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***Ampelisca eschrichtii* Krøyer, 1842 (*Ampeliscidae*) of the Sakhalin Shelf in the Okhotsk Sea starve in summer and feast in winter**

Valentina B. Durkina ¹ , John W. Chapman ^{Corresp., 2} , Natalia L. Demchenko ³

¹ Laboratory of Physiology, National Scientific Center of Marine Biology FEB RAS, 690041, ul. Palchevskogo 17, Vladivostok, Russia

² Department of Fisheries and Wildlife, Oregon State University, Newport, Oregon 97365, United States of America

³ Laboratory of Marine Ecosystem Dynamics, National Scientific Center of Marine Biology FEB RAS, 690041, ul. Palchevskogo 17, Vladivostok, Russia

Corresponding Author: John W. Chapman

Email address: john.chapman@oregonstate.edu

Ampelisca eschrichtii Krøyer, 1842 of the Sakhalin Shelf of the Okhotsk Sea, Far Eastern Russia, comprise the highest known biomass concentration of any amphipod population in the world and are a critically important prey source for western gray whales. The high prevalence of atrophied ovaries, undersized and damaged oocytes, undersized broods of embryos and the absence of terminal phase males or females brooding fully formed juveniles among these populations in late spring and early fall are consistent with trophic stress and starvation. *A. eschrichtii* therefore appear to starve in summer and grow and reproduce in late fall and winter. In summer, these populations, occur below water strata containing the bulk of phytoplankton biomass and appear more likely to receive their trophic sources with vertical mixing that occurs in winter.

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5 **Valentina B. Durkina¹, John W. Chapman², and Natalia L. Demchenko³**

6 ¹**Laboratory of Physiology, National Scientific Center of Marine Biology FEB RAS,**
7 **690041, ul. Palchevskogo 17, Vladivostok, Russia**

8 ²**Department of Fisheries and Wildlife, Hatfield Marine Science Center, Oregon State**
9 **University, 2030 SE Marine Science Dr., Newport, Oregon, United States of America**

10 ³**Laboratory of Marine Ecosystem Dynamics, National Scientific Center of Marine**
11 **Biology FEB RAS, Vladivostok, Russia**

12 Corresponding author:

13 Valentina B. Durkina¹

14 Email address: vdurkina@mail.ru

15 **ABSTRACT**

16 *Ampelisca eschrichtii* Krøyer, 1842 of the Sakhalin Shelf of the Okhotsk Sea, Far Eastern Russia,
17 comprise the highest known biomass concentration of any amphipod population in the world and are
18 a critically important prey source for western gray whales. The high prevalence of atrophied ovaries,
19 undersized and damaged oocytes, undersized broods of embryos and the absence of terminal phase
20 males or females brooding fully formed juveniles among these populations in late spring and early fall
21 are consistent with trophic stress and starvation. *A. eschrichtii* therefore appear to starve in summer
22 and grow and reproduce in late fall and winter. In summer, these populations, occur below water strata
23 containing the bulk of phytoplankton biomass and appear more likely to receive their trophic sources with
24 vertical mixing that occurs in winter.

25 **INTRODUCTION**

26 The densest known gammaridean amphipod populations in the world occur in the “Offshore” feeding
27 grounds of the critically endangered western gray whale, *Eschrichtius robustus* (Lilljeborg, 1861) (IUCN,
28 2008), at 40–60 m depths and approximately 52.0°N and 143.7°E on the northeastern Sakhalin Island
29 Shelf (Demchenko et al., 2016). These amphipods consist primarily of *Ampelisca eschrichtii* Krøyer,
30 1842. The production and growth of their populations are of international concern for both gray whale
31 conservation and for understanding high latitude benthic ecosystem dynamics. However, estimates of their
32 productivity have remained complicated due to irregular sampling over time within years and due to the
33 absence of any sampling between late fall and early spring (winter from here on). Demchenko et al. 2016
34 partially solved this problem by integrating comparisons of *A. eschrichtii* size density modes and female
35 brood development stages between late spring and early fall (summer from here on) among six sampling
36 years between 2002 and 2013. They discovered that Sakhalin Shelf *A. eschrichtii* are gonochoristic,
37 iteroparous, mature at body lengths greater than 16 mm, have a predominantly two-year life span and a
38 low incidence of individuals surviving to 3 years.

39 Demchenko et al. (2016) noted also that brooding females in their summer samples were rare, that
40 brooding females with 0-age juveniles ready for release were absent, that terminal phase reproductive
41 males were absent and that length density modes of these populations did not increase over time. The
42 preliminary histological analyses of Demchenko et al. (2016) also revealed vitellogenic oocytes that
43 appeared to be undergoing lysis and resorption. Oocyte lysis and resorption is a condition that has been
44 associated with “spent” or starving fish, decapods and amphipods (Sheader, 1983; Santos et al., 2005,

45 2009). Demchenko et al. (2016) concluded from these signs of starvation that *A. eschrichtii* are food
46 limited in summer and, by default, that growth and reproduction of these populations does not occur in
47 summer and must therefore occur in winter.

48 Demchenko et al.'s 2016 samples nevertheless, contained juveniles as small as 3.8 mm in length that
49 might not be expected to occur during non-reproductive periods. Moreover, their histological sample,
50 which included only 8 reproductive size females from October of 2013, was small and limited to a single
51 period in time. Demchenko et al.'s 2016 proposals of winter growth and production are also counter
52 to previous reports of summer growth and production in North Pacific ampeliscid populations (Coyle
53 et al., 2007). Winter samples, that would allow direct tests of Demchenko et al.'s 2016 winter growth
54 proposal, have not been possible due to the remote location of the Offshore area that is covered by ice
55 and frequented by severe weather in winter (Fadeev, 2012). We therefore test Demchenko et al.'s 2016
56 winter production hypothesis herein by expanded histological examinations of male and female gonads
57 and oocytes, embryo and brood development in summer. We also compare these life history characters of
58 *A. eschrichtii* with other amphipod populations in the world.

59 The conditions of gonads, oocytes and sperm are readily apparent in histological sections (Hastings,
60 1981; Sainte-Marie, 1991; Johnson et al., 2001; Demchenko et al., 2016). Mature males produce fully
61 formed spermatophores that are stored in the *vas deferens* and develop terminal phase morphologies
62 adapted for pelagic mating (Hastings, 1981; Johnson et al., 2001). Females produce oogonia from mitotic
63 division of primary oogonia. Oogonia develop into vitellogenic oocytes through stage of the previtellogenic
64 oocytes (Charniaux-Cotton, 1985). Females deposit batches of mature oocytes into the marsupium through
65 the oviducts in pereonite 5, immediately after molting, while the new exoskeleton is still flexible enough
66 to allow their passage (Hyne, 2011). The oocytes are fertilized in the marsupium from spermatophores
67 which mating males deposit at the same time as the arriving oocytes (Johnson et al., 2001).

68 Lipids are critical for energy storage, for construction of cell organelles and for egg production of
69 aquatic organisms (Parrish, 2013) and thus provide useful measures energetic exchanges. Crustaceans
70 can survive extended periods of low food abundance on trophic reserves, including lipids in particular
71 (Lawrence, 1976). Moreover, *Ampelisca macrocephala* Liljeborg, 1852, a similar species to *A. eschrichtii*,
72 can survive in aquaria for 5 months without food (Kannevorff, 1965). Lipids that are concentrated in
73 vitellogenic oocytes of reproductive amphipods can be resorbed (Charniaux-Cotton, 1985). Growth or
74 atrophy of vitellogenic oocytes and losses of embryos are therefore useful predictors of reproductive
75 competence (Sheader, 1996). The numbers of embryos and the sizes and condition of reproductive cells
76 also provide directly visible indices of amphipod energetics due to the large stores of lipids required for
77 their production.

78 The lecithotrophic amphipod embryos develop, hatch and emerge from the female marsupium fully
79 formed. The externally brooded embryos can not receive additional nourishment from the parent and thus
80 cannot increase in biomass after deposition. The mature oocyte biomass therefore must equal or exceed
81 the biomass required to produce a viable embryo (Charniaux-Cotton, 1985). The immediate reproductive
82 competence of females therefore can also be determined from oocyte size relative to the viable sizes of
83 embryos. The lack of specialized larval dispersal stages permit direct sampling of all life history stages
84 from benthic samples.

85 Van Dolah and Bird (1980), Nelson (1980), Sainte-Marie (1991) and Johnson et al. (2001) summarized
86 over 200 amphipod species life histories from around the world. Their summaries of brood size and embryo
87 dimensions relative to female length revealed common patterns of variation in embryo diameter and brood
88 size among amphipod species. Their life history summaries permit independent comparisons with *A.*
89 *eschrichtii* life history characteristics relative to most amphipods. Water uptake with the conversion of
90 yolk reserves into structural elements can increase amphipod embryo dimensions as they mature (Sheader,
91 1996). However, early stage amphipod embryo diameters vary closely with amphipod body lengths
92 (Nelson, 1980). We therefore compared the reproductive morphologies of *A. eschrichtii*, gonads, oocytes,
93 early stages embryo development and brood sizes with other amphipod species and populations in the
94 world additionally to test Demchenko et al.'s 2016 default conclusion of winter growth and production.

95 METHODS

96 Reproductive competence of *A. eschrichtii* females is a function of oocyte and embryo development
97 and brood size. *A. eschrichtii* male reproductive competence can be assessed to a lesser extent from
98 development terminal phase swimming morphologies adapted for pelagic mating characteristic of the

99 genus (Borowsky and Aitken-Ander, 1991) and mature sperm. We predicted 4 life history characteristics
100 of *A. eschrichtii* that we used to test whether they reproduce and grow in summer:

- 101 1. presence of all brood development stages, and embryo development stages occurring over the
102 summer months;
- 103 2. reproductive effort equal to similar sized amphipods;
- 104 3. sufficiently large oocytes to produce viable embryos and;
- 105 4. males fully developed for reproduction.

106 We assumed that each supported prediction is evidence that *A. eschrichtii* growth and reproduction occurs
107 primarily in summer. We assumed that evidence counter to each prediction is evidence of winter growth
108 and reproduction.

109 Life history

110 Our classification of brood development follows Tzvetkova's 1975 criteria:

- 111 *FO*—rudimentary oostegites lacking egg retention setae;
- 112 *FI*—brooding uncleaved embryos and oostegites with fully developed embryo retention setae;
- 113 *FII*—brooding cleaved embryos;
- 114 *FIII*—brooding fully formed juveniles;
- 115 *FIV*—developed oostegites with embryo retention setae and an empty brood pouch.

116
117 Amphipod embryo sizes, brood numbers and brood biomass increase with amphipod size (Sainte-
118 Marie, 1991). We estimated reproductive effort from the number of embryos times their average weight,
119 relative to amphipod size. We estimated *A. eschrichtii* size from their lengths measured from the anterior
120 end of the head to the base of the telson. We estimated embryo diameter from the average of length and
121 width (Sainte-Marie, 1991).

122 Summer embryo viability is relative to the sizes of winter embryos. The absence of *FI* and *FIII*
123 females in our selected sample herein and Demchenko et al.'s 2016 samples could result if these brood
124 development stages are of brief duration relative to the other brood development stages in summer. We
125 therefore compared the sizes and conditions of vitellogenic oocytes, which must grow rapidly to replace
126 broods of embryos that mature and are released when trophic stress is low.

127 We assume large oocytes occur during periods of high reproductive competence and short embryo
128 replacement times and that the observed summer juveniles were progeny of the observed *FIV* females
129 in our samples. We therefore compared *A. eschrichtii* embryo weight relative to 0-age juvenile weight
130 to determine whether summer embryos are large enough to produce the smallest observed summer
131 juveniles. We also compared the sizes of summer *A. eschrichtii* embryos relative to embryos of similar
132 sized amphipods to determine whether they are likely to produce normal size juveniles.

133 The close similarities among general amphipod demographics and life histories (Van Dolah and Bird,
134 1980; Nelson, 1980; Sainte-Marie, 1991; Johnson et al., 2001) permit estimates of oocyte, embryo
135 and brood sizes among reproductive *A. eschrichtii* populations independent of our restricted summer
136 observations. Brood and embryo sizes of other *Ampelisca* species are within the range of other similar
137 sized amphipod species (Sainte-Marie, 1991) and thus, the life history of *A. eschrichtii* is likely to be
138 similar to the life histories of other amphipod species.

Our first estimate of minimum viable embryo size is based on *A. eschrichtii* embryo biomass relative to
the smallest 0-age juveniles observed in summer. This estimate requires that early stage, undifferentiated,
embryos are of similar specific gravities [approximately 1.146 g ml⁻¹ for crustaceans (Spaargaren, 1979)].
The volume per weight of a peracaridean crustacean is approximately 1/1.146= 0.8726. An *Ampelisca*
oocyte diameter required to produce an embryo diameter (*D*) of sufficient weight (g) for a minimum
length (*L*) (zero age) *A. eschrichtii* juvenile can therefore be estimated from length-weight relationships.
Demchenko et al.'s 2016 summary of *A. eschrichtii* weight per length provided our estimate the zero age
weight where: $g = 1.49E-5 * L^{3.0605}$. The weight of a zero-age juvenile thus converts to the volume (*V*) of
a spherical oocyte by the relation:

$$V = 0.8726g = \frac{4}{3}\pi\left(\frac{D}{2}\right)^3. \quad (1)$$

139 and therefore by substitution, the oocyte or embryo diameter (D) (required for a zero-age *A. eschrichtii*)
140 can be estimated by the relation:

$$D = 2\sqrt[3]{\frac{0.6545g}{\pi}}. \quad (2)$$

141 Our second estimate of embryo viability rests on whether *A. eschrichtii* embryos are similar in size to
142 embryos of other similar sized amphipod species (Van Dolah and Bird, 1980; Nelson, 1980; Sainte-Marie,
143 1991). These two estimates of viable embryo size were necessary to assess *A. eschrichtii* reproductive
144 effort (embryo size times embryo number) in summer relative to an expected reproductive effort of other
145 similar size amphipods (prediction 2).

146 Histology

147 We examined *A. eschrichtii* oocytes (prediction 3) and sperm (prediction 4) viability and condition by
148 histological methods. Forty reproductive sized females and 14 reproductive sized males were selected
149 from Offshore area samples collected in October 2013, July 2015 and October 2015 for these analyses to
150 permit comparisons of reproductive condition over time. These data nevertheless remain insufficient for
151 comparisons of overall population structures and population dynamics summarized in Demchenko et al.
152 (2016).

153 Females and males from each collection date were separated into six length groups, spanning approxi-
154 mately 3 mm each, and prepared together in batches. The specimens were soaked in fresh water for 24 h,
155 dehydrated, cleared in xylene and then infiltrated with melted paraffin. The paraffin was cooled into blocks
156 that were cut into 10 μm thick sections for mounting on microscope slides. Sections containing gonad
157 tissue were stained using hematoxylin and eosin and permanently mounted on glass slides. The histology
158 slides and whole dissected specimens for these analyses are deposited in the museum collections of the
159 National Scientific Center of Marine Biology FEB RAS. We photographed the prepared slides to illustrate
160 cell and tissue conditions (Figs. 1-3, 5). We measured reproductive cell and gonad dimensions from the
161 photographs using Videotest (<http://www.videotest.ru>). We include a key to cell anatomy abbreviations in
162 the supplemental materials (Table S1). Oogonia, oocytes and embryos were assumed to be elliptical for
163 estimates of their volumes or diameters.

164 We assessed oocyte viability from their development and structure and by comparing their sizes
165 to our assessed viable size of embryo size. We classified ovaries with normal vitellogenic oocytes as
166 “undamaged” (normal), “partial” lysed (partial lysis) in which the lysed and normal vitellogenic oocytes
167 co-occurred in the same ovary and “total” lysed in which all vitellogenic oocytes were damaged.

168 RESULTS

169 Females

170 We included only 16 mm and greater length females (Table 1) in our analyses, which produce vitellogenic
171 oocytes.

Table 1. Reproductive development stage frequencies among females bearing vitellogenic oocytes by collection dates and length group.

	October 2013			July 2015			October 2015		
Lengths	F0	FII	FIV	F0	FII	FIV	F0	FII	FIV
16-18	2	0	0	0	0	0	0	0	0
19-21	3	0	0	0	0	0	1	0	0
22-24	4	4	0	2	2	1	3	2	3
25-27	1	4	0	0	2	1	0	0	2
31-33	1	0	2	0	0	0	0	0	0
Totals	11	8	2	2	4	2	4	2	5

172 Vitellogenic oocytes in 16-18 mm and greater length females (Fig. 1A) grow within a single-layer of
173 secondary follicular epithelium. We found normal (Figs. 1A, 1C) and lysed vitellogenic oocytes (Fig. 1B).

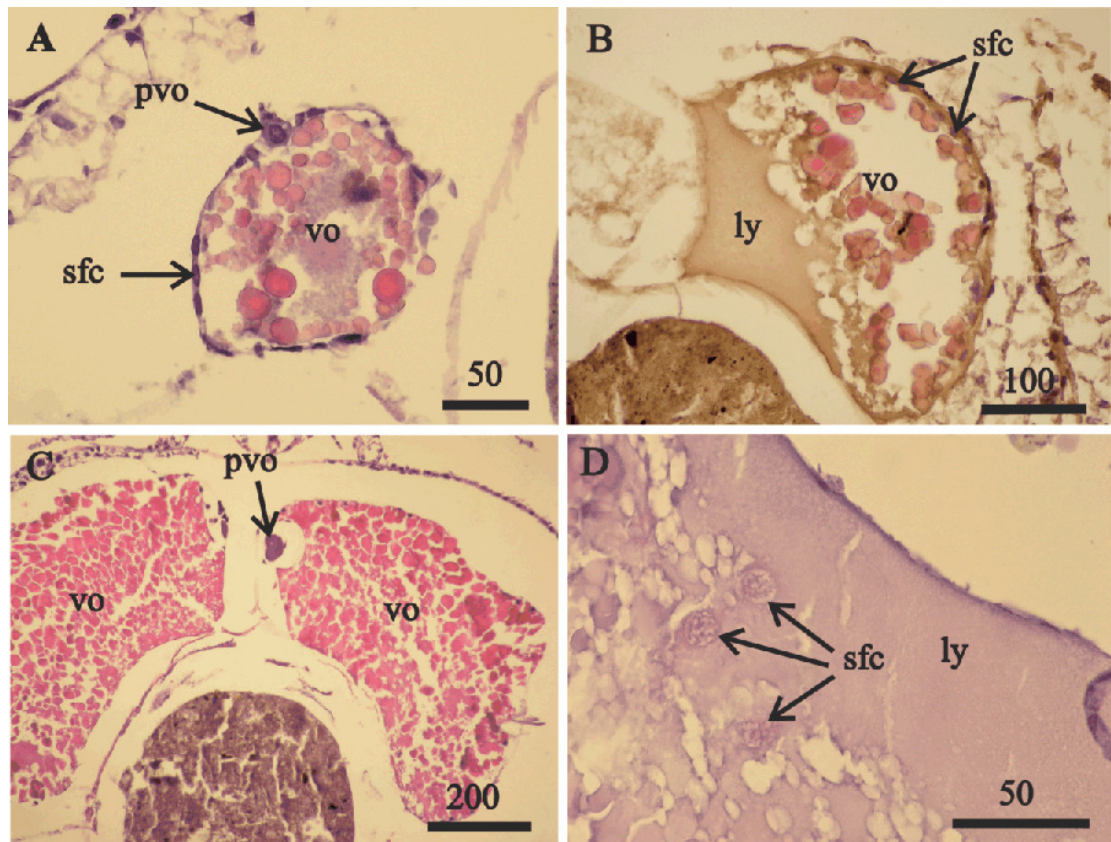


Figure 1. Stage *F0* *A. eschrichtii* ovaries. (A) – a 17 mm female with previtellogenic oocytes (pvo), vitellogenic oocytes (vo) and secondary follicle cells (sfc); (B) – a 21 mm female with undergoing lysis (ly) of vitellogenic oocytes; (C) – a 24 mm female with mature vitellogenic oocytes (vo) and, (D) – secondary follicular cells among lysed yolk of a vitellogenic oocyte. All scales are in μm .

174 Oocyte lysis was accompanied by increases in the diameters of the nuclei of the surrounding follicular
 175 cells from 0.010 to 0.016 mm (Fig. 1D). The frequencies of reproductive stages *F0*, *FII* and *FIV* did not
 176 vary with collection date or body length (Table 1). The ovaries with normal vitellogenic oocytes, with
 177 partial lysis or with complete lysis of vitellogenic oocytes also did not vary with collection date (Table 2)
 178 or with body length or reproductive development (Table 3).

Table 2. Frequencies of *A. eschrichtii* containing normally developing, partially lysed, lysed, with atrophied or regenerated ovaries by date.

Date	Normal	Partial lysis	Lysis	Atrophy	Regenerated
October 2013	4	4	9	2	2
July 2015	1	4	3	0	0
October 2015	3	0	8	0	0
Totals	8	8	20	2	2

179 Normal vitellogenic oocytes (Fig. 2A) were prevalent in the anterior ovary sections and disintegrating
 180 vitellogenic oocytes (Fig. 2B) were increasingly prevalent in posterior ovary sections of *FII* females
 181 brooding early stage embryos (blastula). All vitellogenic oocytes of *FII* females brooding segmented
 182 embryos were undergoing lysis and resorption (Figs. 2C, 2D). Oocyte resorption was accompanied by
 183 mass mortalities of follicular epithelium cells. Chromatin did not effectively stain the nuclei of these
 184 epithelial cells, the cells swelled and then destroyed (Fig. 2E).

185 After apparent resorption, only expanded tubes of fibrous connective tissue and remnants of previtel-
 186 logenic and vitellogenic oocytes remained in two *FII* females (Fig. 2F) (one 24 and one 26 mm length).

Table 3. Frequencies of *A. eschrichtii* female length classes and reproductive stages containing normally developing, partially lysed, lysed, with atrophied, or regenerated ovaries by date.

Stage	Size group, mm	Normal	Partial Lysis	Total lysis	Atrophied	Regenerated
<i>F0</i>	16-18	2	0	0	0	0
	19-21	0	0	4	0	0
	22-24	3	3	3	0	0
	25-27	0	1	0	0	0
	31-33	1	0	0	0	0
<i>FII</i>	16-18	0	0	0	0	0
	19-21	0	0	0	0	0
	22-24	2	2	3	1	0
	25-27	0	2	3	1	0
	31-33	0	0	0	0	0
<i>FIV</i>	16-18	0	0	0	0	0
	19-21	0	0	0	0	0
	22-24	0	0	4	0	0
	25-27	0	0	3	0	0
	31-33	0	0	0	0	2
	Totals	8	8	20	2	2

187 We classified their ovaries as atrophied (Tables 2, 3).

188 The amphipod ovary wall is composed of fibrous connective tissue (basal membrane). The anterior
 189 ovary sections of the first 32 mm *FIV* female in our sample were reduced to empty tubes composed of
 190 the basal membrane (Fig. 3A). A germinal zone occurred in the middle ovary sections of this female
 191 that contained mesoderm cells and sparse, large primary oogonia (Fig. 3B). The oogonia and their nuclei
 192 diameters were, respectively, 0.04 mm and 0.029 mm. Transformation of the mesoderm cells into follicular
 193 cells was apparent in their ovary germinal zones (Fig. 3C). The middle ovary sections of this female
 194 also contained oogonia in the prophase, anaphase and telophase of mitosis (Fig. 3D) and 0.026 mm
 195 diameter primary oogonia with 0.019 mm diameter nuclei (Fig. 3E). Posterior ovary sections included
 196 previtellogenic oocytes of variable sizes (Fig. 3F) of that contained large granules of chromatin in their
 197 nuclei (first prophase of meiosis) and cells of primary follicular epithelium. The overall structure this
 198 female ovaries indicated that they were recovering *de novo* after atrophy and that regeneration began at
 199 the posterior end (opening into pereonite 5) and was advancing to the anterior sections (near pereonite 2).
 200 The ovaries of the second 32 mm *FIV* female contained previtellogenic oocytes with large granules of
 201 chromatin in their nuclei in anterior sections and small vitellogenic oocytes that appeared to be new in
 202 posterior sections. The ovaries of these two 32 mm *FIV* females (Tables 2, 3) therefore appear to have
 203 "regenerated".

204 The 32 mm *F0* female in our sample (Table 3) contained 0.49 mm diameter vitellogenic oocytes.
 205 Moreover, reproductive development of this *F0* female, with vitellogenic oocytes, indicates that she was
 206 also regenerating but at an advanced reproductive development relative to the two 32 mm *FIV* females.

207 The 16 to 32 mm length range of the 8 females with entirely undamaged (normal) vitellogenic oocytes
 208 was overlapped by the 19 to 27 mm length range of females with partially or totally lysed vitellogenic
 209 oocytes (Table 3). Our sample size is insufficient for resolving whether the frequencies of total or partially
 210 lysed vitellogenic oocytes between *F0* and *FII* and *FIV* females were different. However, a greater range
 211 of vitellogenic oocyte diameters occurred among 16 to 32 mm *F0* females than among the 22 to 32 mm
 212 length *FII* and *FIV* females (Table S2, Fig. 4).

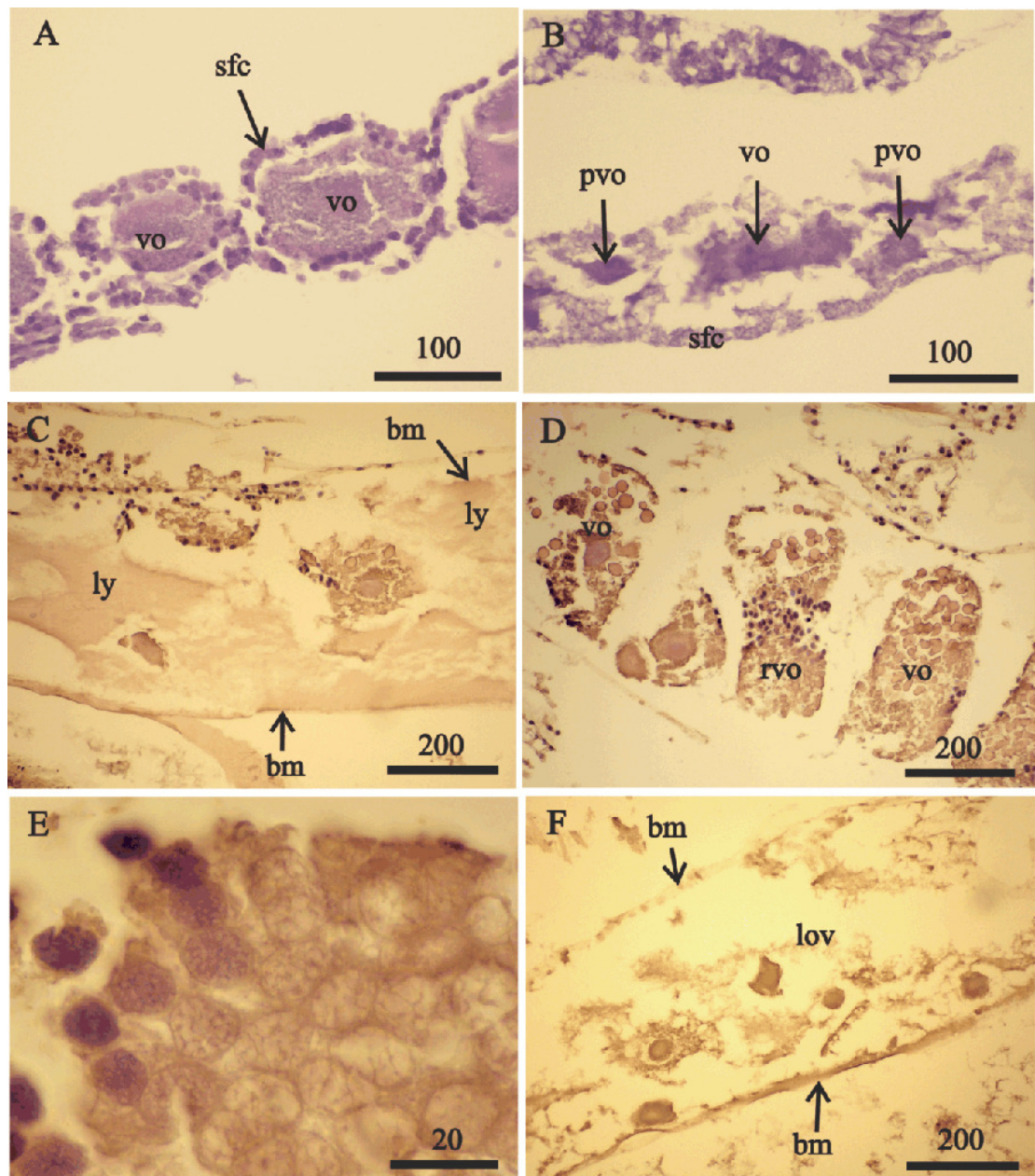


Figure 2. Stage *FII* *A. eschrichtii* ovaries. (A) – a 23.5 mm female with normal oocytes in anterior section and (B) – degraded oocytes in posterior section; (C) – a 24 mm female with lysed yolk of vitellogenic oocytes inside of the ovary, the wall of ovary is composed of the basal membrane (bm); (D) – resorption of vitellogenic oocytes by follicle cells (rvo); (E) – destruction of follicle cells in process of resorption of vitellogenic oocyte; (F) – remnants of oocytes in ovary lumen (lov). All scales are in μm .

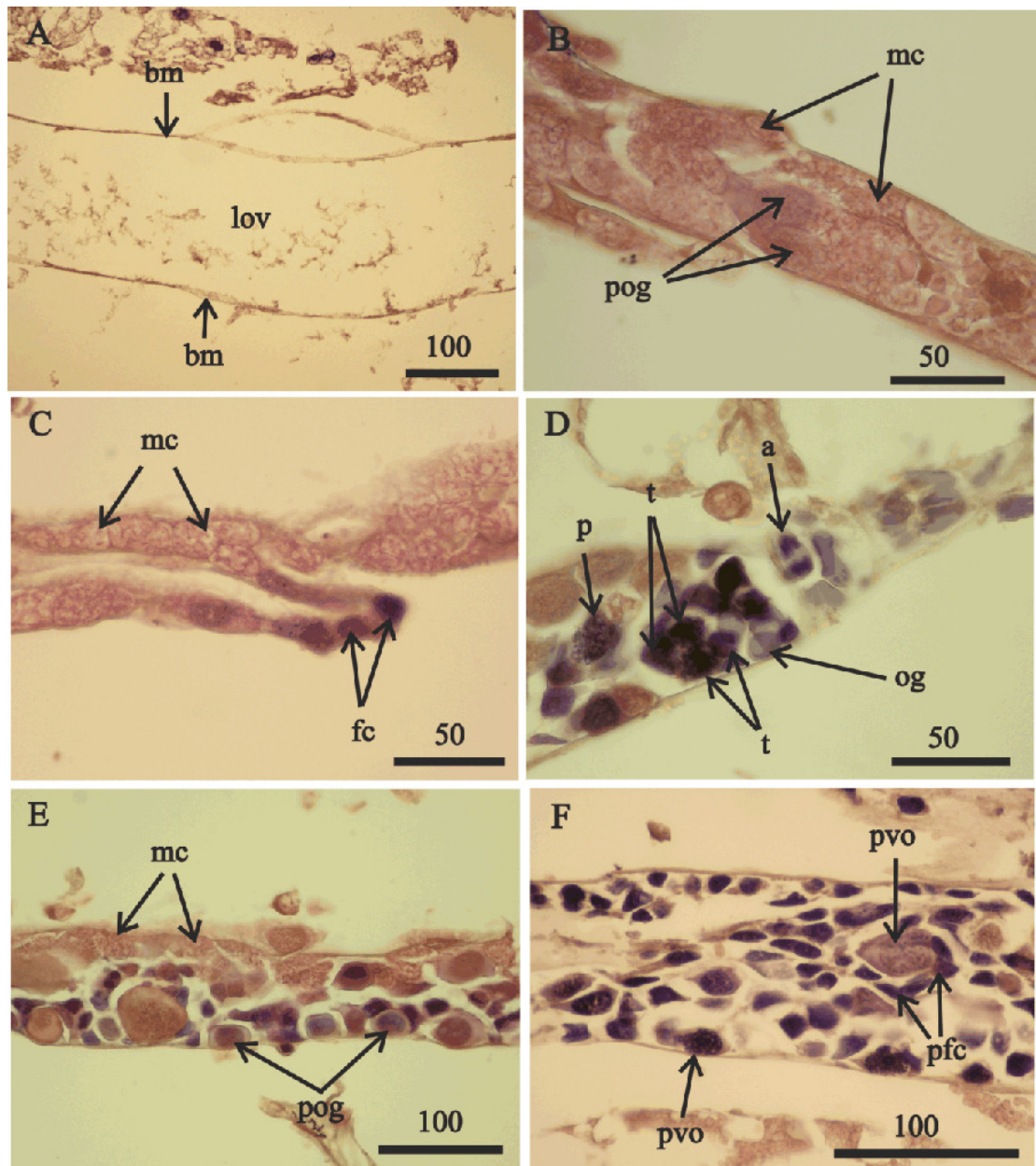


Figure 3. Stage FIV 32 mm *A. eschrichtii* ovary in *de novo* recovery. (A) – the empty anterior section; (B) – large primary oogonia (pog) in the germinal zone; (C) – mesodermal cells (mc) transforming into follicular cells (fc); (D) – oogonia in anaphase, prophase and telophase of mitosis (a, p and t, respectively); (E) – small primary oogonia in the germinal zone; (F) – posterior section with previtellogenic oocytes. All scales are in μm .

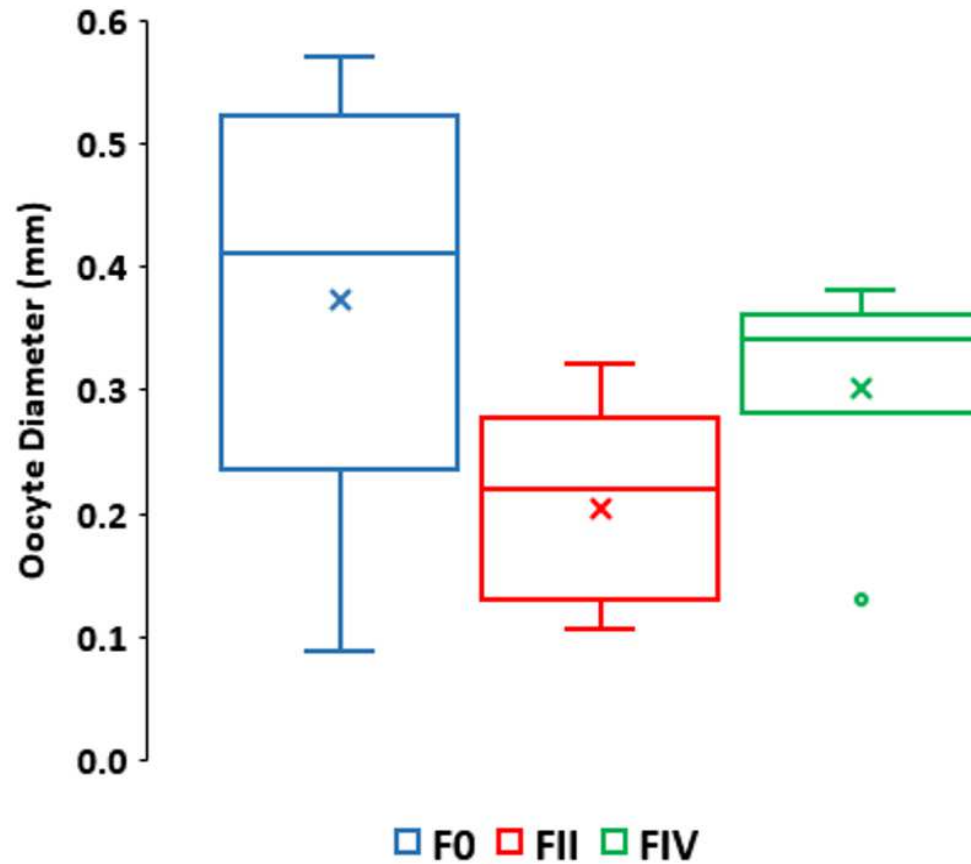


Figure 4. Range, upper and lower quartile (box) mean (x), and median (solid line) of vitellogenic oocyte diameters in *FO*, *FII* and *FIV* females (N = 17, 13 and 7, respectively) with larger oocytes and complete overlap of *FO* females with *FII* and *FIV* females and significantly larger oocytes in *FIV* females than in *FII* females (ANOVA, $F = 6.75$, $p < 0.02$, $df = 2$).

213 The largest diameter oocytes among *FO* females and the smallest diameter oocytes among *FII* females
 214 were expected with normal active reproduction (Fig. 4). The absence of *FI* and *FIII* females however
 215 indicates that the observed size variation in oocytes was due instead to delayed reproductive development.

216 Males

217 Reproductive development advanced among males with increasing size. Based on the presence of
 218 spermatophores, greater than 21 mm in length, male testes were reproductively competent. The testes
 219 primordia (two narrow cords of mesoderm cells (mc)) occurred in 16.5 mm length males (Fig. 5A) and
 220 rare spermatogonia with nuclei that stained with hematoxylin, occurred on the periphery of the cords.

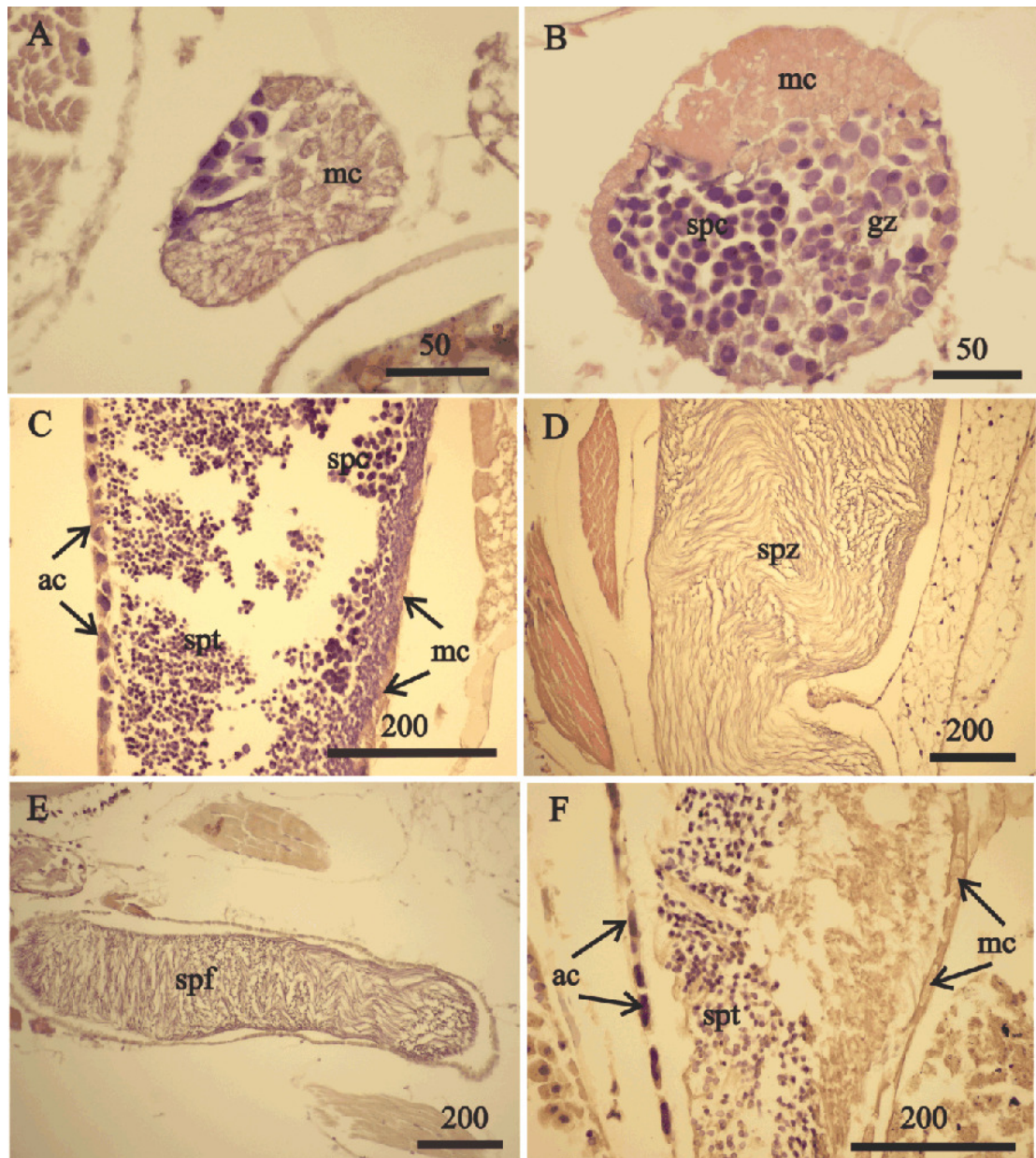


Figure 5. *A. eschrichtii* testes. (A) – cord of mesodermal cells (mc) of a 16.5 mm male previous to functional testis; (B) – germinal zone (gz) and spermatocytes (spc) of a 18 mm male testis; (C) – accessory cells (ac) and spermatids (spt) of a 21 mm male testis; (D) – spermatozoa (spz) in seminal vesicle of a 21 mm male; (E) – spermatophore (spf) in *vas deferens* of a 21 mm male; (F) – atrophied spermatids (spt) of a 26 mm male testis. All scales are in μm .

221 The testes of 18 mm males, in addition to the mesoderm cells, contained well developed germinal
 222 zones with spermatogonia and spermatocytes outside the germinal zone (Fig. 5B). Testes of 20 mm males
 223 also contained numerous spermatocytes. We found numerous spermatids - the product of meiotic division
 224 of spermatocytes, in the lumen of the testes of 21 mm of males along with accessory cells (ac) (Fig. 5C).
 225 Accessory cells are associated with the transformation of spermatids into spermatozoa (Charniaux-Cotton,
 226 1985). Seminal vesicles of 21 mm of individuals contained numerous spermatozoa (Fig. 5D), and within
 227 the *vas deferens*, spermatozoa were packed into a spermatophore (Fig. 5E). The testes of greater than 21
 228 mm males lacked germinal zones and the testes walls of these males were lined with rare mesodermal
 229 cells. Testes of 24 and 26 mm males contained few spermatocytes or spermatids. The flattened accessory
 230 cells and rare mesodermal cells of the testis of these males (Fig. 5F), indicates they were atrophied. The
 231 spermatozoa in the seminal vesicles and spermatophores in the *vas deferens* these greater than 21 mm
 232 males indicates they would be competent to mate only once more.

233 Life history

234 We found only *F0*, *FII*, and *FIV* stage females in July and October 2015 (Table 1), consistent with
 235 Demchenko et al.'s 2016 observations from 2002-2013 samples. The *FII* embryos of July 2015 were in
 236 the blastula stage in contrast to the segmented embryos in the *FII* females of October 2013 and 2015.
 237 The small differences in embryo development between July and October are consistent with delayed
 238 development in contrast to rapid replacement or turnover expected with active reproduction.

239 We used the antilog of Sainte-Marie's (1991, Table 9) equation for our estimate of ampeliscid brood
 240 size (*BS*) with body length (*BL*) [$BS = 1.227 * BL^{1.335}$, $r^2 = 0.49$, $n = 24$] (Fig. 6).

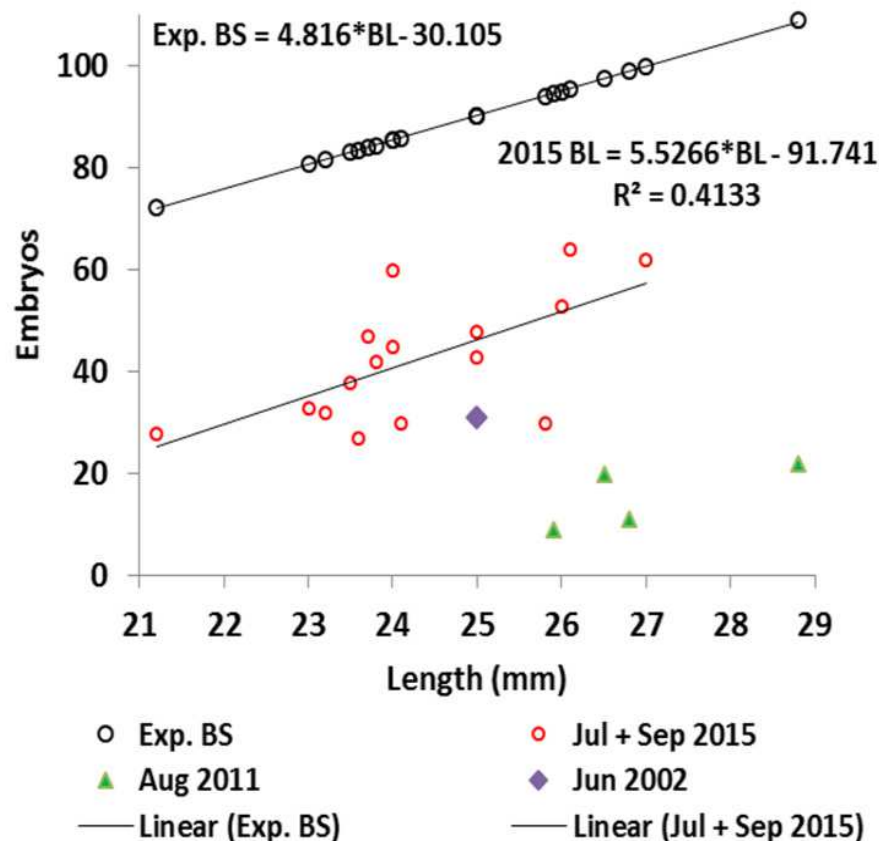


Figure 6. Expected brood sizes (Exp. *BS*) (black line and circles) and observed *A. eschrichtii* brood sizes of July and October 2015 (2015 *BS*) (red circles), June 2002 (purple diamond) and August 2011 (green triangles) with body length (*BL*). (Note: observed embryo with body length equation (Obs) includes only the 2015 population.)

241 Our one sample from 2002, four samples from 2011 and fifteen samples from 2015 (Fig. 6) were
 242 respectively, 34%, 15% and 49% of the expected size adjusted ampeliscid brood size and thus, counter to
 243 prediction 2 for viable brood size with summer reproduction.

244 From equations (1) and (2), an embryo with sufficient weight to produce the smallest length *A.*
 245 *eschrictii* that we found in our samples (a 3.8 mm juvenile) would be 1.14 mm in diameter. A 1.14 mm
 246 diameter embryo is within the range of both observed *A. eschrictii* embryo diameters and the embryo
 diameters estimated from other gammaridean amphipod species (Sainte-Marie, 1991) (Fig. 7).

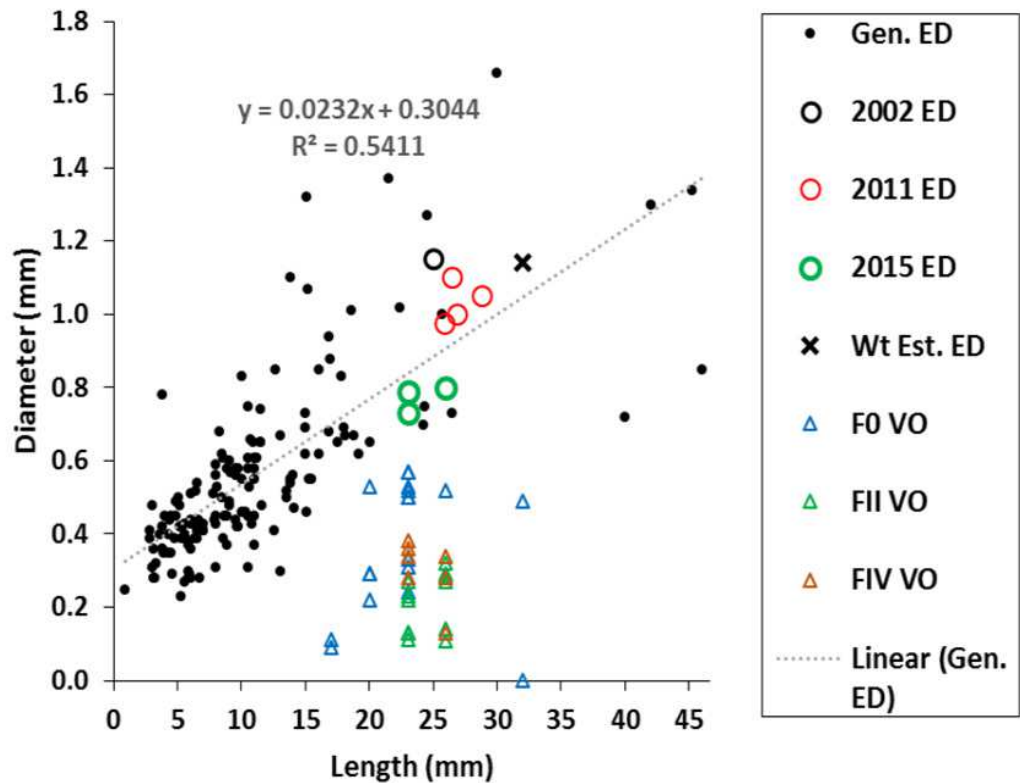


Figure 7. Embryo diameters (ED) with body length among 123 gammaridean amphipod species of the northern hemisphere (black dots, Sainte-Marie (1991), appendix Table 1), Sakhalin Shelf *A. eschrictii* embryo diameters from 2002 (black circle), 2011 (red circles) and 2015 (green circles), estimated embryo diameter for a 3.8 mm juvenile *A. eschrictii* (black X) and observed oocyte diameters for *F0*, *FII* and *FIV* females (blue, green and red triangles, respectively).

247 The 2002, 2011 and 2015 *A. eschrictii* embryo diameters (Fig. 7, red and green circles), ranged
 248 between 0.76 and 1.15 mm and were from females averaging 23.6 mm in length. These observed *A.*
 249 *eschrictii* embryo diameters also were within the range of embryo diameters expected for a 23.6 mm
 250 generalized amphipod.
 251

252 We assume a minimum oocyte diameter required to produce a viable juvenile *A. eschrictii* (prediction
 253 3) is the same as for embryos. However, we did not find oocyte diameters as large as the observed or
 254 estimated minimum sizes of embryos (Fig. 7). Thus, we did not find viable oocytes for reproduction in
 255 our samples counter to prediction 4 for summer reproduction.

256 DISCUSSION

257 A lack of evidence for our predicted summer reproduction life history characters and our new discoveries
 258 of ovary atrophy strongly indicate that *A. eschrictii* starve in summer and feast in winter, as Demchenko

259 et al. (2016) proposed. Counter to prediction 1, not all brood and embryo development stages were found
260 and reproductive development of gonads advanced incompletely with increasing female size.

261 Counter to prediction 2, reproductive effort was less than expected among similar sized reproductive
262 females of other species. The lower than expected *A. eschrichtii* embryo numbers were not compensated by
263 larger embryo sizes. The low embryo counts are consistent with cannibalism under starvation conditions
264 observed in other amphipods. Amphipod oocytes released into the marsupium when copulation does
265 not occur are not fertile. They do not develop and they disappear from the marsupium within a few
266 days (Hyne, 2011). Sheader (1996) experimentally demonstrated embryo losses due to cannibalism in
267 *Gammarus insensibilis* and that the oocytes of females that do not ovulate are resorbed. Oocyte lysis and
268 resorption are thus likely to be common responses of amphipods to food abundance and starvation. We
269 are unaware of previous reports of ovary atrophy or regeneration that we observed here.

270 The high prevalence of lysed oocytes in all sample periods and the small oocyte diameters relative
271 to the observed and predicted viable embryo diameters were counter to prediction 3. Consistent with
272 an expected summer cycle of oocyte development the largest diameter oocytes we found were among
273 *F0* females, and the smallest diameter oocytes were among *FII* females (Fig. 4). Depending on how
274 close they were to oviposition and transition into reproductive stage *F0*, females contain the smallest
275 (young) and largest (mature) oocytes. Also consistent with prediction 3, the recent embryos of stage *FII*
276 females are expected to have depleted the largest oocytes from their ovaries as they were deposited into
277 the marsupium. However, counter to prediction 3, the oocytes of stage *FIV* females, were too small to
278 produce viable embryos. *FIV* oocytes were also not as large as the largest *F0* oocytes. The relatively
279 small *FIV* oocytes thus were not large enough to produce viable embryos for these females, that were
280 ready to carry new embryos. These undersize oocytes indicate food stress was occurring in these *FIV*
281 females and are counter to prediction 3.

282 The mature sperm in the *vas deferens* of the largest males are consistent with active summer reproduc-
283 tion (prediction 4). Sperm are not rich in lipids and thus, are poor indicators of trophic stress. However,
284 counter to prediction 4, males with terminal phase pelagic mating morphologies were not found in these
285 samples or any previous summer samples (Demchenko et al., 2016).

286 CONCLUSIONS

287 Atrophied ovaries of two (24 and 26 mm) *FII* females indicate starvation and maximum use of the content
288 of the ovaries can occur as a source of energy for the needs of the organism. The depletion of *A. eschrichtii*
289 ovaries may be an extreme adaptation to starvation and is inconsistent with active summer reproduction.
290 We assume that restoring the ovaries after they atrophy is a lengthy process. We found 22-24 and 25-27
291 mm *FII* females with atrophied ovaries, two 32 mm *FIV* females with regenerated ovaries and one 32
292 mm *F0* female with restored ovaries. The presence of a 32 mm *F0* female with large vitellogenic oocytes
293 without signs of lysis indicates the successful functioning of the restored ovaries.

294 The winter based life history adaptations of *A. eschrichtii* inferred here are consistent with previous am-
295 phipod life history observations. Adaptations to low temperatures and to winter growth and reproduction
296 are prevalent among amphipods (i.e., Kusano et al. (1987), Jakob et al. (2016)). Amphipod reproduction
297 occurs when food is abundant and amphipod juveniles are commonly released when maximum food
298 sources are present (Sagar, 1980; Sutcliffe, 1993). Moreover, juveniles of the North European *Ampelisca*
299 *macrocephala* emerge in coincidence with the maximum phytoplankton abundances while the adults can
300 survive for months without food (Kanneworff, 1965).

301 Trophic stress among Sakhalin Shelf *A. eschrichtii* populations in summer is also consistent with
302 Sakhalin Shelf oceanography. Phytoplankton biomass, consisting mostly of diatoms, is concentrated in
303 summer over the Sakhalin Shelf at the upper boundary of a thermocline ranging from the surface to 10–15
304 m (Sorokin and Sorokin, 1999; Sorokin, 1997; Rutenko and Sosnin, 2014; Prants et al., 2017). Vertical
305 mixing and down-welling of Sakhalin Shelf waters is prevalent in winter (Leonov et al., 2007). The
306 40-60 m depth ranges of the Offshore benthos are below the high surface concentrations of phytoplankton
307 in summer and more likely to receive most of their autotrophic food sources in winter when vertical
308 mixing carries phytoplankton to their depths. Winter surveys of these amphipod populations in the
309 Offshore gray whale feeding area are needed to resolve their life history and ecology and to understand
310 the oceanographic mechanisms of production for western gray whale prey stocks. These surveys would
311 also also increase our understanding of high latitude benthic community production.

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392 SUPPLEMENT MATERIALS.

Table S1. Abbreviations to Figs. 1-3, 5.

a	anaphase of mitosis
ac	accessory cells
bm	basal membrane
fc	cells of follicular epithelium
gz	germinal zone
lov	lumen of ovary
ly	lysed yolk
mc	mesodermal cells
og	oogonia
p	prophase of mitosis
pfc	cells of primary follicular epithelium
pog	primary oogonia
pvo	previtellogenic oocyte
rvo	resorption of vitellogenic oocyte by follicle cells
sfc	cells of secondary follicular epithelium
spc	spermatocytes
spf	spermatophore
spt	spermatids
spz	spermatozoa
t	telophase of mitosis
vo	vitellogenic oocyte

Table S2. Vitellogenic oocyte (Vo) maximum, upper quartile, mean, median, lower quartile and minimum diameters among *F0*, *FII* and *FIV* stage females with complete ovaries.

VO Diameters	<i>F0</i>	<i>FII</i>	<i>FIV</i>
Maximum	0.57	0.32	0.38
Upper Quartile	0.52	0.27	0.35
Mean	0.37	0.20	0.30
Median	0.33	0.22	0.34
Lower Quartile	0.23	0.13	0.28
Minimum	0.00	0.11	0.13
N	18	13	7