Jordan Journal of Natural History, 10 (2), 2023 Pages: 32-51

The Reproductive Cycle of the Female Cushion Sea Star *Patiriella regularis*

Khalid M. Alqaisi,[1,5*] Miles D. Lamare,[2] Dave R. Grattan,[3] Khalil I Altaif,[4] P. Mark Lokman[1]

[1]Department of Zoology, University of Otago, 340 Great King Street; [2] Department of Marine Science, University of Otago, 310 Castle Street; [3] Department of Anatomy, University of Otago, 270 Great King Street, Dunedin 9016, New Zealand; [4] Department of Pharmacy, Faculty of Pharmacy, Middle East University, [5] Department of Medical laboratory Sceinces, Faculty of Allied Medical Sceinces, Al-Ahliyya Amman University, 19328, Amman, Jordan

Received: October 15, 2023; Revised: November 25, 2023; Accepted: November 29, 2023

Abstract

Patiriella regularis or the New Zealand Sea Star spawns small eggs (150 μm) and has indirect development through the bipinnaria and brachiolaria larvae, typical for asteroids. While *P. regularis* has been used in studies related to reproduction and embryo development, the gametogenic cycle in this species has not been studied in terms of histological analysis or division into stages. The current study aims to describe the ovarian cycle and oogenesis of *P. regularis* in terms of organ indices and histological and ultrastructural analysis of the ovary using light and electron microscopy. The reproductive cycle in the female *P. regularis* showed high similarity to that in many other studied asterinid species and was divided into five growth/gametogenic stages: I) The recovery stage, II) The growing stage, III) The maturing stage, IV) The partly- spawned stage, and V) The spent stage. While oocytes at different growing phases were found in the ovary throughout the reproductive cycle, the majority of the oocytes developed synchronously and indicated that *P. regularis* spawns during summer. The outcome of this study provides new insight into the gametogenesis of the *P. regularis* ovary throughout the reproductive cycle, and presents new details pertaining to oogenesis in a major invertebrate phylum that is rarely studied.

Keywords: Reproductive cycle, Oogenesis, Asteroids, Sea star.

*Corresponding author: k.alqaisi@ammanu.edu.jo

Introduction

The female reproductive cycle in marine invertebrates consists of a series of events that include oogenesis, gonad activation and growth, spawning of gametes, regression of gonadal activity, and a resting period (Giese, 1959). Oogenesis is a complex process that results in the production of a fully mature egg (ovum) from a primordial germ cell. This process involves cell growth, synthesis of organelles, and preparation of a highly specialised cell for fertilisation (Song *et al.*, 2006). In oviparous animals, in which the females deposit eggs that develop and hatch outside the maternal body, the gamete must be prepared for the energy and nutritional requirements of the future embryo (Smiley, 1990; Blackburn, 1999). Oogenesis is the major event during the reproductive cycle. Thus, during oogenesis, nutrients which mainly consist of yolk proteins accumulate in the oocyte, the genes encoding ribosomal ribonucleic acid (rRNA) are amplified, and many types of RNA are synthesised and stored in their inactive form until there is a need to use them by the developing embryos (Carlson, 2009).

Offspring survival is the main criterion for reproductive success. Therefore, reproduction is often synchronised with environmental conditions that will be most favourable for the success of the offspring (Lawrence, 1987). The synchronisation of gametogenesis and spawning within the populations of marine invertebrates including echinoderms is controlled by various factors.

These factors are generally categorised into two main groups: *i)* exogenous factors and, *ii)* endogenous factors (Giese and Pearse, 1974). The exogenous factors include environmental variables such as temperature, photoperiod, and food availability (Pearse *et al.*, 1986). For example, it was found that shifting the photoperiod regime six months out of phase resulted in shifting the gametogenic cycle by a similar time in the sea stars *Pisaster ochraceus* (Pearse and Eernisse, 1982) and *Asterias vulgaris* (Pearse and Walker, 1986). This shift also resulted in a change of the gonad index and oocyte size in the sea star *Sclerasterias mollis* (Xu and Barker, 1990b).

The reproduction of asteroids has been studied extensively. Like most other marine invertebrates, echinoderms show diversity in reproductive strategies (Carvalho and Ventura, 2002). For example, in the family Asterinidae, *Meridiastra gunnii*, *M. calcar* and *Cryptasterina pentagona* (previously *Patiriella gunnii*, *P. calcar* and *P. pseudoexigua*, respectively) are broadcasters, spawn large eggs, and develop directly through non-feeding brachiolaria larvae (Lawson-Kerr and Anderson, 1978; Grice and Lethbridge, 1988; Byrne, 1991, 1996; Chen and Chen, 1992). In contrast, *Patiriella regularis* spawns small eggs that develop indirectly through feeding bipinnaria and brachiolaria larvae (Crump, 1971; Byrne and Barker, 1991). Unlike the above-mentioned species, *Parvulastra vivipara* and *Parvulastra parvivipara* (previously *Patiriella vivipara* and *P. parvivipara*, respectively) are viviparous, i.e. fertilisation occurs inside the gonad and they brood their embryos in their gonads (Chia, 1976; Chia and Walker, 1991; Byrne, 1996).

P. regularis is one of New Zealand's most common rocky shore sea stars (Morton and Miller, 1973), and embryo development in this species represents a typical asteroid developmental pattern (Byrne and Barker, 1991). *P. regularis* has been used in a number of studies related to reproductive biology (Crump, 1971; Byrne and Barker, 1991; Styan *et al.*, 2005; Prowse and Byrne, 2012; Alqaisi *et al.*, 2016). Crump (1971) studied seasonal changes in gonad and pyloric caeca indices and changes in the oocyte sizes of *P. regularis* in three geographically separated populations and provided useful information regarding the reproductive cycle and spawning time of this species. However, Crump (1971) did not provide a full description of the gametogenic cycle and of cellular changes in gonads during the reproductive cycle. In addition, the reproductive cycle of *P. regularis* remains to be divided into stages that can facilitate future studies on the reproductive biology of this species.

Studies related to reproduction are multidisciplinary; from molecular biology to reproductive ecology. Understanding the cellular processes associated with the reproductive cycle is essential for all studies on reproductive biology. Furthermore, understanding the different reproduction-related processes, such as vitellogenesis, and the factors that regulate reproduction is based on describing the reproductive cycle and how the reproductive status changes in relation to these factors (Chia and Walker, 1991). In general, the reproductive cycle in echinoderms is described by measuring temporal changes in the relative gonad size (i.e. gonad indices) and the histological analysis of the gonads at different reproduction phases (Schoenmakers et al., 1984). The gonad index provides information of relative gonad size and can be helpful to visualise the relationship between the gonad and other body organs. For example, in most studied asteroids, an inverse relationship is found between the pyloric caeca and gonad indices over the course of a reproductive cycle, indicating a transfer of nutrients from the pyloric caeca to the gonads during gametogenesis (Crump, 1971; Byrne, 1992; Chen and Chen, 1992; Carvalho and Ventura, 2002; Georgiades et al., 2006). The histology of gonads is another fundamental approach to describing the reproductive cycle. Staging the reproductive cycle and providing a detailed description of vitellogenin deposition in the ooplasm and of the relationship between the

developing gametes and the surrounding somatic cells in the gonads can be made using histological analysis (Schoenmakers et al., 1981; Beijnink et al., 1984; Schoenmakers et al., 1984; Reimer and Crawford, 1995).

Studies on the oogenesis of invertebrates cover only 1% of the described species in each of major invertebrate's phyla (Eckelbargera and Hodgson, 2021). Thus, studying the reproductive cycle of *P. regularis* in more detail will be useful in future comparative studies on the reproductive biology of other asterinid sea stars and marine invertebrates more broadly. In this respect, the current study aims to describe the female reproductive cycle in a population of the sea star *P. regularis* over one year. In this study, the authors describe the reproductive stages based on: *i)* ovary and pyloric caeca index analysis, and *ii)* a detailed histological and ultrastructural analysis in the ovary throughout the annual reproductive cycle using light and electron microscopy.

Materials and Methods

Animal sampling

Patiriella regularis was collected monthly between March 2010 and February 2011 from a population in the Otago Harbour, New Zealand (45° 48' S; 170° 38' E). Due to difficulties in collecting a large number of samples from one location for all sampling events, samples were collected from one of two locations: Portobello Peninsula or Harrington Point (Figure 1). The samples from the selected locations have similar reproductive potential (Crump, 1971). Around thirty samples were collected each month from a depth of five to eight meters by SCUBA diving. After collection, the samples were returned to the Department of Zoology Aquaria, University of Otago, and were maintained overnight in 30 L glass tanks of sea water from Otago Harbour at ambient temperature and photoperiod during the time of each sampling.

Figure 1. Map of New Zealand (upper left) and Otago Harbour. Labelled red points with numbers represent the sampling locations; 1: Portobello Marine Laboratory wharf; and 2: Harrington Point. Google Map.

Dissection of animals

Only sea stars with a ray (arm) length (measured from the oral opening to the tip of the ray) of more than 30 mm were selected for dissection. Prior to the dissection, the total body wet weight of animals was taken after the animal was gently drained of excess water. Animals were dissected and pieces from gonads were mounted on a slide for a microscopic examination of sex, for this sea star is not sexually dimorphic. Based on the number of females found in each, between eleven and seventeen females were found between the collected sea stars dissected during each monthly sampling. Pyloric caeca and gonads were removed, weighed to the nearest mg, and kept in filtered sea water (FSW) at 4 °C. The ovary index and the pyloric caeca index were calculated for each sample as organ wet weight /total body wet weight * 100 (Broertjes *et al.*, 1984). Ovarian tissues were used for histological examination and staging as shown in the following section.

Histology

Light microscopy analysis

During dissection, fragments of ovary tissues were drained and divided into small pieces (10 – 15 mm each) and were immediately fixed in 1.3 ml of 4 % paraformaldehyde (PFA) in FSW for at least twenty-four hours. the samples were then washed three times with 70 % ethanol for two hours and stored in 70 % ethanol at room temperature until processing. Later, the samples were dehydrated in a graded ethanol series as follows: 80 % for 1 hr, 95% for one hour and 100 % two times for thirty minutes. Subsequently, the dehydrated tissues were embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer GmbH, Germany) as described by the manufacturer and were sectioned at 2 µm thickness using a Reichert-Jung microtome (Cambridge Instruments GmbH, Germany). Two slides were prepared containing serial sections from each sample; one slide was stained with haematoxylin and eosin (H&E) and the other with periodic acid-Schiff (PAS) reagent. The slides were viewed with an Olympus-BX51 microscope and the images were taken with a camera (Olympus-SC100, Japan) connected to a computer and were viewed using Cell Sens (Olympus) software. The images from ovary sections were used for measuring oocyte diameters using the image-analysis software ImageJ (https://imagej.net/ij/) as follows: The area of the biggest thirty oocytes from each sample was measured, and the diameter was calculated by the software as the longest distance between two points along the oocyte area. This method was used to give an estimation of the oocyte diameter since oocytes were not spherical in shape in all stages of the reproductive cycle. To ensure that the oocytes were measured from the centre, only oocytes with visible nucleolus were measured. Ovary sections of five females from each monthly sampling were randomly selected and used for this analysis.

Electron microscopy analysis

Fragments from the ovary were divided into small pieces, approximately 1 mm^3 each and were immediately fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer with 1.68 % NaCl overnight at 4 °C. The samples were sent to the Otago Centre for Electron Microscopy, University of Otago, where they were processed as follows: The fixed samples were washed in 0.1 M cacodylate buffer with 1.68 % NaCl three times, for ten minutes. Subsequently, the samples were post-fixed in 1 % osmium tetroxide in 0.1 M cacodylate buffer with 1.68 % NaCl for one hour, washed in the same buffer three times, ten minutes each, and were stored at 4 °C until further analysis. Prior to subsequent dehydration, the samples were washed in de-ionised water three times for ten minutes, en bloc stained in 1 % uranyl acetate in de-ionised water for one hour and rewashed in de-ionised water two times for five minutes each. The samples were dehydrated in ethanol and were infiltrated with Quetol 651 resin and embedded and polymerised at 60 °C. Finally, the samples were sectioned at 80 nm using a Reichert-Jung Ultracut E ultramicrotome (C. Reichert AG, Vienna, Austria) and were

stained with uranyl acetate and lead citrate for transmission electron microscopy (TEM). The sections were viewed using a Philips CM100 BioTWIN transmission electron microscope (Philips/FEI Corporation, Eindhoven, The Netherland), and images were taken with a MegaView lll digital camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany) connected to a computer.

Data analysis

The statistical analysis of changes in ovary and pyloric caeca indices during the reproductive cycle was done using GraphPad Prism 6 (GraphPad Software, Inc., USA). Graphs were prepared using GraphPad Prism 6 or Office Excel 2010 (Microsoft®). Data were tested for normal distribution using D'Agostino and Pearson omnibus normality tests and were subjected to log-transformation where needed. In doing so, the data still did not achieve normality after log transformation, and therefore, the monthly variations of ovary index or pyloric caeca index were analysed using a nonparametric test, Kruskal-Wallis, followed by Dunn's multiple comparisons test. Regression analysis was used to establish the relationship between ovary index and pyloric caeca index throughout the reproductive cycle. Day length and sea temperature data in Otago Harbour were kindly provided by the Department of Marine Science at the University of Otago, New Zealand.

Results

Organ indices

Monthly variations in ovary index and pyloric caeca index in *P. regularis* varied significantly throughout the reproductive cycle (Kruskal-Wallis test, $P < 0.001$ for ovary and pyloric caeca) (Figure 2). The ovary index peaked in the early austral summer and reached approximately 25 % in December. This was followed by a gradual decrease from January to March during which the ovary index reached its minimum value of about 5 %. The ovary index remained stable during the austral autumn (March to May) with no significant change, then increased

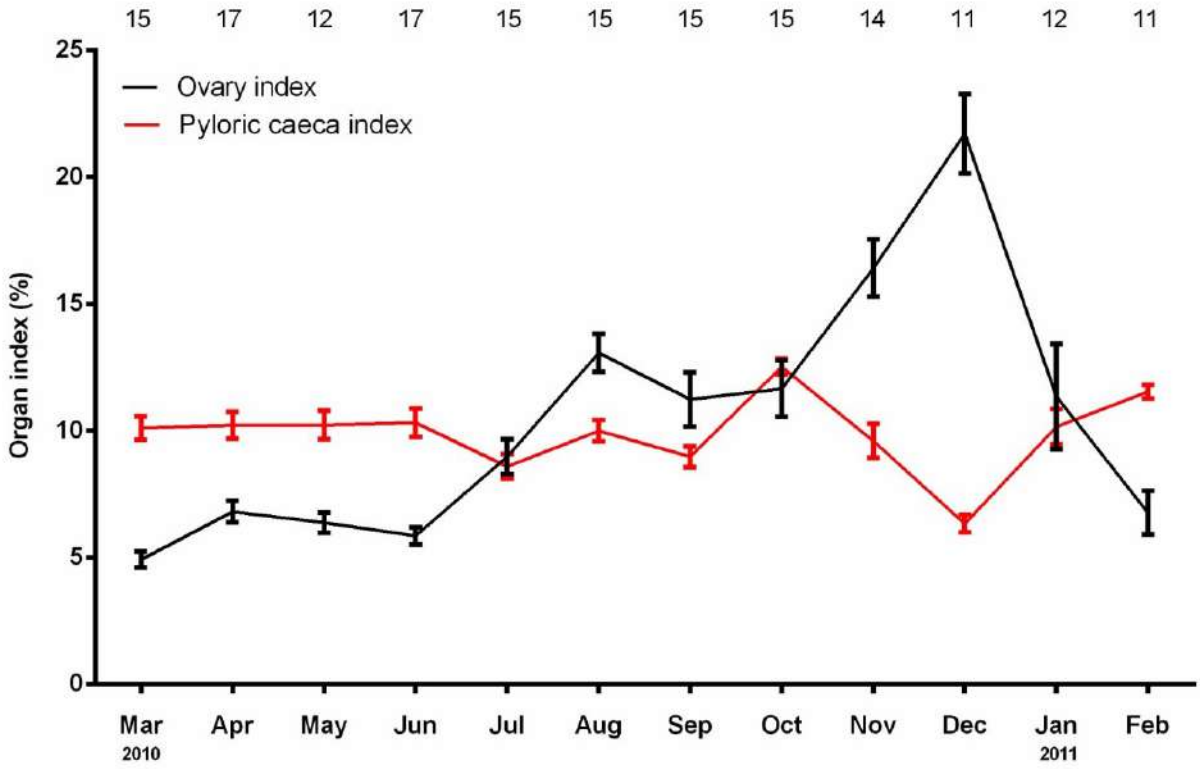


Figure 2. Monthly ovary index and pyloric caeca index (mean ± standard error of the mean) of female Patiriella regularis between March 2010 and February 2011. Numbers across the top indicate sample number for females sampled each month.

gradually through winter, from June to August (Figure 2). Significant differences were found between mean ovary index in August, March, and July (Dunn's multiple comparisons test, α: 0.05). The ovary index increased approximately two-fold during spring and early summer (from 11.5 % in October to 22 % in December).

The pyloric caeca index was stable with no significant change during autumn and winter. It peaked in October during the spring season to 13 % (Figure 2), then decreased and reached its minimum values in December (6 %). In December, the mean pyloric caeca index was significantly different from that in all other months except for July and September (Dunn's multiple comparisons test, α: 0.05). The pyloric caeca index increased during late summer and became stable during autumn. There was no clear inverse relationship between pyloric caeca index and ovary index (r^2= 0.03, $P < 0.02$; Supplementary Figure 1).

Histological analysis and the stages of the reproductive cycle

Based on the histological and ultrastructural analysis of the ovary and ovary index, the reproductive cycle of *P. regularis* was divided into five stages. The following criteria were used to define each stage: the abundance of oocytes at each developmental stage (i.e., young oocytes, growing oocytes and fully-developed oocytes), the thickness of the haemal system (HS), and the presence of HS invaginations in the ovary lumen, the presence/absence of unspawned and degenerating oocytes and the abundance of somatic cells. The stages were named after the previously described other asterinid species by Byrne (1992) as follows: Stage I: The recovery stage; Stage II: The growing stage; Stage III: The maturing stage; Stage IV: The partly-spawned stage; and Stage V: The spent stage. Ovary and oocyte descriptions and terminology for each stage used in the present study were based on Byrne (1992) and Schoenmakers et al. (1981).

Stage I: The recovery stage

Young oocytes were found at this stage lining the ovary wall or attached to HS invaginations into the ovary lumen (Figure 3 (b)). The young oocytes were spherical to oval-shaped, and their diameter reached up to 40 µm (Figure 3 (b)). These oocytes had a large spherical nucleus, their cytoplasm was basophilic and mainly PAS-. Few PAS+, and electron dense granules indicative of yolk deposition were found in the cytoplasm of young oocytes (Figure 3 (d), Figure 3 (e)). Oogonia were found in clusters with young oocytes, forming cell nests (Figure 3 (b)). These cell nests were attached to the ovary wall or HS invaginations. More developed oocytes were pear-shaped, and their size increased to 40 – 100 µm in diameter. These oocytes were attached to the ovary wall and were the most abundant type of primary oocytes in the ovary during this stage (Figure 3 (a)). Pear-shaped oocytes were characterised by PAS+ staining and eosinophilic cytoplasm (Figure 3 (a), Figure 3 (c), Figure 3 (d)); the PAS+ staining reflected yolk deposition in the ooplasm (Schoenmakers et al., 1981; Byrne, 1992). Ultrastructurally, the number of Golgi complexes increased in these growing oocytes and the cytoplasm became more granular and included a large number of electron dense granules (yolk granules). Young oocytes and pear-shaped oocytes were surrounded by somatic cells, mainly follicle cells (Figure 3 (b), Figure 3 (c)).

The lumen of the ovary was devoid of oocytes or contained eosinophilic and PAS+ cell debris resulting from the reabsorption of degenerated unspawned oocytes. Many phagocytes were found dispersed in cell debris and were characterised by a dense basophilic round nucleus (Figure 3 (a)). Many somatic cells were dispersed between young and growing oocytes (Figure 3 (b)). The HS, which appeared to have proliferated from previous stages, was thick and projected into the ovary lumen through invaginations (Figure 3 (b), Figure 3 (c), Figure 3 (d)). The HS and its invaginations included somatic cells that were characterised by densely stained basophilic nuclei (Figure 3 (b)).

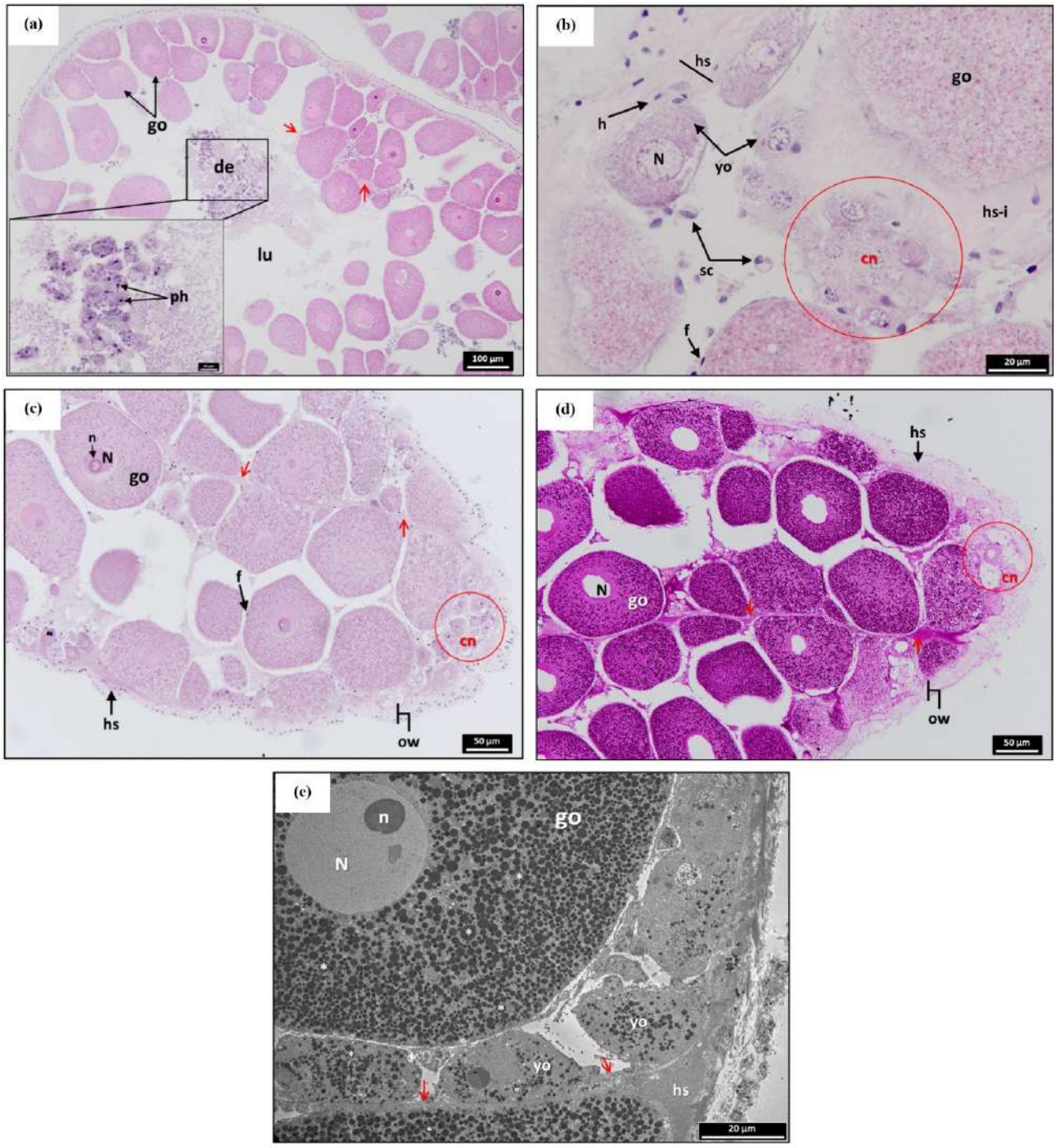


Figure 3. Histology of sea star Patiriella regularis ovaries at recovery stage (Stage I) using light microscopy (a – d) and transmission electron microscopy (e). (a) General section in ovary; growing pear-shaped oocytes (go) are attached to the ovary wall; ovary lumen (lu) contains debris (de); phagocytes (ph) are dispersed in cell debris (enlarged); red arrow heads indicate haemal system invagination into ovarian lumen. (b) Cell nest (cn) containing oogonia and early young oocytes; haemal system (hs) is proliferated and forming invaginations into ovarian lumen (hs-i); somatic cells (sc) scattered between oocytes; young oocytes (yo) and growing oocytes (go) are attached to ovary wall or haemal system invagination; follicle cells (f) surrounding oocytes; h: cell in haemal system; N: nucleus. (c) and (d) are serial sections stained with H&E and PAS respectively; early young oocytes are PAS- and growing oocytes are PAS+; the haemal system (hs) is PAS+; ovary wall (ow). Red arrow heads indicate haemal system invagination in ovary lumen. (e) Section in ovary at late Stage I; young oocytes are attached to the haemal system invagination (red arrow heads), asterisks indicate yolk granules.

Stage II: The growing stage

The ovary at this stage was characterised by increasing numbers of pear-shaped growing oocytes (Figure 4 (a), Figure 4 (c)). Cell nests and young oocyte numbers decreased, and few aggregations were found attached to HS invaginations or the ovary wall (Figure 4 (c), Figure 4 (d)). Somatic cells, including phagocytes, decreased in number at this stage and the HS became attenuated. Toward the end of this stage, the pear-shaped oocytes grew more and became spherical in shape, their size reaching up to 150 µm in diameter. These oocytes detached from the ovary wall and they started to fill the ovarian lumen (Figure 4 (b)). As the oocytes grew and became spherical in shape, the number of basophilic granules increased in their cytoplasm and also, their cytoplasmic PAS+ staining became more intense (Figure 4 (c), Figure (d)). In addition, cortical granules were observed, dispersed throughout the cytoplasm, and they were recognised as a vesicle containing a mix of electron dense and translucent material. The large spherical-in-shape oocytes were still surrounded by follicle cells. Increases in oocyte size at this stage coincided with a gradual increase in the ovary index. Organelle numbers including Golgi complex and mitochondria, increased at this stage (Figure 4 (e)), and electron-dense granules (yolk granules) were abundant in the oocyte cytoplasm (Figure 4 (e)).

Stage III: The maturing stage

During this stage, fully-developed oocytes were the most abundant in the ovary (Figure 5 (a)). These large oocytes (around 200 µm in diameter) were packed in closely together without any spaces between them, and they occupied the entire ovarian lumen (Figure 5 (a)). The fully-developed oocytes were characterised by having a large peripheral nucleus and their cytoplasm was PAS+, but the PAS intensity was less compared to that in growing oocytes (Figure 5 (b), Figure 5 (c), Figure 5 (d)). At the ultrastructural level, the cytoplasm included a large number of electron-dense granules and the cortical granules can be seen arranged under the oocyte cytoplasm (Figure 5 (e), Figure 5 (f)). Many young oocytes and growing pear-shaped oocytes were found in the ovary at this stage (Figure 5 (c), Figure 5 (d)). Few somatic cells, including phagocytes, were seen at this stage and the HS invaginations in the ovary lumen disappeared. At this stage the ovary index reached its maximum values.

Stage IV: The partly-spawned stage

The ovary lumen at this stage became empty as some eggs had been spawned (Figure 6 (a)). Fully-developed oocytes were loosely packed and their number decreased compared to the previous stage (Figure 6 (a), Figure (b)). Pear-shaped growing oocytes and young oocytes lining the ovary wall were abundant (Figure 6). The HS started to proliferate and the ovary wall became thicker than in the previous stage (Figure 6 (c)). Somatic cell numbers increased. Debris resulting from degenerated eggs or oocytes was evident at this stage (Figure 6 (d)).

Stage V: The spent stage

The ovaries at this stage included few unspawned oocytes that were likely to undergo degradation (Figure 7 (a), Figure 7 (b)). Atretic oocytes were characterised by clumping cytoplasm (Figure 7 (c)). Ultrastructurally, the degenerating ooplasm included a vacuole-like structure that seemed to contain cytoplasmic components including yolk granules (Figure 7 (e)). Somatic cells and phagocytes were abundant and found associated with degrading oocytes and cell debris. Dividing oogonia increased and small cell-nests started to form and were attached to the ovary wall or HS invagination (Figure 7 (d)). Toward the end of this stage, the cell-nests became more abundant. The HS proliferated and was visible at this stage (Figure 7 (a), Figure 7 (b)), forming invaginations into the ovary lumen (Figure 7 (d)).

Oocyte size-frequency distributions and gametogenic stages

The results from the oocyte diameter analysis throughout the reproductive cycle revealed a

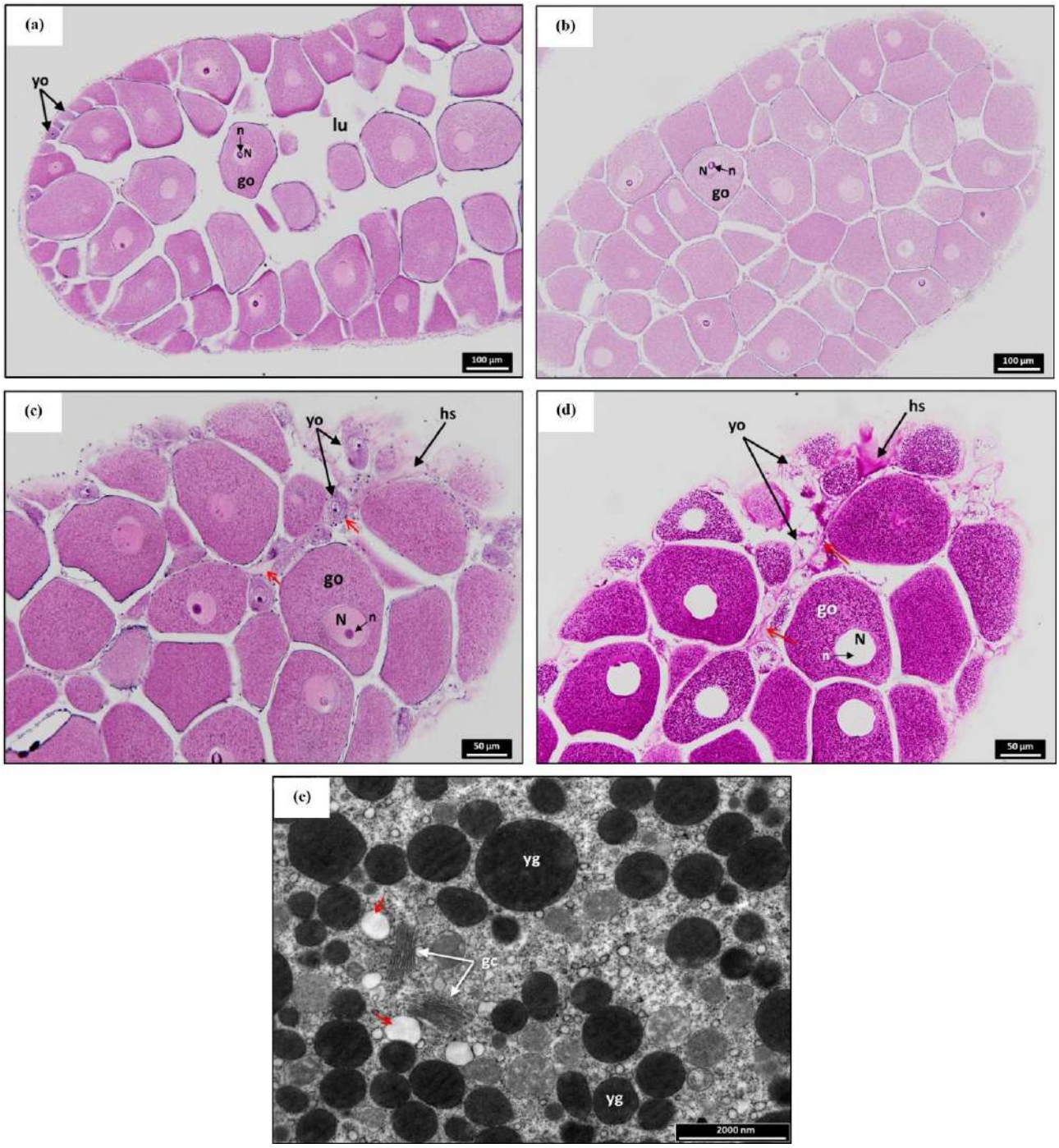


Figure 4. Histology of sea star Patiriella regularis ovaries at growing stage (Stage II) using light microscopy (a – d) and transmission electron microscopy (e). (a) Ovary at early stage II; yo: young oocytes; go: growing oocytes; N: nucleus; n: nucleolus; lu: lumen. (b) Ovary at late stage II; lumen full with oocytes at final growing stage. (c) and (d) are serial sections stained with H&E and PAS respectively; hs: haemal system; red arrow heads indicate haemal system invagination in ovary lumen. (e) Oocyte cytoplasm; gc: Golgi complex; yg: yolk granules, read arrow heads indicate vacuoles.

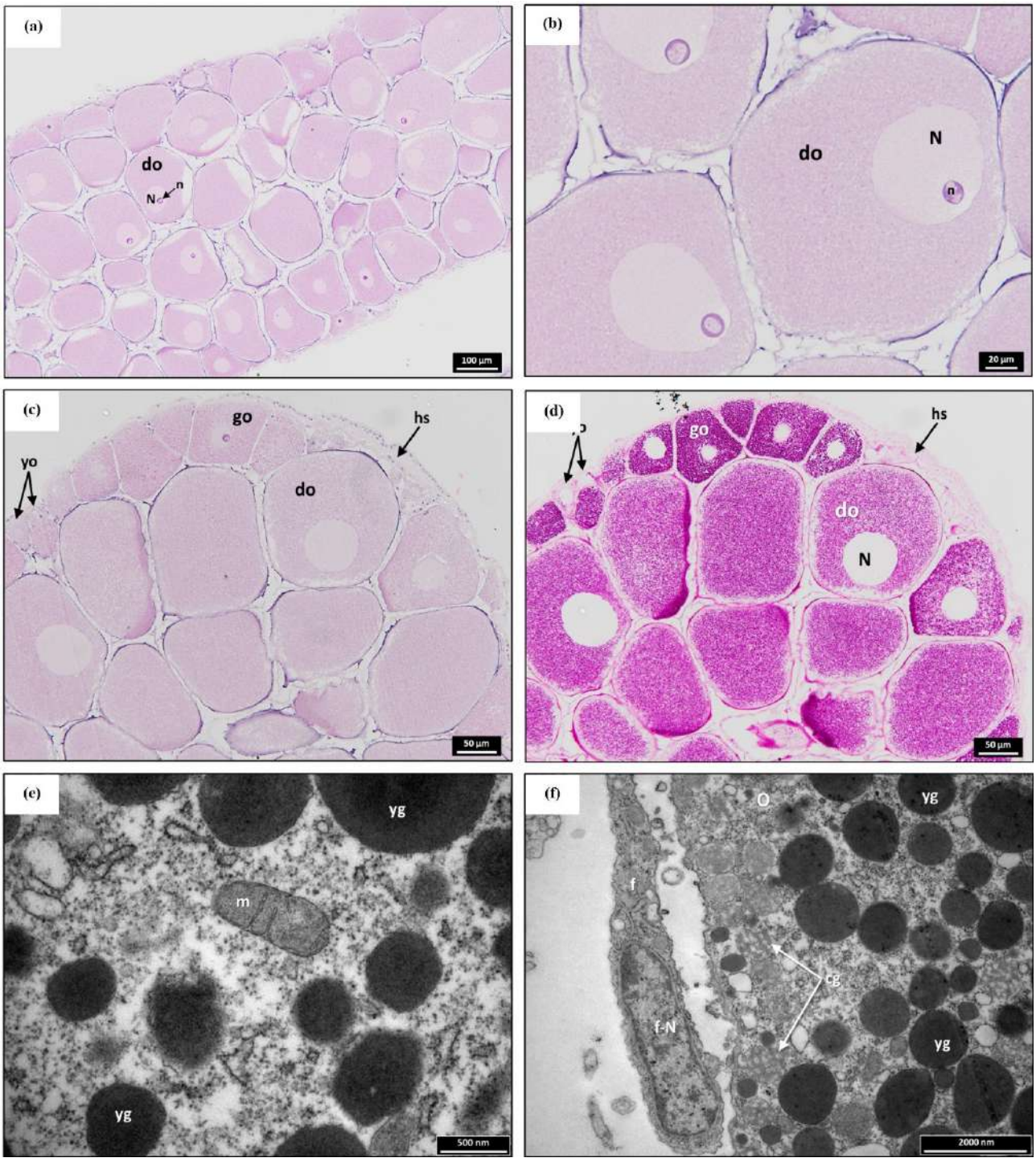


Figure 5. Histology of sea star Patiriella regularis ovaries at mature stage (Stage III) using light microscopy (a – d) and transmission electron microscopy (e and f). (A) Section in ovary showing lumen is full with packed fully-developed oocytes (do); N: nucleus; n: nucleolus. (b) Fully-developed oocytes. (c) and (d) are serial sections stained with H&E and PAS respectively; ovary contains large number of developed oocytes and small number of young oocytes (yo) and growing oocytes (go); hs: haemal system. (e) Ovary cytoplasm; m: mitochondria; yg: yolk granules. (f) Fully-developed oocyte cytoplasm (O) and follicle cells (f) at late Stage III; cortical granules (cg) are abundant at the periphery of oocyte cytoplasm; f-N: follicle cell nucleus.

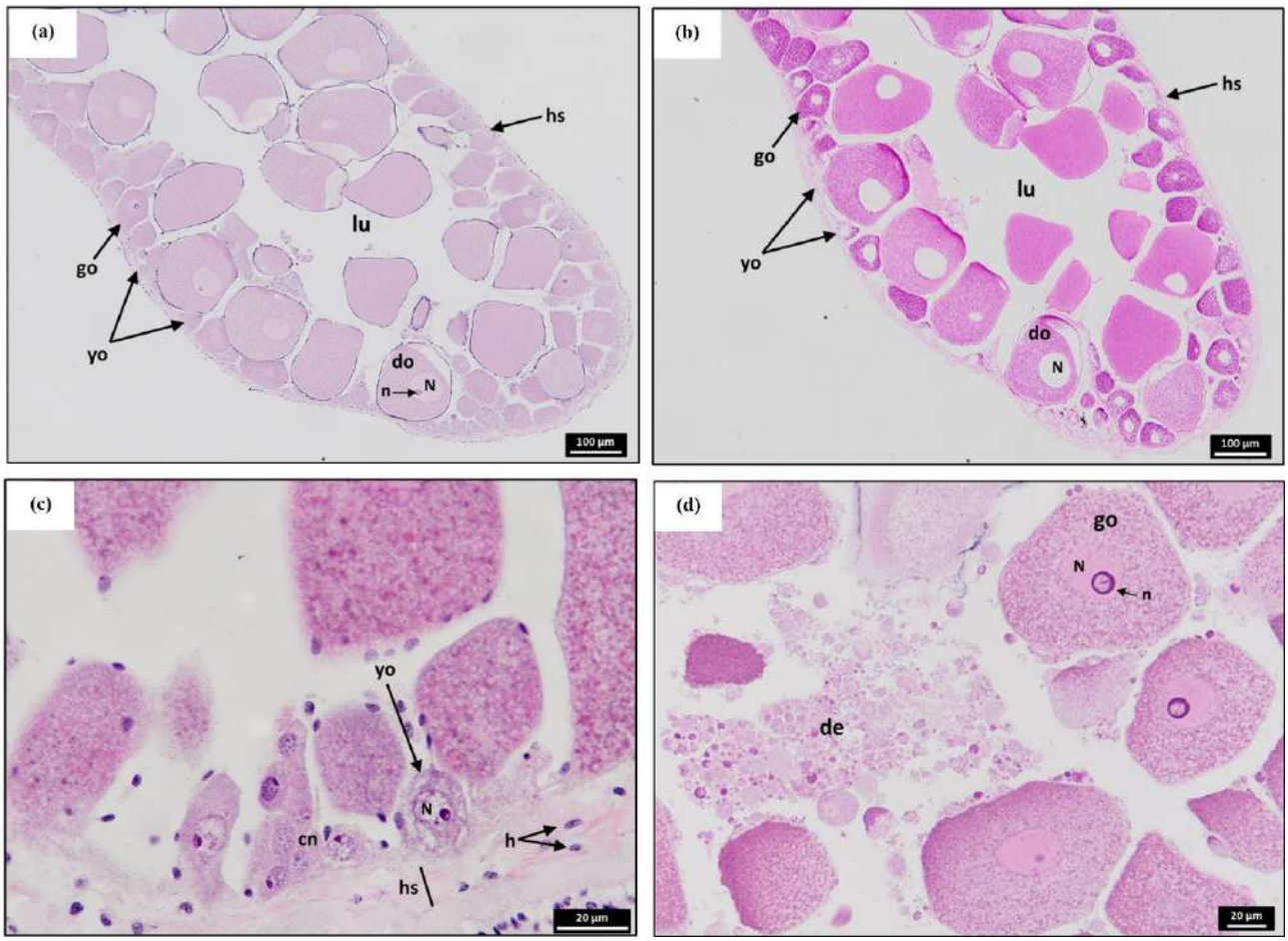


Figure 6. Histology of sea star Patiriella regularis ovaries at partly spawned stage (Stage IV) using light microscopy. (a) and (b) are serial sections stained with H&E and PAS respectively; ovary includes fully-developed oocytes (do) that are not yet spawned; ovary wall is lined with young oocytes (yo) and growing oocytes (go); ovary lumen is clear due to removing fully-developed oocytes during spawning; haemal system (hs) is proliferated; N: nucleus; n: nucleolus. (c) Cell-nest (cn) is abundant with young oocytes at this stage; h: cell in haemal system. (d) Debris (de) in lumen from degenerated oocyte.

seasonal pattern that generally corresponded to the ovary index. The oocyte size-frequency (Figure 8) exhibited a unimodal distribution throughout the annual reproductive cycle, except in the partly spawned stage (Stage IV) due to the abundance of growing oocytes in one cohort and the presence of pre-spawning oocytes on the other. The growing pear-shaped oocytes with diameters between 75 to 150 µm were most abundant between May and July (Figure 8). Most oocyte growth and vitellogenesis occurred during the growing stage in the austral winter between June and September (Figure 9).

From August to December, there was a shift in the most abundant oocytes with diameters between 150 µm to 200 µm (Figure 8). During the same period, there was an increase in ovary index (Figure 2). Females at the mature stage (Stage III) were first observed in late winter, and their number increased toward the summer (Figure 9). During this stage, fully-grown oocytes (175 – 250 µm) were most abundant (Figure 9) which coincided with maximum ovary index values (Figure 2). Spawning did not occur until January in mid-summer when the first partly spawned ovary was observed (Figure 9), and this was followed by a drop in the frequency of large-diameter oocytes (Figure 8). Spawning lasted throughout the summer and early autumn (from January to April). Most females were spent from February to April, by which time most of the ovaries were at the recovery stage of the reproductive cycle.

Young and growing oocytes were found throughout the reproductive cycle which indicated that oogenesis was continuous throughout the year. The majority of oocytes at each stage appeared to develop

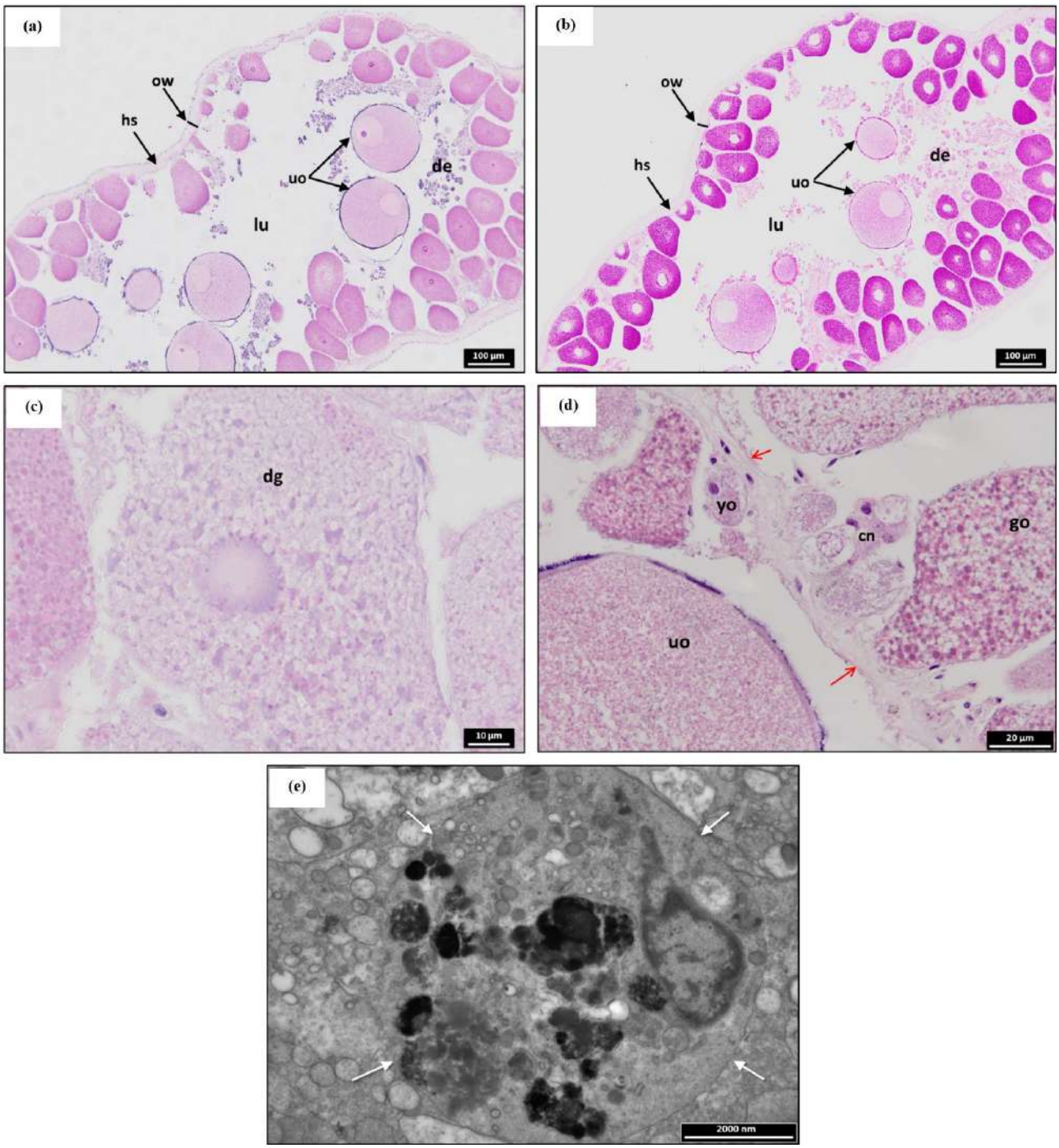


Figure 7. Histology of sea star Patiriella regularis ovaries at spent ovary stage (Stage V) using light microscopy (a – d) and transmission electron microscopy (e). (a) and (b) are serial sections stained with H&E and PAS respectively; ovary lumen (lu) contains few unspawned oocytes (uo) and debris (de) resulted from degenerated oocytes; ow: ovary wall. (c) Degenerating oocytes (dg) with clumping cytoplasm. (d) Cell-nest (cn), young oocytes (yo) and growing oocytes (go) are attached to haemal system invagination (red arrow heads). (e) Cytoplasm of degenerating oocyte; arrow head show the border of vacuole-like structure that contains cytoplasmic components.

synchronously representing one size class. The presence of large oocytes (150 to 200 µm) between May and September resulted from pear-shaped oocytes, diameters being defined as the largest diameter of non-spherical oocytes. Unspawned oocytes (> 200 µm) were present in March and April.

Annual cycle of day length and sea temperature

The annual cycle of day length between March 2010 and February 2011 (Supplementary Figure 2) showed that maximum day length was in late December (approximately 16 hr). This maximum day length just preceded *P.*

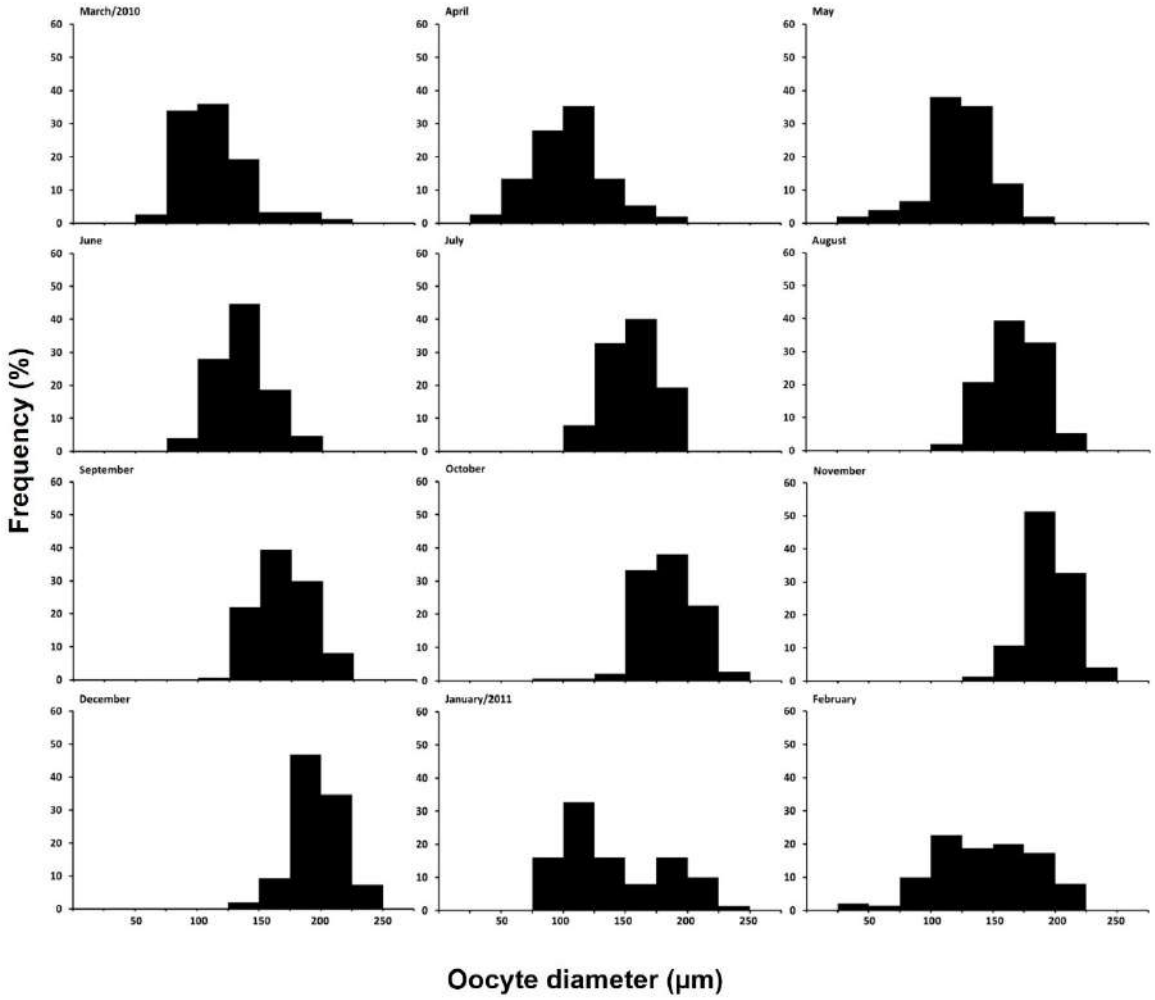


Figure 8. Patiriella regularis. Monthly size-frequency distribution of oocytes from March 2010 to February 2011. n= 150 oocytes per month from across 5 females.

regularis spawning. The shortest day length was on the 21st of June (approximately 9 hr). From August until December, there was an increase in day length which coincided with an increase in ovary index and with the growing stage of the ovary of *P. regularis*. During this period of this study, the maximum sea temperature was in December (average 17.4 °C) when sea stars started spawning. The minimum sea temperature was in July (average 7.9 °C) followed by a gradual increase from August until December.

Discussion

The present study describes the reproductive cycle of the female sea star *P. regularis* based on organ indices and the histological analysis, and the previously described reproductive cycle of other asterinid species (Byrne, 1992). Five stages of the oogenic cycle were defined in *P. regularis*: recovery, growing, maturing, partly-spawned and the spent stage.

A range of reproductive strategies have been reported in the family Asterinidae. For example, *Parvulastra exigua* (previously *Patiriella exigua*) has continuous gametogenesis, whereas *M. gunnii* and *M. calcar* have well-defined seasonal reproductive cycles (Byrne, 1992). *P. regularis*, as in most asteroids, has a distinct annual reproductive cycle (Crump, 1971). This was also evident from the histological analysis of the ovary in the present study. In many temperate asteroids that undergo an annual reproductive cycle, oocyte development occurs mostly during the autumn and winter seasons, such as in *S. mollis* (Barker and Xu, 1991) and *Coscinasterias calamaria* (Crump and Barker, 1985). Similarly, the growing stage of the ovary in *P. regularis* aligned with winter time (Crump, 1971; present study).

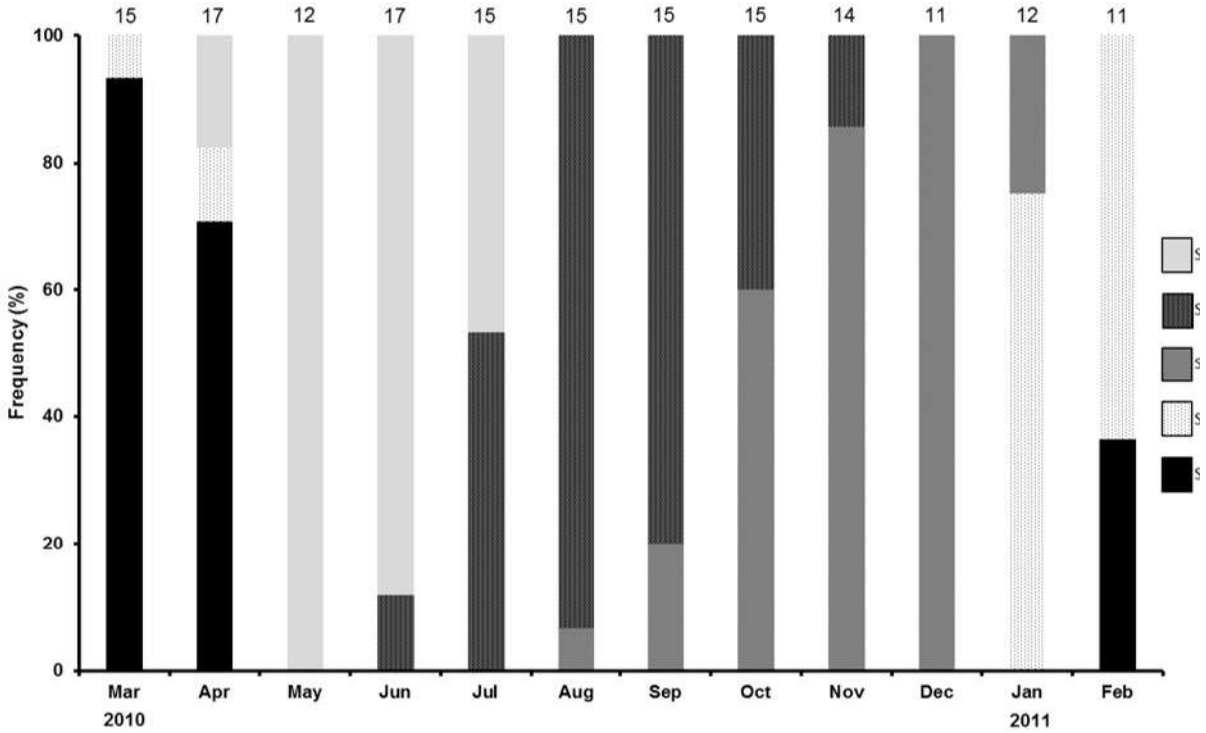


Figure 9. Relative frequencies of ovarian stages in the sea star Patiriella regularis over the annual reproductive cycle. The number of samples for each month is indicated above each histogram. Stage I: recovery stage; Stage II: growing stage; Stage III: mature stage; Stage IV: partly spawned stage; and Stage V: spent stage.

Crump (1971) reported that in *P. regularis*, spawning occurs in mid-summer, between December and February (Southern hemisphere) based on gonadal index. The results from the histological analysis in the present study are in agreement with these previous observations, and showed that spawning occurred in summer, from January to April. However, Crump (1971) noticed a gradual decrease in gonadal index from January to March, which was explained by the presence of ripe and spent animals in the population, and by the assumption that individual sea stars undergo one spawning event. In contrast, the histology of the ovary during the spawning period in the present study revealed the presence of partly-spawned ovaries, including loosely-packed fully-developed oocytes with spaces vacated through spawning. Furthermore, during animal dissection in the present study, shrunken "spent" parts and swollen "mature" parts of ovaries were found in individual sea stars from January to March, with ovary index values being lower than those of the mature ovary and higher than those of the spent ovary. These observations suggest that *P. regularis* is a batch spawner which may explain the gradual decrease in the ovary index during the spawning period. Batch spawning is reported in other asterinids as in *M. calcar* (Byrne, 1992). *P. regularis* spawns over an extended period that lasts for approximately three months (Crump, 1971; present study). Similarly, *M. gunnii* and *M. calcar* spawn for three to four months. Unlike other asterinid species, *C. pentagona* has a restricted spawning season in October (Chen and Chen, 1992).

The reproductive stages described in this study were very similar to those found in other asterinid species studied by Byrne (1992). However, some variation in oocyte size at the onset of vitellogenesis was found between the *P. regularis* and asterinid species studied by Byrne (1992). The histological analysis in the present study revealed that previtellogenic oocytes in *P. regularis* were small, with a diameter of less than 40 µm, and this was evident at higher resolution from electron dense granules in the ooplasm and from PAS+ oocytes. In contrast, the diameter of previtellogenic oocytes in *M. gunnii*, *M. calcar* and *Parvulastra exigua*

ranged from 50 to 100 µm, and oocytes were PAS- and basophilic (Byrne, 1992). The onset of vitellogenesis in these asterinid species occurred when the oocyte diameter was around 100 µm, reflected in the presence of PAS+ oocytes (Byrne, 1992). This comparison shows that vitellogenesis in young oocytes in *P. regularis* started at a smaller size than in *M. gunnii*, *M. calcar* and *Parvulastra exigua*. This may be related to the possibility that in *P. regularis*, oocyte development takes one year (Crump, 1971; Byrne and Barker, 1991; present study), while in *M. gunnii*, *M. calcar* and *Parvulastra exigua*, oocyte development takes two years (Byrne and Barker, 1991; Byrne, 1992).

The ovary wall of *P. regularis* in the present study had a structure similar to previously described asteroids (Walker, 1974; Schoenmakers et al., 1981; Byrne, 1992). In the ovary wall, the haemal system (HS) was observed to have connective tissue characteristics. It consisted of collagen fibers and contained coelomocytes (also called amoeboid cells) (Schoenmakers et al., 1981; Chia and Koss, 1994). In the sea star *Asterias rubens*, the HS proliferates during oocyte growth and vitellogenesis and forms invaginations to which the growing oocytes are attached (Schoenmakers et al., 1981). From late vitellogenesis to spawning, the HS regresses. The same observation was found in the *P. regularis* ovary in the present study, which suggests that HS in asteroids is important for oogenesis, especially during the early stages. Furthermore, vitellogenin was found to be produced in the pyloric caeca and ovaries of *P. regularis* (Alqaisi *et al.*, 2016), and this yolk proteins and other substances are transferred from the pyloric caeca into the ovary through the HS (Beijnink et al., 1984a; Beijnink and Voogt, 1986; Alqaisi *et al.*, 2016). In general, the HS is known to distribute nutrients to different organs in asteroids (Broertjes et al., 1980; Ferguson, 1984; Eckelbargera and Hodgson, 2021).

The asteroid ovaries contain many somatic cells, including follicle cells, phagocytosing cells, and nurse cells, and their abundance depends on the stage of the reproductive cycle (Schoenmakers et al., 1981). In echinoderms, many names have been used to describe somatic cells in the ovary. For example, in the asteroid *Patiria* (*Asterina pectinifera*, the phagocytes were referred to as nurse cells (Aisenshtadt and Vassetzky, 1986), while in echinoids, the somatic cells are known as nutritive phagocytes (Holland and Giese, 1965). Their morphology changes during oogenesis, and functions include the provision of nutrients for germ cells and phagocytosis (Walker et al., 2005; Walker et al., 2007). In the present study, the ovary of *P. regularis* was found to include many somatic cells with varying shapes. Apart from follicle cells that surround oocytes and from the phagocytes that were found scattered within cell debris, it was hard to identify the other somatic cells in the ovary as their function was unclear. Therefore, these cells are referred to as somatic cells in this study. Interestingly, it was noticed in the present study that many cells seemed to originate from the HS and were distributed between the growing oocytes. The fate of those somatic cells and their function are unknown. However, little is known about the somatic cells in the asteroid ovary, and further studies are needed to identify their function. In asteroids, the ultrastructural analysis of oocytes showed an abundance of cortical granules in the mature oocytes that were located close to the plasma membrane (Schoenmakers et al., 1981; Reimer and Crawford, 1995). In addition, yolk granules increased as the oocyte grew, and Golgi complexes increased in abundance during vitellogenesis, indicating a high activity in protein synthesis and processing (Schoenmakers et al., 1981). Similar observations were found in a previous study on *P. regularis* which confirms the abundant expression of vitellogenin genes in ovary and the minor expression of a transferrin-like yolk component termed major yolk protein (MYP) (Alqaisi *et al.*, 2016). The ultrastructural criteria of oocyte atresia in the sea star *P. ochraceus* were studied by Reunov and Crawford (2010). That study showed that oocyte destruction may occur through complex mechanisms that include

elements of necrosis, autophagic cell death, and apoptosis. These atresia patterns were not seen in the present study. This might be because few atretic oocytes were observed.

Oocytes at different stages of development were evident in the *P. regularis* ovary (present study). However, the majority of oocytes grow synchronously at each stage of the ovarian development. The oocyte size-frequency distribution of *P. regularis* during the annual reproductive cycle was studied bimonthly by Crump (1971) and monthly in the present study. The results from both studies are similar and showed general unimodal distribution. Thus, it is concluded that only one oocyte cohort is produced during each reproductive cycle. A similar pattern was found in the sea star *Asterina stellifera* (Carvalho and Ventura, 2002). In comparison, the oocyte size-frequency distributions in *M. gunnii*, *M. calcar*, *Parvulastra exigua* and *C. pentagona* are bimodal which results from the prolonged period of oogenesis that lasts two years (Byrne, 1992; Chen and Chen, 1992).

Pyloric caeca function in digestion and in the storage of nutrients (Lawrence, 1973; Ruppert et al., 2004), in addition to vitellogenin production in *P. regularis* (Alqaisi *et al.*, 2016). The inverse relationship between the gonad index and the pyloric caeca index is commonly observed in asteroids and indicates the transfer of nutrients to the ovary during gametogenesis (Crump, 1971; Barker and Xu, 1991; Byrne, 1992; Chen and Chen, 1992; Carvalho and Ventura, 2002; Georgiades *et al.*, 2006). In the present study, there was no obvious inverse relationship between the pyloric caeca index and the ovary index in *P. regularis* throughout the reproductive cycle. However, the maximum ovary index which was in December coincided with the minimum pyloric caeca index. Crump (1971) reported an approximate inverse relationship between the pyloric caeca index and the ovary index in this same sea star species. Interestingly, while the minimum value for the pyloric caeca index was in December, in Crump (1971) the minimum pyloric caeca value was in March. This difference could reflect differences in food availability and the dependence of ovaries on pyloric caeca food reserves. In this respect, it was suggested that food allocation to different body organs in asteroids is affected by food availability (Harrold and Pearse, 1980), with the ovary being less dependent on stored food reserves in the pyloric caeca when food is readily available (Harrold and Pearse, 1980; Xu and Barker, 1990a).Spawning in asteroids is suggested to be regulated by environmental factors such as photoperiod and temperature (Mercier and Hamel, 2009). In many asteroids, spawning occurs during spring and/or summer when sea temperatures increase seasonally. For example, spawning in *M. gunnii* and *M. calcar* in New South Wales was reported in spring and early summer (August – December) (Byrne, 1992). *P. regularis* in the present study was found to spawn in late December, during the longest period of day length and at maximum sea temperatures. This would be consistent with previous findings in sea stars of photoperiod and sea temperature as cues for maturation and spawning (Pearse and Eernisse, 1982).

In conclusion, the present study has examined the female reproductive cycle of *P. regularis* in terms of ovary histology and organ indices and described five reproductive stages. *P. regularis* has an annual reproductive cycle as previously reported. Oogenesis occurs mainly in the autumn and winter seasons. Ready-to-spawn oocytes are found in late spring season, but spawning did not occur until mid-summer which may suggest a link between temperature, photoperiod, and the onset of spawning. The majority of oocytes develop synchronously, and batch spawning was evident in *P. regularis*. The results from the present study provide a histology index for the ovary of *P. regularis* ovary during the reproductive cycle.

Acknowledgment

The researchers would like to thank the following staff at the University of Otago: former staff at the Department of Zoology (Matthew Downes, Ken Miller and Kim

Garrett), current staff at the Department of Zoology (Dr. Tania King) for their technical help, and the former staff René van Baalen, Dave Wilson and Bev Dickson from the Department of Marine Science for assistance with sample collection. The authors extend their thanks to Gillian Grayston and the technical staff (Microscopy Otago) for processing TEM samples. Finally, special thanks go to Dr. Yuichi Ozaki (Keio University, Japan) and Dr. Erin L. Damsteegt (Department of Zoology) for their technical training and advice.

References

Aisenshtadt, T.B. and Vassetzky, S.G. 1986. Fine structures of oocytes and accessory cells of the ovary in the starfish *Patiria* (*Asterina*) *pectinifera* at different stages of oogenesis and after 1-methyladenine-induced maturation. *Development, Growth and Differentiation,* **28**: 449-460.

Alqaisi, K. M., Lamare, M. D., Grattan, D. R., Damsteegt, E. L., Schneider, W. J. and Lokman, P. M. 2016. A comparative study of vitellogenesis in Echinodermata: Lessons from the sea star. Comparative biochemistry and physiology. *Part A, Molecular & Integrative Physiology,* **198**: 72–86.

Barker, M.F. and Xu, R.A. 1991. Population differences in gonad and pyloric caeca cycles of the New Zealand seastar *Sclerasterias mollis* (Echinodermata: Asteroidea). *Marine Biology,* **108**: 97-103.

Beijnink, F., Broertjes, J., Brands, F. and Voogt, P. 1984a. Immunocytochemical demonstration of vitellogenic substances in the haemal system of the sea star, *Asterias rubens*. *Marine Biology Letters* **5**: 303-313.

Beijnink, F.B. and Voogt, P.A. 1986. The aboral haemal system of the sea star, *Asterias rubens* (Echinodermata, Asteroidea): an ultrastructural and histochemical study. *Zoomorphology* **106**: 49-60.

Beijnink, F.B., Walker, C.W. and Voogt, P.A. 1984b. An ultrastructural study of relationships between the ovarian haemal system, follicle cells, and primary oocytes in the sea star, *Asterias rubens*. *Cell and Tissue Research* **238**: 339-347.

Blackburn, D.G. 1999. Viviparity and oviparity: evolution and reproductive strategies. In: Knobil, E., Neill, J.D. (Eds.), **Encyclopedia of Reproduction**. Academic Press, USA, pp. 994-1003.

Broertjes, J., Posthuma, G., Den Breejen, P. and Voogt, P. 1980. Evidence for an alternative transport route for the use of vitellogenesis in the sea-star *Asterias rubens* (L.). *Journal of the Marine Biological Association of the United Kingdom* **60**: 157-162.

Broertjes, J.J.S., de Waard, P. and Voogt, P.A. 1984. On the presence of vitellogenic substances in the starfish, *Asterias rubens* (L.). *Journal of the Marine Biological Association of the United Kingdom* **64**: 261-269.

Byrne, M. 1991. Developmental diversity in the starfish genus *Patiriella*. In: Yanagisawa, T., Yasumasu, I., Oguro, C., Suzuki, N., Motokawa, T. (Eds.), **Biology of Echinodermata**. Balkema, Rotterdam, pp. 499-508.

Byrne, M. 1992. Reproduction of sympatric populations of *Patiriella gunnii*, *P. calcar* and *P. exigua* in New South Wales, asterinid seastars with direct development. *Marine Biology* **114**: 297-316.

Byrne, M. 1996. Viviparity and intragonadal cannibalism in the diminutive sea stars *Patiriella vivipara* and *P. parvivipara* (family Asterinidae). *Marine Biology* **125**: 551-567.

Byrne, M. and Barker, M.F. 1991. Embryogenesis and larval development of the asteroid *Patiriella regularis* viewed by light and scanning electron microscopy. *The Biological Bulletin* **180**: 332-345.

Carlson, B.M.2009. **Human Embryology and Developmental Biology,** 4th ed. Mosby (Elsevier), USA.

Carvalho, A.L.P.S. and Ventura, C.R.R. 2002. The reproductive cycle of *Asterina stellifera* (Möbius) (Echinodermata:

Asteroidea) in the Cabo Frio region, southeastern Brazil. *Marine Biology* **141**: 947-954.

Chen, B.Y.and Chen, C.P. 1992. Reproductive cycle, larval development, juvenile growth and population dynamics of *Patiriella pseudoexigua* (Echinodermata: Asteroidea) in Taiwan. *Marine Biology* **113**: 271-280.

Chia, F. 1976. Reproductive biology of an intraovarian brooding starfish *Patiriella vivipera* Dartnall. *American Zoologist* **16**: 181.

Chia, F. and Koss, R. 1994. Asteroidea. In: Harrison, F.W. (Ed.), **Microscopic Anatomy of Invertebrates,** vol. 14. *Echinodermata*. Wiley-Liss, USA, pp. 169-245.

Chia, F.-S. and Walker, C.W. (1991). Echinodermata: Asteroidea. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), **Reproduction of Marine Invertebrates**, vol. VI. Echinoderms and Lophophorates. The Boxwood Press, USA, pp. 301-353.

Crump, R.G. 1971. Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. *Journal of Experimental Marine Biology and Ecology* **7**: 137-162.

Crump, R.G. and Barker, M.F. 1985. Sexual and asexual reproduction in geographically separated populations of the fissiparous asteroid *Coscinasterias calamaria* (Gray). *Journal of Experimental Marine Biology and Ecology* **88**: 109-127.

Eckelbarger, K.J. and Hodgson, A.N. 2021. Invertebrate oogenesis – a review and synthesis: comparative ovarian morphology, accessory cell function and the origins of yolk precursors, *Invertebrate Reproduction & Development* **65(2):** https://doi.org/10.1080/07924259.2021.1927861

Ferguson, J.C. 1984. Translocative functions of the enigmatic organs of starfish—the axial organ, hemal vessels, Tiedemann's bodies, and rectal caeca: an autoradiographic study. *The Biological Bulletin* **166**: 140-155.

Georgiades, E.T., Temara, A. and Holdway, D.A. (2006). The reproductive cycle of the asteroid *Coscinasterias muricata* in Port Phillip Bay, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology* **332**: 188-197.

Giese, A.C. 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. *Annual Review of Physiology* **21**: 547-576.

Giese, A.C. and Pearse, J.S. 1974. Introduction: general principles. In: Giese, A.C., Pearse, J.S. (Eds.), **Reproduction of Marine Invertebrates**, vol. I. Acoelomate and pseudocoelomate metazoans. Academic press, USA, pp. 1-49.

Grice, A. and Lethbridge, R. 1988. Reproductive studies on *Patiriella gunnii* (Asteroidea: Asterinidae) in south-western Australia. *Australian Journal of Marine and Freshwater Research* **39**: 399-407.

Harrold, C. and Pearse, J.S. 1980. Allocation of pyloric caecum reserves in fed and starved sea stars, *Pisaster giganteus* (Stimpson): somatic maintenance comes before reproduction. *Journal of Experimental Marine Biology and Ecology* **48**: 169-183.

Holland, N.D. and Giese, A.C. 1965. An autoradiographic investigation of the gonads of the purple sea urchin (*Strongylocentrotus purpuratus*). *The Biological Bulletin* **128**: 241-258.

Lawrence, J.M. 1973. Level, content, and caloric equivalents of the lipid, carbohydrate, and protein in the body components of *Luidia clathrata* (Echinodermata: Asteroidea: Platyasterida) in Tampa Bay. *Journal of Experimental Marine Biology and Ecology* **11**: 263-274.

Lawrence, J.M. 1987. **A Functional Biology of Echinoderms**. Croom Helm Ltd, UK.

Lawson-Kerr, C. and Anderson, D. (1978. Reproduction, spawning and development of the starfish *Patiriella exigua* (Lamarck) (Asteroidea : Asterinidae) and some comparisons with *P. calcar* (Lamarck). *Australian Journal of Marine and Freshwater Research* **29**: 45-53.

Mercier, A. and Hamel, J.-F. 2009. **Advances in Marine Biology: Endogenous and Exogenous Control of Gametogenesis and Spawning in Echinoderms.** vol. 55. Academic Press, UK.

Morton, J.E. and Miller, M.C. 1973. **The New Zealand Seashore**. 2nd ed. Collins, London.

Pearse, J.S. and Eernisse, D.J. 1982. Photoperiodic regulation of gametogenesis and gonadal growth in the sea star *Pisaster ochraceus*. *Marine Biology* **67**: 121-125.

Pearse, J.S., Eernisse, D.J., Pearse, V.B. and Beauchamp, K.A. 1986. Photoperiodic regulation of gametogenesis in sea stars, with evidence for an annual calendar independent of fixed daylength. *American Zoologist* **26**: 417-431.

Pearse, J.S. and Walker, C.W. 1986. Photoperiodic regulation of gametogenesis in a North Atlantic sea star, *Asterias vulgaris*. *International Journal of Invertebrate Reproduction and Development* **9**: 71-77.

Prowse, T.A.A. and Byrne, M. 2012. Evolution of yolk protein genes in the Echinodermata. *Evolution and Development* **14**: 139-151.

Reimer, C.L. and Crawford, B.J. 1995. Identification and partial characterization of yolk and cortical granule proteins in eggs and embryos of the starfish, *Pisaster ochraceus*. *Developmental Biology* **167**: 439-457.

Reunov, A.A. and Crawford, B.J. 2010. An ultrastructural study of oocyte atresia in the starfish *Pisaster ochraceus*. *Zoology* **113**: 295-300.

Ruppert, E.E., Fox, R.S. and Barnes, R.D. 2004. **Invertebrate Zoology: A Functional Evolutionary Approach**. 7th ed. Brooks/Cole-Thomson Learning, Belmont.

Schoenmakers, H.J.N., Colenbrander, P.H.J.M., Peute, J. and Oordt, P.G.W.J. 1981. Anatomy of the ovaries of the starfish *Asterias rubens* (Echinodermata). *Cell and Tissue Research* **217**: 577-597.

Schoenmakers, H.J.N., Goedhart, M.J. and Voogt, P.A. 1984. Biometrical and histological aspects of the reproductive cycle of the ovaries of *Asterias rubens* (Echinodermata). *The Biological Bulletin* **166**: 328-348.

Smiley, S. 1990. A review of echinoderm oogenesis. *Journal of Electron Microscopy Technique* **16**: 93-114.

Song, J.L., Wong, J.L. and Wessel, G.M. 2006. Oogenesis: single cell development and differentiation. *Developmental Biology* **300**: 385-405.

Styan, C.A., Byrne, M. and Franke, E. 2005. Evolution of egg size and fertilisation efficiency in sea stars: large eggs are not fertilised more readily than small eggs in the genus *Patiriella* (Echinodermata: Asteroidea). *Marine Biology* **147**: 235-242.

Walker, C.W. 1974. Studies on the reproductive systems of sea-stars. I. The morphology and histology of the gonad of *Asterias vulgaris*. *The Biological Bulletin* **147**: 661-677.

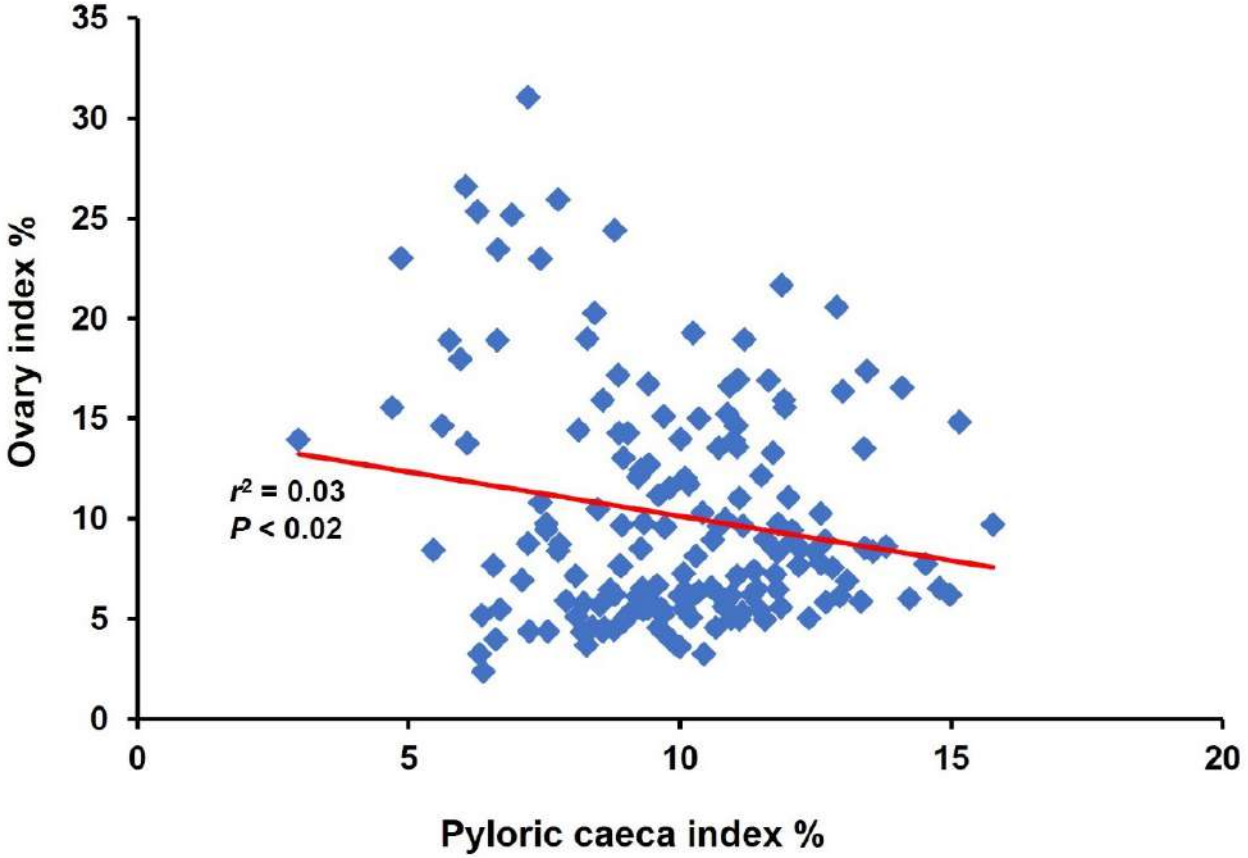
Walker, C.W., Harrington, L.M., Lesser, M.P. and Fagerberg, W.R. 2005. Nutritive phagocyte incubation chambers provide a structural and nutritive microenvironment for germ cells of *Strongylocentrotus droebachiensis*, the green sea urchin. *The Biological Bulletin* **209**: 31-48.

Walker, C.W., Unuma, T., P and Lesser, M. 2007. Gametogenesis and reproduction of sea urchins. In: Lawrence, J.M. (Ed.), **Developments in Aquaculture and Fisheries Science**, vol. 37. Edible sea urchins: biology and ecology. Elsevier, The Netherlands, pp. 11-33.

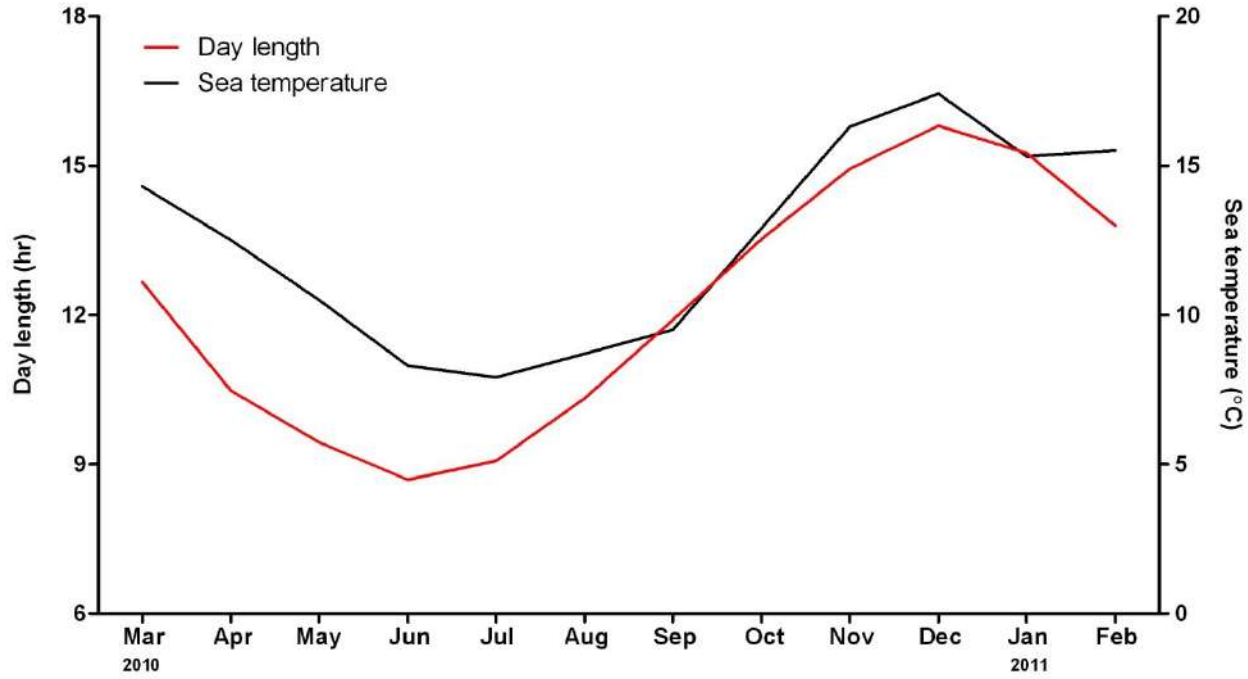
Xu, R.A.and Barker, M.F. 1990a. Laboratory experiments on the effects of diet on the gonad and pyloric caeca indices and biochemical composition of tissues of the New Zealand starfish *Sclerasterias mollis* (Hutton) (Echinodermata: Asteroidea). *Journal of Experimental Marine Biology and Ecology* **136**: 23-45.

Xu, R.A. and Barker, M.F. 1990b. Photoperiodic regulation of oogenesis in the starfish *Sclerasterias mollis* (Hutton 1872) (Echinodermata: Asteroidea). *Journal of Experimental Marine Biology and Ecology* **141**: 159-168.

Supplementary Figures



Supplementary Figure 1. Relationship between the pyloric caeca index and ovary index in sea star Patiriella regularis throughout the reproductive cycle. Regression line equation is y = 14.6 – 0.44 X; r2= 0.03; P < 0.02; n= 169.



Supplementary Figure 2. Annual cycle of day length and sea temperature in Otago Harbour, New Zealand (45° 48' S; 170° 38' E) from March 2010 to February 2011.