Physiological consequences of parasite infection in the burrowing mud shrimp, *Upogebia pugettensis*, a widespread ecosystem engineer

*Michele Repetto* and *Blaine D. Griffen*

*AMarine Science Program, University of South Carolina, Columbia, SC 29208, USA.*  
*BDepartment of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA.*  
*CCorresponding author. Email: bgriffen@biol.sc.edu*

### Abstract

The burrowing mud shrimp, *Upogebia pugettensis*, is an important ecosystem engineer throughout bays and estuaries along the Pacific coast of North America. Populations of *U. pugettensis* have recently declined throughout its range. A likely reason for this decline is the arrival of an invasive bopyrid isopod parasite, *Orthione griffenis*, which has colonised the system and increased in prevalence. We tested the following three hypotheses regarding this host–parasite system: (1) parasite infection is correlated with the volume of water processed by the host; (2) infection negatively affects host’s energetic state; and (3) infection causes feminisation in male hosts. We used several physiological and morphological measures to quantify the effects of this parasite infection on *U. pugettensis*. The parasite appears to have different physiological effects on male and female hosts. Our study provides mixed support for the previous theory that predicted the mechanistic interactions between this host and its new parasite. Recent examples from other systems have demonstrated that invasive parasites can have far-reaching influences when they infect ecosystem engineers. Given the negative effects of *O. griffenis* on *U. pugettensis*, this invasive parasite may have similarly large impacts on Pacific Northwest estuaries throughout its invaded range.

### Additional keywords: host–parasite interactions, *Orthione griffenis*, *Upogebia pugettensis*, Yaquina Bay Oregon.

Received 6 July 2011, accepted 24 September 2011, published online 8 November 2011

### Introduction

Parasites are highly abundant across numerous ecological systems (Hudson 2005; Kuris *et al.* 2008). However, despite their abundance and ecological importance, our mechanistic understanding of the interactions between parasite and host often lags far behind our understanding of other important ecological interactions such as predation and competition. Improving this understanding starts with the fundamental knowledge that, by definition, parasites have a negative impact on their host. However, the strength of these negative impacts can vary widely across different host–parasite pairs. For example, some parasites cause rapid host mortality (e.g. Granath and Esch 1983), others reduce host fecundity (e.g. Brown *et al.* 1994), and others have effects that are only weakly detectable (e.g. Munger and Karasov 1989). These differences may potentially be explained by differential effects of parasites on host energetics. Parasites frequently alter host energy budgets by decreasing energy intake through altering host behaviour (Levri 1999), directly utilising host food and/or energy reserves (Walkey and Meakins 1970), or by altering digestion and metabolism of food by the host (Munger and Karasov 1989). Effects of parasites on host energetics may explain why the impacts of parasites are at times exacerbated when food is scarce (e.g. Schaub and Losch 1989; Brown *et al.* 2000) – reducing energetic efficiency when energy demands are scarcely met should be more detrimental than when energy is abundantly available.

The incidence of invasive parasites may also be increasing alongside other invasive species. As hosts lack evolutionary adaptations enabling them to withstand the effects of these invaders, interactions with novel parasites could have severe consequences for host populations (e.g. Tompkins *et al.* 2003). Negative consequences of invasive parasites may be particularly broad when infected hosts are ecosystem engineers. For example, eastern hemlocks in North America are rapidly succumbing to an invasive aphid-like parasite (hemlock woolly adelgid, *Adelges tsugae*) that originated from Japan (Orwig *et al.* 2002). Because eastern hemlock is an important ecosystem engineering species, its parasite-induced loss can result in the entire restructuring of local forest ecosystems, including the loss of associated fauna (Tingley *et al.* 2002) and changes to soil processes (Jenkins *et al.* 1999). Far-reaching negative impacts when invasive parasites infect ecosystem engineering hosts appear to be a general phenomenon (Ellison *et al.* 2005).

Here, we examine the effects of *Orthione griffenis*, a bopyrid isopod, which is one of the first-documented entirely marine invasive parasites and occurs on the thalassinid burrowing mud shrimp, *Upogebia pugettensis*, a widespread ecosystem engineer.
shrimp, *Upogebia pugettensis*. As an ecosystem engineer, this shrimp species plays an important role in muddy-bottom benthic processes in bays and estuaries along the Pacific coast of North America (MacGinitie 1930; Dumbauld et al. 1996; Griffen et al. 2004; D’Andrea and DeWitt 2009). *U. pugettensis* builds U- or Y-shaped burrows up to 1 m deep through which large volumes of water are filtered from the water column (Thompson 1972; Griffis and Suchanek 1991; Griffen et al. 2004). With densities reaching upwards of 300 m$^{-2}$ (Bird 1982; Dumbauld et al. 1996; Griffen et al. 2004), *U. pugettensis* has widespread impacts on the functioning of the estuarine ecosystem, through its filtration and bioturbation activities that alter suspended particle loads, nitrogen cycling, solute fluxes across the sediment–water interface, and organic matter remineralisation (DeWitt et al. 2004; Griffen et al. 2004; D’Andrea and DeWitt 2009).

Bopyrid isopods are parasites of thalassinid shrimp (Markham 1985, 1988) and they often attach to the gill chamber under the host’s carapace. Effects of these bopyrid parasites on shrimp hosts commonly include reduced growth (Nelson et al. 1986) or eliminated reproduction (O’Brien and Van Wyk 1985) by means of castration (Kuris 1974) and/or feminisation of males (Tucker 1930).

The newly described bopyrid isopod, *Orthione griffenis*, was recently discovered as a parasite of *U. pugettensis* (Markham 2004). This species has also subsequently been found in specimens collected from Chinese waters in the 1950s (Williams and An 2009), and so it is presumed that *O. griffenis* is an invasive parasite that was likely introduced to the western coast of the United States via ballast water from ships originating in Chinese waters. Concurrent with the arrival and increasing prevalence of this new parasite, populations of the shrimp host have declined throughout its range, and evidence suggests that interactions with the parasite are a likely cause (Dumbauld et al. 2011). However, the underlying mechanism causing this host decline is not yet clear. Theoretical work suggests that the most parsimonious mechanism is feminisation of infected male shrimp, with attendant reproductive failure of the host that is induced by energetic costs of carrying the parasite (Griffen 2009), although a previous investigation failed to find any direct evidence of feminisation (Smith et al. 2008).

The goal of the present study was to increase our understanding of the interaction between *U. pugettensis* and *O. griffenis* by testing the following three predictions that were proposed by Griffen (2009): (1) incidence of infection should be positively correlated with shrimp size and inversely correlated with tidal height, consistent with increasing infection with the volume of water pumped through the burrow; (2) parasite infection negatively affects host energetic state and that these impacts become larger as parasite size increases; and (3) parasites feminise male shrimp hosts, thereby eliminating reproduction in the host, and that the incidence of feminisation increases with parasite size.

**Materials and methods**

**Sampling and morphological measurements**

We collected shrimp in August 2009 from a large mud flat (Idaho Flat, 44.62°N, 123.04°W) near the mouth of Yaquina Bay Estuary, Oregon. The tidal range on Idaho Flat is ~2.5 m, with a mean tide level of ~1.3 m above mean low water (MLLW). Sediments on Idaho Flat vary from very fine (68 μm) to medium sand (225 μm) (D’Andrea and DeWitt 2009). Densities of *U. pugettensis* are very high throughout much of this area, approaching 500 m$^{-2}$ in some places. Within this mud flat, we haphazardly sampled 20 individuals from each of 21 randomly selected locations with a yabby pump (a piston-like suction device that extracts shrimp from their burrows). Specific sampling locations were chosen to be within patches of high shrimp density and to cover the range of tidal heights inhabited by the shrimp on this mud flat. Each was separated by ~50–100 m and the tidal heights of each were approximated using a National Oceanic and Atmospheric Administration tide chart for the site. Samples were frozen on collection and were taken to the University of South Carolina for processing.

We collected data on a total of 411 shrimp (197 females, 214 males). For each shrimp, we determined the presence/absence of the parasite as well as the carapace length (CL). We then determined shrimp sex on the basis of the presence of a modified first pleopod (Thölt and Bauer 2007; Smith et al. 2008). This was further verified in the following two ways: (1) by determining the location of the gonopore with a dissecting microscope (females have gonopores at the base of the 3rd walking leg, and males have gonopores at the base of the 5th walking leg); and (2) by measuring cheliped and carapace length to observe the allometric relationship between the two. Cheliped length generally varies between male and female *U. pugettensis* individuals (Dumbauld et al. 1996). Examining modified pleopods, gonopores and chelipeds together, therefore, provided three independent determinants of shrimp sex, allowing us to confidently explore the potential for feminisation of the infected male shrimp. We tested for the effects of parasites on cheliped size by determining the residuals from a regression of log cheliped length on log carapace length. We then used these residuals as the response variable in a linear model, with shrimp sex, parasite infection and sexual maturity treated as categorical variables. We assumed that individuals ≥26-mm CL were sexually mature (Dumbauld et al. 1996). We examined the overall influence of tidal height, sex and shrimp size on parasite infection by using a logistic regression, with infection (yes/no) as a response variable, tidal height and shrimp size as continuous predictor variables, and sex as a categorical predictor variable. All statistical analyses were performed using R v. 2.7.1 (R Development Core Team, Foundation for Statistical Computing, Vienna, Austria).

**Physiological measurements**

We assessed the metabolic impacts of *O. griffenis* on its host. Previous studies have assessed the impacts of this parasite on overall host weight (Smith et al. 2008). However, reproduction in crustaceans is closely tied to the hepatopancreas, because this is the main energy-storage organ (Gibson and Barker 1979). As a result, the mass of the hepatopancreas often increases before reproduction and then declines during vitellogenesis as energy and nutrients for egg production are withdrawn from this organ (Kennish 1997; Griffen et al. 2011). Our intention was, therefore, to analyse the hepatopancreas alone. However, we conducted our sampling before the time of year when most *U. pugettensis* individuals begin the reproductive process.
Parasite effects on ecosystem engineer physiology

Marine and Freshwater Research

(Dumbauld et al. 1996). As a result, we were able to differentiate the hepatopancreas from ovaries only in 27 female shrimp individuals, suggesting that these shrimp had already begun vitellogenesis (we note that none of these 27 females was infected by the parasite). In the remainder of the shrimp, we were unable to separate reproductive organs from the hepatopancreas. We therefore combined these organs for analyses in all shrimp.

Following removal of the gonad and hepatopancreas, we dried these organs (combined), as well as the rest of the shrimp to a constant weight at 60°C. We then calculated the gonado-hepatosomatic index (GHSI) as a measure of physiological condition, which provides a measure of gonad+hepatopancreas size that is independent of differences in overall body size (Kyomo 1988). Previous studies have shown that severe stressors such as starvation (e.g. Sánchez-Paz et al. 2007), toxins (e.g. Pinho et al. 2003), and parasite infection (e.g. Stentiford et al. 2001) can lead to decreased energy storage in the hepatopancreas. We therefore expected the GHSI to decrease with parasite infection. We tested this expectation by using a linear model, with GHSI as the response variable, with tidal height as a continuous predictor variable, and with parasite infection, sex and sexual maturity as categorical variables. For this and all other linear models, we verified that the data met the assumptions of the linear model by visual inspection of residual plots. The three-way interaction between infection, sex and maturity was significant in this analysis. Therefore, to simplify interpretation of the results, we subsequently analysed male and female shrimp separately. We included tidal height in these analyses because we expected GHSI to decrease with tidal height, reasoning that shrimp at higher tidal heights would have less time to forage each day.

Lipids play an important role in crustacean egg production (Cuzon et al. 2008). We therefore also examined the influence of parasite presence on shrimp lipid accumulation in the gonad+hepatopancreas. We extracted the lipids from a subset of 38 shrimp that included infected and healthy individuals and that were sampled from a range of tidal heights. We extracted lipids from a standardised amount of hepatopancreas tissue, by using a modified version of the Folch et al. (1957) method outlined in Hara and Radin (1978). Following lipid extraction, we used a linear model to compare lipid content as a function of tidal height, parasite presence, shrimp sex and sexual maturity.

Our prediction was that lipid content should decrease with tidal height (for the same reasons as hepatopancreas given above) and with parasite infection, and should be higher in females and in mature shrimp.

Finally, if negative effects of parasites on host are affected via negative impacts on host energetics, then these effects may be expected to increase when the size of the parasite increases. To investigate this hypothesis, we divided parasite dry weight by host dry weight. We then used this ratio, together with shrimp sex and maturity, as predictor variables in a linear model, with parasite GHGI as the response variable.

Results

We found that parasite prevalence was greater in female shrimp, with 24% being infected, whereas only 6% of sampled male shrimp were infected. In all cases of infection, parasite load was limited to no more than one female and one male Orthione griffenis present within a single host. We found no evidence for feminisation. Shrimp identified as females on the basis of the presence of modified pleopods always had gonopores located on the third set of walking legs, whereas those identified as males on the basis of the absence of modified pleopods always had gonopores located on the fifth set of walking legs. Finally, we found that male shrimp had larger chelipeds than did females (linear model, $t_{1,386} = 3.98, P < 0.0001, \text{Fig. 1}$), that immature shrimp had larger size-specific chelipeds than did mature shrimp (linear model, $t_{1,386} = 4.27, P < 0.0001$), and that parasite infection had no influence on cheliped length for male or female shrimp (linear model, main effect: $t_{1,386} = -1.11, P = 0.266$; interaction between sex and parasite infection: $t_{1,386} = 0.57, P = 0.57$, interaction between maturity and parasite infection: $t_{1,386} = 0.92, P = 0.36$, interaction between sex and maturity: $t_{1,386} = 6.05, P < 0.0001$, three-way interaction: $t_{1,386} = 1.0, P = 0.32$, Fig. 1).

Incidence of infection increased with shrimp CL (logistic regression, $z = 6.14, P < 0.0001$), with no parasites found infecting shrimp smaller than 20-mm CL. As shrimp CL increased, there was a general increase in the proportion of the population that were females (Fig. 2) and in the proportion of infected individuals, with the highest frequency of infection

Fig. 1. Allometric relationship between cheliped length and carapace length for healthy and infected (a) female and (b) male shrimp.
occurring in the largest females (Fig. 2). Infection was higher for female than male shrimp (logistic regression, \( z = 4.47, P < 0.0001 \), Fig. 2). However, there was no effect of tidal height on parasite presence (logistic regression, \( z = 1.14, P = 0.25 \), Fig. 3).

Our analysis of GHSI for male and female shrimp combined found that females had a larger GHSI than did males (linear model, main effect of sex: \( t_{1,401} = 2.44, P = 0.015 \), Fig. 4). For clarity of interpretation, we reanalysed male and female shrimp separately. For uninfected female shrimp, the effects of parasite infection depended on maturity. GHSI was larger for mature infected than for mature uninfected individuals, whereas infection had no significant effect on GHSI for immature females (linear model, main effect of maturity: \( t_{1,190} = 5.83, P < 0.0001 \), Fig. 4; maturity \( \times \) parasite interaction: \( t_{1,190} = -3.24, P = 0.001 \)). In contrast, for male shrimp, there was no effect of maturity (\( t_{1,210} = 0.55, P = 0.58 \), Fig. 4) or parasite infection (\( t_{1,210} = 0.26, P = 0.80 \), Fig. 4) on GHSI. GHSI strongly declined with tidal height for both sexes (females: \( t_{1,190} = -4.79, P < 0.0001 \); males: \( t_{1,210} = -4.40, P < 0.0001 \), Fig. 5).

We found that the mass of bulk lipids in the hepatopancreas decreased with tidal height (linear model, \( t_{1,35} = -3.55, P = 0.001 \)) and with parasite infection (\( t_{1,35} = -1.99, P = 0.055 \)) and increased with sexual maturity (\( t_{1,35} = 2.39, P = 0.022 \)); however, it was not different for males and females (\( t_{1,35} = 0.17, P = 0.86 \), Fig. 6). Finally, GHSI decreased with the ratio of parasite weight: host weight (i.e. as the size of the parasite increased relative to its host) for female shrimp, but not for male shrimp (linear
model, main effect of parasite:host ratio, $t_{1,54} = -2.47$, $P = 0.017$; main effect of sex, $t_{1,54} = 2.92$, $P = 0.005$; interaction between ratio and sex, $t_{1,54} = 4.08$, $P < 0.0001$, Fig. 7). Shrimp sexual maturity did not influence the GHSI when the ratio of parasite weight : host weight was included in the analysis (main effect of maturity: $t_{1,54} = 0.82$, $P = 0.42$).

**Discussion**

**Tests of predictions**

Our results provided mixed support for the predictions formulated by Griffen (2009). First, we found no evidence that *O. griffenis* is influencing host reproduction through feminisation of male *U. pugettensis*. Specifically, there were no discrepancies in the location of the gonopores and the presence of a modified first pleopod in male or female shrimp. Consistent with Smith et al. (2008), we also found no change in cheliped morphology with infection. Previous theoretical work found that feminisation of infected males was the most parsimonious explanation for the patterns of parasite prevalence in this system (Griffen 2009), as is common in other isopod–shrimp host–parasite systems (e.g. Tucker 1930; Beck 1980). However, our results did not support this explanation. Although feminisation of male hosts does not appear to be responsible for the previously reported patterns that parasite prevalence increases with shrimp size and is higher in female than in male shrimp (Smith et al. 2008; Griffen 2009; Dumbauld et al. 2011), these patterns continue to persist at the present study site (Fig. 2). Although the mechanism causing this pattern remains unclear, this pattern is consistent with the prediction that parasite infection increases with exposure to the planktonic cryptoniscan stage of the parasite, because this exposure should increase with size-dependent pumping rates of shrimp. However, we did not find any relationship between tidal height and parasite infection (Fig. 3), as is also expected if infection is simply a function of exposure to the parasite because shrimp

lower down on the shore will be inundated by the tide for longer periods each day. Perhaps, with an assumption that shrimp size can be directly correlated with age, larger shrimp that are more likely to be infected have had ample time over several years to obtain parasites regardless of intertidal elevation.

Finally, our results provided strong support for the prediction that parasite infection negatively affects host energetic state and that these impacts become larger as parasite size increases. Hepatopancreas lipid content of both infected male and female shrimp was lower than for uninfected shrimp (Fig. 6). This is consistent with previous findings that epibranchial parasitic isopods can change lipid metabolism in their shrimp hosts (Anderson 1975). However, when using GHSI as a metric of individual condition, the influence of the parasites differed for male and female hosts. For female hosts, and especially for females of reproductive size, infection decreased host condition, as expected (Fig. 4). Also as expected, this effect increased as parasite size increased relative to host size (Fig. 7). These results suggest negative impacts of parasite infection on host energy budgets. In contrast, parasites did not negatively affect the condition of male hosts relative to uninfected males (Fig. 4); however, the number of infected males for comparison was low ($n = 12$) and variation was high. Further, for male hosts there was no relation between the size of the parasite relative to the host and the impacts on host condition (Fig. 7). However, we note that negative effects of parasites on female GHSI were only apparent because of the occurrence of large parasites in relatively small shrimp (i.e. parasites that were >10% the biomass of their shrimp host, seen to the right on Fig. 7).

**Differential effects of infection on male and female hosts**

The larger detrimental effects of infection on female hosts than on male hosts reported here suggest that negative impacts of *O. griffenis* on *U. pugettensis* populations may result from consequences to female hosts rather than from male feminisation. Alternatively, detrimental population impacts could stem from lethal effects on male hosts that were not detected here. If so, then this could explain why we did not observe any small male shrimp with relatively large parasites as were found for female shrimp (again, seen on the right on Fig. 7). This would suggest that infection in male shrimp becomes lethal for hosts if parasites get too large relative to host size. In contrast to this reasoning, Dumbauld et al. (2011) found no effect of *O. griffenis* or of shrimp sex on *U. pugettensis* mortality rates when shrimp were held in the laboratory. However, that study examined only the presence/absence of the parasite and did not examine parasite size relative to host size.

One possible explanation for the differential effect of the parasite on male and female hosts could be sex-specific physiological demands associated with host reproductive strategies, as has been shown in previous studies of marine and freshwater crustaceans (Clarke 1984; Plaistow et al. 2001). For instance, Plaistow et al. (2001) found that lipid and glycogen levels both differed between male and female amphipods (*Gammarus pulex*) that were infected with an acanthocephalan parasite. These authors attributed this to different energy requirements for males and females. Females stored greater amounts of energy in the form of lipids for use as a reserve during times
of starvation and reproduction, whereas males required more immediately available energy in the form of glycogen for activities such as mate searching, mate competition and mate guarding behaviours. However, this remains speculative in our study system, given the lack of information on specific \textit{U. pugettensis} reproductive strategies.

Regardless of the specific mechanism involved, it appears that \textit{O. griffenis} infection may reduce or eliminate \textit{U. pugettensis} reproduction via its effects on female physiological condition. Dumbauld et al. (2011) assumed complete reproductive failure of infected \textit{U. pugettensis} females and calculated that this would result in a 68% reduction in overall \textit{U. pugettensis} reproduction within Yaquina Bay. Our observation that none of the infected females examined was engaged in vitellogenesis (whereas 27 non-infected females were) is consistent with the assumption of complete reproductive failure, and our results suggest that such reproductive failure may result from deterioration of female host condition.

The data presented here are correlational only, and therefore unequivocally determining cause and effect is not possible. It could be possible that infection does not impair energetic state, but rather, that poor energetic state facilitates infection. However, the biomass of the hepatopancreas in crustaceans is temporarily variable, depending on season and molt cycle (e.g. Kennish 1997). Bopyrid parasitic isopods such as \textit{O. griffenis} remain with their hosts through successive molt stages. Data on growth rates of \textit{O. griffenis} are unavailable; however, large individuals are likely to have been with their hosts for a long time relative to the time scale of normal variation in hepatopancreas. Thus, although correlational only, it seems likely that the parasite infection is driving the condition of the hepatopancreas and not the other way around. At the same time, the fact that the hepatopancreas varies seasonally suggests that there may be interesting patterns of parasite effect on shrimp energetics that are only observable across seasons. Future studies could benefit from sampling at different times of the year, especially during the reproductive season when clear differentiation of hepatopancreas and gonads would facilitate measuring effects on these two organs separately.

Assuming that \textit{O. griffenis} does indeed cause reproductive failure in \textit{U. pugettensis}, its overall impact on host populations will depend on its prevalence, which may vary temporally because of complex host–parasite population dynamics (Anderson and May 1978; May and Anderson 1978). The prevalence of \textit{O. griffenis} in its host was much lower in our samples than has been found in previous years at this same site. Griffen (2009) reported that in July 2001, 48% of males and 58% of females at this site were infected. Smith et al. (2008) reported that in 2005 at the same site, 57% of males and 80% of females were infected. Here, we report that only 6% of males and 24% of females were infected. This recent decline agrees with data presented by Dumbauld et al. (2011) that showed a decline from 75% overall prevalence in 2006 to 22% overall prevalence in 2009. These researchers also suggested that \textit{O. griffenis} populations remained low in invaded areas following their likely introduction in the early 1980s. Such lag times before rapid population growth, followed by population spikes, are characteristic of invasive species (Mack 1985; Sakai et al. 2001). Lower parasite prevalence reported here may be the result of host–parasite population dynamics and represent reductions to more sustainable levels following the earlier population spikes, although Dumbauld et al. (2011) still reported high prevalence values for other estuaries.

Conclusions

The increasing occurrence of invasive parasites across a variety of ecosystems provides an important area of study, particularly because native hosts of these invaders are often ill-adapted to cope with their negative effects. Parasites also have large impacts by infecting ecosystem engineers in other systems (Thomas et al. 1999). For example, infection of cockles by trematode parasites in intertidal New Zealand waters alters the interactions of species that colonise the hard cockle shells, the only available hard substrate in muddy areas, and thus alters community composition in that system (Thomas et al. 1998). Given the role of \textit{U. pugettensis} as a widespread ecosystem engineer, the local loss or reductions of \textit{U. pugettensis} populations via the impacts of its invasive parasite could have far-reaching implications for estuarine and coastal ecosystem function. Our study has clarified the energetic impacts of \textit{O. griffenis} and has demonstrated differential impacts of this parasite on male and female hosts. Additional work is needed to clarify the reason for differential effects of this parasite on male and female hosts, to clarify how impacts on male hosts change with parasite size, and to further explore the connection between parasite infection and host-population declines.

Acknowledgements

This work was supported by the University of South Carolina and by the Undergraduate Magellan Scholar program. Samples were collected under Oregon scientific taking permit #14365. Thanks go to M. Griffen for assistance in sample collection. Thanks also go to reviewers who greatly improved the manuscript.

References


G

Parasite effects on ecosystem engineer physiology  


