for *Peniagone diaphana* (Barnes et al., 1976), *Kolga nana* (Billett and Hansen 1982; Rogacheva 2012; Gebruk and Krylova 2013), *Elpidia hanseni* and *E. ninae* (Belyaev 1989), *Elpidia* sp., *Scotoplanes globosa* and *Rhipidothuria racovitzai* (Gutt and Piepenburg 1991), *Amperima rosea* (Billett et al. 2010), *Peniagone* sp. and *Elpidia* sp. (Kuhnz et al. 2014). The occurrence of these species in super-abundance at certain times and localities suggests they have reproductive pattern unlike those in many deep-sea echinoderms. Data on the reproduction of Elpidiidae remain fragmentary. Their larval development is completely unknown. Reproductive biology was studied in detail only in five species of Elpidiidae: *Peniagone azorica* and *P. diaphana* (Tyler et al. 1985), *Amperima rosea* (Wigham et al. 2003), *Protelpidia murrayi* and *Peniagone vignoni* (Galley et al. 2008). Although many of deep-sea holothurians are known to reproduce all year round, the latter three elpidiid species were found to reproduce seasonally suggesting opportunistic reproductive patterns dependent on the quality of food. All these species occurred in areas with seasonal food pulses, apparently inducing intensive gametogenesis. Such a reproductive pattern allows populations to increase rapidly in number and to form dense aggregations.

The only two elpidiid species occurring in the Arctic Ocean, *Elpidia heckeri* Baranova 1989 and *Kolga hyalina* Danielssen and Koren 1880 (Figure 1), are very common representatives of the abyssal communities and can reach high population densities (Soltwedel et al. 2003, 2009; MacDonald et al. 2010; Boetius et al. 2013; Taylor et al. 2018). Together with the wide circumpolar distribution of both species in the abyssal Central Artic, Baffin Bay, Greenland and Norwegian Seas, this suggests they have highly successful reproductive strategies. Recent studies in the Nansen and Amundsen Basins revealed the massive seasonal deposition of algae on the abyssal seafloor derived from ice-cover blooms and the presence of algae in the guts of *K. hyalina* (Boetius et al. 2013). The algae would appear to be a valuable food resource for elpidiid holothurians with the potential to affect the intensity of gametogenesis. However, examination of their annual reproductive cycle is complicated by difficult ice conditions affecting sampling most of the year.

While the reproductive biology of *E. heckeri* remains unknown, some data were published for *K. hyalina* such as external morphology of a gonad, gonoduct and genital papilla (Hansen 1975; Danielssen and Koren 1879). Specimens of *Kolga hyalina* in the North Atlantic described originally by Billett and Hansen (1982) have

CONTACT Antonina Kremenetskaia antonina@ocean.ru Shirshov Institute of Oceanology RAS, Moscow, Russia

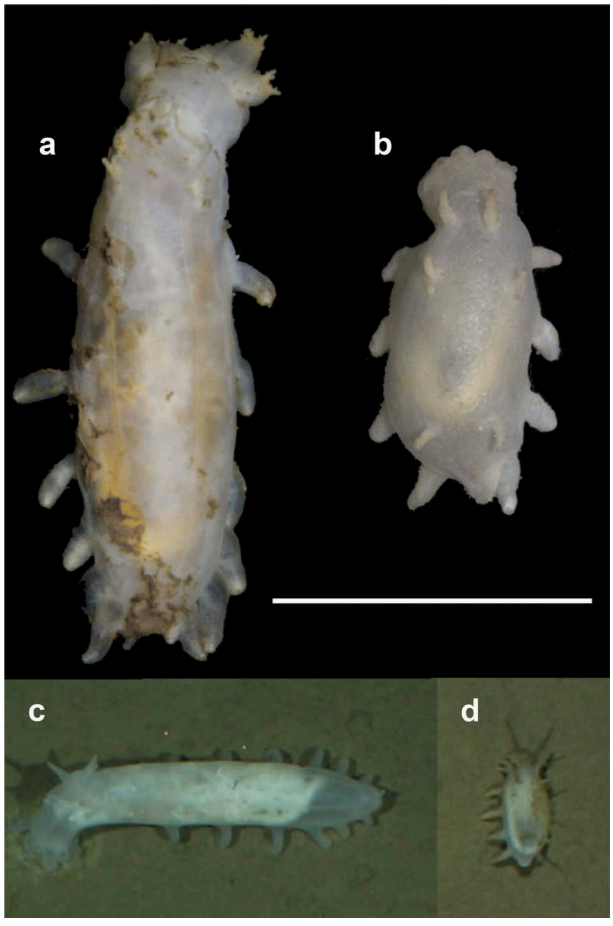

Figure 1. Dorsal view of *Kolga hyalina* (a, c) and *Elpidia heckeri* (b, d): (a, b) – *in vivo* (before preservation), scale 50 mm; (c), (d) – *in situ*.

now been assigned to another species, *Kolga nana* (Théel) (see Rogacheva 2012). Details of the reproductive biology of *K. nana* (as *K. hyalina*) are given in Billett (1988, p. 270–271). Here we present new data on the reproduction of *E. heckeri* and *K. hyalina* collected in the Central Arctic Ocean in the areas under the permanent ice cover and in the area free of ice.

Methods

Material was collected in two cruises from eight stations in the Nansen and Amundsen Basins at depths between 2939 and 4380 m (Table 1, Figure 2). Specimens were collected with Agassiz and Sigsbee trawls and then washed with sea water. Specimens were preserved in 96% ethanol for taxonomic identification. For reproductive studies, they were preserved in 4% borax-buffered formaldehyde in seawater and 2.5% glutaraldehyde solution buffered with 0.5M sodium cacodylate containing 21 mg/ml NaCl.

Gonad internal morphology was examined on the series of histological slides and by examination of preparations of the whole tubules. The latter method allowed studying a greater number of oocytes, and also to observe oocytes in many different tubules. For that purpose, several tubule clusters were dissected, washed in water from a fixative and dehydrated. Before microscopic examination, the tubules were placed in a glycerine drop on the microscope glass and pressed using a cover glass.

Histological slides were prepared using the standard technique of dehydration in ethanol of increasing concentrations (Valovaya and Kavtaradze 1993), followed by embedding the material in paraplast and cutting it into sections 7 µm thick on a microtome (Leica RM 2125). The sections were then stained with hematoxylin, dried and mounted in Canada Balsam.

The external and internal morphology of gonads was examined with the compound and dissection microscopes. Images of histological slides were taken with a

Table 1. Station data for examined samples.

Station number	Location	Gear (Trawl)	Date	Start and End Coordinates	Water depth (m)	Examined specimens of *E. heckeri*	Examined specimens of *K. hyalina*
RV *Polarstern*, Cruise ARK XXVII-3 (IceArc)							
1	Nansen Basin	Agassiz	09.08.2012	84.038° N 30.162° E – 84.038° N 30.188° E	4012 – 4013	♀ - 0 ♂ - 2	♀ – 4 ♂ – 1
2	Nansen Basin	Agassiz	17.08.2012	83.972° N 77.683° E – 83.970° N 77.632° E	3470	♀ – 1 ♂ – 1	♀ – 0 ♂ – 0
3	Nansen Basin, close to Gakkel Ridge	Agassiz	20.08.2012	82.709° N 109.578° E – 82.724° N 109.563° E	3575 – 3576	♀ – 1 ♂ – 1	♀ – 4 ♂ – 5
4	Amundsen Basin	Agassiz	26.08.2012	82.791° N 129.878° E – 82.778° N 129.847° E	4158 – 4159	♀ – 3 ♂ – 0	♀ – 1 ♂ – 1
5	Amundsen Basin	Agassiz	05.09.2012	81.909° N 130.877° E – 81.906° N 130.843°E	4038 – 4039	♀ – 5 ♂ – 3	♀ – 3 ♂ – 2
6	Amundsen Basin	Agassiz	09.09.2012	85°4.35'N 122°42.42'E – 85°4.13'N 122°41.49'E	4353 – 4354	♀ – 3 ♂ – 4	♀ – 6 ♂ – 1
7	Amundsen Basin	Agassiz	19.09.2012	87°53.53'N 59°23.32'E – 87°53.77'N 59°20.81'E	4380	♀ – 1 ♂ – 0	♀ – 5 ♂ – 0
RV *Akademik Mstislav Keldysh*, Cruise 72							
5958	Slope basis of the Laptev Sea	Sigsbee	30.08.2018	78° 57.76'N 125° 44.88'E – 78° 58.68'N 125°49.60'E	3003–2939	♀ – 1 ♂ – 0	♀ – 0 ♂ – 3

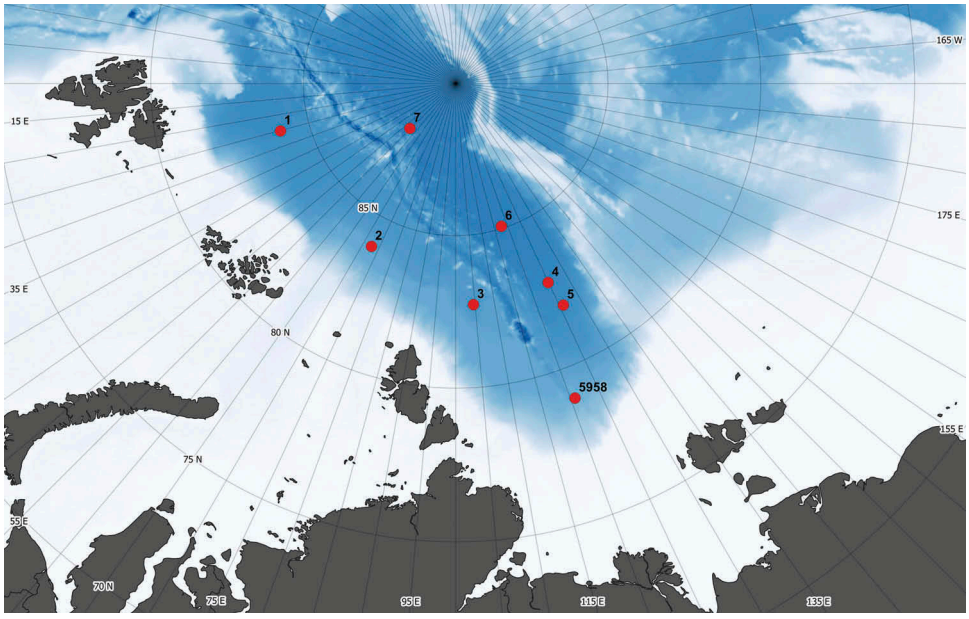

Figure 2. Map of sampling localities.

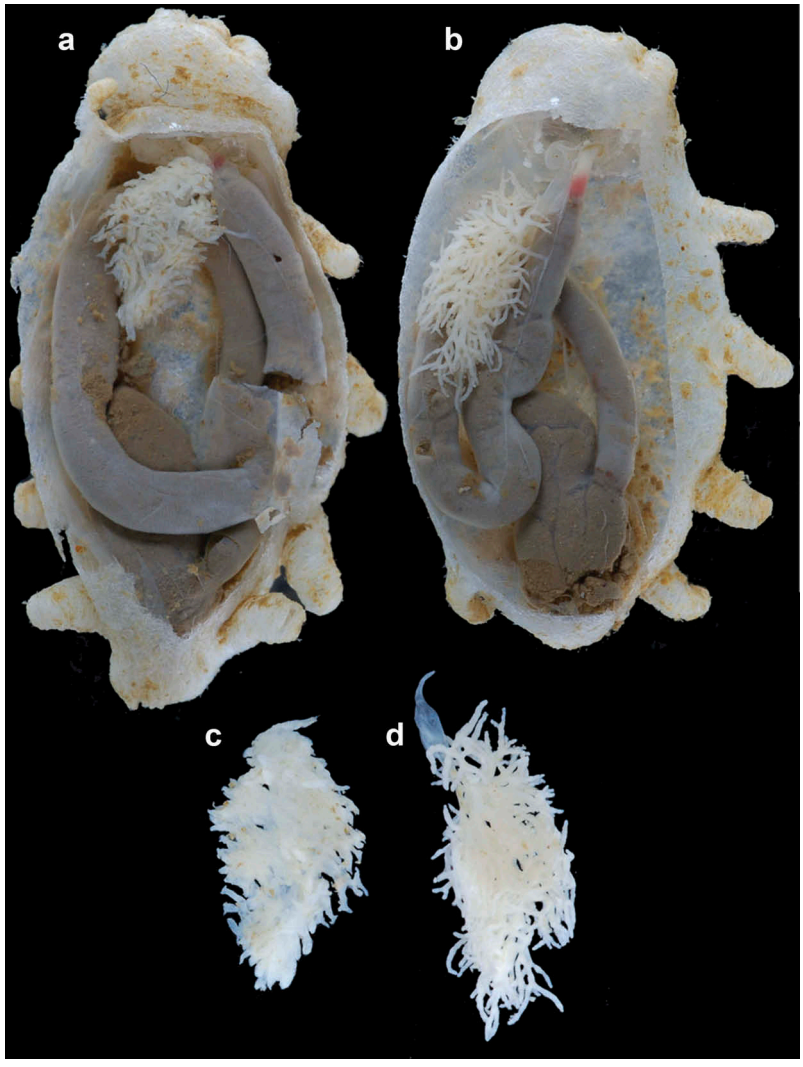

Figure 3. Dissected *Elpidia heckeri* and its gonads: (a), (c) – female; (b, d) – male.

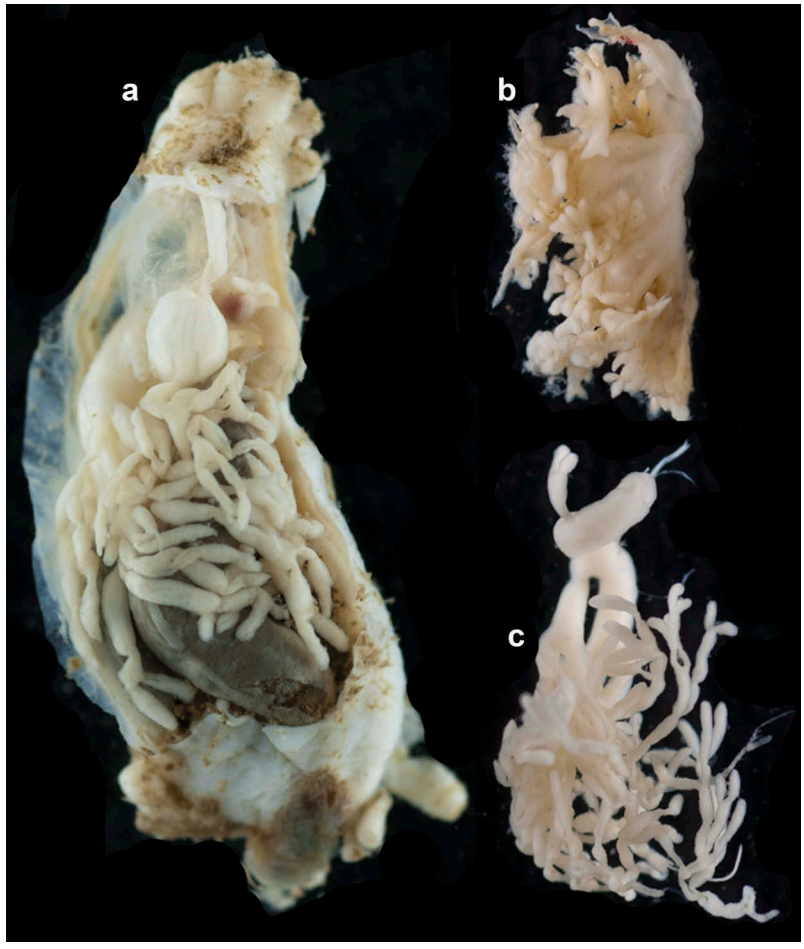

Figure 4. Dissected male of *Kolga hyalina* (a), M – ovary; (c) – testis.

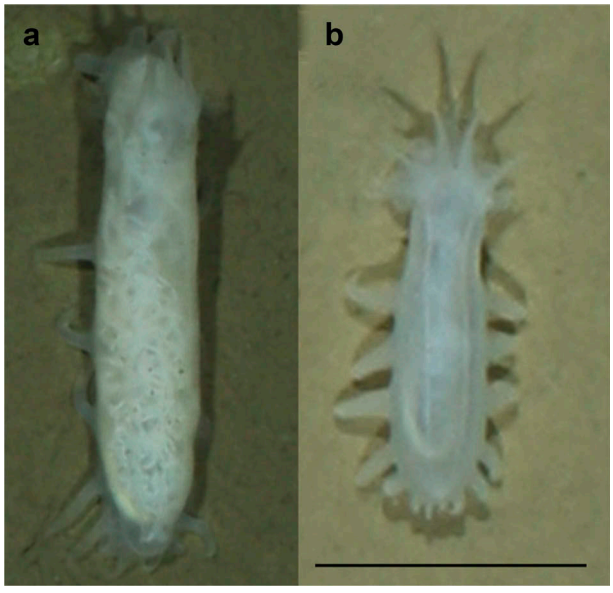

Figure 5. *Kolga hyalina* at Station 5 with well-developed gonad (a) and Station 1 with smaller gonad (b).

Leica DFC490 camera mounted on a Leica DMI4000B compound microscope. Oocyte size was measured with ImageJ software (https://imagej.nih.gov/ij). Feret diameter was determined in oocytes with a visible nucleus.

At some stations, gonad histology was examined only in a single specimen or in one specimen of each sex (Table 1). Although these data are not comprehensive, most of the specimens examined from the same location were at a similar stage of gametogenesis and had a similar oocyte size range, so even data from one specimen could be informative. The oocyte size was measured only in specimens from Stations 1–5 and 5958. Most of the specimens at the post-spawning stage had very few oocytes suitable for measuring because most of the mature and developing oocytes had been resorbed and there were only a few previtellogenic oocytes forming.

Results

External morphology of the gonads

In both species, *E. heckeri* and *K. hyalina*, there was sexual dimorphism in the gonad external morphology.

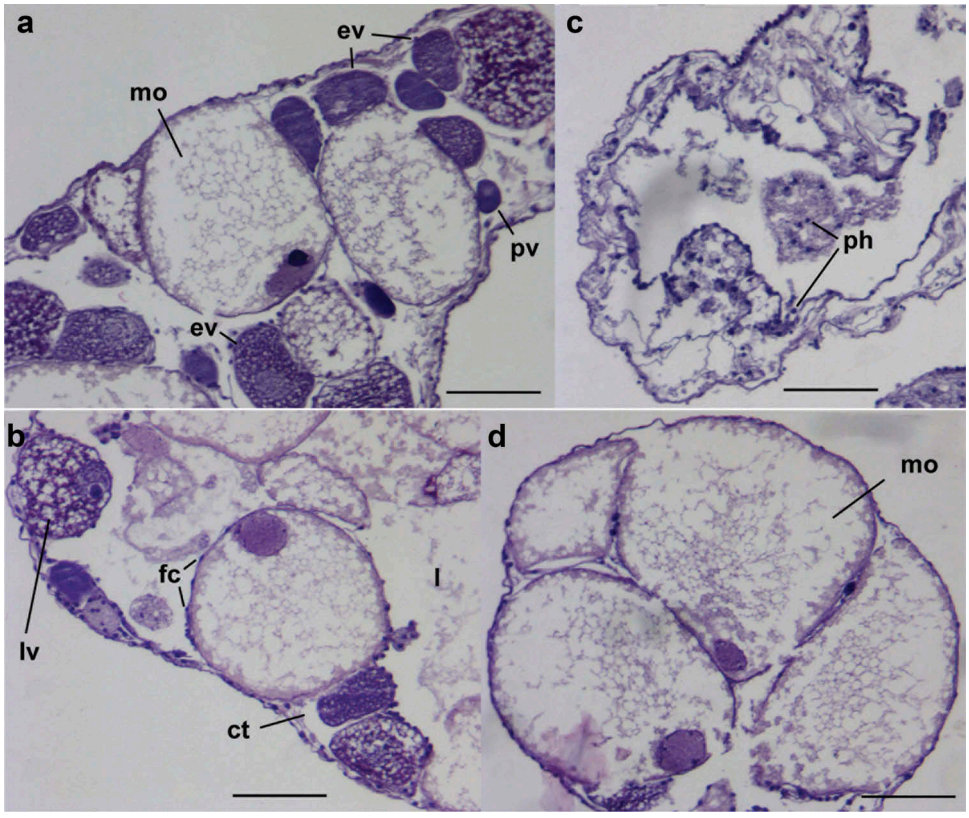

Figure 6. Cross-section of the ovary tubules at pre-spawning (a), spawning stages (b, d) and post-spawning resorption (c) in *Elpidia heckeri*. Scale 100 µm.

ct – connective tissue; ev – early vitellogenic oocyte; fc – follicular accessory cells; l – lumen; lv – later stage vitellogenic oocyte; mo – mature oocytes; ph – phagocytes; pv – previtellogenic oocyte.

Their gonad, unpaired, as well known in elpidiids, lay to the left of the dorsal mesentery. In *E. heckeri* the gonad consisted of a dense cluster of tubules opening to a central duct, which ended in a short gonoduct (Figure 3). The latter opened on the top of the small genital papilla located in the mid-dorsal interradius between the tentacles and the anterior pair of dorsal papillae. In females, there were short ramifications at the ends of tubules, whereas in males these bifurcations were remarkably longer. The tubules were more slender in males.

In mature individuals of *K. hyalina* gonads could fill the entire body cavity (Figures 4–5). In contrast to *E. heckeri* the main duct in *K. hyalina* bifurcated many times, forming long tubules and never forming a dense cluster (Figure 4(c)). The duct opened to a basal sac leading to the gonoduct. The latter opened on the top of the well-developed genital papilla anteriorly of the velum. In females, the main duct was less bifurcated, and the individual tubules were larger and less numerous than in males.

The size of gonads in *K. hyalina* varied greatly, even at stations sampled during the same period. Gonads were relatively small in specimens from Station 1, whereas in specimens from other stations the gonads filled the entire body cavity (Figure 5).

Gametogenesis

Elpidia heckeri

Oogenesis. The perivisceral peritoneum consisted of myoepithelial cells underlined by a thin basal lamina. The connective tissue compartment varied in thickness and was homogenous to irregular in structure (Figure 6, *ct*). It contained fibers and a number of accessory cells, the coelomocytes, apparently having a nutritive value. It also contained haemal lacunas providing nutrition to the growing oocytes. Traces of the membranes left after the egg growing inside of it were spawned or resorbed were also visible (Figure 6(c)). The inner (germinal) epithelium contained oocytes of various stages and accessory cells forming follicles (Figure 6, *fc*). The longitudinal folds were often irregular in shape and often difficult to find.

The smallest oocytes recognized under the light microscope were ~10 µm in diameter. Smaller previtellogenic oocytes (Figure 6, *pv*) were characterized by small amount of basophilic cytoplasm. As they grow, the amount of cytoplasm increased and it became heterogeneous. At

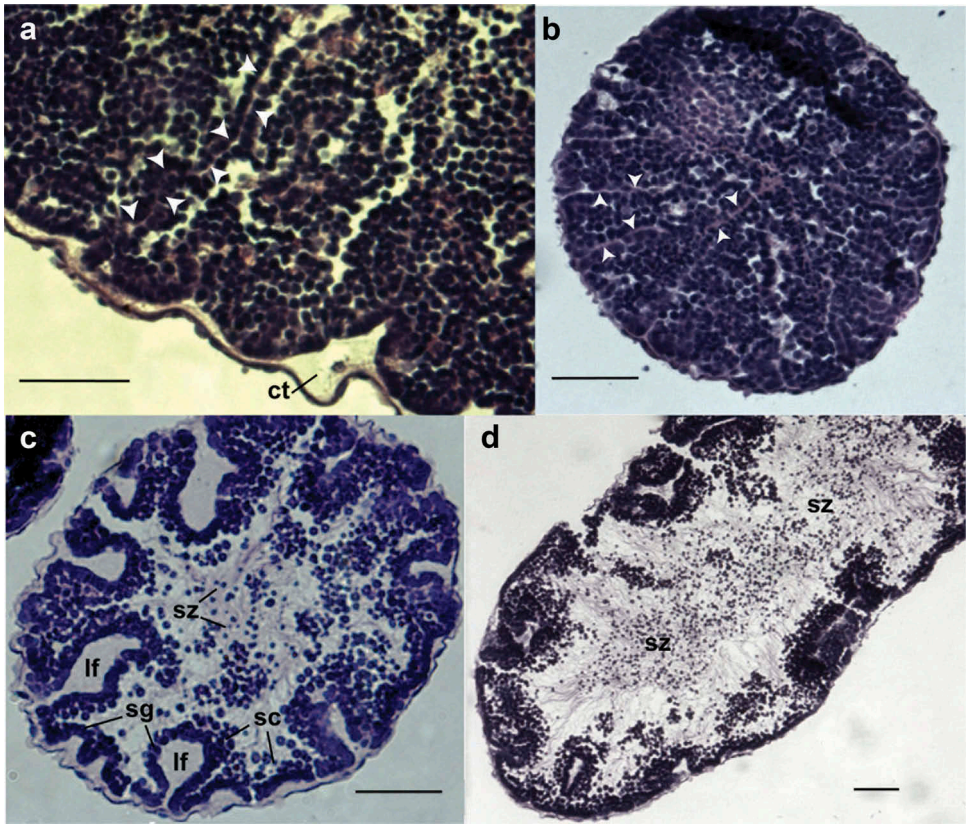

Figure 7. Cross-section of testis tubules in different stages of spermatogenesis in *Elpidia heckeri*. (a) and (b), pre-spawning; (c), (d), spawning. Scale 50 µm. Arrows on (a) and (b) show longitudinal folds.
ct – connective tissue, lf – latitudinal folds; sg – spermatogonia; sc – spermatocytes; sz – spermatozoa.

~50 µm the oocytes became surrounded by accessory cells. At ~70 µm the cytoplasm was highly vacuolated indicating the beginning of vitellogenesis (Figure 6, *ev*). Some of the vacuoles lost their basophily attaining a speckled appearance (Figure 6, *lv*). The oocytes remained vacuolated until reaching the size of ~140 µm. In bigger oocytes the vacuoles started to be destroyed, therefore the oocytes lost basophyly and became mature (Figure 6, *mo*).

Unspawned mature oocytes underwent resorption (Figure 6(c)), apparently affecting vitellogenic and young oocytes as well as the whole tubule wall. The latter was often seen in the smaller-sized tubules at late spawning or postspawning stages.

Spermatogenesis. In comparison to the ovary, the testis wall was characterized by a greater homogeneous connective tissue and more prominent longitudinal folds (Figure 7, *ct, lf*). The latitudinal folds varied in width from filiform (Figure 7(a,b), arrows) to solid structures (Figure 7 (c)). Longitudinal folds were surrounded by spermatogenous cells (spermatogonia and spermatocytes I and II, see Figure 7(c), *sg* and *sc*). In some cases, dividing spermatogonia were found. At the pre-spawning stage, most tubules were filled mainly with spermatocytes and contained narrow longitudinal folds surrounded by accessory cells and dense rows of spermatogonia (Figure 7(b)). Spermatogonia also formed a dense layer along the inner epithelium. At the spawning stage, tubules had a central lumen containing spermatozoa and prominent latitudinal folds surrounded by spermatogonia and less numerous spermatocytes (Figure 7(c,d)).

Kolga hyalina

Oogenesis. In the ovary of *K. hyalina,* the connective tissue compartment and the form of longitudinal folds were more structurally organised (Figure 8, *lf*). The connective tissue compartment was rarely homogenous (Figure 8, *ct*), often contained fibers, lacunas and a number of accessory cells. The latter could be more abundant in narrow parts of the gonad wall.

Cells of inner germinal epithelium were similar in shape to those of perivisceral peritoneum. The youngest oocytes were recognized at the diameter ~15 µm. They were rounded, strongly basophilic and had homogenous cytoplasm (Figure 8, *pv*). At the diameter 30–40 µm the cytoplasm was less basophilic and more vacuolated indicating the beginning of vitellogenesis (Figure 8, *ev*). Unlike development in *E. heckeri*, vitellogenic oocytes were not so

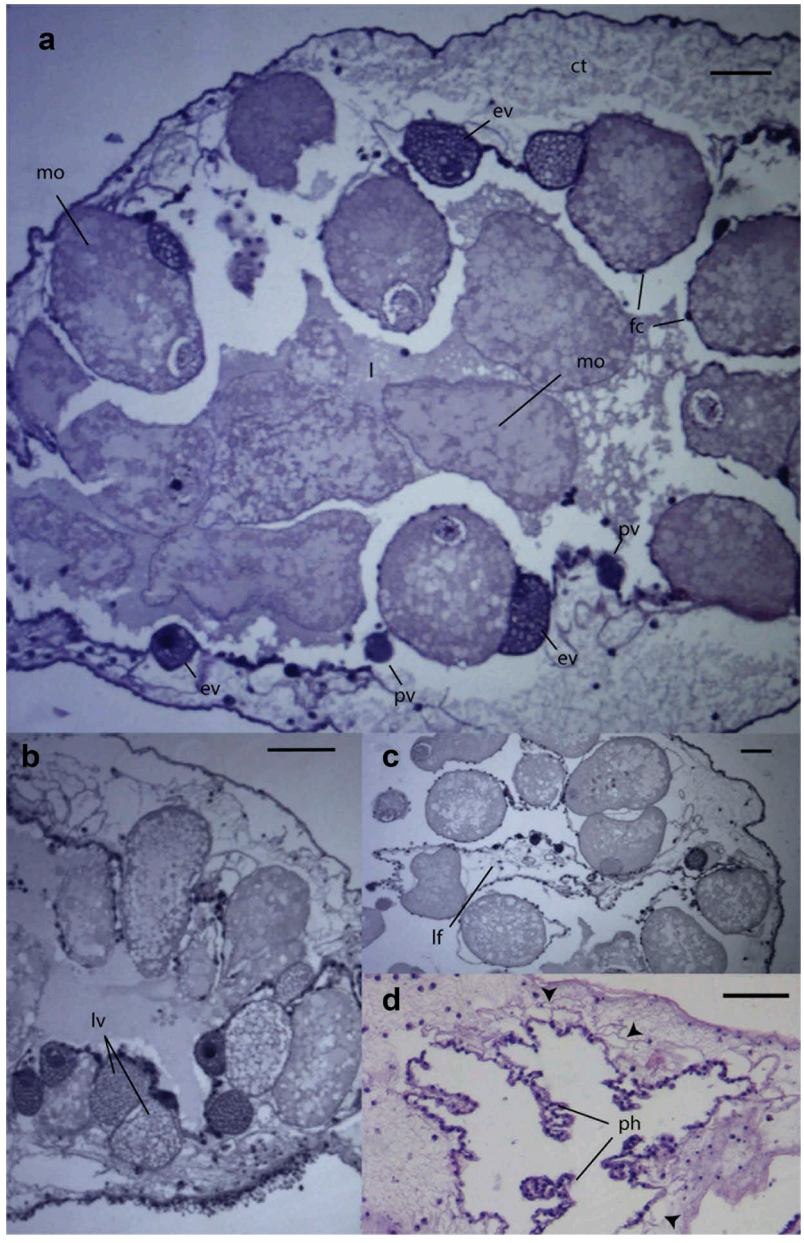

Figure 8. Cross-section of the ovary tubules at spawning stage (a – c) and post-spawning resorption (d) in *Kolga hyalina*. Scale 100 µm. Arrows show a membrane that remains after unspawned oocytes were resorbed.

ct – connective tissue; ev – early vitellogenic oocyte; fc – follicular accessory cells; l – lumen; lf – latitudinal folds; lv – later stage vitellogenic oocyte; mo – mature oocytes; ph – phagocytes; pv – previtellogenic oocyte.

brightly stained in the later stages in *K. hyalina* (Figure 8, *lv*), which suggests a greater amount of lipids in vacuoles. At this stage, the oocytes obtained follicular epithelium (Figure 8, *fc*). At the diameter 100 µm vitellogenic oocytes lost their basophily, the vacuoles started to be destroyed, and the oocytes became mature. In comparison with *E. heckeri*, mature oocytes in *K. hyalina* (Figure 8, *mo*) were more basophilic and contained some vacuoles that suggest higher glycoprotein content. Mature oocytes were numerous in the lumen, whereas in *E. heckeri* they were rare. As in *E. heckeri*, ovary tubules in *K. hyalina* were at different maturation stages: big tubules contained mainly mature oocytes and also some vitellogenic and previtellogenic oocytes, whereas small tubules were at various stages of resorption. Resorbing tubules contained numerous phagocytes (Figure 8, *ph*).

Spermatogenesis. The testis in *K. hyalina* was similar in internal morphology to that in *E. heckeri* but more structured. The gonad wall was wider, ca. 20 µm, and covered by myoepithelium. The connective tissue compartment was homogenous with rare accessory

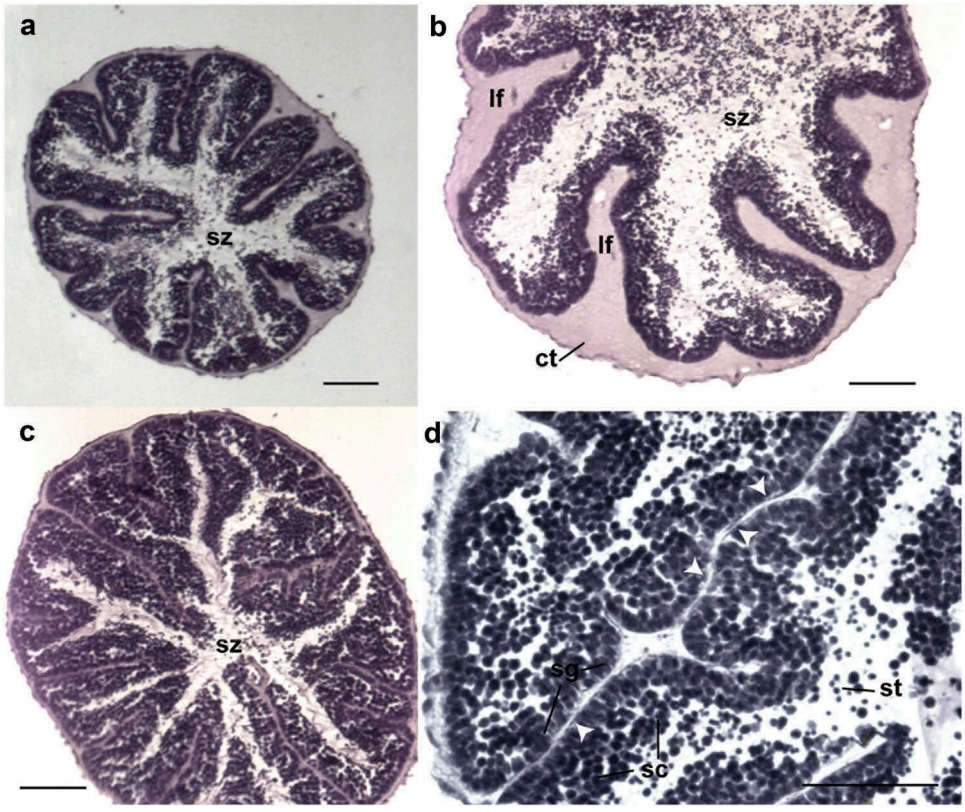

Figure 9. Cross-section of testis tubules in different stages of spermatogenesis in *Kolga hyalina*. (a) – spawning; (b) – late spawning, (c) – pre-spawning/spawning. Scale 100 µm. Arrows on (a) and (b) show longitudinal folds.
ct – connective tissue, lf – latitudinal folds; sg – spermatogonia; sc – spermatocytes; st – spermatids; sz – spermatozoa.

cells, about 10 µm in width (Figure 9(b), *ct* and (d), showed by arrows). Inner epithelium formed prominent well-visible folds (Figure 9(b), *lf*). The folds were surrounded by all the five types of the cells: spermatogonia (Figure 9(d), *sg*), spermatocytes I and II (Figure 9(d), *sc*), spermatids (Figure 9(d), *st*) and spermatozoa (Figure 9, *sz*). The latter were separated from accessory cells lying free in the lumen.

Oocyte size

The maximum oocyte size was 240 µm in *K. hyalina* and 390 µm in *E. heckeri*. According to the box-plot analysis (Figure 10), smaller differences in the egg size mean were found in *E. heckeri*, than in *K. hyalina*. The smallest eggs were in specimens of *E. heckeri* from Station 3, whereas at the Stations 2 and 5 they were the biggest (Figure 10). The oocyte size distribution in *K. hyalina* showed wider mean variation between the examined localities. At the Station 1, oocytes were remarkably smaller than at the other stations. The biggest oocytes were found in specimens from the Station 5 (Figure 10).

Reproductive stage

At the Stations 1–4, males and females of *E. heckeri* were characterized by mixture of some spermatozoa and spermatogeneous cells, and some mature oocytes and numerous smaller oocytes, respectively (Table 2). Absence of empty tubules, and also of the resorbing oocytes suggest the final pre-spawning stage. Oocytes at Station 2 were the biggest, however, there was no evidence of the beginning of spawning. Intensive gametogenesis was also found in the female from Station 5958, however its smaller oocytes indicate even later spawning stage. At Station 5, specimens were obviously at the spawning stage since their tubules were empty or contained mature gametes in higher proportion, or, in females, contained resorbing oocytes. At Stations 6 and 7, specimens were at the post-spawning stage, with gonad tubules containing resorbed oocytes in females and being empty in males. At the same time, few small oocytes and spermatogeneous cells of probably a new generation were present.

Beginning stages of gametogenesis were observed in gonads of *K. hyalina* at Station 1 (Figure 11). In males from the Station 5958, spermatozoa were present in some

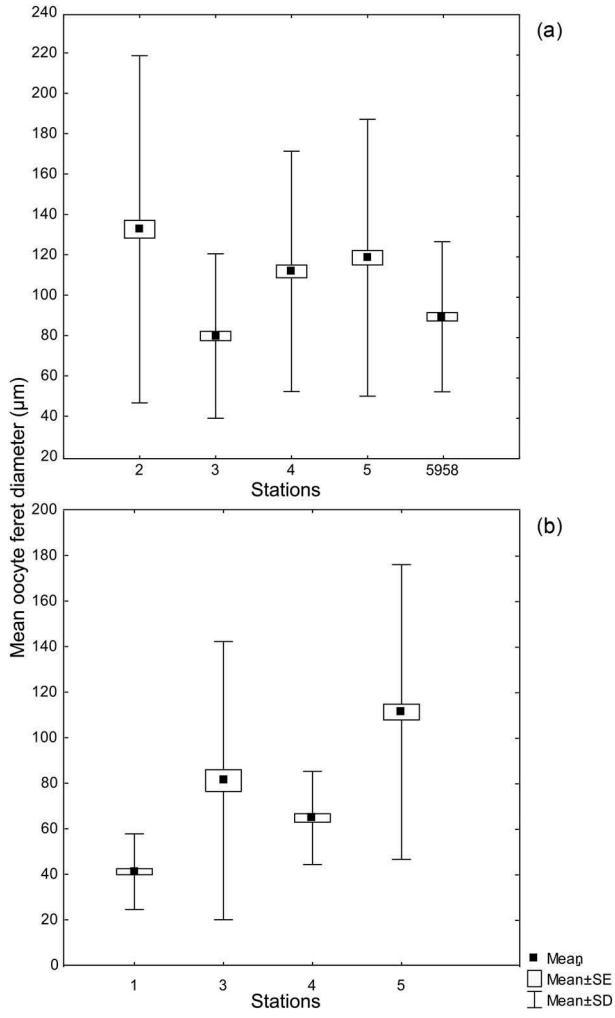

Figure 10. Vitellogenic oocyte size (feret diameter) statistics in *Elpidia heckeri* (a) and *Kolga hyalina* (b).

tubules with others filled with spermatogenic cells at different stages of development suggesting the pre-spawning stage. At Stations 3 and 5, specimens were spawning or at the very beginning of the post-spawning stage. At Stations 4, 6 and 7 specimens were at the post-spawning stage.

Frequency histograms of oocyte size (Figures 12–13) showed a peak at the size of ~40–70 µm in *E. heckeri* and ~30–50 µm in *K. hyalina* both at pre-spawning and spawning stages. At the spawning stage, a number of size clusters increased and size distribution showed another (smaller) peak at ~200 µm in *E. heckeri* and 150–170 µm in *K. hyalina*. At Station 4 *K. hyalina* was observed at the very beginning of the post-spawning stage (Figure 13, bottom left). The oocyte size distribution at this station was similar to the pre-spawning distribution with mature oocytes ejected or resorbed, but most of previtellogenic and vitellogenic oocytes were not fully resorbed.

Discussion

Gonad morphology and oocyte size

The gonad morphology in *E. heckeri* is typical of that in other elpidiids and similar to that described for *Elpidia glacialis* (Théel 1877), *Elpidia belyaevi* (Hansen 1975; identified as *Elpidia glacialis), Amperima rosea* (Wigham 2002) and *Penilpidia desbarresi* (Gebruk et al. 2013). The gonad is located in the anterior body half, on the dorsal side covering the intestine. The gonad of this type consists of a dense cluster of short tubules opening into the gonoduct varying in length. In *K. hyalina* the gonad is of a different deviating type. The same type of gonad is in *Irpa abyssicola* Danielssen and Koren 1879, however these two species might be conspecific (Rogacheva 2007). The gonad in *K. hyalina* consists of a long, ramified duct ending in fertile tubules. In our specimens from some localities, the body cavity was packed with gonad tubules. Observed differences in the gonad size

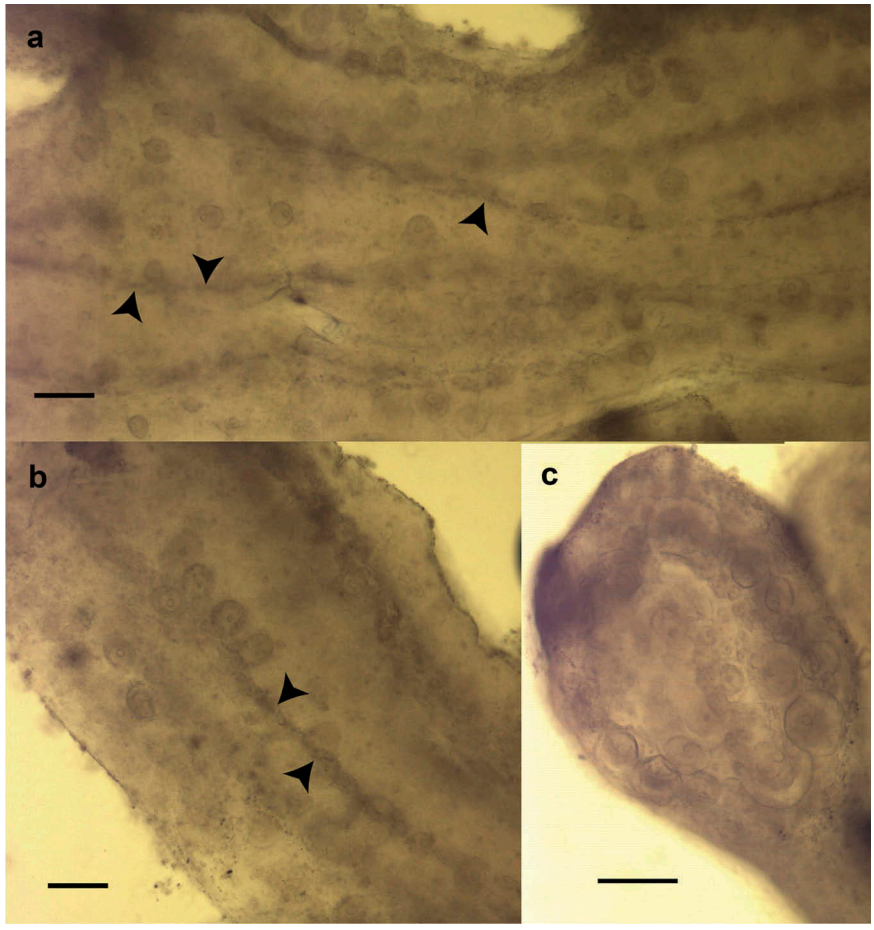

Figure 11. Ovary tubules under the light microscope in *Kolga hyalina* from St. 1. Scale 100 µm. Arrows on (a) and (b) show haemal sinuses, along which the growing oocytes are distributed; (c) – acinus (terminal part of a tubule, where some bigger oocytes occurred).

in *K. hyalina* suggest that gonads are bigger in spawning individuals getting smaller after spawning because of resorption.

Based on the maximum oocyte size, the elpidiids can be divided into three groups (Table 3): 1) with smaller eggs, ~200 µm in ferret diameter (*Elpidia glacialis, Scotoplanes globosa, S. clarki* etc.); 2) with medium-sized eggs, ~300 µm (*Peniagone diaphana* and *Protelpidia murrayi*) and 3) with larger eggs, >400 µm (*Peniagone vignoni*). Maximum oocyte size in *K. hyalina* is smaller than in *E. heckeri* falling into group 1. The same oocyte size was observed by Billett (1991) in *Kolga nana*. The reproductive strategy in the latter species as well as in another species with small eggs, *Amperima rosea*, is opportunistic, i.e. they can quickly respond to increased flux of organic matter and produce a lot of small eggs forming temporary dense aggregations (Billett et al. 2010). It can be suggested that fecundity is higher in *K. hyalina* than in *E. heckeri* because *K. hyalina* is generally bigger with the gonad more branched and smaller eggs.

Reproduction periodicity

Many deep-sea holothurians are known to reproduce continuously with only few species spawning periodically or seasonally. Periodical reproduction was suggested for *Kolga nana* (Billett and Hansen 1982; Billett 1991), *Amperima rosea* (Wigham et al. 2003), *Protelpidia murrayi* and *Peniagone vignoni* (Galley et al. 2008). These species are characterized by significant differences in the oocyte size depending on a season. The annual reproductive cycle remains unclear in our specimens of *E. heckeri* and *K. hyalina* since they were collected during the short period of the year, from the beginning of August to mid-September. However, even this short period of observation revealed remarkable differences in the mean oocyte size in the two species. Differences were more prominent in *K. hyalina*: oocytes were much smaller in specimens from Station 1 than from all other stations. The internal gonad morphology in both species (examined visually) also suggests spawning seasonality, since pre-spawning, spawning and post-spawning stages were found.

Table 2. Visual observation summary of the internal gonad morphology of the specimens from different stations.

Station	Date	Elpidia heckeri	Kolga hyalina
1	09.08.2012	Females: not examined Males: intensive spermatogenesis; some areas contain mature spermatozoa	Females: tubules are mostly empty with smaller-sized oocytes located along the sinuses Males: spermatogeneous cells along the sinuses and empty central part of the tubules
2	17.08.2012	Female: gonad is packed with many smaller-sized oocytes and also big oocytes; some mature oocytes were observed in the lumen Male: intensive spermatogenesis; sinuses are surrounded by spermatogenous cells on different stage of development	Not sampled
3	20.08.2012	Female: intensive oogenesis; some tubules contain large oocytes Male: central part of the tubules are filled with spermatozoa; spermatogenous cells are numerous; some tubules did not contain mature cells	Females: tubules are filled with mature oocytes; some tubules are empty; resorbing oocytes are also present Males: tubules are filled with spermatogenous cells and spermatozoa
4	26.08.2012	Females: intensive oogenesis with few mature oocytes in the lumen Males: not examined	Female: acini mainly empty, some of them contain resorbing oocytes, and few very small oocytes Male: new generations of spermatogeneous cells
5	05.09.2012	Females: many mature oocytes, most of them undergo resorption, some tubules contain only resorbed oocytes Males: spermatozoa and spermatogeneous cells are visible in some big tubules, smaller sized tubules are often empty	Females: many mature oocytes are in the lumen, smaller oocytes and resorbing cells are also present Males: tubule lumen is filled with spermatozoa; on the periphery are spermatogeneous cells on the different stage of development
6	09.09.2012	Female: few resorbing oocytes and a number of small oocytes probably of a new generation Males: tubules are mostly empty, some spermatogeneous cells are developed at the peripheral of the tubules	Females: in the tubules resorbing oocytes and small oocytes probably of a new generation Male: tubules contain spermatogeneous cells at the periphery
7	19.09.2012	Female: tubules contain few resorbing oocytes and small oocytes probably of a new generation Males: not examined	Females: in the tubules resorbing oocytes and small oocytes probably of a new generation Males: not examined
5958	30.08.2018	Female: intensive oogenesis, with mostly smaller oocytes Male: not examined	Female: not examined Male: intensive spermatogenesis with numerous spermatogeneous cells at the different stage of development; many of tubules contain spermatozoa, but they are not numerous

In 2012, spawning took place in the beginning of September in *E. heckeri* and from late August to early September in *K. hyalina*. As in other seasonally reproducing deep-sea holothurians, the time of spawning in *K. hyalina* and *E. heckeri* apparently depends on local environmental factors such as the flux of phytodetritus to the sea floor. This hypothesis can explain peculiar dissimilarities between reproductive stages at different locations on similar dates, or instead, similarities in gametogenesis stages on different dates. Particularly, in 2012 *K. hyalina* was found spawning on August 20 (Station 3) and September, 5 (Station 5), whereas on August 26 it was clearly at the post-spawning stage. Gametogenesis in *E. heckeri* was more consistent. In 2012 this species was found pre-spawning on 9–26 of August, spawning on September 5 and post-spawning on 9–19 of September. The female of *E. heckeri* collected at Station 5958 on 30 August 2018 was expected to be at the same reproductive stage with specimens from Station 4 collected in 2012 also at the end of August. However, oocytes were smaller in the female from Station 5958, suggesting later spawning in 2018 at this locality (Figure 12). We estimate the delay in spawning as ~10 days since the oocyte size at Station 5958 (in 2018) was similar to that at Station 3 (in 2012) and the gametogenesis in *E. heckeri* is relatively consistent.

Gametogenesis in the two species from the same localities was not fully synchronous. Particularly, at Station 3 *K. hyalina* was observed at the spawning stage, whereas *E. heckeri* was pre-spawning. The opposite pattern occurred at Station 1; gametogenesis was very intensive in *E. heckeri* whereas it was at the very beginning stage in *K. hyalina*. At the same time, similar gametogenesis stages were observed at Stations 5–7 (in 2012) and 5958 (in 2018). These dissimilarities can indicate different reproductive and feeding patterns, since strong relationships between the diet and foraging strategy, and the reproductive pattern are known for other elpidiids such as *Kolga nana* and *Amperima rosea* (Billett 1991; Wigham et al. 2003).

Conversely, gametogenesis in specimens of one species at the same locality was visually very synchronous. Differences were mainly observed in tubules of different size in one specimen than between the specimens. Differences between tubules suggest that spawning is not instant, it rather takes several days to complete. The latter can explain why *E. heckeri* was found spawning only at one station, Station 5. At Station 4 taken 9 days earlier, *E. heckeri* was pre-spawning, and at Station 6 taken four days later, specimens were post-spawning. Since gametogenesis stages and rates in *E. heckeri* were relatively consistent, spawning in this species in 2012 at

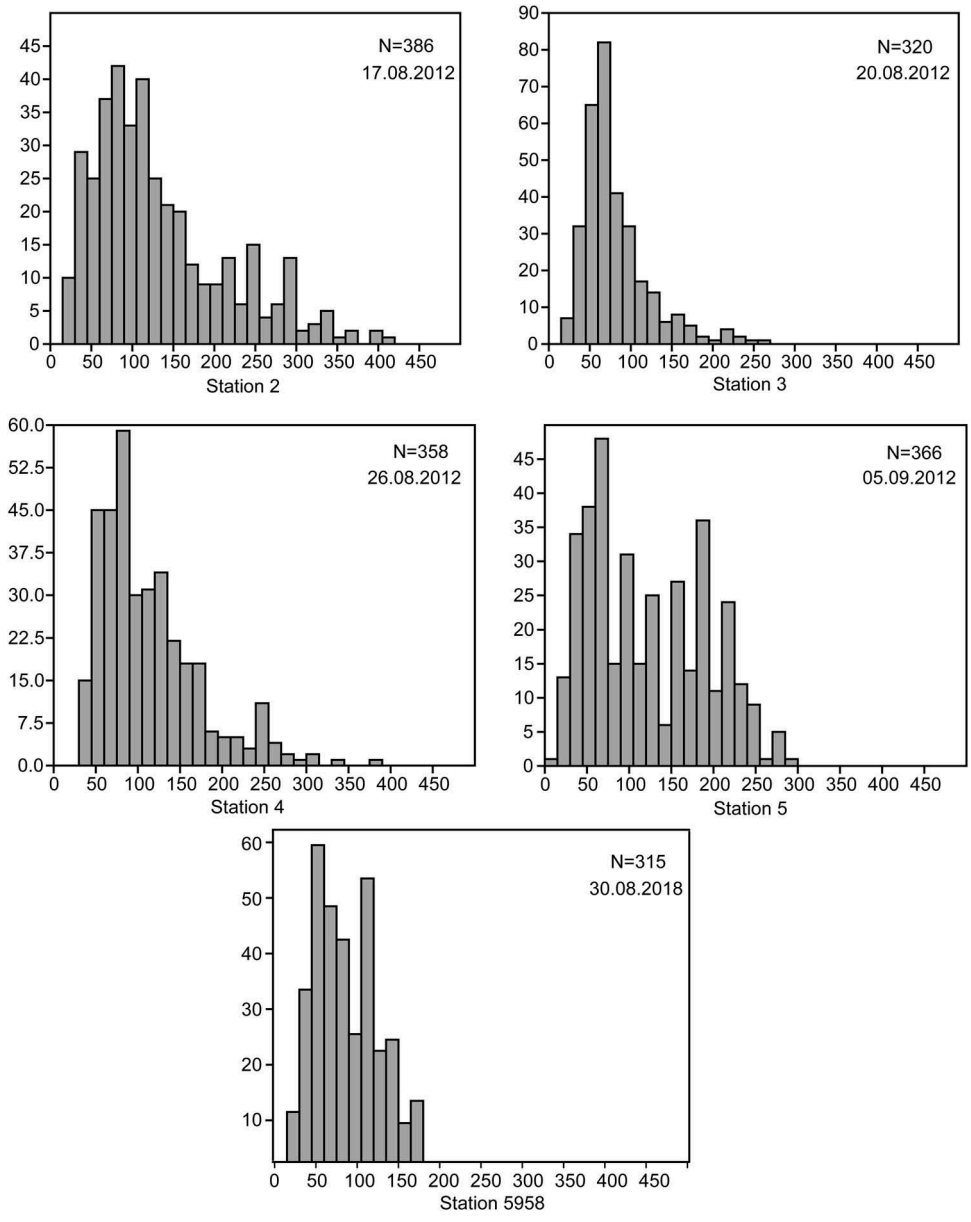

Figure 12. Frequency histograms of oocyte size (feret diameter) distribution in µm in *Elpidia heckeri*.

all localities could occur over a few days in the very beginning of September.

Relation to feeding conditions

Both species in our study, *Kolga hyalina* and *Elpidia heckeri*, were found actively feeding on freshly sunken colonies of ice-algae *Melosira arctica* (Boetius et al. 2013; Rybakova et al. 2019). Abundance of diatom colonies as well as the Chlorophyll *a* concentration in sediment strongly varied between the stations (Table 4). *Elpidia heckeri* occurred at pre-spawning/spawning or post-spawning stages at all the stations. No clear relationship with the phytodetritus abundance was revealed in this species. At the same time, *K. hyalina* was found at the near spawning stage only at the stations with the sunken algae. However, it seems unlikely that *M. arctica* colonies provide a provision for breeding in *K. hyalina*. These diatoms grow on the underside of the sea ice forming dense meter-long filaments with high biomass (Melnikov and Bondarchuk 1987). *Melosira arctica* debris at the seafloor cannot be abundant in areas free of ice in summer-time, such as the locality of Station 5958 where *K. hyalina* was numerous. It is more likely that *K. hyalina* similar to *K. nana* and *Amperima rosea* responds to increased flux of phytodetritus to the seafloor, the latter can be of sea ice or surface water origin. However, no clear relationships between the Chlorophyll *a* concentration, seafloor algal coverage and gametogenesis stage in

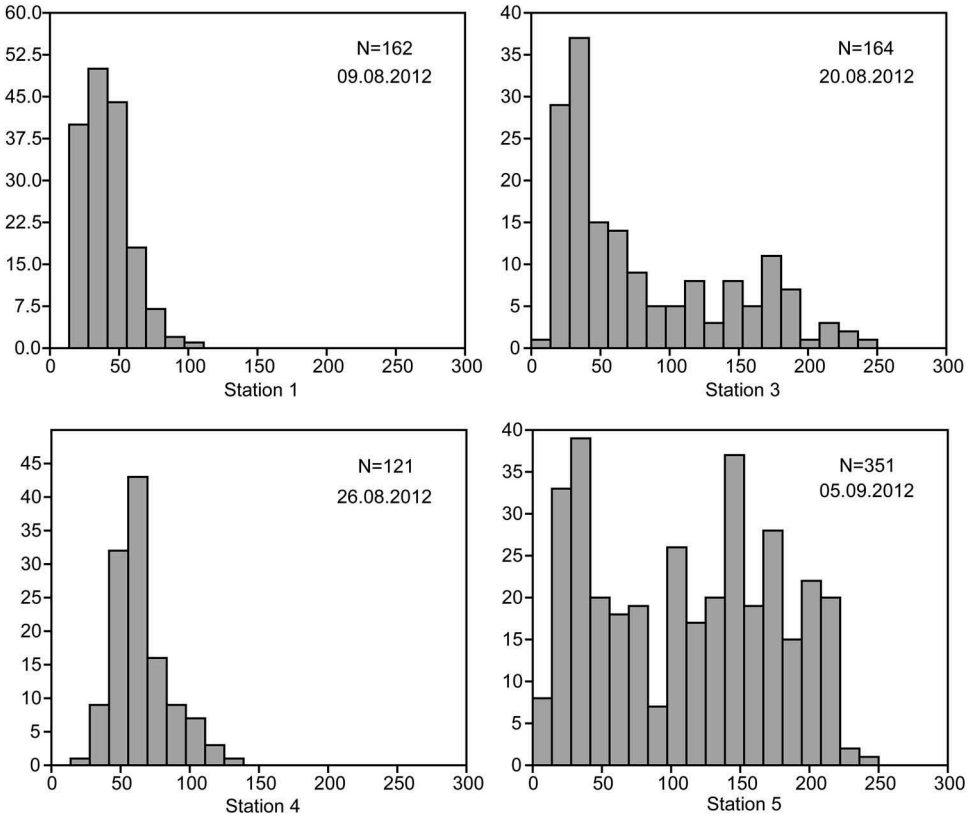

Figure 13. Frequency histograms of oocyte size (feret diameter) distribution in µm in *Kolga hyalina*.

Table 3. Maximum oocyte diameter in Elpidiidae.

Species	Oocyte diameter	Reference
Amperima naresi	200	Hansen (1975)
Amperima rosea	200	Wigham et al. (2003)
Ellipinion molle	200	Wigham et al. (2003)
Elpidia heckeri	390	This study
Elpidia sp. from Baffin Bay	200	Hansen (1975)
Elpidia sp.* from Kermadek Trench	200	Hansen (1975)
Kolga hyalina	240	This study
Kolga nana	240	Billett (1991)
Peniagone diaphana	300	Tyler et al. (1985)
Peniagone azorica	300	Tyler et al. (1985)
Peniagone vignoni	570	Galley et al. (2008)
Peniagone sp.** from Kermadek Trench	200	Hansen (1975)
Protelpidia murrayi	357	Galley et al. (2008)
Scotoplanes globosa	200	Hansen (1975)
Scotoplanes clarki	200	Hansen (1975)

* Identified as Elpidia glacialis, but probably another species.
** Identified as Peniagone azorica, but probably another species.

K. hyalina was found at the stations located in the ice-covered areas and where the sunken algae were observed (Stations 2–7). It can be suggested that algal deposits make up at the seafloor mosaically (especially massive debris) and they are consumed rapidly. However, regular and focused studies are required to understand this phenomenon. At Station 1, oocytes in *K. hyalina* were the smallest, the chlorophyll *a* concentration was low and diatom deposits or their traces were completely absent. Assumingly poor feeding conditions at Station 1 are corroborated by data on megabenthic communities in Rybakova et al. (2019): the biomass and density of megafauna were the lowest at Station 1 among Stations 1–7.

Kolga hyalina was at spawning and post-spawning stages only at stations with higher chlorophyll *a* concentration or obvious algal debris, therefore peculiarities of reproduction in poorer feeding conditions in this species remain unclear. Possible scenarios are fewer eggs or lack of spawning with oocytes undergoing resorption. This question requires further studies.

Conclusions

(1) *Elpidia heckeri* and *Kolga hyalina* are suggested to reproduce seasonally in response to ice cover primary production.
(2) Gametogenesis rate and time of spawning in both *Kolga hyalina* and *Elpidia heckeri* are dependent on the flux of phytodetritus, but more so in *K. hyalina*.

Table 4. Characteristics of the stations (from Boetius et al. 2013; Rybakova et al. 2019; modified).

Characteristic/Station	1	2	3	4	5	6	7	5958
Ice age: first (FYI)/ multi-year ice (MYI)	FYI	FYI	FYI	FYI	FYI	FYI/MYI	FYI/MYI	free of ice
Sea ice coverage (%)	80	80	70	80	60	50	100	0
Sea ice thickness (m)	1.0	1.2	0.7	0.7	1.2	0.9–1.7	1.2–1.8	N/A
Seafloor algal coverage (%) ±SD	0	0.03 ± 0.04	1.6 ± 0.4	0.3 ± 0.2	0.5 ± 0.2	0.4 ± 0.6	2.4 ± 0.7	Not observed
Algal freshness	absent	mostly fresh	mostly fresh, old white degraded patches present	mostly indistinct old white degraded patches	mostly not old white degraded patches	indistinct very old big patches	mostly fresh, old white patches present	N/A
Chlorophyll *a* (Chl *a*) (µg/cm3)	0.07	0.24	0.21	0.22	0.13	0.07	0.08	Not measured
Elpidia heckeri gametogenesis stage	Pre-spawning	Pre-spawning	Pre-spawning	Pre-spawning	Spawning	Post-spawning	Post-spawning	Intensive gametogenesis
Kolga hyalina gametogenesis stage	Early gametogenesis	Not sampled	Spawning	Post-spawning	Spawning	Post-spawning	Post-spawning	Intensive gametogenesis

(3) *Kolga hyalina* has smaller eggs compared to *E. heckeri* and more branched gonads that could lead to higher fecundity.

Acknowledgments

We thank Antje Boetius for organizing the *Polarstern* cruise XXVII-3 and sampling; Ivan Ryzhov, Renate Degen and crew of RV *Polarstern* for their great help in sampling and further work on the deck; Andrey Vedenin for collection of samples in the RV *Akademik Mstislav Keldysh* cruise 72. We are grateful to two anonymous reviewers for their valuable comments to the manuscript. This study was funded by RFBR, project No 17-05-00787 and 18-05-60228. Histological preparation of the material by O. E. was supported by the Russian Science Foundation (project No 18-74-10025).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was funded by RFBR, projects No 17-05-00787 and 18-05-60228. Histological preparation of the material by O. E. was supported by the Russian Science Foundation, project No 18-74-10025.

ORCID

Antonina Kremenetskaia http://orcid.org/0000-0001-8851-3318
Olga Ezhova http://orcid.org/0000-0003-4533-7769

References

Baranova ZI. 1989. A new holothurian species of the genus *Elpidia* from the Arctic Ocean. In: Kalo D, Rozhnov S, Solovev A, Stukalina G, editors. Probl izucheniya Iskop i Sovrem iglokozhikh. Fossil and Rec Echinoderm Res. Tallinn: Academy of Science Estonia, Institute of Geology; 218–222. [in Russian].

Barnes AT, Quetin LB, Childress JI, Pawson DL. 1976. Deep-sea macroplanktonic sea cucumbers: suspended sediment feeders captured from deep submergence vehicle. Science. 194:1083–1085.

Belyaev GM. 1989. Deep-sea oceanic trenches and their fauna. Moscow: Nauka.

Billett DSM 1988. The ecology of deep-sea holothurians [Doctoral Thesis]. Southampton: University of Southampton. https://eprints.soton.ac.uk/id/eprint/384501.

Billett DSM. 1991. Deep-sea holothurians. Oceanogr Mar Biol Annu Rev. 29:259–317.

Billett DSM, Bett BJ, Reid WDK, Boorman B, Priede IG. 2010. Long-term change in the abyssal NE Atlantic: the '*Amperima* Event' revisited. Deep Sea Res Part II Top Stud Oceanogr. 57:1406–1417.

Billett DSM, Hansen B. 1982. Abyssal aggregations of *Kolga hyalina* Danielssen et Koren (Echinodermata: Holothurioidea) in the

north-east Atlantic ocean: A preliminary report. Deep Res. 29:799–818.
Boetius A, Albrecht S, Bakker K, Bienhold C, Felden J, Fernández-Méndez M, Hendricks S, Katlein C, Lalande C, Krumpen T, et al. 2013. Export of algal biomass from the melting arctic sea ice. Science. 339:1430–1432.
Danielssen DC, Koren J. 1879. Fra den norske Nordhavsekspedition. Echinodermer. Nyt Mag Naturvidenskaberne. 24:229–266.
Danielssen DCKoren J. 1880. Fra den norske nordhavsekspedition. Echinodermer. Nyt Mag Naturvidenskaberne. 25:83–140.
Galley EA, Tyler P, Smith CR, Clarke A. 2008. Reproductive biology of two species of holothurian from the deep-sea order Elasipoda, on the Antarctic continental shelf. Deep Sea Res Part II Top Stud Oceanogr. 55:2515–2526.
Gebruk A, Rogacheva A, Pawson DL, Hamel J-F, Macisaac KG, Mercier A. 2013. *Penilpidia desbarresi* sp. nov. (Echinodermata: Holothuroidea: Elasipodida) from the upper slope of Newfoundland and re-description of *P. ludwigi* (von Marenzeller, 1893). Mar Biol Res. 9:1029–1036.
Gebruk AV, Krylova EM. 2013. Megafauna of the Charlie–Gibbs Fracture Zone (northern Mid-Atlantic Ridge) based on video observations. J Mar Biol Assoc United Kingdom. 93:1143–1150.
Gutt J, Piepenburg D. 1991. Dense aggregations of three deep-sea holothurians in the southern Weddell Sea, Antarctica. Mar Ecol Prog Ser. 68:277–285.
Hansen B. 1975. Systematics and biology of the deep-sea holothurians. Galathea Rep. 13:1–262.
Kuhnz LA, Ruhl HA, Huffard CL, Smith KL. 2014. Rapid changes and long-term cycles in the benthic megafaunal community observed over 24years in the abyssal northeast Pacific. Prog Oceanogr. 124:1–11.
MacDonald IR, Bluhm BA, Iken K, Gagaev S, Strong S. 2010. Benthic macrofauna and megafauna assemblages in the Arctic deep-sea Canada Basin. Deep Sea Res Part II Top Stud Oceanogr. 57:136–152.
Melnikov IA, Bondarchuk LL. 1987. To the ecology of the mass aggregations of colonial diatom algae under the Arctic drifting sea ice. Okeanologiya. 27:317–321. [in Russian].
Rogacheva A. 2007. Revision of the Arctic group of species of the family Elpidiidae (Elasipodida, Holothuroidea). Mar Biol Res. 3:367–396.
Rogacheva A. 2012. Taxonomy and distribution of the genus *Kolga* (Elpidiidae: Holothuroidea: Echinodermata). J Mar Biol Assoc United Kingdom. 92:1183–1193.
Rybakova E, Kremenetskaia A, Vedenin A, Boetius A, Gebruk A. 2019. Deep-sea megabenthos communities of the Eurasian Central Arctic are influenced by ice-cover and sea-ice algal falls. PLoS One. 14:e0211009. doi:10.1371/journal.pone.0211009.
Soltwedel T, Jaeckisch N, Ritter N, Hasemann C, Bergmann M, Klages M. 2009. Bathymetric patterns of megafaunal assemblages from the arctic deep-sea observatory HAUSGARTEN. Deep Sea Res Part I Oceanogr Res Pap. 56:1856–1872.
Soltwedel T, Miljutina M, Mokievsky V, Thistle B, Vopel K. 2003. The meiobenthos of the Molloy deep (5600 m), Fram Strait, Arctic Ocean. Vie Milieu Paris. 53:1–13.
Taylor J, Staufenbiel B, Soltwedel T, Bergmann M. 2018. Temporal trends in the biomass of three epibenthic invertebrates from the deep-sea observatory HAUSGARTEN (Fram Strait, Arctic Ocean). Mar Ecol Prog Ser. 602:15–29.
Théel H. 1877. Memoire sur l`*Elpidia*, nouveau genre d`Holothuries. Kongl Sven Vetenskaps Akad Handl. 14:1–30.
Tyler P, Billett DSM, Gage JD. 1985. Life-history biology of *Peniagone azorica* and *P. diaphana*. Mar Biol. 89:71–81.
Valovaya MA, Kavtaradze DN. 1993. Microscopy: principles, techniques, art, and experiment. Moscow: Moscow State University. [in Russian].
Wigham BD 2002. The "*Amperima Event*": analysis of community change in the abyssal Northeast Atlantic Ocean" [PhD Thesis]. Southampton: University of Southampton.
Wigham BD, Tyler PA, Billett DSM. 2003. Reproductive biology of the abyssal holothurian *Amperima rosea*: an opportunistic response to variable flux of surface derived organic matter? J Mar Biol Assoc United Kingdom. 83:175–188.
Young CM. 2003. Reproduction, development and life-history traits. In: Tyler PA, editor. Ecosyst world, Vol 28 Ecosyst Deep Ocean. Amsterdam: Elsevier; p. 381–426.