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HABITAT FILTERING AT MULTIPLE SCALES: A CASE STUDY WITH CRAB-ASSOCIATED MICROBIAL COMMUNITIES

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HABITAT FILTERING AT MULTIPLE SCALES: A CASE STUDY WITH CRAB-ASSOCIATED MICROBIAL COMMUNITIES

by

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Habitat filtering at multiple scales: a case study with crab-associated microbial communities

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Local diversity is the result of colonization from a species pool and local habitat filters. The relative contribution of these processes delineates our ability to predict changes in biodiversity, key in a changing world. Although theory suggests that these interactions are critical for the maintenance of biodiversity, empirical work has been restricted by limitations of studies across biogeographical regions and laboratory microcosms. Alternatively, host-associated microbial communities represent discrete, tractable and replicable assemblages. Hosts provide distinct habitat patches with different habitat filters and are surrounded by environmental bacteria that determine the regional species pools. My dissertation focuses on colonization and habitat filtering processes driving species composition of microbial communities associated with coastal crabs.

First, I investigated colonization from the water column and filtering associated with the striped shore crab (*Pachygrapsus transversus*). Using a field experiment I asked whether species pool size interacts with habitat filters to structure microbial communities. Results indicated that the carapace was a stronger filters than the surrounding surfaces and are thus less influenced by changes in the regional species pool. Next, I examined whether the temporal scale of convergence and divergence in community composition was habitat dependent. In a mesocosm experiment, gut communities converged in

community composition while carapace communities converged at day 8 and diverged thereafter. These findings indicate that the gut represents a strong filter when compared to carapace habitats, whose communities were driven by species interactions during biofilm formation.

Second, I investigated whether multiple colonist pools can influence microbial communities. In a field study, I found that surface and burrow sediment colonize fiddler crab gut and carapace communities To better understand the interaction between multiple colonist pools and habitat filters, I used a mesocosm experiment where I manipulated sediment bacteria. While carapace communities were influenced by burrow sediment, removing bacteria from the surface also impacted carapace microbial composition. In contrast, gut communities responded weakly to colonist pool manipulations suggesting strong filtering.

These findings highlight complex interactions between local communities and colonist pools. Importantly, identifying when multiple colonist pools or habitat filters drives community composition should lead to a more predictive community ecology.

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Chapter 1: Regional species pool size and habitat filters drive community similarity in shore crab-associated microbial communities

ABSTRACT

Community structure results from the interaction between random colonization from the species pool and habitat filters defined by local environmental conditions. When local conditions are beneficial, the number of species available for colonization is hypothesized to limit community diversity, yet few studies have explored how harsh conditions limit recruitment of unfit species and constrain community diversity, regardless of the number of colonists. With a field experiment, we compared the responses of crab carapace and tile surfaces microbial communities to seasonal changes in available colonists from the water column. Since colonization of carapaces is constrained by crab immunity and chitin structure, we expected to find stronger responses to colonist number on tile than on crab-associated communities. Bacterial diversity in the water column increased by 10% as salinity and temperature decreased from early August to late September in the Texas coast. Increased bacterial availability coincided with increased beta diversity in tile but not in carapace-associated microbial communities. Richness in carapace and tile communities was unaffected by bacterial diversity in the water column. Beta diversity responses to regional species pool in tile communities denote the prevalence of colonization over filtering processes while the opposite is true for carapace communities. Our results are consistent with the hypothesis that colonist pool size increases beta diversity but only on habitats with weak filtering.

INTRODUCTION

Central to community ecology is to integrate the traditional view of niche factors shaping community composition with the influence of colonization from the regional species pool (Chase and Myers 2011, Harrison and Cornell 2008, Ricklefs 1987). The niche view of community assembly highlights strong habitat filters that restrict recruitment to

species with sufficient tolerance or competitive traits to maintain viable populations at the local scale (Chesson 2000, Leibold 1995). In contrast, the colonization view describes assembly as a series of random colonization and demographic stochasticity events (Adler et al 2007, Hubbell 2001). While its clear that communities fall along a continuum from the niche to the colonization view, the current challenge lies in identifying the factors mediating the relative contribution of habitat filters and colonization. Theory indicates that communities under weak habitat filters should be more susceptible to changes in the regional species pool than communities under strong habitat filters (Chase 2007, Chase and Myers 2011), yet few studies address the interaction between the size of the species pool and local habitat filtering (Chase and Myers 2011). Given expected changes in species pools due to contemporary extinction and species invasion rates, this represents an important gap in understanding when changes in available species affect local diversity patterns (Cornell and Harrison 2014, Fukami 2004, Grman and Brudvig 2014, Myers and Harms 2009, Questad and Foster 2008).

A useful approach to understand processes underlying community structure is to focus on variation in community composition across sites, known as beta diversity (Chase and Myers 2011). These beta diversity patterns have been fundamental in investigating factors shaping diversity across latitudinal, altitudinal, environmental and productivity gradients (Korhonen et al 2010, Kraft et al 2011, Myers et al 2013, Soininen et al 2007). In addition to these empirical advances, theory makes clear predictions regarding the role of habitat filters and regional species pools in shaping beta diversity patterns. In spatially structured and homogeneous landscapes, theory indicates that, since communities receive a portion of the species pool, larger pools result in additional portions, and thus, in higher beta diversity (Chase and Myers 2011). However, strong habitat filters should constrain beta diversity by selecting only those species able to tolerate local harsh conditions, like temperature extremes, pollutants or low resource availability (Chase 2007). Strong evidence of the interaction between species pool size and habitat filters comes from large

scale observational studies. For example, the latitudinal gradient in diversity, one of the most robust patterns in biology may be explained by a combination of larger species pools at the tropics and stronger environmental filters in temperate regions (Kraft et al 2011, Myers et al 2013, Qian and Ricklefs 2000). However, these observational studies cannot directly test the role of the species pool in driving community structure, and a more useful approach is to use manipulative experiments (Grman and Brudvig 2014).

Although studies that manipulate species arrivals are fundamental to reveal mechanisms driving community structure (Myers and Harms 2009, Tilman 1997, Turnbull et al 2000), most of these studies focus on whether local communities are saturated or dispersal-limited. Thus, seed addition rarely attempts to replicate realistic species pool sizes and instead often doubles or triples local seed density, overestimating the importance of species pools (Myers and Harms 2009, Myers and Harms 2011). In contrast, few studies focus on the influence of realistic species pools and their interaction with local habitat filters.

We used a field experiment to test the hypothesis that bacterial richness in the water column interacts with habitat filters in shaping community structure of microbial communities associated with crab carapaces and tile surfaces. This system provides an alternative to traditional plant and animal communities offering two important advantages. First, hosts provide distinct habitat patches that differ in their habitat filters from the surrounding environment (Christian et al 2015, Sachs et al 2004). For example, bacterial colonization of the crab's carapace is mediated by its chitin structure and the crab's immunity (Moret and Moreau 2012). Second, environmental bacteria around the host represent tractable species pools. In benthic habitats, the pool of bacterial colonists from the water column changes according to the environmental conditions in the water such as salinity and temperature (Apple et al 2006, Bouvier and del Giorgio 2002, Hatosy et al 2013, Sunagawa et al 2015).

We conducted a field experiment to determine the interaction between species pool size and local habitat filters. First, we compare the filtering strength of host-associated habitats to abiotic, tile surfaces. Second, we tested the hypothesis that beta diversity will increase with species pools size, but only under weak habitat filters. Our results support the hypothesis that stronger filters in the crab's carapace preclude beta diversity responses to species pool size, while tile surfaces, under weaker filters showed an increase in beta diversity.

METHODS

To evaluate the interaction between bacterial richness in the water column and host-associated habitat filters, we conducted a field experiment in August and September, 2012, near Port Aransas, Texas (27.8275° N, 97.0722° W). At this location, adult striped shore crabs (*Pachygrapsus crassipes*) were collected from an oyster reef composed of *Crassostrea virginica* and brought to the laboratory where they were kept for 2 weeks in flow-through tanks prior to experimentation. These crabs are common herbivores in the rocky intertidal (Abele et al 1986), where they are exposed to bacterial colonization from the water column.

Experimental design

Our field experiment consisted of 3 species pool and 2 habitat filter treatments. To assess the effect of species pool sizes, we ran the experiment on August 7th, August 30th and September 19th, encompassing one of the periods of most drastic change in salinity and temperature in Texas bays (Applebaum et al 2005). Based on known responses of bacteria to temperature and salinity (Sunagawa et al 2015), we expect these changes in environmental conditions to increase bacterial richness. The effect of these different bacterial pool sizes was assessed on bacterial communities associated with two distinct habitat filters: crab carapaces and tile surfaces. Prior to the experiment, carapaces and tiles were swabbed with alcohol and bleach to disturb the associated bacterial communities. We

sampled these surfaces after the cleaning procedure to assess its effect. To initiate community assembly, crabs and tiles were suspended next to the oyster reef at a depth of 1m, within individual PVC enclosures. Sampling was done after 4 and 8 days of deployment. At each sampling date, 3 crabs and 3 tiles were selected at random and taken to the lab in sterile containers for further processing. In addition to surface samples, we took 3 water samples (400 mL) at each sample date as a measure of the regional species pool. At every sampling date, we determined salinity and temperature as explanatory factors of bacterial diversity in the water using a handheld salinity, conductivity and temperature probe (YSI, Model 30).

Sample processing and DNA extraction was conducted at the Marine Science Institute of the University of Texas. Crabs were rinsed with sterile deionized water to remove unattached bacteria. We swabbed and scraped crab carapaces and tiles for bacterial DNA profiling. Although tiles measured 10 x10 cm, we only sampled a 2.5 x 2.5 cm region to account for area effects on diversity. These dimensions correspond to the average P. transversus adult size (Abele et al 1986). Water samples were filtered using a 0.45 μm pore filter (MoBio) to facilitate filtering of coastal water samples of high particulate content. Although we may lose some bacteria using this larger pore size, 0.45 μm are equally efficient at recovering bacterial cells as smaller pore sized filters (Carter 1996).

We extracted total microbial DNA from swabs and filters using PowerWater DNA extraction kit (MoBio). To eliminate humic acids and reduce PCR inhibition, we used a DNA Clean Up kit (Qiagen) prior to amplification. DNA was amplified with a two-step PCR enrichment of the 16S V4 hypervariable region. This 292 bp long sequence encompasses positions 515-801 in Escherichia coli and specifically avoids amplifying host's DNA (Laurie et al 2010, Wang and Qian 2009). We used an Illumina MiSeq platform for sequencing at the Genomic Sequencing Analysis Facility at the University of Texas at Austin.

To process sequence data, we used the Quantitative Insights Into Microbial Ecology pipeline (QIIME, version 1.8.0, (Caporaso et al 2010). We used FLASH to align the 250 bp paired end reads (Magoc and Salzberg 2011). Sequences were clustered into OTUs (Operational Taxonomic Units) based on 97% similarity in 16S rRNA DNA. We used an open-reference OTU picking protocol based on UCLUST's open reference algorithm (Edgar 2010). The GreenGenes database (version 12.10 (DeSantis et al 2006) was used as reference for OTU picking and taxonomic classification. For analysis, we retained only those OTUs representing more than 0.01% of sequences per sample and occurring in more than 3 samples, to reduce the effect of rare OTUs and sequencing errors (Bokulich et al 2013).

Data analysis

Our analysis consists of three parts. First, we evaluated seasonal changes in environmental conditions and OTU richness. Second, we assessed the strength of the habitat filters. Lastly, and most importantly, we determined the effect of species pool size and habitat filters on community similarity (beta diversity).

To determine seasonal differences in bacterial pools in the water column, we assessed changes in OTU richness and rarefied richness with ANOVA tests. We calculated species accumulation curves to determine whether richness patterns reflect similar sampling effort across samples. We used linear regressions to assess the seasonal change in temperature and salinity.

To assess the strength of habitat filters acting on colonizing bacteria, we compare bacterial diversity on crab carapaces and tile surfaces. Differences in OTU rarefied richness between crab and tile associated communities were assessed with two-way ANOVA tests with habitat filter (tile or carapace) and month as factors. To evaluate differences in sampling depth across habitats, we constructed sample-based rarefaction curves using the specaccum function from the vegan package (Oksanen 2007). We used Redundancy Analysis (RDA) on Hellinger transformed OTU data to assess compositional differences between water species pools, crab carapaces and tile surfaces using temperature and salinity as environmental data (Legendre and Gallagher 2001). This analysis minimizes the effect of rare species and facilitates the visualization of group differences (Legendre and Gallagher 2001). We assessed significance in compositional differences between carapaces,

tiles and water samples using perMANOVA on RDA scores (Anderson and Walsh 2013). We assessed convergence and divergence in community composition in carapaces and tiles using the multivariate homogeneity of group dispersions on Hellinger transformed data, calculated for day 4 and 8. (Anderson 2006). While convergence in community composition through time suggests strong habitat filters acting on local assemblages, divergence suggests weak filters open to colonization.

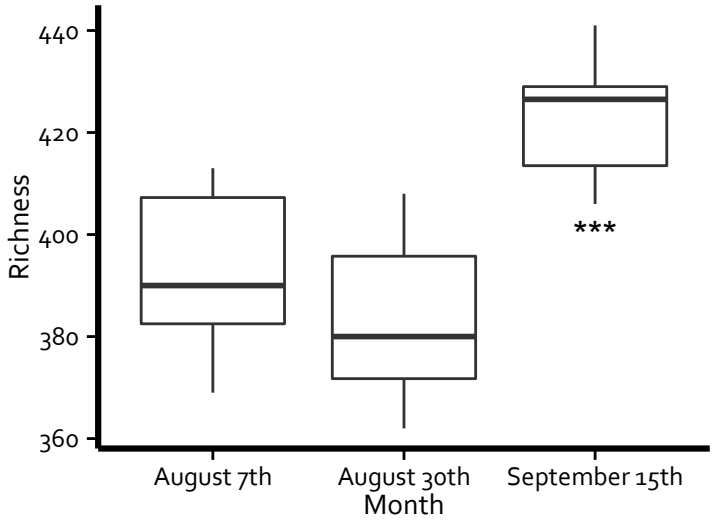

Figure 1.1. Boxplot of seasonal change in bacterial pool richness. *** indicates significant differences from other groups as shown by a Tukey test.

Table 1.1. Summary of an ANOVA on rarefied richness differences between surface habitats (crab carapace and tile surfaces) and month of sampling. Although there is a significant effect of habitat, these differences are lost after a Tukey test.

	d.f.	F	p
Month	2	0.629	0.540
Habitat	1	4.277	0.047*
Month*Habitat	2	1.086	0.350

* indicates significance of $p<0.05$

To evaluate the effect of species pool size and habitat filters on community similarity (beta diversity), we used the multivariate homogeneity of group dispersions on Hellinger transformed relative abundance data (Anderson 2006, Legendre and Gallagher 2001) calculated for each month and each habitat (carapace and tile). We then evaluated the individual taxa driving these community wide responses. We assessed the effect of habitat filter and species pool on relative abundance of individual OTU using two-way ANOVAs.

RESULTS

We obtained 9442± 1080 reads per sample for a total of 660 994 DNA reads. Of the original samples, some were discarded because of low DNA yield (less than 0.5 ng/μL) or low read count (less than 5000 reads), leaving 54 samples suitable for analysis. For example, we did not recover sufficient DNA from crabs or tiles after the cleaning procedure, thus these samples were excluded from the analysis. After accounting for sequence quality and removing low-abundance OTUs, we identified 656 OTUs distributed across 14 phyla and 79 families.

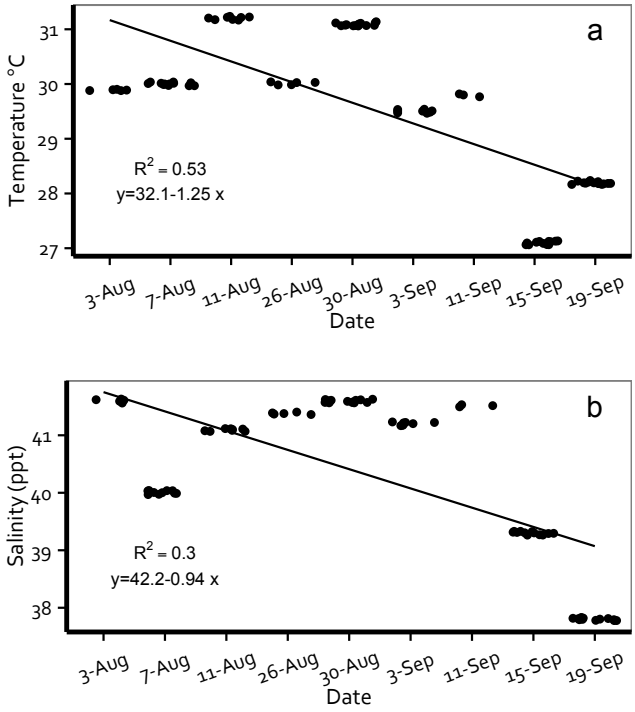

Figure 1.2. Seasonal change in temperature (a) and salinity (b). A summary of the results of a linear regression are indicated for each plot.

The bacterial community in the water column increased from August to September (d.f.=2, F=8.634, p= 0.003, Figure 1.1). We found equivalent patterns from raw richness and rarefied richness, and we only show raw richness results. In the August 7th and 30th experiments, OTU richness averaged 392±13 and 383±15 OTUs respectively while, in September 15th, we found an average of 423±13 OTUs. These changes are paralleled by

salinity and temperature, which decreased drastically between August and September (Figure 1.2).

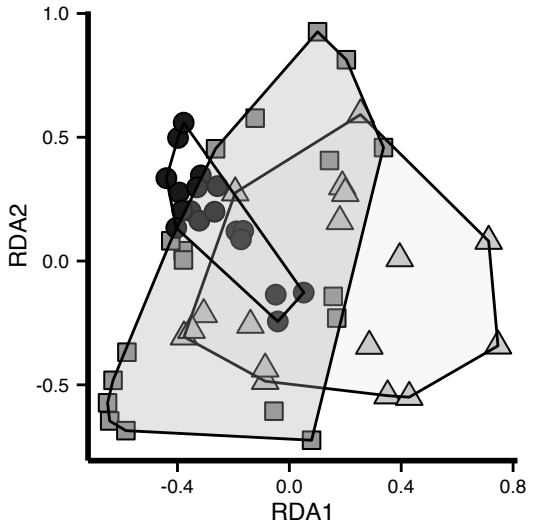

Figure 1.3. Redundancy analysis (RDA) of bacterial communities associated with crab carapaces (light grey triangles), tile surfaces (dark gray squares) and water samples (black circles).

Overall, bacterial communities on crab carapaces, tile surfaces and water samples differed in community composition (Fig 1.3, perMANOVA: d.f.=2, R^2=0.198, p=0.003). These differences were partially due to beta diversity patterns. While water communities remained strongly similar to each other (low beta diversity), carapace-associated communities were intermediate in community composition (intermediate beta diversity), and tile-associated communities were the most variable (high beta diversity, d.f.=2, ANOSIM R=0.778, p=0.001). Differences in diversity between carapaces and tiles were not due to richness differences (Table 1.1, Fig S1.1) or patterns of divergence or convergence in community composition (Fig S1.2). Species accumulation curves from carapace, tile and water samples differed, but they were all near saturated (Fig S1.3). Beta diversity remained unchanged between day 4 and 8 across tile surfaces (d.f.=1, F=2.295, p=0.149), crab carapaces (d.f., F=1.015, p=0.418) and water samples (d.f.=1, F=0.060, p=0.759).

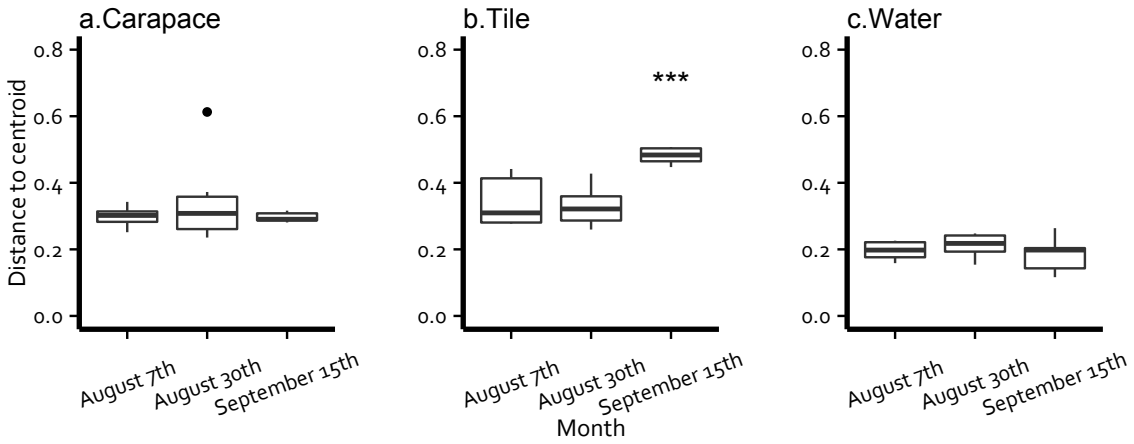

Figure 1.4. Boxplot illustrating seasonal changes in distance to centroid of microbial communities associated with crab carapaces (a) and tile surfaces (b). Distance to group centroid was calculated from hellinger transformed data for each month. *** indicates significant differences from other groups as shown by a Tukey test.

Importantly, beta diversity increased with increasing richness in water bacteria, but only on tile surfaces. Beta diversity across tile-associated bacteria increased from August to September (Fig 1.4, d.f.=2, F=12.69, p=0.0005). In contrast, beta diversity remained constant throughout our study in crab-associated communities (Fig 1.4, d.f.=2, F=0.665, p=0.531) and in the water column (permutes: d.f. =2, F=25.117, p=0.001). OTUs driving these differences in beta diversity were identified among those with average relative abundance higher than 0.005 in either carapace or tiles (Table 1.2, Fig 1.5). We highlight here patterns of selected OTUs as example of some general patterns. For example, OTUs like *Tenacibaculum* sp (Flavobacteriaceae) were consistently higher on the carapace (Fig 1.5a, Table 1.2). Some OTUs, such as *Prochlorococcus marinus*, were consistently higher on tiles (Fig 1.5b, Table 1.2). In contrast, members of Rhodobacteraceae and *Vibrio* sp showed seasonal patterns, particularly on the tiles (Fig 1.5c and 1.5d, Table 1.2).

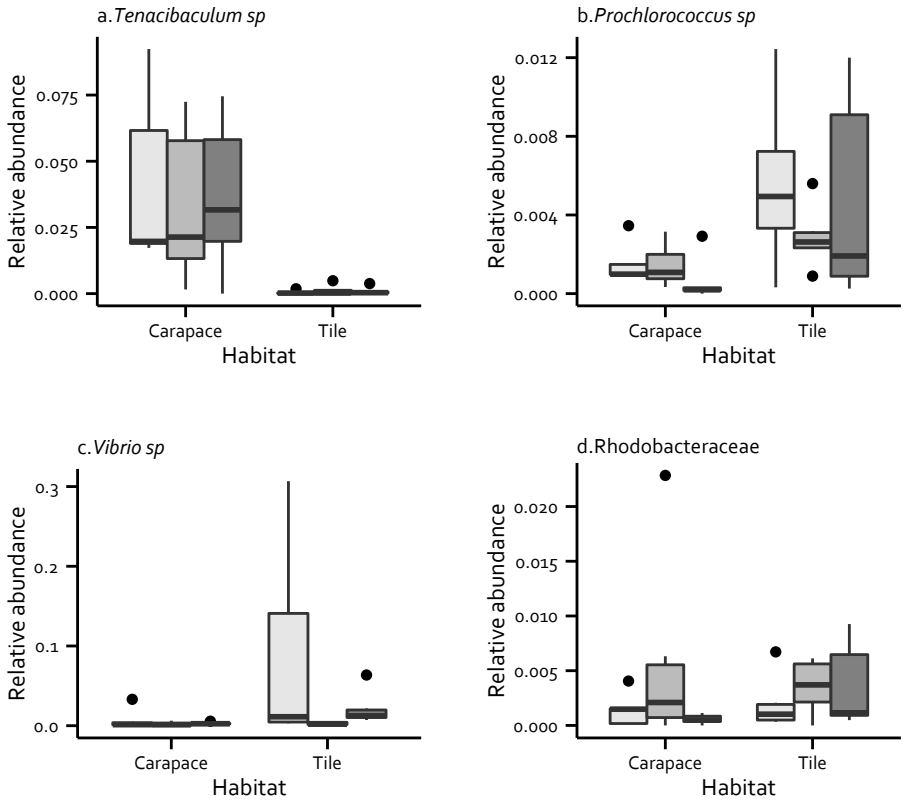

Figure 1.5. Boxplots of relative abundance of selected OTUs. Relative abundance of each OTU is shown for crab carapaces (shape), tile surfaces (shape) and water (shape), across each month of sampling.

DISCUSSION

Although broad biogeographical studies indicate that species pool size and habitat filters interact in driving patterns of community structure (Freestone and Inouye 2015, Kraft et al 2011, Myers et al 2013), few studies address this relationship experimentally (Grman and Brudvig 2014). Our results are consistent with the hypothesis that colonist pool size increases beta diversity but only on habitats with weak filtering. In the September 15th experiment, we found high OTU richness in the water column and increased beta diversity on tile but not on crab surfaces (Fig 1.3). In contrast, in August 7th and 30th, OTU richness in the water column was low and beta diversity on tile surfaces was comparable to crab carapaces.

These results contribute to our understanding of factors mediating the importance of colonization from the species pool and local habitat filters (Chase and Myers 2011, Lessard et al 2012). To our knowledge, only two studies have experimentally tested for this relationship. Grman and collaborators (2014) found that beta diversity was higher when restored prairies were treated with more diverse seed mixes. Their results depended on soil moisture, similar to our findings of habitat-dependent regional species pool effects. Questad and Foster (2008) found that species pool size interacted with disturbance to create higher beta-diversity in grasslands. These studies differ from ours in the choice of species for the species pool. While in Grman and collaborators (2014)'s study, seed cost determined species pool composition and Questad and Foster (2008) built species pools according to species traits, our study provides evidence of habitat filters interacting with natural species pools shaped by seasonal changes in the marine environment.

Seasonal changes in salinity and temperature in the water column may explain shifts in species pool richness (Fig 1.1, 1.2). These variables are major drivers of bacterial diversity in the marine environment (Sunagawa et al 2015, Zinger et al 2011) suggesting that microbial diversity in the water column is decoupled from local community dynamics shaping surface associated biofilms (Harrison and Cornell 2008). This resembles the classic mainland-island model where the species pool is a species reservoir that remains unaffected by processes shaping local communities (MacArthur and Wilson 1967). However, instead of the stable species reservoir portrayed in this classic model, our species reservoir shifts in richness and composition due to seasonal changes in environmental conditions. Likewise, broad climate and landscape patterns can result in shifts in regional species pool diversity for animals and plants (Harrison and Cornell 2008, McPeek and Brown 2000). Whether these shifts have strong impacts on local communities depends on the strength of local habitat filters.

We found that the crab carapace represents a distinct habitat for bacterial communities with stronger filters than found on tile surfaces. Importantly, beta diversity

was consistently lower within crab carapaces than within tile surfaces (Fig 1.3, 1.4). This suggests that filtering mechanisms on the carapace constrain colonist establishment, reducing the influence of random colonization, ecological drift and priority effects on community assembly. While colonization of tile surfaces depends merely on attachment and biofilm development (Jackson et al 2001, Siboni et al 2007), colonization of the carapace is constrained by chitin chemistry and crab immunity (Moret and Moreau 2012). For example, we found that the opportunistic chitin-degrading pathogen *Vibrio* sp (Pruzzo et al 2008) was abundant on tiles in early August, but was excluded from carapace communities (Fig 1.5c). This particular result support the hypothesis that colonization of host-associated microbial habitats is constrained by evolutionarily determined filters that aim at excluding pathogens and enhancing fitness (Robinson et al 2010).

We found little evidence of richness differences in surface-associated microbial communities (Table S1.1, Fig S1.1). Bacteria within the biofilm matrix is protected from physical, chemical and environmental stress by extracellular polymeric secretions (EPS, Decho 2000). Thus, even though factors like temperature or salinity can alter the regional species pool and early biofilm assembly, they should not influence later stages of mature biofilms. Even though richness differences were subtle, community composition differed between surfaces reflecting the strong influence of colonist on weakly filtered tiles. For example, *Prochlorococcus* sp and Rhodobacteraceae, common to the water column, were consistently higher in the tile surfaces (Fig 1.5, Table 1.2). These taxa suggest strong influences from the colonist pools. In contrast, *Tenacibaculum* sp, and other Flavobacteraceae had consistently higher abundances in the carapace (Fig 1.5, Table 1.2).

Using realistic colonist pools drawn from seasonal changes in bacterial richness, our study provides evidence of habitat-dependent effects of species pool size. These findings resonate with studies observing beta-diversity patterns at large spatial scales plant and animals (Freestone and Inouye 2015, Myers et al 2013). Integrating patterns at broad spatial scales with experimental manipulations advances our understanding of the relationship

between colonist species pools and habitat filters (Grman and Brudvig 2014, Questad and Foster 2008).

Chapter 2: Habitat filters determine temporal scale in community assembly of crab-associated microbial communities

ABSTRACT

A useful approach to distinguish between the relative contribution of habitat filters and stochastic processes for community assembly is to study patterns of convergence and divergence in community composition under different conditions. These patterns reveal important trends that can depend on the temporal scale of study. We contrasted temporal patterns in community assembly of host-associated microbial communities on the carapace and gut of the striped shore crab (*Pachygrapsus transversus*) to ask if they differed in the degree to which they diverged or converged from the source pool (water column bacteria) in a controlled mesocosm experiment. Because of the degree of connectance with water column bacteria and likely differences in physical and chemical conditions, we hypothesized that the carapace would act as a weaker habitat filter than the gut. We found that the gut communities differ from the source pool more and converge with each other earlier than carapace communities. They also maintain similar composition throughout the experiment whereas the carapace communities did so only to a lesser degree. In addition to defining local richness and variability in community composition, habitat filters can determine the temporal scale of assembly.

INTRODUCTION

Central to community ecology is to understand the interaction between ecological processes that select for different species during the process of community assembly from those that cause stochastic changes instead (Vellend 2010). The relative contribution of these processes delineates our ability to predict changes in biodiversity, key in a changing

world. At one extreme, communities assemble completely through selection and should often converge towards a predictable species composition (Leibold 1995, Poff 1997). In theory, if we know the species requirements, we can also predict which species should fail to establish viable populations and which species dominate a given environment due to any given habitat filter (defined by local disturbances, stress or low resource availability - (Chase 2003, McGill et al 2006, Samuels and Drake 1997). At the other extreme, communities assemble through stochastic colonization and diverge in species composition through population drift and colonization history, creating many possible outcomes (Hubbell 2001). Stochastic processes counteract the effects of selection and show less convergence to habitat filters. Most communities fall somewhere in between selection and stochasticity (Chase and Myers 2011). The temporal dynamics and degree of convergence among replicate communities following disturbance thus represents an fundamental way to study the relative importance of selection and stochasticity in determining community structure of different habitats.

An important way to study patterns of convergence and divergence among communities is to study ambient levels of variation in species composition between localities with similar environmental conditions, known as beta diversity (Chase 2007, Chase and Myers 2011). This framework has been influential in revealing how ecological processes may differ along environmental gradients and across biogeographical regions (Freestone and Inouye 2015, Myers et al 2013, Soininen et al 2007). Furthermore, this framework assumes that enough time has passed that differences between the effects of stochastic and selection processes on community structure are near steady state. However, these effects can change during the process of community assembly and develop over different time scales. For instance, while long-term vegetation studies following disturbance report that early divergence trends are replaced by convergence in

community composition (Inouye and Tilman 1995), bird communities seem to diverge in the long-term instead (Bengtsson et al 1997). Evidence from microcosm experiments suggests that parameters determining this temporal scale of convergence or divergence in community composition include invasion frequency, disturbance rate, and duration of the study (Grover and Lawton 1994), yet, to our knowledge, the role of habitat filters on temporal changes in convergence and divergence has not been explored.

The concept of habitat filters has a long history in community ecology describing environmental conditions and species interactions as metaphorical sieves that determine which species recruit to a local community (Kraft et al 2015). Central to this concept is the difference in assembly mechanisms that govern communities under strong and weak filters (Leps and Rejmanek 1991). Convergence in harsh conditions (strong filter) depends on the time it takes to eliminate poor competitors with low tolerance (Chesson and Huntly 1997, del Moral 2009). In contrast, divergence under benign conditions (weak filter) depends on colonization rate and ecological drift. Therefore, whether the time scale of convergence and divergence coincide depends on filtering and colonization rates (Leps and Rejmanek 1991). An independent and often underappreciated component of filtering is species interactions (Callaway et al 2002, Kraft et al 2015). This biotic filtering component can operate independently or interact with environmental filtering (Kraft et al 2015).

Community assembly also depends on properties of the species pool, such as its size, composition and the rate of colonization. The size of the species pool (the number of different species) outlines the potential for different assembly trajectories for weak-filtered communities. The composition of the species pool determines the proportion of tolerant species able to survive in strong-filtered communities. The rate of colonization from the species pool may also influence assembly outcomes. While low colonization

rates are conducive to convergence or alternative stable states, high colonization rates lead to transient dynamics (Robinson and Edgemon 1988, Schroder et al 2005). For convergence to occur, initial recruitment of species from the species pool has to be followed by filtering of the least competitive or tolerant species (Macarthur and Levins 1967). In contrast, for divergence to occur, sufficient time has to pass for distinct combinations to accumulate due to random sampling, ecological drift or historical effects. The temporal scale could depend on whether assembly leads to a stable point, such as a climax community or alternative stable states, or to transient dynamics, such as alternative transient states, fluctuating equilibrium or purely stochastic assembly (Robinson and Edgemon 1988, Schroder et al 2005).

Host-associated microbial communities represent an ideal system to explore the interaction between habitat filters and temporal trends in community composition. These communities are highly diverse and assemble rapidly (time frames, refs) making them amenable for experimentation. Each host represents a distinct patch for community assembly and is colonized by tractable species pools. By retaining natural dispersal from this species pool, they differ from traditional microcosm experiments. Within each host, separate habitats available for colonization differ in filtering imposed by host tissue, physiology, immunity and behavior (Robinson et al 2010). For example, studies suggest that strong, selective filters characterize animal guts (Jeraldo et al 2012, Levy and Borenstein 2013), while systems that are more open to colonization, like the human skin, are often driven by stochastic processes (Bouslimani et al 2015, Grice et al 2009).

In the present study, we examine the role of habitat filters in determining the temporal scale of variation in community composition. For this purpose, we used a mesocosm where we tracked the assembly of bacterial communities in the gut and on the carapace of the striped shore crab, *Pachygrapsus transversus*. Because of strong physical

and chemical filtering (Brosing 2010, Vogt et al 1989, Wang et al 2014), microbial communities in the gut are expected to converge in species composition. In contrast, microbial communities in the carapace are open to colonization and expected to diverge in community composition. We hypothesized that convergence in the gut will occur earlier in assembly than divergence in the carapace, indicating fast filtering rates. This hypothesis assumes that the crab's gut and carapace represent stable habitat filters unchanged by time and an alternative hypothesis is that filter strength changes through time due to species interactions or changes in the environmental filter. Our results confirm fast filtering rates that result in quick convergence in gut bacterial communities. However, we found that colonization and filtering interacted in structuring carapace communities, which initially converged and later diverged in composition.

METHODS

Adult female shore crabs (*Pachygrapsus transversus*) were collected on the rocky intertidal along channels west of Port Aransas, TX in May 2013. Upon collection, crabs were transported to the University of Texas Marine Science Institute. For two weeks before the experiment, crabs were kept in a holding tank under flowing seawater and fed sterile shrimp pellets (Brine Shrimp Direct) daily. Seawater was obtained from Corpus Christi shipping channel, strained (0.5cm nylon mesh), allowed to settle and recovered by syphoning to avoid major debris and large marine life. Prior to the beginning of the experiment, crabs were washed with sterile deionized water, swabbed with alcohol (95%) and bleach (90%) and isolated in a sterile container for 48 hours to reduce their initial bacterial load in the carapace and gut. To evaluate these cleaning procedures, we sampled crab carapaces and guts from freshly collected specimens and post-cleaning specimens. Although this cleaning procedure may not eliminate all bacteria, this disturbance is

sufficient to induce colonization from the water column and re-assembly of crab microbial communities.

To assess the effect of time on beta diversity, we established a mesocosm experiment, created as described below, that induced community assembly on crab guts and carapaces. The mesocosm experiment consisted on inserting cleaned crabs in individual sterile containers filled with 1 L of filtered seawater. At 2, 4, 8, 12 and 16 days after the beginning of the experiment, 9 mesocosms were selected at random and samples of the bacterial communities from guts, carapaces and water were taken for a total of 135 samples.

Carapaces were swabbed and scraped with sterile scalpels to mechanically separate bacteria adhering to its surface. After the samples were taken from the carapace, crabs were sacrificed by freezing (10 mins) and dissected to obtain the cardiac and pyloric stomachs. Bacterial communities from water samples were obtained by filtering 400 mL through 0.22 μm polythersulfone filters (MoBio). Samples were stored with 180 μL of ATL Buffer (Qiagen) at -80 °C until downstream processing.

During the experiment, we attempted to control environmental conditions and species pool size as much as possible. Crabs were fed every two days with 4 flakes of autoclaved shrimp pellets (121°C, for 15 minutes). To avoid the accumulation of waste products, we removed crab feces, waste and 100 mL from each container daily. The water level was replenished with UV sterilized filtered seawater to avoid introducing new bacteria to the mesocosm. Mesocosm water was monitored daily to keep temperature and salinity at ambient levels (27 °C, 35ppt).

DNA Extraction and Sequencing

We extracted DNA from water, carapace and gut samples using the Qiagen Blood and Tissue DNA extraction kit with minor modifications that aim at increasing DNA yield (Fierer et al 2008). In short, samples were physically lysed by vortexing at maximum speed for 1 minute with 0.5 mL sterile microbeads and incubated with proteinase K at 57°C for 15 minutes. DNA was amplified with a two-step PCR enrichment of the 16S V4 hypervariable region, a 292 bp long sequence (positions 515-801 in Escherichia coli). We chose this region because it avoids amplifying host's DNA (Laurie et al 2010, Wang and Qian 2009). Libraries were sequenced using the Illumina MiSeq platform at the Genomic Sequencing Analysis Facility at the University of Texas at Austin.

To process sequence data, we used the Quantitative Insights Into Microbial Ecology pipeline (QIIME, version 1.8.0, (Caporaso et al 2010). First, we aligned the 250 bp paired end reads with FLASH (Magoc and Salzberg 2011). Second, sequences with more than 97% similarity in 16S rRNA DNA sequences were clustered into OTUs (Operational Taxonomic Units). We used an open-reference OTU picking protocol based on UCLUST's open reference algorithm (Edgar 2010) and matching sequences against the GreenGenes database (version 12.10 (DeSantis et al 2006). Similarly, using this same database, representative sequences from each OTU were given a taxonomic classification. To reduce the effect of rare OTUs and sequencing errors, we retained only those OTUs representing more than 0.01% of sequences per sample and occurring in more than 3 samples (Bokulich et al 2013).

Although we did not directly profile the functional roles of bacteria in these communities, we explored their functional profiles using Picrust. Assuming that there is an association between gene content and phylogenetic affiliation, this bioinformatics tool

predicts gene family abundances based on 16S data using a genomic database for reference (Langille et al 2013). OTUs were close-reference picked with QIIME to predict the metagenome. Using the nearest sequenced taxon index (NSTI) scores, we checked the availability of closely related reference genomes and excluded samples with low representation (NSTI < 0.15).

Data and statistical analysis

To determine the effect of habitat and time on the variation community composition (beta diversity), we analyzed the similarity of bacterial communities from the mesocosms. Then, we evaluate whether compositional effects are associated with species replacements or richness differences, or with the influence from the species pool in the water column. Finally, we consider the temporal changes in OTUs and Picrust KOGs relative abundance to identify the taxa and functions underlying community assembly.

We assessed the temporal patterns in community similarity with Redundancy Analysis (RDA) on Hellinger transformed data. This analysis facilitates the visualization of group differences (Anderson and Willis 2003, Ramette 2007) and minimizes the effect of rare species (Legendre and Gallagher 2001). To estimate the pure and combined effects of time and habitat on bacterial community composition, we calculated partial RDAs (pRDA) and tested their significance with a Monte Carlo permutation test with 999 permutations (Anderson 2006). We tested the significance of effects of time and habitat on distances to centroid using the multivariate analogue of Levene's test for homogeneity of variances (function betadisper in vegan, (Anderson 2006).

Variation in species composition can be caused by species replacements (also known as turnover) or richness differences (also called nestedness, (Harrison et al 1992,

Williams 1996). To distinguish between replacement and richness effects, we decomposed the total variation in species composition (D) into the replacement (repl) and richness (richdiff) components using Ružička dissimilarities in the Podani family (Legendre 2014). To test for the influence of habitat and time on these components of species composition, we calculated a dbRDA F-test on the root transformed repl and richdiff (Legendre et al 2014). Podani and collaborators (2011) suggest that triangular plots better portray these components given that *repl + richdiff = D* and that the similarity *S = (1-D),* thus *S + repl + richdiff = 1*(Podani and Schmera 2011). Therefore, we calculate S, repl and richdiff for each habitat and represent their relationships in a triangular graph. In addition, we calculated raw and rarefied OTU richness to test its response to habitat and time with a two-way ANOVA. Since we found similar results, only rarefied richness is presented here. Sample-based rarefaction curves were constructed using the specaccum function from the vegan package (Oksanen) to evaluate differences in sampling depth across habitats.

We estimated the influence of the species pools from the multivariate distance from each crab-associated community and the water communities. Given our experimental design, we can measure the distances within each mesocosm and determine the similarity between each crab community and the water column around it. We assessed the effect of time and habitat on the multivariate distance to the pool using a two-way ANOVA.

We tested whether time and habitat influenced the relative abundance of each microbial taxon. Within each habitat, we selected the most abundant OTUs (more than 5% mean relative abundance) for a total of 24 OTU in the carapace, 14 OTUs in the gut and 36 OTUs in the water. To test whether the number of sequences for the OTU depended on time, each independent OTU was evaluated with a GLM with quasibinomial

distribution. Quasibinomial error distribution is less sensitive to outliers and recommended in overdispersed data such as host-associated microbial communities. We used time (days) and abundance in the water as co-factors to determine whether each OTU changed in abundance through time and whether it was influenced by abundance in the water. A second GLM was used on the occurrence of functional groups as described by the PiCrust analysis. We used a partial RDA analysis to test for the contribution of time and habitat to variability in gene relative abundance.

All analyses described in this section were performed in the statistical environment of R, with functions contained in the packages *phyloseq* (McMurdie and Holmes 2013), *vegan* (Oksanen et al 2015) and *mvabund* (Wang et al 2012) as well as custom scripts.

RESULTS

We obtained 8000±1200 reads per sample for a total of 34 958 DNA reads. Of the original samples, 54 were suitable for analysis. Samples were discarded because of crab mortality (17.85%), or low DNA yield (less than 0.5 ng/μL). For example, samples taken from crabs at 2 days after cleaning procedure were consistently low in DNA concentration, thus were not included in the analyses. After accounting for sequence quality and removing low-abundance OTUs, we identified 761 OTUs distributed across 13 phyla and 62 families.

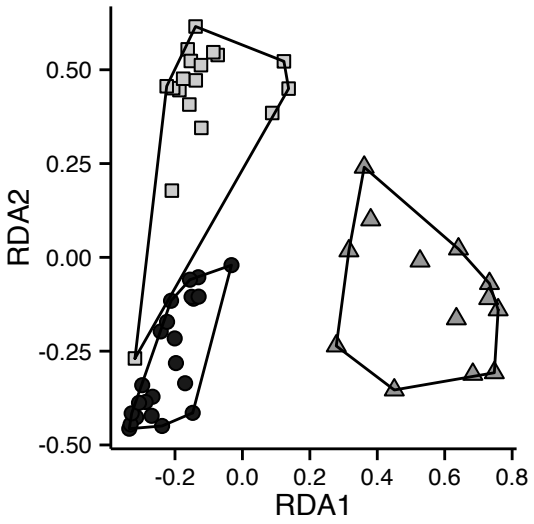

Figure 2.1. Redundancy Analysis (RDA) illustrating similarity in OTU composition between bacterial communities. Each point represents a bacterial community from a crab's carapace (grey squares), gut (dark grey triangles) or from the water column (black circles).

Table 2.1. Percentage contribution of beta diversity components. F-value was calculated from a dbRDA on root transformed repl, richdiff and D data.

	F-value	p	R2
D	9.815	0.001	0.688
Replacement	3.929	0.001	0.616
Richness	22.415	0.001	0.383

Habitat and time influenced the composition of the bacterial communities. While habitat differences explained 30.42% of the variation in community composition, time explained 7.61% (Monte Carlo permutation test, F_3= 7.647, p=0.001). The remaining 61.98% of the variation in community composition was unexplained by time or habitat. These differences in composition were partially due to differences in community similarity, and this effect was habitat dependent. In the carapace, the multivariate distance to group centroid decreased from day 4 to day 8 but increased thereafter (Fig

2.2a, Multivariate test for homogeneity of variances F_3=8.266, p=0.002). In the gut, distance to centroid was constant throughout the duration of this study even though there is slight, albeit not significant, temporal variation (Fig 2.2b, F_3=1.293, p=0.335). In the water, distance to centroid decreased from day 8 to day 12 and increased between day 12 and day 16 (Fig 2.2c, F_3=3.209, p=0.007).

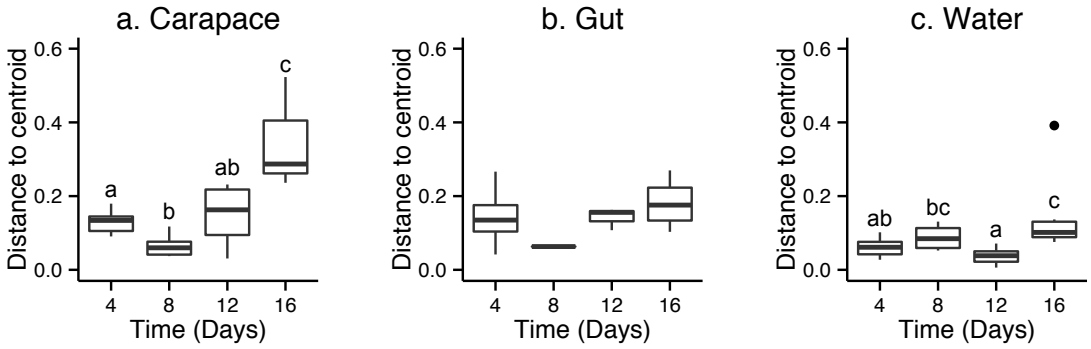

Figure 2.2. Temporal changes in distance to centroid from RDA scores in a) carapace, b) gut and c) water samples. Letters indicate significant differences resulting form a tukeyHSD test.

This variation in community composition was better explained by the replacement than by the richness difference component of similarity. Partitioning of total community similarity reveals that replacement accounts for 61.6% while richness differences only account for 38.3% (Table 2.1, Fig 2.3). This result coincides with the lack of significant temporal change in OTU richness, even though it differed between habitats (Table 2.2, Fig 2.4). The gut had the lowest mean richness value (74.66± 33.04 OTUs) when compared to the carapace (323.33 ± 87.75) or the water (224.63 ± 40.82). Even though a two-way ANOVA indicates that richness differences were driven by habitat and time (Table 2.2) the Tukey HDS test indicated that these differences were only significant for

habitat and not for time. Even though they differed in magnitude, species accumulation from carapace, gut and water were all near saturated, (Fig 2.4a).

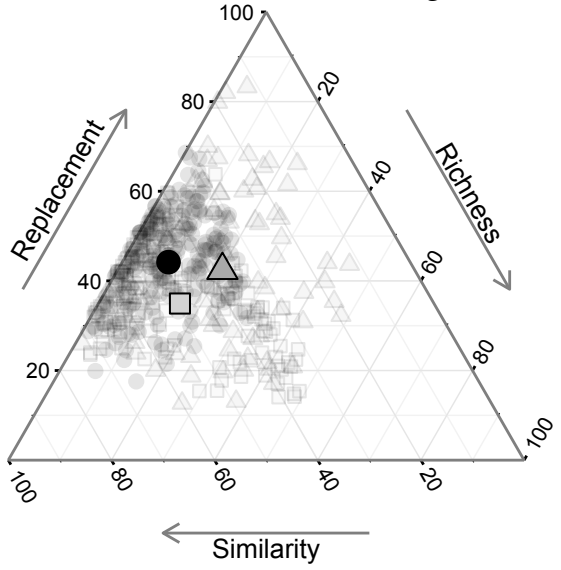

Figure 2.3. Triangular plots representing the components of community variability in carapace (grey squares), gut (dark grey triangles) and water samples (black circles). Each point's position is determined by a triplet of values: similarity (S), replacement (Repl) and richness differences (RichDiff). Small points represent the triplet of values between a pair of samples. Large points represent the means for each habitat.

Table 2.2. Summary of a two-way ANOVA assessing the effects of time and habitat on OTU richness.

	d.f.	F-value	p
Habitat	2	74.737	<0.001***
Time	3	6.087	0.0015
Habitat*Time	6	0.953	0.468

*** indicates significance of p<0.001

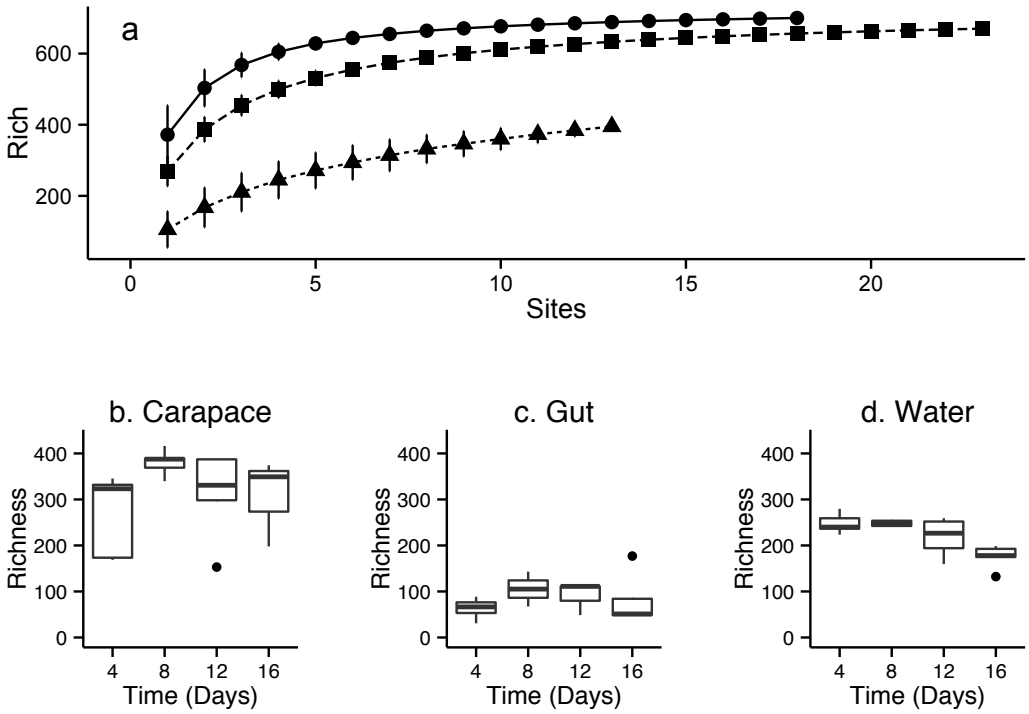

Figure 2.4. Richness differences among habitats. a) species accumulation curves. Box plots of temporal changes in rarefied richness during bacterial assembly in b) carapace, c) gut and d) water samples.

The multivariate distance from crab-associated communities to the water column was habitat-dependent (Fig 2.5). We found that carapace communities were more similar to the species pool in the water than gut communities (Figure 2.5, Table 2.3). This relationship did not change with time (Table 2.3).

We identified OTUs driving differences in community composition among those with average relative abundance higher than 0.001 (Fig 2.6, Table S2.1 and S2.2). For example, members of Mycoplasmataceae, were dominant in the gut and increased smoothly towards day 16 (Fig 2.6a, Table S2). In the carapace, early colonizers found at day four, such as *Vibrio* sp, disappeared sharply by day 8 (Fig 2.6b, Table S2.1). These early taxa are replaced by late succession OTUs such as members of Rhodobacteraceae in

the carapace (Fig 2.6c, Table S2.1) or Oceanospirillaceae in the carapace and gut (Fig 2.6d, Table S2.1 and S2.2). A third group of taxa did not change much in abundance throughout our experiment, such as members of *Vibrio* sp in the gut (Fig 2.6b, Table S2.2). When we evaluated the influence of additional recruitment from the water column, we found significant relationships between abundance in the water and most OTUs abundance in the carapace, but fewer OTUs in the gut (Table S2.1).

Table 2.3. Summary of a two-way ANOVA evaluating the effects of time and habitat on multivariate distance between crab-associated communities to water column assemblages.

	d.f.	F-value	p
Habitat	1	11.244	0.004 *
Time	3	1.015	0.413
Habitat*Time	3	0.448	0.722

* indicates significance of $p<0.05$

Temporal changes in OTU abundance coincided with community functional components as shown by the Picrust analysis. After excluding 4 samples that were not well represented in the databases, we retained 50 samples characterized by 0.097± 0.19 NSTI. Temporal patterns in functional abundance differed in time and across habitats (Table S2.3). Carapaces sampled earlier carried more genes associated with flagellar assembly and less genes associated with oxidative phosphorylation when compared with later carapace communities. In contrast, early gut samples carried more genes associated with nitrogen and methane metabolism, and less DNA and RNA replication genes than late gut communities. Notably, peptidase genes were found to be abundant in the carapace and water throughout the experiment, and to increase in the gut until comparable levels at day 16. Nonetheless, only 16.8% of gene abundance varied

according to time and only 8.25% were driven by habitat (permutation test, F=4.78, df= 7, p=0.01).

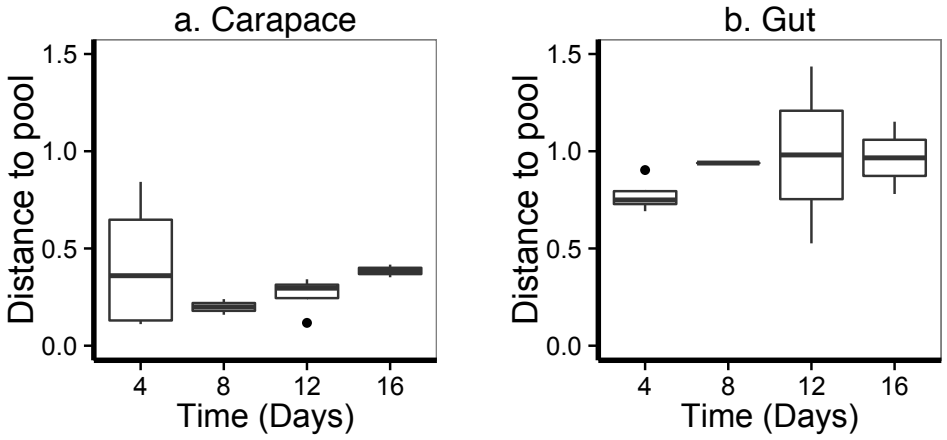

Figure 2.5. Multivariate distance from crab communities and colonist pools in the water column for a) carapace and b) gut assemblages.

DISCUSSION

The study of temporal changes in community structure can reveal the processes underlying ecological assembly. However, the rate of habitat filtering and colonization may differ resulting in disjoint trajectories in community assembly. We found that the time frames of convergence and divergence of crab-associated microbial communities were habitat dependent. Under strong filters, gut communities remained constant throughout the experiment (Fig 2.2). This result suggests early convergence, consistent with a fast and constant filtering rate. In contrast, carapace communities shifted from convergence to divergence at day 8, and diverged thereafter (Fig 2.2). Although differing from our expectations, these patterns in the carapace are coherent with biofilm development driven by the interaction between colonization, interspecific interactions and species interactions with the environment (Jackson 2003).

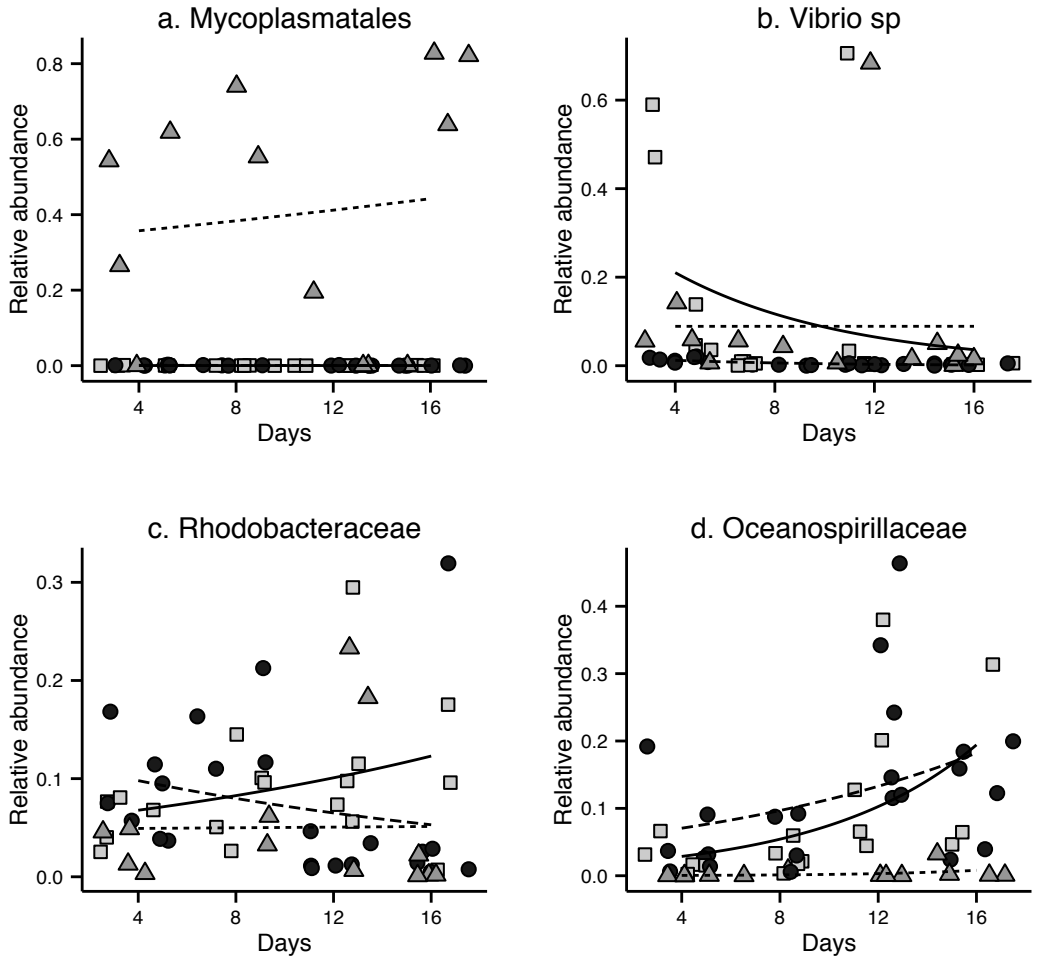

Figure 2.6. Selected examples of temporal changes in microbial relative abundance. Data points represent OTU relative abundance found in carapace (grey squares), gut (dark grey triangles) and water (black circles). Furthermore, lines represent quasibinomial general lineal models as calculated for each type of habitat (carapace with dotted lines, gut with solid lines and water with dashed lines). Titles indicate the lowest taxonomical identification found and were selected to illustrate: a) late colonists in the gut, b) early colonist in the carapace, but constant in the gut, c) early colonist in the gut but late in the carapace and d) late colonists in the carapace and gut.

In our study, both habitats showed three different signs of influence of colonization from the water column. First, shared OTUs between the water column and

crab-habitats represent 93% of species within the carapace and 96.2% within the gut. For example, members of Alteromonadaceae, Oceanospirillaceae and Rhodobacteraceae are common free-living bacteria in the ocean and were found within both carapace and gut communities (Dang and Lovell 2000, Jones et al 2007). Second, temporal changes in OTU relative abundance are influenced by water abundance in both habitats (Table S2.1 and S2.2). For example, the relative abundance of *Methylotenera* sp and members of Oceanospirillaceae and Saprospiraceae in the carapace and *Microbulbifer* sp in the gut are influenced by their abundance in the water. Third, the magnitude of species replacement in the carapace and gut suggests sequential addition of species from the species pool (Fig 2.3). This sequential addition is consistent with species replacements happening within the water column, likely driven by species interactions and changes in nutrient levels (Muscarella et al 2014). Although some studies indicate that sequential arrival should result in divergence in community composition (Lockwood et al 1997), in our study habitat filters were more important in determining assembly trajectory.

The shifting pattern in the carapace suggests that species interactions play an important role in driving community assembly in this habitat. In particular, this pattern coincides with the general model of biofilm development (Jackson et al 2001, Jackson 2003). In brief, initial colonization of submerged surfaces rapidly increases richness and results in strong competition for attachment space and attached organic carbon that subsequently decreases richness (Bruhn et al 2005, Costerton et al 1987, Jackson et al 2001, O'Toole and Kolter 1998, Pruzzo et al 2008, Rao et al 2005, Siboni et al 2007). Next, the resulting competitive community secrete polysaccharides and proteins forming a complex matrix that provides three dimensional habitat and additional niches that facilitate the attachment of new recruits from the water column (Branda et al 2005). In contrast to previous studies that focused on richness patterns (Jackson 2003), we find that

patterns of community similarity and species replacements reflect these interactions (Fig 2.2, 2.3). For example, early colonizers of the carapace such as members of Alteromonadaceae and *Vibrio* sp, known for their successful association with chitin surfaces (Pruzzo et al 2008), are replaced by members of Oceanospirillaceae, Rhodobacteraceae and Saprospiraceae, important taxa in mature biofilms (Yoon et al 2012) Fig 2.6). In the gut, there is a weak yet not significant pattern in similarity that indicates that this process may not be as important (Fig 2.2), potentially overridden by the strength of the filtering processes precluding recruitment. However, we did find evidence of species replacements in the gut (Fig 2.3), likely due to the interplay between species interactions and colonization from free-living environmental bacteria. For example, early *Microbulbifer* sp. and members of Rhodobacteraceae, known free-living sediment oxidizers (Kviatkovski and Minz 2015, Templeton et al 2005) Fig 2.6), were replaced by Mycoplasmatales, permanent members of the arthropod gut (Shinzato et al 2005, Yang et al 2005). These replacements likely contribute to early nitrogen and methane metabolism and later peptidase and glycerol-phospholipid activity detected from 16S sequences (data not shown). Although we lack direct measurements of species interactions, it is clear that species interactions at the local scale can produce distinct assembly trajectories (Kraft et al 2015, Kreyling et al 2011, Ottosson et al 2014). Species interactions have been at the center of ecological theory for filtering unfit species (Macarthur and Levins 1967) but also for facilitating survival of other poor competitors, through direct interaction or by modifying surrounding environmental conditions (Connell and Slatyer 1977). However, this biotic aspect of filtering and its temporal shifting has yet to be fully integrated with environmental filtering (Kraft et al 2015), an important step in understanding the temporal scales of filtering and stochastic processes.

In the wild, carapace and gut undergo complete renewal upon crab molting resulting in reassembly of the associated microbial communities (Becker and Wahl 1996, Givens et al 2013, Middlemiss et al 2015, Trevisan et al 2014). Instead, our study focused on post-disturbance community assembly. Some of the successional patterns we observed may be driven by surviving species, untouched by our disturbance treatment. Although we did not quantify this effect, we found that we were unable to detect bacterial DNA sufficient for sequencing after our disturbance treatments. Therefore, we consider these procedures sufficient disturbance to reduce the bacterial load significantly and promote re-assembly. Even if this re-assembly is representative to post-molt assembly in the wild, crabs undergo molting at different stages resulting in asynchronous crab-associated bacterial communities. This scenario reflects other systems undergoing periodic disturbance and increases variability in community composition relative to synchronous metacommunities (Feio et al 2015), unless its frequency and strength have the effect of harsh conditions and result in community convergence (Hawkins et al 2015, Lepori and Malmqvist 2009).

Other studies focusing on ecological processes driving convergence and divergence provide evidence on the parameters defining assembly trajectory. In controlled environments, microcosm experiments have shown that factors increasing the influence of stochastic assembly are invasion frequency, and sequence of arrival (Drake et al 1993, Grover and Lawton 1994). This line studies has also revealed some of the conditions necessary for alternative stable states, alternative transient states and fluctuating equilibirium (Robinson and Edgemon 1988, Schroder et al 2005). Although these studies have provided fundamental understanding of these factor's isolated effects, they are based on arbitrarily defined colonization levels, which is likely unrepresentative of natural communities and the effects of natural colonization. In contrast, field studies

on community assembly have found evidence of convergence, divergence, and mixed patterns (del Moral 2009, Matthews and Spyreas 2010, Prach et al 2014, Suter et al 2010, Wassenaar et al 2005). Yet these often lack control over environmental factors, which may underlie changes in community composition (Leps and Rejmanek 1991). Our study falls somewhere in between, were we control for changes in salinity and temperature but we allow for natural colonization of host habitats. Our findings highlight the effect of continuous colonization and complex habitat filters on the temporal scale of community assembly (Robinson and Edgemon 1988).

The most significant aspect of our study is that our interpretation of assembly patterns depends on the window of observation. Particularly, our understanding of processes governing assembly on the carapace would have differed if the study was only 8 days long. Our study adds to this long standing discussion (Leps and Rejmanek 1991, Levin 1992) by showing that when comparing across habitat filter effects on community assembly, care must be taken when choosing this observation window. In other systems, less drastic differences in habitat filters have weak early influences but are eventually overridden by colonist pool influences (Prach et al 2014). In particular, the complex filters are known to act on multiple traits and thus alter assembly outcome further than filters acting on a single trait (Trisos et al 2014). For host-associated bacterial communities, habitat filters are complex and encompass a variety of filtering mechanisms (Robinson et al 2010). Our findings are informative when determining the relative temporal scale of other systems with complex filters, such as some anthropogenic effects (Eskelinen and Harrison 2015, Hawkins et al 2015). Studies taking into account the temporal scale of assembly are extremely useful in studying the interactions between colonization rate, environmental filtering rate and species interactions. Future studies

should aim at disentangling the temporal aspect of the interactions between these mechanisms.

Chapter 3: Multiple colonist pools interact with host filters in shaping fiddler crab-associated microbial communities

ABSTRACT

To understand the mechanisms maintaining diversity, there is a need to integrate filtering processes at the local scale and colonization from the regional species pool. Traditionally, the regional species pool concept is based on evolutionary processes, overlooking that distinct sources of colonists colonize many real communities. In this study, we identified distinct colonist pools of bacteria in the surface, subsurface and burrow sediment and their contribution to fiddler crab-associated microbial communities. We expected burrow bacteria to influence the carapace during the crab's burrowing behavior while surface bacteria should influence the gut during crab's feeding from the marsh sediment. Using nearest distance to multivariate pool centroid, we found that burrow sediment bacteria influenced carapace communities, while both burrow and surface sediment bacteria influenced gut communities. The similarity between gut communities and burrow bacteria suggests fiddler crabs, in addition to feeding from the surface, can feed from within these refuges. These findings suggest that a single species pool influences carapace communities while the gut is influenced by multiple species pools. To contrast the influence of colonist pools with local habitat filters, we evaluate the importance of host factors in filtering microbial communities using clustering analysis. Host factors such as sex and species contributed to community structure in the carapace but not in the gut. It remains unclear if community composition in the gut is driven by conserved filters, equal across crab species and sexes, or if the influence from multiple colonist pools interacts with host factors in generating variation in community composition. These findings highlight the interaction between influences from the

colonist pools and local filters in shaping local communities. Importantly, our results suggest that recognizing multiple species pools may expand our understanding of processes driving community structure and diversity.

INTRODUCTION

To identify the mechanisms that control biodiversity, it is fundamental to understand the processes shaping community composition. During all stages of community assembly, colonization from the regional species pool can influence community composition (Freestone and Osman 2011, Fukami 2004, Kraft et al 2011, Ricklefs 1987, Shipley et al 2012). This regional influence was recognized early and popularized when the theory of Island Biogeography described how island species diversity strongly depends on immigration from a mainland species pool (MacArthur and Wilson 1967). Recently, studies on regional pool influences have emphasized how species pools size and composition can regulate local richness (Karger et al 2015, Myers and Harms 2011), influence species composition (Lessard et al 2012), trait distribution (Lessard et al 2012), and ecosystem function (Belmaker and Jetz 2012). These studies highlight the important role of regional influences in understanding disturbance prone communities (Belote et al 2009, Collins et al 2002, Heino et al 2003, Myers and Harms 2011), communities dominated by rare or transient species (Belmaker et al 2008, White and Hurlbert 2010), dispersal limited localities (Roslin 2001) and neutrally assembled communities (Hubbell 2001). Despite this central role of regional influences, empirical evidence has advanced slowly, mostly due to methodological challenges in delineating an operational definition of the regional species pool (Carstensen et al 2013, Lessard et al 2016, Zobel 2016). Overcoming this methodological constraint is important to understand

the contribution of regional influences to local diversity and its interaction with local scale processes, including species interactions and environmental filtering.

The traditional view of the regional species pool emphasizes the role of historical effects, including evolutionary and biogeographical processes (Cornell and Harrison 2014, Godfray and Lawton 2001, Zobel 2016). According to this historical view, the regional species pool is constant over timescales and spatial scales relevant to community assembly. However, this view of an overarching single regional species pool frequently overlooks the effect of dispersal limitation and large-scale environmental filtering in shaping the set of species that arrives to colonize a locality. Many real communities are colonized by multiple colonist pools, defined as sources of colonists shaped by distinct evolutionary and large-scale filtering processes (Zobel 1992). For example, Graves and Gotelli (1983) suggest that bird communities from individual Caribbean islands can be influenced by colonist pools from the West Indies mainland, South American mainland or from other islands and archipelagos (Graves and Gotelli 1983). Each of these colonist pools will differ in species number and composition according to large-scale environmental filters imposed within each mainland or archipelago area (Poff 1997). At the local scale, communities sharing strong connectivity and similar environmental filters with a certain colonist pool will become more similar in community composition (Lessard et al 2012, Partel 2002, Partel et al 2007, Zobel 1992). Because said colonist pool will have strong consequences at the local scale, recognizing these influences is an important goal for basic ecology and applied fields like conservation and restoration.

To accurately quantify the interaction between colonization and ecological processes shaping communities at the local scale, it is first necessary to identify the relevant colonist pools. Traditional approaches to assess regional influences are

unsuitable to assess multiple colonist pool influences because they assume a single and unchanging regional pool (reviewed by (Carstensen et al 2013, Cornell and Harrison 2014, Grace 2001). One way to infer the influence of distinct species pools is to compare geographically replicated experiments focusing on community assembly, but this approach is costly and logistically complex (Lessard et al 2012). Alternatively, controlled laboratory microcosms could be used to test the relationship between multiple colonist pools and local diversity, yet arbitrary choices in dispersal rates and habitat conditions can cause artificial results that differ from natural systems (Drake et al 1993, Grover and Lawton 1994). A third alternative is to focus on discrete, tractable and numerous microbial communities (Srivastava et al 2004) as found on pitcher plants, (Harvey and Miller 1996, Kneitel and Miller 2003), tree holes, (Srivastava and Lawton 1998), pen shells (Munguia 2004), or host-associated microbial communities (Amend et al 2012, Loudon et al 2014).

Host-associated microbial communities are ideal to identify the species pools that influence local communities and to evaluate the interaction between multiple species pools and host filters. Recent advances in sequencing technology have increased interest in the diversity and function of host-associated microbial communities (Mardis 2008). These studies have shown that distinct habitats, such as the human gut (Costello et al 2012, Ngom-Bru and Barretto 2012) or the skin (Bouslimani et al 2015), represent filters equivalent to environmental conditions that structure species composition in animal and plant communities (Keddy 1992). Despite this attention, the extent to which multiple species pools influence community structure has rarely been specifically investigated. Host- associated microbial communities are exposed to colonization from bacterial pools in the surrounding environment. Here, we focus on fiddler crab-associated microbial

communities. Crab carapaces and guts are composed of chitin (Duneau and Ebert 2012) and colonized from distinct pools of bacteria in the surrounding marsh sediment. Based on these crab's natural history, we expect the crab guts to be colonized by bacteria from the surface sediment as the crab feeds on organic matter, microalgae and bacteria that they scrape off the surface sediment (Dye and Lasiak 1987). In contrast, we expect the carapace to be colonized from the sediment within the "J" shaped burrows used for mating and as refuge from predation and extreme temperatures (Kristensen 2008). Importantly, only some bacteria from the sediment establish viable populations on the crab because of local habitat filters within guts and carapaces (Brosing 2010, Vogt et al 1989, Wang et al 2014). Filtering within the host may depend on species-specific habitat filters (Meziane et al 2002, Meziane and Tsuchiya 2002) or sex-specific activity budgets (Caravello and Cameron 1991). Gut and carapace microbial communities should thus be the result of the interaction between local filters and colonization from the sediment pools.

This study aims to identify the role of multiple species pools and local filters in generating local-scale diversity patterns in fiddler crab-associated communities. Specifically, we aim at identifying relevant colonist pool influences to microbial communities on the crab's carapace and gut. Next, we ask whether host factors, such as species and sex, influence community structure by driving habitat filtering. Integrating multiple colonist pools and local scale filtering aspects of community structure should improve our understanding of processes underlying assembly of fiddler crab-associated communities. Results indicate that, colonist source influences community assembly in the gut while host factors drive community composition in the carapace. Importantly, our

results suggest it is important to recognize the role of multiple species pools influencing community diversity and structure.

METHODS

We conducted our study between June and August 2014, in a salt marsh near Aransas Pass, Texas (27° 53' 13.56" N, -97° 7' 0.07 W). These marshes are co-inhabited by two species of fiddler crabs, *Uca panacea* and *Uca rapax*, ideal for the study of multiple species pools influencing host-associated bacterial communities due to their similar behavior, habitat, diet and immunology (Thurman 1987). All samples were collected from a 150x150 m2 marsh area influenced by Redfish Bay waters and vegetated by black mangrove (*Avicenia germinans*), saltmarsh cordgrass (*Spartina alterniflora*) and woody glasswort (*Salicornia* sp).

Adult *Uca panacea* and *Uca rapax* (18 males and 18 females) were collected by hand and stored in individual sterile containers. To profile potential sources of bacterial colonists, we collected 10 samples of each sediment type with sterile spatulas. Approximately 20 gr of surface, subsurface and burrow sediment were collected on sterile containers. Surface sediment was scraped from the top layer (0 to 1 cm deep). For subsurface samples, we mixed sediment from 7 to 15 cm in depth. Although microbial community composition can vary significantly with depth (Bertics and Ziebis 2009), crabs are exposed to all bacteria over this depth range when burrowing. Likewise, burrow samples were obtained from this depth range but along the surface of fiddler crab burrows. We selected only burrows that extended beyond 15 cm in depth and were wider than 2 cm. Crab and sediment samples were transported to the Marine Science Institute from the University of Texas at Austin for further processing.

Upon arrival to the lab, samples were prepared for DNA extraction. Each sample from the sediment was homogenized and 2 gr were separated for DNA extraction. Crabs were rinsed with sterile deionized water to remove debris and unattached microorganisms. Then, carapaces were swabbed and scraped to profile the surface community. To obtain gut samples, crabs were sacrificed by freezing and dissected. To avoid food bolus interference and to remove unattached bacteria, guts were rinsed with sterile deionized water. All samples were kept in MoBio PowerSoil bead tubes at -80°C and were processed within 2 months of collection. DNA was extracted using the PowerSoil DNA extraction kit (MoBio). Samples with less than 0.1 ng/μL DNA yield were excluded from the study to avoid sample bias. To avoid host DNA amplification, we targeted the V4 hypervariable region of bacterial 16S using the 515F/806R primer pair (Ong et al 2013, Wang and Qian 2009). Sequencing was done at the University of Texas Genome Sequencing and Analysis Facility (GSAF) using the MiSeq Illumina platform.

The resulting sequences were processed using custom bash scripts and QIIME (Caporaso et al 2010) with Greengenes as reference databank (DeSantis et al 2006). OTUs (Operational Taxonomic Units) were defined at the 97% sequence similarity and were picked with an open frame. We removed OTUs assigned to Archaea or unassigned and those found in less than 3 times in less than 1% of the samples.

Data analysis

To evaluate the differences between species pools, we compared the OTU diversity of surface, subsurface and burrow sediments. We used an ANOVA to assess the differences in richness and evenness between the sediment communities. To determine whether these results reflect similar sampling effort across samples, we calculated species accumulation curves. To assess compositional differences, the three types of sediment

communities were assessed with CCA analysis (Legendre and Gallagher 2001). Significance of the differences in composition was assessed with a permutational MANOVA based on the CCA scores (Anderson and Walsh 2013).

We determined sediment pool contributions to each crab-associated community based on community similarity. We used the function predict (Oksanen et al 2015) to find the crab sample scores based on the sediment CCA. Based on these scores, we assigned each crab-associated community to a species pool based on the distance to nearest pool centroid in multivariate CCA space. This approach is useful to estimate similarity between crab and sediment samples without the bias of including all samples in the calculation of the original ordination axis (Petraitis et al 2009). Based on these scores, we calculated a perMANOVA to evaluate if crab-associated communities were significantly different in composition when compared to sediment bacteria.

To determine the role of host factors on community structure, we examined richness, evenness, and community composition. We used ANOVAs to assess the role of host factors and pool influences on richness and evenness. These data were square root transformed to meet parametric assumptions. We used cluster analysis to identify significant groups based on community composition and asked whether these clusters correspond to host-factors or pool assignments. This analysis was done separately on carapaces and guts, to emphasize patterns within habitats. We used a hierarchical clustering analysis with the "ward" method (hclust function, (Murtagh and Legendre 2014) and estimated the significant number of clusters using k-means and the gap statistic (Tibshirani et al 2001).

To identify the OTUs that contribute to the above diversity patterns, we first used SIMPER analysis (Clarke 1993) comparing relative abundance between samples from

burrows, surface, carapaces and guts. Based on the SIMPER analysis, we identified OTUs explaining 80 % of the variation between carapace, gut, surface, subsurface and burrow bacteria. We used Picrust to infer whether these patterns correspond to functional diversity. Picrust is a bioinformatics tool used to identify functional diversity based on 16S sequence abundance (Langille et al 2013). Quality of representative pathways was assessed with NSTI scores (Nearest Sequenced Taxon Index) and we discarded samples with less than 0.15 NSTI score. The relative representation of each pathway within carapace, gut, surface and burrow bacteria was tested with an ANOVA.

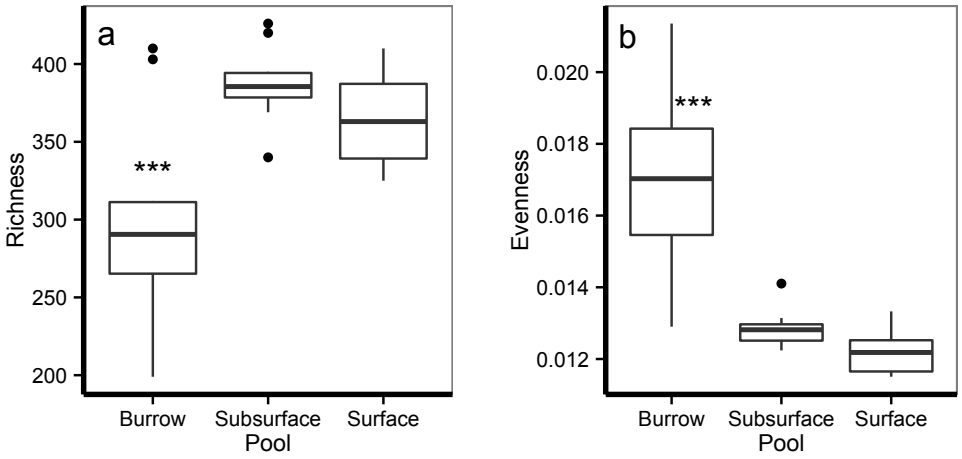

Figure 3.1. Boxplot of OTU richness (a) and evenness (b) in sediment pools. (***) indicates significant differences ($p<0.0001$).

RESULTS

Of the original samples, 65 samples were suitable for analysis. To standardize sampling effort, we discarded samples with low DNA yield (< 0.5 ng/μL) or low read count (< 4000). After accounting for sequence quality and removing low-abundance OTUs, we identified 639 individual OTUs distributed across 17 phyla and 86 families.

Although we had to discard subsurface samples from the Picrust analysis due to low NSTI values, we identified 68 metabolic pathways.

Table 3.1. Summary of ANOVA tests evaluating diversity among sediment bacterial pools (surface, subsurface and burrow).

	d.f.	F-value	p
Richness	2	10.73	0.0003 ***
Evenness	2	22.84	<0.0001***

*** indicates significance of p<0.001

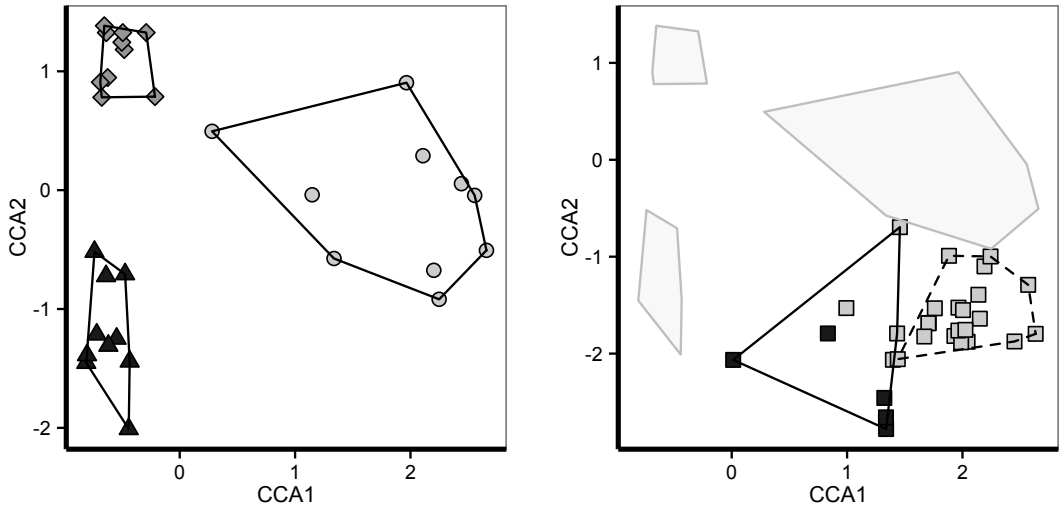

Figure 3.2. CCA of sediment (a) and crab (b) communities. Polygons in (a) indicate samples from different sediment types: surface (black triangles), burrow (light grey circles) and subsurface (dark grey diamonds). Crab community scores in (b) were calculated with the predict function. Grey polygons in (b) show the position of sediment types as reference. Black polygons in (b) indicate distinct crab habitats: gut (solid line), carapace (dashed line). Color in (b) indicates pool assignment for each crab sample: surface (black), or burrow (light grey). No crab samples were assigned to subsurface sediment.

Sediments contained a total of 597 OTUs distributed across three distinct pools differing in richness and composition (Fig 3.1, Table 3.1, Fig 3.2). Burrow sediments had the lowest richness among the pools with only 192 OTUs of which 31 (5.4%) were

unique to the burrow. Surface and subsurface had comparable richness levels with 234 and 228 OTUs respectively. We only found 8 unique OTUs (1.4%) in the subsurface and no unique taxa in the surface. Differences between pools were strongly represented by relative abundance of individual OTUs. For example, burrow communities were more even than in surface or subsurface sediment communities (Fig 3.1, Table 3.1) and the CCA resulted in significant distinctions in OTU composition between sediment types (d.f.= 2, F=183.54, R2=0.92, p=0.01, Fig 3.2). The first two axes of the CCA explained 26.9% of the variation in community composition. Differences in OTU composition between sediment pools was explained by high abundance of Halanaerobiaceae in the surface, high abundance of Bacteroidetes and low abundance of Chloroflexi bacteria in burrow sediments (Fig 3.5, Table S3.1). In contrast, subsurface sediment harbored high abundance of Anaerolinaea and low abundance of Bacteroidetes and Planctomycetes (Fig 3.5, Table S3.1).

Table 3.2. Summary of perMANOVA results on crab samples comparing effect of habitats, host species and host sex.

	d.f.	R2	Pr(>F)
Habitat	1	0.408	0.003 **
Host species	1	0.047	0.053
Host sex	1	0.047	0.054
Habitat*Host species	1	0.018	0.275
Habitat*Host sex	1	0.000	0.970
Host species*Host sex	1	0.058	0.019*
Habitat*Host species*Host sex	1	0.099	0.033*

* indicates significance of p<0.05
** indicates significance of p<0.01

Community composition in the gut and carapace showed significant effects from crab-habitat and interactions between host factors and habitat according to a perMANOVA (Table 3.2). These crab-associated communities were all assigned to

burrow and surface sediment sources, and none to subsurface sediment sources. Carapace communities were all (100%) assigned to burrow communities (Fig 3.2). In contrast, 44 % of the gut-associated communities were assigned to burrow and 56% surface sediments. Crab-associated communities differed from sediment bacteria due to higher relative abundance of Rhodobacteraceae, Saprospiraceae and Vibrionaceae (Fig 3.5, Table S3.1). Carapace communities were characterized by higher Oceanospirillcaeae bacteria. These patterns correspond to decreased carbon fixation and methane metabolism pathways and increased transcription factors, valine and leucine degradation and fatty acid metabolism pathways in crabs, compared to sediment functional profiles (Table S3.1, Fig S3.2).

Cluster analysis showed a contrasting effect of host factors on carapace and gut microbial communities. Gap analysis indicated that carapace communities cluster in 6 significant groups (Fig 3.3). These groups loosely correspond to host factors. For example, the first cluster on the left is composed 80% of males, 80% from *U. rapax*. The two clusters on the far right are composed of females and males, respectively. In contrast, gap analysis indicated only one cluster comprising all gut communities. In other words, neither host factors nor pool assignments were associated with significant clusters in the gut. We did not find any significant relationships between host factors on richness or evenness (Fig 3.4, Table 3.3).

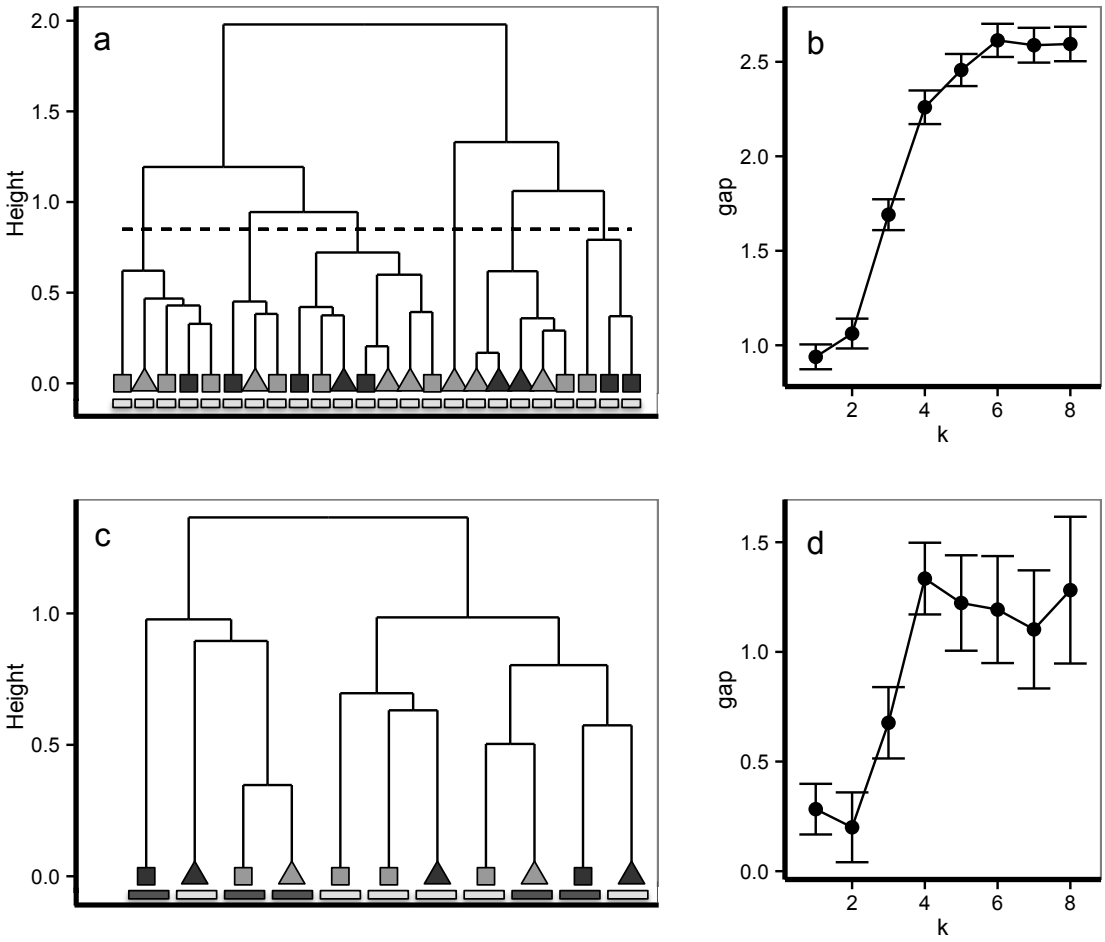

Figure 3.3. Cluster analysis (a, c) and gap statistic calculation results (b, d) for carapace (a,b) and gut (c, d) samples. Height in the y axis represents the distance at which the cluster was formed. Dashed lines show the clustering threshold indicating which sample groups represent significant clusters. Color indicates host species with *U. rapax* in black and *U. panacea* in grey. Shapes indicate host sex: females (triangles), and males (squares). Rectangles at the bottom indicate pool assignment with surface in dark grey and burrow sediment in light grey. The gap statistic plot indicates the gap statistic for different number of clusters (k).

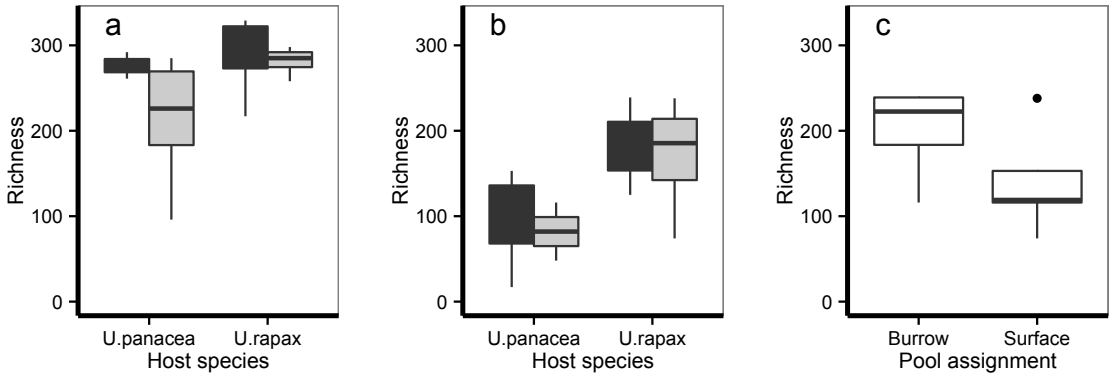

Figure 3.4. Boxplot of OTU richness in carapace (a), and gut samples (b) as well as gut sample richness according to assignment (c). Coloration in panels a and b represent females (dark grey) and males (light grey).

DISCUSSION

Determining the contribution of local environmental conditions, species interactions and regional diversity to community assembly is critical to understand patterns of community structure and diversity (Cornell and Harrison 2014, White and Hurlbert 2010). To accurately quantify the interaction between colonization and ecological filtering shaping communities, it is first necessary to identify the relevant colonist pools. Our results indicate that the influence of multiple species pools is habitat dependent. Specifically, we found that carapace communities were consistently more similar to burrow bacteria than to other sources of sediment bacteria, suggesting that burrow bacteria are the main source of colonists for carapace communities. In contrast, half of gut communities were similar to the surface, but the other half, contrary to our expectations, were similar to burrow sediment bacteria (Fig 3.2). These results suggest that surface and burrow bacteria influence gut communities.

Table 3.3. Summary of ANOVA tests evaluating diversity differences among crab bacterial communities. We include habitat (carapace or gut), host species (*U. panacea* or *U. rapax*) and host sex as factors explaining OTU richness and evenness. Richness and evenness data were square root transformed to meet parametric assumptions.

		d.f.	F-value	p
Richness	Species	1	11.281	0.002 **
	Sex	1	0.196	0.661
	Habitat	1	34.312	<0.001 ***
	Species*Sex	1	0.316	0.579
	Species*Habitat	1	2.386	0.134
	Sex*Habitat	1	0.148	0.703
	Species*Sex*Habitat	1	0.397	0.534
Evenness	Species	1	4.773	0.038 *
	Sex	1	0.264	0.612
	Habitat	1	14.120	<0.001 ***
	Species*Sex	1	0.025	0.875
	Species*Habitat	1	2.706	0.112
	Sex*Habitat	1	0.124	0.727
	Species*Sex*Habitat	1	0.528	0.474

* indicates significance of p<0.05
** indicates significance of p<0.01
*** indicates significance of p<0.001

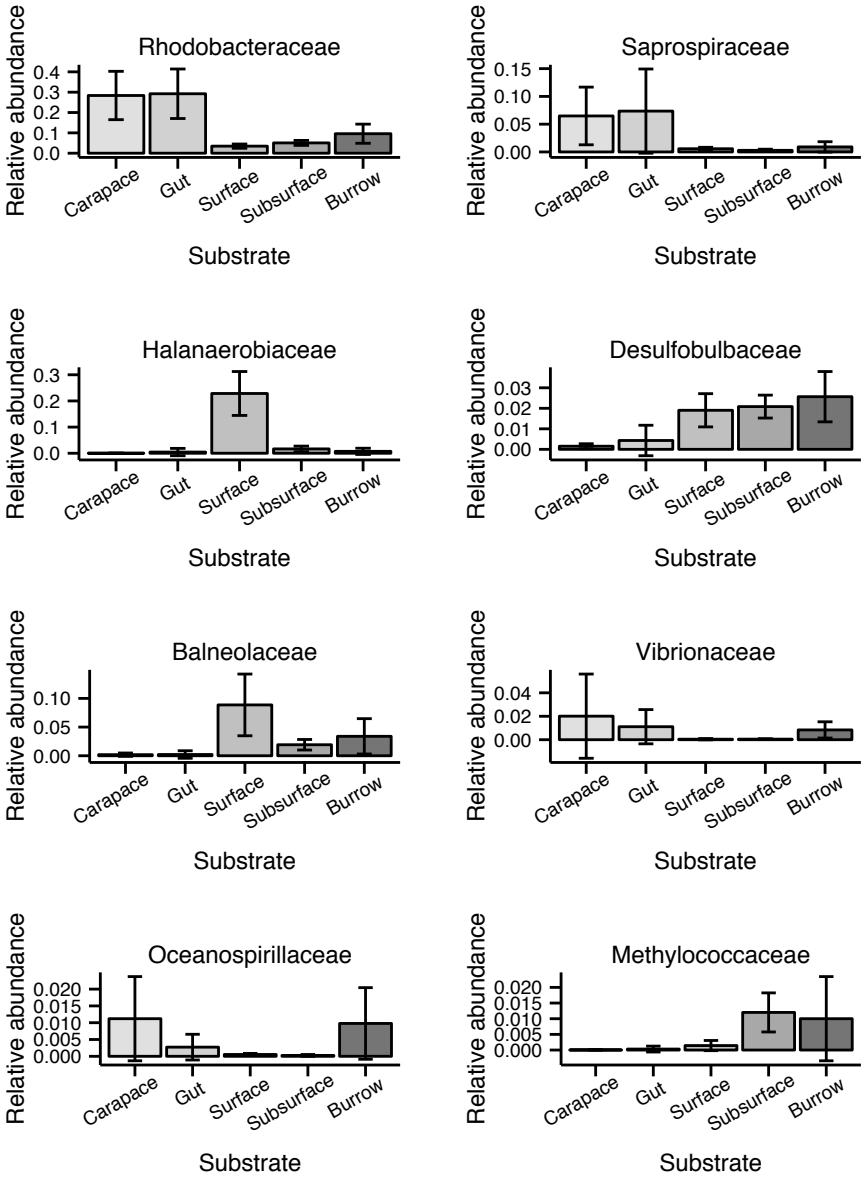

Figure 3.5. Differences in average relative abundance of taxa on carapace, gut, surface and burrow bacteria. Error bars represent standard deviation. Bar color indicate the different bacterial substrates with crab communities in lighter grey and sediment bacteria in darker grey.

Many studies have addressed the impact of colonization on local diversity. Although providing fundamental contributions to our understanding of broad spatial and

temporal scaled processes impacting ecology, the large majority of these studies focus on the traditional evolutionary definition of the regional species pool (Bengtsson et al 1997, Knapp et al 1993, Mora et al 2003). Our findings suggest that distinct colonist pools at small spatial scales can influence local communities. Few studies have addressed colonist pools but they differ substantially from our study. Some studies focusing on plant communities define colonist pools within the same region according to their evolutionary history. In these scenarios, local richness is higher when local conditions match the evolutionary history of the region, such as pH or nutrient availability (Grace 2001, Grace et al 2011, Partel 2002, Partel et al 2007, Zobel 1992). Another group of studies highlights colonist pools with distinct dispersal strategies. For example, plant seeds dispersing in space have stronger influence during community succession after strong disturbances, while seed bank species dispersing in time are more important for community development after small disturbances (Bekker et al 2000, Eskelinen and Virtanen 2005, Kalamees and Zobel 1998, Willems and Bik 1998). However, these studies rarely integrate the joint effect of multiple colonist pools. Our study provides evidence of multiple colonist pools influencing fiddler crab associated communities and suggests that pools shaped by different processes can jointly influence local assembly. Importantly, identifying and tracking changes in each colonist pool may improve our understanding of the interactions between distinct habitat filters and colonist pools.

Processes shaping colonist pools in the sediment differ noticeably from crab-associated habitat filters. The surface, subsurface and burrow were distinct bacterial pools (Fig 3.1, 3.2) concurrent with the expected responses of microbial diversity to these habitats' environmental conditions. Of all the bacteria available in the marsh, the subsets of taxa adapted to survive at the salinity, nutrient availability (Ikenaga et al 2010),

biogeochemistry (Bertics and Ziebis 2009) and bioturbation (Fanjul et al 2015) levels of surface, subsurface and burrow sediments constitute the colonist pool that interacts with the crab's carapace and guts (Fig 5). Other studies have developed the concept of hierarchical filters at different scales, shaping the colonist pool. In stream habitats, environmental filters at the basin and catchment scale shape colonist pools influencing fish and invertebrate communities (Frissell et al 1986, Poff 1997). If colonist pools result from a combination of regional evolutionary and biogeographical forces and filtering at various scales (Cornell and Harrison 2014, Poff 1997, Zobel 2016), multiple colonist pools are likely to appear where distinct filters or evolutionary histories operate on a landscape. Nonetheless, their effect at the local scale depends on how the colonist pool interacts with filters at the local scale (Partel 2002, Zobel 1992).

We found that local, host habitats structure microbial communities associated with the fiddler crabs. Furthermore, host factors structure variability among carapace communities (Fig 3.3a). Consistent with microbiome studies (Smith et al 2015), this result suggests that behavior, immunology and physiology differences associated with crab species and sexual differentiation constitute important filters for bacterial colonization of the carapace. Our results indicate that cluster analysis informs of local processes shaping communities under a single species pool. Although previous work suggested clustering analysis could be used to infer distinct species pools (Carstensen et al 2013), our findings suggest that this method should be used with care when inferring mechanistic explanations of community structure.

Surprisingly, we did not detect any effects of host-factors among gut bacterial communities (Fig 3.3b). Given the gut's important role in digestion and its strong physical and chemical filters (Vogt et al 1989, Wang et al 2014), this result may suggest

that the gut's habitat filter is conserved across fiddler crab sexes and species. Alternatively, this lack of clustering for gut samples can result from the combined effects of multiple species pools and habitat filtering. We didn't find richness or diversity effects on gut communities (Fig 3.4) that would reflect differences in diversity between burrow and surface communities (Fig 3.1). To unravel the interaction between local filters, burrow and surface bacteria on gut communities may require a manipulative approach that reveals the degree of connectivity and overall fit between species traits in the pool and local filters (Zobel 1992, Chapter 4).

Our method uses assignments based on similarity to determine species pool influences. A major limitation of this method is the absence of a direct measure of dispersal, which results in two main issues. First, other sources of colonists may influence fiddler crab-associated communities. Nonetheless, in comparison with tidal seawater, plants, or conspecific interactions, marsh sediment has a stronger relationship with crab-associated habitats suggesting these are the major contributors to colonization. Second, assuming that similarity indicates major contribution neglects the possibility of important colonists recruiting from a species pool that differs in composition. Even with these limitations, the method used here allows us to identify likely sources of colonists from the host's surrounding environment with no previous knowledge from species lists or functional traits.

Our study highlights the importance of developing a species pool definition that embraces multiple colonist pools influencing local communities. These findings add to the work that calls for a standardized method to define colonist pools that make these studies comparable. Our contribution highlights habitat-specific colonization from distinct species pools. Importantly, communities within the same habitat type may

receive colonists from different colonist pools, or jointly, from several species pools. If this represents an important trend, studying the relationship between species pools and local community structure should integrate the mixing of distinct pools (Livingston et al 2013, Rillig et al 2015), differential dispersal rates (Munguia 2015) and habitat filters (Kraft et al 2015). Widening the regional species pool framework to include contribution from different forms of dispersal and distinct colonist pools will improve our understanding of regional contributions to community diversity and structure.

Chapter 4: The relative contribution of multiple colonists pools is regulated by habitat filters in fiddler crab-associated microbial communities

ABSTRACT

Community structure can be influenced by multiple sources of colonists shaped by different large-scale ecological and evolutionary processes. The consequences of multiple colonist pools for local community diversity should depend on the relative contribution of each colonist pool and the strength of the local habitat filter. To identify the contribution of distinct colonist pools of bacteria to fiddler crab-associated microbial communities, we manipulated bacterial pools in surface and subsurface sediments and compared the community-level responses of carapace and gut microbial assemblages. We combined normal and sterilized surface and subsurface sediment in a factorial mesocosm experiment. After four days of crabs feeding from and burrowing in the sediment, we obtained samples of carapace, gut, surface sediment and burrow sediment and performed next generation sequencing on the 16S ribosomal region. We evaluate two aspects of colonist pools influences on local communities: (1) community-level response to colonist pool manipulation and (2) similarity in community composition between local communities and colonist pools. Compared to carapace-associated communities in mesocosms with normal sediment, composition changed and became less similar between one carapace and the other in mesocosms with modified surface and subsurface sediments. In contrast, the composition of gut-associated microbial communities showed no response to bacterial pool manipulation. In mesocosms with normal sediment, carapace and gut community composition was more similar to burrow than to surface sediment bacteria. We conclude that burrow sediment bacteria influences carapace and gut communities. However, surface bacteria also influenced carapace communities

suggesting this secondary pool provides key colonists that contribute to community assembly. In contrast, limited response of gut communities suggests strong filters characterize this habitat. These findings provide a first step to understand how multiple colonist pools influence community assembly and to incorporate dynamic and multiple species pools into the contemporary concept of species pools.

INTRODUCTION

A central goal of community ecology is to understand how patterns in diversity result from interactions between local processes like resource availability, abiotic conditions and species interactions, and large-scale processes like regional evolutionary and biogeographical history (Ricklefs 1987, Zobel 1997). In an effort to describe the influence of regional and historical processes on local diversity, the species pool concept is defined as the set of all species available to colonize local communities within a region (Cornell and Harrison 2014, Taylor et al 1990, Zobel 2016). Local community deviations in terms of richness and composition from regional species pool can be interpreted as local processes operating as filters and only species with traits required for survival are able to colonize and recruit (Chase and Myers 2011). Despite the potential usefulness of the concept, a consensus on a clear definition and use of the species pool is missing (Lessard et al 2016, Zobel 2016). A potential reason behind this disagreement is the underlying assumption that the species pool is a static entity (Cornell and Harrison 2014). Evidence shows that species pools can vary along gradients in latitude, altitude, heterogeneity and abiotic conditions (Freestone and Osman 2011, Graves and Rahbek 2005, Kraft et al 2011, Lessard et al 2012, Myers et al 2013, Partel 2002, Pither and Aarssen 2005, Tello et al 2015), as well as due to contemporary evolution (Fukami et al 2007, Fukami 2015, Loeuille and Leibold 2008, Pantel et al 2015, Terhorst et al 2010). A

definition of a dynamic species spool may contribute to a complete integration in our understanding of local and regional aspects of community ecology.

A useful approach proposed by Frissell (1990) and later developed by Poff (1997), recognizes that, of all the species available for colonization in a region, only a subset of colonists arrives at each location. This framework views the colonization process as a series of filters at different scales (Frissell et al 1986, Poff 1997). Environmental conditions characteristic of each level represent filters, which species with traits matching the environment can pass (Leibold 1995, Tonn et al 1990). For example, when describing stream communities, large-scale filters operate at the watershed level, while lower level filters will define each catchment and pool (Poff 1997). Ultimately, the colonist pool that interacts with the local-scale depends on the traits that have been filtered at higher scales. Although this approach seems straightforward in the context of stream systems, local communities can also be exposed to colonist pools shaped by distinct large-scale filters. For example, Gotelli and Graves (1983) suggested that individual Caribbean islands could be influenced by colonist pools from the West Indies mainland, South American mainland or from other islands and archipelagos (Graves and Gotelli 1983). Each of these colonist pools will differ in species number and composition according to large-scale environmental filters imposed within each mainland or archipelago area (Poff 1997). Defined as sources of colonists shaped by distinct evolutionary and large-scale filtering processes (Zobel 1992), multiple colonist pools could influence many natural communities such as plant communities resulting from an interaction between seed rain and seed banks (Bekker et al 2000, Eskelinen and Virtanen 2005, Kalamees and Zobel 1998, Willems and Bik 1998) and multiple sources of bacteria colonizing host-associated microbial communities (Chapter 3). Because each pool can

have strong consequences on diversity and structure at the local scale, recognizing multiple colonist pool influences is an important goal for basic ecology and applied fields like conservation and restoration.

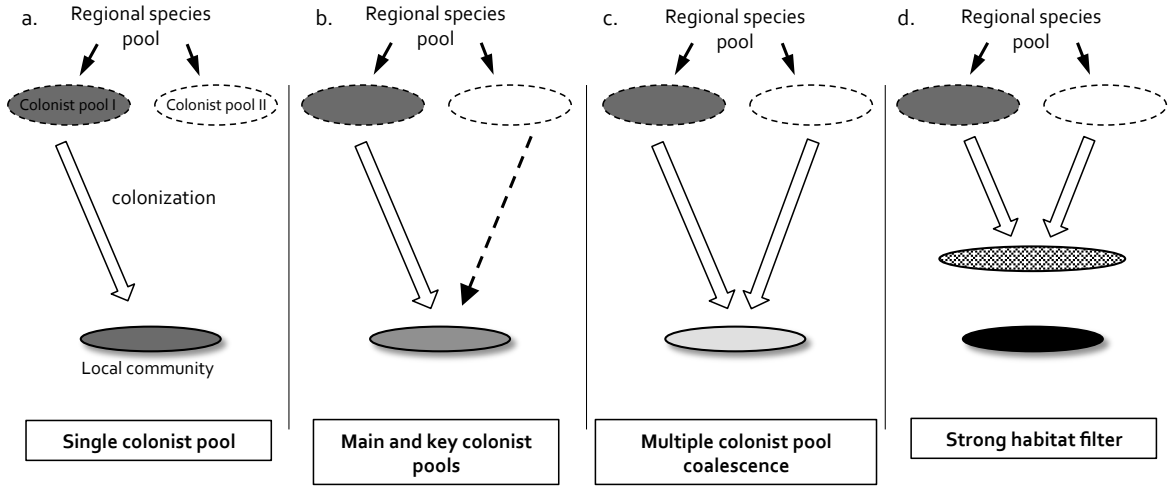

Figure 4.1. Conceptual model of multiple colonist pool influences on local communities. Regional species pools, shaped by evolutionary and biogeographical processes, are filtered at large scales, resulting in 2 distinct colonist pools. Differences in composition between colonist pools are represented by distinct colors (dark grey and white). Colonization is illustrated with arrows connecting the colonist pools and the local community. Colonization rate and frequency are represented by the arrow type with white, thick arrows denoting frequent and abundant colonization and black, dashed arrows denoting weak or infrequent colonization. Coloration at the local scale indicates community composition and its similarity with each colonist pool. A strong filter is represented in (d) by a sieve blocking colonization. Each scenario describes one of the four types of multiple colonist pool influence.

The effect of colonization from a regional species pool depends on the frequency of arrival and how the colonist pool interacts with filters at the local scale (Partel 2002, Zobel 1992). In addition to arrival frequency and habitat filters, consequences of multiple colonist pools on local community assembly depend on each pool's degree of contribution. In this study, we focus in four alternative scenarios based on (1) the effect on local communities of manipulating colonist pools, and (2) the similarity between

colonist pools and local communities (Fig 4.1). First, if dispersal limitation or strong habitat filtering constrain colonization of other colonist pools, a single pool will influence local communities even if multiple sources of colonist co-occur (Fig 4.1a). In this simple scenario, once the relevant colonist pool has been identified, evaluating the influence of species pools can proceed with traditional methods (Carstensen et al 2013, Chase and Myers 2011). Second, if two colonist pools influence community assembly but their relative contribution is unequal, a main species pool will supply most colonists and an accessory species pool will supply few, but key colonists (Fig 4.1b). This scenario resembles the keystone species concept (Paine 1995) where a species causes a disproportionate effect on the community's diversity given its low abundance. A colonist pool that contributes few species that are fundamental for community assembly and radically impact local diversity is a key colonist pool. Third, if two or more colonist pools jointly contribute colonists for assembly, the resulting local community will be a mixture of the two sources shaped by habitat filters and species interactions (Fig 4.1c). Predicting the outcome of community assembly in this case depends on the similarity between the large scale and the local filters, the temporal dynamics of colonization and the relative contribution of each pool (Fukami 2015, Rillig et al 2015). Fourth, under strong local habitat filters, local assemblages are expected to maintain the same composition and structure regardless of the source of colonists (as long as the main components are present, Fig 4.1d). Although these may not represent all possible relationships between multiple species pools and local communities, we focus on these in an attempt to investigate the colonization of fiddler crab gut and carapace microbial communities from distinct colonist pools within the marsh sediment.

Fiddler crab-associated microbial communities are colonized by bacteria from distinct colonist pools of bacteria in the surface and burrow marsh sediment (Chapter 3). These bacterial pools in the sediment are amenable for experimental manipulation (Cuellar-Gempeler and Munguia 2013), ideal to identify their relationship with local, crab-associated communities. In addition, crab carapace and gut represent distinct microbial habitats. While the carapace is open to colonization, the gut represents a strong, selective filter (Chapter 2, Brosing 2010, Vogt et al 1989, Wang et al 2014). Furthermore, in a prior study, it was found that burrow bacteria influenced the crab's carapace while both surface and burrow sediment were influential for the gut (Chapter 3). However, these findings were based exclusively on compositional similarity between local communities and colonist pools.

Our objective in this study is to evaluate the relative contribution of multiple colonist pools to local community composition. To distinguish between the four proposed scenarios of multiple colonist pool influence on fiddler crab-associated microbial communities, we combined normal and sterilized surface and subsurface sediment in a factorial mesocosm experiment. We evaluated (1) local community responses to colonist pools manipulation, and (2) similarity in composition between colonist pools and local communities. Based on prior findings and on fiddler crab natural history, we hypothesize that carapace communities will be influenced by a single colonist pool (Fig 4.1a). Therefore, carapaces should remain similar to burrow sediment bacteria and respond to burrow but not to surface sediment sterilization. In contrast, gut communities are expected to respond to both sterilization procedures and to either be the result of the interaction between the main and a key colonist pool (Fig 4.1b) or of colonist pool coalescence (Fig 4.1c). Alternatively, the strong filter in the gut could result in

communities that remain mostly unaffected by the colonist pool treatments (Fig 4.1d). Our results conflicted with our expectations for carapace and gut microbial communities. Carapace assemblages responded to pool manipulations in the surface and burrows while gut assemblages were robust to the treatments. These findings highlight the importance of the habitat filter in regulating the influence of multiple colonist pools.

METHODS

Adult female fiddler crabs (*Uca panacea*) and sediment were collected from a 150 x150 m2 salt marsh area near Aransas Pass, Texas (27° 53' 13.56" N, -97° 7' 0.07 W) in August 2014. Crabs were taken to the Marine Science Institute at the University of Texas were they were kept in a holding tank under flowing seawater. The tanks contained sand from the collection site and were inclined such that one-quarter of the tank was submerged. Crabs were fed every two days with sterile shrimp pellets (Brine Shrimp Direct) and were added to the mesocosms 2 weeks after collection. We collected sediment samples from the same marsh area for the mesocosm the before the start of the mesocosm experiment. Sediment was collected from the surface (0 to 2cm deep) and subsurface (5 to 15 cm deep) of the marsh and stored in sterile autoclave bags for transport to the laboratory. We aimed at maintaining the distinct pools from the marsh for our mesocosm experiment.

Experimental design

We used a mesocosm where we experimentally removed bacterial pools from the sediment to determine the influence of surface and burrow bacterial pools on fiddler crab-associated microbial communities. To replicate these distinct bacterial pools, each mesocosm consisted of a tank (30.2 cm length, 19.8 cm width, and 20.5 cm depth) filled

with a 7 cm deep subsurface sediment layer and a 2 cm deep surface sediment layer. The sterile surface treatment (-\+) had a subsurface layer of normal sediment while the surface sediment layer was sterile. The sterile subsurface treatment (+\-) had a sterile subsurface layer and a normal surface layer. In addition, we had a sterile control treatment (-\-) with sterile surface and subsurface, and a normal control treatment, with normal sediment layers (+\+). Fifteen tanks were set up for each treatment for a total of 60 mesocosms.

Although we manipulate subsurface sediment directly, we focus instead on the influence of burrow sediment bacteria, because these have distinct bacterial assemblages and direct influence on crab-associated communities (Chapter 3). Burrow sediment bacteria result from crab burrowing on experimental subsurface sediment.

Bacteria were removed from the species pool by autoclaving with a 20 min, 121°C solid cycle. The autoclave procedure consisted on laying a shallow layer (3cm) of sediment within an autoclave bag and onto a metal sheet. Each set of sediment was autoclaved 3 times, and mixed thoroughly in between cycles. This procedure improves the sterilization of sediments and soil (Trevors 1996).

Prior to the beginning of the experiment, crabs were washed with sterile deionized water, swabbed with alcohol (95%) and bleach (90%) and isolated in a sterile container for 48 hours to reduce their initial bacterial load in the carapace and gut. To evaluate these cleaning procedures, we sampled crab carapaces and guts from freshly collected specimens and post-cleaning specimens. Although this cleaning procedure may not eliminate all bacteria, this disturbance is sufficient to induce colonization from the sediment and re-assembly of crab microbial communities. After the cleaning procedure, we were unable to recover sufficient bacterial DNA for sequencing from crab carapaces or guts.

Crabs were allowed to burrow and interact with the sediment for 4 days. Daily, we added 10 mL sterile water to the surface and bottom of the mesocosm. This procedure enhances crab feeding (Dye and Lasiak 1987, Robertson and Newell 1982) and reduces mortality. To add water to the bottom of the mesocosm, we placed a sterile rubber tube (0.5 cm diameter) on one corner of each tank.

Samples were taken after 4 days. Crabs were rinsed with sterile deionized water to remove unattached bacteria. Crabs were swabbed and scraped to obtain carapace bacterial communities. Then they were sacrificed by freezing and dissected under sterile conditions to obtain gut samples. To avoid food bolus interference and to remove unattached bacteria, guts were rinsed with sterile deionized water. Sediment samples consisted of 5 gr of sediment from the surface or burrow sediment. This sample was homogenized and 2 gr were used for DNA extraction.

All samples were kept in MoBio PowerSoil bead tubes at -80°C and were processed within a month of collection. DNA was extracted using the PowerSoil DNA extraction kit (MoBio). Samples with less than 0.1 ng/μL DNA yield were excluded from the study to avoid sample bias. To avoid host DNA amplification, we targeted the V4 hypervariable region of bacterial 16S using the 515F/806R primer pair (Ong et al 2013, Wang and Qian 2009). Sequencing was done at the University of Texas Genome Sequencing and Analysis Facility (GSAF) using the MiSeq Illumina platform.

The resulting sequences were processed using custom bash scripts and QIIME (Caporaso et al 2010) with Greengenes as reference databank (DeSantis et al 2006). OTUs (Operationa Taxonomical Units) were defined at the 97% sequence similarity and were picked with an open frame. We removed OTUs assigned to Archaea or unassigned and those found in less than 3 times in less than 1% of the samples.

Data analysis

To determine the influences of multiple species pools on crab carapace and gut communities we evaluated the effect of the sterilization treatments on the sediment species pools and on crab-associated microbial communities. First, we evaluate the treatment effects on diversity and composition of the sediment and then on the crab-associated communities. Lastly, to determine the similarity between sediment pools and crab-associated assemblages, we calculated the multivariate distance from each crab-associated community to the sediment community within its own experimental mesocosm tank.

To determine the effect of the sterilization treatments on sediment bacteria, we evaluated diversity in the 2 layers of sediment throughout the treatments. OTU richness was evaluated with a two-way ANOVA. Community composition effects of our treatments were assessed with an RDA on Hellinger transformed data (Legendre and Gallagher) and evaluated with a perMANOVA on RDA scores (Anderson et al 2006).

To evaluate the effect of our treatments on crab-associated communities, we calculated richness and compositional effects. We used a two-way ANOVA to estimate the effect of the sterilization treatments on OTU richness of crab-associated communities. We used the function predict (Oksanen et al 2015) on crab-associated communities Hellinger transformed data to find crab sample ordination scores based on the sediment RDA. We used a perMANOVA on the scores resulting from the predict function to determine effects of sterilization treatments on OTU composition in crab communities. To tease out these results we looked into three response variables - effects along the first and second RDA axis and community similarity effect. We assessed effects along the RDA axis with two-way ANOVAs and the similarity effect with a multivariate

homogeneity of group dispersions test (Anderson et al 2006) on Hellinger transformed OTU relative abundances.

We calculated the distance from each crab-associated community to the sediment bacterial pools for each mesocosm tank. We tested the effect of he sterilization treatments on carapace and gut communities with two-way ANOVA.

We compared patterns in OTU relative abundance using SIMPER analysis (Clarke 1993) to identify taxa contributing to differences between carapace, gut, surface, subsurface and burrow bacteria. We show OTU explaining 90% of the variation between these substrates.

Analyses in this section were performed using R statistical environment. Most of the analyses were performed using functions contained in the packages phyloseq (McMurdie and Holmes 2013), vegan (Oksanen et al 2015) and mvabund (Wang et al 2012) as well as custom scripts.

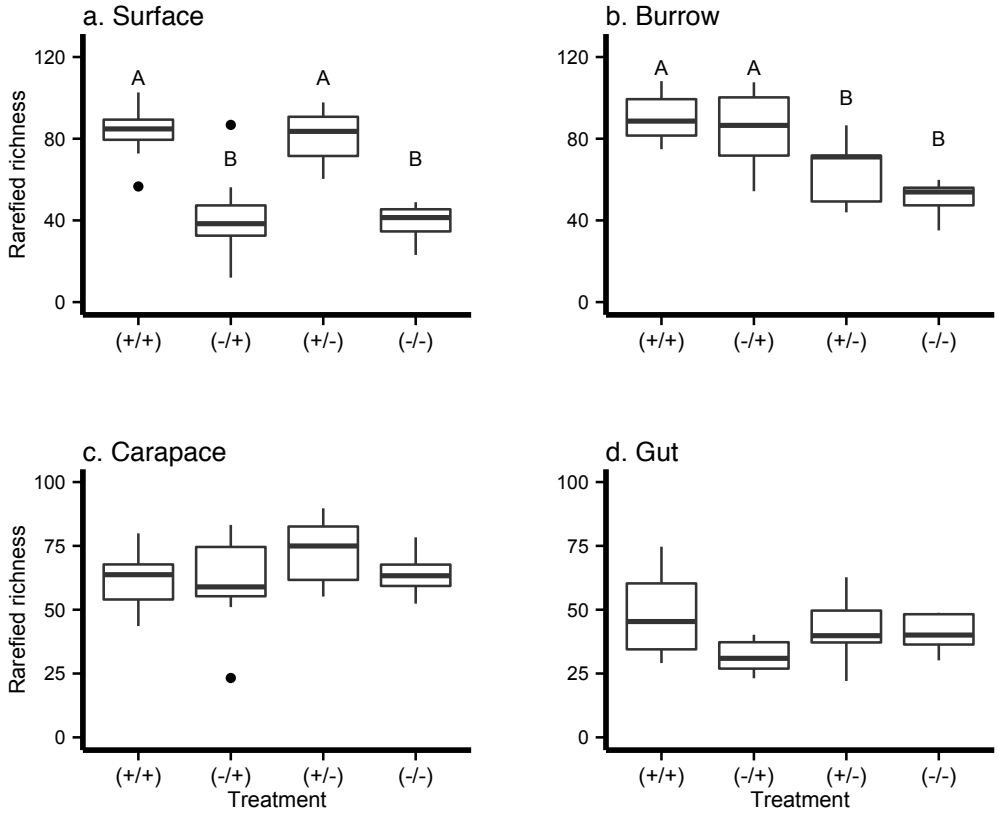

Figure 4.2. Boxplot of rarefied richness effects of treatments for sediment pools (a, b) and crab-associated communities (c, d). Letters denote significant differences between groups as indicated by a Tukey HSD test.

Table 4.1. Summary of results from a two-way ANOVA assessing the effects of the sterilization treatments on sediment (surface and burrow) and crab-associated (carapace and gut) substrates rarefied richness.

	d.f.	F	p
Treatment	3	13.425	<0.001 ***
Substrate	3	29.878	<0.001 ***
Treatment*Substrate	9	7.221	<0.001 ***

*** indicates significance of p<0.001

RESULTS

We obtained 2593±1314 reads per sample for a total of 350054 DNA reads distributed across 135 samples that were suitable for analysis. Samples were discarded because of crab mortality (43.75% of total samples), or low DNA yield (less than 0.1 ng/μL). After accounting for sequence quality and removing low-abundance OTUs, we identified 311 individual OTUs distributed across 12 phyla and 50 families.

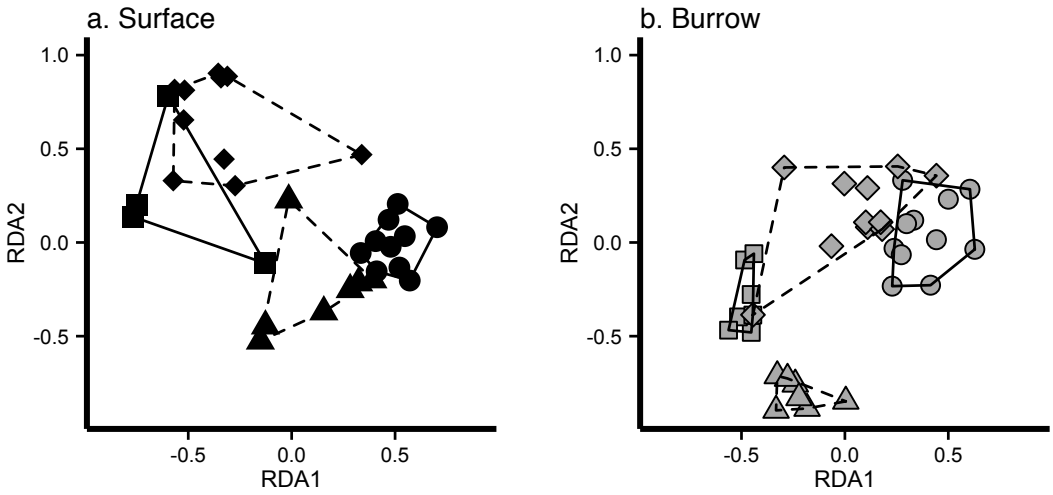

Figure 4.3. RDA of OTU composition responses to treatments in the sediment. Effects of sterile surface (-/+, diamonds), sterile subsurface (+/-, triangles), sterile control (-/-, squares) and normal control (+/+, circles) are shown for surface (a, black) and burrow (b, grey). RDA scores for surface and burrow are presented separately for better visualization.

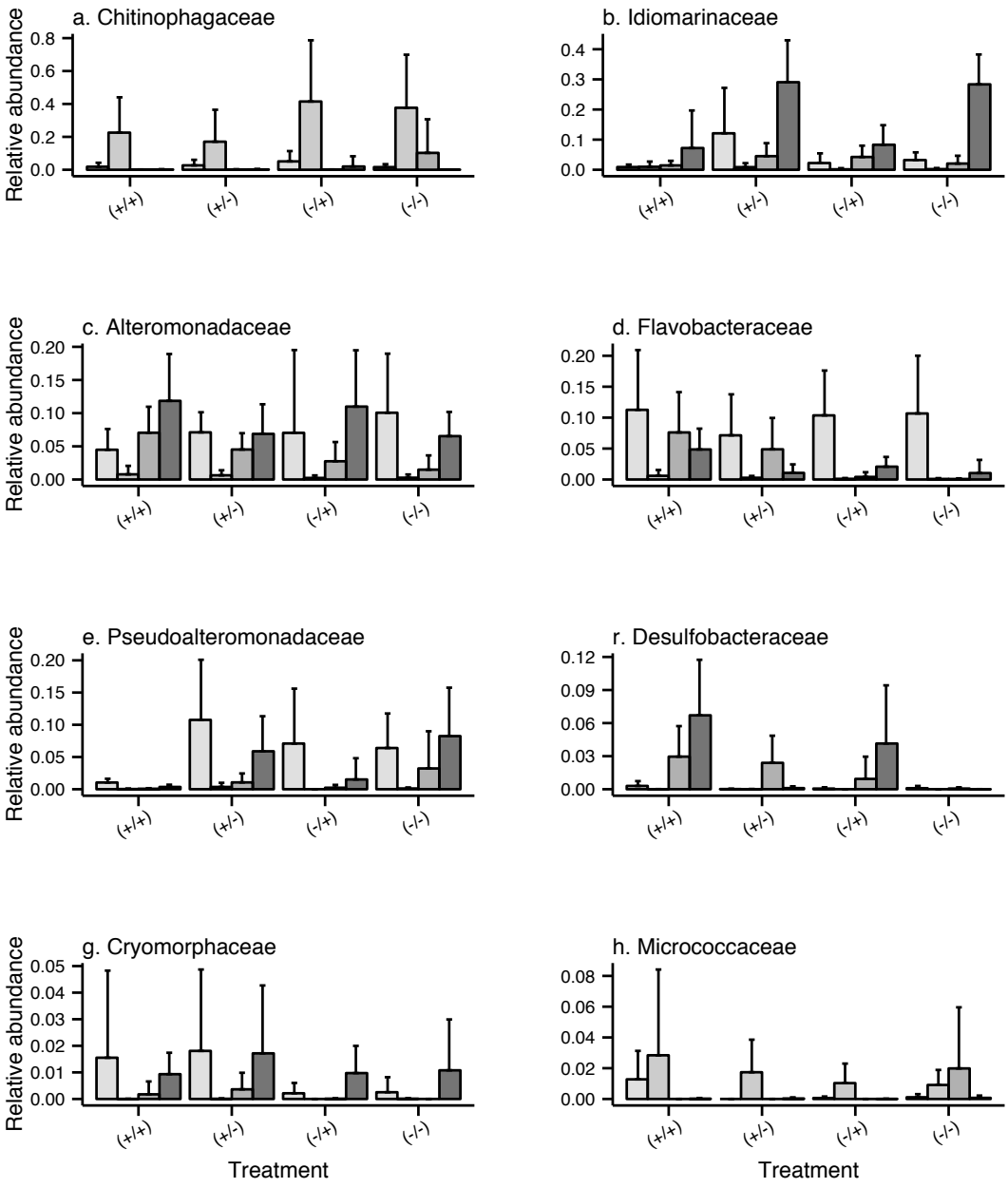

Figure 4.4. Average relative abundance of selected taxa on carapace, gut, burrow and surface. Bars indicate standard error.

The sterilization treatments had effects on the richness and OTU composition of sediment pool bacteria (Fig 4.2a, 4.2b). While there was insufficient DNA for sequencing

in sediments after the 3rd autoclave cycle, we found reduced richness in treated sediment at the end of the mesocosm experiment (Table 4.1). Sterilized surface and sterile control had, in average, 62.4±20.8 and 35.2±6.75 OTUs respectively, while normal surface sediment had an average of 77.6±13.3 OTUs. Burrow sediment had an average of 92.8±11.5 OTUs in the normal control while sterilized burrow and control had 77.7±15.9 and 59.3±8.37 OTUs respectively. Sterilization treatments also had significant effects on OTU composition in the sediment (Fig 4.3, Table 4.2). For example, Desulfobacteraceae bacteria had strong relative abundance responses to sterilization treatments (Fig 4.4e, Table S4.1). However, taxa like Pseudoalteromonadaceae increased their abundances in treated sediment (Fig 4.4f, Table S4.1).

Table 4.2. Summary of results from a perMANOVA assessing the effects of the sterilization treatments on sediment (surface and burrow) and crab-associated (carapace and gut) substrate OTU composition.

	d.f.	F	R2	p
Treatment	3	3.314	0.063	0.01 *
Substrate	3	10.398	0.198	0.01 *
Treatment*Substrate	9	1.452	0.083	0.01 *

* indicates significance of $p<0.05$

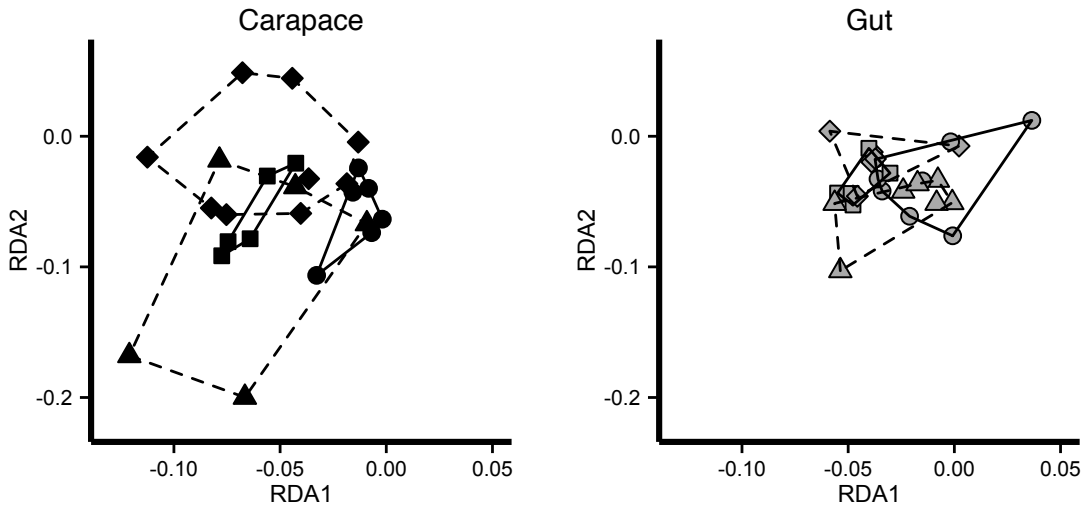

Figure 4.5. RDA scores for crab samples as obtained with the function predict. Effects of sterile surface (-/+, diamonds), sterile subsurface (+/-, triangles), sterile control (-/-, squares) and normal control (+/+, circles) are shown for carapace (a, grey) and burrow (b, black). RDA scores for carapace and gut samples are presented separately for better visualization.

Sediment sterilization treatments had compositional but no richness effects on crab-associated communities (Fig 4.2c, 4.2d, Fig 4.5). Rarefied richness was higher in the carapace (64.14±14.52 OTUs) than in the gut (41.57±26.33 OTUs) but was not affected by the sediment sterilization treatments (Table 4.1). Overall, richness in crab-associated communities was significantly lower than in the sediment (Fig 4.2). Sterilization treatments had significant effects on OTU composition in the crab-associated communities (Fig 4.5, Table 4.2). Some OTUs were not affected by sediment treatments, like Chitinophagaceae in the gut (Fig 4.4a) or Flavobacteraceae in the carapace (Fig 4.4d). In contrast, taxa like Cryomorphaceae or Micrococcaceae decreased in relative abundance when sediment bacterial pools were manipulated (Fig 4.4g, 4.4h).

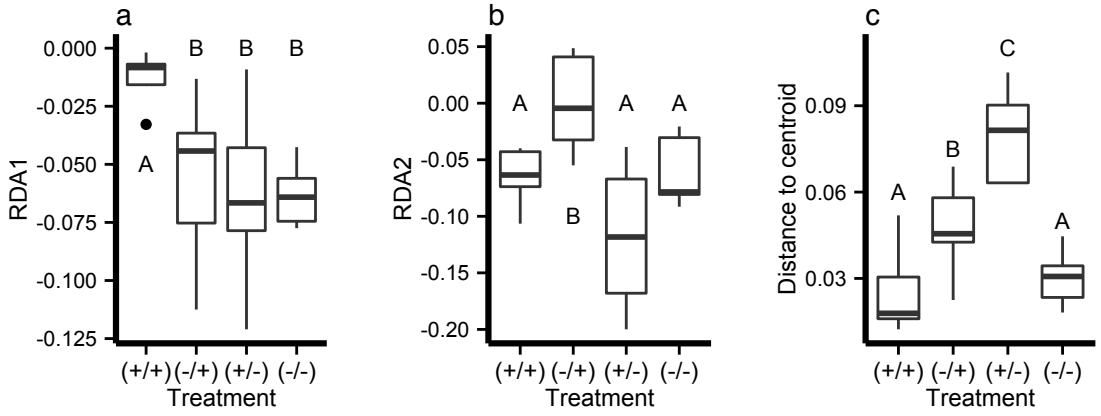

Figure 4.6. Carapace-associated communities response variables to sediment sterilization treatments. Boxplots are shown for a) RDA1, b)RDA2, c) distance to centroid.

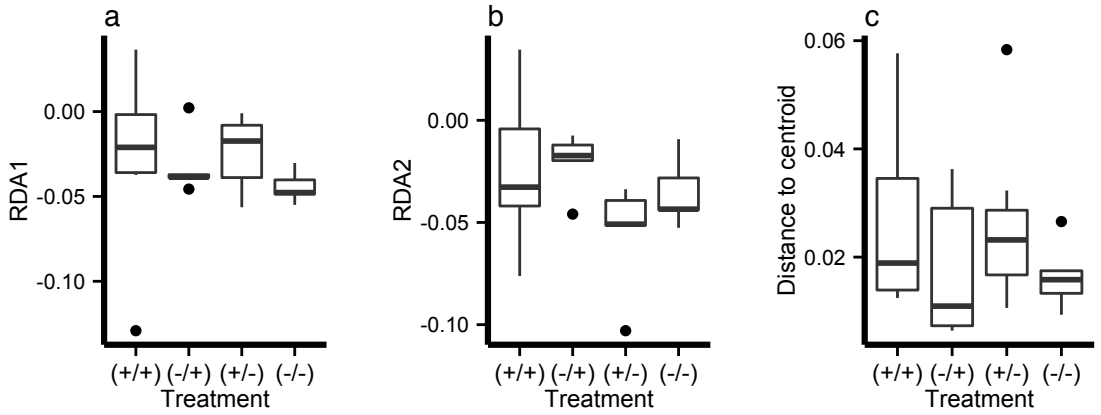

Figure 4.7. Gut-associated communities response variables to sediment sterilization treatments. Boxplots are shown for a) RDA1, b) RDA2, c) distance to centroid.

Sterilization treatments affected the community response variables in the carapace assemblages but not in the gut (Fig 4.6, 4.7). In carapace communities, RDA scores along the first axis were higher in the control (+/+, -0.01±0.01) than when any of the sediment pools were removed (Fig 4.6a, Table 4.3). RDA scores along the first and second axes were higher when the surface sediment pool was removed (-/+, -0.01±0.04) than in the

other treatments (Fig 4.6b, Table 4.3). Compared with both controls (+/+, -/-), variation in carapace community composition doubled when surface sediment pool was removed (-/+) and tripled when the burrow sediment pool was removed (+/-, Fig 4.6c, d.f.=3, F=15.72, p=0.001). In contrast, gut communities were unresponsive to the sediment sterilization treatments (Fig 4.7). There were no significant differences in the first RDA axis scores (Fig 4.7a, Table 4.3), the second RDA axis scores (Fig 4.7b, Table 4.3) or in variation in community composition (Fig 4.7c, d.f.=3, F=0.987, p=0.386).

Table 4.3. Summary of two-way ANOVA evaluating the community composition response to sterilization treatments along the first and second RDA axis.

		d.f.	F	p	
RDA1	Treatment	3	2.932	0.044	*
	Substrate	1	3.263	0.077	
	Treatment*Substrate	3	1.857	0.151	
RDA2	Treatment	3	9.625	<0.001 ***	
	Substrate	1	6.293	0.016	*
	Treatment*Substrate	3	3.473	0.024	*

* indicates significance of p<0.05
*** indicates significance of p<0.001

Table 4.4. Summary of results from a two-way ANOVA assessing Euclidean distance between crab-associated substrate (carapace and guts) and sediment pools (burrow and surface). Data was inversely transformed to meet parametric assumptions.

	d.f.	F	p	
Pool	1	10.387	0.004	**
Substrate	1	0.131	0.720	
Pool*Substrate	1	0.057	0.813	

** indicates significance of p<0.01

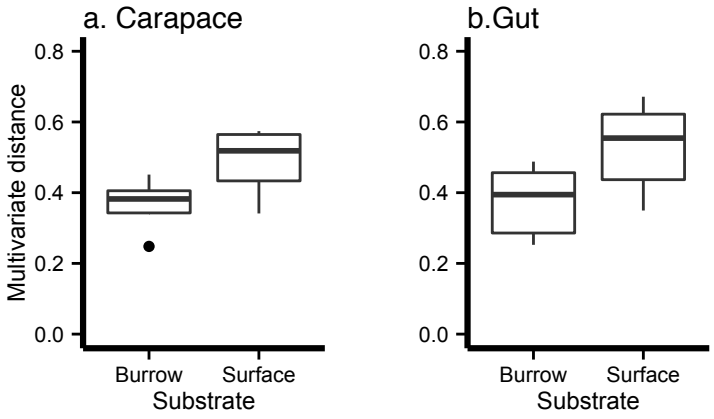

Figure 4.8. Boxplot of multivariate distance between crab-associated communities and bacterial sediment pools. Results are shown for carapace (a) and gut (b) samples.

Crab-associated communities were more similar in composition to burrow than to surface sediment bacteria (Fig 4.8, Table 4.4). Although the differences between groups were lost after the Tukey HSD test, this trend is consistent with the percent overlap between carapace and gut with burrow communities. While carapace and gut communities share 79.3% and 81.9% unique OTUs with burrow sediment, respectively, they only share 63.0% and 70.3% OTUs with the surface sediment. This is most evident with OTUs with high relative abundance in the carapace and burrow sediments, such as members of Alteromonadaceae and Flavobacteraceae (Fig 4.4c, 4.4d, Table S4.1).

DISCUSSION

Regional influences on community structure are increasingly recognized as a fundamental aspect in the maintenance of diversity (Lessard et al 2016, Zobel 2016). Colonists are traditionally considered to conform to a single regional species pool, yet many natural communities are influenced by distinct colonist pools shaped by different

large-scale filters (Chapter 3, (Kalamees and Zobel 1998, Partel 2002, Zobel 1992). The consequences of multiple colonist pools at the local scale should depend on the relative contribution of each colonist pool and the strength of the local habitat filter (Fig 4.1). Our results show that carapace associated communities are more similar in composition to burrow than to surface sediment bacteria, but were influenced by manipulation of both bacterial pools (Fig 4.4, 4.6). In contrast, the gut communities showed little influence from the experimental treatments, suggesting strong habitat filters in the gut structure microbial community assembly (Fig 4.5). Results from gut and carapace communities contrasted with our expectations based on previous findings and fiddler crab natural history, suggesting complex interactions between multiple colonist pools in sediment bacteria and fiddler crab-associated microbial communities. Importantly, weak habitat filters in the carapace and strong filters in the gut were strong indicators of colonist pool influences.

Responses of carapace communities suggest that multiple colonist pool influences are stronger where local habitat filters are weak and open to colonization from the sediment. When sediment bacterial pools were manipulated, carapace communities showed responses along both RDA axes and in community similarity (Fig 4.4, 4.6, Table 4.3, 4.4). OTU relative abundance of taxa like Cryomorphaceae reflected patterns in surface sediment (Fig 4.8g), while members of Alteromonadaceae responded to patterns in burrow sediments (Fig 4.8c). These are common members of microbial communities in seawater and coastal sediments, thus likely representing common patterns of colonization (Bowman 2014, Lopez-Perez and Rodriguez-Valera 2014). This finding echoes previous work emphasizing strong regional influences in more benign conditions, known as weak habitat filters (Chase 2007, Chase and Myers 2011). The strength of habitat filters

depends on the degree to which large-scale filters shaping the colonist pool matches local filters (Zobel 1992). For example, high pH soils have been common evolutionary drivers for plant species due to glacial refugia and thus, for the resulting regional species pool in temperate regions, low pH represents strong filters, while the opposite is true for tropical forests due to the effect of carbonate leaching from the soil (Partel et al 2007). Conditions on the crab's carapace are a better match to the nutrient availability, biochemistry and other environmental conditions in the sediment (Bertics and Ziebis 2009, Ikenaga et al 2010) than conditions in the gut. Nonetheless, colonizing carapace surface requires attachment and is mediated by chitin structure and crab's immunity (Moret and Moreau 2012). OTUs like Flavobacteriaceae, common environmental bacteria, were consistently dominant in the carapace, regardless of the treatment (Fig 4.8d, Bernardet and Nakagawa 2006). Although differing in the degree of filtering, both habitats constrained colonization from the sediment resulting in less variation in community composition than found in the sediment (Fig 4.3, 4.4).

Microbial communities in the gut responded weakly to colonist pool manipulation, coherent with expectations based on strong physical and chemical filtering in this bacterial habitat (Brosing 2010, Vogt et al 1989, Wang et al 2014). This result contrasts with previous findings where the gut was influenced by both surface and burrows, and with the crab's natural history that suggests feeding is exclusive to the surface (Chapter 3, Meziane et al 2002, Thurman 1987). There are two possible explanations for these inconsistencies. First, lab mesocosms were depleted in OTU richness when compared to sediment from the marsh (Chapter 3), which was reflected in low richness within the gut (Fig 4.2d). Second, some OTUs could have survived our

cleaning procedure and driven community assembly. Nonetheless, these scenarios still highlight the limited influences from colonization on gut community assembly.

Surprisingly, we found that the manipulation of a sediment bacterial pool had a strong effect on the other. Surface and burrow pools had 216 OTUs common across all treatments. We found that, when we manipulated a sediment layer by autoclaving, several OTUs that were rare in the control mesocosm were able to successfully colonize sterilized sediment. For example, members of Pseudoalteromonadaceae and Idiomarinaceae become dominant within treated sediment (Fig 4.8b, 4.8e). Although it is possible that these bacteria survived three autoclave cycles, bacteria from the unaltered pool likely colonized the open habitat. Sterilized sediment retains resources like organic carbon and can thus represents open, empty habitat, ready for colonization. Migration from one pool to another can result from interstitial water flow (Bernhard et al 2015), bioturbation (Fanjul et al 2015) and crab feces (Cuellar-Gempeler and Munguia 2013). This finding suggests that colonist pools can have different degrees of interactions. The importance of multiple colonist pool effects should be expected to be stronger in more independent colonist pools.

Community composition in the carapace responded to manipulation of bacteria in surface and burrow sediment (Fig 4.4) and was more similar to burrow bacteria (Fig 4.6a). These results suggest that the carapace is influenced by a main bacterial source from the burrow and key colonist pool from the carapace (Fig 4.1b). It is possible for surface bacteria to colonize the carapace when the crab burrows through the surface layer or when the surface sediment is disturbed. However, influences from one colonist pool to the other indicate that burrow bacteria mediated carapace community responses to surface pool manipulation. In other words, changes in surface bacterial composition

impacts bacteria in the burrow sediment, which, in turn, influences carapace communities. Feedbacks between sediment pools may be important ecological processes shaping bacterial community structure. For example, sediment chemistry and bacterial composition can be altered by environmental conditions like drought or pollution (Tam 1998), resulting in altered carapace microbial communities. Regardless of whether the influence of surface bacteria occurs directly or indirectly, our results suggest that bacterial composition of two distinct colonist pools have consequences for local carapace communities. For example, relative abundance of OTU like Cryomorphaceae diminished when the surface sediment was removed (Fig 4.8g). Importantly, colonist pool influences can manifest directly as relative abundance patterns of contemporary coexistent taxa or indirectly, through the impact on assembly of taxa that later become locally extinct (Miller et al 2009).

Many studies have addressed regional effects on local diversity, yet few recognize that colonist pools are often shaped by distinct large-scale processes. Few studies have addressed colonist pools but they differ substantially from our study. Focusing on hierarchical scales of environmental filtering, some studies show that the influence of colonist pools depends on the mobility of focal organisms (Bajer et al 2015, Poff 1997, Sydenham et al 2015). Yet these studies define a single overarching pool as opposed to multiple colonist pools. Another group of studies highlights colonist pools contrast plant seeds dispersing in space with seed bank species dispersing in time. Although these studies rarely integrate the joint effect of multiple colonist pools, they show that seed rain dispersing in space has a stronger influence during community succession after strong disturbances, while seed bank species dispersing in time are more important for community development after small disturbances, such as herbivore activity (Bekker et al

2000, Eskelinen and Virtanen 2005, Kalamees and Zobel 1998, Willems and Bik 1998). Our results coincide with other work suggesting that distinct sources of colonists are rarely completely independent. Although this effect is likely a result of our experimental design, coexisting multiple colonist pools can fall along a gradient of independence, from completely filtered and independent to a single regional species pool. Understanding the degree of pool independence would improve our understanding of the interaction between multiple colonist pools and local habitat filters in driving communities composition.

Our study provides evidence of multiple species pools interactions with local habitat filters. We demonstrated multiple species pool influences on communities under weak habitat filters in the carapace, and contrasted this effect with robust filtering in the gut. Although many systems may be colonized from a single and coherent evolutionary regional species pool, we argue that the effects of hierarchical filters, dispersal limitation and local-regional feedback may be more complex in most natural communities (Cornell and Harrison 2014). Importantly, multiple colonist pool influences will be important when pools are distinct and local habitat filters are weak. To fully integrate these processes and make studies comparable, a definition of the colonist pool that encompasses all the effects of dispersal on community diversity and structure. These advances are bound to have critical implications for basic ecology, leading towards a more predictive field. Furthermore, acknowledging the role of multiple colonist pools may contribute to conservation ecology, leading to a better integration of dispersal on management and restoration plans.

Appendix

CHAPTER 1: SUPPLEMENTAL INFORMATION

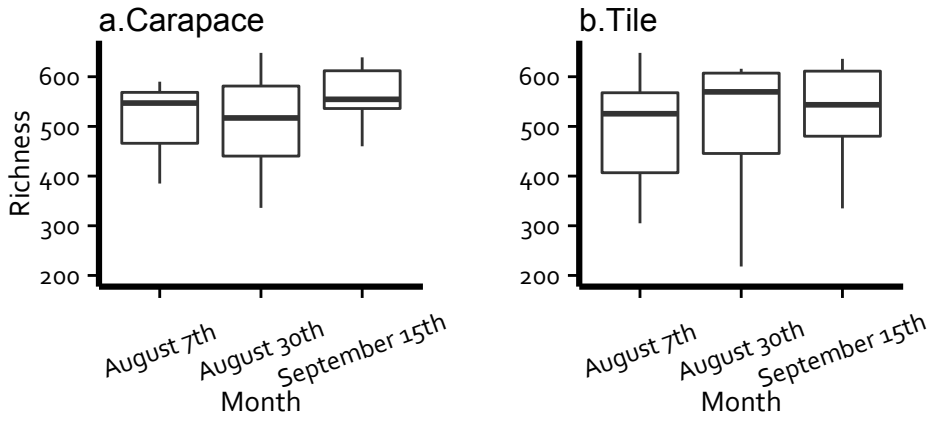

Figure S1.1 Boxplot illustrating OTU rarefied richness of microbial communities associated with crab carapaces (a) and tile surfaces (b).

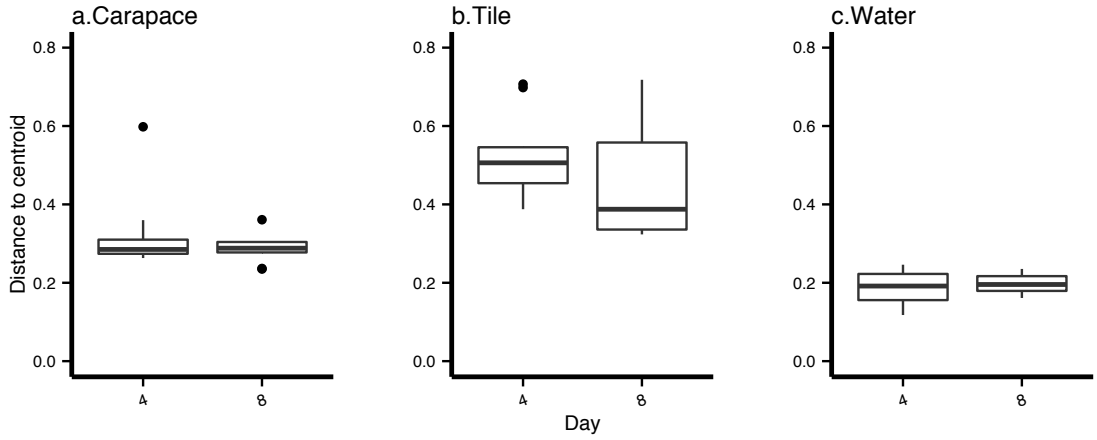

Figure S1.2. Boxplot illustrating temporal changes in distance to centroid of microbial communities associated with crab carapaces (a) and tile surfaces (b). Distance to group centroid was calculated separately from hellinger transformed data for samples collected on day 4 and 8.

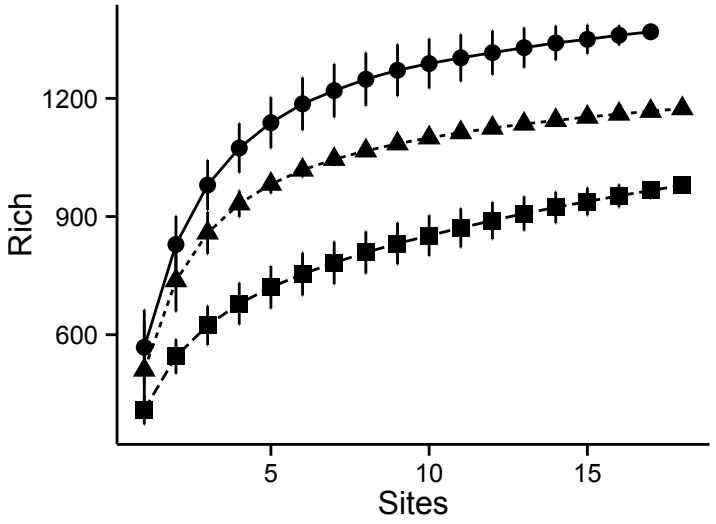

Figure S1.3 Species accumulation curves for carapace (circles), tiles (triangles) and water samples (squares).

Table S1.1. Summary of two-way ANOVAs assessing the effect of month and habitat type on OTU relative abundance. Results are shown for OTUs with more than 0.005 average relative abundance in either carapaces or tile.

			Av. relative abundance		Month			Habitat			Month*Habitat		
Phylum	Family	Genus	Carapace	Tile	df	F value	p	df	F value	p	df	F value	p
Bacteroidetes	Flavobacteriaceae	Tenacibaculum	0.0439	0.0011	2	0.062	0.940	1	26.564	0.000	2	0.167	0.847
Proteobacteria	Erythrobacteraceae	-	0.0425	0.0005	2	0.375	0.690	1	6.780	0.014	2	0.370	0.694
Proteobacteria	Alteromonadaceae	Alteromonas	0.0307	0.0023	2	1.859	0.174	1	8.176	0.008	2	1.478	0.245
Proteobacteria	Rhodobacteraceae	-	0.0236	0.0024	2	4.277	0.024	1	22.215	0.000	2	6.713	0.004
Proteobacteria	Rhodobacteraceae	-	0.0148	0.0022	2	0.656	0.527	1	11.601	0.002	2	0.843	0.441
Bacteroidetes	Flavobacteriaceae	-	0.0134	0.0001	2	3.529	0.042	1	9.683	0.004	2	3.340	0.050
Proteobacteria	Erythrobacteraceae	-	0.0131	0.0009	2	0.697	0.506	1	16.601	0.000	2	0.625	0.542
Cyanobacteria	Synechococcaceae	Prochlorococcus	0.0130	0.0010	2	0.911	0.413	1	0.920	0.345	2	0.978	0.388
Cyanobacteria	-	-	0.0126	0.0062	2	0.705	0.503	1	1.985	0.169	2	0.168	0.846
Cyanobacteria	-	-	0.0126	0.0062	2	0.705	0.503	1	1.985	0.169	2	0.168	0.846
Bacteroidetes	Flavobacteriaceae	-	0.0121	0.0023	2	2.220	0.127	1	7.577	0.010	2	0.979	0.388
Actinobacteria	Intrasporangiaceae	-	0.0112	0.0000	2	1.063	0.359	1	2.080	0.160	2	1.047	0.364
Bacteroidetes	Flavobacteriaceae	-	0.0111	0.0004	2	0.940	0.402	1	6.183	0.019	2	0.957	0.396
Bacteroidetes	Flavobacteriaceae	-	0.0102	0.0001	2	0.237	0.790	1	6.153	0.019	2	0.202	0.818
Proteobacteria	-	-	0.0096	0.0004	2	3.469	0.045	1	25.291	0.000	2	4.364	0.022
Proteobacteria	Erythrobacteraceae	-	0.0095	0.0001	2	0.723	0.494	1	2.161	0.152	2	0.707	0.501
Bacteroidetes	Flavobacteriaceae	-	0.0088	0.0644	2	1.112	0.343	1	7.564	0.010	2	0.820	0.450
Proteobacteria	Erythrobacteraceae	-	0.0072	0.0202	2	1.523	0.235	1	22.627	0.000	2	1.080	0.353
Proteobacteria	Rhodobacteraceae	-	0.0061	0.0268	2	0.511	0.605	1	3.761	0.062	2	0.645	0.532
Proteobacteria	Rhodobacteraceae	-	0.0056	0.0082	2	3.193	0.056	1	0.710	0.406	2	1.017	0.374

Table S1.1. Continued

			Av. relative abundance		Month			Habitat			Month*Habitat		
Phylum	Family	Genus	Carapace	Tile	df	F value	p	df	F value	p	df	F value	p
Cyanobacteria	-	-	0.0054	0.0134	2	0.255	0.777	1	2.812	0.104	2	1.048	0.363
Cyanobacteria	-	-	0.0053	0.0155	2	0.839	0.443	1	2.289	0.141	2	1.136	0.335
Proteobacteria	Vibrionaceae	Vibrio	0.0047	0.0431	2	2.545	0.096	1	3.149	0.086	2	1.623	0.215
Proteobacteria	Rhodobacteraceae	-	0.0032	0.0331	2	0.468	0.631	1	4.078	0.053	2	0.401	0.673
Proteobacteria	Rhodobacteraceae	-	0.0029	0.0247	2	0.220	0.804	1	3.538	0.070	2	0.244	0.785
Proteobacteria	Vibrionaceae	Vibrio	0.0022	0.0146	2	1.381	0.267	1	1.243	0.274	2	0.455	0.639
Bacteroidetes	Flavobacteriaceae	-	0.0019	0.0115	2	1.601	0.219	1	29.936	0.000	2	0.831	0.446
Proteobacteria	-	-	0.0018	0.0097	2	1.648	0.210	1	2.126	0.156	2	0.513	0.604
Proteobacteria	Rhodobacteraceae	-	0.0017	0.0087	2	1.211	0.312	1	1.237	0.275	2	0.910	0.414
Bacteroidetes	Flavobacteriaceae	-	0.0017	0.0078	2	3.398	0.047	1	7.603	0.010	2	1.331	0.280
Cyanobacteria	-	-	0.0011	0.0076	2	0.976	0.389	1	1.200	0.282	2	0.633	0.538
Proteobacteria	Colwelliaceae	Thalassomonas	0.0009	0.0143	2	1.088	0.350	1	1.511	0.229	2	0.992	0.383
Proteobacteria	Alteromonadaceae	-	0.0005	0.0091	2	1.202	0.315	1	1.822	0.188	2	1.122	0.339
Proteobacteria	Erythrobacteraceae	Erythrobacter	0.0003	0.0098	2	1.001	0.380	1	1.091	0.305	2	0.887	0.423
Proteobacteria	Rhodobacteraceae	-	0.0002	0.0100	2	0.573	0.570	1	3.059	0.091	2	0.571	0.571

CHAPTER 2: SUPPLEMENTAL INFORMATION

Table S2.1. Summary of GLMs assessing the effect of time and water abundance on OTU abundance on the carapace.

			Average	Intercept			Days			Water		
Phylum	Family	Genus	rel.abund	Estimate	s.e	p	Estimate	s.e	p	Estimate	s.e	p
Proteobacteria	Vibrionaceae	Vibrio	0.470	-1.510	2.563	0.569	-0.068	0.207	0.748	46.3	123.3	0.715
Proteobacteria	Alteromonadaceae	Alteromonas	0.130	-0.981	0.963	0.333	-0.439	0.175	0.031	116.7	329.3	0.730
Proteobacteria	Rhodobacteraceae		0.068	-3.616	0.533	0.000	0.101	0.039	0.026	4.2	3.2	0.219
Proteobacteria	Rhodobacteraceae		0.042	-2.736	0.376	0.000	-0.271	0.078	0.006	2.8	35.5	0.939
Proteobacteria	Rhodobacteraceae		0.032	-3.256	0.593	0.000	-0.254	0.073	0.006	12.3	6.2	0.077
Proteobacteria	Rhodobacteraceae		0.032	-1.233	0.963	0.230	-0.164	0.063	0.027	-17.0	14.5	0.269
Proteobacteria	Rhodobacteraceae		0.027	-3.224	0.684	0.001	-0.220	0.062	0.005	25.9	39.8	0.530
Proteobacteria	Methylophilaceae	Methylotenera	0.013	-7.796	0.911	0.000	0.280	0.061	0.001	3991.3	705.7	0.000
Bacteroidetes	Flavobacteriaceae		0.005	-3.886	1.367	0.017	-0.193	0.137	0.189	60.9	57.0	0.311
Bacteroidetes	Flavobacteriaceae		0.004	-3.459	0.521	0.000	-0.241	0.078	0.007	-	-	-
Bacteroidetes	Flavobacteriaceae	NA	0.003	-4.761	0.471	0.000	-0.010	0.042	0.822	12.8	6.6	0.080
Proteobacteria	Oceanospirillaceae		0.002	-4.043	0.824	0.001	0.047	0.075	0.546	5.6	1.9	0.015
Bacteroidetes	Flavobacteriaceae		0.002	-5.244	0.859	0.000	0.005	0.083	0.956	-	-	-
Proteobacteria	Colwelliaceae	Thalassomonas	0.002	-4.889	0.492	0.000	-0.048	0.053	0.388	14.0	13.0	0.305
Proteobacteria	Erythrobacteraceae		0.002	-4.108	0.458	0.000	-0.055	0.049	0.277	-	-	-
Bacteroidetes	Flavobacteriaceae	Kordia	0.002	-3.383	1.011	0.007	-0.034	0.149	0.824	1.6	5.6	0.787
Proteobacteria	Rhodobacteraceae		0.001	-3.994	0.503	0.000	-0.070	0.055	0.223	-	-	-
Bacteroidetes	Flavobacteriaceae		0.001	-6.567	1.222	0.000	-0.020	0.121	0.873	51.9	14.4	0.005
Proteobacteria	Oceanospirillaceae		0.001	-8.502	2.230	0.003	-0.013	0.221	0.955	37.1	6.2	0.000

Table S2.1. Continued.

Phylum	Family	Genus	Average rel.abund	Intercept Estimate	s.e	p	Days Estimate	s.e	p	Water Estimate	s.e	p
Bacteroidetes	Flavobacteriaceae		0.000	-6.263	0.845	0.000	0.152	0.067	0.039	-	-	-
Proteobacteria	Rhodospirillaceae		0.000	-5.721	0.551	0.000	0.063	0.049	0.218	-	-	-
Proteobacteria	Rhodobacteraceae		0.000	-5.262	0.609	0.000	0.075	0.053	0.178	-	-	-
Bacteroidetes	Saprospiraceae		0.000	-6.796	0.903	0.000	0.203	0.064	0.010	787.0	230.1	0.007
Proteobacteria	Alteromonadaceae		0.000	-5.225	0.975	0.000	0.037	0.090	0.688	-	-	-

Table S2.2. Summary of GLMs assessing the effect of time and water abundance on OTU abundance in the gut.

Phylum	Family	Genus	Average rel.abund	Intercept Estimate	s.e	p	Days Estimate	s.e	p	Water Estimate	s.e	p
Tenericutes	-	-	0.618	-2.825	1.328	0.071	0.105	0.097	0.312	2905.1	1239.0	0.051
Proteobacteria	Vibrionaceae	Vibrio	0.142	-2.379	3.143	0.474	0.038	0.215	0.866	-10.6	166.5	0.951
Proteobacteria	Rhodobacteraceae	-	0.128	4.967	3.598	0.210	-0.402	0.198	0.082	-73.7	55.2	0.223
Proteobacteria	Alteromonadaceae	Microbulbifer	0.039	-4.114	1.126	0.008	-0.118	0.106	0.300	1166.7	480.1	0.045
Proteobacteria	Rhodobacteraceae	-	0.029	-0.369	1.709	0.835	-0.574	0.321	0.117	-1.7	14.0	0.906
Proteobacteria	Rhodobacteraceae	-	0.013	-2.876	1.230	0.052	0.060	0.098	0.560	-8.8	9.1	0.366
Proteobacteria	Rhodobacteraceae	-	0.003	-4.701	1.025	0.003	0.067	0.097	0.515	-54.4	491.5	0.915
Bacteroidetes	Flavobacteriaceae	-	0.000	-6.276	2.575	0.033	0.108	0.195	0.592	-	-	-
Planctomycetes	Planctomycetaceae	Planctomyces	0.000	-6.023	1.476	0.002	0.085	0.115	0.477	-	-	-
Cyanobacteria	-	-	0.000	-13.432	7.341	0.095	0.623	0.466	0.208	-	-	-
Proteobacteria	Rhodobacteraceae	-	0.000	-5.900	1.644	0.004	0.066	0.131	0.628	-	-	-
Tenericutes	-	-	0.000	-6.611	2.221	0.013	0.127	0.165	0.457	-	-	-
Tenericutes	-	-	0.000	-12.030	2.697	0.003	0.241	0.165	0.186	20330.5	3298.1	0.000

Table S2.3. Summary of GLMs assessing the effect of time and substrate (carapace and gut) on functional pathways as indicated by the Picrust analysis.

	Intercept		Days		Carapace		Gut	
Pathway	Estimate	p value	Estimate	p value	Estimate	p value	Estimate	p value
Benzoate degradation	-5.109	0	-0.013	0.094	-0.01	0.896	-0.255	0.003
beta Alanine metabolism	-5.155	0	-0.006	0.178	0.001	0.99	-0.241	0
Fructose and mannose metabolism	-5.309	0	-0.002	0.531	0.034	0.246	0.178	0
Fatty acid biosynthesis	-5.053	0	-0.006	0.364	-0.041	0.508	-0.55	0
Pantothenate and CoA biosynthesis	-5.129	0	-0.005	0.126	-0.023	0.491	-0.218	0
Glycerophospholipid metabolism	-5.478	0	0.012	0.025	0.024	0.662	0.403	0
Peptidoglycan biosynthesis	-5.000	0	-0.008	0.185	-0.054	0.343	-0.566	0
Bacterial chemotaxis	-5.094	0	-0.011	0.316	0.15	0.169	-0.159	0.203
Protein export	-5.314	0	0.009	0.088	-0.058	0.31	0.251	0
Lysine biosynthesis	-4.989	0	-0.004	0.468	-0.034	0.573	-0.512	0
Histidine metabolism	-4.984	0	-0.003	0.222	-0.063	0.027	-0.389	0
Flagellar assembly	-4.987	0	-0.028	0.008	0.32	0.003	0.089	0.437
Lysine degradation	-4.960	0	-0.004	0.422	-0.059	0.228	-0.382	0
Phenylalanine tyrosine and tryptophan biosynthesis	-4.886	0	-0.005	0.376	-0.03	0.611	-0.529	0
DNA replication	-5.425	0	0.021	0.045	-0.076	0.519	0.608	0
Replication recombination and repair proteins	-5.091	0	0.009	0	-0.031	0.079	-0.054	0.005
Pentose phosphate pathway	-5.146	0	0.005	0.268	0.02	0.693	0.304	0
Membrane and intracellular structural molecules	-4.855	0	-0.007	0.188	0.045	0.389	-0.313	0
Nitrogen metabolism	-4.736	0	-0.014	0.043	-0.01	0.874	-0.345	0

Table S2.3. Continued.

Pathway	Intercept		Days		Carapace		Gut	
	Estimate	p value	Estimate	p value	Estimate	p value	Estimate	p value
Tryptophan metabolism	-4.774	0	-0.005	0.175	-0.052	0.174	-0.312	0
Valine leucine and isoleucine biosynthesis	-4.877	0	-0.001	0.504	-0.023	0.252	-0.057	0.008
Fatty acid metabolism	-4.761	0	-0.007	0.197	0.02	0.715	-0.231	0.001
Lipid biosynthesis proteins	-4.807	0	-0.001	0.816	-0.004	0.863	-0.234	0
Glyoxylate and dicarboxylate metabolism	-4.694	0	-0.015	0.024	0.025	0.695	-0.153	0.037
Cysteine and methionine metabolism	-4.795	0	-0.001	0.384	-0.021	0.221	-0.173	0
Homologous recombination	-5.076	0	0.015	0.068	-0.057	0.526	0.419	0
Transcription machinery	-4.901	0	0.01	0.037	-0.003	0.947	0.071	0.154
Translation proteins	-4.845	0	0.008	0.052	-0.083	0.058	0.017	0.709
Alanine aspartate and glutamate metabolism	-4.558	0	-0.006	0.202	-0.034	0.476	-0.415	0
Chaperones and folding catalysts	-4.709	0	0.005	0.037	-0.009	0.724	0.004	0.882
Butanoate metabolism	-4.464	0	-0.009	0.053	0.011	0.811	-0.198	0
Methane metabolism	-4.585	0	-0.003	0.029	0.026	0.1	0.05	0.002
Propanoate metabolism	-4.491	0	-0.004	0.149	-0.011	0.708	-0.176	0
Carbon fixation pathways in prokaryotes	-4.418	0	-0.005	0.152	-0.005	0.889	-0.297	0
Glycolysis Gluconeogenesis	-4.715	0	0.01	0.055	-0.024	0.669	0.348	0
Glycine serine and threonine metabolism	-4.276	0	-0.012	0.017	-0.042	0.373	-0.325	0
Pyruvate metabolism	-4.448	0	-0.001	0.17	-0.015	0.061	0.049	0
Arginine and proline metabolism	-4.199	0	-0.005	0.295	-0.049	0.293	-0.4	0
Oxidative phosphorylation	-4.394	0	0.005	0.001	0.037	0.016	0.007	0.678
Bacterial motility proteins	-4.213	0	-0.013	0.239	0.227	0.035	-0.076	0.524
Peptidases	-4.237	0	0.005	0.003	-0.021	0.199	-0.138	0

CHAPTER 3: SUPPLEMENTAL INFORMATION

Table S3.1. Pairwise comparisons between carapace and gut communities, and surface and burrow sediment bacteria using SIMPER analysis. Average relative abundance is shown for each sediment type (first column corresponds to the first type in the compared pair, second column for the second). Taxonomic classification are shown to the deepest assignment for each OTU. OTUs representing approximately 30% of the variation are shown fro each comparison.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Surface vs. burrow	Firmicutes	Halanaerobiaceae	-	0.229	0.007	0.163	0.264
	Proteobacteria	Desulfobacteraceae	Desulfobacterium	0.053	0.113	0.055	0.353
	Bacteroidetes	Balneolaceae	KSA1	0.089	0.034	0.048	0.431
	Proteobacteria	Rhodobacteraceae	-	0.035	0.096	0.048	0.507
	Proteobacteria	Desulfohalobiaceae	Desulfovermiculus	0.036	0.001	0.026	0.550
	Bacteroidetes	Flavobacteriaceae	-	0.030	0.052	0.021	0.584
	Proteobacteria	Helicobacteraceae	-	0.000	0.020	0.015	0.640
	Proteobacteria	OM60	-	0.001	0.019	0.013	0.662
	Proteobacteria	Alteromonadaceae	Marinobacter	0.010	0.022	0.011	0.679
	Proteobacteria	Halomonadaceae	Halomonas	0.006	0.019	0.011	0.697
	Proteobacteria	Desulfobulbaceae	-	0.019	0.026	0.010	0.712
	Firmicutes	Clostridiaceae	-	0.013	0.007	0.009	0.727
	Proteobacteria	Thiohalorhabdaceae	-	0.013	0.000	0.009	0.742
	Proteobacteria	Syntrophobacteraceae	-	0.002	0.012	0.008	0.755
	Proteobacteria	Rhodospirillaceae	Inquilinus	0.014	0.015	0.008	0.769
	Proteobacteria	Methylococcaceae	-	0.001	0.010	0.008	0.781
	Bacteroidetes	Rhodothermaceae	Salisaeta	0.012	0.005	0.007	0.792
	Thermi	Deinococcaceae	Deinococcus	0.013	0.004	0.007	0.803

Table S3.1. Continued.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Surface vs. Gut	Proteobacteria	Rhodobacteraceae	-	0.035	0.292	0.178	0.208
	Firmicutes	Halanaerobiaceae	-	0.229	0.005	0.152	0.386
	Bacteroidetes	Balneolaceae	KSA1	0.089	0.002	0.059	0.455
	Bacteroidetes	Saprospiraceae	-	0.006	0.074	0.048	0.511
	Bacteroidetes	Flavobacteriaceae	-	0.030	0.086	0.046	0.564
	Proteobacteria	Desulfobacteraceae	Desulfobacterium	0.053	0.002	0.036	0.606
	Proteobacteria	Xanthobacteraceae	-	0.003	0.042	0.028	0.639
	Proteobacteria	Desulfohalobiaceae	Desulfovermiculus	0.036	0.001	0.024	0.667
	Proteobacteria	Caulobacteraceae	Caulobacter	0.000	0.031	0.021	0.692
	Proteobacteria	Enterobacteriaceae	-	0.000	0.022	0.015	0.709
	Proteobacteria	Erythrobacteraceae	-	0.001	0.019	0.012	0.724
	Proteobacteria	Desulfobulbaceae	-	0.019	0.004	0.011	0.737
	Proteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	0.000	0.015	0.011	0.750
	Proteobacteria	Rhodospirillaceae	Inquilinus	0.014	0.001	0.009	0.761
	Proteobacteria	Thiotrichaceae	Thiothrix	0.000	0.013	0.009	0.771
	Firmicutes	Clostridiaceae	-	0.013	0.001	0.009	0.781
	Thermi	Deinococcaceae	Deinococcus	0.013	0.000	0.009	0.791
	Tenericutes	Anaeroplasmataceae	-	0.000	0.012	0.009	0.801

Table S3.1. Continued 2.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Surface vs. Carapace	Proteobacteria	Rhodobacteraceae	-	0.035	0.284	0.175	0.205
	Firmicutes	Halanaerobiaceae	-	0.229	0.000	0.162	0.395
	Bacteroidetes	Balneolaceae	KSA1	0.089	0.002	0.062	0.467
	Bacteroidetes	Flavobacteriaceae	-	0.030	0.100	0.052	0.527
	Bacteroidetes	Saprospiraceae	-	0.006	0.065	0.042	0.577
	Proteobacteria	Desulfobacteraceae	Desulfobacterium	0.053	0.002	0.037	0.620
	Proteobacteria	Desulfohalobiaceae	Desulfovermiculus	0.036	0.000	0.026	0.651
	Proteobacteria	Alteromonadaceae	Marinobacter	0.010	0.040	0.024	0.678
	Proteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	0.000	0.029	0.019	0.700
	Proteobacteria	Erythrobacteraceae	-	0.001	0.027	0.018	0.722
	Proteobacteria	Vibrionaceae	Vibrio	0.000	0.020	0.013	0.737
	Proteobacteria	Desulfobulbaceae	-	0.019	0.002	0.013	0.752
	Proteobacteria	Xanthobacteraceae	-	0.003	0.016	0.012	0.766
	Bacteroidetes	Cryomorphaceae	-	0.000	0.016	0.010	0.778
	Proteobacteria	Rhodospirillaceae	Inquilinus	0.014	0.001	0.010	0.789
	Firmicutes	Clostridiaceae	-	0.013	0.000	0.009	0.800

Table S3.1. Continued 3.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Burrow vs. Gut	Proteobacteria	Rhodobacteraceae	-	0.096	0.292	0.145	0.200
	Proteobacteria	Desulfobacteraceae	Desulfobacterium	0.113	0.002	0.079	0.309
	Bacteroidetes	Saprospiraceae	-	0.009	0.074	0.047	0.375
	Bacteroidetes	Flavobacteriaceae	-	0.052	0.086	0.040	0.430
	Proteobacteria	Xanthobacteraceae	-	0.001	0.042	0.028	0.468
	Bacteroidetes	Balneolaceae	KSA1	0.034	0.002	0.022	0.499
	Proteobacteria	Caulobacteraceae	Caulobacter	0.001	0.031	0.021	0.528
	Actinobacteria	koll13	-	0.032	0.002	0.021	0.557
	Proteobacteria	Desulfobulbaceae	-	0.026	0.004	0.015	0.579
	Proteobacteria	Enterobacteriaceae	-	0.000	0.022	0.015	0.599
	Proteobacteria	Helicobacteraceae	-	0.020	0.000	0.014	0.619
	Proteobacteria	Alteromonadaceae	Marinobacter	0.022	0.010	0.014	0.638
	Proteobacteria	Erythrobacteraceae	-	0.005	0.019	0.012	0.655
	Proteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	0.004	0.015	0.011	0.687
	Proteobacteria	Halomonadaceae	Halomonas	0.019	0.006	0.011	0.702
	Proteobacteria	Rhodospirillaceae	Inquilinus	0.015	0.001	0.010	0.717
	Proteobacteria	Thiotrichaceae	Thiothrix	0.000	0.013	0.009	0.729
	Proteobacteria	Syntrophobacteraceae	-	0.012	0.000	0.009	0.741
	Tenericutes	Anaeroplasmataceae	-	0.000	0.012	0.009	0.753
	Proteobacteria	Vibrionaceae	Vibrio	0.008	0.011	0.009	0.765
	Cyanobacteria	Synechococcaceae	Prochlorococcus	0.009	0.008	0.008	0.776
	Planctomycetes	Pirellulaceae	-	0.013	0.009	0.008	0.787
	Proteobacteria	Methylococcaceae	-	0.010	0.000	0.007	0.797
	Proteobacteria	Comamonadaceae	-	0.000	0.010	0.007	0.807

Table S3.1. Continued 4.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Burrow vs. Carapace	Proteobacteria	Rhodobacteraceae	-	0.096	0.284	0.141	0.203
	Proteobacteria	Desulfobacteraceae	Desulfobacterium	0.113	0.002	0.083	0.322
	Bacteroidetes	Flavobacteriaceae	-	0.052	0.100	0.043	0.384
	Bacteroidetes	Saprospiraceae	-	0.009	0.065	0.041	0.444
	Proteobacteria	Alteromonadaceae	Marinobacter	0.022	0.040	0.027	0.482
	Bacteroidetes	Balneolaceae	KSA1	0.034	0.002	0.023	0.515
	Actinobacteria	koll13	-	0.032	0.002	0.022	0.546
	Proteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	0.004	0.029	0.018	0.573
	Proteobacteria	Desulfobulbaceae	-	0.026	0.002	0.017	0.598
	Proteobacteria	Erythrobacteraceae	-	0.005	0.027	0.017	0.622
	Proteobacteria	Helicobacteraceae	-	0.020	0.000	0.015	0.643
	Proteobacteria	Vibrionaceae	Vibrio	0.008	0.020	0.013	0.663
	Proteobacteria	Halomonadaceae	Halomonas	0.019	0.007	0.012	0.680
	Proteobacteria	OM60	-	0.019	0.004	0.011	0.697
	Proteobacteria	Rhodospirillaceae	Inquilinus	0.015	0.001	0.011	0.713
	Proteobacteria	Xanthobacteraceae	-	0.001	0.016	0.011	0.728
	Bacteroidetes	Cryomorphaceae	-	0.001	0.016	0.010	0.743
	Proteobacteria	Syntrophobacteraceae	-	0.012	0.000	0.009	0.756
	Proteobacteria	Oceanospirillaceae	Marinomonas	0.010	0.011	0.008	0.768
	Actinobacteria	-	-	0.000	0.011	0.008	0.779
	Planctomycetes	Pirellulaceae	-	0.013	0.003	0.008	0.790
	Proteobacteria	Thiotrichaceae	Thiothrix	0.000	0.011	0.008	0.801

Table S3.1. Continued 5.

Comparison	Phylum	Family	Genus	Average abundances		Contribution (%)	Cumulative contribution
Gut vs. Carapace	Proteobacteria	Rhodobacteraceae	-	0.292	0.284	0.092	0.191
	Bacteroidetes	Saprospiraceae	-	0.074	0.065	0.047	0.289
	Bacteroidetes	Flavobacteriaceae	-	0.086	0.100	0.041	0.373
	Proteobacteria	Xanthobacteraceae	-	0.042	0.016	0.034	0.444
	Proteobacteria	Alteromonadaceae	Marinobacter	0.010	0.040	0.025	0.495
	Proteobacteria	Pseudoalteromonadaceae	Pseudoalteromonas	0.015	0.029	0.021	0.538
	Proteobacteria	Caulobacteraceae	Caulobacter	0.031	0.004	0.020	0.580
	Proteobacteria	Erythrobacteraceae	-	0.019	0.027	0.016	0.614
	Proteobacteria	Enterobacteriaceae	-	0.022	0.003	0.015	0.645
	Proteobacteria	Vibrionaceae	Vibrio	0.011	0.020	0.014	0.674
	Bacteroidetes	Cryomorphaceae	-	0.008	0.016	0.011	0.698
	Actinobacteria	-	-	0.010	0.011	0.010	0.719
	Proteobacteria	Thiotrichaceae	Thiothrix	0.013	0.011	0.009	0.739
	Tenericutes	Anaeroplasmataceae	-	0.012	0.002	0.009	0.757
	Proteobacteria	Comamonadaceae	-	0.010	0.008	0.007	0.772
	Actinobacteria	Propionibacteriaceae	Propionibacterium	0.010	0.007	0.007	0.786
	Proteobacteria	Oceanospirillaceae	Marinomonas	0.003	0.011	0.007	0.800

Table S3.2. Summary of ANOVA tests performed on each pathway assessing the differences in relative representation in carapace, gut, surface and burrow sediments. Subsurface sediments were removed due to low NSTI scores. Average relative representation and standard deviation is shown for each pathway.

Pathway	d.f.	F	p	Average	Standard Deviation
Transporters	3	1.548	0.218	0.056	0.008
ABC transporters	3	1.476	0.236	0.036	0.006
DNA repair and recombination proteins	3	3.468	0.025	0.022	0.001
Purine metabolism	3	21.006	0.000	0.020	0.001
Two component system	3	14.721	0.000	0.019	0.002
Ribosome	3	53.006	0.000	0.018	0.002
Function unknown	3	11.871	0.000	0.017	0.002
Secretion system	3	4.584	0.008	0.015	0.002
Peptidases	3	1.117	0.354	0.015	0.001
Pyrimidine metabolism	3	53.261	0.000	0.014	0.002
Bacterial motility proteins	3	2.009	0.129	0.014	0.002
Oxidative phosphorylation	3	29.745	0.000	0.014	0.001
Transcription factors	3	13.440	0.000	0.014	0.001
Arginine and proline metabolism	3	8.043	0.000	0.013	0.001
Amino acid related enzymes	3	24.435	0.000	0.013	0.001
Chromosome	3	7.700	0.000	0.012	0.001
Pyruvate metabolism	3	0.175	0.913	0.011	0.000
Ribosome Biogenesis	3	1.245	0.307	0.011	0.001
Carbon fixation pathways in prokaryotes	3	24.737	0.000	0.011	0.001
Glycine serine and threonine metabolism	3	1.503	0.229	0.011	0.001
Methane metabolism	3	76.452	0.000	0.011	0.001
Butanoate metabolism	3	5.756	0.002	0.011	0.001
Others	3	5.171	0.004	0.011	0.001
Porphyrin and chlorophyll metabolism	3	1.772	0.168	0.011	0.001
Other ion coupled transporters	3	19.586	0.000	0.010	0.001
Glycolysis Gluconeogenesis	3	113.405	0.000	0.010	0.001
Valine leucine and isoleucine degradation	3	32.638	0.000	0.010	0.002
Propanoate metabolism	3	23.416	0.000	0.010	0.001
Aminoacyl tRNA biosynthesis	3	127.957	0.000	0.010	0.001
Alanine aspartate and glutamate metabolism	3	11.120	0.000	0.009	0.000

Table S3.2. Continued.

Pathway	d.f.	F	p	Average	Standard Deviation
Amino sugar and nucleotide sugar metabolism	3	20.386	0.000	0.009	0.001
Energy metabolism	3	5.695	0.002	0.009	0.001
Chaperones and folding catalysts	3	3.068	0.039	0.009	0.001
DNA replication proteins	3	28.171	0.000	0.009	0.001
Glyoxylate and dicarboxylate metabolism	3	22.080	0.000	0.008	0.001
Protein folding and associated processing	3	12.453	0.000	0.008	0.000
Fatty acid metabolism	3	29.394	0.000	0.008	0.001
Cysteine and methionine metabolism	3	215.15 2	0.000	0.008	0.001
Transcription machinery	3	39.762	0.000	0.008	0.001
Lipid biosynthesis proteins	3	19.529	0.000	0.008	0.000
Citrate cycle TCA cycle	3	67.099	0.000	0.008	0.001
Valine leucine and isoleucine biosynthesis	3	80.605	0.000	0.007	0.000
Translation proteins	3	18.640	0.000	0.007	0.001
Nitrogen metabolism	3	0.380	0.768	0.007	0.000
Replication recombination and repair proteins	3	31.002	0.000	0.007	0.001
Phenylalanine tyrosine and tryptophan biosynthesis	3	69.961	0.000	0.007	0.001
Tryptophan metabolism	3	33.874	0.000	0.007	0.001
Pentose phosphate pathway	3	18.076	0.000	0.007	0.000
Bacterial secretion system	3	12.533	0.000	0.007	0.001
Homologous recombination	3	4.706	0.007	0.006	0.001
Membrane and intracellular structural molecules	3	55.803	0.000	0.006	0.001
Lysine biosynthesis	3	147.24 2	0.000	0.006	0.001
Histidine metabolism	3	15.350	0.000	0.006	0.000
Mismatch repair	3	5.271	0.004	0.006	0.000
Lysine degradation	3	37.371	0.000	0.006	0.001
Pantothenate and CoA biosynthesis	3	2.572	0.068	0.006	0.000
Fructose and mannose metabolism	3	61.950	0.000	0.006	0.001
Flagellar assembly	3	13.319	0.000	0.006	0.001
Peptidoglycan biosynthesis	3	17.033	0.000	0.006	0.001
Benzoate degradation	3	16.820	0.000	0.005	0.001
Bacterial chemotaxis	3	3.523	0.024	0.005	0.001
Fatty acid biosynthesis	3	18.244	0.000	0.005	0.000

Table S3.2. Continued 2.

Pathway	d.f.	F	p	Average	Standard Deviation
Carbon fixation in photosynthetic organisms	3	11.236	0.000	0.005	0.000
beta Alanine metabolism	3	37.858	0.000	0.005	0.001
One carbon pool by folate	3	9.586	0.000	0.005	0.000
Starch and sucrose metabolism	3	8.593	0.000	0.005	0.001
Terpenoid backbone biosynthesis	3	47.305	0.000	0.005	0.000
Protein export	3	19.415	0.000	0.005	0.000

CHAPTER 4: SUPPLEMENTAL INFORMATION

Table S4.1. Pairwise comparisons between carapace, gut, surface and burrow sediment bacteria using SIMPER analysis. Average relative abundance is shown for each sediment type (first column corresponds to the first type in the compared pair, second column for the second). Taxonomic classification is shown to the deepest assignment for each OTU. OTUs representing approximately 30% of the variation are shown fro each comparison.

VS	Phylum	Family	Relative abundance		Contribution (%)	Cumulative contribution
Surface vs. Gut	Bacteroidetes	Chitinophagaceae	0.0139	0.3140	0.1725	0.1889
	Firmicutes	Bacillaceae	0.2270	0.0203	0.1291	0.3303
	Proteobacteria	Halomonadaceae	0.1714	0.0265	0.1016	0.4416
	Proteobacteria	Rhodobacteraceae	0.0446	0.1320	0.0810	0.5302
	Bacteroidetes	Balneolaceae	0.0952	0.0034	0.0581	0.6733
	Firmicutes	Staphylococcaceae	0.0062	0.0706	0.0408	0.7180
	Firmicutes	Halanaerobiaceae	0.0587	0.0008	0.0347	0.7559
	Actinobacteria	Corynebacteriaceae	0.0126	0.0517	0.0339	0.7931
	Proteobacteria	Alteromonadaceae	0.0437	0.0044	0.0248	0.8202
	Bacteroidetes	Flavobacteriaceae	0.0370	0.0027	0.0227	0.8451
	Proteobacteria	Idiomarinaceae	0.0312	0.0049	0.0173	0.8640
	Proteobacteria	Rhodospirillaceae	0.0246	0.0006	0.0152	0.8807
	Proteobacteria	Desulfobacteraceae	0.0180	0.0006	0.0112	0.8929
	Actinobacteria	Micrococcaceae	0.0026	0.0142	0.0091	0.9029

Table S4.1. Continued.

VS	Phylum	Family	Relative abundance		Contribution (%)	Cumulative contribution
Burrow vs. Surface	Firmicutes	Bacillaceae	0.2270	0.1190	0.1423	0.1994
	Proteobacteria	Halomonadaceae	0.1714	0.0858	0.0894	0.3248
	Proteobacteria	Idiomarinaceae	0.0312	0.1590	0.0810	0.4384
	Bacteroidetes	Balneolaceae	0.0952	0.0276	0.0576	0.5191
	Proteobacteria	Alteromonadaceae	0.0437	0.0959	0.0401	0.5754
	Firmicutes	Halanaerobiaceae	0.0587	0.0214	0.0352	0.6246
	Proteobacteria	Rhodobacteraceae	0.0446	0.0438	0.0334	0.6715
	Bacteroidetes	Flavobacteriaceae	0.0370	0.0253	0.0247	0.7061
	Proteobacteria	Desulfobacteraceae	0.0180	0.0334	0.0224	0.7375
	Proteobacteria	Pseudoalteromonadaceae	0.0076	0.0332	0.0198	0.7653
	Proteobacteria	Oceanospirillaceae	0.0044	0.0310	0.0171	0.7892
	Proteobacteria	Rhodospirillaceae	0.0246	0.0103	0.0148	0.8100
	Proteobacteria	Halothiobacillaceae	0.0102	0.0186	0.0123	0.8272
	Bacteroidetes	Chitinophagaceae	0.0139	0.0070	0.0115	0.8433
	Proteobacteria	Chromatiaceae	0.0087	0.0121	0.0100	0.8713
	Cyanobacteria	Synechococcaceae	0.0114	0.0077	0.0095	0.8847
	Actinobacteria	Corynebacteriaceae	0.0126	0.0027	0.0086	0.8967
	Proteobacteria	Methylococcaceae	0.0090	0.0047	0.0074	0.9071
Surface vs. Carapace	Proteobacteria	Rhodobacteraceae	0.0446	0.2542	0.1270	0.1548
	Firmicutes	Bacillaceae	0.2270	0.0039	0.1231	0.3048
	Proteobacteria	Halomonadaceae	0.1714	0.0267	0.0853	0.4087
	Bacteroidetes	Balneolaceae	0.0952	0.0050	0.0557	0.4767
	Bacteroidetes	Flavobacteriaceae	0.0370	0.1081	0.0535	0.5418
	Firmicutes	Halanaerobiaceae	0.0587	0.0058	0.0335	0.5826
	Proteobacteria	Alteromonadaceae	0.0437	0.0685	0.0325	0.6222
	Proteobacteria	Pseudoalteromonadaceae	0.0076	0.0543	0.0311	0.6601
	Actinobacteria	Corynebacteriaceae	0.0126	0.0443	0.0294	0.7331
	Proteobacteria	Idiomarinaceae	0.0312	0.0349	0.0241	0.7625
	Bacteroidetes	Chitinophagaceae	0.0139	0.0311	0.0239	0.7916
	Firmicutes	Staphylococcaceae	0.0062	0.0284	0.0178	0.8133
	Proteobacteria	Oceanospirillaceae	0.0044	0.0293	0.0171	0.8341
	Bacteroidetes	Saprospiraceae	0.0000	0.0260	0.0153	0.8527
	Proteobacteria	Moraxellaceae	0.0000	0.0281	0.0150	0.8710
	Proteobacteria	Rhodospirillaceae	0.0246	0.0014	0.0147	0.8888
	Proteobacteria	Desulfobacteraceae	0.0180	0.0010	0.0106	0.9018

Table S4.1. Continued 2.

VS	Phylum	Family	Relative abundance		Contribution (%)	Cumulative contribution
Burrow vs. Gut	Bacteroidetes	Chitinophagaceae	0.3140	0.0070	0.1795	0.1945
	Proteobacteria	Idiomarinaceae	0.0049	0.1590	0.0929	0.2951
	Proteobacteria	Rhodobacteraceae	0.1320	0.0438	0.0856	0.3878
	Firmicutes	Bacillaceae	0.0203	0.1190	0.0738	0.5505
	Proteobacteria	Halomonadaceae	0.0265	0.0858	0.0582	0.6135
	Proteobacteria	Alteromonadaceae	0.0044	0.0959	0.0570	0.6752
	Firmicutes	Staphylococcaceae	0.0706	0.0035	0.0423	0.7210
	Actinobacteria	Corynebacteriaceae	0.0517	0.0027	0.0311	0.7547
	Proteobacteria	Desulfobacteraceae	0.0006	0.0334	0.0213	0.7778
	Proteobacteria	Pseudoalteromonadaceae	0.0009	0.0332	0.0197	0.7991
	Proteobacteria	Oceanospirillaceae	0.0008	0.0310	0.0180	0.8186
	Bacteroidetes	Balneolaceae	0.0034	0.0276	0.0168	0.8368
	Bacteroidetes	Flavobacteriaceae	0.0027	0.0253	0.0151	0.8532
	Firmicutes	Halanaerobiaceae	0.0008	0.0214	0.0135	0.8679
	Proteobacteria	Halothiobacillaceae	0.0002	0.0186	0.0123	0.8812
	Actinobacteria	Micrococcaceae	0.0142	0.0003	0.0085	0.8904
	Proteobacteria	Chromatiaceae	0.0015	0.0121	0.0083	0.8994
	Firmicutes	Streptococcaceae	0.0123	0.0002	0.0077	0.9078
Gut vs. Carapace	Bacteroidetes	Chitinophagaceae	0.3140	0.0311	0.1669	0.2089
	Proteobacteria	Rhodobacteraceae	0.1320	0.2542	0.1494	0.3959
	Bacteroidetes	Flavobacteriaceae	0.0027	0.1081	0.0648	0.5612
	Firmicutes	Staphylococcaceae	0.0706	0.0284	0.0456	0.6183
	Actinobacteria	Corynebacteriaceae	0.0517	0.0443	0.0424	0.6713
	Proteobacteria	Alteromonadaceae	0.0044	0.0685	0.0393	0.7205
	Proteobacteria	Pseudoalteromonadaceae	0.0009	0.0543	0.0326	0.7613
	Proteobacteria	Halomonadaceae	0.0265	0.0267	0.0261	0.7940
	Proteobacteria	Idiomarinaceae	0.0049	0.0349	0.0211	0.8204
	Proteobacteria	Moraxellaceae	0.0100	0.0281	0.0208	0.8465
	Proteobacteria	Oceanospirillaceae	0.0008	0.0293	0.0178	0.8687
	Bacteroidetes	Saprospiraceae	0.0034	0.0260	0.0163	0.8891
	Firmicutes	Bacillaceae	0.0203	0.0039	0.0126	0.9048

Table S4.1. Continued 3.

VS	Phylum	Family	Relative abundance		Contribution (%)	Cumulative contribution
Burrow vs. Carapace	Proteobacteria	Rhodobacteraceae	0.0438	0.2542	0.1351	0.1737
	Proteobacteria	Idiomarinaceae	0.1590	0.0349	0.0859	0.2840
	Firmicutes	Bacillaceae	0.1190	0.0039	0.0673	0.3705
	Bacteroidetes	Flavobacteriaceae	0.0253	0.1081	0.0552	0.4415
	Proteobacteria	Alteromonadaceae	0.0959	0.0685	0.0456	0.5001
	Proteobacteria	Halomonadaceae	0.0858	0.0267	0.0443	0.5570
	Proteobacteria	Pseudoalteromonadaceae	0.0332	0.0543	0.0364	0.6038
	Proteobacteria	Oceanospirillaceae	0.0310	0.0293	0.0265	0.6767
	Actinobacteria	Corynebacteriaceae	0.0027	0.0443	0.0258	0.7098
	Bacteroidetes	Chitinophagaceae	0.0070	0.0311	0.0206	0.7363
	Proteobacteria	Desulfobacteraceae	0.0334	0.0010	0.0205	0.7626
	Firmicutes	Staphylococcaceae	0.0035	0.0284	0.0174	0.7850
	Proteobacteria	Moraxellaceae	0.0012	0.0281	0.0162	0.8058
	Bacteroidetes	Saprospiraceae	0.0001	0.0260	0.0160	0.8264
	Bacteroidetes	Balneolaceae	0.0276	0.0050	0.0158	0.8467
	Firmicutes	Halanaerobiaceae	0.0214	0.0058	0.0137	0.8642
	Proteobacteria	Halothiobacillaceae	0.0186	0.0013	0.0114	0.8789
	Bacteroidetes	Cryomorphaceae	0.0113	0.0083	0.0088	0.8902
	Proteobacteria	Chromatiaceae	0.0121	0.0000	0.0076	0.8999
	Proteobacteria	Vibrionaceae	0.0012	0.0119	0.0074	0.9094

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