A peer-reviewed version of this preprint was published in PeerJ on 22 June 2018.

View the peer-reviewed version (peerj.com/articles/4841), which is the preferred citable publication unless you specifically need to cite this preprint.

Durkina VB, Chapman JW, Demchenko NL. 2018. *Ampelisca eschrichtii* Krøyer, 1842 (*Ampeliscidae*) of the Sakhalin Shelf in the Okhotsk Sea starve in summer and feast in winter. PeerJ 6:e4841 https://doi.org/10.7717/peerj.4841

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.

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[2], and 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, Hatfield Marine Science Center, Oregon State University, 2030 SE Marine Science Dr., Newport, Oregon, United States of America**
[3]**Laboratory of Marine Ecosystem Dynamics, National Scientific Center of Marine Biology FEB RAS, Vladivostok, Russia**

Corresponding author:
Valentina B. Durkina[1]

Email address: vdurkina@mail.ru

ABSTRACT

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.

INTRODUCTION

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

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

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

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

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

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

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

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

METHODS

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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

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

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

Life history

Our classification of brood development follows Tzvetkova's 1975 criteria:
F0–rudimentary oostegites lacking egg retention setae;
FI–brooding uncleaved embryos and oostegites with fully developed embryo retention setae;
FII–brooding cleaved embryos;
FIII–brooding fully formed juveniles;
FIV–developed oostegites with embryo retention setae and an empty brood pouch.

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

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

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

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

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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

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

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

Histology

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

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

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

RESULTS

Females

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

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

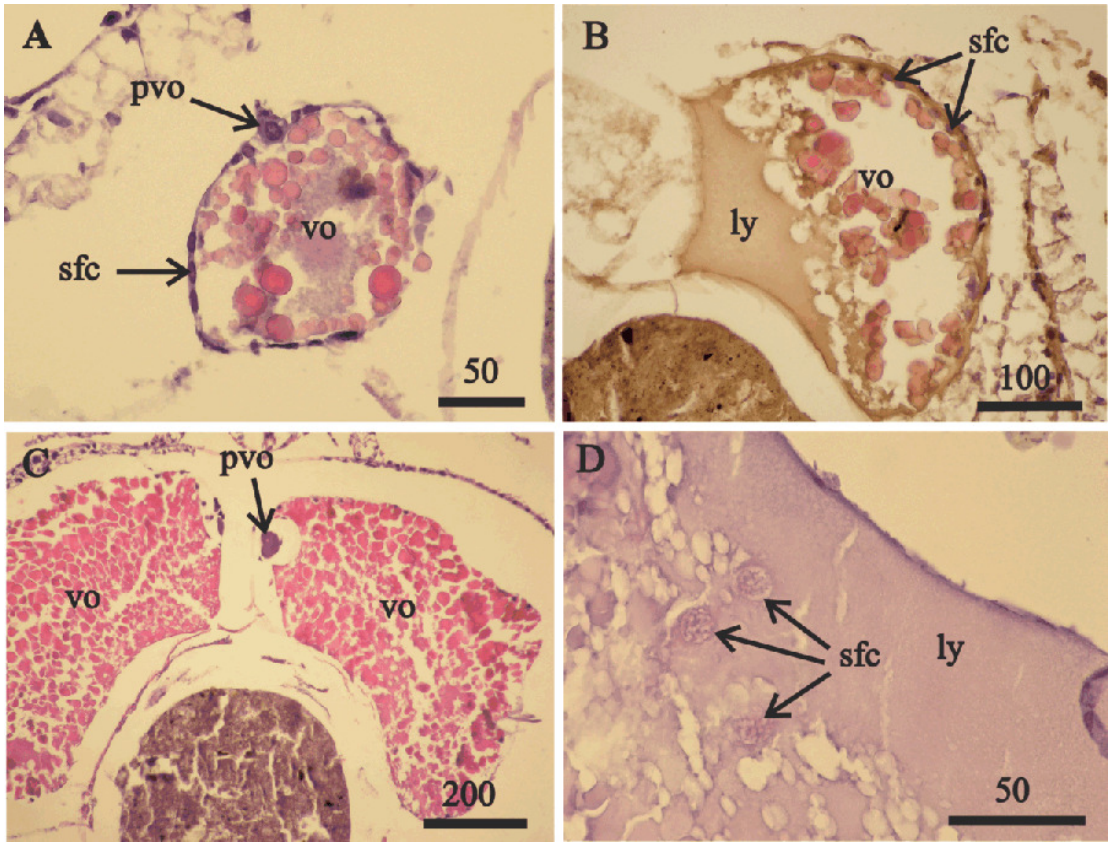

Figure 1. Stage *F0 A. eschrichtii* ovaries. (A) – a 17 mm female with previtellogenic oocytes (pvo), vitellogenic oocytes (vo) and secondary follicle cells (sfc); (B) – a 21 mm female with undergoing lysis (ly) of vitellogenic oocytes; (C) – a 24 mm female with mature vitellogenic oocytes (vo) and,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

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

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

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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
F0	**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
FII	**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
FIV	**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**

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

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

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

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

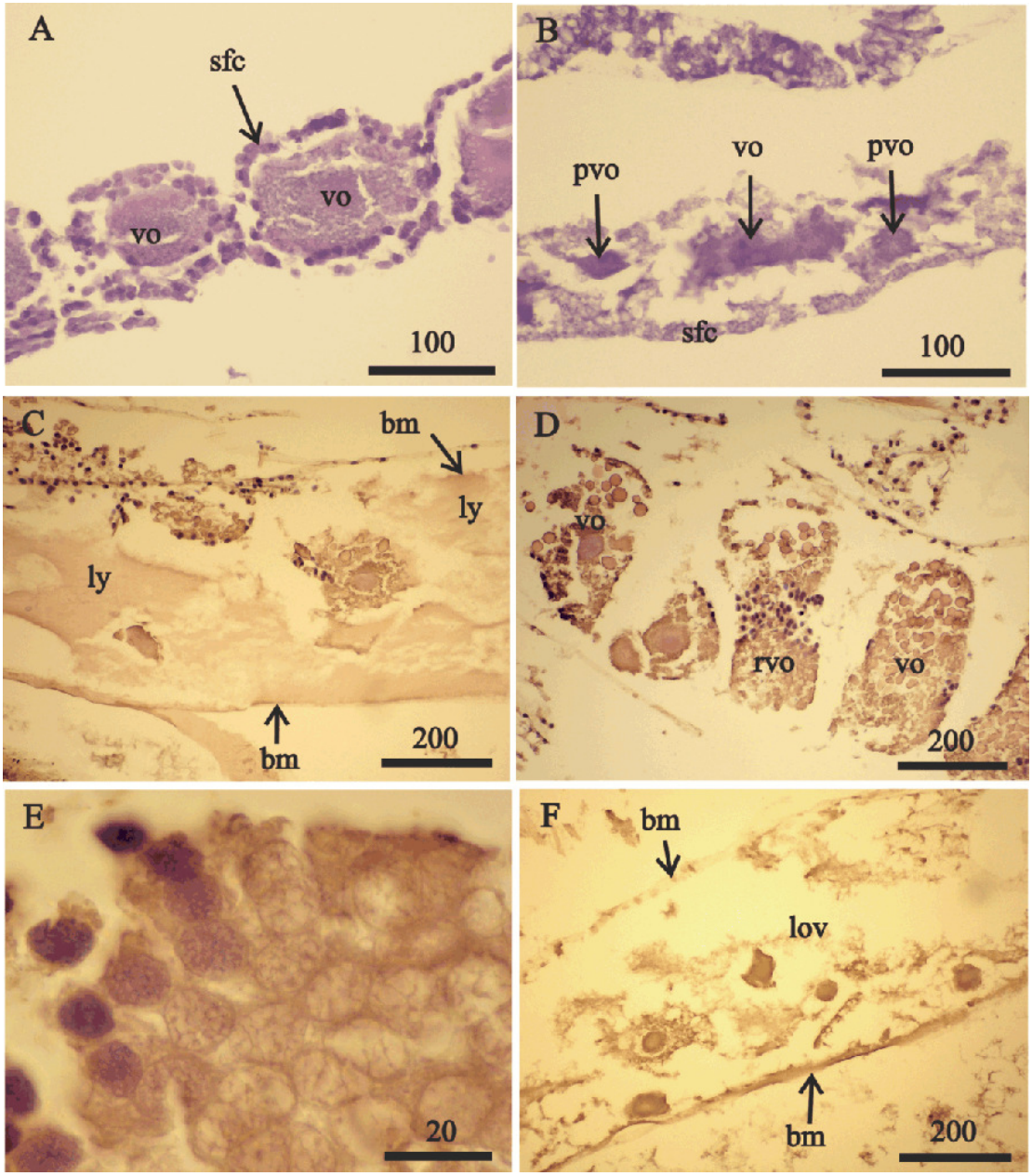

Figure 2. Stage *FII A. eschrichtii* ovaries. (A) – a 23.5 mm female with normal oocytes in anterior section and (B) – degraded oocytes in posterior section; (C) – a 24 mm female with lysed yolk of vitellogenic oocytes inside of the ovary, the wall of ovary is composed of the basal membrane (bm); (D) – 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

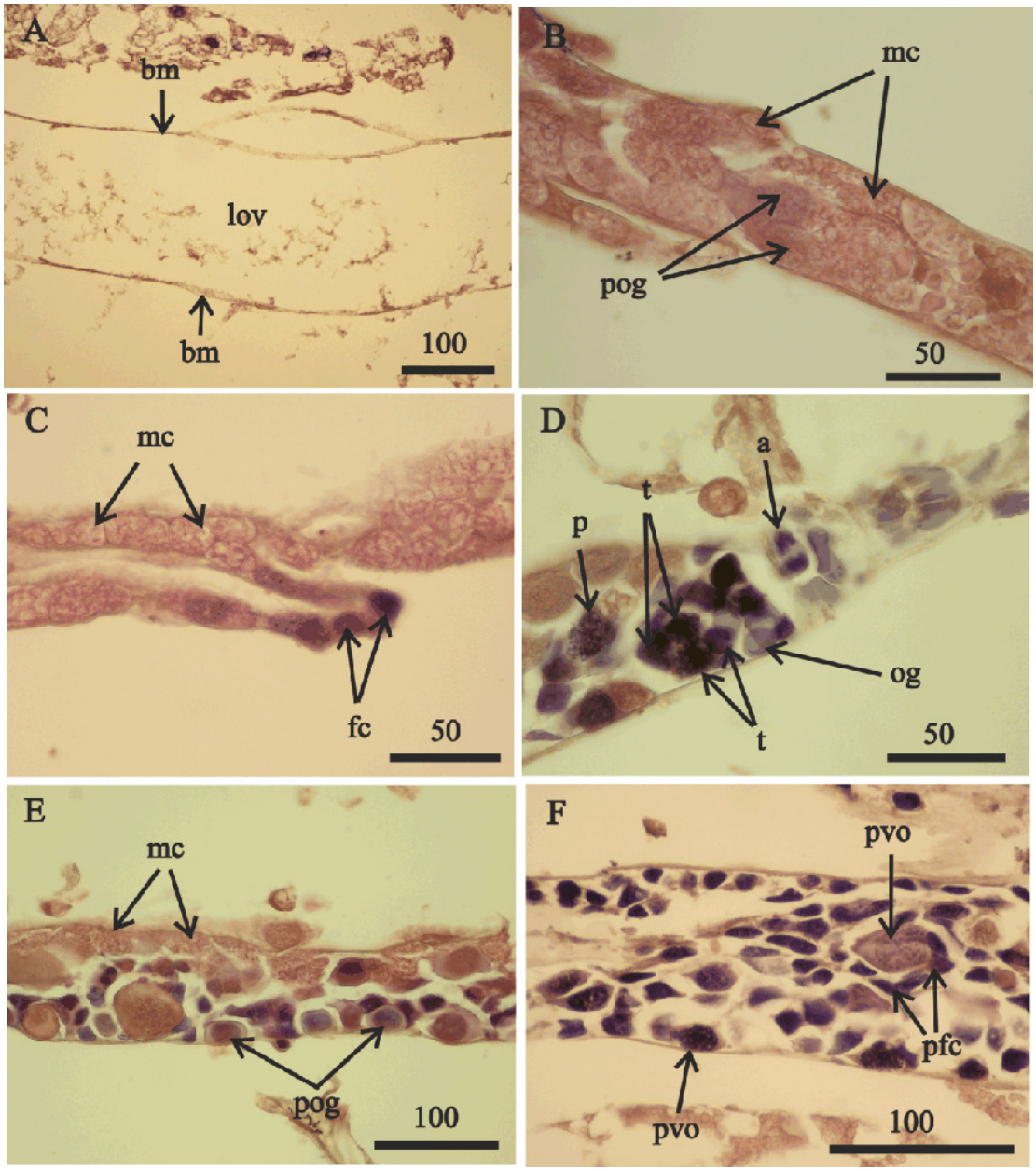

Figure 3. Stage *FIV* 32 mm *A. eschrichtii* ovary in *de novo* recovery. (A) – the empty anterior section; (B) – large primary oogonia (pog) in the germinal zone; (C) – mesodermal cells (mc) transforming into follicular cells (fc); (D) – oogonia in anaphase, prophase and telophase of mitosis (a, p and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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

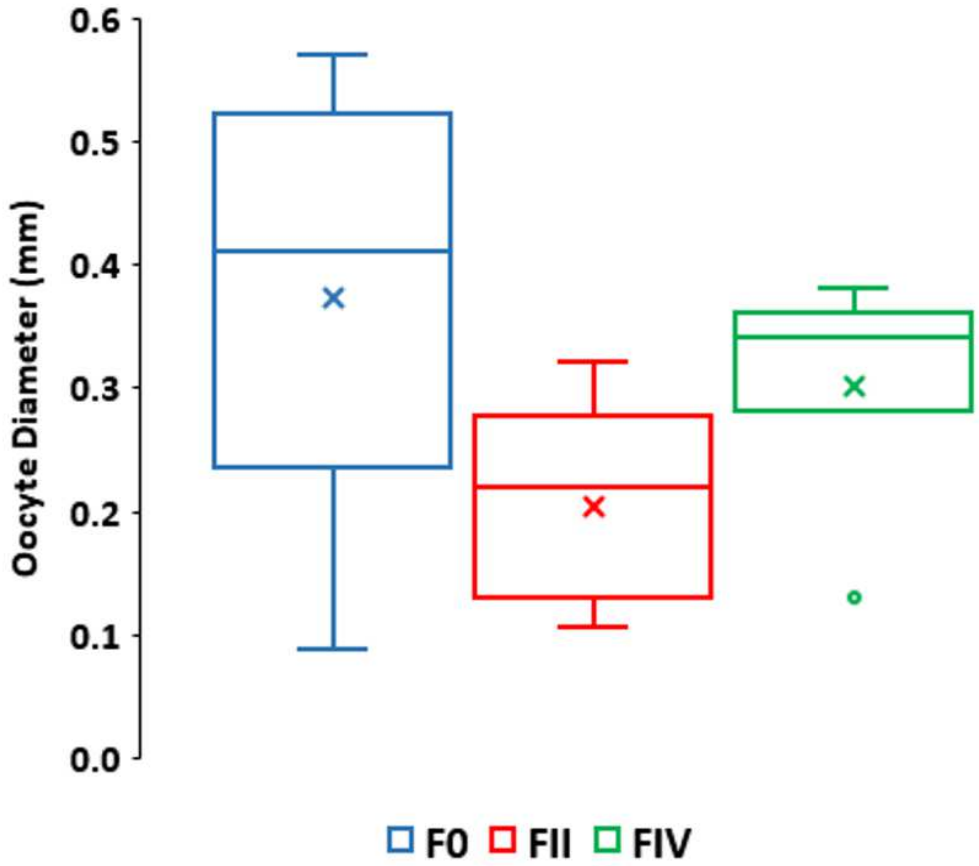

Figure 4. Range, upper and lower quartile (box) mean (x), and median (solid line) of vitellogenic oocyte diameters in *F0*, *FII* and *FIV* females (N = 17, 13 and 7, respectively) with larger oocytes and complete overlap of *F0* females with *FII* and *FIV* females and significantly larger oocytes in *FIV* females than in *FII* females (ANOVA, $\boldsymbol{F} = 6.75$, $\boldsymbol{p} < 0.02$, $\boldsymbol{df} = 2$).

The largest diameter oocytes among *F0* females and the smallest diameter oocytes among *FII* females were expected with normal active reproduction (Fig. 4). The absence of *FI* and *FIII* females however indicates that the observed size variation in oocytes was due instead to delayed reproductive development.

Males

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

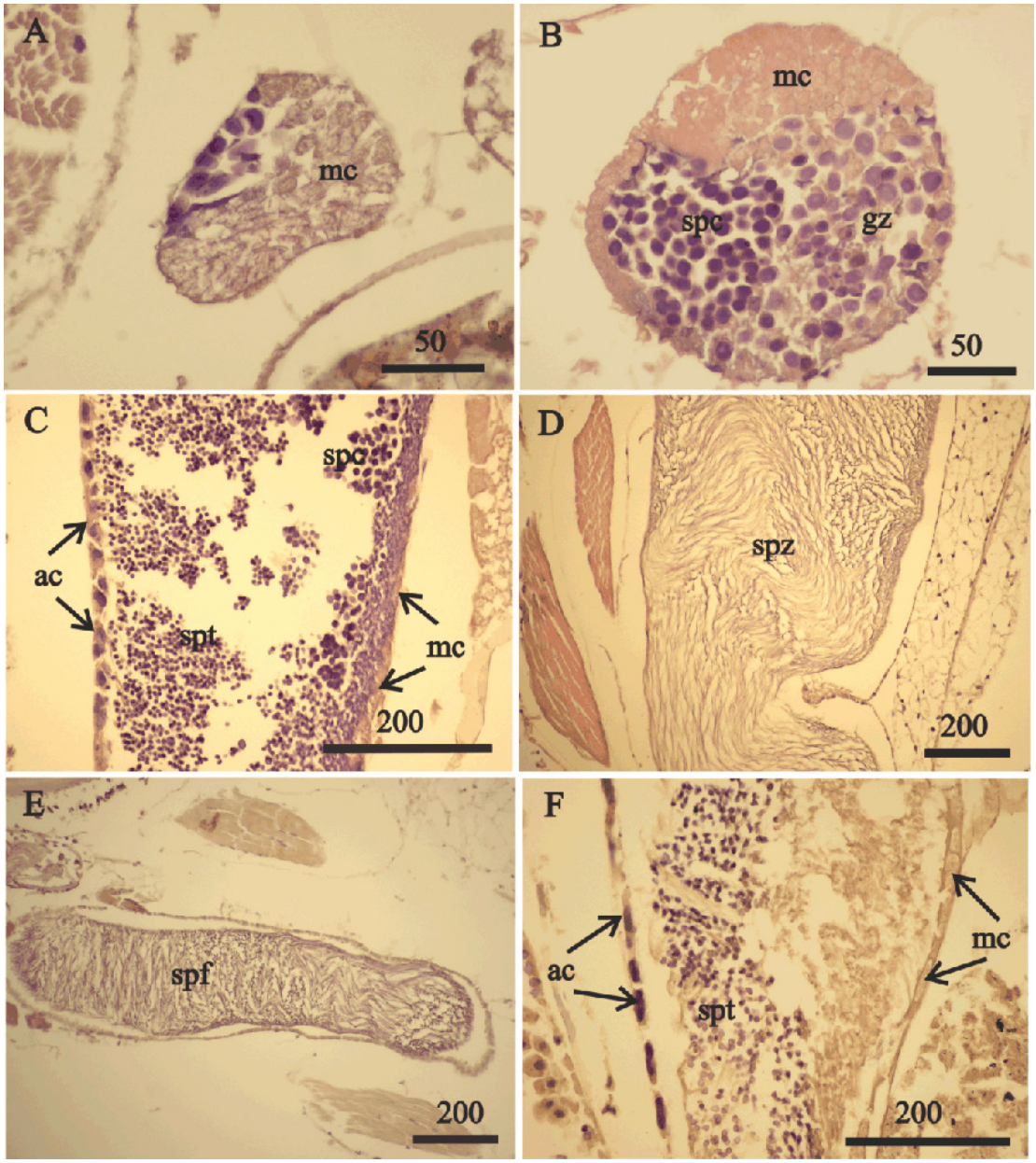

Figure 5. *A. eschrichtii* testes. (A) – cord of mesodermal cells (mc) of a 16.5 mm male previous to functional testis; (B) – germinal zone (gz) and spermatocytes (spc) of a 18 mm male testis; (C) – accessory cells (ac) and spermatids (spt) of a 21 mm male testis; (D) – spermatozoa (spz) in seminal vesicle 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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

Life history

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

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

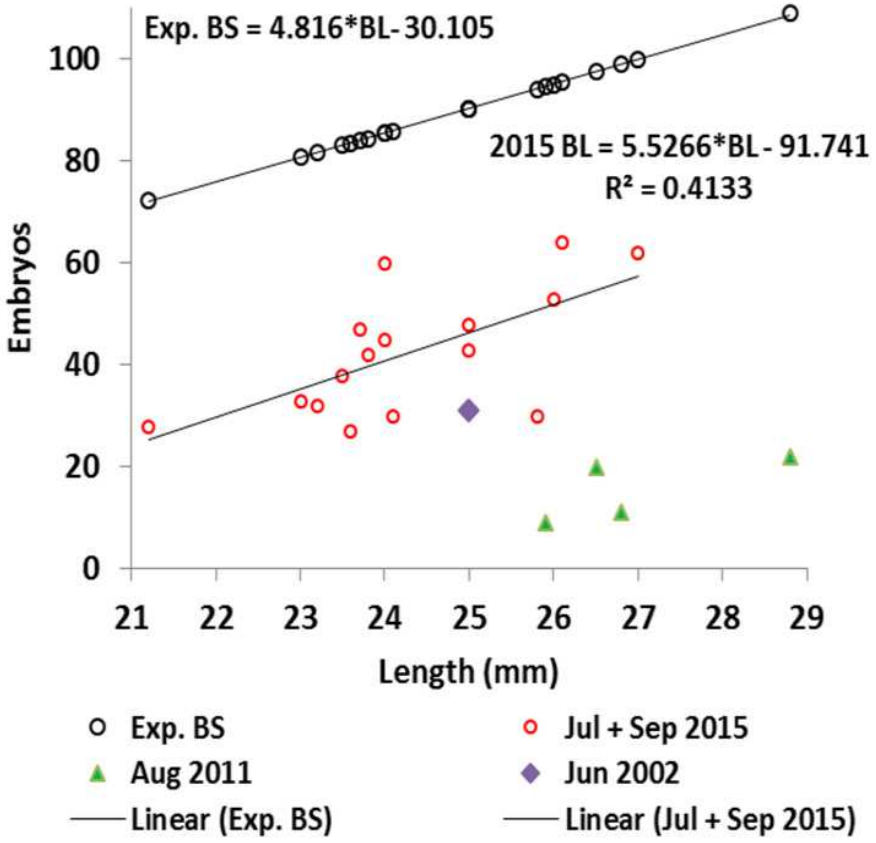

Figure 6. Expected brood sizes (Exp. *BS*) (black line and circles) and observed *A. eschrichtii* brood sizes of July and October 2015 (2015 BS) (red circles), June 2002 (purple diamond) and August 2011 (green triangles) with body length (*BL*). (Note: observed embryo with body length equation (Obs)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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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

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

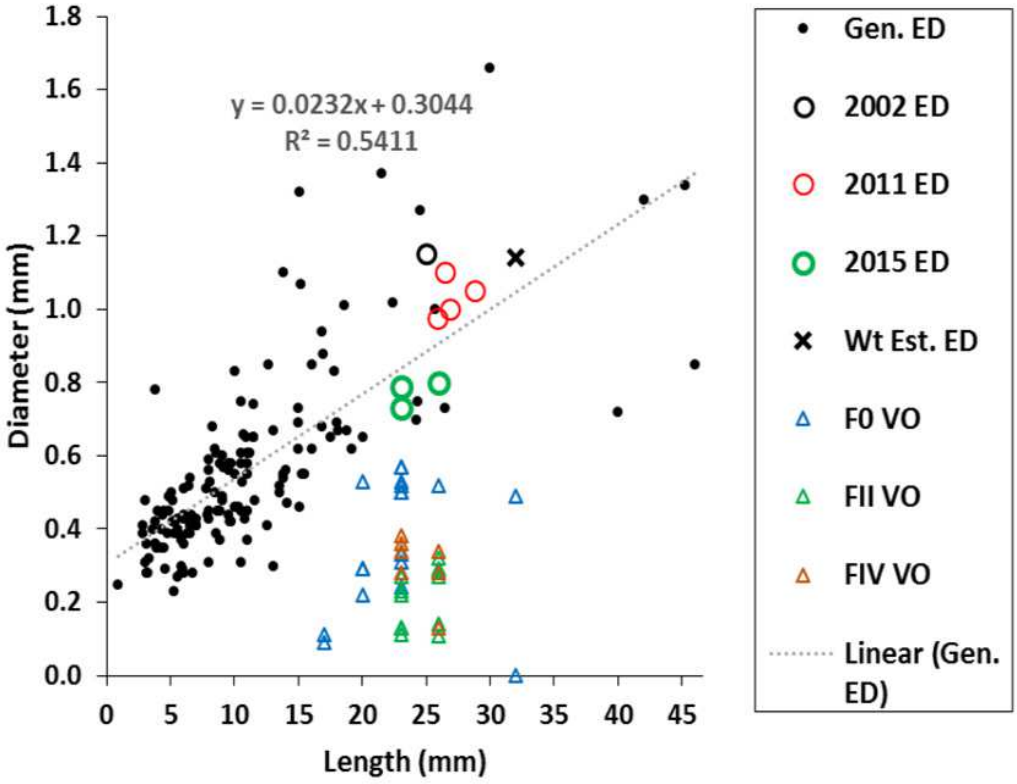

Figure 7. Embryo diameters (ED) with body length among 123 gammaridean amphipod species of the northern hemisphere (black dots, Sainte-Marie (1991), appendix Table 1), Sakhalin Shelf *A. eschrichtii* embryo diameters from 2002 (black circle), 2011 (red circles) and 2015 (green circles), estimated embryo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

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

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

DISCUSSION

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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

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

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

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

CONCLUSIONS

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

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

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

ACKNOWLEDGMENTS

VBD, JWC and NLD accept all responsibility for the integrity and validity of the data collected and analyzed. We thank E.P. Dats (Vladivostok State University of Economics and Service, VSUES) for assistance with Overleaf, editing formulas and calculations.

REFERENCES

Borowsky, B. and Aitken-Ander, P. (1991). Sexually dimorphic free-swimming behaviour in the amphipod crustacean Ampelisca abdita. *Journal of the Marine Biological Association of the United Kingdom*, 71:655–663.

Charniaux-Cotton, H. (1985). Vitellogenesis and Its Control in Malacostracan Crustacea. *American Zoologist*, 25(1):197–206.

Coyle, K., Bluhm, B., Konar, B., Blanchard, A., and Highsmith, R. (2007). Amphipod prey of gray whales in the northern Bering Sea: Comparison of biomass and distribution between the 1980s and 2002–2003. *Deep Sea Research Part II: Topical Studies in Oceanography*, 54(23-26):2906–2918.

Demchenko, N. L., Chapman, J. W., Durkina, V. B., and Fadeev, V. I. (2016). Life History and Production of the Western Gray Whale's Prey, Ampelisca eschrichtii Krøyer, 1842 (Amphipoda, Ampeliscidae). *PLOS ONE*, 11(1):e0147304.

Fadeev, V. (2012). Chapter 3. Benthos studies in feeding grounds of the western population of gray whales, 2011. Technical report, Vladivostok.

Hastings, M. (1981). Intersex specimens of amphipod Ampelisca brevicornis (Costa). *Crustaceana*, 41(2):199–205.

Hyne, R. (2011). Review of the reproductive biology of amphipods and their endocrine regulation: identification of mechanistic pathways for reproductive toxicants. *Environmental toxicology and chemistry*, 30(12):2647–2657.

IUCN (2008). Eschrichtius robustus: Reilly, S.B., Bannister, J.L., Best, P.B., Brown, M., Brownell Jr., R.L., Butterworth, D.S., Clapham, P.J., Cooke, J., Donovan, G.P., Urbán, J. & Zerbini, A.N.: The IUCN Red List of Threatened Species 2008: e.T8097a12885255. Technical report, International Union for Conservation of Nature. itemType: dataset DOI: 10.2305/IUCN.UK.2008.RLTS.T8097A12885255.en.

Jakob, L., Axenov-Gribanov, D. V., Gurkov, A. N., Ginzburg, M., Bedulina, D. S., Timofeyev, M. A., Luckenbach, T., Lucassen, M., Sartoris, F. J., and Pörtner, H.-O. (2016). Lake Baikal amphipods under climate change: thermal constraints and ecological consequences. *Ecosphere*, 7(3):e01308.

Johnson, W., Stevens, M., and Watling, L. (2001). Reproduction and development of marine peracaridans. *Advances in Marine Biology*, 39:105–260.

Kanneworff, E. (1965). Life cycle, food, and growth of the amphipod Ampelisca macrocephala Liljeborg from the Øresund. *Ophelia*, 2(2):305–318.

Kusano, H., Kusano, T., and Watanabe, Y. (1987). Life History and Reproduction of Jesogammarus spinopulps (Anisogammaridae : Amphipoda) Inhabiting a Lowland Pond in Tokyo City. *Jpn. J. Limnol*, 48(2):117–126.

Lawrence, J. M. (1976). Patterns of Lipid Storage in Post-Metamorphic Marine Invertebrates. *American Zoologist*, 16(4):747–762.

Leonov, A., Mogil'nikova, T., Pishchal'nik, V., and Zenkin, O. (2007). Characteristic of microalgae development in the Sea of Okhotsk in winter and modeling of their annual dynamics in Aniva Bay. *Water Resources*, 34(2):184–194.

Nelson, W. (1980). Reproductive patterns of gammaridean amphipods. *Sarsia*, 65(2):61–71.

Parrish, C. C. (2013). Lipids in Marine Ecosystems. *International Scholarly Research Notices*, page 16. DOI: 10.5402/2013/604045.

Prants, S. V., Andreev, A. G., Uleysky, M. Y., and Budyansky, M. V. (2017). Mesoscale circulation along the Sakhalin Island eastern coast. *Ocean Dynamics*, 67(3):345–356.

Rutenko, A. and Sosnin, V. (2014). Hydrodynamic processes on the Sakhalin shelf in the coastal Piltun area of the grey whale feeding and their correlation with atmospheric circulation. *Russian Meteorology and Hydrology*, 39(5):335–349.

Sagar, P. M. (1980). Life cycle and growth of the Antarctic gammarid amphipod Paramoera walkeri (Stebbing, 1906). *Journal of the Royal Society of New Zealand*, 10(3):259–270.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

Sainte-Marie, B. (1991). A review of the reproductive bionomics of aquatic gammaridean amphipods: variation of life history traits with latitude, depth, salinity and superfamily. *Hydrobiologia*, 223(1):189–227.

Santos, C. M., Lima, G. V., Nascimento, A. A., Sales, A., and Oshiro, L. M. Y. (2009). Histological and histochemical analysis of the gonadal development of males and females of Armases rubripes (Rathbun 1897) (Crustacea, Brachyura, Sesarmidae). *Brazilian Journal of Biology = Revista Brasleira De Biologia*, 69(1):161–169.

Santos, R. N., Andrade, C. C., Santos, A. F. G. N., Santos, L. N., and Araújo, F. G. (2005). Hystological analysis of ovarian development of the characiform Oligosarcus hepsetus (Cuvier, 1829) in a Brazilizn Reservoir. *Brazilian Journal of Biology*, 65(1):169–177.

Sheader, M. (1983). The reproductive biology and ecology of Gammarus duebeni (Crustacea: Amphipoda) in southern England. *Journal of the Marine Biological Association of the United Kingdom*, 63:517–540.

Sheader, M. (1996). Factors influencing egg size in the gammarid amphipod Gammarus insensibilis. *Marine Biology*, 124(4):519–526.

Sorokin, Y. (1997). Primary production in the Sea of Okhotsk. In *Complex Studies of Ecosystem of the Sea of Okhotsk*, pages 103–110. VNIRO, Moscow. Edited by Prof. V.V. Sapozhnikov.

Sorokin, Y. and Sorokin, P. (1999). Production in the Sea of Okhotsk. *Journal of Plankton Research*, 21(2):201–230.

Spaargaren, D. (1979). Hydrodynamic Properties of Benthic Marine Crustacea.I. Specific Gravity and Drag Coefficients. *Marine Ecology Progress Series*, 1:351–359.

Sutcliffe, D. W. (1993). Reproduction in Gammarus (Crustacea, Amphipoda): female strategies. *Freshwater Forum*, 3(1):26–64.

Tzvetkova, N. (1975). *Pribrezhnye gammaridy severnykh i dal'nevostochnykh morei SSSR i sopredel'nykh vod [Genera Gammarus, Marinogammarus, Anisogammarus, Mesogammarus (Amphipoda, Gammaridea)]*. Nauka, Leningrad.

Van Dolah, R. and Bird, E. (1980). A comparison of reproductive patterns in epifaunal and infaunal gammaridean amphipods. *Estuarine and Coastal Marine Science*, 11(6):593–604.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018

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	***F0***	***FII***	***FIV***
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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.3496v2 | CC BY 4.0 Open Access | rec: 20 Apr 2018, publ: 20 Apr 2018