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# Arrangement and size variation of intra-gonadal offspring in a viviparous asterinid sea star

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## Abstract

Sibling competition and developmental asynchrony may greatly influence the arrangement and size of offspring of marine invertebrates that care for their young. In *Parvulastra parvivipara*, an asterinid sea star that incubates its young in the gonads, sibling cannibalism supports post-metamorphic development. Offspring size varies within (coefficient of variation, CV = 22.6 %) and among (CV = 17.7%) the gonads. Confocal microscopy was used to visualize early embryos and oocytes, and revealed the presence of several developmental stages within individual gonads. The eggs were a mean diameter of 84 µm. The observation of a gastrula at 86 µm smaller than the largest egg observed (134 µm) suggests that terminal egg size varies. The appearance of early embryos surrounded by somatic cells suggests that they may receive nutrients through histotrophy. Sibling competition intensifies once the digestive tract is functional in the tiny juveniles which then start to consume siblings. The arrangement of the offspring in the gonads was observed using micro-computed tomography. The juveniles were oriented with their oral surface facing each other, presumably as a defensive strategy to protect themselves from being eaten. Periodic release of offspring in single or several cohorts indicates continual reproduction. Released and retained juveniles varied in size. It is not known what initiates birth but it may be mediated by sibling competition. Larger adults had a greater allocation to female reproductive output than smaller adults.

**Key words:** adelphophagy, brooding, viviparity, lecithotrophy, matrotrophy, micro-CT

## Introduction

Diverse modes of parental care have evolved independently in marine invertebrate lineages (McClary & Mladenov 1990; Byrne 1991; Trumbo 1996; Ostrovsky *et al*. 2015). Some species care for their offspring internally (gonads, bursae, gastric pouches), whereas others have their young on or under the body surface (Strathmann & Strathmann 1982; Trumbo 1996; Gillespie & McClintock 2007; Larson 2017). Internal care of offspring is considered to have evolved to provide a 'safe harbour' for offspring to enhance their survival by providing protection from predators, UV radiation and pathogens (Shine 1978; Trumbo 1996; Gillespie & McClintock 2007). Incubation chambers also serve important functions such as supplying dissolved oxygen and nutrients to offspring and removing excretory materials (Strathmann & Chaffee 1984; Strathmann 1995; Gillespie & McClintock 2007). As there are limitations in the space and resources that can be provided to offspring (Bernardo 1996; Kamel *et al*. 2010A), marine invertebrates that care for their young adopt diverse strategies to arrange or package their offspring to maximize reproductive output and offspring fitness. For example, early developmental stages in the sea urchin, *Amphipneustes lorioli*, are partitioned in the deeper recesses of the brood pouches to reduce overcrowding, an adaptation to limited space (Galley *et al*. 2005). In the clam, *Transennella tantilla*, the smallest embryos are positioned dorsally adjacent to the oviduct and, as

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they develop, progressively move towards the ventral surface of the gill that facilitates continuous reproduction and hatching (Kabat 1985). The maternal provisioning strategy may play a role in determining the arrangement and size of the offspring in the incubation space (Carrasco & Phillips 2014).

For species that care for their young internally, offspring interact with the parent and may compete with siblings for limited resources; these interactions may influence size variation at birth (Schrader & Travis 2009; Kamel *et al.* 2010B). Parent-offspring conflict and sibling competition are more intense in species that provide extra-embryonic nutrition, a mode of parental care called matrotrophy (Frick 1998; Schrader & Travis 2009; Mercier *et al.* 2016; Ostrovsky *et al.* 2015; Kamel & Williams 2017). Extreme sibling competition may result in cannibalism where the progeny ingest eggs (oophagy) or siblings (embryophagy/adelphophagy) (Lesoway *et al.* 2014; Ostrovsky *et al.* 2015). Sibling cannibalism greatly increases size variation of offspring in some gastropods, sharks and sea stars (Rivest 1983; Smith & Reay 1991; Byrne 1996; Collin & Spangler 2012). Thus, adelphophagic matrotrophic species provide an opportunity to assess how sibling competition mediates offspring size variation and how the young are arranged in the incubation space.

Non-invasive imaging techniques such as micro-computed tomography (μ-CT) has been used to observe offspring inside the brood chamber non-destructively in situ in ophiuroids (Landschoff & Griffiths 2015). The asterinid sea star, *Parvulastra parvivipara*, exhibits a highly derived mode of matrotrophic incubation, sibling cannibalism, and a high level of offspring size variation (Byrne 1996; Khan *et al.* 2019). This species care for its young within the gonads to an advanced juvenile stage that varies in size from 0.5–2.5 mm in diameter (Byrne 1996; Khan *et al.* 2019). Adelphophagy provides significant nourishment for the juveniles, and gonad histology shows that cannibalism may mediate the position of juveniles within the gonads (Byrne 1996).

In *P. parvivipara*, the size differentiation of offspring starts post-metamorphosis due to sibling cannibalism and to asynchronous gamete maturation and fertilization (Byrne 1996; Byrne & Cerra 1996). The extent to which the offspring size varies among and within gonads has not been characterised. *Parvulastra parvivipara* is a simultaneously hermaphroditic self-fertile species and has young in the gonad year-round with a range of developmental stages from embryo to juvenile (Byrne 1996: Khan *et al.* 2019). The observation that the parents die after releasing all of the juveniles (Keough & Dartnall 1978) requires confirmation. There are several features of the reproductive biology of this species that are yet to be determined including the minimum and maximum size at birth, and whether parents release all of the juveniles at the same time or in cohorts. It is also not known if adults release larger juveniles over a certain size range and retain the smaller juveniles for a longer period. These questions are addressed here.

## Materials and methods

### Sample collection

*Parvulastra parvivipara* were collected from the Smooth Pool (32′54″S, 134′04″E), Eyre Peninsula, South Australia (Ministerial Exemption from the Ministry of Agriculture, Fisheries and Forestry, Government of South Australia- ME9902902) and transported to the University of Sydney. Each *P. parvivipara* (*n* = 54) was kept in a separate plastic beaker that was checked daily for newly released juveniles. Released juveniles were photographed using a camera connected to a dissection microscope (Olympus SZX10) to measure juvenile diameter using the software Micropublisher 3.0. The juveniles were weighed using a microbalance (Mettler H35AR) to 0.0001 g and then preserved in 70% ethanol in a separate Eppendorf tube for each individual. If the parents did not release offspring, the juveniles were collected from the dissected gonads of the parent. Then the juveniles were photographed, counted, measured, weighed, and preserved in 70% ethanol. Before dissection, the arm radius (R) of the parent was measured from the center of the mouth to the tip of one arm and wet weight was measured.

A total of 500 gonads were dissected from 50 *P. parvivipara* and 491 progeny (juveniles and embryos) were counted and measured to compare the size of the released (*n* = 197) and retained (*n* = 294) juveniles. The coefficient of variation (CV) in offspring size within gonads was determined for 64 gonads from 22 adults. Among gonad CV within individual stars was also determined. The CV in offspring size was calculated from the gonads with more than one developing young using the following formula: CV = Standard deviation/mean × 100. The relationship between within gonad CV and number of offspring was analysed using linear regression to determine whether the number of offspring influences CV in offspring size within a gonad.

The relationship between the total number of offspring and adult size was analysed using linear regression. The length of the gonads was measured from the base of the gonad to the farthest tip (or longest length) of the gonad from the photographs taken using Micropublisher 3.0. The relationship between the number and size of gonads that contained offspring per adult and adult size was analysed using linear regression. It was noted if gonads were predominantly testes. The relationship between the number and size of the male gonads (which only had sperm) and the parent size was also analysed. These data were used to determine relative investment in the male (testes) and female (brooding) function using linear regression. Prior to analyses, data were tested for normality with Shapiro-Wilk test and for homogeneity of variance with Levene's test. All linear regressions were analysed using IBM SPSS statistics 24 and significance was assigned at the 5% level.

### Micro-computed tomography and confocal microscopy

One *P. parvivipara* was preserved in 70% ethanol. Micro-CT scans of this individual was performed using Skyscan 1172 with isotropic voxel resolution 2.94 µm. The specimen was firmly positioned within an Eppendorf tube using polystyrene foam and then scanned. A scan of 11 hours at 23 kV and 200 µA resulted in 1170 images that visualized the skeleton. The images were analysed using the software Avizo 9.5 version. Manual "draw tool" and "magic wand" were applied to each image stack to isolate each of the juveniles. A different color was applied to each juvenile within a single gonad to visualize them separately. The position and orientation of the juveniles were visualized by applying three-dimensional (3-D) reconstruction and by making the parent body virtually transparent.

For confocal microscopy, *P. parvivipara* were fixed in 2.5% glutaraldehyde in 1 µm filtered seawater (FSW) for 24 hours and then stored in 70% ethanol. Individual sea stars were then dissected, and each gonad was isolated and dehydrated in an ethanol series (70–95 %). The gonads were cleared with benzyl alcohol and benzyl benzoate, 2:1 or 1:1 (v/v) (Sigma-Aldrich Co.) and mounted on a glass slide. The glutaraldehyde auto-fluorescence was used as a signal for analysis using a Leica SPE-2 Confocal Laser Microscope or ZEISS LSM 800 plus Spectral Confocal Microscope. The samples were excited at 488 nm wavelength with an argon laser contrasting separate gain and offset values for each sample. Images were taken from the different focal planes at 1024×1024 pixel array with a frame average that varied from 4 to 12 based on sample response to excitation. A series of images were stacked for viewing 3D view along the Z axis (Z-stack). The diameter of egg and embryo were measured using confocal microscope online assistance.

## Results

### Biology of viviparity in *Parvulastra parvivipara*

*Parvulastra parvivipara* with young in the gonads were a range of sizes (R = 1.8–5.2 mm, 0.0094–0.1234 g). The gonads are attached by the gonoduct to the aboral body wall and the juveniles emerge through the gonopore (Fig. 1a). The gonads varied in size and shape (Fig. 1b–c). Predominantly male gonads were usually tubular and branched and had occasional oocytes (Fig. 1c). Gonads that contained juveniles were oval to round. In gonads with one juvenile, the gonad shape reflected the juvenile form (Fig 1c). Only two of the 50 adults had juveniles in all ten of their gonads. The gonads ranged in size from 120–2,630 µm ($\bar{x}$ = 941, SD = 624, $n$ = 49) due to the wide size range of juveniles. Predominantly male gonads were small ($\bar{x}$ = 422, SD = 18, Range = 147–940 µm, $n$ = 56 gonads from 27 adults) with a mean of 2.11 testes per individual (SD = 0.96, Range = 0–4, $n$ = 28). The number of male gonads was not related to adult size ($r^2 = 0.079$, $F_{1,26} = 2.242$, $P > 0.05$), but the size of these gonads was larger in the bigger adults ($r^2 = 0.388$, $F_{1,25} = 16.156$, $P < 0.05$). The number of gonads that contained juveniles was positively related to adult size ($r^2 = 0.466$, $F_{1,38} = 33.107$, $P < 0.05$).

Offspring within gonads ranged from developing gastrulae, brachiolaria larvae, and metamorphic juvenile to fully developed juveniles (Fig 1f). Late brachiolaria ranged from 146–247 µm length ($\bar{x}$ = 197 µm, SD = 28, $n$ = 6) and pre-metamorphic juveniles ranged from 243–326 µm diameter ($\bar{x}$ = 277 µm, SD = 35, $n$ = 5).

There was a marked variation in the size of the juvenile that were released in the laboratory ($\bar{x}$ = 1311 µm diameter, SD = 474 µm, Range = 473–3,013 µm, $n$ = 197) (Fig. 2). The juveniles that remained in the gonad also varied in size ($\bar{x}$ = 860 µm diameter, SD = 408, Range = 146–2,514 µm, $n$ = 294) (Fig. 2). *Parvulastra parvivipara* often retained juveniles larger or equal to the largest released juvenile (Fig. 2). Among the 54 adults monitored, 47 released juveniles at least once, 12 released juveniles more than once (2–5 different time points), six released all of their juveniles in a single event, and seven of them did not release juveniles, even though large juveniles ($\bar{x}$ = 960, SD = 47 µm, Range = 219–2,514 µm diameter, *n* = 115) were present in the gonads (Fig. 2). The largest number of offspring (50 juveniles ranging from 418 to 1,280 µm diameter) were isolated from an adult R = 4.7 mm. The smallest adult had only one juvenile (1,397 µm diameter), whereas the largest adult (R = 5.2 mm) had 31 juveniles, of which 16 emerged through the gonopores at five different time points over 14 days, while the remaining 15 juveniles were dissected from the gonads.

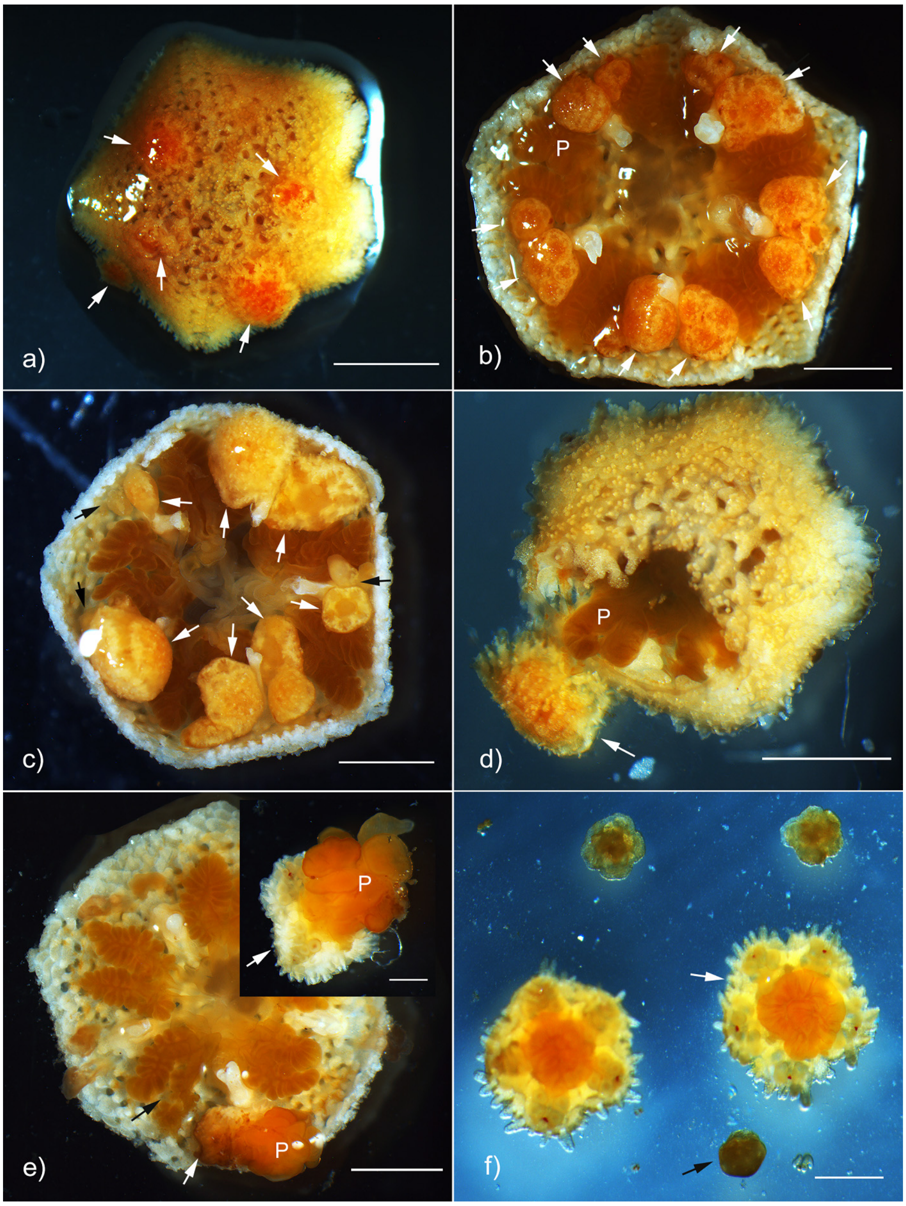

**FIGURE 1.** *Parvulastra parvivipara*: a) five juveniles emerging through the gonopores (white arrows); b) ten gonads with juveniles (white arrows); c) asynchronous development with juveniles (white arrows) at variable in size and predominantly male gonads (black arrows); d) a juvenile (white arrow) crawling out after rupturing its parent's body; e) a juvenile (arrow) eating adjacent torn (black arrow) pyloric caeca (P), inset is the larger view (scale 0.5 mm); f) pre-metamorphic (black arrow), newly metamorphic (upper top) and fully developed (white arrow) juveniles from a gonad. Scale: a and c = 4.0 mm, b = 2 mm, d = 1.5 mm, e = 3.0 mm and f = 0.5 mm.

Four adults died after releasing all of their young. These were small adults (R = 2.5–3.2 mm). In two other adults, the body wall was ruptured by the emerging juveniles but the parents were still alive (Fig 1d, e). In one adult (R = 1.8 mm), the juvenile (1,398 µm diameter) was 37 % of the parent size. In the second adult (R = 4.2 mm), the juvenile (1,443 µm) was around 18 % of the parent diameter. One juvenile was observed eating the pyloric caeca of the parent (Fig. 1e). Total brood weight was significantly related to adult size ($r^2 = 0.367$, $F_{1,52} = 30.101$, $P < 0.05$) and weight ($r^2 = 0.414$, $F_{1,46} = 32.616$, $P < 0.05$).

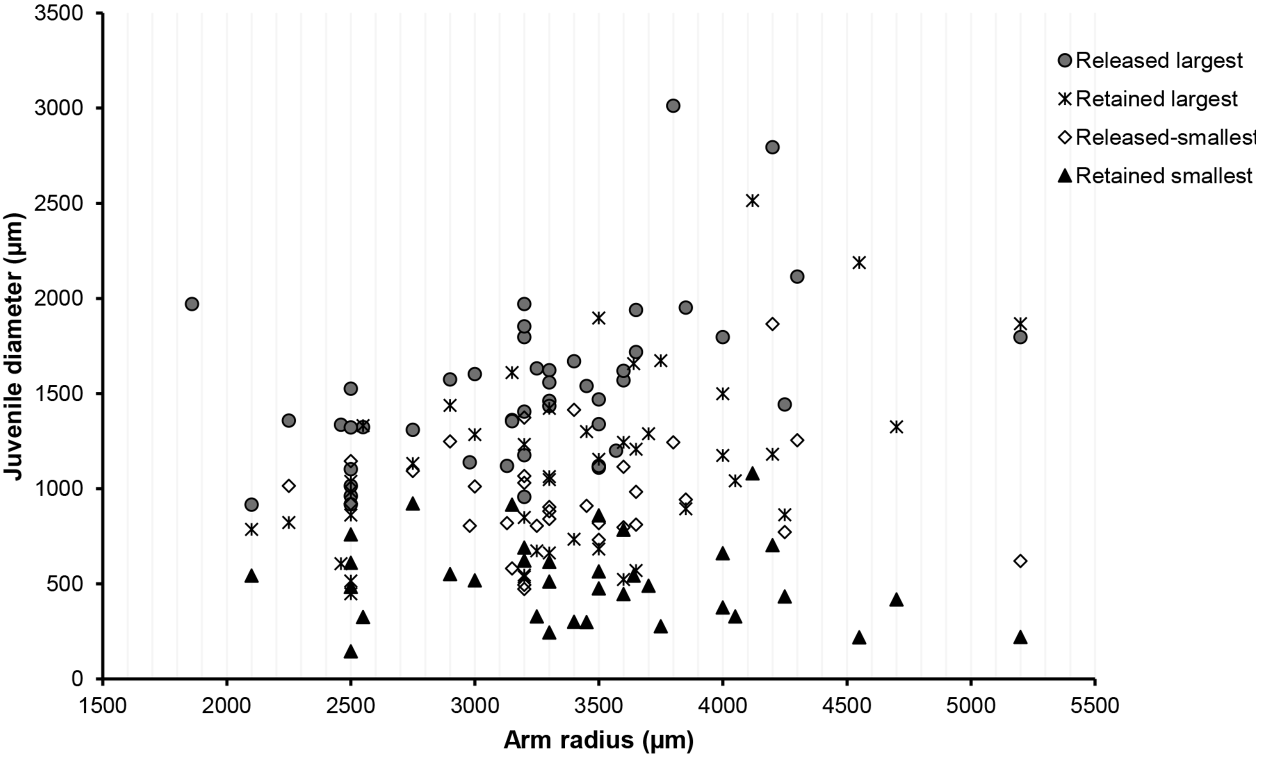

**FIGURE 2.** Size of released and retained juveniles in *Parvulastra parvivipara* in the laboratory from 54 parents having different arm radius (µm). The size of the retained juveniles was determined after dissecting the parent.

The CV in offspring size within an individual gonad was 24.7 % (SD = 24.18, Range = 0.14–112.57, $n$ = 64 gonads from 22 adults). Among gonad CV within an adult was 19.5 % (SD = 14.56, Range = 1.91–39.01, $n$ = 22 adults 64 gonads). The largest number of juveniles in a single gonad was nine.

## Confocal Microscopy

The gonads are sac-like structures with outer and inner epithelial layers (Fig. 3a). Developing eggs are attached to the germinal epithelium by a basal stalk-like structure and were surrounded by a layer of follicle cells (Fig. 3b). Egg development is continuous. The mean egg size was 84 µm (SD = 21, Range = 54–134 µm, $n$ = 43) and the largest oocyte observed was 134 µm diameter. The gonads contained a range of developmental stages including gastrula, brachiolaria larva and juveniles (Fig. 3a–i). The eggs and early embryos may remain surrounded by somatic cells (Fig. 3a–b). Juveniles remain orally apposed to each other (Fig. 3e). Amorphous material was observed adjacent to brachiolaria and metamorphic juvenile within the gonadal lumen which may be haemal fluid (Fig. 3i). The mean diameter of the gastrula was 91 µm (SD = 4, $n$ = 3), early brachiolaria was 112 µm (SD = 6, $n$ = 4), late brachiolaria was 145 µm (SD = 13, $n$ = 3), and pre-metamorphic juvenile was 218 µm (SD = 4, $n$ = 3). The presence of eggs and offspring at different stages of development indicates asynchronous reproduction (Fig. 3a–i).

Among 63 gonads observed from 13 adults, four were predominantly male with no oocytes (Fig. 3f) and offspring, 32 contained juveniles, five with embryos only, four with embryos and juveniles, and 18 had developing oocytes. All of the gonads except four male gonads (Fig. 3f) had oocytes of variable stages and sizes. The smallest early embryo encountered was 86 µm diameter (early gastrula). Gonads with developing oocytes were tubular or dome-shaped and become oval or round with increasing size and number of juveniles. The profile of gonads that had only one large juvenile reflected the shape of the juvenile (Fig. 3c). Juveniles may extrude their stomach into the lumen (Fig. 3i).

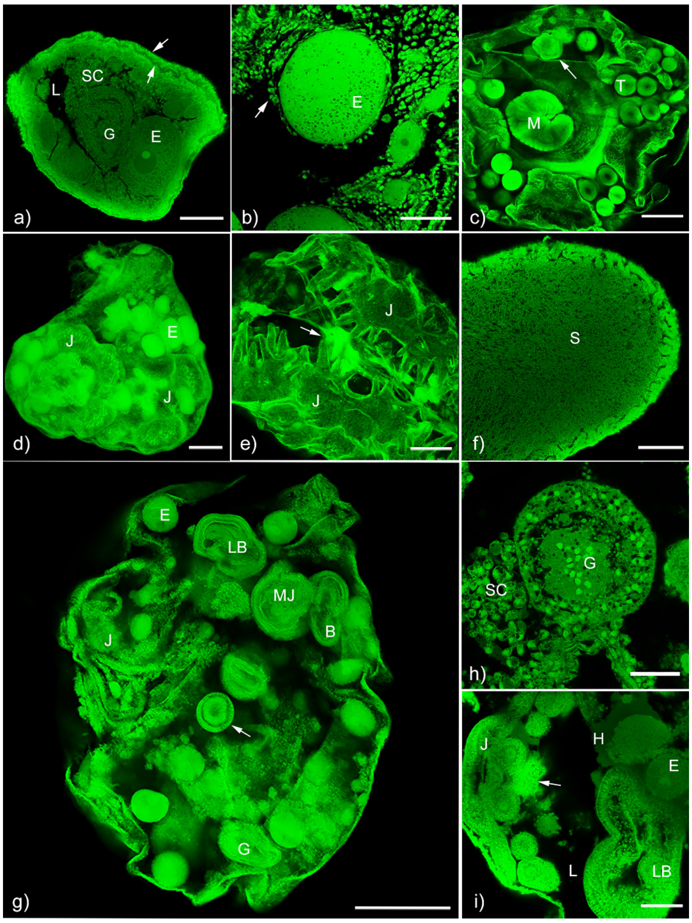

**FIGURE 3.** Confocal microscopy of *Parvulastra parvivipara* gonads: a) a gonad containing a gastrula surrounded by somatic cells in the lumen adjacent to an egg, outer and inner gonad wall (arrows); b) eggs surrounded by follicle cells (arrow); c) oral face of a large juvenile with two pairs of tube feet in each arm contrasting in size with a small metamorphosing juvenile (arrow); d) a 3-D view of a gonad containing two juveniles and eggs; e) a 3-D view of two juveniles with their oral faces (arrow) opposite to each other; f) sperm in a predominantly male gonad; g) gonad containing eggs, early gastrula (arrow), gastrula, brachiolaria, late brachiolaria, and metamorphic juvenile and juvenile; h) flocculent material and somatic cells adjacent to a gastrula; i) juvenile with stomach extruded (arrow), amorphous material (AM) next to the late brachiolaria and around egg that may be haemal fluid. E, egg; G, gastrula; B, brachiolaria; LB, late brachiolaria; MJ, metamorphic juvenile; J, Juvenile; L, lumen; SC, somatic cells; M, mouth; S, sperm; T, tube feet. Scale: a, b, f and i = 50 µm; c, e and g = 200 µm, d = 100 µm, h = 20 µm.

### Offspring arrangement using µ-CT

The 3D tomographic video of *P. parvivipara* shows 13 juveniles (392–1,620 µm diameter) in the gonads (Fig. 4a–b). Four gonads each contained one juvenile, two gonads each contained two juveniles, one gonad had five juveniles and three gonads had no juvenile (Fig. 4a–b). In gonads with two juveniles these were facing orally opposite to each other (Fig. 4c). In the gonad that had five juveniles, the largest juvenile was 926 µm diameter and the smallest juvenile was 721 µm diameter. All five juveniles were oriented with their oral face opposite to each other and they had their oral face towards the center of the gonad (Fig. 4d). The position and orientation of juvenile had no specific pattern in the gonads that had a single juvenile. The largest juvenile was more than four times larger than the smallest solitary juvenile.

## Discussion

*Parvulastra parvivipara* exhibits a unique spatial arrangement of its young in the gonads that is influenced by maternal provisioning strategy and sibling cannibalism. The juveniles usually position their oral surface opposing one another, an orientation that may defend them from being preyed upon by siblings (Byrne 1996). Juveniles that achieve an advantageous position and face the aboral surface of another juvenile may successfully cannibalize their sibling (Byrne 1996). Large juveniles appear to be relatively stationary in the gonads potentially to prevent rupture of the gonad wall. Juveniles often had their stomachs extruded, indicative of predation and perhaps using gonadal fluid and eggs as sources of nutrient.

The 3-D reconstruction of *P. parvivipara* offspring add a new dimension in observing brooding in marine invertebrates. There is one previous three-dimensional reconstruction (μ-CT) of the offspring in the brood chamber of echinoderms, those of the ophiuroids, *Ophioderma wahlbergii* and *Amphipholis squamata* (Landschoff & Griffiths 2015). In these ophiuroids, the juveniles had their oral surface pressed against the bursal sinus, which provides nutrients to developing young (Byrne 1991; Hendler & Tran 2001). In *P. parvivipara*, amorphous material in the gonad may be haemal fluid and a potential source of nutrients for the juveniles. For this species, however, adelphophagy is the main source of nutrients that supports for substantial post-metamorphic growth.

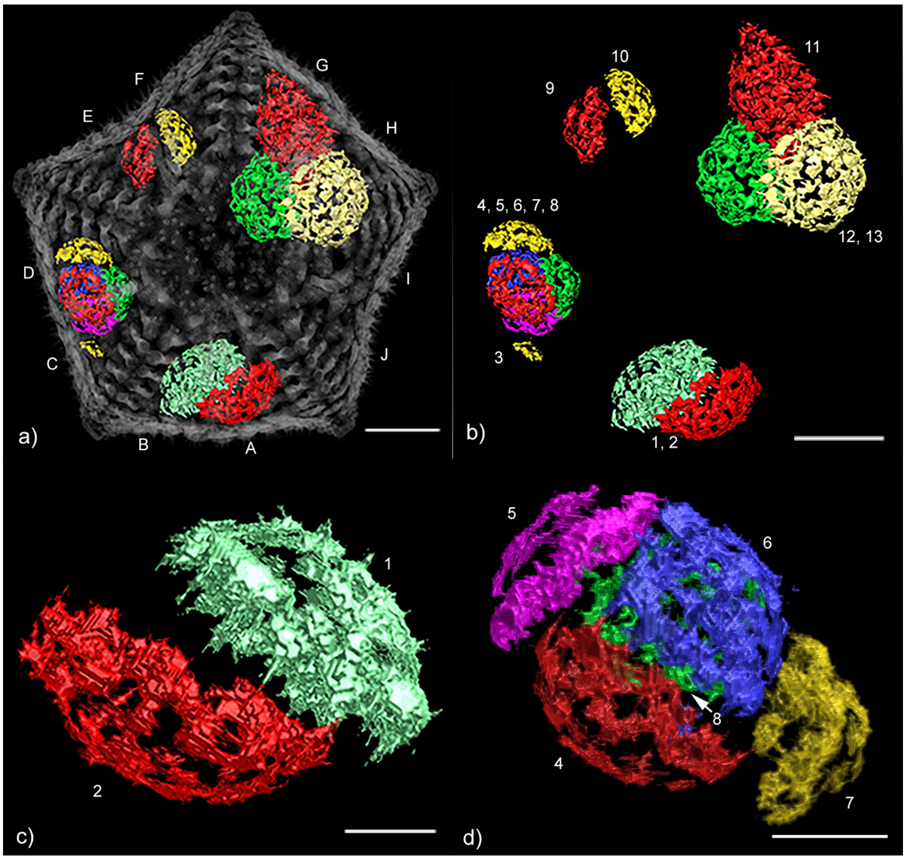

**FIGURE 4.** Micro-Computed tomography of *Parvulastra parvivipara*: a) 3-D image showing the position of 13 juveniles (coloured differently) within the gonads (A–J), gonad B, I and J had no juveniles; b) juveniles (coloured differently) with parent body removed virtually, separate numbers indicates juveniles from separate gonads (A–H); c) two juveniles orally apposed to each other in a gonad; d) position of five juveniles (coloured differently) within a gonad oriented with their oral surfaces opposed. Scale: a and b = 1.0 mm, b and c = 0.5 mm.

Studies that relate sibling competition to the arrangement of offspring in the incubation chamber in marine invertebrates are rare. This unusual mode of maternal provisioning is also reported in the holothuroid *Leptosynapta clarki* and the sea star *Pteraster militaris*, and *P. vivipara*, (McClary & Mladenov 1990; Byrne & Cerra 1996; Sewell *et al.* 2006). Sibling cannibalism is common in marine gastropods and polychaetes (Cable & Tinsley 1991; Cubillos *et al.* 2007; Carrasco & Phillips 2014). Sibling cannibalism has evolved to support embryonic nutrition and generation of large offspring size in sharks and mollusks (Wourms 1981; Smith & Reay 1991; Strathmann 1995; Cubillos *et al.* 2007). For example, in sand tiger sharks, *Carcharias taurus*, 9–10 embryos begin development in each oviduct, but only one gigantic offspring survives (Wourms

1981). Similarly, several mollusks place hundreds of embryos within a single egg case to support few large hatchlings that grow through cannibalism (Strathmann & Strathmann 1995; Carrasco & Phillips 2014). As the juveniles of *P. parvivipara* develop from a very small secondarily reduced egg (84 µm diameter), intragonadal sibling cannibalism may have evolved in parallel to a decrease in egg size to support post-metamorphic nutrition and producing large offspring size (Byrne 1996; Byrne & Cerra 1996). Developing embryos and juveniles may also get nutrition from surrounding somatic cells, gonadal fluid and haemal fluid. These extra-embryonic sources remain to be investigated.

Sibling cannibalism may be an adaptation to reduce the number of embryos competing for oxygen, as in the mollusk *Acanthina monodon*, where adelphophagy increases with increasing oxygen demand (Lardies & Fernandez 2002). It is not known whether sibling cannibalism in *P. parvivipara* decreases embryonic development time as it does in several muricid gastropods (e.g. *Crepipatella fecunda*) (Spight 1975; Cubillos *et al*. 2007). Adelphophagy should be favoured if structural and energetic constraints limit maximum egg size (Strathmann 1995). This may be the case for *P. parvivipara*, which is the smallest known sea star. The offspring produced by *P. parvivipara* are much larger than the juveniles released by the closely related asterinid brooders that have large eggs and lecithotrophic development (Byrne & Cerra 1996).

Sibling cannibalism in *P. parvivipara* may be an adaptation to produce large offspring size (>1000 µm) to enhance their success in the harsh high intertidal environment occupied by this species (Byrne 1996; Trumbo 1996; Gillespie & McClintock 2007). The largest juveniles produced by *P. parvivipara* are nearly reproductively mature (Byrne 1996). Large offspring may be selected for early reproductive maturity, high survival and stress tolerance (Rivest 1983; Moran & Emlet 2001; Olivera-Tlahuel *et al*. 2015).

As seen here for *P. parvivipara*, the presence of several development stages in the gonad space also occurs for the young in the bursa of the ophiuroid, *Amphiura carchara* (Hendler & Tran 2001), bivalves *Transennella tantilla and T. confusa* (Kabat 1985; Russell *et al*. 1992), sponge *Rhopaloeides odorabile* (Whalan *et al*. 2007), and annelid *Diopatra marocensis* (Arias *et al*. 2013). Asynchronous development of several ontogenic stages or superfetation commonly occurs in live-bearing fishes (Clinidae, Zenarchopteridae, Poeciliidae) and was thought to be restricted to fishes (Wourms 1981; Frick 1998). Frick (1998) first used the term 'superfetation' in the marine invertebrate literature to describe viviparity in the holothuroid *Synaptula hydriformis* that cares for multiple cohorts of embryos in the perivisceral coelom. In *P. parvivipara*, The presence of an early gastrula at 86 µm diameter, which is smaller than the largest egg observed (134 µm diameter), suggests that the terminal egg size may vary.

Superfetation is thought to be evolutionarily linked with matrotrophy in many live-bearing poeciliid fishes (Scrimshaw 1944; Pollux *et al*. 2014; Olivera-Tlahuel *et al*. 2015) and is advantageous with respect to energetics and fecundity, because a parent can produce small eggs with the outcome of producing large juveniles freeing the adult from fixed initial cost to produce a large egg (Olivera-Tlahuel *et al*. 2015). This strategy results in many small early-stage embryos and few large-late stage embryos, avoids the constraints limiting the number of offspring due to maternal size, and allows production of large hatchlings (Scrimshaw 1944; Wourms 1981; Trexler 1997; Olivera-Tlahuel *et al*. 2015). Thus, asynchronous viviparity may also be evolutionarily linked to matrotrophy (sibling cannibalism) in *P. parvivipara* where producing a small egg (80 µm) freed from fixed energy investment at the onset of embryo development, supported continuous reproduction and large offspring to maximize energetic efficiency (Travis *et al*. 1987).

Continuous production of offspring may be a mechanism to reduce overcrowding and utilize limited space available to accommodate offspring in the small-bodied sea star *P. parvivipara,* similar to the sea urchin *A. lorioli* (Galley *et al*. 2005). According to the energetic hypothesis, this tiny sea star may not have sufficient energy to produce more and larger eggs to invest all of its resources at a time (Chia 1974). However, larger adults of *P. parvivipra* are more capable of allocating a greater amount of energy at a time for female functioning or offspring care than smaller adults.

*Parvulastra parvivipara* exhibits substantial among and within gonad variation in offspring size. Size variation of offspring among broods often reflect a bet-hedging strategy influenced by the environment on maternal provisioning or availability of the resources (Bernardo 1996; Moran & Emlet 2001; Marshall & Keough 2008). Selection favours parents investing optimum resources for all offspring (Vance 1973; Smith & Fretwell 1974), but individual offspring will gain a fitness benefit by securing a greater share of parental resources (Trivers 1974; Kamel & Williams 2017). Thus, offspring will compete with their siblings and the parent. In cases where outcrossing occurs, individual offspring are more closely related to themselves than to siblings or the parent (Parker *et al*. 2002; Kamel & Williams 2017) and this may promote competition. In contrast, in *P. parvivipara* and other viviparous asteroids, the parent and offspring have the same genotype due to self-fertilization (Keever *et al*. 2013). As all juveniles are genetically identical to each other, sibling relatedness or mating system has a little role in sibling competition and size variation.

In species with asynchronous viviparity, early hatched larger embryos/juveniles out compete less-developed siblings (asymmetric competition) due to their physical superiority or greater competitive ability (Olivera-Tlahuel *et al*. 2015). Hence, they will be larger at birth, as occurs in some live-bearing fish (Schrader & Travis 2012). In *P. parvivipara*, asynchronous vivparity initiates an asymmetric competition among early developing embryos, and dynamic sibling cannibalism reduces parental control on offspring size (Pollux & Reznick 2011) and intensifies the size variation of offspring at the post-metamorphic stage. Within brood variation in offspring size in *P. parvivipara* may also be an adaptation to an unpredictable intertidal environmental (bet-hedging) (Marshall & Keough 2008), or some offspring may simply obtain slightly more resources (randomly) in the early development stages (Rivest 1983; Moran & Emlet 2001).

In the laboratory, *P. parvivipara* released all of its juveniles in a single cohort or at several time points. *Parvulastra parvivipara* may retain juveniles in the gonads which are larger than those released. This species does not normally die after birth of offspring, counter to early reports (Keough & Dartnall 1978). This species releases offspring at a diverse size range (0.4–3.0 mm diameter) and retains juveniles up to 2.5 mm in diameter.

*Parvulastra parvivipara* releases juveniles through the aboral gonopore associated with softening of the surrounding collagenous tissue (Byrne 1996), but is not known if this process is controlled by the parent or if it is mediated by a signal from the offspring. As the adults retain juveniles that are larger than the released juveniles, the parent may have little control over juvenile release or influence on offspring size at birth. Some small juveniles may vacate the gonad to avoid being preyed on by the larger siblings (Byrne 1996) or may emerge as a group with other siblings. Large single juveniles may remain in the gonad to a very advance stage and occasionally may tear the gonad and body wall of the parent. In extreme cases, they may cause major damage to the parent, possibly when gonad has reached its maximum capacity. This resulted in occasional adult mortality and was most commonly seen in the smallest adults.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

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