

Research Article

No evidence that the introduced parasite *Orthione griffenis* Markham, 2004 causes sex change or differential mortality in the native mud shrimp, *Upogebia pugettensis* (Dana, 1852)

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Abstract

Dramatic, rapid, population declines of the native North American burrowing shrimp *Upogebia pugettensis* (Dana, 1852) are associated with intense infestations by the introduced Asian bopyrid isopod parasite, *Orthione griffenis* Markham, 2004. However, expected host weight losses with increasing parasite weights do not occur, even among apparently castrated females. The prevailing assumption that energetic losses cause host castration have thus remained open to question, and the mechanism(s) resulting in castration and consequent population declines of *U. pugettensis* have remained unclear. Proposed alternative explanations for these declines, which have been based on a dramatically greater prevalence of *O. griffenis* among *U. pugettensis* females, include parasite induced sex change, increased male mortality, and differential tidal exposure of sexes to settling *O. griffenis* larvae. We examined 508 *O. griffenis* infestations from 2,014 shrimp collected from 26 stations in 5 Oregon estuaries to test these alternative hypotheses. We expected greater infestation frequencies among females than among males and a close association of *O. griffenis* infestations with intersex shrimp in the overall population if feminization occurs. We also expected covariation in sex ratio with tide exposure if *O. griffenis* settlement is sex linked. Instead, we found an overall 1:1.07 sex ratio, a lack of association of intersex *U. pugettensis* with *O. griffenis* infestations, and an unchanging sex ratio with tidal exposure, precluding parasite induced sex change, male mortality, or tidal immersion effects on infestations. The most likely mechanism driving *U. pugettensis* declines thus remains castration due to host energetic losses. This energetic interaction is likely to be resolved quantitatively through controlled experiments and increasingly detailed field surveys over time.

Key words: bopyrid isopod, gebiid shrimp, co-evolution, feminization, castration, energetic losses, mudflat

Introduction

The native burrowing mud shrimp *Upogebia pugettensis* (Dana, 1852) once ranged from Morro Bay, California, to Prince William Sound, Alaska. *Upogebia pugettensis* are geochemical ecosystem engineers that have large impacts on the benthic intertidal mudflats of Pacific Northwest (PNW) estuaries. The community composition of PNW mudflats is often structured around these shrimp (Posey et al. 1991; Griffen 2002; Smith et al. 2008;

D'Andrea and DeWitt 2009; Ferraro and Cole 2011). Beds of *U. pugettensis* previously covered thousands of estuary hectares and comprised thousands of tonnes of biomass (Chapman and Carter 2014), but *U. pugettensis* beds have declined drastically since 1998, and appear to be locally extinct in many areas (Dumbauld et al. 2011). Population collapses and extinctions are occurring in association with intense infestations of the introduced Asian bopyrid isopod parasite, *Orthione griffenis* Markham, 2004. Excepting five Oregon populations that appear to be slowly

declining, all *U. pugettensis* populations within the species range appear to have declined to functional or absolute extinction since the arrival of *O. griffenis* in the 1980s (Smith et al. 2008; Dumbauld et al. 2011; Chapman et al. 2012; Chapman and Carter 2014). The striking variation in *U. pugettensis* declines between Washington, Oregon, and California after the arrival of *O. griffenis* (Chapman et al. 2012) reflect persistent differences among northeast Pacific estuary ecosystems, that are altering the local host-parasite population dynamics and responses of *U. pugettensis* populations to *O. griffenis* invasions. Resolving how those regional differences occur is critical for conservation and requires deep knowledge of how the host-parasite population dynamics occur. The interactions of this non-coevolved marine bopyrid and its new hosts are of particular interest for invasion ecology because native bopyrid isopod/host interactions presumed in other systems may not apply to *O. griffenis* in North America.

Reduced female reproduction (Nelson et al. 1986), or effective castration (O'Brien and Van Wyk 1985; Smith et al. 2008; Dumbauld et al. 2011), has been the most commonly reported effect of bopyrid parasites on shrimp hosts. However, alternative effects, including feminization or male sex change (Tucker 1930; Griffen 2009) and increased male mortality (Repetto and Griffen 2012), have also been proposed. Gonochorism (distinct sexes within a species) and sequential, or possibly simultaneous, hermaphroditism have been identified among the few mud shrimp for which sexual development has been resolved (Tucker 1930; Dumbauld et al. 1996; Smith et al. 2008; Griffen 2009; Nanri et al. 2010). Intersex individuals (bearing characteristics of both sexes) have been observed among *Upogebia major* (De Haan, 1841) (Nanri et al. 2010), but the cause of hermaphroditism in these individuals was unclear. Smith et al. (2008) found that *O. griffenis* infestations are consistently less common among *U. pugettensis* males than among females. Feminization is a common explanation of unequal infestations observed among sexes in other crustacean host/parasite systems (e.g. Tucker 1930; Beck 1980).

The gram per gram energetic exchange between bopyrid isopods and their decapod hosts that results in effective castration of females is the most fundamental parameter for population analyses, if host-parasite dynamics are energetically driven. Experimental resolution of energetic exchanges by *in situ* experiments, or in laboratory cultures of vulnerable stage *U. pugettensis* (i.e. Anderson 1977) are, so far, impossible. Reproductive sized *U. pugettensis* that are vulnerable to *O. griffenis* infestations are incompetent at re-burrowing (Thompson 1972; Jackson et al.

2011) and survive poorly in culture. Smith et al. (2008) therefore compared weights of infested and uninfested *U. pugettensis* to estimate energy dynamics, and while infested shrimp of comparable size weighed less than un-infested shrimp, no correlations were observed between *O. griffenis* weights and weight loss of either *U. pugettensis* sex. Griffen (2009) and Repetto and Griffen (2012) recognized that this lack of correlation between host weight loss and *O. griffenis* weight placed the energetic basis for this interaction in doubt. They expanded on Smith et al.'s (2008) survey and re-examined alternative host-parasite interactions with potentials to control the sex ratios and the dynamics of *U. pugettensis*.

Griffen (2009) compared 500 *U. pugettensis* that were collected from five intertidal elevations ranges across a Yaquina Bay tide flat using a yabby pump (a modified suction pump [Griffen 2009; Dworschak 2015]) in August 2008. He distinguished male and female *U. pugettensis* by the modified first pleopods occurring on mature females (as had Dumbauld et al. 1996 and Smith et al. 2008) and found increasing *O. griffenis* prevalence among ≥ 12 mm carapace length (CL) *U. pugettensis*, along with the previously observed increased proportion of *O. griffenis* prevalence among large females. Dumbauld et al. (2011) also found this pattern in Willapa Bay, Washington. Griffen (2009) concluded that sex change and tide exposure could control infestation success and that sex change was more likely to create sex biased infestations than male mortality.

Griffen (2009) proposed 4 testable but non-exclusive hypotheses to explain *O. griffenis* infestation patterns observed in *U. pugettensis*: 1) infestation risk for *U. pugettensis* increases as more water is pumped through their burrows; 2) infestation by *O. griffenis* increases male *U. pugettensis* mortality; 3) *O. griffenis* infestations feminize *U. pugettensis* males, with increasing probability as infestation time and tidal height increase (but also, that feminization is unlikely before *O. griffenis* grows to at least 100 mg) and, if any of the previous hypotheses should prove correct, that; 4) *U. pugettensis* is reproductively capable only in specific intertidal locations that minimize infection risk or maximize nutrient availability. Griffen's (2009) analyses depended on the quality of sex identifications and the sex ratio estimates across the entire tide range occupied by *U. pugettensis* and complete samples of the male and female size frequencies in the *U. pugettensis* meta-population. The secondary sexual characteristic of first pleopods that are present only on females, which Griffen (2009) used to determine shrimp sex, can be modified by environmental and biological factors (i.e. Nanri et al. 2010) and immature *U. pugettensis* females lack first

pleopods. Additionally, where sexes do not occur in uniform size distributions, under-sampling small size classes (via yabby pump collections) can bias overall sex ratio estimates (personal observation). Yabby pump samples also damage shrimp and separate parasites from the host more frequently than core or excavation samples (Dworschak 2015). The less reliable methods for sex determination and possibly biased sampling methods limited the power of Griffen's (2009) analyses. Griffen (2009) emphasized in his conclusions the importance of expanded sampling to test his hypotheses and the value of increasing the precision of sex determinations by using gonopores rather than modified first pleopods for shrimp <12 mm CL.

Repetto and Griffen (2012) re-examined the potential for feminization, increased mortalities among males, and tidal differences in *U. pugettensis* vulnerability to *O. griffenis* among 197 female and 214 male *U. pugettensis* they collected from Yaquina Bay in August 2009. Repetto and Griffen (2012) expanded their vertical and spatial sampling to 21 sites located over the range of tidal elevations inhabited by *U. pugettensis*. Their samples were again collected by yabby pump; however, they distinguished *U. pugettensis* sexes using ovipores, which occur on the coxae at the base of the 3rd walking leg of females (Figure 1), and spermatopores, which occur on the distal medial coxa of the 5th walking leg of males (Figure 2). Ovipores and spermatopores are primary external reproductive structures of thalassinid shrimps (Tóth and Bauer 2007; Smith et al. 2008). The expanded sample design and improved sex determinations of all shrimp sizes revealed, again, more *O. griffenis* infestations occurring among reproductive-sized females, and no evidence of feminization or tide level related differences in parasite prevalence.

Repetto and Griffen (2012) found poor correlations between their gonado-hepatosomatic index and host weight loss with parasite weight among females, and no correlation in these indices among males. Thus, again, how population declines of *U. pugettensis* could be related to energetic losses due to *O. griffenis* infestation remained unclear. Repetto and Griffen (2012) concluded that lethal effects on male hosts could contribute to female biased infestations if *O. griffenis* size becomes excessive relative to male host size. Dumbauld et al. (2011) however, had inadvertently tested this latter hypothesis in a 100-day starvation experiment that included parasitized and parasite free male and female *U. pugettensis*. Dumbauld et al. (2011) did not find male biased mortality effects of *Orthione* or evidence of sex change. Repetto and Griffen (2012) proposed that Dumbauld et al. (2011) examined only the presence/absence of the parasite rather than parasite size relative

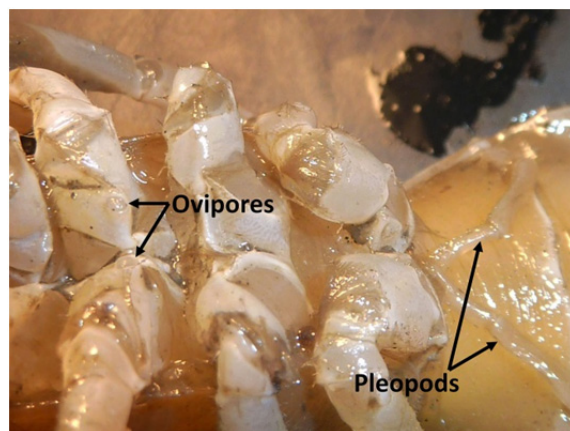


Figure 1. Ventral *Upogebia pugettensis* female ovipores on the third walking legs and pleopods on the first abdominal segment (arrows). Photo D. Asson.

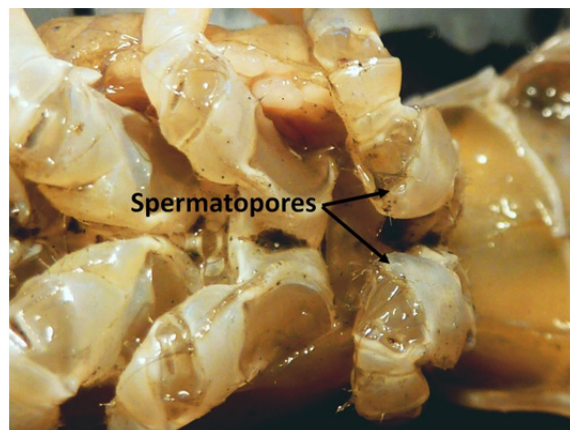


Figure 2. Ventral *Upogebia pugettensis* male with spermatopores on the fifth walking leg (arrows). Photo D. Asson.

to host size. However, Dumbauld et al.'s (2011) treatments included only large *Orthione* and thus created a sensitive test for sex-linked *Orthione*-induced mortality, but did not find it.

We expanded on Griffen's (2009) and Repetto and Griffen's (2012) tests of alternative castration mechanisms to energetic loss. All size/age classes of both sexes must be included in static samples used to test for sex change, or for differential mortality among sexes. Complete population structure is needed to control for differences in growth rates among sexes. All females that Repetto and Griffen (2012) identified by the presence of gonopores also bore modified first pleopods. Repetto and Griffen (2012) could have under-sampled <20 mm CL female age classes (which lack first pleopods) for their comparisons.

Although *U. pugettensis* < 12 mm CL are not infested by *O. griffenis* (Smith et al. 2008), their inclusion in samples of population structure is critical to achieve accurate estimates of sex ratios required to resolve meta-population dynamics. Isolated samples are insufficient to search for growth effects unless they include juveniles and all size classes. Dumbauld et al. (2011) and Repetto and Griffen (2012) thus lacked or under-sampled critical juvenile *U. pugettensis* size classes needed to test whether shrimp sex ratios change over the shrimp life history.

The mechanism(s) causing increased *O. griffenis* frequencies among female hosts are critical to resolve for understanding how this introduced parasite affects *U. pugettensis* population dynamics. Whether *O. griffenis* feminize *U. pugettensis* males or castrate females, for instance, would produce different effects on the remaining populations. Smith et al. (2008) and Dumbauld et al. (2011), for example, overestimated the prevalence of castration among females if male *U. pugettensis* are feminized by *O. griffenis*, and underestimated the extended impact of *O. griffenis* on their hosts if *O. griffenis* increase male mortality. We therefore expanded the spatial scale and magnitude of samples, and increased the detail of population analyses, to more clearly define the populations sampled. We predicted significant deviations from a 1:1 sex ratio where sex change either occurs naturally or is induced by parasites, and expected a high coincidence of intersex shrimp with *O. griffenis* infestations if infestation-induced sex change occurs. We tested for *O. griffenis* induced mortality among *U. pugettensis* by comparing the sex ratios among infested and uninfested populations and predicted a negative association of male *U. pugettensis* frequencies with *O. griffenis* frequencies if significant parasite induced mortality occurs.

Methods

We sampled *U. pugettensis* from 26 stations among 11 populations on 9 mudflats in 5 estuaries along the central Oregon, USA, coast between 25 May and 14 July 2010 (Figure 3, Table 1) to permit more rigorous tests of parasite associated feminization and differential mortality on *U. pugettensis* at a meta-population scale. We collected shrimp within each estuary at up to 3 stations (i.e. georeferenced locations, Table 1) along transects positioned over a tidal gradient to test for tidal interactions with infestations. The determination of upper-, mid-, and lower- intertidal was based on the distance from the station to the low tide level, not a specific elevation. Transects were spaced across several hundred meters of mudflat within each estuary. The sample sites at Garibaldi (Tillamook Bay),

Siletz Bay, and Coos Bay were too small for more than one station or for sampling over tidal gradients.

We attempted to collect at least 60 shrimp at each station to ensure representative samples of sizes and sexes. We collected ten samples at each station using a 12.5 cm wide by 40 cm deep core. We inserted the corer to its maximum depth (40 cm) into the sediments for the first sample section, and then inserted a second core into the bottom of the initial hole to recover sediments falling out of the first core. Our coring method extend samples to at 80 cm or greater depths. We dug excavations in the same areas as the initial cores to ≥ 80 cm depths by ≥ 1 m diameters using the corers and shovels (Dworschak 2015). Sediments from core samples and excavations were deposited on the deck of a mud-sled where they were broken apart to expose and collect all contained shrimp. Our excavations quickly filled with water which permitted recovery of swimming shrimp by hand or by seining using a 3 mm mesh dip-net (Dworschak 2015). Intact *U. pugettensis* burrows in the core and excavation samples indicated that minimum sediment disruption occurred in the collection process and that damage to the shrimp and parasite losses were minimal.

Less than 60 shrimp were recovered from the low density Garibaldi station in Tillamook Bay (Figure 3, point B) even after coring and excavation. We therefore collected by yabby pump several days later from an undisturbed area adjacent to the initial sample station and incorporated all shrimp sampled from this station into a single count. The possible bias due to yabby pump collection of shrimp in this sample did not affect the overall *U. pugettensis* sex ratio or infestation frequency (Figure 4).

Samples were frozen or refrigerated and processed within 3 days of collection. We measured carapace lengths (CL) of all *U. pugettensis* to the nearest 0.1 mm from the anterior tip of the rostrum to the posterior carapace edge using analog calipers. We distinguished *U. pugettensis* females by the modified first pleopods and by ovipores (Figure 1) and *U. pugettensis* males by the absence of the modified first pleopods and by the presence of spermatopores (Figure 2). We examined both branchial chambers of each *U. pugettensis* for *O. griffenis* under 6 \times magnification.

Orthione griffenis only infest *U. pugettensis* > 12 mm CL (Smith et al. 2008; Dumbauld et al. 2011; Chapman et al. 2012). We therefore estimated the effective *U. pugettensis* population available to *O. griffenis* as a function of length-classes > 12 mm CL multiplied by the numbers of shrimp per length class. We tested for differences in sex ratio and isopod infestation rates using Chi-squared tests, and used heterogeneity tests and contingency tables with

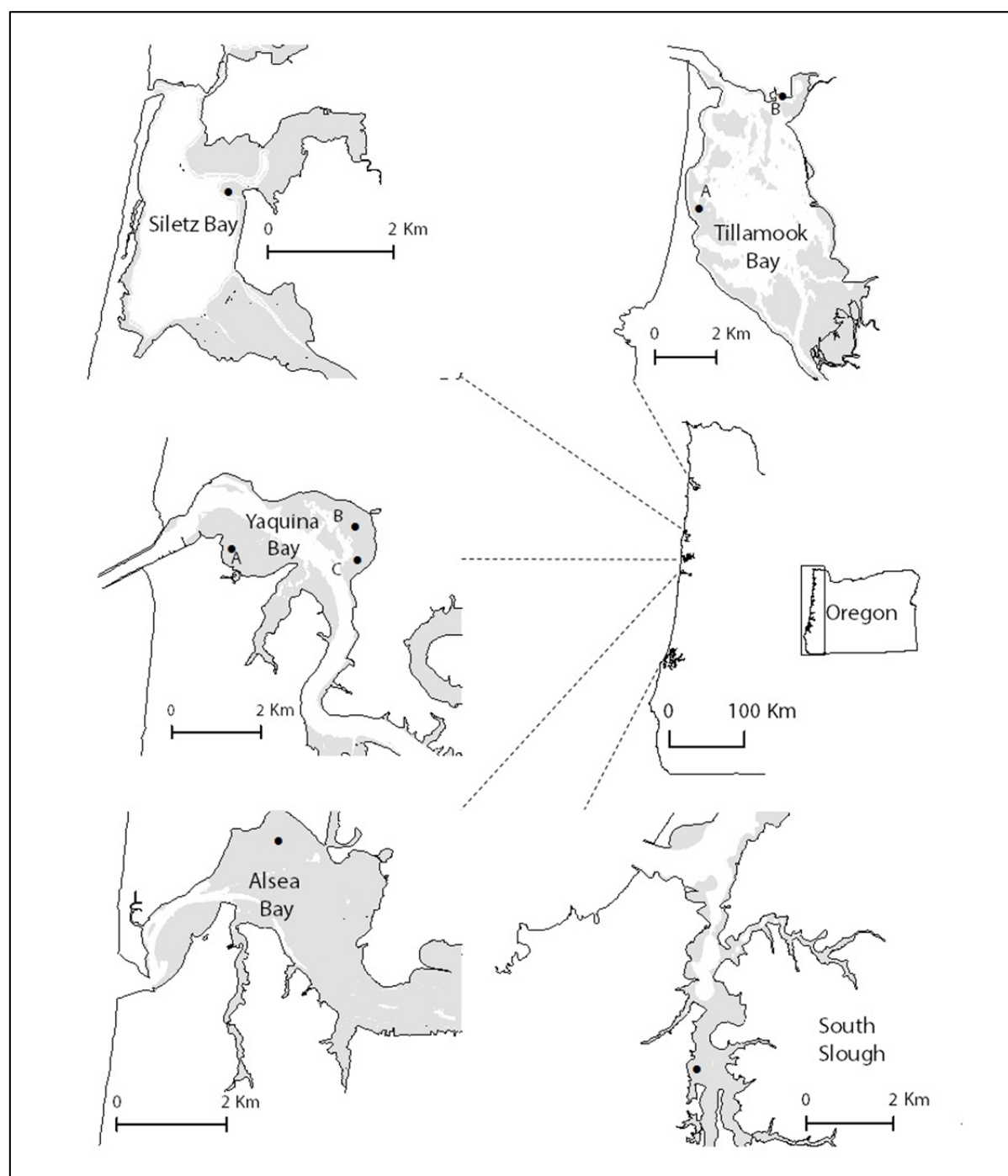


Figure 3. Survey areas (black dots are 1–3 stations each) for *Upogebia pugettensis* in Siletz Bay, Tillamook Bay, Yaquina Bay, Alsea Bay, and South Slough, Coos Bay, Oregon, USA (see Table 1 for coordinates).

Table 1. Number of shrimp and SR (sex ratio, M/F) by station, transect, and estuary (* = significantly different from 1:1 M/F ratio, ** = $p < 0.001$). Station: S = Seaward, M = Middle, L = Landward.

Estuary	Transect	Station	Longitude	Latitude	Females	Males	Total shrimp	SR/ station	SR/ transect	SR/ estuary
Yaquina	Idaho Point 1	S	44°26'32.55"N	124°02'58.69"W	35	44	230	1.26	1.21	
		M	44°26'40.19"N	124°02'55.84"W	38	37		0.97		
		L	44°26'48.74"N	124°02'52.83"W	31	45		1.45		
	Idaho Point 2	S	44°26'36.12"N	124°02'18.34"W	41	34	217	0.83	0.89	
		M	44°26'32.81"N	124°02'23.08"W	36	37		1.03		
		L	44°37'05.53"N	124°02'24.16"W	38	31		0.82		
	Idaho Point 3	S	44°37'02.04"N	124°02'29.63"W	51	36	267	0.71	0.84	0.95
		M	44°37'04.02"N	124°02'25.99"W	49	42		0.86		
		L	44°37'01.39"N	124°02'20.05"W	45	44		0.98		
	Sally's Bend 1	S	44°36'59.53"N	124°02'20.83"W	45	51	269	1.13	1.09	
		M	44°36'57.02"N	124°02'22.55"W	43	44		1.02		
		L	44°36'51.79"N	124°01'55.69"W	41	45		1.10		
Alsea	Alsea Bay 1	S	44°36'46.17"N	124°00'41.14"W	35	36	219	1.03	0.99	1.03
		M	44°36'46.08"N	124°00'35.74"W	34	42		1.24		
		L	44°37'12.70"N	124°00'42.46"W	41	31		0.76		
	Alsea Bay 2	S	44°37'16.53"N	124°00'37.40"W	32	28	96	0.88	1.13	
		M	44°37'21.44"N	124°00'32.45"W	13	23		1.77		
		L	44°37'21.44"N	124°00'32.45"W	13	23		1.77		
Tillamook	Tillamook 1	S	45°31'18.38"N	123°56'26.08"W	47	23	245	0.49*	0.61**	0.75*
		M	45°31'20.18"N	123°56'18.02"W	45	32		0.71		
		L	45°31'22.62"N	123°56'15.62"W	60	38		0.63*		
	Garibaldi 1	N/A	45°33'23.81"N	123°55'08.67"W	46	56	102	1.22	1.22	
Coos	Coos 1	N/A	44°55'24.24"N	124°00'57.37"W	41	22	63		0.54*	
Siletz	Siletz 1	N/A	43°19'50.52"N	124°19'38.89"W	32	63	95		1.97*	
TOTAL					1039	975	2014		0.94	

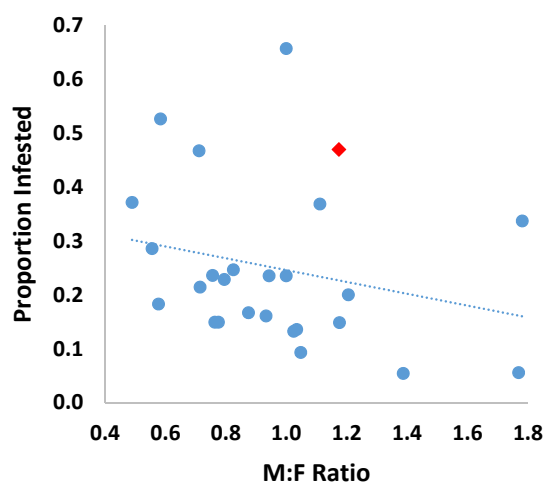


Figure 4. Infestation prevalence of ≥ 12 mm carapace length *Upogebia pugettensis* relative to the M:F sex ratio among 26 sample stations. Yabby shrimp additions to the Garibaldi sample (red point) did not appear to alter the proportion infested relative to sex ratio outside of the range of the other samples. Infested = $-0.09 \times \text{SR} + 0.34$. $R^2 = 0.03$, $df = 24$, NS.

a Mantel-Haenszel test to evaluate tidal height and independence amongst sampling stations. The relationship between parasite prevalence and host sex ratio was evaluated using least squares linear regression. We assumed significant differences where $p < 0.05$.

Results

Twenty-six percent of the 2,015 *U. pugettensis* collected were infested by *O. griffenis*. Sample sizes among stations ranged from 63 to 269 shrimp (Table 1) and the samples included a broad range of size classes (5–36 mm CL). We found a single hermaphrodite (bearing ovipores, pleopods, and complete spermatopores [Figure 5]). The hermaphrodite, which was not infested, was recovered from the landward station of the Idaho Point transect 3 population in Yaquina Bay (Figure 3, Table 1), where the *U. pugettensis* sex ratio was 1:1 and the infestation prevalence was 14%. The hermaphrodite was not included in the total population used for analyses, resulting in 2,014 shrimp used in calculations. We found three possible partial-intersex males (bearing

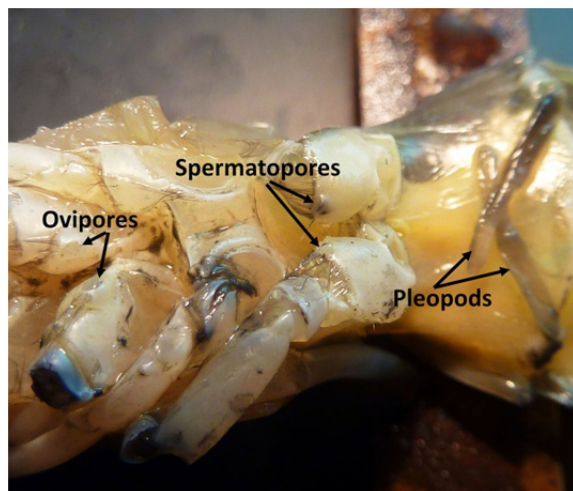


Figure 5. *Upogebia pugettensis* hermaphrodite with female ovipores on the third walking leg coxae, spermatopores on the fifth walking leg coxae and pleopods on the first abdominal segment (arrows). Photo D. Asson.

characteristics of each sex but not fully hermaphroditic) in Alsea Bay. Two of these males had complete spermatopores on the fifth walking leg and a single pleopod on the first abdominal segment, and one male had complete spermatopores and a single ovipore. The four shrimp bearing characteristics of both sexes accounted for 0.2% of the population. Only the male with a single ovipore was infested by *O. griffenis*. We assumed the partial-intersex shrimp were functional males and included them in comparisons of population structures.

Consistent with previous surveys, 67% of infestations were among female *U. pugettensis* (Figure 6A). Maximum *O. griffenis* size increased with carapace lengths of both *U. pugettensis* sexes but small *O. griffenis* infested all vulnerable host sizes (Figure 6B). The majority of infestations were among the largest size classes of both sexes (Figure 7A and B). Consistent with Smith et al.'s (2008) and Griffen's (2009) observations, male *U. pugettensis* were more frequent in the 5–22 mm CL classes, females were more frequent among >22 mm CL classes (Figure 7B) and 95% of infestations occurred among shrimp with ≥ 20 mm CL. *O. griffenis* prevalence ranged between 5% to 65% among stations, 13% to 47% among transects, and 18% to 46% among estuaries (Table 2).

Where data was taken at locations along transects (e.g. seaward – landward), tidal gradient did not significantly influence either overall sex ratio ($\chi^2 = 32.049$, $df = 22$, $p = 0.076$, Figure 8A), which was not significantly different from 1:1 (Table 1), nor infestation prevalence among >12 mm CL shrimp (Mantel-Haenszel $m^2 = 0.43038$, $df = 2$, $p = 0.8064$,

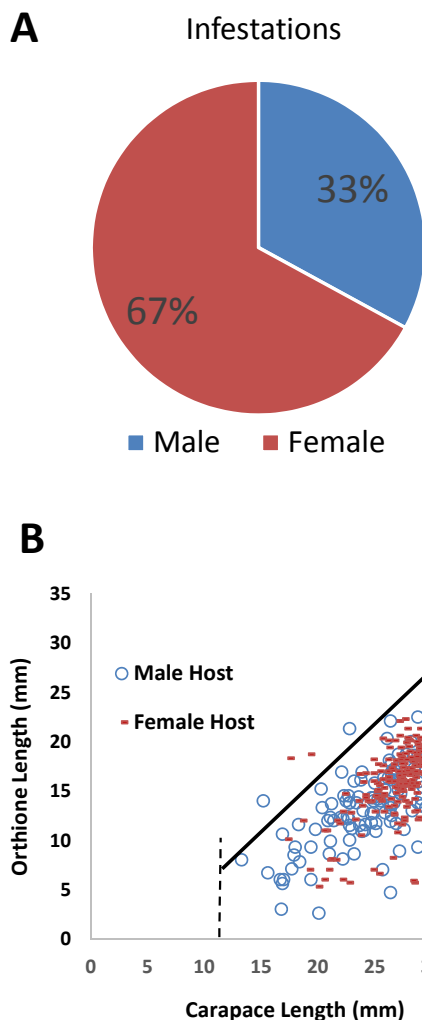


Figure 6. **A** – Proportions of infested male and female *Upogebia pugettensis* ($n = 508$) and **B** – Increasing *Orthione griffenis* lengths with carapace lengths of infested *U. pugettensis* from all collections including a dashed line at the lower 12 mm CL limit (below which *O. griffenis* infestations do not occur) and a solid line indicating the approximate upper limit of *O. griffenis* length per *U. pugettensis* carapace length.

Figure 8B). *Orthione griffenis* prevalence varied widely within tide levels but the highest prevalence was among seaward stations (Figure 8B). The sex ratio of >12 mm CL *Upogebia pugettensis* did not vary with *O. griffenis* prevalence ($r^2 = 0.03$, $df = 24$, NS; Figure 4) or with tide level/distance from channels (Table 1). Sex ratio of infested shrimp also did not vary with tide level (Figure 8C). We found no evidence of different host sex ratios or infestation prevalence among estuaries and therefore pooled the data to permit meta-population level analyses.

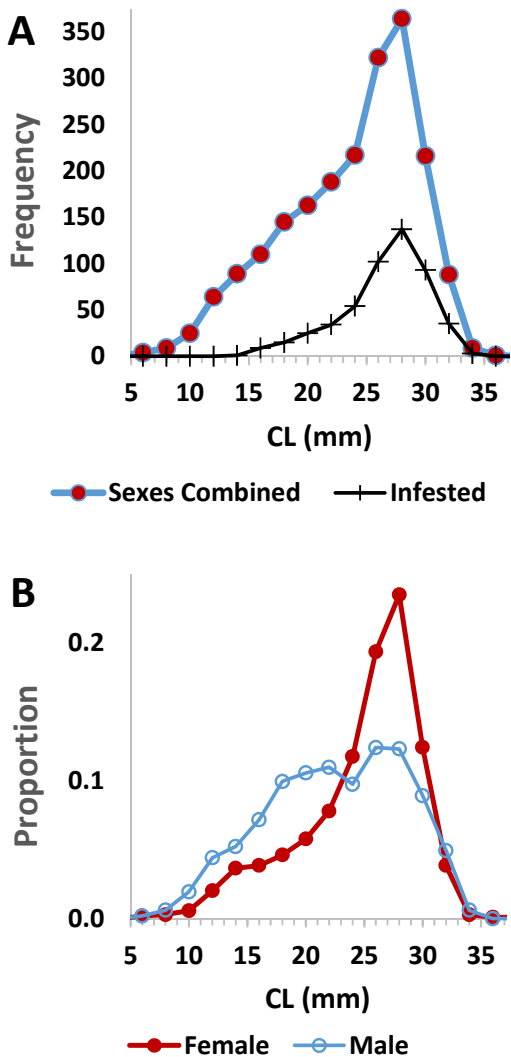


Figure 7. A – Frequencies of all *Upogebia pugettensis* and all infested *U. pugettensis* among 2 mm carapace length classes; and; B – proportions of the 1039 female and 975 male *U. pugettensis* among 2 mm carapace length classes.

The *U. pugettensis* male/female sex ratio among all samples (Table 1) was 0.94 (with 66 more females than males). A 1:1 sex ratio was common at most sampling locations (Table 1) and amongst >12mm CL shrimp most vulnerable to infestation by *O. griffenis* (Table 2). The average infestation prevalence among vulnerable size classes was 26% but varied widely among individual stations, transects, and estuaries (Table 2). Infestations were significantly higher among females than males ($\chi^2 = 68.208$, $df = 1$,

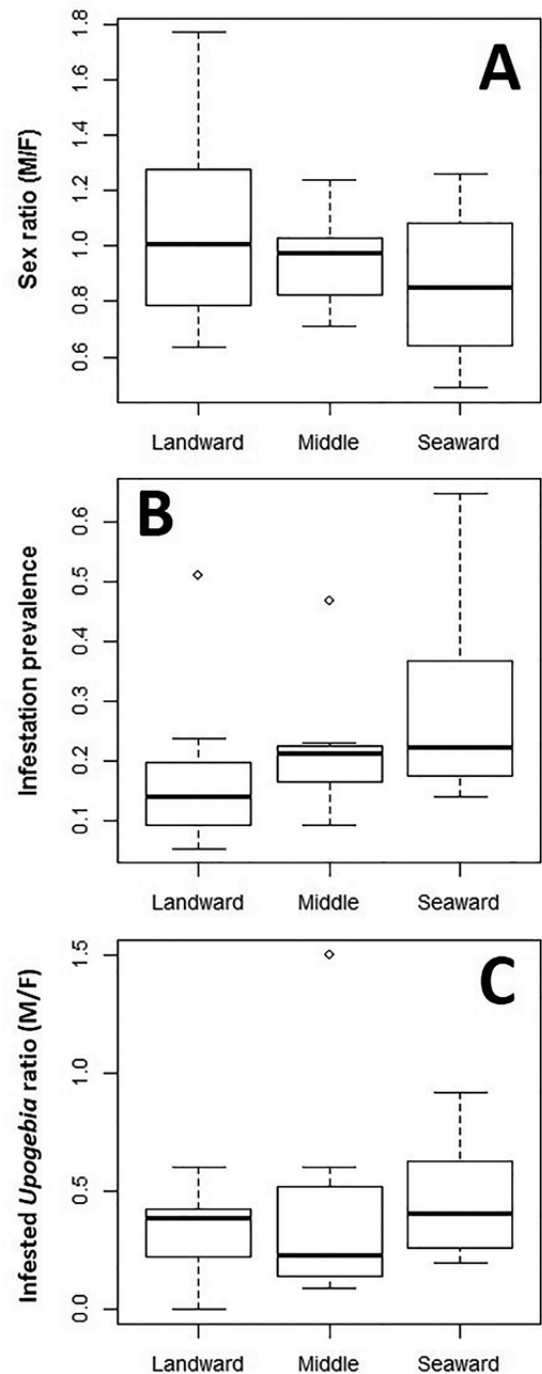


Figure 8. Maximum and minimum, upper and lower quartiles and medians of: A – *U. pugettensis* sex ratio among stations with tidal level; B – prevalence of *O. griffenis* among >12 mm CL *U. pugettensis* among stations with tide level; and; C – sex ratio (M/F) among infested *U. pugettensis* among stations with tide level.

Table 2. Number of shrimp and SR (sex ratio, M/F) by station, transect, and estuary for all shrimp with a carapace length ≥ 12 mm (* = significantly different from 1:1 M/F ratio, ** = $p < 0.001$; + = significantly different infestation frequency than the overall frequency of 26%, ++ = $p < 0.001$). Station: S = Seaward, M = Middle, L = Landward.

Estuary	Transect	Station	Total shrimp	SR/station	SR/transect	SR/ estuary	Infested females	Infested males	% infested/ station	% infested/ transect	% infested/ estuary
Yaquina	Idaho Point 1	S		1.17			9	2	14 ⁺		
		M	220	0.94	1.16		13	3	23	14 ⁺	
		L		1.39			3	1	5 ⁺⁺		
	Idaho Point 2	S		0.85			13	5	24		
		M	212	1.00	0.88		14	2	23	21	
		L		0.79			9	1	15 ⁺		
	Idaho Point 3	S		0.72			15	3	21		
		M	261	0.78	0.81	0.92	11	1	14 ⁺	17 ⁺	18 ⁺⁺
		L		0.96			10	4	16 ⁺		
	Sally's Bend 1	S		1.13			22	13	36 ⁺		
		M	268	1.05	1.09		5	3	9 ⁺⁺	20	
	Sally's Bend 2	L		1.10			8	3	13 ⁺		
		S		0.58*			9	6	18		
		M	211	0.79	0.76*		14	2	23	18 ⁺	
		L		1.03			5	3	14 ⁺		
Alsea	Alsea Bay 1	S		1.03			24	22	65 ⁺⁺		
		M	218	1.21	0.98		6	9	20	36 ⁺	
		L		0.76		1.02	12	5	24		29
	Alsea Bay 2	S		0.88			7	3	17 ⁺		
		M	96	1.77	1.13		2	0	6 ⁺	13 ⁺	
Tillamook	Tillamook 1	S		0.49*			20	6	37 ⁺		
		M	242	0.71	0.59*		25	11	47 ⁺⁺	46 ⁺⁺	
		L		0.58*		0.73*	35	15	53 ⁺⁺		46 ⁺⁺
	Garibaldi 1	N/A	101	1.20	1.20		28	19	47 ⁺⁺		
Coos	Coos 1	N/A	56		0.56*		10	6		29	
Siletz	Siletz 1	N/A	91		1.84*		10	20		33	
Total			1976		0.92		340	168		26%	

$p < 0.001$) and totals included 340 *U. pugettensis* females versus 168 males (Table 2). Assuming unbiased *O. griffenis* settlement among sexes, our expected excess females with sex change (the number of *U. pugettensis* that could have changed sex, if sex change occurs) was therefore: $(340 - 168)/2 = 86$.

Discussion

Our results indicate that bopyrid isopod induced feminization (Griffen 2009), differential mortality among sexes (Griffen 2009; Repetto and Griffen 2012), and differential exposure to isopods driven by tidal position (Griffen 2009) did not occur in *U. pugettensis*. We recovered more infested females than males from vulnerable reproductive-sized *U. pugettensis* in our sample while the sex ratio was not significantly different from 1:1.

Previous to the occurrence of abundant *O. griffenis* in Willapa Bay, Dumbauld et al. (1996) found approximately 43% males and 57% females among *U. pugettensis* (a sex ratio of 0.75). Repeating the comparisons among >12 mm CL shrimp (sufficient

size for reliable sex determinations based on pleopods) before 1998 when no shrimp were infested, the female to male sex ratio was 0.49 and did not change among infested and uninfested shrimp after 1998. Thus, females were more common than males at the Willapa Bay sample location, but the Willapa Bay sex ratios were within the range of sex ratios we found in this study (Table 2). *O. griffenis* thus did not appear to alter the *U. pugettensis* sex ratio in Willapa Bay.

We also found no evidence of *O. griffenis* induced sex change. Among the 26 stations sampled in 2010, sex change, with random *O. griffenis* settlement among *U. pugettensis* sexes, would have created 86 additional females and 86 less males in the sample $([340 - 168]/2 = 86)$ and would have altered the sex ratio to $902.5/1,074.5 = 0.84$, significantly different from 1:1 ($\chi^2 < 0.0001$, $df = 1$). *O. griffenis* induced male mortality requires that the 172 males were lost from the larger population. With random *O. griffenis* settlement the initial population would have been $1,977 + 172 = 2,149$ and the expected females would have been, $2,149/2 = 1,074.5$. Expected males would have been, $1,074.5 - 172 = 902.5$ and thus, the sex

bias toward females would again be 0.84, significantly different from 1:1 ($\chi^2 < 0.0001$, $df = 1$). Thus, the observed 0.92 sex ratio is also significantly different from the expected 0.84 sex ratio with sex change or male mortality ($\chi^2 < 0.04$, $df = 1$).

Moreover, the one *U. pugettensis* hermaphrodite that we found was not infested, and only one of the 3 other male shrimp with female characteristics was infested. The 25% infestation frequency among the 4 intersex shrimp was therefore the same as among the overall population. Thus, not even the rare intersex shrimp were likely results of *O. griffenis* infestations. In common with many other gebiid shrimp, *U. pugettensis* appear to be gonochoristic, and to maintain a nearly equal sex ratio that is unaffected by bopyrid infestations.

The approximate 1:1 M:F ratio was consistent across all stations, regardless of tidal position or infestation rate (Tables 1 and 2). Infestations were more prevalent in seaward populations than middle or landward stations while landward and middle stations were similar. However, there was a wide variance in infestation rates among all stations; seaward stations in particular experienced infestation rates from 14%–65% (Figure 8B), indicating the infestation rates are more likely a result of multiple factors rather than tidal location alone. This pattern may warrant further investigation.

A lack of settling stage *O. griffenis* in our samples precluded analyses of parasite recruitment (exposure) and survival with the prevalence of *O. griffenis* with tide level, or with distance from channels. The greater *O. griffenis* prevalence at deeper or seaward stations that we found could result from greater host pumping times, volumes, and exposure, as suggested by Griffen (2009), or from greater *O. griffenis* survival. We found no evidence of isopods lost to host molting (Dumbauld et al. 2011). The greater prevalence of *O. griffenis* among *U. pugettensis* females also appears more likely to result from the greater prevalence of females in the largest and most vulnerable size classes, rather than selection among sexes (Figure 7B). We suggest that female and male *U. pugettensis* experience differential growth with females growing more quickly to vulnerable sizes where they live longer than males. The differential growth could account for the skewed sex ratio among infested shrimp.

Increasingly expanded surveys, observations, and integrations of results over multiple studies were required to isolate and test these alternative hypothesis (Smith et al. 2008 and herein; Griffen 2009; Dumbauld et al. 2011; Repetto and Griffen 2012). Other extensive comparisons of bopyrid infestations

among Gebiid shrimp sexes have included *Gyge branchialis* in *Upogebia pusilla* (Petagna, 1792) (as *U. littoralis* [Ngoc-Ho 2003]) (Tucker 1930; Pascal et al. 2016). *Gyge branchialis* Cornalia and Panceri, 1861 infested all sizes of *U. pusilla* but were most prevalent among middle host size classes (Tucker 1930). Tucker (1930) found 26% more infested males than females. However, Pascal et al. (2016) found equal infestations among sexes which, in general, corresponds to our results. Both Tucker (1930) and Pascal et al. (2016) may have under-sampled small shrimp that possibly were not infested by *G. branchialis*. Ubaldo et al. (2013) found high intersex frequencies (44%) among Japanese *Upogebia major* populations infested by *Gyge ovalis* (Shiino, 1939), but these were not associated with *G. ovalis* infestations.

By elimination of alternative hypotheses, *O. griffenis* appear to effectively castrate their hosts by energetic loss as previously assumed for coevolved bopyrids and hosts (Tucker 1930; Callan 1940; Ubaldo et al. 2013; Sherman and Curran 2015). Modified secondary sex characteristics including feminized chelae of male *U. pugettensis* and larger, masculinized chelae in infested females are however associated with *O. griffenis* infestations (Woods et al. 2007). These changes could also result from energetic losses or hormonal changes. The bodies of infested female *U. pugettensis* could also possibly shrink faster than their chelae. Repetto and Griffen (2012) compared the differentiation of the female ovaries from the hepatopancreas, which is a main energy-storage organ of crustaceans (Gibson and Barker 1979). The hepatopancreas increases in size before reproduction and declines during vitellogenesis as energy and nutrients for egg production are withdrawn (Kennish 1997). Repetto and Griffen (2012) concluded that their shrimp had initiated vitellogenesis because they could differentiate ovaries from the hepatopancreas in 27 of the 197 female *U. pugettensis* they examined. The absence of *Orthione* infestations among the 27 differentiated females when 24% of all females were infested by *Orthione* is strong evidence of effective castration and likely energy loss among the parasitized females. We thus concur with Repetto and Griffen (2012) that energetic exchanges from *U. pugettensis* to *O. griffenis* are more likely observable over time, spanning reproductive periods, seasons, or even the lifespans of these hosts and their parasites.

Chapman et al. (2012) reported six “abundant” *U. pugettensis* populations (Tillamook Bay, Netarts Bay, Sand Lake, Siletz Bay, Yaquina Bay and Alsea Bay) remaining in the 18 estuaries within their 1,660 km (Morro Bay, California to Bamfield, British Columbia)

survey of 2008. Those six abundant populations occurred within a 124 km coastline of Oregon (7.5% of the survey range). The Siletz population has since collapsed (personal observations) and thus 9 years later, 5 “abundant” populations remain and the decline of all populations appears to continue (Chapman and Carter 2014). The nonindigenous origins of *O. griffenis* in North America preclude all assumptions of host resilience, equilibrium domains (Gunderson 2000), or other presumptions of long-term persistence of this host-parasite system. Energetic losses to *O. griffenis* can only have three outcomes: unpredictable extinction of *O. griffenis*, coexistence, or *U. pugettensis* extinction. The absence of alternative hosts has not prevented *O. griffenis* from driving *U. pugettensis* populations to local extinction. Immediate, urgent responses to resolve the dynamics of this host-parasite system that permit either *U. pugettensis* persistence or rapid functional or complete extinction are critical for conserving the remaining *U. pugettensis* populations.

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