

## The reproductive ecology of the Antarctic bivalve *Aequiyoldia eightsii* (Protobranchia: Sareptidae) follows neither Antarctic nor taxonomic patterns

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1 **The reproductive ecology of the Antarctic bivalve *Aequiyoldia eightsii* (Protobranchia:**  
2 **Sareptidae) follows neither Antarctic nor taxonomic patterns**

3

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22 **Abstract**

23 The accepted paradigm for reproduction in Antarctic marine species is one where oogenesis  
24 takes 18 months to 2 years, and a bimodal egg-size distribution where two cohorts of eggs  
25 are present in female gonads throughout the year. These slow gametogenic traits are driven  
26 by low temperature and/or the restriction of resource availability because of extreme  
27 seasonality in the marine environment. Here we present data on the reproductive ecology of  
28 the common Antarctic bivalve *Aequiyoldia eightsii* (Jay, 1839) (Protobranchia: Sarepidae)  
29 from monthly samples collected between January 2013 and May 2014 at Hangar Cove,  
30 Rothera Point on the West Antarctic Peninsula. These data show that *A. eightsii* is unusual  
31 because it does not follow the typical pattern expected for reproduction in Antarctic marine  
32 invertebrates, and differs also from closely related nuculanid protobranch bivalves with  
33 respect to gametogenic duration and reproductive periodicity. Continuous oogenesis,  
34 evidenced by the year-round occurrence of previtellogenic, vitellogenic, and ripe oocytes in  
35 female gonads, is supplemented by a seasonal increase in reproductive intensity and  
36 spawning in Austral winter (April-May), evidenced by the loss of mature spermatozoa and  
37 ripe oocytes from males and females respectively. The simultaneous occurrence of these  
38 contrasting traits in individuals is attributed to a flexible feeding strategy (suspension and  
39 deposit feeding) in response to seasonal changes in food supply characteristic of the  
40 Antarctic marine environment. Asynchrony between individual females is also notable. We  
41 hypothesise that the variability may represent a trade-off between somatic and reproductive  
42 growth, and previously reported internal interannual cycles in shell growth.

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44 Keywords: Antarctic; marine; invertebrate; bivalve; interannual; reproduction;

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## 48 **Introduction**

49 Marine invertebrate reproductive ecology influences patterns of species biogeography,  
50 population persistence, and species survival (Ramirez-Llodra 2002; Lester et al. 2007).  
51 Investigating the reproduction of a particular species therefore provides insight into its  
52 adaptive capacity and potential resilience to environmental change (Byrne 2011; Sanford and  
53 Kelly 2011). As the global ocean is under increasing threat from climate change, especially  
54 warming and acidification (Byrne 2011), evaluation of a species' reproductive success is key  
55 to understanding vulnerability to future climate scenarios (Grange et al. 2004). In the  
56 Southern Ocean, the waters around the West Antarctic Peninsula have experienced  
57 pronounced warming relative to the global average (see review by Mayewski et al. 2009),  
58 with sea surface summer temperatures in the Bellingshausen Sea on the west of the  
59 Peninsula increasing by  $\sim 1^{\circ}\text{C}$  since the 1950s (Meredith and King 2005). Antarctic shallow-  
60 water invertebrates are particularly susceptible to the effects of climate change, as warming  
61 causes perturbations in sea ice dynamics, which could subsequently lead to ecological  
62 regime shifts in benthic systems (Barnes and Conlan 2007; Barnes and Souster 2011;  
63 Barnes 2016). Presently, there is a lack of knowledge specific to the recovery potential of the  
64 benthos after these disturbance events (Clark et al. 2015). Additionally, many studies have  
65 demonstrated the physiological constraints and vulnerability of Antarctic benthic  
66 invertebrates to subtle temperature increases (e.g. Peck et al. 2004a; Pörtner et al. 2007;  
67 Peck et al. 2010; 2014). Benthic assemblages in Antarctica are taxonomically diverse and  
68 abundant, with more than 7137 species identified, which accounts for  $\sim 88\%$  of the total  
69 number of identified species in the Southern Ocean (De Broyer and Danis 2011). However,  
70 the reproductive ecology and related paradigms regarding invertebrate life history and  
71 ecology are far from fully understood (Pearse and Lockhart 2004), with details of  
72 reproductive biology, especially in terms of variations between the years, or longer term  
73 trends, rarely published.

74 The reproductive patterns of Antarctic shallow-water benthos are often characterised by a  
75 prolonged reproductive cycle (18-24 months), lecithotrophy, and direct development, low  
76 fecundity and seasonal reproductive patterns (Clarke 1979; Brey and Hain 1992; Chiantore  
77 et al. 2002; Grange et al. 2004, 2007; Higgs et al. 2009; Pearse et al. 2009; Reed et al.  
78 2013a). However, a few species also demonstrate shorter gametogenic periods (12 months  
79 or less) (Clarke 1988; Chiantore et al. 2002; Tyler et al. 2003; Grange et al. 2011),  
80 planktotrophic larvae (Stanwell-Smith et al. 1999; Bowden et al. 2009), and relatively high  
81 fecundity (McClintock and Pearse 1986; Bosch and Pearse 1990; Grange et al. 2004; Orejas  
82 et al. 2007). Moreover, the reproductive ecology of Antarctic shallow-water and deep-sea  
83 species are similar in several benthic taxa, including asteroids (Bosch and Pearse 1990) and  
84 octocorals (Orejas et al. 2012). Several authors have suggested that the abiotic and biotic  
85 characteristics shared between the polar and deep-sea environments attribute similarities in  
86 reproductive ecology between Antarctic shallow-water and the deep-sea benthos (Bosch and  
87 Pearse 1990; Ramirez-Llodra 2002; Orejas et al. 2012). Both realms are characterised by  
88 low stable temperatures, low light, and ice scour, and functionally similar benthic  
89 communities with slow population turnover rates (Bosch and Pearse 1990; Aronson et al.  
90 2007; Clark et al. 2015). Furthermore, the continental shelf in Antarctica is depressed by the  
91 weight of the ice-sheet, such that its average depth is around 500 m compared to around 200  
92 m elsewhere in the world (Heywood et al. 2014). This feature facilitates the connection  
93 between shallow and deep areas of the Southern Ocean across the continental shelf, with  
94 submerging Antarctic bottom water and emerging circumpolar deep water that may have  
95 enhanced colonisation of shallow fauna to deep waters (and vice versa) during glacial cycles  
96 of shelf ice advance and retreat (Berkman et al. 2004; Strugnell et al. 2011). Many Antarctic  
97 invertebrate taxa including Bivalvia, Gastropoda, Amphipoda, and Decapoda are reported to  
98 have a significantly higher degree of eurybathy compared to their temperate counterparts,  
99 often being distributed from surface water to hundreds of meters (Brey et al. 1996).

100 Protobranchia is a subclass of small deposit feeding bivalves commonly found in soft  
101 sediment environments with approximately 750 species distributed globally (see review by  
102 Zardus 2002). With a Cambrian origin (Carter et al. 2000; Sharma et al. 2013), this subclass  
103 has diversified and colonised the deep sea, representing ~50% and ~90% of bivalve species  
104 on the continental slope and abyssal plain, respectively (Allen 1978). Although protobranch  
105 bivalves are important bioturbators in the deep sea (Zardus 2002), they remain one of the  
106 least studied molluscan groups (Sharma et al. 2013; Reed et al. 2014). Protobranch bivalves  
107 are well represented in the Antarctic, but information regarding the ecology of Protobranchia  
108 in the Southern Ocean is limited as most species are found only in deeper water (Reed et al.  
109 2013b). One exception is the common shallow-water species *Aequiyoldia eightsii* (Jay 1939)  
110 (previously *Yoldia eightsii*), which has been used as a model bivalve species in several  
111 investigations. While studies have examined its abundance and ecophysiology (described  
112 below), the reproductive ecology and gametogenic cycles in this species are poorly  
113 understood, constrained by the problems associated with seasonal sampling of organisms in  
114 the Southern Ocean.

115 Here, we investigated the reproductive ecology of an Antarctic shallow-water protobranch  
116 bivalve *A. eightsii*, which is one of the most abundant benthic species in the maritime  
117 Antarctic. Previous records show densities of *A. eightsii* of up to 1540 m<sup>-2</sup> at Signy Island  
118 (Peck and Bullough 1993), and biomass contributions equivalent to 56.6% of the total  
119 macrofaunal biomass at Faro station in Potter Cove (Pasotti et al. 2015a). The geographical  
120 distribution of *A. eightsii* is patchy around the Antarctic continent (Dell 1990), but is extended  
121 to sub-Antarctic regions including King George Island (62° 10'S) and the Magellan Strait (53°  
122 28'S) (González-Wevar et al. 2012). With a bathymetric distribution ranging from intertidal  
123 sand flats to deep waters (824 m), *A. eightsii* is most commonly found at depths shallower  
124 than 100 m (Dell 1990; Peck and Bullough 1993). *Aequiyoldia eightsii* has an opportunistic  
125 feeding strategy, which typically exhibits deposit feeding but can alternate to a suspension  
126 feeding mode when phytoplankton is present in the water column (Davenport 1988). Deposit

127 feeding and burrowing in *A. eightsii* involve intensive locomotion, where this species is one of  
128 the fastest burrowing of all Antarctic marine benthic groups (Peck et al. 2004b). Previous  
129 observations have also shown a single specimen can expel sediment up to 13 times its dry  
130 tissue mass within 24 hours (Davenport 1988). Given its high abundance and wide  
131 distribution within Antarctica, *A. eightsii* is likely to be one of the major bioturbators among  
132 the Antarctic shallow-water benthic communities. Moreover, *A. eightsii* has frequently  
133 featured in physiological studies as a comparative species with other Antarctic benthos (Peck  
134 and Conway 2000; Peck et al. 2004b, 2014; Fattorini et al. 2010; Román-González et al.  
135 2017), and as a model species in studies designed to analyse the impact of climate change  
136 on Antarctic seafloor communities (McClintock et al. 2009; Peck et al. 2009, 2010; Clark et al.  
137 2016). A recent study identified an innate endogenous growth rhythm in this species and  
138 suggested the pattern may be attributed to the reallocation of energy resources between  
139 cycles of somatic and reproductive investment (Román-González et al. 2017). Owing to the  
140 use of *A. eightsii* in a range of Antarctic ecological studies, the reproductive data reported  
141 here should inform future studies focused on interpreting the biological and ecological role of  
142 the species, and the ecological processes of the Protobranchia and Antarctic benthos as a  
143 whole.

144 The objectives of this study were to: (1) describe the reproductive ecology and general  
145 anatomy of *A. eightsii* from a shallow-water site at Rothera Point off the West Antarctic  
146 Peninsula using wax histology, (2) to compare the reproductive condition of *A. eightsii* with  
147 other Antarctic shallow water benthos and protobranch bivalves, and (3) to evaluate, where  
148 possible, what may be driving these reproductive patterns.

## 149 **Materials and Methods**

### 150 **Sample collection**

151 Monthly samples of *A. eightsii* were scuba diver-collected between January 2013 and May  
152 2014 at 13 to 16 m depths in Hangar Cove, adjacent to the British Antarctic Survey's Rothera  
153 Research Station on Rothera Point, Adelaide Island, off the West Antarctic Peninsula (67° 33'

154 52 S, 68° 07' 43 W) (Fig. 1). Fifteen to 20 individuals were collected per month and fixed in  
155 4% buffered formaldehyde in the field. Formaldehyde-preserved samples were then  
156 transported to the University of Southampton, UK for storage. Poor ice conditions and  
157 inclement weather prevented field sampling in February and June 2013.

### 158 **Histological preparation**

159 For each specimen, maximum shell length, height, and width were measured using a digital  
160 caliper ( $\pm 0.01$  mm) and the soft tissue separated from the shell using a scalpel. Photographs  
161 of specimens before and after shell removal were taken for anatomical study.

162 *Aequiyoldia eightsii* with shell length  $> 20$  mm have been reported to be reproductively  
163 mature (Peck et al. 2000). In an effort to maintain consistency, only individuals with a shell  
164 length between 20 and 25 mm were selected for processing. None of the bivalves collected  
165 in March or August 2013 measured between 20 and 25 mm and were therefore discounted  
166 from further study. A total of 95 individuals were selected for histological analysis. Sex of  
167 individuals was determined from histological sections.

168 The gonad of *A. eightsii* is not a discrete organ and the germinal tissues are infiltrated in the  
169 visceral mass. Therefore, whole specimens were examined using standard wax histology  
170 techniques following the protocol outlined in Higgs et al. (2009). Preliminary studies  
171 demonstrated the denser foot muscle to be more sensitive to the process of clearing when  
172 compared to other soft tissues. This tissue was therefore dissected and processed  
173 separately. Bivalves were dissected in a longitudinal direction through the body just below  
174 the digestive gland, removing the foot muscle from the main body.

175 Both dissected parts of soft tissue were dehydrated in a graded series of isopropanol,  
176 cleared in three washes of histoclear (CellPath, UK) and embedded in wax blocks. Serial  
177 sections of 7 $\mu$ m thick were mounted and stained with Haematoxylin Z (CellPath, UK) and  
178 Eosin Y (CellPath, UK), and immediately cover-slipped using a DPX mounting medium



179 (Sigma-Aldrich, UK). Note that the gonads of six female individuals were damaged during  
180 histological preparation and were therefore excluded from reproductive analysis.

## 181 **Data analysis**

### 182 ***Gonad morphology***

183 General anatomical observations of *A. eightsii* were made with respect to gonad morphology  
184 from the serial sectioning of both dissected parts of animals under a light microscope  
185 (Olympus BHS (BH-2), and the macrophotography images of soft tissues after shell removal.

### 186 ***Female reproductive analysis***

187 Preliminary analysis indicated that a sexually mature female could simultaneously carry in  
188 excess of 3000 oocytes. Therefore, only eight histological sections, evenly distributed across  
189 the gonad-digestive complex per specimen (around 1.5 mm apart), were taken for image  
190 analysis. Thin tissue sections were photographed using a Nikon D5000 digital camera  
191 mounted on a light microscope (Olympus BHS (BH-2)). Oocyte size, measured as Equivalent  
192 Circular Diameter (ECD), of individual females was quantified using the 'Area' tool in ImageJ  
193 v1.48 (Rasband 1997-2016). ECD assumes the best-fit spherical size of each oocyte and is  
194 equivalent to Oocyte Feret Diameter (OFD) used in previous studies (e.g. Reed et al. 2013a,  
195 2014). Only oocytes with a visible nucleus were measured to ensure the size of each oocyte  
196 was approximated at, or very close to its maximum cross-sectional area. The equation used  
197 for calculating ECD from the traced area of each oocyte was:

$$198 \quad \text{ECD} = 2 \sqrt{\frac{A}{\pi}} \quad (1)$$

199 where ECD is Equivalent Circular Diameter ( $\mu\text{m}$ ), and A is the area of an individual oocyte  
200 ( $\mu\text{m}^2$ ).

201 Female maturity was evaluated based on the overall oocyte cellular appearance and  
202 description of marine bivalve ovary development outlined in Morse and Zardus (1997).

203 Oocytes  $< 50 \mu\text{m}$  ECD, with basophilic cytoplasm and a large nucleus were defined as  
204 previtellogenic. Oocytes between 50 and 80  $\mu\text{m}$  ECD, with eosinophilic cytoplasm and yolk

205 droplets were identified as vitellogenic, whereas oocytes > 80 µm ECD with an outer vitelline  
206 envelope and jelly coat were defined as ripe oocytes (Fig. 1 in Online Resource 1).

### 207 **Male reproductive analysis**

208 Eight histological sections evenly distributed across the gonad-digestive complex of each  
209 male *A. eightsii* were photographed for image analysis. Five stages of gonad maturity were  
210 identified according to the rationale for the overall development and distribution of  
211 spermatozoa in marine bivalve testes development described in Morse and Zardus (1997)  
212 (Fig. 2 in Online Resource 1).

213 **Stage I (early developing stage):** Spermatogonia develop along the follicle wall.

214 **Stage II (mid developing stage):** Spermatogonia divide into spermatocytes and move away  
215 from the follicle wall. The spermatocytes have increased in number and occur in clusters.

216 **Stage III (late developing stage):** Spermatocytes divide into spermatids, which then  
217 differentiate into spermatozoa. The division and differentiation process leads to spermatozoa  
218 being placed in the centre of the lumen in chained form.

219 **Stage IV (ripe stage):** Spermatozoa increase in number and occupy the entire lumen.  
220 Testes expand in size and are fully developed.

221 **Stage V (spent stage):** Spawned spermatozoa; testes are reduced in size with empty  
222 spaces visible in follicles.

### 223 **Statistical analysis**

224 A *chi-square test of independence* was conducted between both month using pooled females  
225 and individual females within each month, and oocyte size frequency distribution to  
226 determine whether there was a statistically significant association between these variables.  
227 For instances where expected cell frequencies were less than five, neighbouring oocyte size  
228 bin ranges were collapsed and the procedure re-run until this assumption was met. Statistical  
229 significance is reported at  $p < 0.05$ . For instances where a statistically significant association  
230 was reported, the strength of association between variables was evaluated using *Cramer's V*

231 (Cohen 1988), with a value of 0 and 1 indicating “no association” and “complete association”,  
232 respectively (i.e. small association = 0.1, moderate association = 0.3 and large association =  
233 0.5). In addition, the deviation of individual cells from independence was reported using  
234 adjusted standardised residuals according to Agresti (2007), where values > 3 were deemed  
235 indicative of cells that significantly deviate from independence.

236 All statistical analyses were performed using IBM SPSS Statistics version 24 for Mac OS X.

## 237 **Results**

### 238 **Gonad morphology**

239 The overall anatomy of *A. eightsii* is presented in Fig. 2. The gonad envelops the centre of  
240 the alimentary canal. A thin layer of gonadal tissue (up to 3-4 cells thick) covers the outer  
241 layer of the digestive gland and envelops the stomach where the gonad tissue thins to  
242 approximately 1 cell thick. A strip of gonadal cells is located within the anterior adductor in  
243 the upper body (Fig. 2b and d; Fig. 3a).

244 While the diameter of the stomach reduces as it penetrates the centre of the foot muscle, the  
245 gonadal tissue layer around the stomach thickens (up to 10-12 cells thick; Fig. 3b). The  
246 adjacent section of hindgut in the foot muscle is also surrounded by gonadal tissue, but fewer  
247 cells are present (around 5-6 cells thick). No gonadal cells are found near the base of the  
248 half gut loop in the foot muscle.

### 249 **Sex ratio**

250 All specimens (n = 95) selected for histological analysis had distinguishable gonads and  
251 were separately sexed. Of the 95 individuals, 48 (50.5%) were female and 47 (49.5%) were  
252 male.

### 253 **Female reproductive analysis**

254 Qualitative assessment of the female gonad revealed the presence of oocytes of all  
255 developing stages throughout the study period. Previtellogenic oocytes (and other smaller  
256 oocytes) were observed developing along the follicle walls next to both vitellogenic and ripe  
257 oocytes throughout all months, and in 39 of the 42 females sampled between January 2013

258 and May 2014 (Fig. 4). The absence of ripe oocytes was observed in the gonad of only three  
259 females, comprising single specimens from July 2013 (shell length = 20.95 mm), October  
260 2013 (shell length = 20.40 mm) and May 2014 (shell length = 24.62 mm). The measured  
261 oocyte diameters ranged from 12.5 to 176.7  $\mu\text{m}$ . A spawning event occurred between April  
262 and May in 2013 and 2014, indicated by a reduction in average oocyte size ( $\pm\text{SD}$ ), from  $97.4$   
263  $\pm 23.4 \mu\text{m}$ ,  $n = 3549$  to  $59.3 \pm 14.3 \mu\text{m}$ ,  $n = 740$ , and from  $93.0 \pm 28.7 \mu\text{m}$ ,  $n = 2164$  to  $54.2 \pm$   
264  $14.5 \mu\text{m}$ ,  $n = 208$ , respectively.

265 Although oocytes of all maturity stages were observed in the gonad throughout the study  
266 period, the distribution of oocyte sizes varied between months (Fig. 5). A *chi-square test of*  
267 *independence* was conducted between month, using pooled females, and oocyte size  
268 frequency distribution. All expected cell frequencies were greater than five. There was a  
269 statistically significant association between month and the frequencies of oocytes distributed  
270 across the range of oocyte size ( $\chi^2 = 8156.288$ ,  $df = 36$ ,  $p < 0.0001$ ). The association was  
271 moderately strong (Cohen, 1988), *Cramer's V* = 0.349, and 46 of 52 cells had adjusted  
272 standardised residuals > 3 (Table 1) indicating a significant deviation from independence.

273 Statistically significant associations between individual females, sampled in the same month,  
274 and their oocyte size frequency distributions were also recorded, where the strength of  
275 associations was generally moderate ( $p < 0.05$ ; see Table 1 in Online Resource 2).

276

### 277 **Male reproductive analysis**

278 Seasonal changes in male maturity stage were interpreted to show a spawning event in  
279 April-May, as indicated by spent testicular acini (stage V) in 2013 and newly developed  
280 spermatogonia (stage I) in 2014 (Fig. 6). Testes matured from an early developmental (stage  
281 I) to mature stage (stage IV) between July 2013 and April 2014, and from a late  
282 developmental (stage III) to mature stage (stage IV) between January and April 2013.

### 283 **Discussion**

284 The present study of the reproductive ecology of the Antarctic shallow-water protobranch  
285 bivalve, *A. eightsii* demonstrates a reproductive mode that differs from previously described  
286 protobranch species and other Antarctic shallow-water invertebrates. Our results indicate  
287 that the Hangar Cove population of *A. eightsii* exhibits continuous oogenesis, with a period of  
288 increased reproductive intensity and spawning between April and May, with asynchrony in  
289 oocyte development among females. Although we acknowledge reduced sample sizes in  
290 some months, this was to ensure all samples were comparable with respect to shell lengths  
291 and therefore sexual maturity, based on previous studies (Peck et al. 2010). Of those  
292 specimens which fit our selection criteria, there was no control over gender at the point of  
293 collection. Within the individual females measured, efforts were made to measure in excess  
294 of 1000 oocytes where possible to reduce the weighting of inherent variability. All specimens  
295 in this study were dioecious with an overall female to male ratio approximating unity, and with  
296 no evidence of hermaphroditism found.

297 Continuous investment in oogenesis was evidenced by the simultaneous, year-round  
298 occurrence of previtellogenic, vitellogenic, and ripe oocytes in the female gonads. This  
299 reproductive trait contrasts with observations of seasonal oogenic development reported in  
300 most shallow-water Antarctic marine invertebrates studied to date (e.g. Chiantore et al. 2001;  
301 Ahn et al. 2003; Tyler et al. 2003; Grange et al. 2004, 2007, 2011; Kang et al. 2009; Reed  
302 2013a), and other closely related protobranch bivalves including the cold water *Yoldia*  
303 *hyperborea* (Jaramillo and Thompson 2008), where cohorts of oocytes distinguishable in  
304 their maturity stage dominate the gonad at different times of year. Continuous oogenesis is  
305 observed however in some deep-sea protobranch bivalves (Tyler et al. 1992; Zardus 2002;  
306 Scheltema and Williams 2009; Reed et al. 2014). Significant associations between individual  
307 females and their oocyte size frequency distribution were also observed indicating  
308 asynchrony in oogenesis between individuals sampled in the same month. This asynchrony  
309 could be attributed to an aseasonal reproductive rhythm. However, we did not observe any  
310 evidence for aseasonality in the form of multiple spawning events in the females (Fig. 6), nor

311 male maturity stage (Fig. 2 in Online Resource 1). Instead, we observed a seasonal  
312 periodicity in the reproduction of both male and female *A. eightsii* (described below). In the  
313 case of the females studied, this seasonal periodicity was observed simultaneously to the  
314 continuous investment in oocyte development described above. We therefore propose that  
315 the asynchrony observed between individual females is consistent with an innate, long-term  
316 multi-year reproductive cycle, related to the partitioning of energetic resources between  
317 somatic and reproductive investment (as hypothesised in Román-González et al. 2017 and  
318 discussed below).

319 Observed simultaneously to the continuous oocyte development described above was a  
320 seasonal increase in reproductive intensity (spawning) during early Austral winter. This was  
321 demonstrated by an investment in the size and presence of ripe oocytes and their  
322 subsequent loss from the gonad, and a statistically significant association between month  
323 and oocyte size frequency distribution. Despite this observation, previous studies have  
324 identified very few bivalve veligers or larvae in winter in shallow waters adjacent to Rothera  
325 Research Station (Bowden et al. 2009). However, the pericalymma larva of protobranchs  
326 differs from the typical veliger larva of most bivalves in identifying features, being barrel-  
327 shaped and cloaked with an outer test enveloping the larva (Zardus and Morse 1998). This  
328 atypical appearance and the well-established difficulties associated with field identification of  
329 larvae, render this larval type susceptible to misidentification, underestimation and/ or being  
330 missed entirely in plankton samples. Winter spawning events have been previously reported  
331 for other Antarctic shallow benthic invertebrates, including the seastar *Odontaster validus*  
332 (Pearse 1965), the ascidian *Cnemidocarpa verrucosa* (Sahade et al. 2004), and the  
333 octocoral *Malacobelemnion daytoni* (Servetto and Sahade 2016), and confirmed in some  
334 cases by the presence of larval stages in the water column (e.g. for *O. validus*; Bowden et al.  
335 2009). These events have been attributed to flexible feeding, energy storage, and/or  
336 extended gametogenic periods. In the case of *A. eightsii*, based on observations in this study,  
337 there is no evidence of energy storage or an extended gametogenic period, however flexible  
338 feeding in the form of both suspension and deposit feeding has been reported (Davenport

339 1988), and we speculate that the peculiar reproductive ecology of the bivalve is underpinned  
340 by a long-term internal cycle related to energy allocation.

341 Feeding plasticity may overcome the food limitation that characterises shallow Antarctic  
342 waters and poses resource limits for many species during winter, and may also facilitate the  
343 continuous oogenesis observed in *A.eightsii*. *Aequiyoldia eightsii* exploits phytoplankton in  
344 the summer by suspension feeding, and deposit feeds on organic material in the sediment,  
345 mostly composed of microphytobenthos and macroalgal fragments all year round (Davenport  
346 1988; Corbisier et al. 2004, Pasotti et al. 2015b). Fragments of phytoplankton/algae were  
347 observed in the stomach and hindgut of all specimens in the current study, supporting  
348 continuous feeding throughout the year. These findings are consistent with previous  
349 laboratory experiments where specimens aged younger than 24 years fed primarily on  
350 phytoplankton < 20 µm in diameter (Chris and Priddle 1995). As the *A. eightsii* specimens  
351 analysed in the current study had shell lengths between 20 – 25 mm, representative of 10 –  
352 33 years of age (Peck and Bullough 1993), it is likely that the phytobenthos is the primary  
353 food source. Nanoplankton concentrations in the waters around Rothera Research Station  
354 peak in the late summer (~March), and are suggested to have an important influence on the  
355 ecology of benthic grazers in the area (Clarke et al. 2008). The settlement and subsequent  
356 deposit feeding on nanoplankton in sediment therefore could trigger a reproductive response  
357 in *A. eightsii*, such as spawning in the Hangar Cove population, which could explain the  
358 increased reproductive intensity and spawning detected in April and May.

359 While feeding plasticity enables *A. eightsii* to sustain continuous oogenesis, and the  
360 seasonal availability of food, i.e. nanoplankton production, acts as a selective pressure  
361 driving a superimposed seasonal reproduction, recent evidence of a multi-year cycle in shell  
362 growth of the protobranch bivalve (Román-González et al. 2017) suggests the reproductive  
363 asynchrony observed in females is not underpinned by environmental factors. Nor can this  
364 reproductive pattern be explained by the accepted paradigm of an 18-24 month period  
365 needed for oogenesis in several other Antarctic invertebrates (Peck et al. 2005; for examples  
366 see Grange et al. 2004, 2007; Brockington et al. 2007; Orejas et al. 2007). The reproductive

367 ecology of *A. eightsii* also differs from other shallow-water and deep-sea nuculanid  
368 protobranch bivalves that have a 12-month gametogenic cycle, often with seasonal  
369 investment in reproduction (Tyler et al. 1992; Nakaoka 1994; Jaramillo and Thompson 2008;  
370 Scheltema and Williams 2009). Instead the asynchrony observed here may be linked to an  
371 innate, endogenous growth rhythm (e.g 9.06 year) that is asynchronous between individuals,  
372 not keyed with environmental variability, and attributable to cycles in reproductive output  
373 (Román-González et al. 2017).

374 The maximum egg size of *A. eightsii* measured in this study was 176.7 µm. In marine  
375 invertebrates, larval development mode is often inferred from egg size (Moran and McAlister  
376 2009). However, in Protobranchia, egg sizes can vary extensively within individual modes of  
377 development and even within closely related species (Gustafson and Reid 1986; Scheltema  
378 and Williams 2009). This subclass is known to only exhibit two modes of larval development  
379 to date; pelagic lecithotrophy and brooding (Zardus 2002; Scheltema and Williams 2009).  
380 Since gametogenesis of *A. eightsii* shows synchrony in seasonality between males and  
381 females, the loss of a relatively large proportion of gametes between April and May, and the  
382 absence of brooded embryos suggests this bivalve is a broadcast spawner with  
383 lecithotrophic larvae. Winter spawning of lecithotrophic larvae may facilitate the avoidance of  
384 predators feeding on larvae associated with the summer bloom (Sahade et al. 2004), ice-  
385 mediated disturbance (Barnes and Souster 2011), and a greater availability of free  
386 substratum for larval settlement (Bowden 2005). As adult *A. eightsii* can inhibit larval  
387 settlement via density-dependent control (Peck and Bullough 1993), larvae spawned during  
388 periods with more available habitat could increase the chance of settlement.

389 The reproductive ecology of *A. eightsii* exhibits continuous oogenesis, with a period of  
390 increased reproductive intensity and spawning between April and May, and asynchrony  
391 between females. These characteristics contrast with the short-term, seasonal reproductive  
392 traits reported for many shallow-water Antarctic invertebrates and closely related protobranch  
393 bivalves. We hypothesise that the asynchrony documented between females is underpinned



394 by a multi-year periodicity attributed to an innate, endogenous rhythm related to energetic  
395 allocation between somatic and reproductive investment. Whereas the ability to switch  
396 between suspension and deposit feeding modes, according to the quality and quantity of  
397 food available, facilitates a continuous investment into oogenesis, whilst being responsive to  
398 the seasonal pulse in primary production. This study provides key information on a  
399 scientifically important species of bivalve that has been commonly used for baseline  
400 ecological investigations in Antarctica. Knowledge of its reproduction provides insight into the  
401 biological flexibility of the species and contributes to our wider understanding of adaptations  
402 in polar marine environments.

### 403 **Compliance with Ethical Standards**

404 The authors declare they have no conflict of interest. All applicable institutional and/or  
405 national guidelines for the care and use of animals were followed and the necessary permits  
406 for collection of Antarctic animals obtained.

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631 **Figures**

632 **Fig. 1** Location of sampling site for *Aequiyoldia eightsii* in Hangar Cove, Adelaide Island,  
633 West Antarctic Peninsula (67° 33' 52 S, 68° 07' 43 W).

634 **Fig. 2** Anatomy of a female *Aequiyoldia eightsii* collected in April 2013 from Hangar Cove,  
635 Adelaide Island, West Antarctic Peninsula (67° 33' 52 S, 68° 07' 43 W). (a) Photograph of  
636 soft tissue after shell removal (left side); (b) General anatomical structure (left side). Gonad  
637 envelopes *dg* digestive gland shown in orange; (c) Photograph of soft tissue after shell  
638 removal (right side). (d) Configuration of the alimentary canal and associated gonad (right  
639 side). *a* anus, *aa* anterior adductor, *dg* digestive gland, *es* exhalant siphon, *f* foot, *ct*  
640 ctenidium, *h* hinge, *hg* hindgut, *is* inhalant siphon, *o* oesophagus (attaches to *pl*), *pa* posterior  
641 adductor, *pl* palp lamella, *pp* palp proboscis, *r* rectum, *st* stomach. Scale bar = 1 cm.

642 **Fig. 3** Transverse histological sections of a female *Aequiyoldia eightsii* collected in April 2013  
643 at Hangar Cove, Adelaide Island, West Antarctic Peninsula (67° 33' 52 S, 68° 07' 43 W). (a)  
644 Horizontal section through the median level of the visceral mass. (b) Horizontal section  
645 through the top level of foot muscle. *fm* foot muscle, *o* oocytes. Scale bar = 500 µm. For  
646 other abbreviations see Fig. 2.

647 **Fig. 4** Oocyte ripening in *Aequiyoldia eightsii* from Hangar Cove, Adelaide Island, West  
648 Antarctic Peninsula (67° 33' 52 S, 68° 07' 43 W) between January 2013 and May 2014. *DG*  
649 digestive gland, *HG* hindgut, *MO* ripe oocytes, *OG* oogonia, *PVO* previtellogenic oocytes, *VO*  
650 vitellogenic oocytes. Scale bar = 200 µm.

651 **Fig. 5** Average monthly oocyte size-frequency distributions measured as Equivalent Circular  
652 Diameter (µm) of *Aequiyoldia eightsii* from Hangar Cove, Adelaide Island, West Antarctic  
653 Peninsula (67° 33' 52 S, 68° 07' 43 W) between January 2013 and May 2014. Box-whisker  
654 plots for each month; diamond = mean, line = median, box = 25<sup>th</sup> to 75<sup>th</sup> percentile range,  
655 and whiskers = 1.5 times the value of the interquartile range. Oocyte diameter = equivalent  
656 circular diameter. *N* = number of females, *n* = number of oocytes measured. Error bars = ±  
657 1SE.

658 **Fig. 6** Testes maturation in *Aequiyoldia eightsii* from Hangar Cove, Adelaide Island, West  
659 Antarctic Peninsula (67° 33' 52 S, 68° 07' 43 W) between January 2013 and May 2014. *DG*  
660 digestive gland, *HG* hindgut, *LM* lumen, *RS* residual sperm, *SC* spermatocytes, *SG*  
661 spermatogonia, *SS* spent acini, *SZ* spermatozoa. Scale bar = 200 µm.

68°09 W

68°08 W

68°07 W

Adelaide  
Island

Hangar  
Cove

Rothera Point

*Aequiyoldia  
eightsii*



67°33'40 S

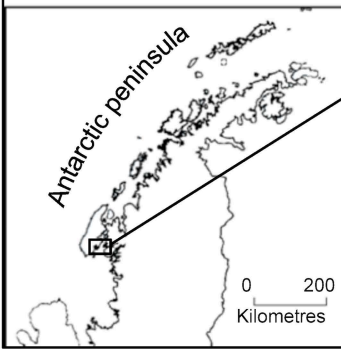
67°34'00 S

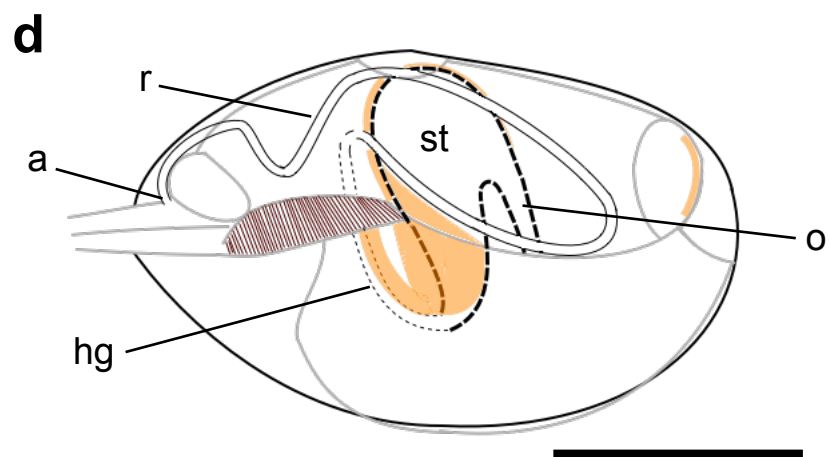
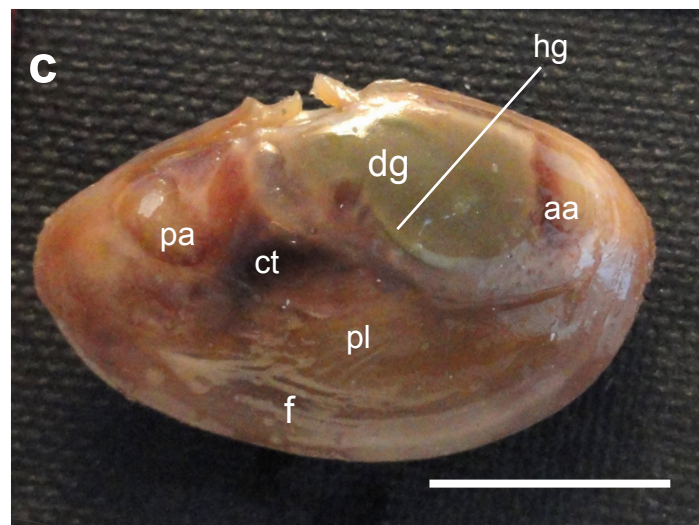
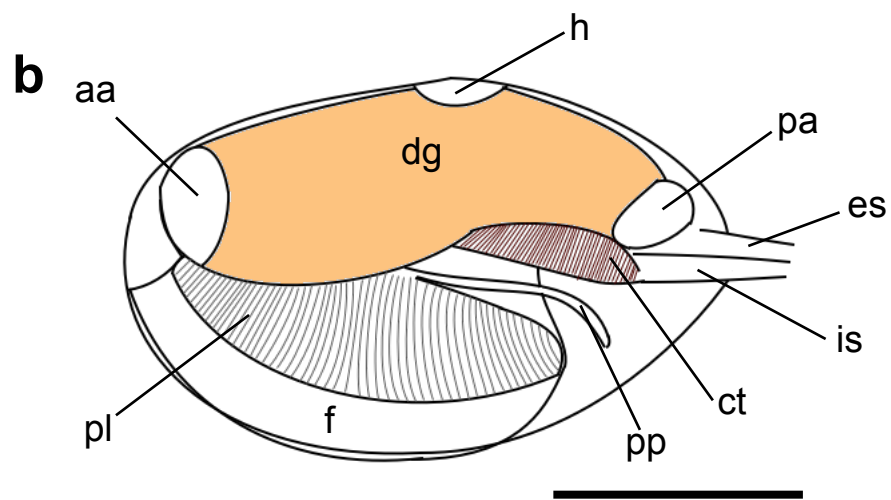
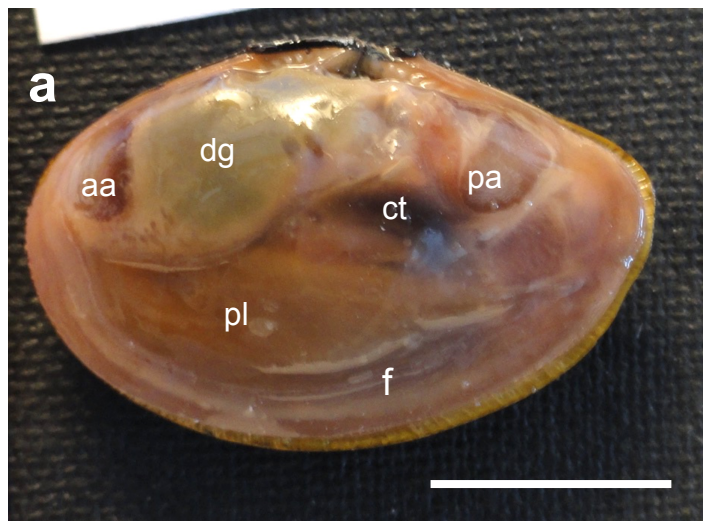
67°34'20 S

Antarctic peninsula

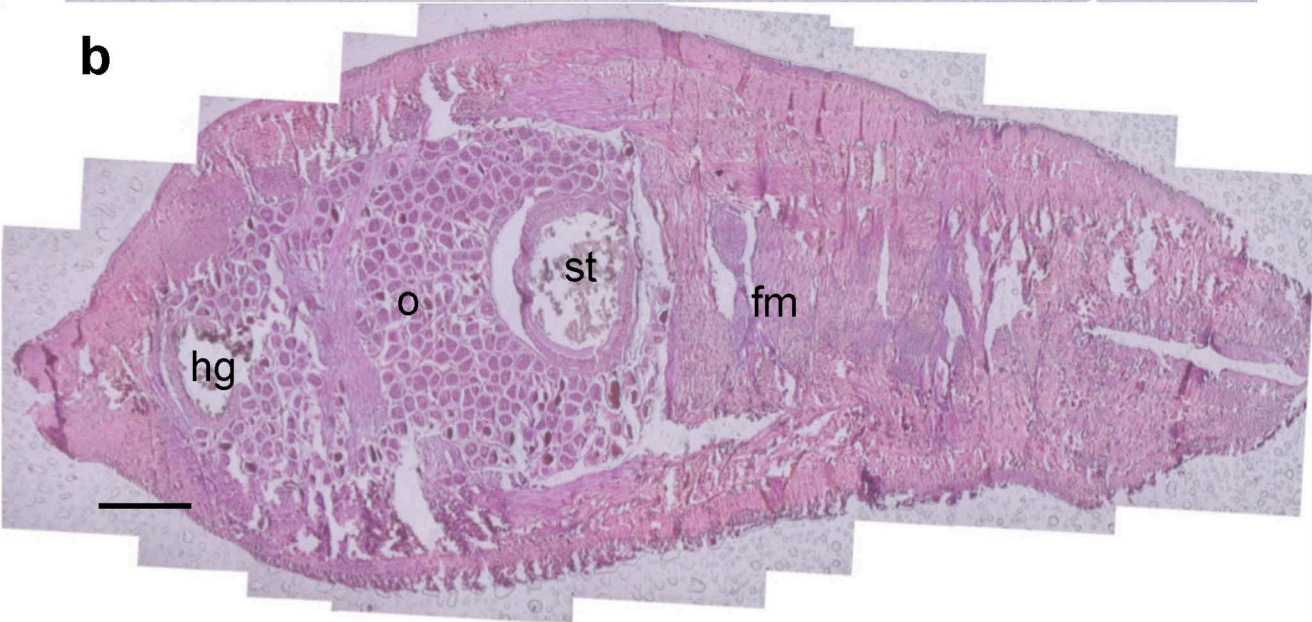
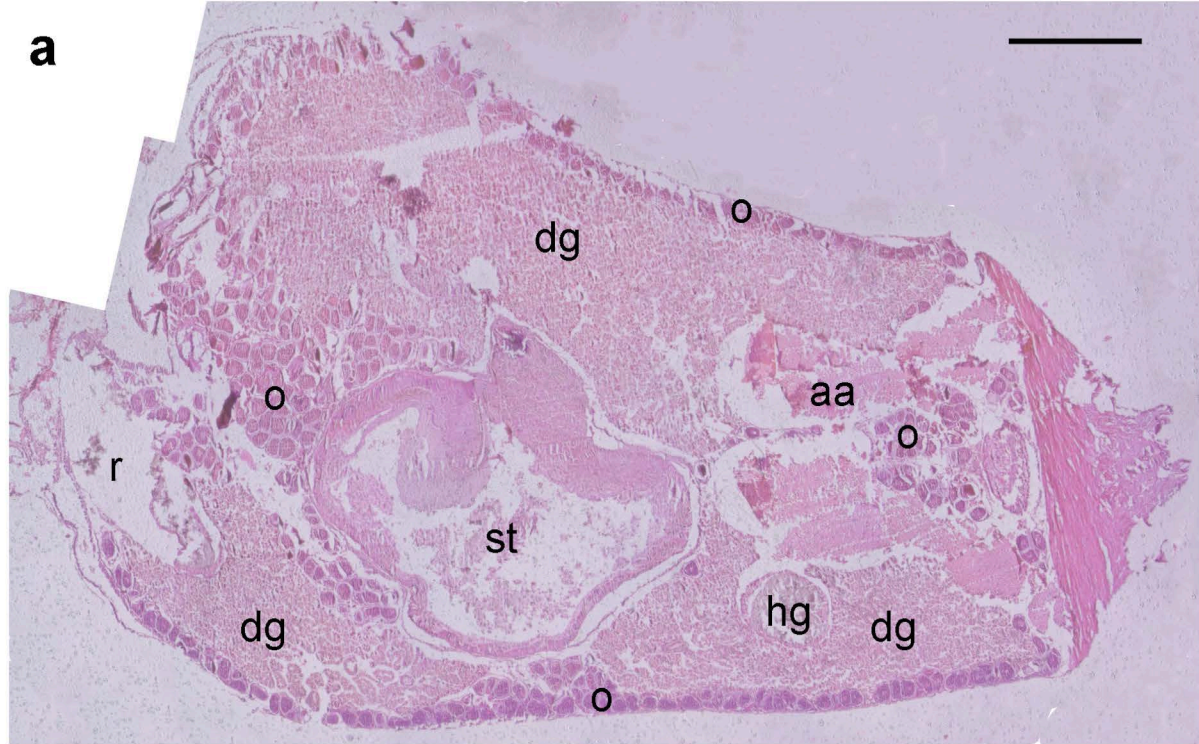
0 200  
Kilometres

0 300  
Metres

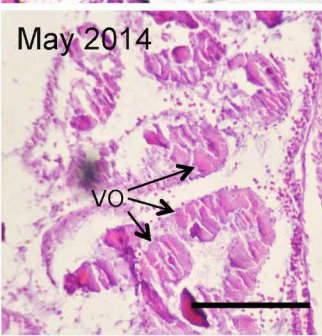
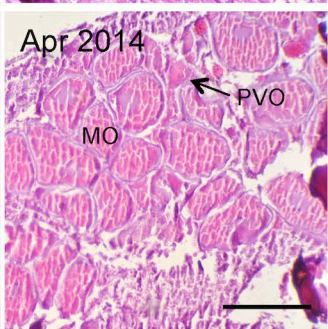
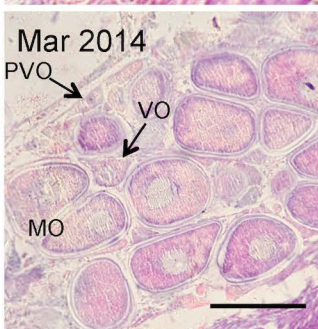
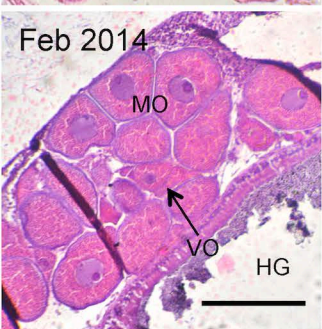
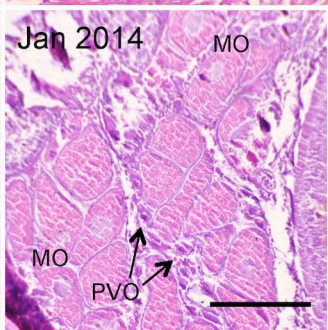
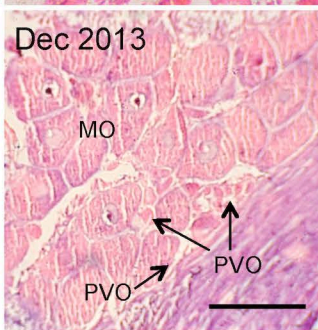
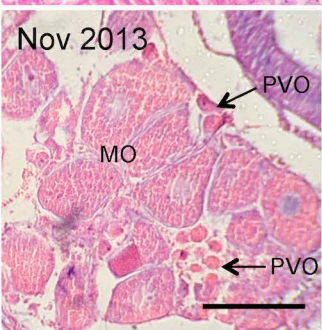
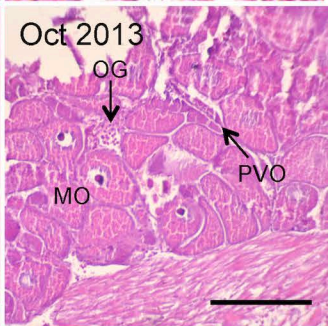
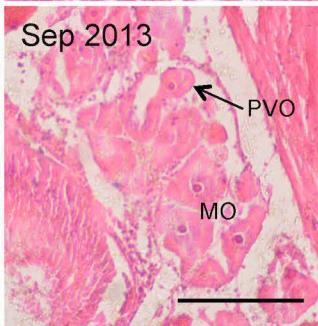
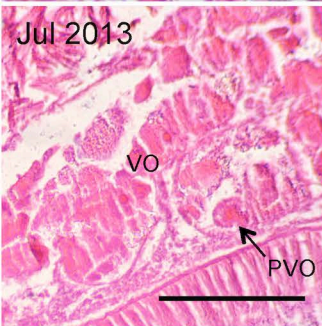
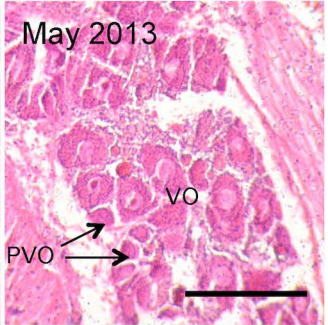
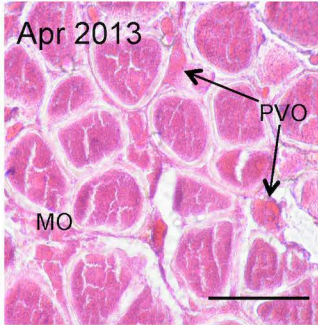






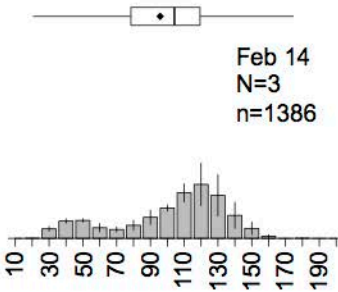
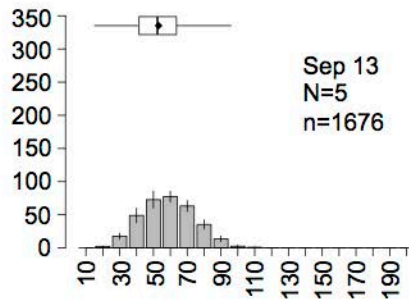
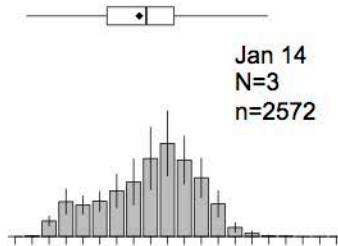
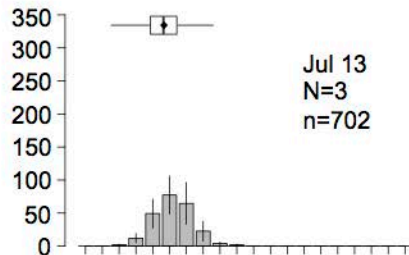
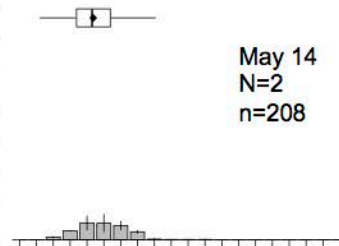
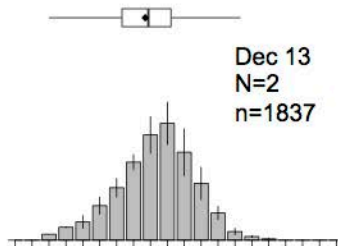
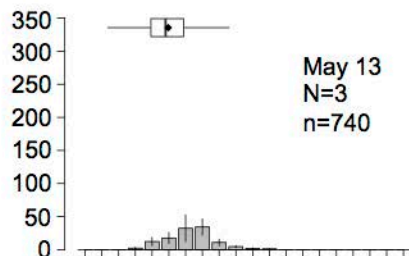
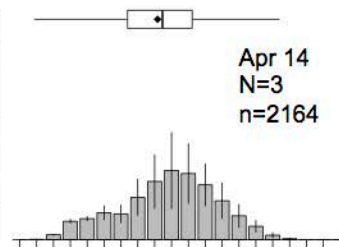
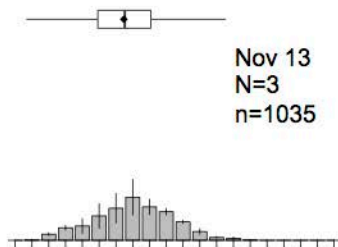
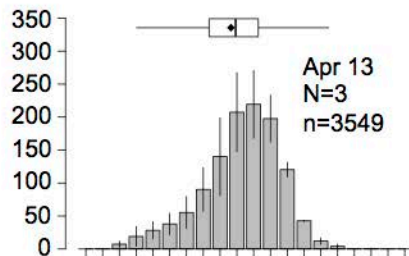
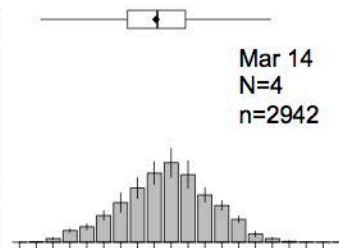
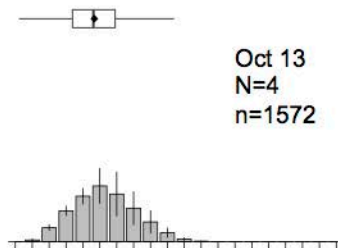
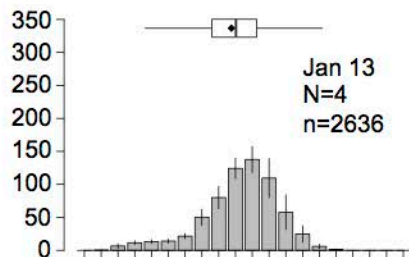








Count frequency



Oocyte size ( $\mu\text{m}$ )

