Behavioral changes in the grass shrimp, *Palaemonetes pugio* (Holthuis), induced by the parasitic isopod, *Probopyrus pandalicola* (Packard)

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**Abstract**

Preliminary observations indicated that the grass shrimp, *Palaemonetes pugio*, parasitized by the isopod, *Probopyrus pandalicola*, captured significantly fewer prey items (*Daphnia*) and exhibited reduced activity compared to unparasitized conspecifics. Further research focused on elucidating the factors involved in altering the shrimp’s behavior. When viewed from above in an opaque container, activity levels of parasitized shrimp were considerably lower than unparasitized shrimp; however, when viewed from the side in a glass container, differences in activity were also seen, but not under all circumstances. In response to the observer above, the parasitized shrimp reduced their activity disproportionately. When (in the opaque tank) prey capture of *Artemia* was examined, there was no difference between parasitized and unparasitized shrimp’s capture rate. This is attributable to the greater activity of *Artemia*, which makes them more likely than *Daphnia* to encounter a relatively inactive predator. There was not a significant difference in prey capture when *Cyprinodon* larvae were used as prey and tanks were not observed from above. When predator avoidance was studied using mummichogs (*Fundulus heteroclitus*), we expected that the more conspicuous parasitized shrimp would be preyed upon more, but this was not the case. Their lowered activity in the presence of a predator apparently reduced their conspicuousness, so that they were not preyed on more than the unparasitized shrimp. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Activity; Behavior; Isopod; Parasite; Shrimp; Predation

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1. Introduction

Predator–prey relations are a major force influencing the structure and dynamics of many communities (Sih, 1987). Since predators may intimidate prey into decreasing their feeding activity, prey growth can be impaired. The amount of time an organism takes to recover after confronting a predator is important. If recovery is fast, the predator may still be in the vicinity thus increasing predation rates. If slow, the organism will decrease its potential foraging time unnecessarily (Von Buskirk and Yurewicz, 1998). Additionally, if an individual is hungry, it may accept a greater risk in order to obtain food (Ryer and Olla, 1998). The more risk the individual is inclined to take, the more prone to predation it will become.

Parasites can alter predator–prey relationships of their host. Mesa et al. (1994), in a review paper, concluded that in 73% (27 out of 37) of the studies reviewed, ‘substandard’ prey (e.g. sick or parasitized individuals) were captured in higher than expected proportions. Most research indicates that stressed or substandard prey are more vulnerable to predation, and that predators typically capture these individuals disproportionately (Temple, 1987).

Parasites exploit the energetic resources of their hosts. Parasitic infections are frequently associated with reduced growth, fecundity, and survival (O’Brien and Van Wyk, 1985; Oppliger and Clobert, 1997), and altered host behaviors such as unusual levels of activity, disorientation, and increased conspicuousness (Holmes and Bethel, 1972), which may lead to differential predation. Any noticeable change in behavior or morphology has the potential to stimulate an increase in predator attacks and lead to differential predation (Bams, 1967). Most previous research concentrated on infected intermediate hosts whose behaviors are altered by the parasites in ways which increase their transmission to their final definitive hosts (Lafferty and Morris, 1996). Less work has been done on behavioral alterations in the definitive host, which could affect predator–prey interactions.

The grass shrimp, *Palaemonetes pugio* (Holthius) (Crustacea; Decapoda), is a vital part of the estuarine community due to its role as an opportunistic omnivore in estuarine food webs (Odum and Heald, 1972; Morgan, 1980; Posey and Hines, 1991). It is an important prey item to numerous resident and migratory estuarine species, especially the killifish, *Fundulus heteroclitus* (Nixon and Oviatt, 1973; Welsh, 1975; Kneib, 1987; Posey and Hines, 1991). Consequently, it is a very important trophic link between the intertidal marsh and open estuarine water. Juveniles mature when they are 1.5–2 months old and about 15–18 mm long. *P. pugio*’s overall lifespan ranges anywhere from 6 to 13 months and adults may reach a maximum size of up to 50 mm (Alon and Stancyk, 1982).

*Probopyrus pandalicola* (Packard) (Epicaridea; Bopyridae) is a hematophagous ectoparasitic isopod that infests palaemonid shrimps, including *P. pugio* (Pike, 1960; Walker, 1977; Cash and Bauer, 1993). Based primarily on observations of field-collected adult hosts, some studies concluded that the vitality of infected hosts is unimpaired (Morris, 1948; Van Wyk, 1982). On the other hand, Anderson (1990) demonstrated that experimental infections by *P. pandalicola* are detrimental to young hosts, with up to 100% mortality resulting within a few days after infection in some groups.

In this study, we investigated the effects of this parasite on predator–prey interactions
Fig. 1. Photograph of *Palaemonetes pugio* with *Probopyrus* parasite on its right side.

and activity of the grass shrimp. We hypothesized that since the parasite forms a large obvious deformation on the side of the host (Fig. 1), it might reduce the host’s activity level, coordination, and ability to capture live food. Since we planned prey capture experiments with different prey species that required different salinities, we also tested for effects of salinity itself on the activity level of the grass shrimp. We also hypothesized that the large, opaque, parasite would make the transparent host more conspicuous to predators. In view of prior work showing that stressed prey are more vulnerable to predators, we hypothesized that the parasite would reduce the host’s ability to escape from predators.

2. Materials and methods

A tidal marsh system located in Tuckerton, New Jersey (TK), was the study site. TK is a high-salinity (mean, 28 ppt) salt marsh that is tidally inundated daily. Parasitized (P)
and unparasitized (U) grass shrimp (24–33 mm) were collected from tide pools using umbrella nets (side², 106.68 cm; 5 mm mesh) and transferred to the lab where they were allowed to acclimate for several days. All shrimp were measured to the closest mm from the tip of the rostrum to end of the telson.

2.1. Shrimp activity level and salinity

A grid of lines 2 cm apart was drawn on the bottom of opaque plastic containers (length, 10 cm; height, 19 cm) which were filled with 200 ml water at salinity of 5, 15, or 26 ppt using commercial sea salts. One shrimp, either P (28 mm±0.43 SE) or U (28 mm±0.60 SE), was randomly selected, measured, placed in the container, and allowed to acclimate for 5 min. The number of lines crossed (each line crossed, 2 cm) was noted after 1 and 3 min. Trials were run with 20 P and 20 U at 15 ppt, and 10 P and 10 U at both 5 and 26 ppt. These salinities were chosen to check whether salinity itself could alter activity levels of the shrimp, since prey capture experiments (see below) were run at these different salinities. Two-sample t-tests were used to compare the activity level (number of lines crossed) of P vs. U shrimp at each salinity (5, 15 and 26 ppt) after 1 and 3 min. One-way ANOVA compared P and U shrimp separately at each salinity.

2.2. Shrimp activity and hunger

A 9.5-l clear glass tank filled with 4 l of 28 ppt sea water was used as an observation tank. Three sides of the tank were covered with paper. One shrimp, either P (26 mm±0.33 SE) or U (27 mm±0.34 SE), was randomly selected and measured, placed in the tank and allowed to acclimate for 5 min. Three variables (walking, swimming, and resting) were measured concurrently over a 5-min period using three stopwatches. The role of hunger was investigated by testing shrimp: (a) after being fed 1 h prior to test; (b) after being starved for 1 day; and (c) after being starved for 2 days. Trials were run with 13 P and 13 U under condition (a) and 10 P and 10 U for both (b) and (c). Data were analyzed by t-tests and ANOVA.

2.3. Shrimp responses to an observer

In order to ascertain the reaction of the shrimp to the observer, a 9.5-l clear glass tank filled with 4 l of 15 ppt sea water was used. All four sides of the tank were covered with paper. One shrimp (P or U) was randomly selected and allowed to acclimate for 5 min. Once the shrimp was deemed active (either swimming or walking), the observer would stand up and look down directly into the tank (to mimic a potential predator above). All shrimp ‘froze’ and ceased activity immediately. The time taken to resume activity in the presence of the ‘predator’ was then recorded. Observations were made on 20 P and 20 U shrimp. Data were analyzed by t-tests.

2.4. Prey capture

Grass shrimp (mean, 29 mm) were starved for 2 days, and placed in opaque observation tanks (length, 10 cm; height, 19 cm) filled with 200 ml water. One grass
shrimp (P or U) was placed in the container and allowed to acclimate for 5 min. Ten adult *D. magna* (Straus) were then added as prey. Salinity was 4 ppt since *Daphnia* are basically freshwater species but can tolerate low salinity (Schuytema et al., 1997). The number of prey eaten was recorded after 5 and 10 min. Trials were run with 13 P and 13 U grass shrimp. The experiment was repeated with 10 adult *Artemia salina* (L.) as prey, with the salinity at 28 ppt. Trials were run with 20 P and 20 U grass shrimp. Another set of trials was done using 10 1-week-old *Cyprinodon variegatus* (Lacepede) larvae as prey, with salinity at 15 ppt. These fish larvae are a more natural food item, which grass shrimp are likely to find in their normal habitat, unlike *Daphnia* or *Artemia*. Trials were run with 20 P and 20 U shrimp. Since fish larvae exhibit predator avoidance behavior and are more difficult to catch than the two other species, trials lasted 2 h, and the test tanks were left alone (not watched) until the 2 h had elapsed.

### 2.5. Predator avoidance

Adult mummichogs, *F. heteroclitus* (L.) from TK (70–80 mm SL) were starved for 2 days and groups of three fish were allowed to acclimate in an 80-l test tank for 1 h prior to testing. The test tank had sand on the bottom, and three large rocks for prey refuge. The experimental procedures used in Smith and Weis (1997) were followed with minor modifications. Six *P. pugio* (3 P and 3 U, mean 28 mm) were introduced into the test tank. The number and order in which the shrimp were captured was recorded over 30 min. Eight trials were run. Data were analyzed by $\chi^2$.

### 2.6. Statistical procedures

All statistical analyses (ANOVA, *t*-tests and $\chi^2$) were executed using Statistix® for Windows analytical software.

### 3. Results

#### 3.1. Shrimp activity and salinity

At 5 ppt, P shrimp were less active than U shrimp (Fig. 2), but differences were not significant ($P > 0.05$). At 3 min, differences were still not significant. However, at 15 ppt, highly significant differences ($P < 0.001$) were found between P and U individuals after 1 and 3 min. At 26 ppt, significant differences were also found after 1 min ($P < 0.001$) and 3 min ($P < 0.01$), with U shrimp exhibiting higher activity than P shrimp (Fig. 2).

One-way ANOVA compared P and U shrimp separately at each salinity. P shrimp had no significant differences among the three salinities at 1 ($F = 1.96, P > 0.10$) or 3 min ($F = 1.34, P > 0.20$). Similarly, there were no significant differences among the three salinities after 1 min ($F = 2.93, P > 0.05$), or 3 min ($F = 2.12, P > 0.10$) for U shrimp.
3.2. Shrimp activity and hunger

We compared P vs. U shrimp activity after (a) having just been fed, (b) having been starved for 1 day, and (c) having been starved 2 days. Shrimp that had just been fed had highly significant differences in activity budgets, with U shrimp having greater activity. P shrimp spent about 33% of the time resting, 10% walking, and 57% swimming, while U shrimp spent only 7% of the time resting, 7% walking, and 86% of the time swimming. $\chi^2$ (two by two) comparing active (time swimming + walking) versus inactive was 13.3 ($P < 0.01$). Shrimp that had been starved for 1 day showed significant differences. P shrimp spent 15% of the time resting, 10% walking, and 74% of the time swimming, while U shrimp spent 3% of the time resting, 4% walking and 93% of the time swimming. Thus, both groups increased their time swimming and decreased their time resting. This may represent increased searching for food. $\chi^2$, comparing time active versus time inactive was 4.1 ($P < 0.05$). After being starved 2 days, the same relationships were seen among the different activities, but differences were no longer significant. P shrimp spent 29% of the time resting, 10% walking, and 61% swimming, while U shrimp spent 16% of the time resting, 10% walking, and 74% of the time swimming. $\chi^2$ for time active versus time inactive was 2.2 ($P > 0.05$). The active time for both groups was decreased, which may be due to reduced energy reserves after the 2 days of starvation.

Fig. 2. Activity level (number of lines crossed) for parasitized and unparasitized shrimp at three different salinities.
3.3. Shrimp response to the observer

When the observer stood up, all shrimp immediately ceased activity and went to the bottom. The time taken to resume activity was significantly greater \( t = 2.40, P < 0.05 \) for P shrimp \( (142 \pm 38 \text{ s}) \) than U shrimp \( (38 \pm 21 \text{ s}) \). Since P shrimp took much longer than U shrimp to resume activity, the activity differences we observed previously in the opaque tank can be interpreted as decreased activity in response to the observer.

3.4. Prey capture

Significant differences were found by \( t \)-tests between U and P shrimp in mean number of Daphnia captured (Table 1). After 5 min, there was a significant difference \( (P < 0.02) \) in the number of \( D. \text{magna} \) captured, with P shrimp catching far fewer than U shrimp. After 10 min, the difference became highly significant \( (P < 0.001) \) with P shrimp capturing far fewer prey than U shrimp. However, when Artemia was used as prey, there was no difference between the number consumed by U and P shrimp (Table 1) after 5 min \( (t = 0.17, P > 0.50) \) or 10 min \( (t = 0.38, P > 0.50) \). In the trials with the Cyprinodon larvae, P shrimp captured \( 0.27 \pm 0.08 \) (SE), and U shrimp captured \( 0.39 \pm 0.09 \) larvae. These capture rates are not significantly different \( (t = 1.0, P > 0.05) \).

Since the differences in capture rates of the crustacean prey may be due to different swimming behaviors and activity exhibited by the two species, we analyzed their spontaneous activity. Daphnia tend to move vertically rather than horizontally in the water, whereas Artemia generally move horizontally. Activity of these two crustaceans was measured by recording the number of lines individual Daphnia \( (n = 10) \) or Artemia \( (n = 10) \) crossed after 1 and 3 min. Artemia crossed more lines than Daphnia. Significant differences were seen after 1 min \( (t = 5.53, P = 0.001) \) and 3 min \( (t = 4.43, P = 0.001) \) using a two-sample \( t \)-test. After 1 min, Artemia crossed \( 34 \pm 4 \) SE lines and Daphnia crossed only \( 8 \pm 1 \) lines. This difference was seen again at 3 min with Artemia crossing significantly more lines \( (90 \pm 15 \text{ SE}) \) than Daphnia \( (22 \pm 3 \text{ SE}) \). The greater horizontal activity of Artemia makes them much more likely to encounter an inactive predator (i.e. P shrimp) than the Daphnia. In the trials with Cyprinodon larvae as prey, the shrimp’s activity was not affected by the observer, since tanks were left alone for 2 h. Far fewer fish larvae were captured than either Artemia or Daphnia despite the longer time.

<table>
<thead>
<tr>
<th>Shrimp condition</th>
<th>Artemia</th>
<th>Daphnia magna</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Parasitized (P)</td>
<td>3.8±0.46</td>
<td>5.0±0.37</td>
</tr>
<tr>
<td>Unparasitized (U)</td>
<td>3.7±0.38</td>
<td>4.9±0.37</td>
</tr>
</tbody>
</table>

*\( n = 20 \) for Artemia tests; \( n = 13 \) for Daphnia tests.
*Indicates significant difference \( (P < 0.05) \) between parasitized and unparasitized shrimp.
Table 2
Sequence and number of P. pugio preyed on by F. heteroclitus in 30 min (no statistically significant differences)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Parasitized (P)</th>
<th>Unparasitized (U)</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>U, P, U, P</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>U, U, P, U</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>U, P, P</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2</td>
<td>U, U, P</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td>U, P</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1</td>
<td>P, P, U</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>P, P, P, U</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0</td>
<td>P</td>
</tr>
</tbody>
</table>

3.5. Predator avoidance

There was no difference ($t = 1.8$, $P > 0.05$) between the mean number of U (1.38±0.35 SE) versus P (1.75±0.27 SE) shrimp captured by F. heteroclitus. When the order in which shrimp were eaten was examined, U shrimp were captured first 63% of the time versus 38% for P shrimp (Table 2). This may be due to the greater activity of U shrimp. However, this difference is not significant by $\chi^2$.

4. Discussion

In an opaque tank with the observer above, P shrimp were less active than U shrimp. The differences are partly due to the fact that the P shrimp reduce their activity to a greater degree in response to an observer viewing from above. When a seated observer stood up, P shrimp took much longer to recover and resume activity than U shrimp. Thus, the differences seen in activity in the opaque tanks are partly due to the experimental set-up. When the observer looked horizontally through the glass of a clear tank, differences in activity of P and U shrimp were again seen, under some conditions. Differences were highly significant shortly after the shrimp had been fed, were significant after shrimp had been starved for 1 day (when both groups were more active, perhaps searching for food), and were not significant after shrimp had been starved for 2 days (when both groups were less active, perhaps conserving energy).

The prey capture experiments also emphasize the importance of test conditions, as well as knowledge of the behavior of the prey species. Based solely on the data collected using Daphnia, one would conclude that P shrimp are poorer predators than U shrimp. However, these tests were performed in the opaque tank with the observer above. Because of the P shrimp’s disproportionately decreased activity combined with the low activity of Daphnia, the number of encounters between P shrimp and their prey was greatly reduced. When a more active prey species was used (Artemia), the number of encounters (and captures) by P shrimp rose, and were equivalent to those of U shrimp. Our salinity/activity experiments demonstrated that these differences in prey capture
were not due to the differential salinity at which these trials were run, but rather were
due to the prey’s behavior. Thus, P shrimp are not necessarily poorer predators, but only
were when provided with inactive prey. It is interesting to note that the shrimp did not
‘freeze’ in response to movements of the observer on the outside of the glass tank, but
only to an observer above. This may reflect a situation in which natural predators
approach grass shrimp from above. In the trials with the Cyprinodon larvae as prey, the
shrimp were not watched from above. The larvae tended to remain motionless on the
bottom of the tank most of the time. However, unlike the crustacean prey, they did
display predator avoidance behavior; when a shrimp approached, they darted away
rapidly. Capture rates were much lower than the captures of the crustaceans, and were
comparable for P and U shrimp. Since predation trials were run after shrimp had been
starved for 2 days, P and U shrimp would not be expected to have significantly different
activity levels under these circumstances, as seen in our hunger/activity experiments.

Effects of parasites on foraging have been studied by Giles (1987), who reported that
parasitized sticklebacks (Gasterosteus aculeatus) ate more Tubifex worms than un-
parasitized fish. This is contrary to findings of Milinski (1985) in which heavily
parasitized G. aculeatus ate less than unparasitized fish. In both studies, parasitized
individuals foraged more often and took a wider variety of prey than unparasitized ones.
Barber and Ruxton (1998) found that parasitized sticklebacks captured fewer prey items
under some circumstances (when prey were in locally dense patches) but equal amounts
under other circumstances (when prey was presented sequentially). Thus, in this study as
well as ours, P animals captured either fewer or an equal amount of prey, but not more.

If parasitized shrimp have lower (or equal) prey capture, and decrease their feeding
activity disproportionately after observing a predator they can incur energetic deficits,
since the metabolic demands of the parasite are added to the host’s needs. Walker (1977)
estimated that a parasitized grass shrimp lost up to 25% of its hemolymph volume daily
to the isopod. Anderson (1977) estimated that up to 10% of the daily energy intake of P.
pugio went to the bopyrid. Thus, the isopods are a significant energy drain for the
shrimp. The lower activity level of P shrimp in the glass tank, (when not responding to
an observer above) may reflect conserving their energy reserves. The differences in
activity were greatest shortly after being fed, the condition most likely to occur in
nature, where shrimp can easily graze on meiofauna and detritus without exerting much
energy.

Upon appearance of a predator, aquatic organisms usually reduce their feeding rate
and reduce movement (Gilliam et al., 1989; Turner and Mittelbach, 1990), which lowers
the risk of predation (Lima, 1998). Our results imply that reduced activity was useful to
P. pugio in avoiding predation by F. heteroclitus, a visual predator that, contrary to
expectations, did not capture significantly more parasitized than unparasitized shrimp,
and often captured the more active unparasitized shrimp first. The lower activity of P
shrimp may make them less conspicuous, and thereby compensate for their greater
visibility and reduced agility. Similarly, Tierney et al. (1993) found that cestodes
(Schistocephalus solidus) increased the time sticklebacks (G. aculeatus) spent motion-
less. They suggest that the parasites may induce predator-avoidance behaviors, thereby
decreasing the probability of predation on the fish. Similar behavioral changes appear to
be evoked in Palaemonetes by the bopyrid.
References


