

**LOCAL FACTORS AFFECTING  
MORPHOLOGICAL ABNORMALITIES IN  
THE MUD SHRIMP, UPOGEBIA MAJOR, INHABITING  
MUD TIDAL FLATS OF THE SETO INLAND SEA**

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**Jonathan Pino Ubaldo**

**The Graduate School of Natural Science and Technology  
(Doctor Course)**

**OKAYAMA UNIVERSITY**

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## I. ABSTRACT

Estuarine and coastal areas are some of the environments most severely affected by anthropogenic activities. Animal communities found in these habitats face the greatest risk of exposure to altered environments such as increased levels of pollution from terrestrial sources and effluents. Thus, these organisms are potential indicators of the level of degradation in the local ecosystem.

*Upogebia major* is a burrow-dwelling and mainly filter-feeding gonochoristic Thalassinidean widely distributed throughout mud flats in Japan. Amongst *U. major* surveyed in the Seto Inland Sea, the population in Kasaoka Inlet was found to have relatively higher frequencies of intersex male morphology consisting of a single or a pair of first pleopods normally found in the female but absent in the male (Nanri *et al.*, 2011). However, the cause has not yet been confirmed.

Mud flats are not homogeneous environments but have spatial complexity (i.e. tidal gradients, variation in sediment composition); however, information on how abnormal morphologies are distributed spatially in the habitat is lacking. Intersex features may have been elicited by the advancing sediment contamination in the area or by modification of host mud shrimp sex characters by the branchial epicaridean parasite, *Gyge ovalis*, which was prevalent in this population.

This study investigated the occurrence patterns of intersex morphologies within the Kasaoka Inlet population of *U. major* with regards to life history, relationship with tidal level position, and co-occurrence with parasitic infection. A total of 1,623 males and

877 females were sampled from 10 points in the mud flat, from both upper and lower tidal areas.

Intersex features were not correlated with total body length or the distance from the tip of the rostrum to the telson (GLM,  $n=1,623$ ,  $P=0.714$ ). These appeared at any size of the mud shrimp and were permanent once they emerged. In contrast, parasitic infection prevalence was highest in small specimens (70 to 80% in specimens with TL <50 mm) and decreased with larger host mud shrimp, being almost nonexistent in hosts with TL >100 mm, in both sexes. Castration of the host was evident, especially in females, and chela size was reduced in males but slightly increased in large females. However, infection was not a significant predictor of intersex (GLM,  $n=1,623$ ,  $P=0.212$ ). Males that were both intersex and infected ( $n =18$ ) were much fewer than those having only one of either condition (intersex,  $n =143$ ; infected,  $n =261$ ). Nevertheless, trends in infection prevalence suggest that early infection followed by recovery from the parasite in the course of growth could be associated with the intersex morphologies and may account for majority of cases that were intersex but parasite-free.

On the other hand, intersex occurrence was correlated with deletions of the cuticular ridge (CRD) between the first and second abdominal segments (GLM,  $n=1,289$ ,  $P<0.01$ ), and with tidal level position (GLM,  $n=1,623$ ,  $P<0.01$ ). Lower tidal zone (LTZ) mud shrimp were three and four times more likely to be intersex and exhibit CRD, respectively, than those in the upper tidal zone (UTZ). *G. ovalis* prevalence in small males (TL <70 mm) in the LTZ were around twice that in the UTZ. Thus, higher rates of early infection may explain the higher frequency of intersex in the lower tidal areas. In addition, the preliminary survey showed much higher levels of the developmental

toxicant organotin, tributyltin, in the fine sediments found near the lower edges of *U. major* habitat, when compared to the sediment in the area where burrows can be found. Consequently, their proximity to contaminated fine sediments and longer periods of submersion (i.e. longer time for filter feeding) place mud shrimp inhabiting the lower tidal areas at greater exposure to sediment-bound contaminants. This may lead to higher rates of intersex and CRD in *U. major* occupying the LTZ.

## II. INTRODUCTION

Intersex individuals that possess both male and female characteristics are frequently reported among crustaceans including branchiopods (Bowen & Hanson, 1962; Mitchell, 2001), copepods (Gusmão & McKinnon, 2009), isopods (Rigaud & Juchault, 1998) and amphipods (Kelly *et al.*, 2004; Yang *et al.*, 2011). In decapod crustaceans, intersexuality has been reported in hermit crabs (Fantucci *et al.*, 2007; Turra, 2004), lobsters (Farmer, 1972), crabs (Johnson & Otto, 1981; Zou & Fingerman, 2000), crayfish (Curtis & Jones, 1995; Sagi *et al.*, 1996; Rudolph, 2002), and mud shrimps (Tucker, 1930; Tunberg, 1986; Pinn *et al.*, 2001).

Causes of intersex that have been suggested include incomplete feminization by cytoplasmic parasites in isopods (Juchault *et al.*, 1991; Rigaud & Juchault, 1998) and amphipods (Kelly *et al.*, 2004), and variation in androgenic gland development in crayfish (Sagi *et al.*, 1996; Rudolph *et al.*, 2001) and fiddler crab (Zou & Fingerman, 2000). Pollutants have also been increasingly reported to cause sexual disruption in copepods (Moore & Stevenson, 1991), freshwater crabs (Takahashi *et al.*, 2000; Ayaki *et al.*, 2005) and several other crustaceans (Depledge & Billingham, 1999; Olmstead & LeBlanc, 2007). Hence, the manifestation of intersex individuals is a potential biomarker of environmental endocrine or other forms of sexual disruption (LeBlanc, 2007). However, given the number of other factors that may cause intersex, there is a need to ascertain whether the real etiological factor is an environmental pollutant. Ford (2012) argued that more field-based studies should be conducted to test whether these abnormalities are occurring in the wild against background population variability, and whether intersex individuals are products of transitional phases normal in the life history

of sequential hermaphrodites (i.e. protandric or protogynous species). Furthermore, parasitic infections may elicit feminizing and demasculinizing effects (Tucker, 1930; Reinhard, 1956). Therefore, in a given field population with intersex individuals, the interactions between pollution, intersex and parasitism need to be clarified.

Intersex has only recently been reported in the burrow-inhabiting gonochoristic thalassinidean, *Upogebia major* (Nanri *et al.*, 2011). Some males have a single or pair of pleopods on the first abdominal segment that are normally only found on females, but retain other normal male characters (i.e. gonopore position, chela size, testes). *U. major* is widely distributed in Japan and intersex is rare except in Kasaoka Inlet, Seto Inland Sea where the advancing contamination of sediments in the area is a concern (Fukue *et al.*, 2003). A zone of very fine sediments, which are often reservoirs of sediment-bound pollutants, surrounds the lower tidal areas of the habitat in Kasaoka. Thus, pollution may be a possible influencing factor particular to this habitat, which may lead to the emergence of intersex.

On the other hand, *U. major* is also a definitive host of the branchial epicaridean *Gyge ovalis* (formerly *Metabopyrus ovalis*) (Shino, 1939; Markham, 2001; Itani, 2004; An *et al.*, 2009). Some of these parasites can partially or completely castrate and modify the external secondary sex characters of their decapod crustacean hosts (i.e. chela size reduction), especially in the male (Reinhard, 1956; O'Brien & Van Wyk, 1985; Dumbauld *et al.*, 2011). Males with first pleopods were reported to be very rare in congeneric *Upogebia deltaura* (Tunberg, 1986) and *Upogebia stellata* (Pinn *et al.*, 2001), but were common in at least one population of *Upogebia pusilla* in which infection by an epicaridean, *Gyge branchialis* was widespread (Tucker, 1930). In *U. major*, the

prevalence of bopyrid infection, effects of the parasite on host sexual development and reproduction, and their relation to intersex are unknown.

In this study, we focused on the population of *U. major* in Kasaoka previously found by Nanri et al. (2011) to have relatively high proportion of intersex males, and sought to understand the factors particular to this habitat that may lead to intersex. We examined the prevalence of *G. ovalis* and its effects on the general morphology and growth of *U. major*. We tested whether (1) being a host made an individual more likely to be intersex and (2) prevalence of infection varied together with intersex occurrence according to host life history (i.e. size and age) and according to tidal level. Moreover, we measured levels of tributyltin (TBT), a sediment-bound potential endocrine disruptor (Ohji et al, 2002) and developmental toxicant (Weis and Kim, 1988) in various points within the habitat and in fine sediments of the lower tidal zone to evaluate whether variation in TBT levels coincided with intersex.

### III. MATERIALS AND METHODS

#### **Sample Collection**

*Upogebia major* specimens were collected from a mud flat area in Kasaoka Inlet (N 34° 28' 33", E 133° 31' 43"), Okayama Prefecture, along the Seto Inland Sea of Japan (Figure 1A and 1B). Sampling occurred twice per month during periods of the lowest low tides, from late November 2011 through April 2012 concurrent with the breeding period in *U. major*. Samples were taken from five stations in the lower (L1 to L5), and five stations in the upper (U1 to U5) intertidal zones (Figure 1B). The relative position of each station was estimated from its perpendicular distance from the low water line ( $LWL_{est}$ , Figure 1B). We estimated the  $LWL_{est}$  of the advancing tidal edge using image overlay feature in Google Earth (version 6.2.2.7373; build date: 6/25/2012) and the distance of each station at right angles from  $LWL_{est}$  using ImageJ software (Rasband, 1997-2012).

Within a 10 m radius of each station, two to three holes of about 2 m in diameter were excavated to 80 cm depth in order to sample all sizes of the mud shrimp. Larger specimens were collected by hand and smaller specimens were sampled using a 2 x 2 mm fine wire mesh. Specimens were transported to the laboratory, inspected individually, and stored in -30 degrees Celsius deep freezer.

Some normal specimens and some with morphological abnormalities (i.e. abnormal pleopods, cuticular lesions and deletions) from the first two collections were placed inside artificial U-shaped PVC burrows submerged in rectangular polyethylene tanks 2m long x 1m wide x 0.3m deep with aerated and flowing seawater. For 3 months,

these specimens were checked twice a week for ecdysis, exuviae were collected and newly ecdysed mud shrimp were re-examined for changes in the external morphology, especially those individuals with abnormalities.

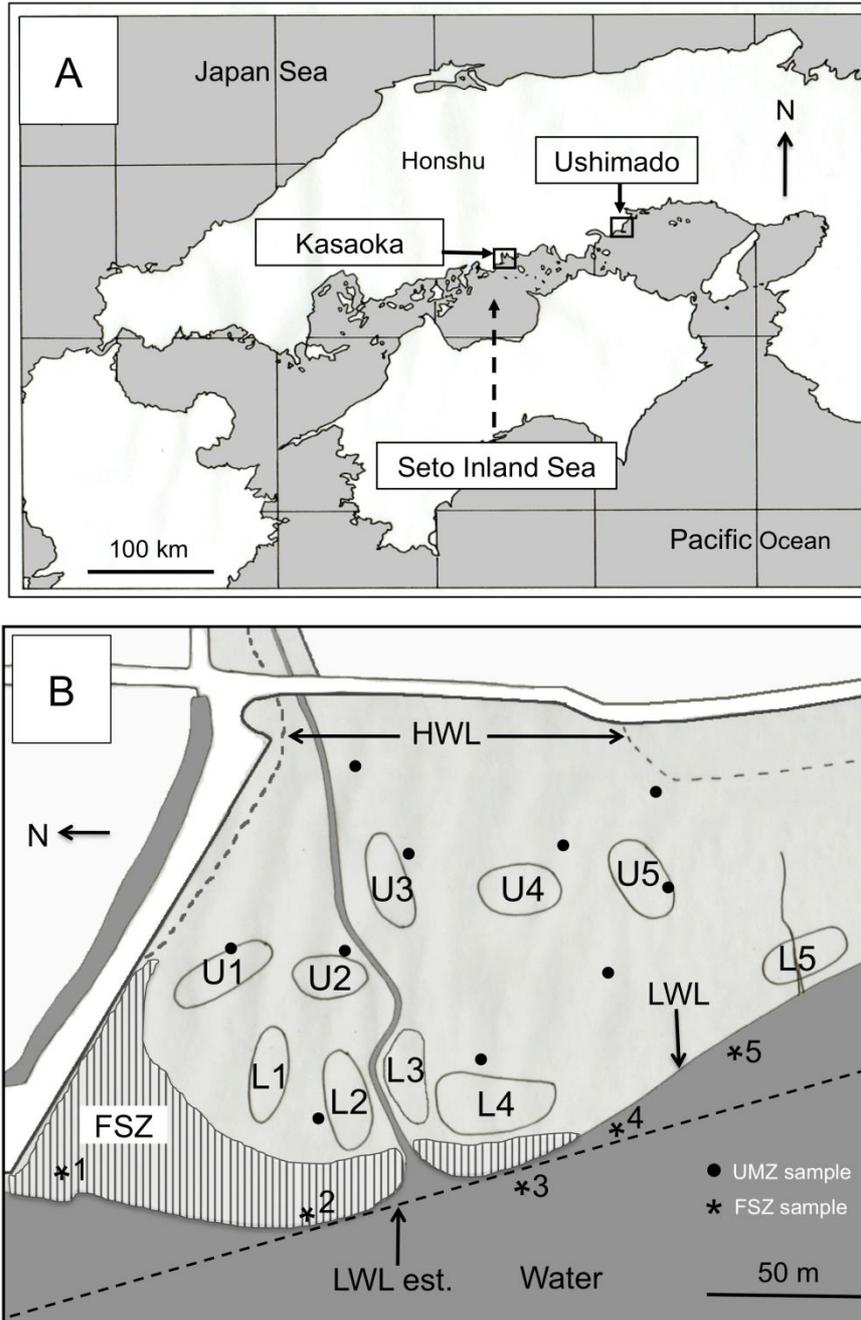


Figure 1. Study area maps: (A) Sampling sites of *Upogebia major* in the Seto Inland Sea, Japan; (B) Kasaoka Inlet, lower tidal zone (L1 to L5) and upper tidal zone (U1 to U5) sampling locations. Areas in stripes indicate fine sediment zones (FSZ); HWL, high water line; LWL, low water line. Sediments were sampled for organotin content analysis within the mud shrimp habitat (UMZ sample, solid dots) and in the FSZ (FSZ sample, asterisks).

## **Shrimp morphology and parasites**

Specimens were classified as male or female on the basis of: (1) the presence of the first pair of pleopods (Ple I) in normal females and its absence in the normal male (Figure 2A), (2) the gonopores located at the base of the fifth and third coxae in males and in females, respectively, and (3) the sexually dimorphic characteristics of the chela. In males, a row of knobs line the outer side of the dactylus while 2 to 3 distinct ridges can be found on its inner surface. In contrast, females have an almost smooth dactyl on both inner and outer surfaces.

Total length (TL) from the tip of the rostrum to the edge of the tail fan was measured by a digital caliper (accuracy of 0.1 mm). Propodus width (PW) was measured at the widest point of the propodus. Ple I lengths of intersex males and normal females were measured for comparisons of normal female and intersex male Ple I growth. We did not find newly settled males and females that yet lacked Ple I. Thus, supplementary data on the size at which Ple I normally appears in the females were derived from small females collected from another habitat in Ushimado, located around 60 km NE of Kasaoka (Fig. 1A).

The gill chambers of each specimen were checked for the parasite, which was identified as the epicaridean, *Gyge ovalis*. Infected individuals were externally marked by a swollen branchiostegite on the occupied gill chamber. In five males and five females, the gill chambers were empty but distended like in other infected specimens, and castration in different degrees was evident. Thus, we elected to include these individuals under the infected group in the analysis. Other abnormalities in the external morphology were noted such as blackened cuticular lesions, deletions in the cuticular ridge of the

ventral junction of the first and second abdominal segments (CRDs), and the presence or absence of the Ple I and other abnormal appendages.

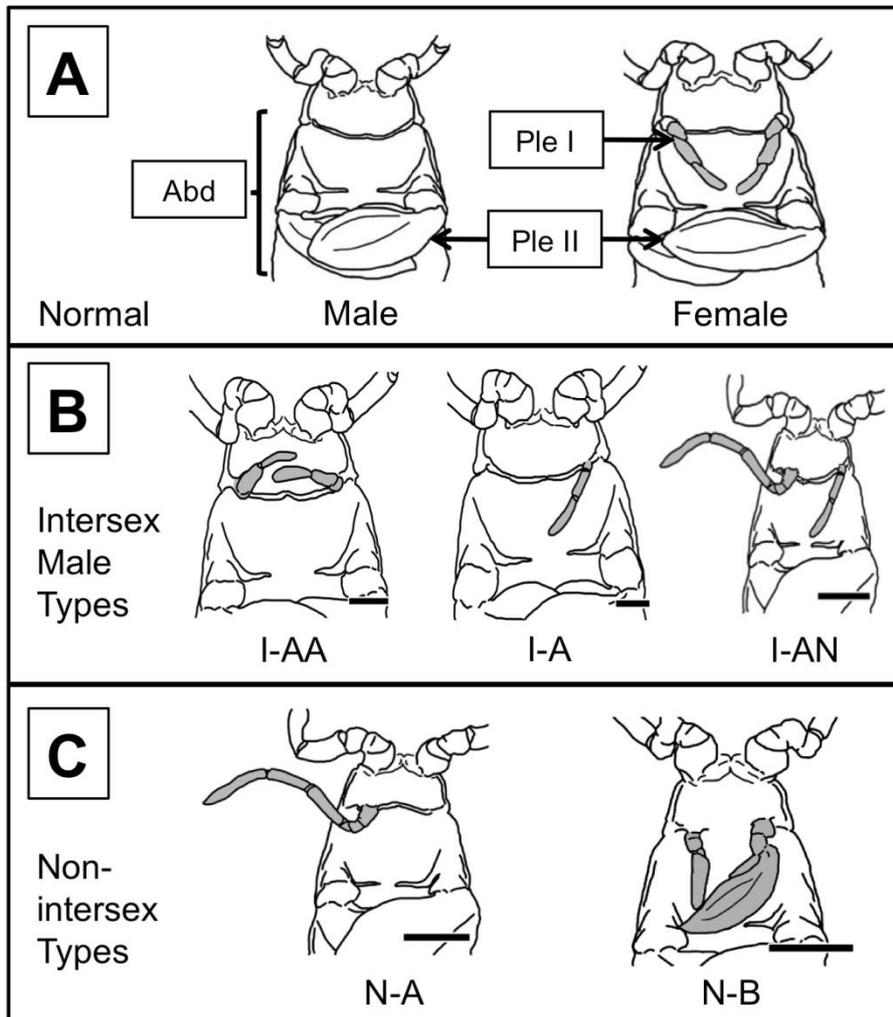


Figure 2. Classification of pleopod disorders with reference to a normal male and female: (A) normal: Abd, abdomen; Ple I, first pleopod; Ple II, second pleopod; (B) intersex types: Type I-AA, males with first pleopods like normal females; Type I-A, single pleopod on either side; Type I-AN, pleopod and an appendage that resembles the pereopod; (C) non-intersex types: Type N-A, with appendage similar to pereopods; Type N-B, appendage similar to pleopods 2 to 5. Scale bars: 5 mm.

### **Effects of parasite and intersex on allometric growth**

We inspected the secondary sex characters of individuals, especially with regards to being infected or intersex, including temporary characters associated with the breeding period such as the presence of ovigerous hair normally found in the Ple I of mature females. Visual evaluation showed that the presence of the parasite might affect the development of chela dimorphism. To quantitatively determine this effect, we examined the allometric growth relationship between the PW and TL in normal specimens and compared these with parasitized specimens. In addition, we also checked whether the allometric growth of intersex individuals also deviated from normal, parasite-free specimens.

### **Sampling and measurement of organotin levels in the habitat sediment**

We sampled both surface and deep (50 cm below the tidal flat surface, using a handheld corer) sediments in 10 points across the tidal flat in the general area where mud shrimp could be found (UMZ in Figure 1B). The number of specimens we collected sharply decreased to almost none at the point in the lower tidal areas where the sediment drastically changed from a mix of sand and mud into fine, soft sediments. Sheltered areas at the periphery of the lower tidal zone also had this type of sediment and *U. major* was very rarely found. These areas are collectively indicated as the fine sediment zone (FSZ) in Figure 1B. Similarly, surface and deep sediments were collected in 5 points located in the FSZ. All 30 samples were individually sealed within small glass jars, kept in cold condition, and sent to a commercial laboratory where tributyltin (TBT) and triphenyltin

(TPT) levels were analyzed using Gas Chromatography/Mass Spectroscopy- Selective Ion Monitoring (GC/MS-SIM) methods.

## **Analysis**

Data analysis was carried out in R version 2.15.0 (R Development Core Team, 2008). A main effects generalized linear model (GLM) with binomial error distribution was used to test the significance of the following predictors for the categorical response variable, intersex: TL for body size, parasite infection (GO), and LWL<sub>est</sub> distance. We only realized the significance of CRDs when we noted that they were retained after shrimp molted in aquaria even amongst non-intersex individuals. Specimens used for observation in aquaria could not be rechecked, thus we constructed models from two data sets. Model 1 included all data (n=1,623), but without CRD as predictor due to missing values, while Model 2 included only specimens wherein CRDs were thoroughly checked (n=1,289). We selected the final models using Akaike Information Criterion (AIC) value.

A method modified from Eberhard et al. (1991, 2000) was used for allometric growth analysis and consisted of two steps: (1) testing for linearity and (2) estimation of the “switch point” in the allometric growth relationship between propodus width and TL. Analysis of Covariance (ANCOVA) was then applied to the piecewise regression to determine the effects of parasitism and intersex on the allometric growth. Relationships between body measurements were analyzed using linear model and correlation test functions. Differences in the incidence of parasite infection between sexes, as well as the size distribution of males between UTZ and LTZ, were tested using Kolmogorov-Smirnov test. We pooled the data from all five stations each for the LTZ and UTZ due to

insufficient numbers of parasitized specimens in some of the sampling stations. We used a two-sample chi square test to compare the proportion of intersex individuals between infected (GO+) and uninfected (GO-) males within the same zone (UTZ or LTZ), and between GO+ or GO- of different zones. Finally, we used relative risk analysis to compare the likelihood of developing intersex and CRD relative to tidal zone location.

## IV. RESULTS

### **Intersex and other morphological abnormalities**

We collected 1,623 males and 877 females from Kasaoka Inlet (Table 1). Males were 2.1 and 1.5 times the number of females in the upper and lower tidal zones, respectively. All females and 91.2% of males were normal (non-intersex) (Figure 2A). Intersex males made up the remaining 8.8% ( $n=143$ ) of the total male sample. Out of 8.8%, 5.4% ( $n=87$ ) had Ple I on one side (type I-A), 3.3% ( $n=54$ ) had a complete pair of Ple I (type I-AA), and 0.1% ( $n=2$ ) had a single Ple I with a contralateral side bearing an appendage that resembled the walking legs (type I-AN) (Figure 2B). Two males were non-intersex but had abnormal growths instead of Ple I: One had an appendage with segmentation similar to walking legs (type N-A), while the other had an appendage that was broad and flat like the second to fifth swimming pleopods (type N-B) (Figure 2C).

Ple I in normal females first appeared at 20 – 23 mm TL and growth was bilaterally symmetrical (Figure 3A, 3B). Ple I length was positively correlated with TL on both the right (Pearson's  $r = 0.94$ ,  $df=89$ ,  $P < 0.001$ ) and the left (Pearson's  $r = 0.92$ ,  $df=89$ ,  $P < 0.001$ ). In intersex males, the length of the abnormal Ple I showed only moderately positive correlation with TL and Ple I were markedly more variable in length when compared to females of similar body size. Intersex males did not bear ovigerous setae even in the breeding season. Male gonopores were in their usual position at the coxae of the fifth walking legs; gonads appeared to be indistinct from normal males. Abnormal Ple I were consistently retained after each ecdysis in intersex specimens kept in aquaria.

Cuticular ridge deletions (CRD) were found in both sexes. In normal specimens, a continuous cuticular ridge is present ventrally in the junction of the first and second abdominal segments in both sexes (Figure 4A and 4D). We found that 5.0% of males and 4.8% of females had discontinuities or deletions in this structure. In males with CRD, breaks were partial and intermittent (Figure 4B) and often appeared with the abnormal Ple I (Figure 4C). The presence of CRD was significantly correlated with intersex (GLM,  $n=1,289$ ,  $P<0.05$ ; Table 2). Intersex CRD males were 35.1% (27 out of 77) of all intersex males and 41.5% (27 out of 65) of all males with CRD in the sample set where all specimens were checked for CRD (see Table 1). Female CRD was associated with loss of the Ple I on one side when deletion was partial (Figure 4E), or on both sides when the deletion was extensive (Figure 4F). Individuals with cuticular damages and black patches on the exoskeleton, as well as missing or injured Ple I, were relatively common but these damages were repaired after one or two moult cycles. In contrast, CRDs were consistently retained even after several moult cycles.

Table 1. *Upogebia major* from Kasaoka Inlet in November 2011 to April 2012, with the prevalence of infection by *Gyge ovalis* and the morphological abnormalities observed.

Location	Site	Total sample		Intersex M only	with GO		Intersex+GO M only	CRD		Intersex+CRD M only
		M	F		M	F		M	F	
Lower Tidal Zone	L1	47	18	5	8	13	1	2	2	1
	L2	188	133	32	24	18	2	ND	ND	ND
	L3	50	63	11	4	3	2	9	6	5
	L4	146	94	34	10	16	3	ND	ND	ND
	L5	111	42	11	44	28	3	18	3	7
<b>Sub total (%)</b>		<b>542</b>	<b>350</b>	<b>93 (17.2)</b>	<b>90 (16.6)</b>	<b>78 (22.3)</b>	<b>11 (2.0)</b>	<b>29 (13.9)*</b>	<b>11 (8.9)*</b>	<b>13 (6.3)*</b>
Upper Tidal Zone	U1	386	177	18	65	61	4	2	7	0
	U2	85	19	16	4	2	2	14	2	7
	U3	271	164	2	49	38	0	1	4	0
	U4	242	121	7	37	24	0	12	5	4
	U5	97	46	7	16	11	1	7	2	3
<b>Sub total (%)</b>		<b>1,081</b>	<b>527</b>	<b>50 (4.6)</b>	<b>171 (15.8)</b>	<b>136 (25.8)</b>	<b>7 (0.6)</b>	<b>36 (3.3)</b>	<b>20 (3.8)</b>	<b>14 (1.3)</b>
<b>Overall (%)</b>		<b>1,623</b>	<b>877</b>	<b>143 (8.8)</b>	<b>261 (16.1)</b>	<b>214 (24.4)</b>	<b>18 (1.1)</b>	<b>65 (5)</b>	<b>31 (4.8)</b>	<b>27 (2.1)</b>

M= Males, F= Females; ND= No data; CRD= Cuticular ridge deletion; GO= infected by *G. ovalis*

\*Percentage based on only sampling events where CRD was checked.

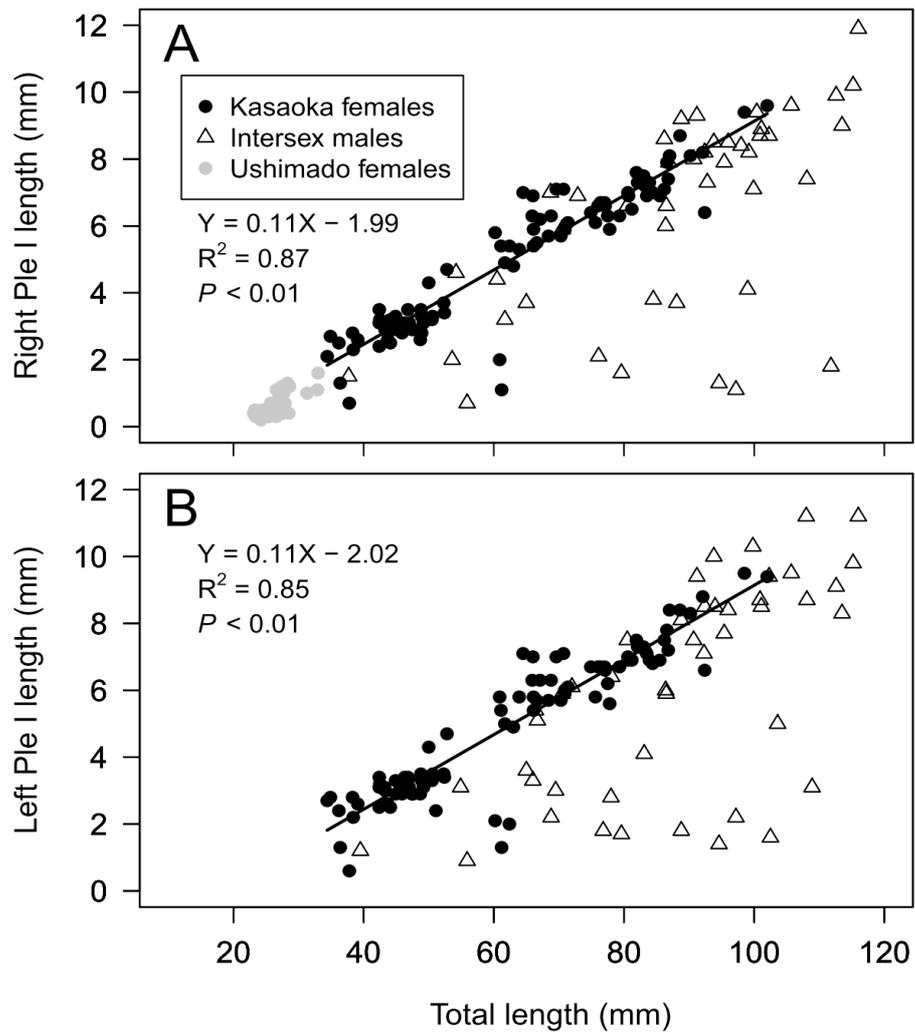


Figure 3. Comparison of allometric growth relationships between total length (TL) and length of the right (A) and left (B) first pleopod (Ple I length) in normal females and intersex males. Measurements from small specimens of Ushimado are shown in gray dots (in A). Regression equations,  $R^2$  and  $P$  values ( $H_0: a = 0$ ) are also shown.

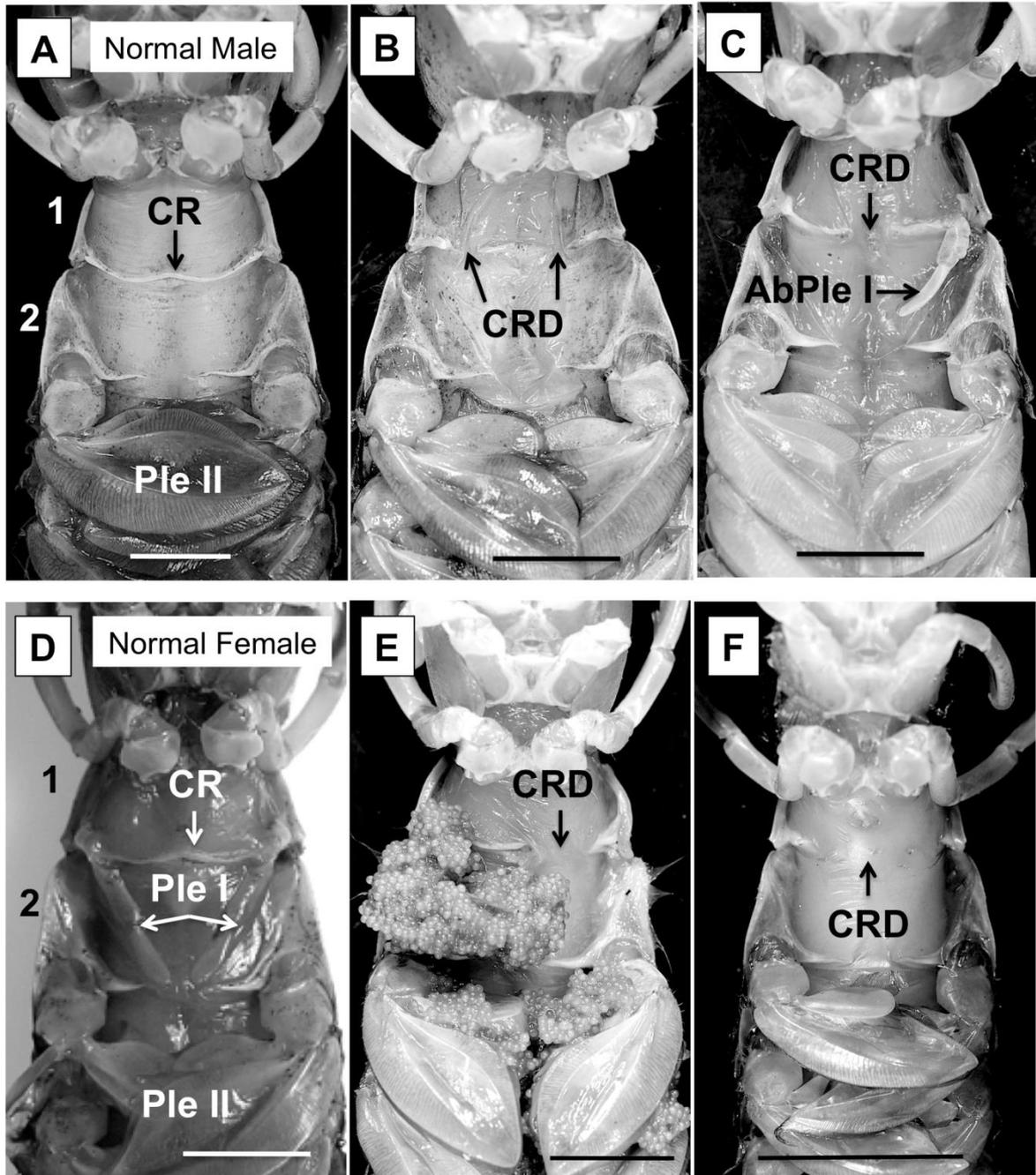


Figure 4. Ventral view of male (A–C) and female (D–F) abdomen showing intact cuticular ridge (CR), and deletions (CRD): (A) normal male: 1, first abdominal segment; 2, second abdominal segment; Ple II, second pleopod; (B) male with CRD in two points; (C) male with both CRD and abnormal first pleopod (AbPle I); (D) normal female; (E) gravid female with CRD on one side; (F) female with extensive CRD. Scale bars: 10 mm.

**Effects of parasitism and intersex on secondary sex characteristics**

Parasitized mud shrimp were distinguishable from parasite-free individuals by the distended branchiostegite of the occupied gill chamber (Figure 5A). The external morphology of the parasite *Gyge ovalis* is shown in Figure 5B and 5C.

Another feature of infected mud shrimp was the retarded development of gonads and secondary sex characters. Among large males, there was a visually striking difference between infected and parasite-free individuals; infected males tend to resemble the relative size and form of the female chela, although finer morphological features such as those in the dactylus are retained. A comparison between the chela of a normal and infected male of similar body sizes (TL), and a large normal female is shown in Figure 5D.

A quantitative evaluation of this chela reduction effect of parasitism was done through analysis of allometric growth relationship between TL and propodus width (PW). Allometric growth switch points were estimated to be at a TL of 80 mm and 85 mm for females and males, respectively. In normal and parasite-free specimens, allometric growth beyond this point climbed steeper, while in females, the slope slightly decreased. As initially expected through visual inspection, a significant effect on the chela was detected with the analysis of covariance (ANCOVA) of allometric growth regression lines. In the males (Figure 6A), the slope of allometric growth in both juvenile (ANCOVA,  $df=468$ ,  $P=0.01$ ) and mature (ANCOVA,  $df=1109$ ,  $P=0.007$ ) mud shrimp were significantly lower in infected specimens compared to non-infected counterparts. Reduction in PW was significantly evident in juveniles, but was even more pronounced in mature males.

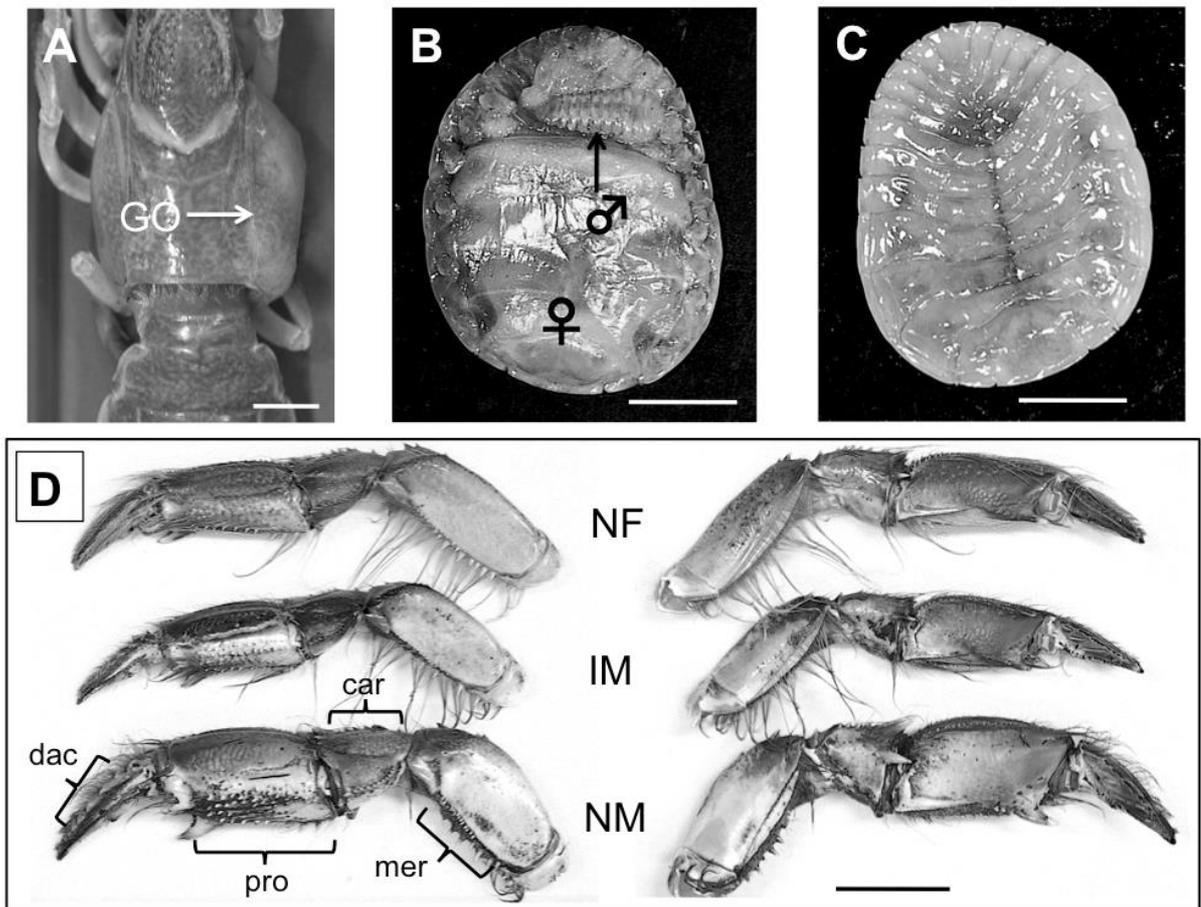


Figure 5. Modifications in the morphology of the host mud shrimp by *Gyge ovalis*. (A) *Upogebia major* with bulging right branchiostegite occupied by *G. ovalis* (GO). (B) Dorsal view of GO showing a diminutive male (♂) attached to the much larger female (♀); (C) ventral view, female GO. (D) Comparison of the left chela size and morphology among healthy female (HF, TL=121 mm), and infected male (IM, TL=101 mm) and healthy male (HM, TL=103 mm). Left panel shows the outer side of the merus (mer), carpus (car), propodus (pro) and dactylus (dac); right panel, the inner side. Scale: 10 mm.

In females (Figure 6A), PW was unaffected in juveniles as indicated by non-significant difference in the slope of allometric growth regression lines (ANCOVA,  $df=413$ ,  $P=0.72$ ) (Figure 6B). However, among mature females, a significant difference in the allometric growth slopes of infected and non-infected specimens was discernable, but showed a trend opposite of the male. Whereas parasitic infection caused depression in the allometric growth in males, the apparent effect in the female was an upward shift in the allometric growth (Figure 6B). Infected mature females had significantly larger PW relative to TL than non-infected ones.

Although the chela of infected mature males approached the general shape and relative size of the females, these remained significantly larger (ANCOVA,  $df=437$ ,  $P<0.001$ ) than females of comparative size. It should also be noted that the response of individual host to infection showed relatively high variation in mature specimens, especially in males where a minority of specimens had PW that were indistinguishable from uninfected males (Figure 6A).

With regards to other characters affected by the parasite, infected females of reproductive size did not bear any ovigerous setae, showed no accumulation of yolk in the ovaries, and visual inspection showed undeveloped or poorly developed gonads in both sexes even in larger, supposedly mature specimens. Furthermore, we could not collect ovigerous females that were infected by the epicaridean parasite, throughout the whole breeding season.

In contrast to parasitized males, the allometric growth of intersex males that were parasite-free was indistinguishable from normal males in both small (ANCOVA,  $df=254$ ,  $P=0.804$ ) and large males (ANCOVA,  $df=1068$ ,  $P=0.718$ ) (Figure 7).

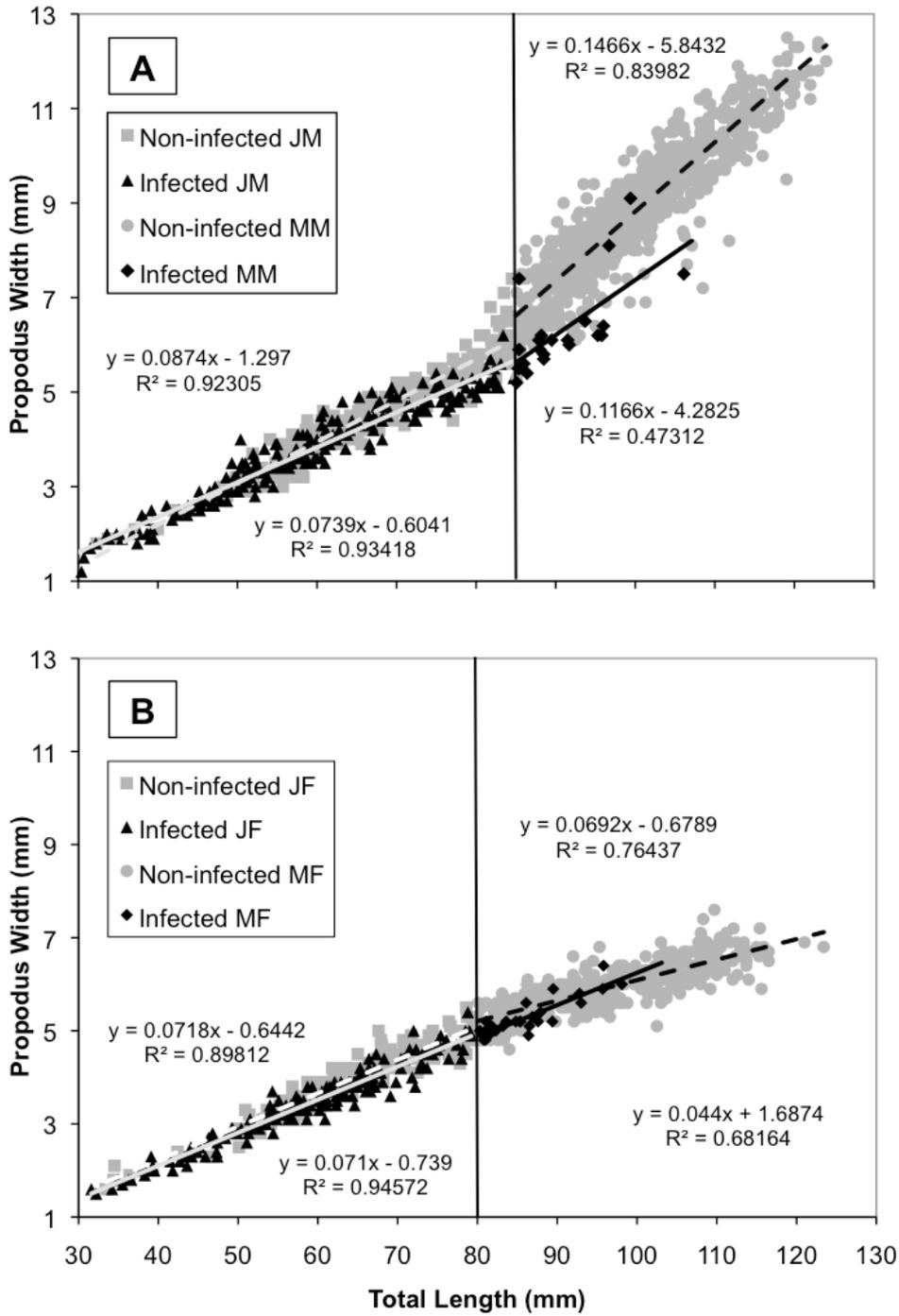


Figure 6. Comparison of allometric growth relationships of propodus width and total length between (A) infected and non-infected small and large males, and (B) infected and non-infected small and large females. Regression lines are shown for infected (broken) and non-infected (solid) mud shrimps. Vertical line marks switch point in the regression.

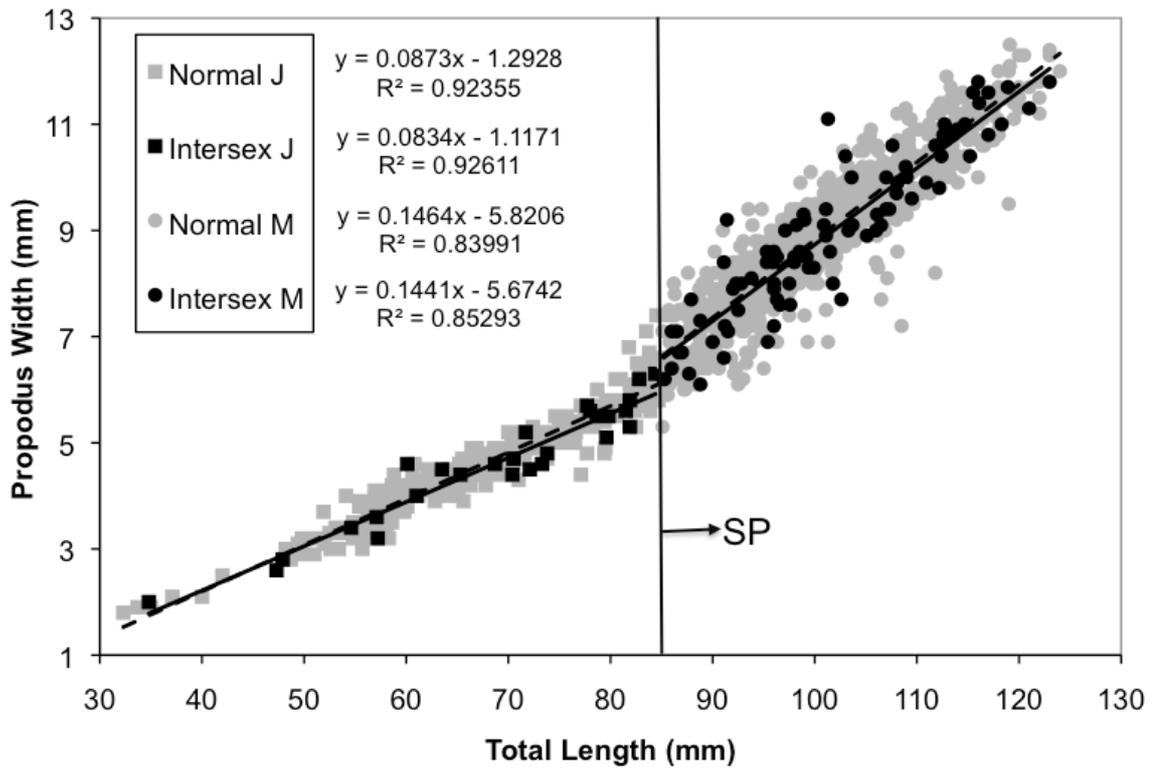


Figure 7. Allometric growth relationships of propodus width and total length in normal small (gray squares) and large (gray dots) males, and in small (solid squares) and large (solid dots) intersex males. Regression lines for normal (solid) and intersex (broken) are also shown, along with regression equations and R<sup>2</sup> values; SP, switch point in the segmented regression.

## Relationships with host size

Infection by *G. ovalis* was lower in male *U. major* (16.1%) than among females (24.4%) (Table 1). However, when controlled for host size, no significant difference in the infection rates between sexes was apparent (Kolmogorov-Smirnov test,  $P=0.41$ ) (Figure 8A). The incidence of parasitic infection peaked at 70 to 80% in both sexes in specimens with  $TL < 50$  mm (Figure 5A) and incidence of infection decreased among larger shrimp to 0.2% ( $n=468$ ) in males with  $TL > 100$  mm, and all females ( $n=156$ ) of the same size group were parasite-free. Infected large males had female-sized chelae, but denticulation of the dactylus remained characteristically male. Infected females of reproductive size did not bear ovigerous setae and lacked yolk in their ovaries. One ovigerous female that had fewer eggs than normal had a deformed but empty right gill branchiostegite.

Intersex males ranged from 35.0 to 123.0 mm in TL and in contrast to the incidence of infection, intersex appeared in all host size groups (Figure 5A, heavily-shaded bars). Size class relative frequency of intersex males ranged from 3.6 to 13.4%, but overall, TL was not a significant predictor of intersex (GLM,  $n=1,623$ ,  $P=0.714$ ; Table 2). In addition, being host to *G. ovalis* was not a significant predictor of intersexuality (GLM,  $n=1,623$ ,  $P=0.212$ ; Table 2). Intersex males made up only 6.9% (18 out of 261) of infected males, and infected males comprised only 12.6% (18 out of 143) of all intersex males (Table 1).

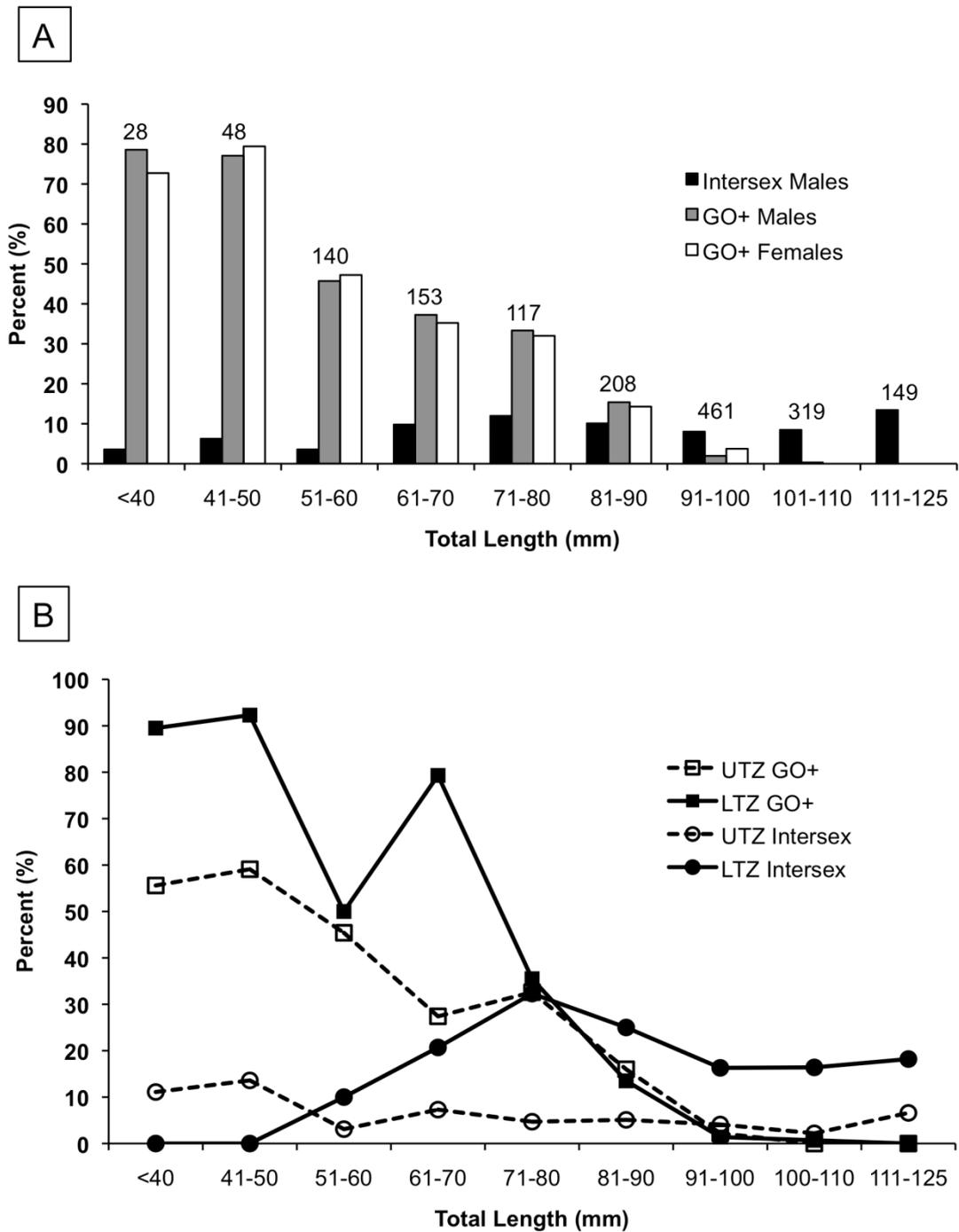


Figure 8. Incidence of intersex and parasites. (A) Incidence (in %) of intersex males, and *Gyge ovalis* prevalence (GO+) in both sexes with total length (TL). Numbers above bars indicate the number of males per size class. (B) Incidence of intersex (circles) and infection (squares) in the upper tidal zone (UTZ, broken line and open symbols) and lower tidal zone (LTZ, solid lines and symbols) according to TL.

## Relationships with tidal level

*U. major* in Kasaoka can be found from the upper to the lower intertidal area, spanning about 150 m at the widest point of the tidal flat perpendicular to the shore (Figure 1B). Large males were more frequent in the lower tidal zone (LTZ) than the upper tidal zone (UTZ) (Kolmogorov-Smirnov test,  $P<0.01$ ). Thus, comparisons of intersex and infection incidence in males between the tidal zones were made according to TL (Figure 8B). Although male infections decreased with larger host size for both UTZ and LTZ, infection rates in LTZ (89.5 to 92.3%) were almost twice that of the UTZ (55.6 to 59.1%) in the smallest males (TL<50 mm). On the other hand, among males with TL<50 mm, 11.1 to 13.6% of the UTZ males were intersex but none were found in the LTZ. However, intersex frequencies were higher in the LTZ (10.0 to 32.3%) compared to the UTZ (2.2 to 7.3%) among males with TL>50 mm.

Mud shrimp size (TL) and infections were not significant predictors of intersex males (see Table 2), but infections were negatively correlated with host size (Figure 8A). We therefore compared the incidence of intersex between the UTZ and LTZ by separately analyzing parasitized and unparasitized groups (Figure 9).

Within the LTZ, intersex occurred in 11 out of 90 (12.2%) parasitized males (GO+), compared to 82 out of 452 (18.1%) parasite-free males (GO-); the difference was not statistically significant (Chi square test,  $P=0.23$ ). In the UTZ, 4.1% (7 out of 171) of GO+ males were intersex and not so different from 4.7% (43 out of 910) of intersex GO- males (Chi square test,  $P=0.87$ ). The proportion of intersex males was, however, significantly greater in both GO+ (Chi square test,  $P=0.03$ ) and GO- (Chi square test,  $P<0.01$ ) males in the LTZ compared to those in the UTZ. When compared to mud shrimp

in the UTZ, those in the LTZ were 3 times (Relative risk analysis, 95% CI=1.2, 7.4,  $P=0.02$ ) and 3.8 times (Relative risk analysis, 95% CI=2.7, 5.5,  $P<0.01$ ) more likely to develop intersexuality, for GO+ and GO- groups, respectively.

On the other hand, CRD was not only a significant overall predictor of intersex males (Table 2), but also varied with tidal level similar to intersex (Figure 9). Thirty-six out of 1,081 (3.3%) UTZ males had CRD compared to 13.9% (29 out of 208) of the LTZ male sample (Chi square test,  $P<0.01$ ). LTZ males were 4.3 times more likely to have CRD than those in the UTZ (Relative risk analysis, 95% CI=2.7, 6.9,  $P<0.01$ ). The proportion of females with CRD was significantly higher at 8.9% (11 out of 123) in the LTZ compared to 3.8% (20 out of 527) in the UTZ (Chi square test,  $P=0.03$ ). CRD rates were not significantly different between males and females in both the LTZ (Chi square test,  $P=0.24$ ) and the UTZ (Chi square test,  $P=0.74$ ). We found that the rate of intersex increased as distance from the LWL<sub>est</sub> decreased (Figure 10), independent of parasite infection and TL.

Table 2. Main effects Generalized Linear Model (GLM) results of predictors of intersex in male *Upogebia major*.

Model	Coefficients <sup>a</sup>	Estimate	Std. Error	P value
<b>A. All data used (n = 1, 623)</b>				
(i) Intersex ~ TL + GO + LWLD	(Intercept)	0.048	0.634	0.940
	TL	-0.005	0.006	0.411
	GO	-0.423	0.339	0.212
	LWLD*	-0.030	0.003	< 2x10 <sup>-16</sup>
Minimum AIC model:				
(ii) Intersex ~ LWLD	(Intercept)*	-0.476	0.200	0.017
	LWLD*	-0.029	0.003	2x10 <sup>-16</sup>
<b>B. Only data with CRD checked (n = 1, 289)</b>				
(i) Intersex ~ TL+ GO + CRD + LWLD	(Intercept)	-0.702	0.887	0.428
	TL	-0.007	0.007	0.351
	GO	-0.456	0.405	0.260
	CRD*	2.465	0.307	9x10 <sup>-16</sup>
	LWLD*	-0.022	0.006	2x10 <sup>-4</sup>
Minimum AIC model:				
(ii) Intersex ~ CRD + LWLD	(Intercept)*	-1.476	0.470	0.002
	CRD*	2.474	0.305	5x10 <sup>-16</sup>
	LWLD*	-0.021	0.006	3x10 <sup>-4</sup>

TL = Total length (mm); GO = *Gyge ovalis* infection; LWLD = Low water line distance (m); CRD = Cuticular ridge deletion; AIC = Akaike Information Criterion

<sup>a</sup>\* Significant at  $P < 0.05$

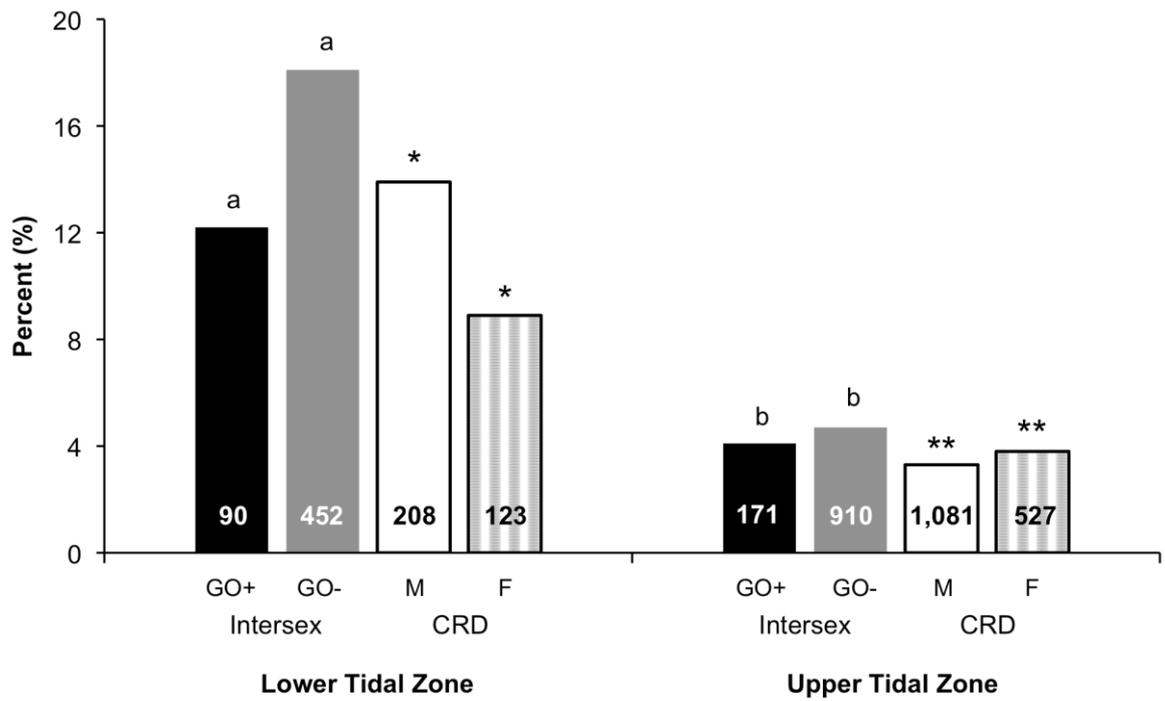


Figure 9. Frequencies of infected and uninfected intersex males and cuticular ridge deletions (CRD) of males and females by tidal zone. GO+, infected; GO-, uninfected; M, male; F, female. Different alphabets (intersex), or number of asterisks (CRD) indicate significantly different values.

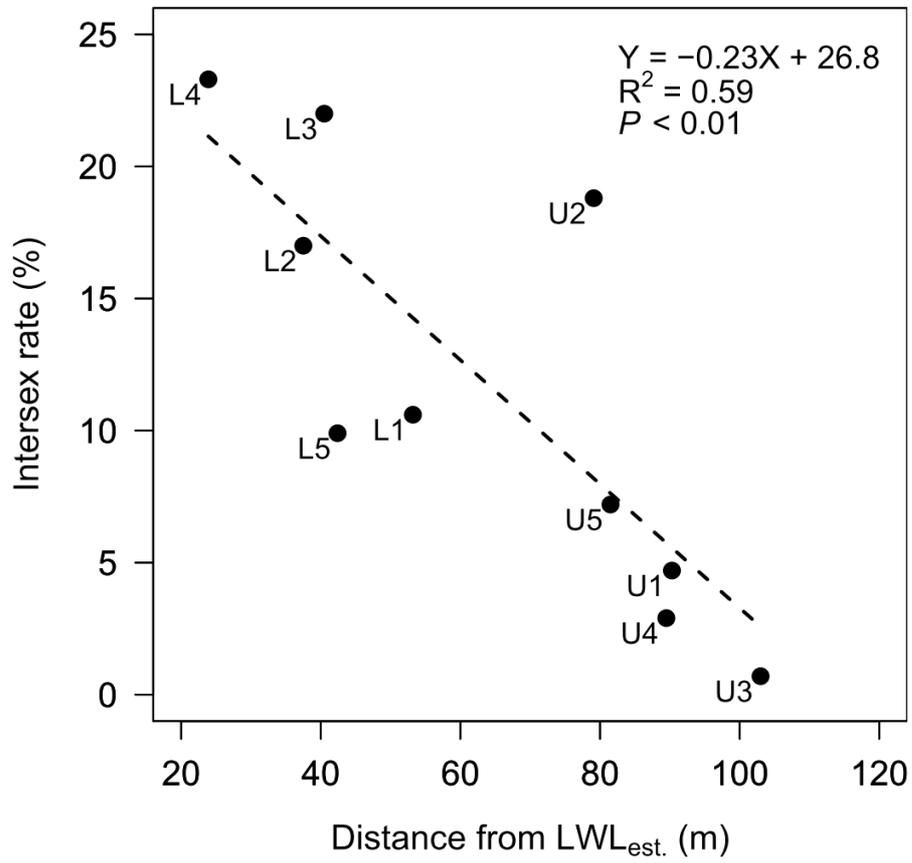


Figure 10. Intersex prevalence and tidal level relative to distance from the estimated low water line (LWL<sub>est.</sub>). Regression equation,  $R^2$  value and  $P$ -value ( $H_0: a = 0$ ) are also shown.

### **TBT and TPT levels associated with sediment distribution on the tidal flat**

Both surface and deep sediment samples taken from the area of the tidal flat that were inhabited by *U. major* (see Figure 1B) contained uniformly low levels of TBT and TPT (Table 3). Mean surface sediment TBT content was  $1.1 \pm 0.35 \mu\text{g kg}^{-1}$  and surface TPT levels were below detection limit ( $<1 \mu\text{g kg}^{-1}$ ). Deep sediments contained mean levels of  $1.4 \pm 1.3 \mu\text{g kg}^{-1}$  and  $1.0 \mu\text{g kg}^{-1}$ , for TBT and TPT, respectively.

On the other hand, organotin levels were highly variable in the fine sediment zone (FSZ, Figure 1B), but were also higher than in the zone inhabited by *U. major*. Surface sediment TBT levels ranged from 2 to  $12 \mu\text{g kg}^{-1}$  while deep sediment levels were from 2 to  $68 \mu\text{g kg}^{-1}$  in the more open areas of the FSZ (sampling points 2 to 5, Figure 1B). TPT levels were barely detectable ( $\leq 1 \mu\text{g kg}^{-1}$ ) in both surface and deep samples taken from the same points as above. Samples from the FSZ in the sheltered area near the seawall (sampling point 1, Figure 1B) contained the highest levels of TBT (surface:  $110 \mu\text{g kg}^{-1}$ ; deep:  $190 \mu\text{g kg}^{-1}$ ) and TPT (surface:  $6 \mu\text{g kg}^{-1}$ ; deep:  $24 \mu\text{g kg}^{-1}$ ).

Table 3. Sediment organotin (tributyltin and triphenyltin) levels in the immediate habitat of *Upogebia major*, and the adjacent fine sediment zone.

Sampling Point	Surface sediment		Deep sediment (50 cm below)	
	TBT ( $\mu\text{g kg}^{-1}$ )	TPT ( $\mu\text{g kg}^{-1}$ )	TBT ( $\mu\text{g kg}^{-1}$ )	TPT ( $\mu\text{g kg}^{-1}$ )
<b>Fine sediment zone</b>				
1	110	6	190	24
2	12	BDL	5	1
3	5	1	2	BDL
4	9	BDL	68	1
5	2	BDL	2	BDL
<b><i>Upogebia major</i> zone</b>				
	<b>Mean (SD)</b>			
10 sampling points <sup>a</sup>	1.1 (0.35)	BDL	1.4 (1.3)	1

BDL = value below detectable limit

<sup>a</sup> Data pooled from 10 separately analyzed samples across the area inhabited by *U. major*

## V. DISCUSSION

Nanri *et al.* (2011) reported a significantly higher proportion of intersex *Upogebia major* in Kasaoka Inlet than elsewhere in the Seto Inland Sea, Japan. We confirmed similar occurrence rates of intersex, but also found other morphological abnormalities and a relatively high prevalence of the branchial bopyrid isopod parasite *Gyge ovalis* in this population (Table 1). We explored the relationship between intersex and parasite prevalence, time and the onset of intersex and parasitization, and finally whether tidal level was a factor in either condition.

### **Relationship between intersex and parasitization**

Tucker (1930) argued that the appearance of the female-like Ple I in male *U. pusilla*, in addition to the “feminization” of the chela, was an inversion of sex characters resulting from early infestation by *G. branchialis*. Although lacking quantitative treatment of the degree of association between parasitic and intersex incidence, they were implied to co-occur in the majority of cases. We found no significant association between the parasite and Ple I in males (Table 2); however, infected males had reduced PW and castration of infected females appeared to be complete (i.e. undeveloped ovaries, absence of ovigerous setae) similar to *U. pusilla*. Prevalence of *G. ovalis* was similar in both sexes (Figure 8A). Intersex males made up 8.8% of the total males (see Table 1). Contrary to our expectation, parasite infection was not significantly correlated to intersex (GLM, Table 2), and only 12.6% of all intersex males were parasitized. Further, there

was no significant difference between the 6.9% of parasitized males displaying intersex characteristics and 9.2% of unparasitized intersex males.

In contrast to infection, cuticular ridge deletion (CRD) often co-occurred with and was a significant predictor of intersex (Tables 1 and 2). The mechanism that links CRD and intersex is unknown but the rate at which they occur together in the same specimen may suggest common factors leading to the development of both intersex and CRD. CRDs occur on the same region of the abdomen where female-like Ple I appear (Figure 4), and like intersex, also are more common in the lower tidal zones (Figure 9).

### **Timing of parasitization and intersex**

Whereas epicaridean infection and higher prevalence in adult host decapods have also been described (Smith *et al.*, 2008; Conner & Bauer, 2010; Repetto & Griffen, 2012), a prevailing observation is that infection is most successful in young hosts when frequency of ecdysis is high and the cuticles and branchiostegite are soft and can accommodate the fast growth of the parasite (Tucker, 1930; Pike, 1960). Some hosts become increasingly resistant to the infective cryptoniscus with age (Anderson, 1990). Consistent with these past findings, the smallest specimens of *U. major* (TL < 50 mm) had the highest prevalences of *G. ovalis* at 73 to 79% (Figure 8A). Infections decreased to < 1% in hosts with TL > 100 mm in both sexes. At least two possible factors causing this decreasing trend are possible and each may not preclude the other. They are: (i) increased mortality in infected specimens and (ii) parasite loss over the course of host growth and ecdysis. Our data cannot distinguish the relative contributions of each factor. However, in studies of the strategies used by epicaridean parasites to cope with host ecdysis, loss of

the epicaridean parasite has also been documented (Anderson, 1990; Cash & Bauer, 1993). Likewise, we found at least 10 specimens with recently vacated gill chambers, and a few more that seemed to be in various states of recovery from gill infection.

A high rate of early infection followed by recovery may explain how a majority (87.4%) of intersex males were parasite-free. This is consistent with our observations that while Ple I are likely permanent once they appear (Figure 3A and 3B), the castration effects of the parasite may be reversible. Males with parasites removed gradually regained normal male chela proportions (Ubaldo, Nanri & Saigusa, pers. obs.), and an ovigerous female with eggs fewer than normal showed clear signs of recent infection. Hence, a male in which growth of Ple I was triggered by early infection, the parasite was eventually lost, and gonads and chela recovered, would appear normal in all respects other than the presence of the Ple I. Interestingly, while infection occurs almost exclusively in small hosts (Figure 8A), intersex may appear at almost any size of *U. major* (Figure 3A and 3B). Thus, even if early infection caused intersex, the appearance of the Ple I may not be concurrent with infection. Moreover, it is plausible, given the 93.1% of infected non-intersex males, that the effective period of infection that can trigger intersex development may be restricted to a limited period in very small males. In a similar host and parasite pair investigated by Repetto and Griffen (2012), *Upogebia pugettensis* and *Orthione griffenis*, the latter only infected large hosts and no male secondary sex character modifications were observed.

The controls for Ple I development are unknown. In females however, Ple I buds normally appear around a TL of 20 mm (Figure 3A) and infection, when it happens earlier or around this period, might interfere with normal development in the males.

### **Tidal level variations in infection and intersex**

The infective stage of epicarideans, the cryptoniscus, is pelagic (Beck, 1980b; Boyko & Williams, 2009). Naturally, *U. major* in the lower tidal areas that are immersed for longer periods would also experience greater exposure to settling cryptoniscans, and a corresponding higher risk for infection. Infection prevalence was higher in LTZ males (Figure 8B) as we expected, in contrast to the findings of Repetto and Griffen (2012) where tidal height had no effect the prevalence of *O. griffenis* in *U. pugettensis*. Consistent with the early infection hypothesis of intersex, a greater portion of small males infected in the LTZ would then result in a higher incidence of intersex among males that occupy the LTZ (Figure 8B, 9 and 10).

However, the presence of trait-altering pollutants should not be disregarded. Fine sediments accumulate in less turbulent zones in Kasaoka Inlet such as near breakwaters and in lower tidal areas below the lower limits of *U. major* distribution (Fine sediment zone, Figure 1B). These zones are often reservoirs of organic and hydrophobic contaminants that adhere to fine particles (Harkey *et al.*, 1994; Lee *et al.*, 2006). Weis and Kim (1988) found the organotin tributyltin (TBT), which has been shown to associate with fine sediments (Langston & Pope, 1995), to elicit deformed regenerated limbs in the fiddler crab. Itow *et al.* (1998) reported abnormal eye embryos in horseshoe crab exposed to organotin. We found in our preliminary surveys relatively higher levels of TBT in the FSZ surrounding the *U. major* habitat when compared with the sediment within the area where mud shrimp burrows can be found (Table 3).

*U. major* mainly feed by pumping overlying water into their Y- or U-shaped burrows and filtering suspended particles with the “basket” formed by the setal structures

of the first and second pereopods and the maxillipeds (Mukai & Koike, 1984). Upogebiids selectively ingest the smaller particles (Dworschak, 1987; Pinn *et al.*, 1998). Moreover, *U. major* burrows also function as traps for organic matter, portions of which are incorporated into burrow walls (Kinoshita *et al.*, 2003). Thus, their proximity to contaminated fine sediments and the longer periods of submersion (i.e. longer time for filter feeding) place *U. major* in the LTZ where greater exposure to sediment-bound contaminants is likely. These factors may lead to higher rates of intersex and CRD in *U. major* of the LTZ (Figure 6 and 7).

Whether differences in rates of early infection, exposure to sediment-bound pollutant, or the additive effect of both factors have resulted in the variation of intersex incidence between upper and lower tidal areas remains unclear. On the other hand, there is little evidence for sex change in *U. major*. Hence, differential mortality and dispersal between the sexes might result to the overall preponderance of males (i.e. male:female = 1.9:1; Table 1) in addition to the effects of parasite and pollution.

### ***Possible mechanisms of modification of external morphology***

Bopyrid epicarideans are widely known to affect their hosts in a variety of ways, most notable among them being their role as parasitic castrators, and modifiers of the host external morphology (Williams and Boyko, 2012). In general, these parasites affect the gonads of the female more severely than the male, while males also suffer from alterations of external secondary sex characteristics to a larger extent than the females (Reinhard, 1956; Walker, 1977; O'Brien and Van Wyk, 1985; Calado *et al.*, 2005; Conner and Bauer, 2010).

In female *U. major*, castration may proceed to its full extent, as throughout the sampling we could not collect infected females of reproductive size that were ovigerous, or even bearing ovigerous setae that normally appears in Ple I and the other pleopods of mature females during the breeding season. Moreover, infected mature-sized females possessed undeveloped ovaries upon visual inspection. Temporary female external characters such as ovigerous setae have been experimentally demonstrated in pericarideans (Charniaux-Cotton and Payen, 1985), and decapods (Nagamine and Knight, 1987) to be under the control of an ovarian factor referred to as a temporary ovarian hormone (TOH), and are expressed when the ovary is undergoing secondary vitellogenesis (Suzuki and Yamasaki, 1991). Aside from accounting for the condition in infected *U. major* females, this could also explain why intersex males with Ple I never produce the ovigerous setae seen in normal females during the breeding season.

In the males, it is harder to confirm to what extent infection has impacted reproduction without delving into histological work, which we did not cover in the current study. Certain irregularities in comparison to uninfected specimens can however be observed with visual inspection of the gonads. *Upogebia major* has a peculiar gonadal structure as a gonochoristic shrimp whose males possess a “testis proper” and posterior to this, a more prominent “ovarian part” that also shows seasonality in activity and in size (Ishikawa, 1891; Oka, 1941; Kang *et al.*, 2008). We observed in infected mature-sized males a similar pattern of undeveloped ovarian part of testis, and thin and translucent, almost nonexistent testis proper, as opposed to more salient yellowish ovarian part of testis and semi-translucent testis proper in healthy males of comparable size. Tucker (1930), working on the congeneric mud shrimp *Upogebia pusilla* infested by the

epicaridean *Gyge branchialis*, also described similar effects on the host's gonads, although males of this species do not have the ovarian part of testis. Ovarian development was totally arrested in infected females, and histological work on the testis of infected males revealed these to be in various states of degeneration both in mass and spermatogenetic activity. This is also most likely true for *U. major*.

Previously, Nanri *et al.* (2011) found that the gonadal index of parasite-free males collected from the same site bearing the abnormal Ple I were indistinguishable from normal males. Likewise, we could not differentiate between parasite-free, intersex males and normal males of comparative size upon visual inspection of the gonads. These indicate that gonadal development in uninfected intersex males may proceed normally and remain functional for reproduction.

On the other hand, less cryptic than the castration effect in males is the more readily observable and quantifiable impact of infection on external male secondary sex characters. Our results show that infected males possessed relatively smaller chela, with narrower propodus in particular (Figure 5 and 6) compared to parasite free males of comparative total length (TL), and this effect was even more pronounced in mature-sized males. These specimens end up having chela of intermediate size between normal males and females, and can thus be described as demasculinized, or in this case, "juvenilized" by virtue of their resemblance to the undifferentiated form in immature males (Beck, 1980a). In contrast with the effects of infection by the epicaridean, intersex males without the parasite possessed chelae that are indistinguishable in size and form from normal, parasite-free males. The denticulation of the dactylus, which very early on differentiates between the sexes and could be used as basis to separate the smallest

specimens into males and females, remain characteristically male even in both infected and intersex specimens, as well as the position of the gonopores. These primary sex characteristics are not likely affected by the parasite, as well as the unknown factors that lead to development of abnormal Ple I in males.

Although here we are drawing contrasts between the observable changes in gonads and external male secondary characters in infected males against the apparent lack of these associated with intersex males, it can be inferred that the underlying mechanisms affected by the parasite resulting to these modifications may not necessarily involve that which gives rise to intersex male features in the later developmental stages. There is a lack of published work on endocrinology specific to upogebiids or even thalassinideans, but relevant studies on sex differentiation done on other model crustaceans provide insight on the mechanisms responsible for the development of these traits. Malacostracan sex determination may be genetic, but the agent of sex differentiation into males from the default female development is the androgenic gland (AG) which represses ovarian differentiation and accounts for both primary and secondary male characters in amphipods and isopods (Charniaux-Cotton and Payen, 1985), and also being increasingly successfully demonstrated in decapods (see review by Ventura *et al.*, 2011).

As for the control of traits most related to our current study, it has been demonstrated that secondary male sex characters in dimorphic gonochoristic species such as the transformation of the second gnathopod in *Orchestia* into powerful claws (Charniaux-Cotton, 1957), the development of greater relative propodus width and a characteristic red patch on the chela in crayfish *Cherax quadricarinatus* (Khalaila *et al.*,

2001), and the longer and more robust chelae in mature males of the nonhermaphroditic decapod prawn, *Macrobrachium rosenbergii* (Nagamine *et al.*, 1980a, 1980b), are controlled by the AG. Likewise, the enlargement of the chelae as the male *U. major* matures is most likely under the influence of the gland.

Aside from its influence on the mature male chela, the AG has also been shown to control other male secondary sex characteristics including those that involve the pleopods. For instance, it has been revealed to stimulate differentiation of the second pleopod endopodites into appendices masculina in *M. rosenbergii* (Nagamine *et al.*, 1980b), and transform the Ple I into the enlarged and robust sexualized form in the crayfish, *Procambarus clarkii* (Taketomi and Nishikawa, 1996). The stark difference in *U. major* is the lack of these sexually differentiated structures involving the pleopods, apart from Ple I being absent in the male. Thus, the AG may promote the development of these secondary sex characters in the pleopod structures in both *M. rosenbergii* and *P. clarkii*, but in *U. major* it might suppress the growth of Ple I instead. In the female mud shrimp, Ple I is not a secondary sex character but appears quite early just a few molts after metamorphosis when specimens have a TL of about 20 to 23 mm, while sexual maturity is attained much later at TL~80 mm.

Recent studies on suspected AG hormones that are yet to be chemically identified known as insulin-like androgenic gland factors (IAGs) have revealed such to be expressed *in situ* in the AG much earlier than previously thought, as soon as 8 days after separation from the mother in the crayfish *Cherax quadricarinatus* (Manor *et al.*, 2007) and only 20 days post metamorphosis in *M. rosenbergii*, even before external male characters appeared (Ventura *et al.*, 2011). Thus, it is not inconceivable for the AG or its

early stages to already function in suppressing Ple I growth. At about the same time in the female, a possible early ovarian factor counterpart stimulates the growth of the Ple I, in a process similar to the induction of oostegite growth by the primary follicular cells of the ovary in the amphipod, *Orchestia gammarella* (Charniaux-Cotton, 1955). In the experiment mentioned, the oostegites did not appear in males that have been merely andrectomized, but required the grafting of an ovary into these AG-deprived males. However, another possibility is that the growth of Ple I is spontaneous in the same way permanent female characteristics of isopods differentiate without being mediated by a hormone, but are inhibited by AGH in the male (Charniaux-Cotton and Payen, 1985).

In the light of our observations in *U. major*, the first explanation is more likely since co-occurrence of infection and intersexuality in males is relatively rare. If the only condition required for the development of Ple I were the removal of the inhibiting effect by the AGH, then infected males that show feminization in the chela would most likely also grow Ple I, but this does not seem to be the case. Unfortunately, these are mere speculations until we get a clearer picture of not only how these traits are controlled in the host, but also the mechanisms by which the parasite affects its host mud shrimp.

It is still not completely understood how bopyrid isopod parasites interfere with the host system in order to effect feminization or inhibit the development of male secondary sex characters. However, the general view is that bopyrid isopods, in contrast to closely related rhizocephalan parasites that castrate their hosts through chemical means (Boyko *et al.*, 2009), can accomplish this by energy drain or possibly creating deficiencies in the reproductive hormones (Reinhard, 1956; Anderson, 1977; Walker, 1977; Beck, 1980a). Both pathways are plausible considering that branchial bopyrid

parasites feed on the hemolymph of the host shrimp. Parasitological models have shown that energy drain by itself may explain parasitic castration if the hosts have the tendency to make up for the loss of energy by sacrificing reproduction first over other biological functions (Lafferty and Kuris, 2009). This may well explain why female gonads which require greater amounts of energy to maintain are often more severely affected, and male sexual function may not be completely impaired as in the case of simultaneously hermaphroditic shrimp *Lysmata sp.* (Calado *et al.*, 2005; Calado *et al.*, 2006).

In *U. major*, castration also appears to be more absolute in females while effects on the male may be more varied, if the minority of infected mature specimens that retained normal-looking chelae is an indication. With the differentiation of chelae being under AG control through a yet to be chemically identified AGH, it is most likely that the parasite disrupts this mechanism though it is presently unknown which endocrinological pathway is being directly affected. It is even possible that hormonal interference starts more upstream in the endocrine axis between the eyestalk ganglia, where the X-organ sinus gland complex is located, and the AG which is negatively regulated by the former (Khalaila *et al.*, 2002; Sook Chung *et al.*, 2011). Future studies that elucidate the endocrinology of both host and parasite should greatly improve our understanding of the phenomenon of parasitic castration, and the appearance of the intersex features.

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## VIII. APPENDIX

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