Feedback in marine soft-sediments: Symbiosis, ecosystem engineering and feedbacks

**Keywords:** Ecosystem engineering, Axidea, Symbiosis, Marine soft-sediments, Feedback

**Abstract:**
Ecosystem engineering by burrowing species is one of the most influential determinants of community structure in marine sedimentary ecosystems. However, per capita ecosystem engineering rates displayed by burrowers are variable, and thereby contribute to enhancing local biotic and abiotic variability. Lacking in current understanding of processes influencing variability in ecosystem engineering rates by burrowers and consequent feedbacks to assemblages are interactions occurring between burrowers and their burrow-symbionts. In this paper, we quantify behaviour responses of burrowing sandprawns (*Callichirus kraussi*; Crustacea: Axiidea) to burrow symbionts (*Betasus jucundus*; Crustacea: Alpheidae) using controlled laboratory experiments. We demonstrate that sandprawns display discrete behaviours in response to *B. jucundus* with distinct levels of tolerance. Such variability in tolerances of sandprawns to *B. jucundus* is consistent with the idea that the outcomes of symbiotic relationships are variable, depending on processes that shift the costs and benefits involved for partners. Importantly, these discrete tolerances were associated with significant changes to time spent by sandprawns on ecosystem engineering activities (sediment turnover, burrow irrigation) with intolerant sandprawns increasing sediment turnover rate by 50% and decreasing irrigation by 30%. In a second experiment seeking to determine if variability in sandprawn responses to *B. jucundus* could be due to differences in sandprawn sexes, we demonstrate that male and female sandprawns respond differently to the presence of *B. jucundus*, with differences in time spent on ecosystem engineering in the presence of *B. jucundus*. Taken collectively, our results highlight the complex behaviours occurring within burrows in marine sediments and their potential to influence ecosystem activities of burrowers.

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1. Introduction

Ecosystem engineering, the modification of habitats by organisms (Jones et al., 1994), is one of the most influential determinants of ecosystem functioning and self-structuring in marine soft sediment systems (Reise, 2002; Lohrer et al., 2004; Bouma et al., 2005; Pillay and Branch, 2011). Through biological activities and the production of structures, engineers modulate the availability of local resources, which ultimately feeds back to affect engineers and sympatric species (Jones et al., 1994; Bouma et al., 2009). Engineering by burrowers has received particular research attention in marine soft-sediment ecosystems, given that burrowers often occur in very high densities over large spatial areas, can burrow down to depths in excess of 1 m in sediments, display very high per capita engineering rates and can modulate multiple resource flows to species simultaneously (Reise, 2002; Berkenbusch et al., 2000; Berkenbusch and Rowden, 2003; Pillay and Branch, 2011).

Burrowers physically and chemically alter resource flows in marine sediments through three main pathways, which are driven by their burrowing activities (Rowden and Jones, 1993) allied with feeding and respiration behaviours. Firstly, sediment turnover from depth to the sediment-water interface enhances sediment erodibility, thereby generating negative effects on surface fauna (Rhoods and Young, 1970; Pillay et al., 2007a, b), while disrupting settlement/recruitment of juvenile invertebrates (Pillay et al., 2007c). Sediment turnover has also been shown to negatively impact vascular plants and their recruitment (Hughes et al., 2000). Secondly, the elaborate burrow systems burrowers create enhance habitat complexity and provide key habitat for a range of species. Burrow walls are colonised by various microbes and invertebrates (Branch and Pringle, 1987; Papaspyrou et al., 2005a, b), while burrows are often inhabited by various symbionts (Bromley, 1996; Anker et al., 2005; Campos et al., 2009; Lavesque et al., 2016). Burrowing increases sediment porosity and can positively influence burrowing infauna (Siebert and Branch, 2005, 2007; Pillay et al., 2011). Lastly, irrigation and ventilation of burrows oxygenate sediments and transfer nutrients from the sediment-water interface to...
deep sediments and vice-versa (Koike and Mukai, 1983; Pillay et al., 2011; Pascal et al., 2016).

Given the importance of feedbacks generated by physical engineering activities of burrowers to sympatric assemblages (Bouma et al., 2009), studies have sought to understand spatial and temporal conditions that alter per capita ecosystem engineering rates of burrowers. Such studies provide key information required for fine-tuning understanding of contextual dependencies responsible for altering the strength of engineers on co-occurring residents (Berkenbusch and Rowden, 1999; Gutiérrez and Iribarne, 2004). Various physical and biotic variables have been identified as important determinants of ecosystem engineering rates, but engineer behaviour is the ultimate regulator of engineering rates, which in turn is affected by various biotic and abiotic processes (Roskosch et al., 2012). For example, increasing temperature has been shown to increase sediment turnover rates by burrowers; however, the latter is mediated by increasing temperature enhancing metabolic activity of the engineer, which therefore increases burrowing rate (Berkenbusch and Rowden, 1999).

Currently lacking in our understanding of processes impacting per capita ecosystem engineering rates and hence feedbacks to sympatric assemblages, are interspecific interactions occurring within burrow systems. Because of the refuge provided by burrows against physical and biological stressors and trophic resources contained therein, burrows often serve as attractants for other species (MacGinitie, 1934, 1939; Pohl, 1946; Anker et al., 2001, 2005). This results in shared use of burrows, and the evolution of symbiotic relationships in some cases, leading to complex behavioural interactions between burrowers and symbionts (Preston, 1978; Anker et al., 2001, 2005; Thompson, 2004; Pillay, 2010). Such interactions have the potential to alter net rates of ecosystem engineering by shifting the time allocated to burrow construction and maintenance to compensate for time spent interacting with burrow symbionts. Generally though, the latter has not been well researched.

Due to the viscous nature of marine sedimentary habitats, the majority of organisms live and interact within this habitat matrix, relative to hard substrata. However, because of the opaque nature of sediments, direct observations of organismal interactions are rarely possible in situ, resulting in a general dearth of knowledge of behavioural interactions occurring within burrows (Baesa, 2015). The little information that is available is largely descriptive (Anker et al., 2005; Murphy and Williams, 2013; Goto et al., 2014; but see Peiró et al., 2013 and Baesa et al., 2010), leading to a paucity of quantified data on basic behavioural interactions occurring between symbionts and engineers, the conditions that alter the strength of the symbiont-engineer interactions, and how both of the above influence net ecosystem engineering rates.

In this paper, we quantify behavioural responses of an axiidean crustacean (Callichirus kraussi; Crustacea: Axiidea) to the presence of a burrow symbiont (Betaeus jucundus; Crustacea: Alpheidae). We also quantify the effects of symbiont presence on time spent by C. kraussi on ecosystem engineering activities, to assess potential effects of symbiont presence on shifts in ecosystem engineering activities of C. kraussi. C. kraussi is a burrowing crustacean that commonly occurs in estuaries and sheltered marine sedimentary systems in South Africa at very high densities (Pillay et al., 2007a, b). Locally, C. kraussi are referred to as “sandprawns” and globally as “ghost shrimp”. Studies have shown that ecosystem engineering by C. kraussi affects a wide range of assemblages including bacteria, meiofauna, macrofauna, fish and seagrasses. Locally, ecosystem engineering by C. kraussi is one of the most influential determinants of ecosystem functioning and community structure (Siebert and Branch, 2006; Pillay et al., 2008, 2012; Pillay and Branch, 2011).

2. Materials and methods

Controlled laboratory experiments were employed to test the effects of the presence of the burrow symbiont Betaeus jucundus on sandprawn (Callichirus kraussi) behaviour and subsequent effects on time spent by sandprawns on engineering activities. Laboratory experiments are powerful tools capable of identifying causal relationships in marine sedimentary ecology. Moreover, these techniques offer researchers a controlled environment in which organismal behaviour can be rigorously monitored and quantified (Stamhuis et al., 1996). The latter would be logistically difficult using field surveys or experiments. C. kraussi and B. jucundus were collected from intertidal sediments of Oosterwal (33.121652° S, 18.053201° E), Langebaan Lagoon, on the west coast of South Africa (see Pillay et al., 2011 for site details). Symbiont cores were collected using a stainlessrawn pump (= yabby pump; Manning, 1975. Length = 90 cm, diameter = 10 cm), and sieved on a 1 mm mesh. Retained C. kraussi and B. jucundus were then transported to the laboratory where they were held separately in aerated seawater (salinity = 35‰, temperature = 20 °C) until commencement of experiments for a maximum of 6 h, without being fed.

Two experiments were performed sequentially in this study. The first experiment aimed to quantify differences in sandprawn behaviour in the presence and absence of B. jucundus. Based on the results of the first experiment, which showed that sandprawns displayed three discrete behaviours in response to the presence of B. jucundus, a second experiment was undertaken to determine whether some of the results obtained from Experiment 1 could be accounted for by differences in sandprawn sex.

2.1. Experimental design

A closed-circulation system consisting of four narrow glass aquaria (length = 50 cm, height = 40 cm, width = 0.7 cm) contained within a shallow (15 cm) reservoir tray (length = 1.5 m, width = 1 m) was used for the experiment (Fig. 1). The narrow widths of the aquaria restricted movement of burrowing prawns and symbionts to 2 dimensions, thus permitting direct observations of interactions between experimental individuals occurring within burrows and quantification of the time spent by sandprawns on particular behaviours. Observations were made from a single side of each tank, as indicated in Fig. 1, with the contents of entire burrow systems being visible. The tray containing the aquaria was filled with seawater (salinity = 35‰) and acted as a water reservoir in this closed circulation system. Water was pumped (HQ water pump; maximum output = 3000 L/h) from the reservoir tray into each of the four aquaria via PVC pipes (2 cm diameter) allowing water within the four aquaria to overflow into the reservoir tray. Flow rate of water from the reservoir to each aquarium was standardised.

![Fig. 1. Overview of experimental setup used for observations of sandprawn (C. kraussi) - responses to its burrow symbiont (B. jucundus) in Experiments 1 and 2. Tanks 1–4 indicate observational tanks. Arrows indicate direction of water flow. WF = water pump; AP = air pump. Solid filled arrows indicate direction of observations.](image-url)
by using flow regulator valves positioned directly over each aquarium. Three Elite 802 air pumps (output = 1500 cm³/phot) were evenly interspersed within the reservoir tray together with an Elite Jet-Flo water filter in order to oxygenate and clean water circulating through the system. Aquaria were placed on wooden stilts (10 cm) to raise them above the water level in the reservoir tray in order for all behavioural interactions occurring within aquaria to be visible.

Individual aquaria were filled to a depth of 30 cm with sediment collected from the middle intertidal zone of a sandprawn dominated sandflat at Oosterwal, Langebaan Lagoon. Sediments used in the experiment were neither sieved nor de-faunated prior to use in experiments. Following additions to aquaria, sediment was allowed to settle for 10–20 min before a single sandprawn (carapace width = 5.5 mm ± 1.1 SD) was introduced into each of the aquaria. Aquaria containing sandprawns and sediments were left for 3 days in order for sandprawns to construct burrows and acclimatise to their settings prior to observations.

2.2. Ethogram development

Before commencing formal data gathering, preliminary observations were made in order to develop an ethogram of rigorously-defined behaviours displayed by sandprawns in the presence and absence of *B. jucundus* that would form the basis of observations in final experiments. In order to develop the ethogram, the behaviours of five different sandprawns were observed before and after the addition of *B. jucundus* into sandprawn burrows for 45 min. The behaviours exhibited by sandprawns that were quantified in Experiments 1 & 2 (see descriptions below) are listed in Table 1.

2.3. Experimental observations

In Experiment 1, the effect of the symbiotic shrimp *B. jucundus* on sandprawn behaviour was explored. In Experiment 2, the effect of sandprawn sex on responses to *B. jucundus* was tested. In both experiments, the time spent by sandprawns on specific behaviours (Table 1) was quantified using paired observations on a single sandprawn in the presence (45 min) and absence (45 min) of *B. jucundus*. For Experiment 1, behavioural responses of 12 different sandprawns were quantified (i.e. 12 paired before-after observations). For Experiment 2, 10 observations were carried out comprising paired before-after observations on 5 male and 5 female sandprawns. A stopwatch (Oregon Scientific, precision = 0.001 s) was used to quantify time spent by sandprawns on particular behaviours. To avoid temporal factors confounding the results of experiments, all observations were initiated at the same time of day (10:30 am). Similar sized *B. jucundus* (= 5 mm length from rostrum to telson ± 0.9 SD) were used for all experiments. The sample sizes and duration of observations in the experiment were severely constrained by the instability of burrows under laboratory conditions, brought on by the low organic content of sediments used in experiments. Low concentrations of organic matter are characteristic of sediments inhabited by sandprawns.

### Table 1

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burrow irrigation</td>
<td>Back and forth movement of plesiopods by sandprawns.</td>
</tr>
<tr>
<td>Feeding</td>
<td>Transfer of burrow wall sediment to the mouthparts, followed by manipulation of sediment grains by mouthparts.</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Movement of sandprawn through burrow complex.</td>
</tr>
<tr>
<td>Tunneling</td>
<td>Extension of burrow tunnels</td>
</tr>
<tr>
<td>Sediment turnover</td>
<td>Ejection of sediment from burrows to form mounds</td>
</tr>
<tr>
<td>Cleaning</td>
<td>Removal of sediment grains from body through lateral movement of pereiopods.</td>
</tr>
<tr>
<td>Pursuit</td>
<td>Pursuit of <em>B. jucundus</em> through burrows.</td>
</tr>
<tr>
<td>Burrow sealing</td>
<td>Collecting sediment from within the burrow to close off the burrow opening.</td>
</tr>
<tr>
<td>Acceptance</td>
<td>Contact between host and symbiont followed by non-aggressive behaviour or burrow sealing.</td>
</tr>
<tr>
<td>Intolerance</td>
<td>Contact between host and symbiont followed by pursuit of symbiont out of the burrow.</td>
</tr>
</tbody>
</table>

2.4. Data analysis

Two approaches were used to analyse data obtained from Experiments 1 and 2. Firstly, multivariate analyses were undertaken in PRIMER 6.1.11, in which component behaviours (individual behaviours identified in Table 1) were aggregated to assess effects of different treatments on overall sandprawn behaviour. Non-metric multidimensional scaling (nMDS) was used to visually assess differences in sandprawn behaviour between experimental treatments. nMDS plots were generated from resemblance matrices produced by Bray-Curtis similarities after data (square root) transformation. PERMANOVA (permutational analysis of variance) was used to statistically confirm patterns observed in nMDS plots, with pair-wise tests used for inter-treatment differences. The second analytical approach included the use of univariate statistical testing to identify significant differences in sandprawn component behaviours among treatments, using SPSS (Statistical Package for the Social Sciences). Due to observations of behaviours before and after the addition of *B. jucundus* being carried out on the same sandprawns, the assumptions of sample independence were not upheld (Quinn and Keough, 2002). Therefore, repeated measures ANOVA (analysis of variance) was used to test for differences in sandprawn behaviours in the presence and absence of *B. jucundus* with post-hoc Tukey tests for inter-treatment differences. Prior to univariate testing, one sample Kolmogorov-Smirnov and Levene’s tests were performed to test for data normality and equality of variances respectively. In instances in which assumptions for parametric testing were not met, data were transformed (log x + 1). A significance level of 0.05 was used in all statistical tests.

3. Results

3.1. Experiment 1: effect of *B. jucundus* presence on *C. kraussi* behaviour

nMDS plots detected a clear distinction in sandprawn behaviours in the presence and absence of *B. jucundus* and greater variability in sandprawn behaviours in the presence of *B. jucundus* (Fig. 2A). PERMANOVA statistically confirmed the discrete behavioural responses exhibited by sandprawns in the presence and absence of *B. jucundus* (PERMANOVA, pseudo-F = 12.025, P = 0.001, Fig. 2A).

Based on observations of sandprawn responses to *B. jucundus* during ethogram development behaviours of sandprawns to the presence of *B. jucundus* were divided into three categories (viz. intolerance, semi-tolerance and tolerance), based on the proportion of time spent on the behaviours listed below. Intolerant sandprawns spent the majority of time interacting with *B. jucundus* by pursuing it out of burrows and then sealing off burrows by infilling openings with sediment. Semi-tolerant sandprawns, spent the greater part of their interactions with *B. jucundus* by pursuing it out of burrows, but did not display any burrow sealing behaviour, thus allowing *B. jucundus* to return. Tolerant individuals, initially pursued *B. jucundus*, but then made contact with *B. jucundus* (heads of sandprawns and *B. jucundus* touch, followed by contact of *B. jucundus* by sandprawn antennae), followed by *B. jucundus* moving freely within the
burrow system. Visual examination of the nMDS plots indicated that each of the three behaviours above were well defined and discrete (Fig. 2B). Differences in behavioural categories were statistically confirmed by PERMANOVA (pseudo-F2,11 = 28.489, P = 0.001) and pairwise tests (P < 0.01).

Several patterns regarding individual sandprawn behaviours emerged in Experiment 1. Firstly, time spent on sediment turnover differed significantly between tolerant, semi-tolerant and intolerant sandprawns in the presence of *B. jucundus* (Fig. 3A, repeated measures ANOVA, F2,11 = 4.29; P = 0.026; Table 2A), with intolerant sandprawns spending more time on sediment turnover than both tolerant and semi-tolerant sandprawns (Fig. 3A, post hoc Tukey, P < 0.01). Secondly, time spent by sandprawns on burrow irrigation differed significantly in the presence and absence of *B. jucundus* (Fig. 3B, repeated measures ANOVA, F1,23 = 12.36; P < 0.001; Table 2A) with time spent on irrigation being depressed in the presence of *B. jucundus* (post hoc Tukey, P = 0.014). Sandprawn behavioural category, symbiont presence and their interaction did not influence time spent by sandprawns on tunnelling, locomotion and cleaning (Table 2A; Fig. 4).

![Fig. 2](image)

**Fig. 2.** Non-metric multidimensional scaling (nMDS) ordination illustrating variability in (A) *C. kraussi* responses to the presence of *B. jucundus* and (B) differences in *C. kraussi* behaviour in the presence and absence of *B. jucundus*.

![Fig. 3](image)

**Fig. 3.** Variation in time (mean ± SE) spent by intolerant (n = 3), semitolerant (n = 4) and tolerant (n = 5) *C. kraussi* on (A) sediment turnover, (B) burrow irrigation and (C) feeding in the absence (black bars) and presence (grey bars) of *B. jucundus*.

<table>
<thead>
<tr>
<th>[A]</th>
<th>Behavioural category</th>
<th>Symbiont presence</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment turnover</td>
<td>4.29</td>
<td>0.026</td>
<td>3.56</td>
</tr>
<tr>
<td>Irrigation</td>
<td>1.23</td>
<td>0.31</td>
<td>12.36</td>
</tr>
<tr>
<td>Feeding</td>
<td>2.32</td>
<td>0.12</td>
<td>2.36</td>
</tr>
<tr>
<td>Tunnelling</td>
<td>1.86</td>
<td>0.17</td>
<td>1.26</td>
</tr>
<tr>
<td>Locomotion</td>
<td>2.13</td>
<td>0.14</td>
<td>1.96</td>
</tr>
<tr>
<td>Cleaning</td>
<td>2.26</td>
<td>0.13</td>
<td>1.61</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[B]</th>
<th>Behaviour</th>
<th>Symbiont presence</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression</td>
<td>6.53</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Burrow sealing</td>
<td>5.69</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Acceptance</td>
<td>4.56</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Pursuit</td>
<td>3.63</td>
<td>0.042</td>
<td></td>
</tr>
</tbody>
</table>

Values in bold denote statistical significance.
Time spent by sandprawns on behaviours exhibited exclusively in the presence of *B. jucundus* differed significantly between individuals that were classified as intolerant, semi-tolerant or tolerant (Fig. 5, Table 2B). Time spent on burrow sealing (repeated measures ANOVA, $F_{2,11} = 5.69; P = 0.009$; Table 2B), aggression (repeated measures ANOVA, $F_{1,11} = 6.53; P = 0.005$) and pursuit (repeated measures ANOVA, $F_{1,11} = 3.63; P = 0.042$) generally increased from tolerant to intolerant sandprawns at varying degrees (Fig. 5). However, the reverse pattern was observed for time spent on acceptance behaviour (repeated measures ANOVA, $F_{1,11} = 4.56; P = 0.021$; Table 2B).

3.2. Experiment 2: effect of *C. kraussi* sex on their responses to *B. jucundus*

nMDS plots indicated no behavioural differences between male and female *C. kraussi* in the absence of *B. jucundus* (pseudo-$F_{1,9} = 0.9941, P = 0.431$) but distinct behaviours between males and females in the presence of *B. jucundus* (pseudo-$F_{1,9} = 2.9815, P = 0.027$).

Except for time spent feeding (which was greater in females than males; repeated measures ANOVA, $F_{1,4} = 3.65; P = 0.042$; Table 3A), the sex of sandprawns did not affect its behavioural responses to *B. jucundus* (Table 3A, Figs. 7 & 8, repeated measures ANOVA $P > 0.1$), but the presence and absence of *B. jucundus* did influence time spent on burrow sealing (repeated measures ANOVA, $F_{1,11} = 5.69; P = 0.009$; Table 2B).

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by sandprawns on burrow irrigation (repeated measures ANOVA, \( F_{1,4} = 3.56; P = 0.042; \text{Fig. 7B} \)), which was depressed in the presence of \( B. jucundus \). There was a significant interaction between \( C. kraussi \) sex and symbiont presence on time spent by \( C. kraussi \) on sediment turnover (repeated measures ANOVA, \( F_{1,4} = 4.6; P = 0.02 \)), with sediment turnover activities being similar in the presence and absence of symbionts for females, but being disproportionally depressed in males compared to females when in the presence of symbionts.

In terms of behaviours exhibited by sandprawns exclusively in the presence of \( B. jucundus \), males generally spent more time interacting with \( B. jucundus \), but results were only significant for time spent by sandprawns on aggression (repeated measures ANOVA, \( F_{1,4} = 4.6; P = 0.02 \)) and pursuit (repeated measures ANOVA, \( F_{1,4} = 3.56; P = 0.042; \text{Fig. 9} \)) of \( B. jucundus \), while time spent on acceptance behaviour was marginally non-significant (repeated measures ANOVA, \( F_{1,4} = 3.35; P = 0.053; \text{Fig. 9, Table 3B} \)).

### 4. Discussion

This study aimed firstly to broaden our understanding of behavioural interactions occurring between burrowing ecosystem engineers and

### Table 3

Results of Repeated Measures Analysis of Variance testing the effects of \( C. kraussi \) sex, symbiont presence and the interactions on times spent by \( C. kraussi \) on behaviours exhibited in the (A) presence and absence and (B) presence only of \( B. jucundus \).

<table>
<thead>
<tr>
<th></th>
<th>( C. kraussi ) sex</th>
<th>Symbiont presence</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( F )</td>
<td>( P )</td>
<td>( F )</td>
</tr>
<tr>
<td>Sediment turnover</td>
<td>( 1.63 )</td>
<td>0.21</td>
<td>2.46</td>
</tr>
<tr>
<td>Irrigation</td>
<td>( 1.69 )</td>
<td>0.20</td>
<td>( 6.32 )</td>
</tr>
<tr>
<td>Feeding</td>
<td>( 3.65 )</td>
<td>( 0.042 )</td>
<td>1.32</td>
</tr>
<tr>
<td>Tunnelling</td>
<td>( 2.13 )</td>
<td>0.14</td>
<td>1.59</td>
</tr>
<tr>
<td>Locomotion</td>
<td>( 1.23 )</td>
<td>0.31</td>
<td>1.69</td>
</tr>
<tr>
<td>Cleaning</td>
<td>( 1.65 )</td>
<td>0.21</td>
<td>1.89</td>
</tr>
</tbody>
</table>

|         | \( F \)  | \( P \) |
| Aggression | \( 5.63 \) | \( 0.01 \) |
| Acceptance | 3.35 | 0.053 |
| Pursuit | 4.98 | 0.016 |

Values in bold denote statistical significance.

Fig. 7. Variation in time (mean ± SE) spent by male \((n = 5)\) and female \((n = 5)\) \( C. kraussi \) on (A) sediment turnover, (B) burrow irrigation and (C) feeding in the absence (black bars) and presence (grey bars) of \( B. jucundus \).

Fig. 8. Variation in time (mean ± SE) spent by male \((n = 5)\) and female \((n = 5)\) \( C. kraussi \) on (A) tunnelling, (B) locomotion and (C) cleaning in the absence (black bars) and presence (grey bars) of \( B. jucundus \).
their symbionts. Observations on the behavioural interactions among symbiotic partners within burrows (or host body cavities) have rarely been conducted given logistic difficulties (Ocampo et al., 2014). The second goal was to quantify the potential for symbiont-engineer interactions to alter ecosystem engineering activities of burrowing engineers. The study was motivated by the scarcity of knowledge on behavioural interactions occurring within burrows in marine sedimentary systems and their consequences for ecosystem functioning (Pillay, 2010; Pillay and Branch, 2011). Overall, our results indicate that the behaviour of burrowing engineers is altered by the presence of symbionts, with engineers demonstrating varying behaviours and degrees of tolerance to symbionts. Moreover, we have demonstrated that the sex of engineers also influences their responses to symbionts. Both results indicate that symbiont-engineer interactions are variable, and can influence the time spent by engineers on ecosystem engineering activities, which may alter the strength of feedbacks to sympatric assemblages.

In our first experiment, multivariate approaches indicated that sandprawn behaviours were different in the presence and absence of the symbiont Betaeus jucundus. Unexpectedly though, the behaviours exhibited in the presence of B. jucundus were more variable than those exhibited in the absence of this symbiont, with sandprawns displaying discrete degrees of tolerances to B. jucundus. We then conducted a second experiment in which the sex of C. kraussi was manipulated, in order to determine whether responses of sandprawns in Experiment 1, (particularly those of intolerant and tolerant sandprawns), could be ascribed to sandprawn sex. In Experiment 1, intolerant sandprawns increased sediment turnover by roughly 50%, presumably in an effort to prevent B. jucundus from re-entering burrows once evicted. In Experiment 2, however, male sandprawns responded to the presence of B. jucundus by reducing sediment turnover by roughly 30%. Thus, sandprawn sex does not explain the increase in sediment turnover displayed by intolerant sandprawns in Experiment 1 to the presence of B. jucundus. Differences in sediment turnover between Experiments 1 and 2 could also be due to the proportion of intolerant sandprawns being lower in Experiment 2 and possibly being lower in males than females. Time spent on irrigation was reduced in the presence of B. jucundus in intolerant and tolerant sandprawns in Experiment 1, but also for both male and female sandprawns in the presence of B. jucundus in Experiment 2, again suggesting that sandprawn sex may not be responsible for differences in irrigation rates observed in Experiment 1. In terms of behaviours exhibited exclusively in the presence of symbionts, time spent by sandprawns on aggression and pursuit increased from tolerant to intolerant individuals – a pattern also evident between female and male sandprawns. While the direction of interactions was similar between Experiments 1 and 2, the magnitude of these differences was much greater in Experiment 1 than 2. Lastly, in Experiment 1, time spent on burrow sealing activities increased in tolerant sandprawns relative to intolerant ones, but in Experiment 2, no burrow sealing behaviour was exhibited by both male and female sandprawns. Taken collectively, our results indicate that sandprawn sex may partially explain the discrete behaviours and variable tolerance levels displayed by sandprawns to symbionts in Experiment 1.

The three discrete responses of sandprawns (ranging from tolerance to intolerance) to the presence of B. jucundus were an unexpected but interesting finding, and were consistent with the idea that the outcomes of symbiotic interrelationships can be flexible (Bronstein, 1994; Lee et al., 2009; Brown et al., 2012; Heil, 2013). Indeed, the outcomes of symbiotic interactions have been reported to be conditional; depending upon varying local biotic and abiotic conditions that define environmental contexts within which symbionts interact. Such variability can alter the costs and benefits for each partner involved in symbiotic relationships (Bronstein, 1994), and can ultimately cause symbiotic associations to switch from being mutually beneficial (e.g., mutualism) to antagonistic (e.g., parasitism; Bronstein, 1994).

Betaeus jucundus has been suggested to be involved in a mutualistic relationship with the sandprawn C. kraussi (Pillay and Branch, 2011), but the exact nature of the costs and benefits involved in this relationship has not been quantified. Irrespective of the underlying symbiotic association, discrete conditions (biotic or abiotic) within experimental tanks that we were not able to recognize and control for could have altered the costs and benefits underlying the association between B. jucundus and C. kraussi, resulting in the three levels of tolerance displayed by C. kraussi to the presence of B. jucundus. While studies have demonstrated that variability in abiotic conditions can influence the outcome of symbiotic interactions (Setala et al., 1997, Rico-Gray et al., 1998; Kersch and Fonseca, 2005), abiotic variability is an unlikely explanation for the varying tolerances displayed by sandprawns to symbionts in Experiment 1, given that environmental conditions between experimental tanks were controlled and standardised. However, the possibility does exist that microscale variability within tanks that were not directly controlled, could have contributed to the variable tolerances displayed by C. kraussi to B. jucundus.

It is also plausible that variable responses of sandprawns to B. jucundus were driven by differences in biotic traits of sandprawns and symbionts used in Experiment 1, given that apart from sizes of sandprawns and symbionts, other biological traits could not standardise due to the small sizes of organisms. Studies have shown that physical, genetic, and life history traits of partners (Thompson, 1988; Cushman and Whitham, 1989; Bronstein, 1994; Saikonen et al., 1998) can influence the outcomes of mutualistic interactions, due to partner traits influencing the quality of benefit they provide. Heil (2013) argues that host individuals can identify potential partners with particular characteristics (e.g., either behavioural or physiological) and preferentially associate with those providing the highest quality of benefits (partner choice) or cease a partnership if partners do not provide an expected, high-quality benefit (host sanctioning). It is possible that traits other than the size of sandprawns and symbionts may have influenced the quality of benefits required and/or provided respectively, thus resulting in the three discrete levels of tolerance displayed by sandprawns to symbionts.

4.1. Symbiont-engineer interactions: implications for ecosystem functioning

Burrowing ecosystem engineers influence resource flows to other species via multiple pathways, but their (1) sediment turnover activities (which enhances sediment instability at the sediment water interface) and (2) burrow irrigation (which alters nutrient fluxes across the sediment-water interface) are among the most important processes that influence the functioning of marine sedimentary systems (Lohrer et al., 2004, Pillay and Branch, 2011). Our findings suggest that the above resource flow pathways are influenced by sandprawn interactions with the symbiont Betaeus jucundus in various ways. Given (1) the range of
symbionts reported to occur in burrows, (2) their patchy spatial distribution ([MacGinitie, 1934, 1939; Pohl, 1946; Anker et al., 2005; Moyo, 2014], (3) the variable responses of engineers to symbionts (see above), and (4) ability of symbiont presence to alter time spent by engineers on ecosystem engineering activities, burrower-symbiont interactions may be an under-appreciated determinant of spatial variability in physical (sediment turnover) and chemical (irrigation rates) processes occurring in marine sedimentary ecosystems. In general, factors responsible for generating physico-chemical heterogeneity in marine sediments have been well-researched, but interactions occurring within burrows have generally been neglected.

We have demonstrated that sandprawns categorised as “intolerant” generally responded to the presence of B. jucundus by increasing their tunnelling and burrow sealing activities, resulting in a 50% increase in sediment turned over to the sediment water interface. In addition, male sandprawns diminished the time spent turning over sediment in the presence of B. jucundus by roughly 30%, which is probably a response to the increased time spent by males on interacting with the symbionts (relative to conspecific females). Our results also indicate that both tolerant and intolerant sandprawns spent less time on burrow irrigation, with intolerant sandprawns demonstrating a 30% reduction in time spent irrigating burrows.

The effects of B. jucundus presence in altering sandprawn ecosystem engineering activities may at first glance appear to be of minor consequence in feeding back to sympatric assemblages. However, it must be borne in mind that the strength of ecological feedbacks arising from ecosystem engineering is dependent on thresholds at which engineering take effect (Bouma et al., 2009). If these ecological thresholds are sharp, then relatively small changes to ecosystem engineering levels can generate disproportionately large feedbacks to sympatric assemblages. The latter is especially pertinent for species with traits that predispose them to engineering effects. For example, suspension-feeders are generally thought to be particularly susceptible to ecosystem engineering by deposit feeders (Rhoads and Young, 1970; Pillay et al., 2007a). This is because traits such as their immobility and need to continuously filter water for feeding prevent them from overcoming high levels of suspended sediments in the water column generated through sediment reworking (Rhoads and Young, 1970). On a similar note, our previous work quantifying impacts of a deposit-feeding sandprawn on a suspension feeding bivalve (Eumarcia paucaperta) demonstrated that the performance (body condition) of the bivalve was best related to sediment turnover by sandprawns as a negative exponential model ($y = 0.001x^{-1.45}$), in which a threshold of roughly 27% of the maximum sandprawn sediment turnover resulted in a 78% depression in E. paucaperta performance (Pillay et al., 2007a). Therefore, in the context of the present study, the seemingly minor effects of symbiont presence in altering sandprawn ecosystem engineering may not necessarily translate to minor feedbacks to sympatric assemblages; those that are predisposed (both positively and negatively) to sandprawn ecosystem engineering may be disproportionately affected.

5. Conclusion

This study has shed light on the complexity of behavioural interactions that occur among symbiotic partners within burrows in marine sedimentary ecosystems, and the potential for such interactions to influence ecosystem engineering activities by burrow builders. Given the range of symbionts reported to occur in burrows and the variety of interactions reported ([MacGinitie, 1934, 1939; Pohl, 1946, Anker et al., 2005]), it would be valuable for future studies to broaden sample sizes and duration of observations to more rigorously quantify these interactions. It would also be important for studies to focus on understanding costs and benefits underlying such relationships as well as biotic and abiotic processes responsible for shifting the cost-benefit balance experienced by symbiotic partners. This study is an attempt to move research on burrow symbionts beyond descriptive accounts and we argue in favor of similar studies attempting to link interactions occurring between engineers and symbionts with ecosystem effects. Such studies will improve our understanding of factors influencing ecosystem engineering by burrowing species and potential effects on ecosystem heterogeneity and feedbacks to sympatric assemblages.

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