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BIO4000W Project 1

# Drivers of burrow symbiont distribution in a soft-sediment system: host abundance or burrow trophic environment?

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# Abstract

The Langebaan Lagoon sandflats are dominated by the burrowing activities of thalassinid shrimps. Their burrows are home to various burrow symbionts including a commensal shrimp (*Betaeus jucundus*), a six-legged crab (*Spiroplax spiralis*) and a scaleworm (*Antinoe lactea*). Little work has been conducted on these burrow symbionts, and the mechanisms influencing their abundance and distribution are unknown. To test whether host abundance or the burrow trophic environment (i.e. food availability) is the dominant force shaping patterns of burrow symbiont distribution, samples of host and symbiont abundances as well as chlorophyll-a and extracellular polymeric substance (EPS) measurements were taken from three sites in Langebaan Lagoon over spring and autumn. Clear signals emerged in contradiction of the hypothesis that burrow symbiont abundances peak in areas associated with high abundances of hosts. Host abundances peaked at Bottelary (10.18 counts/site ± 1.02 SE), a site where recreational activities and thalassinid shrimp bait collection are prohibited. In contrast, peak *B. jucundus* abundance (6.56 counts/site ± 0.37 SE) occurred at Oesterval during September – the muddy sediment of the site resulted in high sedimentary food retention and the September spring phytoplankton bloom resulted in peak chlorophyll-a (234.12 mg chl-a/g sediment ± 42.74 SE) and EPS (0.13 mg EPS/g sediment ± 0.008 SE) concentrations. Regression analyses confirmed that food availability was the best explanation of the patterns observed in *B. jucundus* distributions, over and above that of host distributions. *S. spiralis* and *A. lactea* did not show this pattern, the result of the low counts of these species in the collected samples or their reliance on food sources different to those depended on by *B. jucundus*. These results are of consequence in changing the way we think about symbiont distributions relative to that of the hosts, in that the two may not be linked directly, but rather influenced by larger scale trophic changes such as the availability of food within the burrow.

# Plagiarism Declaration

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This project is my own work. I have not and will not allow anyone to copy my work with the intention of passing it off as his or her own.

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# Acknowledgements

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# Introduction

It was Charles Darwin who first noticed the effects that earthworm burrowing had on sediment properties (1881), describing his observations as “merely curious and of little real importance”. Today however, the study of sediment disturbance encompasses a broad range of disciplines including archaeology, geomorphology and ecology because burrowing organisms affect the sediment of the planet on an enormous scale (Meysman et al, 2006). The reworking and modification of sediments through organism activities such as burrowing, ploughing or food excavation is known as bioturbation (Pillay, 2010). This disturbance of sediments is an important process in determining the structure and function of both terrestrial and marine ecosystems because, over the long term, a population of bioturbators can alter systems by changing the dominant habitat type in a particular area (Hansell, 1993). Reise (2002) argues that the biological disturbance of sediment is as important a process as classical trophic interactions in the functioning of these systems.

The burrows created by organisms are just one example of bioturbation. In both a marine and terrestrial context, burrowing organisms change the properties and physical structure of sediment and in so doing, modify existing habitats and create novel ones. This changes resource availability for other species within the system, and often renders burrowing species “ecosystem engineers” (Jones et al, 1994).  Ceblallos et al (1999) showed that burrowing prairie dogs had a significant impact on plant succession, nutrient cycling and flux, the physical and chemical composition of the soil, turnover rates and hydrology as well as the physical architecture of the environment (Ceblallos et al, 1999).

Thalassinid shrimps are marine burrowing crustaceans (Figure 1) that create extensive burrow networks in soft sediment sandflat systems (Griffis and Sachanek, 1991). Their burrowing activities define and modify niches for themselves and for other species (Olding-Smee et al, 1996). Like prairie dogs, their burrowing results in dramatic changes in sedimentary and biochemical properties and processes. These changes, in turn, impact many different levels of the food chain both positively and negatively, from microbes and microalgae to seagrasses and even fish and birds (Pillay and Branch, 2011). Some thalassinid species are deposit feeders that consume microfauna growing on the sediment interface of the burrow walls (Coelho, 2004) and their ‘gardening’ of these microbes allows for a build up of organic matter and thus, the concentration of food resources. Other thalassinid species create U-shaped burrows to maximise water circulation efficiency (Pillay, 2010), reducing the required expenditure of energy during feed by the filter feeding organisms hidden within (Bromley, 1996). Thalassinid burrowing increases sediment permeability and porosity, improving oxygenation and benefiting other burrowing meiofauna (Pillay and Branch, 2011). The burrows also provide protection from predators, turbulence and low tide exposure. However, the rapid sediment turnover as a result of thalassinid burrowing decreases the presence of microbial films at the sediment-water interface. These films provide food, sediment stability and biochemical cues for larval settlement and their removal negatively affects epibenthic grazers and filter feeders reliant on the sediment-water interface for food (Pillay and Branch, 2011). Thalassinid burrowing also affects marine vegetation growth, in some cases completely excluding seagrasses (Pillay and Branch, 2011).

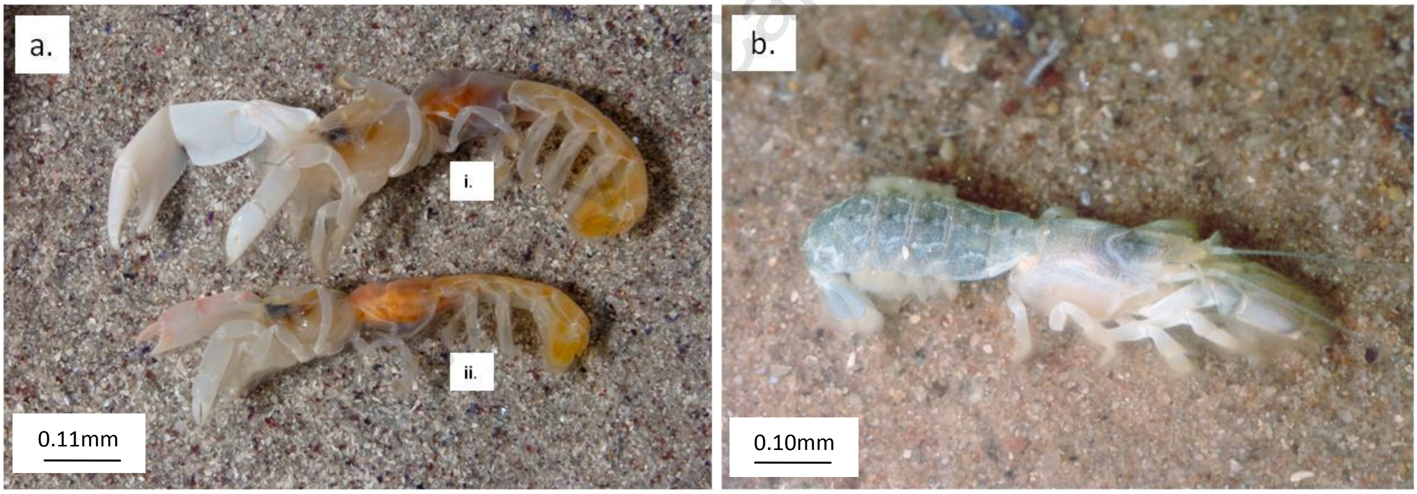

**Figure 1:** South African **(a)** Sandprawn *C. kraussi* male (i) and female (ii) and **(b)** Mudprawn *U. africana.* Both species are collected locally as bait (Griffiths CL)

Tunnels and burrows affect both habitat heterogeneity and diversity (Pillay and Branch, 2011) with a variety of species assemblages occupying the burrow alongside the burrow host. There are a broad range of these symbiotic relationships, from facultative to obligate (Itani, 2004). Some examples include assemblages found within the burrows of the innkeeper worm host (*Urechis unicinctus*), so named because of the diverse range of co-inhabiting fauna, such as goby species, scaleworms and crabs (Anker et al, 2005). Thalassinid burrows also play host to a variety of burrow symbiont species, and areas with thalassinids have been shown to have higher species richness than areas without the burrowers present (Griffis and Suchanek, 1991). Likewise, Ceblallos et al (1999) showed that the presence of burrowing prairie dogs enhanced regional species diversity, with habitat heterogeneity linked directly to burrow density. Thus, areas with the burrowers have a greater species richness and abundance than areas without, with some species found exclusively in the areas occupied by the burrowing hosts (Ceblallos et al, 1999).

Hansell (1993) provides a model to explain why there is higher species richness and diversity in areas with burrows in comparison to areas without (Figure 2). Spatial heterogeneity is created by the physical modification of the environment as a consequence of burrowing, allowing microhabitats to develop. Species are drawn into a shared use of the burrow because of the advantages these microhabitats offer, such as protection against predators, protection from the external environment and improved sediment porosity and oxygenation as well as the accumulation of food resources (Hansell, 1993). Long term occupation of burrows results in the radiation of species able to exploit the spatial and temporal heterogeneity in and around the burrows over evolutionary time (Odling-Smee et al, 2003).

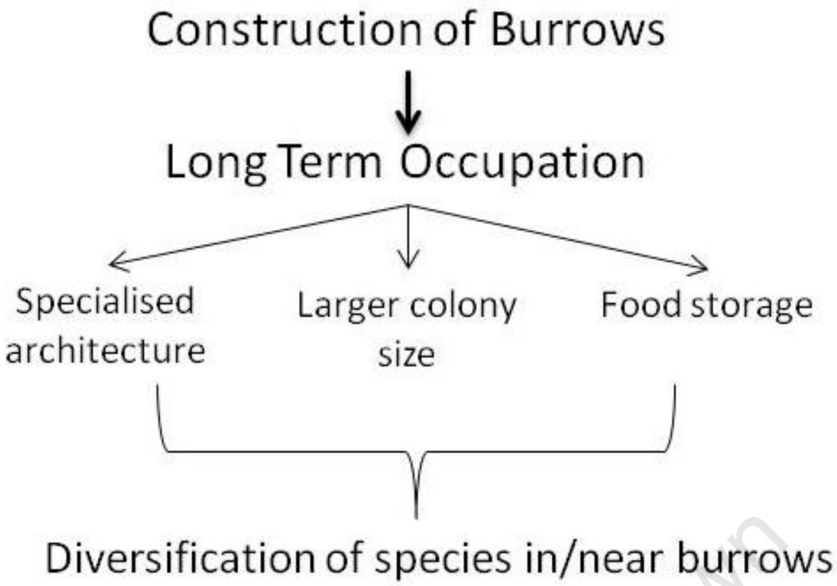

**Figure 2:** Broad evolutionary trends resulting from the creation of burrows. The construction of burrows results in habitat modification and the physical burrow space provides protection from predators and the environment. The burrows thus offer advantages for species able to exploit these advantages, which results in the long term occupation of these species in the burrow. This long term occupation promotes the development of specialised architecture, larger colony sizes as well as food storage within the burrow system. These changes result in the diversification of microhabitats within and around the burrow system which leads to a diversification of species able to occupy the novel niches opened up within and around the burrow (modified from Hansell, 1993)

Such a model can be applied to both a marine and a terrestrial context and thus, burrowing thalassinid shrimps have effects beyond the scale of ecosystem modification - they are drivers of evolutionary change in soft sediment ecosystems (Jones et al, 1994). The changes in sediment properties brought about through thalassinid burrowing act as powerful selective agents for novel morphology, behaviour and social interactions in co-occurring, burrow species (Pillay and Branch, 2011). Thalassinid burrows are, like the terrestrial prairie dog burrows, ‘elite structures’ (Bromley, 1996) that attract sedimentary and pelagic species into a shared use of the burrow, allowing the speciation of these symbiotic burrow associates with differing degrees of dependence on both the burrow structure and the host (Pillay and Branch, 2011). The presence of burrowers appears to thus be a driving force behind the development of burrow symbiont and host interactions (Hansell, 1993; Bromley, 1996; Eisenberg and Kinlaw, 1999; Hafner et al, 2000).

This role of marine bioturbators in micro-evolutionary processes remains unexplored (Pillay, 2010). Although well documented in the literature, there has been little attempt to move beyond simple description to a quantitative understanding the mechanisms driving the distributions of these burrow symbionts. For example, Anker et al (2005) described at length the assemblages found within the burrows of the innkeeper worm host *U. unicinctus*, but did not delve into the forces dictating how the diverse range of co-inhabiting symbiotic fauna is distributed. It is simply assumed that where there are more hosts, more burrow symbionts will be found. It is important to understand how the thalassinid hosts, the burrow environment and the burrow symbionts are related spatially and temporally because it may hold clues as to how these symbiotic relationships evolved.

With this in mind, this study seeks to uncover the predominant driving force, be it the abundance of hosts or the food availability within the burrow, shaping the spatial and temporal distributions and abundances of burrow symbionts within the Langebaan Lagoon sandflat system. Both *Callichirus* and *Upogebia* are present in Langebaan Lagoon, and there are three burrow symbionts of primary interest. These include a shrimp symbiont (*Betaeus jucundus*) that undertakes apparent warning behaviour to alert the host to a treats or an invasion of the burrow (Moyo, pers. com.), and two non-mutualistic burrow symbionts (the six-legged crab *Spiroplax spiralis*, and the scaleworm *Antinoe lactea*).

Given the reasons behind the attraction of species into a commensal use of the burrows and the lack of further work on the burrow symbionts themselves, it is hypothesised that host abundance will be the primary driving force behind the patterns of burrow symbionts distribution. To unravel this, three objectives were investigated, namely (1) whether symbiont abundance is linked to host abundance (2) whether symbiont and host abundances are affected by the trophic environment, particularly food availability within the burrow and (3) if seasonality affects these trends.

# Methods

## *Study Site*

Langebaan Lagoon ($33^0$ 11’ 27’’ S; $18^0$ 07’ 37’’E) is a large saltwater lagoon on the West Coast of South Africa (Figure 3). The 15km lagoon forms part of the West Coast National Park, and is divided into three zones limiting the recreational and harvesting activities that may take place in different areas of the lagoon.

Three sites (Klein Oesterval, Oesterval and Bottelary) were sampled during autumn (April) to determine the distribution and abundance of burrowing prawn hosts and the burrow co-inhabitants. Data from the spring season (September) had already been collected using identical methods, and thus can be compared to determine whether a seasonal trend is present.

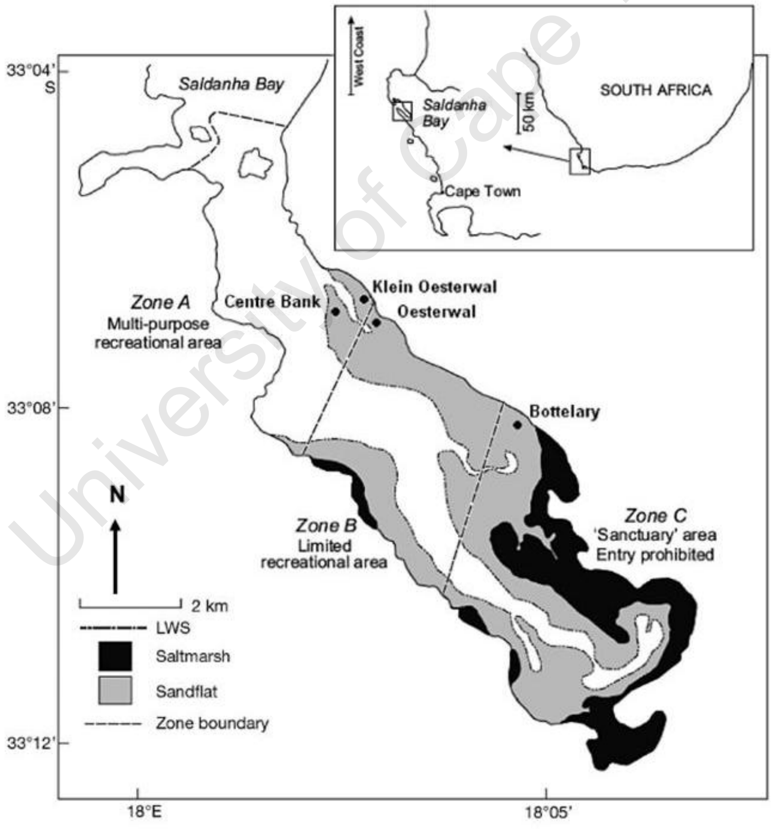

**Figure 3:** Langebaan Lagoon, showing its geographic position on the South African coastline, as well as sampling sites and park zones. Sites Klein Oesterval, Oesterval and Bottelary are indicated. Tidal rage at sampling sites is approximately 1.8 m. LWS: low water spring (from Pillay et al, 2010)

## *Sampling Design*

A nested sampling design was used (Figure 4), with three randomly chosen sub-sites (50m apart) sampled from the high to the low water mark at each major site. The distance from the high to the low water mark at Klein Oesterval and Oesterval was 300m, and at Bottelary it was 500m. Three line transects 10m apart were taken at each sub-site from the high to the low water mark. Samples were collected at five equidistant stations down the transect line, with five samples collected at each point with ‘prawn pumps’. The volume of the “prawn pump” used was 1473 $cm^3$.

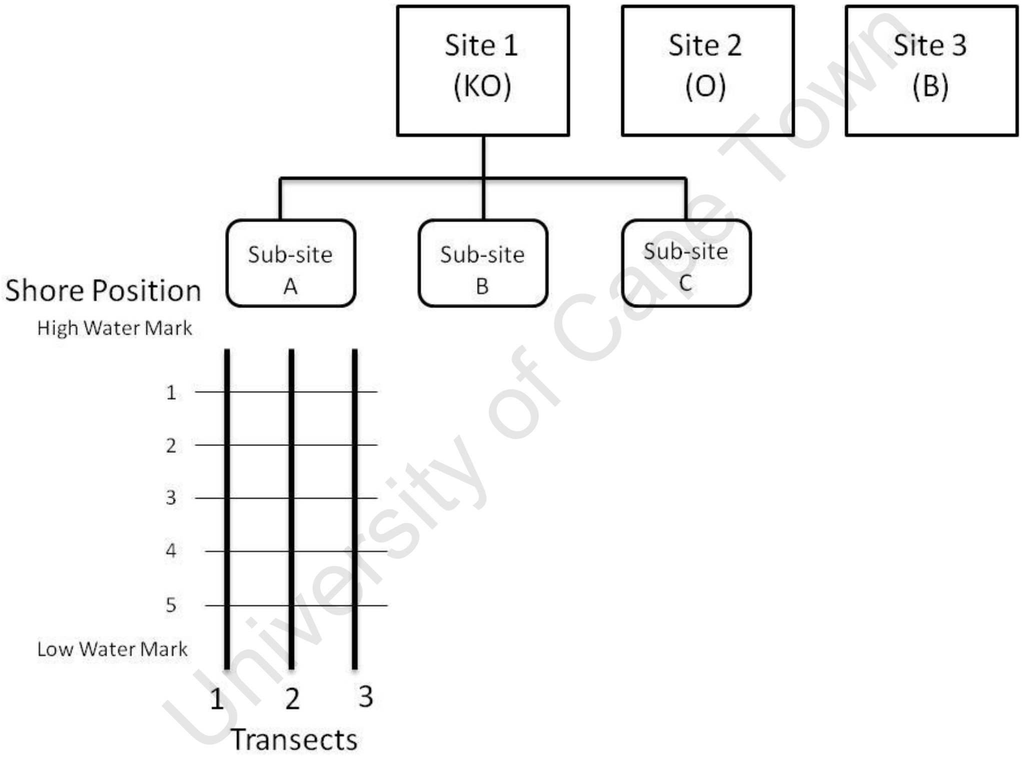

**Figure 4:** Sampling design showing nested sampling at each of the three sites, with three sub-sites and three transects per sub-site from high to low water mark (where KO = Klein Oesterval, O = Oesterval and B = Bottelary)

### *Measurables*

Given the sampling design, 15 samples were taken at each subsite and 45 samples were taken overall for each of the three sites. For each season, 135 samples were taken. The contents of the prawn pump at each sampling position were emptied and sieved using a 1000µm sieve, and the hosts and burrow symbionts identified and counted.

The trophic environment in the burrows was analysed at the mid-water mark, since previous research indicated that the highest density of burrow symbionts occurred at this position (Moyo, pers. com). To analyse chlorophyll a (chl-a) and extracellular polymeric substances (EPS) concentrations, the burrows were split and the sediment from the burrow walls was scraped off and weighted. For each season, 10 sediment samples from the burrow walls were collected at three sub-sites within the three sites (a total of 90 samples per season) and analysed for both chl-a and EPS.

The chl-a was extracted by placing the collected samples in 30ml 90% acetone, and keeping them in a dark freezer for 48 hours. A sub-sample of the homogenised sample was analyzed using a fluorometer (Turner Designs Trilogy) to measure the chl-a concentration per gram of sediment.

Once collected, EPS samples were placed in a freezer for 48 hours before analysis. EPS per gram sediment was measured using the Underwood et al (1995) phenol-sulphuric acid assay on a sub-sample of the homogenised sediment samples – 2ml distilled water was added, followed by 1ml of 5% phenol and 5% concentrated sulphuric acid. This phenol-sulphuric mix was then be diluted 10 fold and measured using a spectrophotometer (Merck Spectroquant Pharo 100) against a reagent blank.

### *Statistical Analysis*

The software package SPSS was used in the analysis of the univariate data obtained, and a p value of 0.05 was used in all statistical tests.

The design of the sampling required the application of a nested analysis of variance (ANOVA) to determine the influence of spatial and temporal variables on the relative abundances of hosts and symbionts, as well as EPS and chl-a measurements. Temporal significance was tested between April and September to the scale of “site” due to the intrinsic variation found at the levels subsite, transect and station. Spatial significance was also tested for at the level of “site”, and each season was run separately. Post-hoc tests were used to determine the within treatment effects.

The ANOVA was run without any transformation of the data, since no transformation resulted in the required normal distribution of data, and no non-parametric equivalent was available. However, Zuur et al (2009) indicates that the nested ANOVA design would be powerful enough to detect differences despite any non-normality.

Linear regression analyses were conducted to determine whether host abundance or the trophic environment was the more important factor influencing the abundance of the three different symbionts, and the strength of these relationships.

# Results

## *Patterns of Distribution*

### a) Hosts

There was no significant temporal variation in abundance between April and September for Sandprawns, Mudprawns or Total Host abundance (Table 1).

**Table 1:** Summary statistics of nested ANOVA testing the effects of temporal variation on host abundance

| Variable | Mean Abundance | SS | *df* | MS | Fs | p - value |
|---|---|---|---|---|---|---|
| Sandprawns | 3.64 | 234.2 | 1 | 919.0 | 16.0 | 0.20 |
| Mudprawns | 0.352 | 2.315 | 1 | 2.315 | 2.65 | 0.11 |
| Total Hosts | 3.99 | 2.504 | 1 | 2.504 | 0.448 | 0.50 |

There was significant spatial heterogeneity in sandprawn abundance (Figure 5a) (Fs = 16.3, *df* = 2, $p < 0.01$). A post-hoc Tukey HSD test showed that the Bottelary site contributed most to this spatial heterogeneity, being significantly different to both the Klein Oesterval and the Oesterval sites ($p < 0.05$).

Mudprawn distribution (Figure 5b) showed no significance in terms of spatially heterogeneity (Fs = 0.639, *df* = 2, p = 0.53) while total host abundance (Figure 5c) showed the same significant pattern of spatial distribution as that of sandprawn abundance (Fs = 16,1, *df* = 2, $p < 0.01$). A post hoc Tukey HSD test showed that the Bottelary site was again the main contributor to this heterogeneity in total host abundance ($p < 0.05$), being significantly different to both the Klein Oesterval and Oesterval sites.

### b) Burrow Symbionts

Significant temporal variability was observed between April and September for both shrimp and six-legged crab abundances (Table 2). This temporal variability was significant at the Oesterval site for the shrimp symbiont (Figure 6a) and at the Klein Oesterval site for the six-legged crab (Figure 6c).

**Table 2:** Summary statistics of nested ANOVA testing the effects of temporal variation on symbiont abundance

| Variable | Mean Abundance | SS | *df* | MS | Fs | p - value |
|---|---|---|---|---|---|---|
| Shrimp | 1.64 | 161.781 | 1 | 161.8 | 19.99 | **< 0.01** |
| Scaleworm | 0.111 | 0.370 | 1 | 0.370 | 3.448 | 0.07 |
| Six-Legged Crab | 0.633 | 3.559 | 1 | 3.559 | 4.878 | **0.03** |

In terms of spatial variability, the shrimp symbiont showed significant differences in abundance between the three sites (Fs = 37.97, *df* = 2, $p < 0.01$), with a post hoc indication that abundances at the Oesterval site were significantly different to both the Klein Oesterval ($p < 0.05$) and Bottelary sites ($p < 0.05$).

Neither the scaleworm nor the six-legged crab abundances (Figure 6b) showed any significance difference in spatial distribution on a site scale, although the results were marginally non-significant (Fs = 3.059, *df* = 2, p = 0.051 and Fs = 0.065, *df* = 2, p = 0.94 respectively).

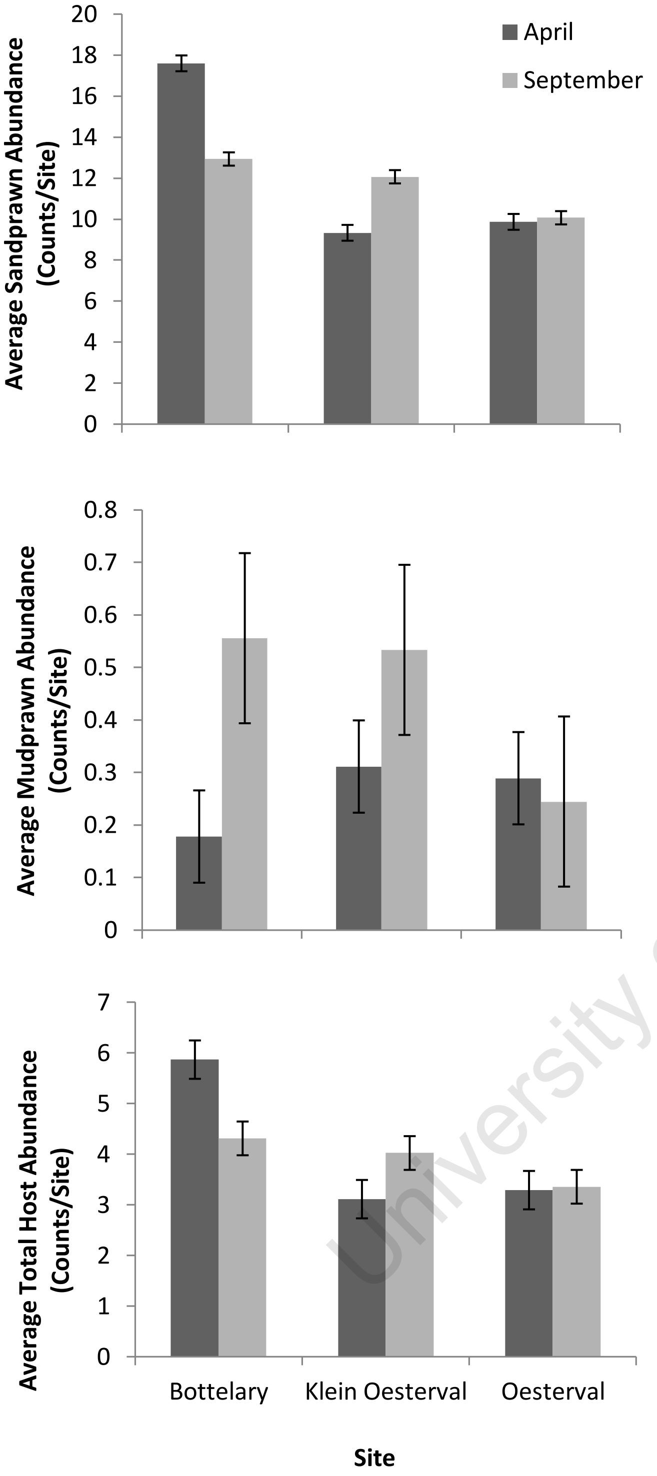

**Figure 5:** Spatial variation in abundance of **(a)** sandprawns **(b)** mudprawns and **(c)** total hosts for each of the three sites ±SE

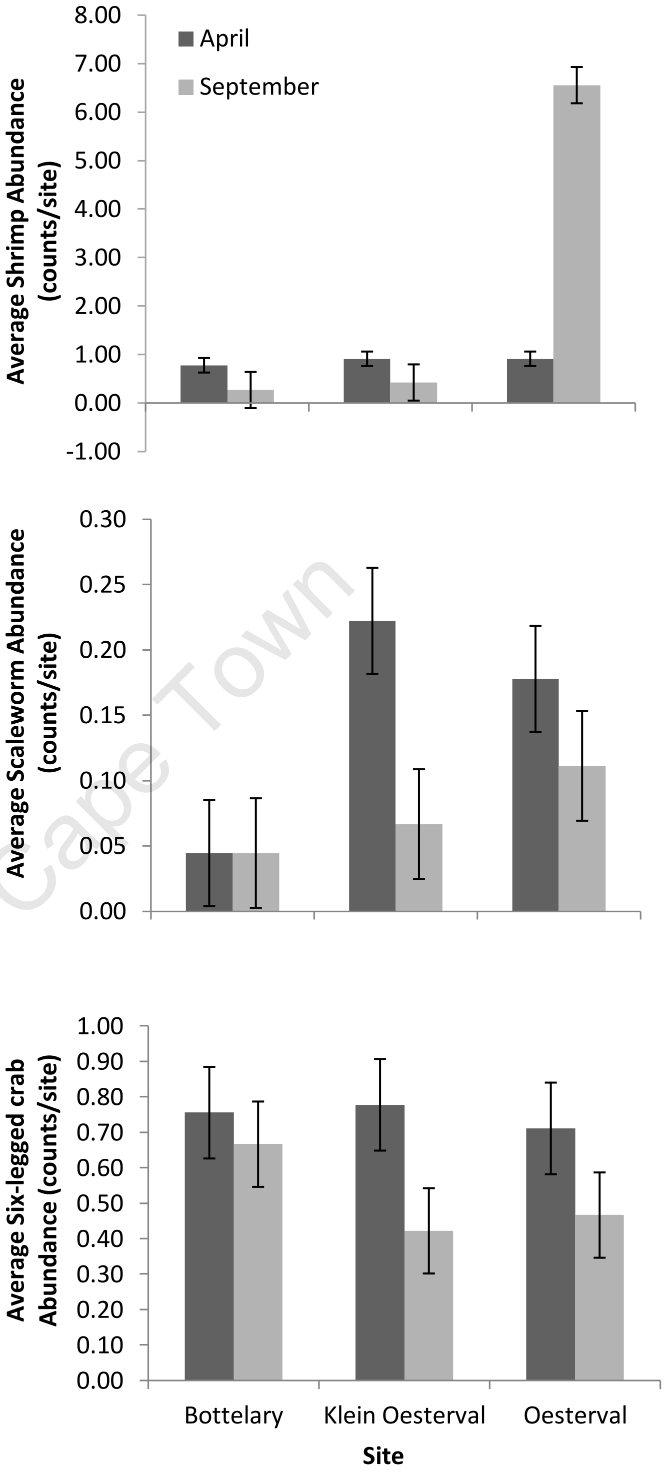

**Figure 6:** Spatial variation in abundance of **(a)** shrimps **(b)** scalewoms and **(c)** six-legged crabs for each of the three sites ±SE

## *Patterns of Food Availability*

### a) EPS

There was a significant temporal variation in EPS concentrations at a site scale (F = 14.84, *df* = 2; $p < 0.01$). A post hoc analysis of within treatment effects showed that the source of this variation came from the measurements at the Oesterval site, which was significantly different to both Klein Oesterval ($p < 0.05$) and Bottelary ($p < 0.05$). Figure 7a shows that a peak in EPS concentrations occurs during September at Oesterval.

### b) Chlorophyll-a

There was a significant temporal variation in chl-a concentrations at a site scale (F = 40.422, *df* = 2; $p < 0.01$). A post hoc analysis of within treatment effects showed that the source of this variation came from the measurements at both the Oesterval and Bottelary sites, both of which were significantly different to each other and to Klein Oesterval ($p < 0.05$). Figure 7b shows that chl-a concentrations peak in September for both Oesterval and Bottelary, with little change between the seasons for Klein Oesterval.

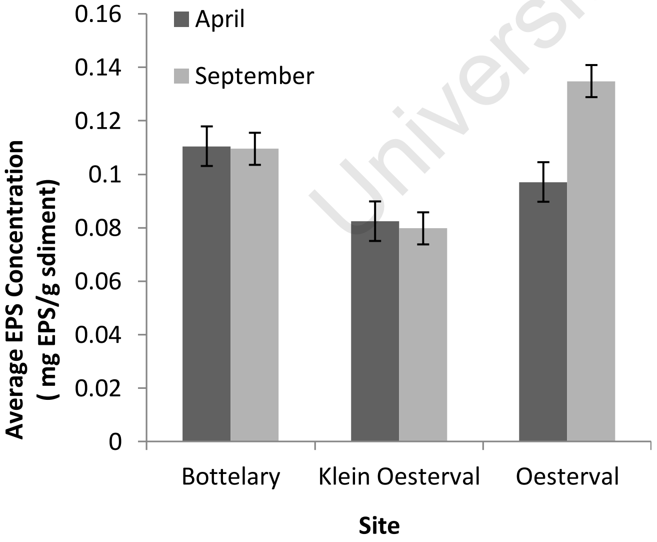

**Figure 7a:** Spatial variation in average EPS concentrations at the three sites ±SE (n = 90)

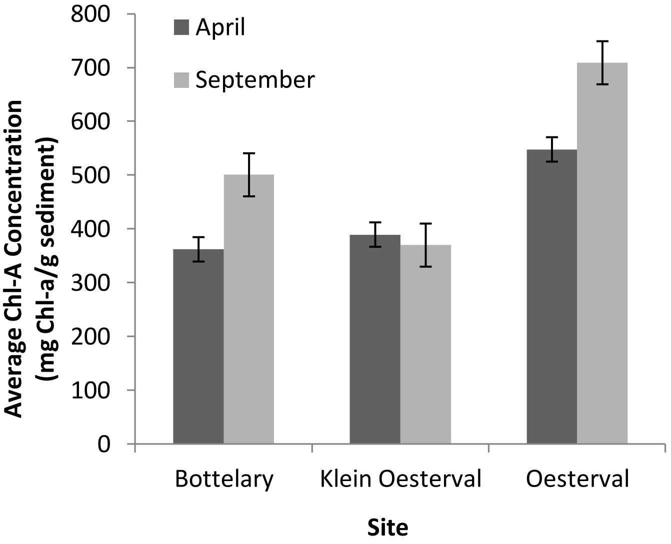

**Figure 7b:** Spatial variation in average chl-a concentrations at the three sites ±SE (n = 90)

## *Drivers of Symbiont Abundance*

### a) Hosts

The host abundance data was poorly fitted to the burrow co-inhabitant abundance data, with no clear pattern emerging for either season. The relationship between shrimp abundance and sandprawn abundance (Figure 8a) was not significant for either season, increasing for April (F = 1.411, *df1* = 1, *df2* = 25, p = 0.25) and decreasing during September (F =3.589, *df1* = 1, *df2* = 25, p = 0.07). Mudprawn abundance was a weak predictor of shrimp abundance (Figure 8b) for both April (F = 0.10, *df1* = 1, *df2* = 25, p = 0.76) and September (F = 0.556, *df1* = 1, *df2* = 25, p = 0.46), as was total host abundance (Figure 8c) with an increasing relationship in April (F =1.491, *df1* = 1, *df2* = 25, p = 0.23) and a decreasing relationship in September (F = 2.856, *df1* = 1, *df2* = 25, p = 0.10).

Scaleworm abundance showed no relationship with sandprawn, mudprawn or total host abundance ($R^2$ = 0). The dependent variable was a constant for September, and thus no curves were fitted.

A general trend of increasing six-legged crab abundance with increasing host abundance was noted for both April and September (Table 3). There was no significant relationship between the six-legged crab abundance and sandprawn, mudprawn or total host abundances (Table 3). The relationship between sandprawn and six-legged crab abundance for September was marginally non-significant, as was the relationship in September between total host and six-legged crab abundance (Table 3).

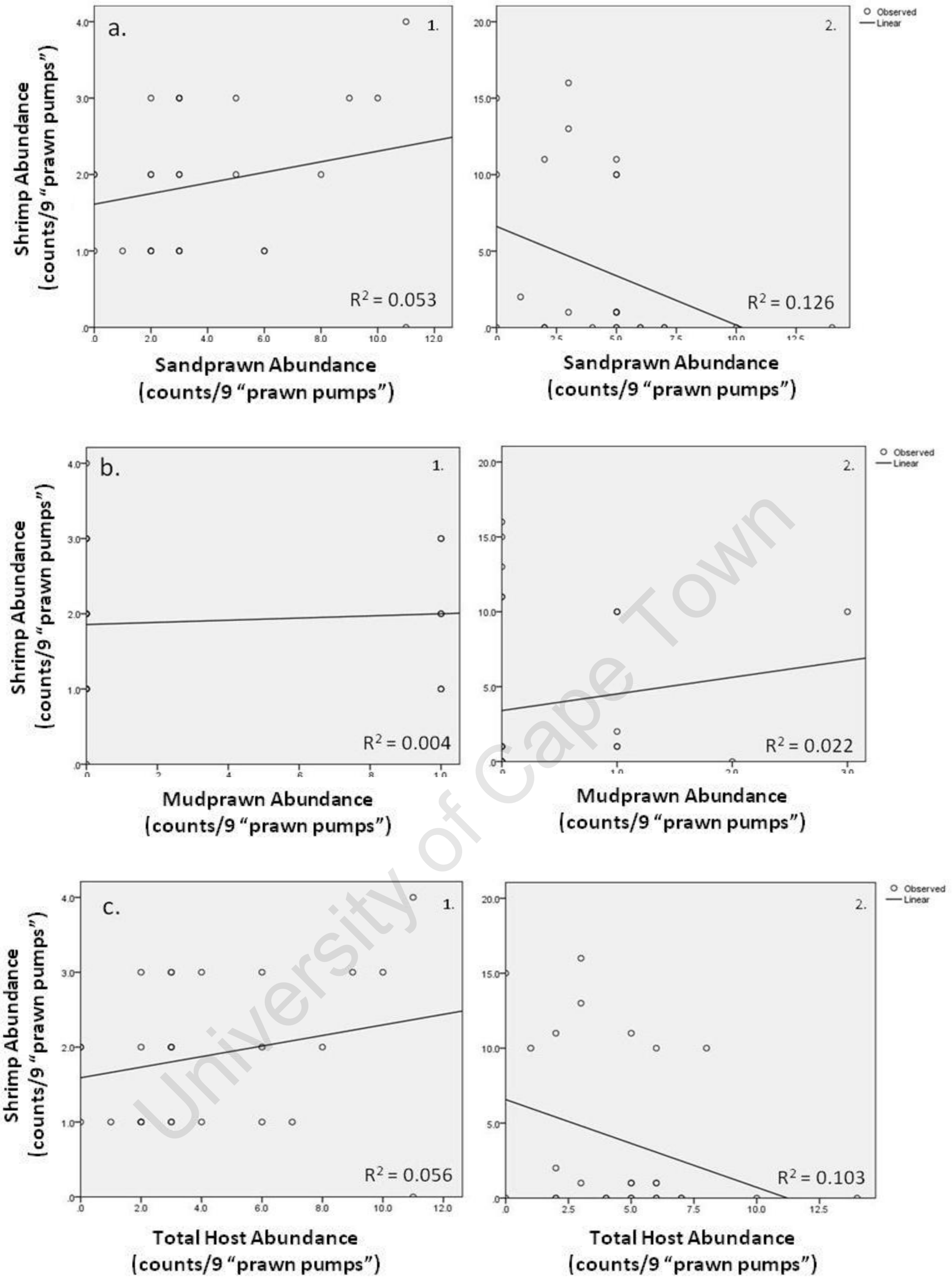

**Figure 8:** Relationship between shrimp abundance and **(a)** sandprawn **(b)** mudprawn **(c)** total host abundances for April **(1)** and September **(2)**. $R^2$ values indicate the coefficient of determination and indicate how well the data fits the curve

**Table 3:** Model summary for regression analysis for the relationship between six-legged crab abundance and host abundances

| **Variable** (abundance) | **Variable** (abundance) | **Date** | **Equation** | **Model Summary** | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $R^2$ | F | *df1* | *df2* | p-value |
| Six-Legged Crab | Sandprawn | April | Linear | 0.050 | 0.126 | 1 | 25 | 0.73 |
| | | Sept. | Linear | 0.124 | 3.555 | 1 | 25 | 0.07 |
| | Mudprawn | April | Linear | 0.000 | 0.007 | 1 | 25 | 0.93 |
| | | Sept. | Linear | 0.002 | 0.051 | 1 | 25 | 0.82 |
| | Total Host | April | Linear | 0.005 | 0.132 | 1 | 25 | 0.72 |
| | | Sept. | Linear | 0.134 | 3.855 | 1 | 25 | 0.06 |

### b) Food Availability

EPS level within the burrow and shrimp symbiont abundance showed no a significant relationship for April (F = 0.480, *df1* = 1, *df2* = 7, p = 0.51), but a significant relationship (F = 12.98, *df1* = 1, *df2* = 7, p = 0.009) was shown for September (Figure 9a). No significant relationship was found between chl-a levels and shrimp abundance for April (F =0.489, *df1* = 11, *df2* = 7, p = 0.511), but the relationship was again significant for September (Figure 9b) (F = 12.78, *df1* = 1, *df2* = 7, p = 0.01).

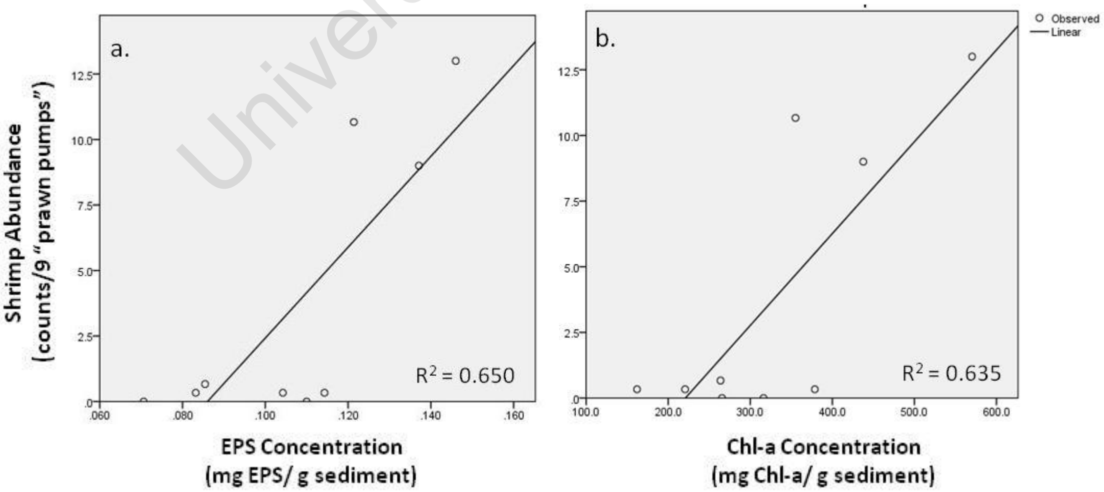

**Figure 9:** Relationship between shrimp abundance and **(a)** EPS **(b)** chl-a for September. $R^2$ values indicate the coefficient of determination and indicate how well the data fits the curve

EPS/g showed an increasing linear relationship with six-legged crab abundance, although this relationship was not significant for either April or September (Table 4). There was no significance in the relationship between chl-a availability and six-legged crab abundance for the linear relationship during April or September (Table 4).

Regression analysis for the scaleworm data was not possible. Scaleworm abundance showed no relationship to food availability for either EPS or chl-a ($R^2 = 0$). The analysis could not be conducted for September because the dependent variable was a constant, and thus no curves could be fitted (Table 4).

**Table 4:** Model summary for regression analysis for the relationship between burrow co-inhabitant abundance and of food availability

| **Dependent Variable** (abundance) | **Independent Variable** (mg per g sediment) | **Date** | **Equation** | **Model Summary** | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | $R^2$ | F | *df1* | *df2* | p-value |
| Six-Legged Crab | EPS | April | Linear | 0.136 | 1.105 | 1 | 7 | 0.33 |
| | | Sept. | Linear | 0.278 | 2.695 | 1 | 7 | 0.15 |
| | Chl-a | April | Linear | 0.136 | 1.100 | 1 | 7 | 0.33 |
| | | Sept. | Linear | 0.295 | 2.934 | 1 | 7 | 0.13 |
| Scaleworm | EPS | April | Linear | - | - | - | - | - |
| | | Sept. | Linear | - | - | - | - | - |
| | | | | - | - | - | - | - |
| | Chl-a | April | Linear | - | - | - | - | - |
| | | Sept. | Linear | - | - | - | - | - |

# Discussion

The broad aim of this study was to contribute to the understanding of mechanisms driving burrow symbiont abundances in the sandflat system of Langebaan Lagoon. The fundamental question investigated was whether the primary driving force behind symbiont distributions was food availability within the burrow, or the abundance of the thalassinid hosts. Currently there is very little known about symbiotic relationships in soft sediment systems when compared to that known in other systems such as coral reefs. Studies previously undertaken have mostly focused on the role of thalassinid shrimps as ecosystem engineers, whose burrowing activities affect sediment properties and affect community structure. Little work has been focused on the ecology of these co-inhabiting burrow assemblages, such as how these burrow symbionts occupy a space known to be occupied by burrowing bioturbators (Widdicombe et al, 2004).

In a system with a heterogeneous distribution of hosts, burrow symbionts could follow two distinct gradients. Either, symbiont abundance and distributions will be directly linked those of hosts (in an area with more hosts there will be more symbionts), or the abundances will follow a distribution influenced rather by the trophic environment within the burrow, where factors such as food availability are more powerful determinants than host abundance. The Langebaan sandflat system has very few areas of refuge, with large expanses of open sand upon which burrow symbionts such as the shrimp *B. jacundus* would be an easy target for predators such as birds. Host burrow systems are also utilised by symbionts like *B. jacundus* for protection from the physical environment and the pursuit of food resources (Hansell, 1993). The burrow symbionts thus exist only within the burrow systems of the thalassinid hosts, and it can therefore be argued that host abundance is the predominant determinant of burrow symbiont abundances. However, this study has shown that there is a separation of the mechanisms within the soft-sediment system that drive patterns of burrow symbiont distribution – it is the trophic environment within the burrow, namely food availability, that is a significant force driving burrow symbiont abundances over and above that of host abundances.

There is evidence for this mismatch between host and burrow symbiont abundances in that peaks in abundance occurred during different seasons and at different sites. If burrow symbiont abundances were exclusively linked to host distribution and abundance, we would expect a corresponding pattern. The April peak in total host as well as sandprawn abundances at Bottelary does not appear to follow either food availability proxy measured in this study (both EPS and chl-a peak in September not April). This peak in abundance may simply be the result of the timing in thalassinid spawning, compounded by the prohibition of bait collection (the thalassinid hosts are collected locally as bait) at Bottelary, the only site within the limited access “Sanctuary” portion of the lagoon. High host abundances may not lead to high abundances of burrow symbionts because the rapid turnover of sediment by thalassinid burrowing negatively affects the ability of planktonic symbiont larvae to settle on the sediment. The higher the abundance of hosts, the higher the level of sediment turnover and thus burial and the reduction of microalgal biofilms that provide biochemical cues for larval settlement would reduce recruitment of burrow symbionts in an area (Pillay and Branch, 2011).

Since this study has shown that it is burrow trophic environment, and specifically food availability, that determines the distribution of burrow symbionts, the question must be asked as to what drives spatial and temporal food availability within the burrows. Langebaan Lagoon is located on the western coast of South Africa in the southern Benguela, a system characterised by strong upwelling cycles between August and May (Monteiro and Largier, 1999). The inflow of cold, upwelled water into the lagoon is an important driver in the ecology of the lagoon as it brings in nutrients and thus imposes a control on production (Monteiro and Largier, 1999). These seasonal upwelling cycles produce marked differences in gross primary production rates within the system over the year, increasing from 261,6 mgC/m$^3$/day in winter to 675,4 mgC/m$^3$/day in spring and to a maximum of 885,3 mgC/m$^3$/day in summer (Henry et al, 1977). This pattern is confirmed in this work, with significantly higher concentrations of sediment chl-a at two of the three sites during September (spring) relative to April (autumn).

This study has shown that there is also spatial variability in sedimentary chlorophyll concentrations within the lagoon system with higher chl-a measurements at Oesterval, as confirmed by work by Monteiro and Brundrit (1990) and Henry et al (1977). This spatial variation in sedimentary chl-a concentrations may be due to local hydrodynamic patterns, local upwelling events, retention time and the degree of shelter and stability of the sediment (Fielding et al, 1988). In addition, it is hypothesised that sediment type in an area has a relatively large effect in determining sediment chl-a concentrations (Herman et al, 2001). Sediment granulometry, and in particular, grain size, determines the size of the interstitial spaces between the grains, as well as the potential for resuspension. Finer sediment, although easily resuspended in the water column, allows for the build up of microalgal biomass (Herman et al, 2001). There is very little wave action in the sheltered space of Langebaan Lagoon, and so very little sediment is resuspended allowing for a high retention time and thus, higher chlorophyll concentrations within finer sediment (Herman et al, 2001). Oesterval has a muddy sediment type (Moyo, pers. Com) consisting of very fine sediment and very small grain sizes and, with the lack of wave action and resuspension, has higher sedimentary chl-a concentrations that the other sites with more coarse sediment types (Moyo, pers. com.). Water is drawn down into the burrow from the water column through the activities of the thalassinid hosts (Bromley, 1996) and thus a peak in water column productivity such as that during spring upwelling (Henry et al, 1977) would result in a peak in microalgae abundance within the burrows.

Extracellular polymeric substances (EPS) are a carbohydrate proxy measure of bacterial abundance on the surface of the burrow wall (Dawson and Pillay, 2011). EPS are exuded by micro-organisms such as microalgae and bacteria that live on the surfaces of sediments (Wotton 2004a), and by larger invertebrates (such as *Parvulastra exigua*) in faeces and mucous produced by feeding on sedimentary microalgae (Dawson and Pillay, 2011). EPS coats the sediment surface and creates biofilms which play an important ecological role in the provision of food for invertebrates (Decho, 1990) and the stabilisation of sediments (Paterson and Hagerthey, 2001). EPS biofilms also impact the successful settlement and recruitment of many benthic species (Pillay et al, 2007). The observed temporal variation in EPS may be a result of the spring peak in production within the system - more microalgae is brought into the burrows and this increases EPS production by the microalgae itself, the decomposition of this increased production by bacteria as well as through invertebrate grazing. This effect is compounded by the fine sediment at Oesterval, which retains the microalgae better than a more course sediment (Herman et al, 2001).

The effects of the seasonality in terms of EPS and chlorophyll-a concentrations were found to be significant in determining burrow symbiont abundances. The abundance of the commensal shrimp *B. jucundus* showed an increase in at Oesterval during September, with a number of explanations for this increase. Firstly, while correlation may not imply causation, an improvement in the survival of recruits would result in a large cohort of juvenile *B. jucundus* reaching maturity, and result in the observed increase in adult shrimp abundance. A large cohort of surviving juveniles would have been detected had monthly samples been taken. A second explanation is that the observed pattern may have been due to a simple concentrating effect, where improved conditions and resources in one area attract individuals from other areas increasing the abundance within a local site. Another explanation is that there may be a linkage in the time of spawning of the shrimp to coincide with higher food availability within the system. The higher EPS concentrations measured as Oesterval during September may have positively influenced shrimp larvae settlement and recruitment, as EPS biofilms act as important determinant in planktonic larval settlement (Pillay et al, 2007). A further potential explanation may be that the hosts simply have a slower generational turnover time than the shrimp symbionts, rendering the hosts unable to exploit rapid increases in food availability as effectively as the burrow symbionts. Sampling over numerous years would reveal whether the patterns observed for a particular year are a true reflection or due to stochastic events.

Interestingly, no patterns were detected for the other burrow symbionts, namely the six-legged crab *S. spiralis* and the scaleworm *A. lacteal*. This lack of clear signal between food availability and burrow symbiont abundances may be accounted for by the extremely low counts of these species within the samples taken. Alternatively, the scaleworm and six-legged crab may simply depend on a food source different to that of the deposit feeding shrimp *B. jucundus*. For example, the symbiotic blind burrow goby *T. californiensis* (a species not present in Langebaan Lagoon) consumes large free-floating particles that are brought into the burrow with the currents created by the burrow host, rather than feeding on the EPS and microalgae growing on the sediment surface (MacGinitie, 1939). It may be that the six-legged crab and scaleworm follow a similar mode of feeding as the goby in that their abundances do not depend on sedimentary EPS or chl-a within the host burrow. The scaleworm *A. lacteal* is recorded as having a diet based on small invertebrates (Branch et al, 2010), and while little is known about the diet of the six-legged crab *S. spiralis*, it is assumed to be able to interchange between deposit feeding and scavenging of larger particles brought into the burrow (Branch et al, 2010). Both the scaleworm and the six-legged crab would thus have abundance distributions independent to the trophic environment and food availability within the host burrow.

There are broad scale implications of this work beyond that of soft sediment systems in both marine and terrestrial symbiont ecology. These results are of consequence in changing the way we think about symbiont distributions relative to that of the hosts, in that the two may not be linked directly, but rather influenced by larger scale trophic changes such as the availability of food within the burrow.  This is the first step towards understanding the broader ecology of these symbiotic relationships, since it is these relationships and interactions between the burrowers and the burrow symbionts (such as whether there is mutualism) that dictate resource use and partitioning within the system. For example, deposit feeding by high abundances of *B. jucundus* may impact alterations in microbial transformations of important nutrients at the sediment–water interface (Laverock et al, 2010). In addition, it is well understood and acknowledged that burrows improve regional species diversity (Ceblallos et al, 1999), and Pillay (2010) describes how burrowers indirectly influence the development of soft-sediment burrow symbiont relationships on a micro-evolutionary scale. The driving force behind the distribution of burrow symbionts in a system reveals insight into the potential mechanisms of how the symbiotic relationships may have come to evolve – it reveals what dominant reason for the occupation of burrows by burrow symbionts, and thus deeper insight into symbiotic relationship formation and maintenance.

There is very little known about the burrow symbionts themselves, with current research predominantly focused on these symbionts as a source of food for other species within the sandflat system. The literature remains poor as to the diets and life histories of the burrow symbionts, and conclusions are therefore difficult to draw with certainty. Inferences have been made with almost no understanding of the behaviour and interactions between the burrow symbionts, their hosts or amongst each other, and thus research is required into the core biology of these species. For example, the question must be asked as to whether the different burrow symbionts show discrete patches of distribution within the system. Investigations must be made as to the nature of the relationship between these burrow symbionts and their thalassinid hosts, particularly the extent and dependency of the symbiotic relationship – for example, is there true mutualism between the shrimp and the host? The study is also limited by the lack of sediment granulometry measurements to confirm hypothesised mechanisms of increased food availability at a site. Increasing sampling effort would also increase collections of the rarer burrow symbionts (the scaleworm and six-legged crab), to potentially detect a clear signal as to the drivers of their abundance distributions.

Despite these limitations, clear signals emerge. This study has refuted the hypothesis made that host abundances are the dominant force in shaping burrow symbiont distributions in terms of abundance within a sandflat system, over and above the influence of food availability within the burrow. Host abundance was not dependent on food availability within the burrow, and the relationship between host abundance and symbiont abundance was very weak. Instead, chl-a and EPS concentrations were significant predictors of burrow symbiont abundances, and seasonality had a strong effect on these patterns.

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