Potential effects of different salinities on the survival of the mangrove crab, *Uca urvillei* and its associated chemoautotrophic bacterial symbionts

by

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ABSTRACT

The ability of natural populations to maintain fitness is important to their long-term persistence and has further relevance in the light of climate change scenarios. Fitness is however also influenced strongly by interactions with other species of the community. It is, therefore, important to focus on how environmental change can alter key biological interactions. The present study aimed to investigate the effects of different salinity levels on one species of mangrove crab, *Uca urvillei* and the associated chemoautotrophic bacterial symbionts, from the Mngazana estuary, South Africa. To examine the influence of different salinities over time on the symbiotic bacterial community, salinity experiments were set up, choosing three salinity exposures (5, 20 and 35‰) and four time exposures (3, 7, 14 and 21 days). The results showed that microbial community associated with *U. urvillei* was generally stable throughout the three salinity treatments, while the survival of the host (crab), was influenced by high salinities, particularly after day 14. Overall, the results of this study suggest that over time, environmental salinity (35‰) has the potential to affect significantly the physiology of *U. urvillei*, but this might not necessarily be the case for the associated microbial communities.

The modes at which the significant symbionts are transferred from mother to offspring were also investigated by analysing the bacterial profiles from eggs, ovigerous and non-ovigerous females, along with mud. The results were fairly complex, but with significant differences in the bacterial communities of eggs from mud and females. These differences were driven mostly by two dominant phyla: Actinobacteria and Proteobacteria. The presence of both these phyla throughout the categories (even though in different percentages) suggest that *U. urvillei* might employ a mixed mode strategy of acquiring and maintaining the bacterial symbionts. Overall, this study contributes to further understand the dynamic and complex effects of environmental

change on symbiotic communities, with overall potential cascading repercussions to the persistence of mangrove systems.

Declaration

I, Tumeka Mbobo with student number 200907045, hereby declare that the treatise/dissertation/thesis for "my qualification" is my own work and that it has not previously been submitted for assessment to another university or for another qualification.

Signature: Date: June 2015

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Ethics statement

All samples were collected following ethical regulations for collection of animals samples (SAIAB Ethics Clearance, reference number: 2014/07).

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Chapter 1: General introduction

Natural ecosystems are governed by the persistence of species that have adapted over millennia to certain climatic limits (Midgley *et al.*, 2005). The alteration of such limits would, at times, result in increased extinction rates and consequently lead to the collapse of entire ecosystems (Hoegh-Guldberg and Bruno, 2010). Climate change, on the other hand, is as old as the atmosphere itself, where the earth has swung through a variety of states in which life has prospered or experienced catastrophic declines (Lugo, 2000). Because of climatic unpredictability, species have evolved a certain range of tolerance to various abiotic climate-related factors, and such adaptations contributed to the persistence of such species in their landscape (Spicer and Gaston, 1999). The current changes in global climate between now and the next decades are, however, likely to be dominated by the influence of greenhouse effects caused by increasing concentrations of greenhouse gases (Simas *et al.*, 2001; Ramanathan and Feng, 2009). This will have a significant impact, and represents one of the most distinct threats to several ecosystems (Hughes, 2003; Blenckner, 2005; Pittock *et al.*, 2008; Gillanders *et al.*, 2011; Spalding *et al.*, 2014). These changes will likely create physical and biological conditions not previously experienced in the evolutionary history of most organisms (Franks and Hoffman, 2012). Thus, global climate change is recognised as one of the greatest challenges that humans are facing this century (Stern *et al.*, 2006). It incorporates changes in temperature, rainfall and evaporation rates, sea level rise and storm frequency (Roessig *et al.*, 2004).

The ecological consequences of global climate change have become apparent in the last decades, with noticeable changes in all levels of ecological organization: population and life history changes, shifts in geographic range, changes in species composition of communities, and changes in the structure and functioning of ecosystems (McCarty, 2002; Parmesan and

Yohe, 2003; Thomas *et al*., 2004; Blenckner, 2005; Adger *et al.*, 2005; Both *et al.*, 2006; IPCC, 2013; Tylianakis *et al.*, 2008; Yang ad Rudolf, 2010; Hoffmann and Sgró, 2011).

Every species on earth is involved directly or indirectly in one or more symbiotic partnership, where different kinds of organisms often play a very important ecological role in each other's lives (Kiers *et al.*, 2010). Examples of symbioses include those from reef building corals and their associated dinoflagellate algae; the diverse array of pollinators that mediate sexual reproduction in many plant species; even down to the myriad nutritional symbionts that fix nitrogen and aid digestion (e.g Herre *et al.*, 1999; Douglas, 2010; Kiers *et al.,* 2010). Symbiotic associations between bacteria and multicellular organisms date back to as early as approximately 0.9 billion years ago and, today, they are common in nature (Oliver *et al.,* 2003). They are found in numerous ecosystems, described across many taxa, with both partners interacting in ways that are vital to the functioning of both organisms (Dimijian, 2000; Ruby et al., 2004; Gilbert *et al.* 2010; Dmytrenko *et al*., 2014). The interactions between two symbiotic organisms have had central roles in the evolution of eukaryotic life and life itself (Nussbaumer *et al.*, 2006; Douglas, 2010; Roeselers and Newton, 2012), and are ecologically important, and are profoundly influential at all levels of biological organization (Herre *et al*., 1999). Symbiotic interactions in nature are central to the survival and reproduction of multitudes of organisms, providing essential ecosystem functions, such as seed dispersal, and they are involved in constituting critical components of global carbon and nutrient cycles (Kiers *et al.*, 2010). De Bary (1878, in McFall-Ngai 2002) was the first to coin the term symbiosis to describe these close associations and defined it as the living together of unlike named organisms. This is the more inclusive definition of the term, where symbiosis is viewed as an umbrella concept that applies to all types of bacteria-animal associations (McFall-Ngai, 2002), irrespective of the influence that one organism might have on the other (Taylor *et al*., 2001).

Nonetheless, over the past years, confusion has afflicted the definition (Douglas, 1994; Margulis and Chapman, 1998; Moran and Dunbar 2006; Martin and Schwab, 2012), with researchers arguing that symbiosis is a continuum from mutualism to commensalism; others maintaining the common restrictive definition (that symbiosis means mutualism). Davy *et al.,* (2012) further report that more than often the boundary lines between the two proposed symbiotic categories are not clear and that there are frequent transitions between them. Additionally, Carrapico (2010) emphasized that symbiosis should not only be viewed as a beneficial process for both organisms involved, but rather as a complex, continuous and dynamic equilibrium of relations such as mutualism, parasitism and commensalism.

The degree of interaction between the symbiont and its host can vary from very "loose" and temporary, to highly specific and permanent associations (Moya *et al.*, 2008). The temporal and "loose" associations are often termed facultative symbiosis where the organisms give each other a greater chance of survival, but the interaction is not absolutely necessary (Oliver *et al*., 2010). In obligate symbiosis, on the other hand, the organisms rely on the symbiotic relationship in order to survive (Byler *et al.*, 2013; Hosokawa *et al.*, 2015). For example, almost all aphids possess *Buchnera aphidicola*, an obligate endosymbiotic bacterium that provides the host with essential amino acids and other nutrients (Koga *et al.*, 2003; 2012; Shigenobu and Wilson, 2011), and without the symbionts aphids suffer sterility and/or death (Houk and Griffiths, 1980). The interdependence of symbiotic partners has, over the past, led to diverse evolutionary opportunities; however, the cost it carries is very high (Prado *et al.,* 2006; Kiers *et al.*, 2010). Colwell *et al*. (2012) report that the extinction of a single species is rarely an isolated event and, because species in ecological communities build complex webs of interactions, any change in one species severely threatens the ecology and evolution of the involved partners, as well as the associated communities (McFall-Ngai *et al*., 2013; Ellison *et*

al., 2005). In symbiotic associations, the dependent parasites, commensals and mutualistic partners face the risk of extinction as their partners or hosts decline or fail. It is predicted that the decline or extinction of one symbiotic partner can lead to the reduction in fitness or in extreme cases, co-extinction of its partner, particularly where the association is highly specific and obligate (Colwell *et al.*, 2012; Dattilo, 2012). This, in turn, can potentially trigger a cascade of linked extinctions through the ecological community (Dunn *et al.*, 2009; Colwell *et al.*, 2012). Symbioses are, however, remarkably persistent evolutionarily, including through major climatic changes (Douglas, 2007; 2010), with a certain host species consistently associating with a specific symbiont. The current anthropogenically driven changes of the environment are, however occurring at much faster rates than previous climatic alterations causing local decline of species, altering community composition and, consequently, ecosystem functioning (Bertrand *et al.*, 2011). Several studies suggest that these environmental changes may disturb the balance between interacting species, leading to the decline or extinction of one or more species (Crozier *et al.*, 2005; Biesmeijer *et al.*, 2006; Tylianakis et al., 2008; Ockendon *et al.*, 2014), because how and when these species respond might not always be parallel (Crozier *et al.*, 2005). Moreover, how one species responds to an environmental disturbance depends on how it interacts ecologically with other species in the ecosystem (Harmon *et al.*, 2009). For example, if a competitively dominant species is sensitive to a disturbance, then a competitively subordinate species may benefit indirectly from the disturbance through the exclusion of the dominant species (Harmon *et al.*, 2009). At the current rate of climate change, systems with species involved in symbiotic interactions have been altered, in most cases with devastating effects on ecosystems (Eakin *et al.*, 2008). For example, the symbiosis between reef-building corals and their associated dinoflagellate microalgae, (Jones *et al.*, 2008) has been threatened by increase in coral bleaching and mortality, associated with rising seawater temperatures (Hoegh-Guldberg, 1999). Future environmental changes are likely to be even more severe and

have the potential to enhance or reduce the fitness of partners involved in interactions (Six, 2009). Given such scenario, it is reasonable to expect a similar fate for important ecosystems, such as mangrove forests and the associated fauna. In response to sea level rise for example, mangroves are predicted to move inland, with further environmental alterations and forcing, but what will happen to the associated mangrove biota? Both *et al.* (2006) report that even with moderate climate changes, the ability of several organisms to migrate and adapt will be challenging. Now the question is: can the mangrove biota move fast enough to keep track with their associated habitat or are they likely to adapt to the local environmental conditions? Hoffmann and Sgrò (2011) suggest that, in order for species to persist despite climate change, they need to disperse rapidly enough to track moving climate conditions, adapt to local conditions or respond through plasticity. This is true for individual species that interact with other species and where the interaction does not necessarily determine the survival chances of either or both (Dáttilo, 2012), but can species in obligate symbiosis (host and endosymbiont) co-adapt fast enough and at the same speed, or do they face co-extinction? For example, logically the mangrove biota is expected to move with the mangroves or at least try to keep track, but it if the associated bacterial symbionts will move at the same speed, especially if they are to be acquired occasionally from the surrounding environments remains unclear. Furthermore, it is expected that species that are strictly tolerant to saline or freshwater conditions be more impacterd by these changes (Rahel and Olden, 2008), should the estuarine environment dominated by mangrove ecosystem become more or less saline due to sea level rise and/or changes in freshwater input. This set of threats represents an important challenge for ecologists, especially in a context of conservation.

The present thesis aimed to partially address some of these important questions and concepts, investigating the response of mangrove brachyurans, and their associated bacteria, to changes in salinity and the possible modes of transfer of bacteria symbionts.

Aim and hypotheses of the present study

Using molecular techniques, the study primarily aims at investigating and documenting temporal changes in the structure of the bacterial community residing within the gills of an ocypodid mangrove crab (*Uca urvillei*) and to relate this to changes in environmental conditions (salinity).

It was hypothesised that: the bacterial community harboured within the gill and hindgut tissues of *U. urvillei* undergoes temporal changes in abundance, composition and diversity driven by changes in salinity, and additionally,

if *U. urvillei* undergoes vertical transmission of bacterial symbionts, then the gills sampled from gravid females should have the same bacterial community as the eggs and be similar to that from gills of non-gravid females, whereas,

if *U. urvillei* undergoes horizontal transmission of bacterial symbionts, then the gills sampled from females, along with mud and water samples should have the similar bacterial strains.

Thesis outline

The thesis consists of four linked, but independent chapters. The two central chapters include respective introduction, materials and methods, results and discussion sections. Wherever the same methodology was used for the research reported in the empirical chapters, reference is made to their initial description.

Chapter 1: General introduction. This chapter provides the rationale for the study and the structure of the thesis.

Chapter 2: In this chapter, the effect of different time exposures to different sets of salinities on selected species of mangrove brachyurans, *Uca urvillei* and associated chemoautotrophic bacteria was examined.

Chapter 3: This chapter includes a study to investigate the possible modes of transfer of bacteria symbionts in *U. urvillei*.

Chapter 4: General discussion. The key findings are summarised, integrated and discussed within the broader topic introduced in Chapter one, on the links and possible effects of symbiosis and climate change.

Chapter 2: Potential effects of different salinities on the survival of the mangrove crab, *Uca urvillei* and its associated chemoautotrophic bacterial symbionts

Introduction

Mangroves are coastal ecosystems which occupy intertidal settings along estuaries or sheltered creeks in tropical, subtropical and temperate regions of the world (Duke, 1995; Alongi, 2002, 2008; Kristensen, 2008; Rajkaran and Adams, 2010). Mangroves are amongst the most productive and biologically important coastal systems (Giri *et al*., 2011), supporting a variety of benthic invertebrates including gastropods, bivalves, barnacles and crustaceans (Cannicci *et al.,* 2008; Nagelkerken *et al.,* 2008). They provide primary nursery areas for commercially and ecologically important species of fish and crustaceans, as well as habitat for insects. They afford critical refuge from predators and foraging opportunities for wildlife, fishes and invertebrates, supporting commercial and recreational livelihoods of many communities (Scavia *et al*., 2002; Satheeshkumar and Khan, 2012). The dominant macrofaunal inhabitants of mangroves, in terms of both numbers and biomass, are crabs (Smith *et al.,* 1991; Emmerson, 1994; Cannicci *et al*., 2008). In this context, any environmental disturbance, depending on its frequency and intensity, caused either by natural or anthropogenic factors, may put mangroves and their associated inhabitants in an unsustainable situation, harming the key life support processes (Duke *et al*., 2007; Gilman *et al*., 2008; Spalding *et al*., 2014; Alongi *et al*., 2014). Located at regions where marine and fresh waters meet, these areas experience great environmental variation (Harrison and Whitfield, 2006; *Whitfield et al*., 2008). They are governed by the interaction of various factors, including, wind, pH, temperature, tidal action, turbidity, currents and salinity, and are exposed to harsh conditions, including cyclical

flooding, high temperature variability and wide salinity fluctuations (Doyle *et al.,* 2003; Gomes *et al.*, 2008; Ghizelini *et al*., 2012). Each of these factors play an integral part in mangroves and can affect mangrove dynamics differently. Of these, salinity is considered an obvious, dominant and very important ecological parameter within these environments (Navarro, 1988; Teske and Wooldridge, 2003), with the potential to drastically influence the composition and dynamics of aquatic ecosystems (Carrasco and Perissinotto, 2012).

In estuaries, salinity determines the fauna and flora that can live in different zones of the system (Whitfield, 1995, 1998; Rowe, 2002). For mangrove crabs, their occurrence and distribution within an estuary is dictated by specific environmental parameters, including pH and temperature, but mostly salinity (Icely and Jones, 1978; Lawal-Are and Kusemuji, 2010). Salinity affects growth and survival of larvae of numerous decapod species. For example, zoeae of the fiddler crab, *Uca pugnax,* show high survival at high salinities (20 and 30‰), and comparatively low survival at 10‰ (O'Connor and Epifanio, 1985). Nurdiani and Zeng (2007) showed that larvae of *Scylla serrata*, a crab that spends most of its life in brackish, saltwater, generally tolerates a broad range of salinity. However, when zoea larvae are exposed to decreased salinities and either low or high temperature, survival rate decreases drastically, leading to mass mortalities (Nurdiani and Zeng 2007). Adult crabs, on the other hand, are believed to tolerate a wide range of salinities (Lawal-Are and Kusemuji, 2010). For example, adult burrowing ocypodid crab, *Paratylodiplax blephariskios* was shown to tolerate salinities of up to approximately 55 ppt (Owen and Forbes, 2002). The burrowing mangrove crab, *Neosarmatium africanum* (=*meinerti*, Ragionieri *et al.*, 2012), shows high survival rates at salinities between 16-65‰ (Gillikin *et al*. 2004). This suggests that there are broadly-adapted species that can acclimate to different salinities, ranging from fresh to sea water, especially as adults. Even so, organisms inhabiting estuarine environments have to adapt to unpredicted fluctuations between low and high salinities.

Salinity is an important ecological parameter in estuaries. It is amongst the most widely fluctuating physico-chemical parameters in coastal systems (O'Connor and Epifanio 1985; Whitfield 1998). In estuaries, the fluctuations can be sudden or gradual and are attributed to several factors including increased/decreased freshwater inflows, tide cycles and unpredicted rainfalls (droughts and/or floods) (Whitfield, 1998, Gardner and Thompson, 2001). This irregular rise and fall of salinity play a major part in the functioning of the entire estuarine system, and can drastically affect organismal physiological activities such as osmoregulation, respiration and excretion (Navarro, 1988). For example, Almada-Villela (1984), showed that fluctuating salinities resulted in depressed shell growth of small coastal mussel, *Mytilus edulis*. Rysgaard *et al.* (1999), demonstrated that NH_4^+ desorption from the Randers Fjord estuary sediment increased with increasing salinities, suggesting that fluctuating salinity in estuarine sediments plays a vital role in controlling adsorption capacity of the sediment. For many crustaceans, salinity is crucial for their survival. The effects of salinity changes have been observed in delayed development, reduced survival, as well as extended or suspended moulting cycles, metabolic shifts in energy partitioning resulting in altered behaviour (Anger *et al.*, 1998; Torres *et al.*, 2011).

With direct and indirect effects driven by climate change, such as global warming (Alongi, 2002) overexploitation and habitat degradation, coastal systems are at risk. The 2007 and 2013 Intergovernmental Panels on Climate Change (IPCC) confirm that warming of the climate is unambiguous with global air and water temperatures increasing, global melting of snow and ice, and worldwide rising of sea level. In estuarine environments, sea level rise (SLR) coupled with lack of freshwater, is among the main factors with the greatest effect on the functioning of natural systems.

With climate change, it is predicted that salinity fluctuations will further intensify as the rate of SLR accelerates, pushing more saline water into estuarine systems and exposing habitats to increasing inundation, erosion and saltwater intrusion (Kennedy *et al.* 2002; Scavia *et al.* 2002). Further changes in freshwater availability, precipitation and patterns of river flow (Scavia *et al*. 2002; Alber 2002; Doyle *et al*. 2003) may lead to major shifts in mangrove forest composition and structure, altered productivity and function of the entire ecosystems (Scavia *et al.* 2002; Doyle *et al*. 2003).

Fiddler crabs (Family Ocypodidae, genus *Uca*) characteristically dominate mangrove systems (Hartnoll *et al.*, 2002) and are widely distributed throughout the tropics, subtropics and temperate regions of the world (Crane, 1975; Rosenberg, 2001; Lim and Diong, 2003). They are essential biota of the mangroves, as they are considered bioturbating and bioengineering components of the mangrove fauna (Cannicci *et al*., 2008; Andreetta *et al*., 2014). Through burrow digging and maintenance activities, they create aerobic soil conditions (Kristensen and Alongi, 2006), and they convert intertidal organic matter into small sizes for several predatory organisms that are both of marine and terrestrial origin (Litulo, 2004). As estuarine inhabitants, these crabs are frequently subjected to changing salinities over short (tidal) and long (seasonal) cycles. Because mangroves are rich in hydrogen sulphide, a toxic chemical that inhibits aerobic respiration, their inhabitants require several adaptations to thrive in these rather challenging habitats. These include respiratory, morphological and/ or physiological adaptations. The fiddler crab, *Uca urvillei* is a mangrove associated crab adapted to semi-terrestrial life (Santos *et al.*, 1987; Anger, 1995) that is diurnally active, only retreats from the burrows at low tide (Crane, 1975; Kristensen, 2008), and use their burrows as refuge when environmental conditions become unfavourable, for example, at high tide. It has been recently found that *U. urvillei* harbour bacterial symbionts in the gills and hindguts, suggesting that these bacteria aid in the midst of H_2S (Fusi, 2014). These bacteria, which clustered primarily within the

Proteobacteria and Actinobacteria, seem to be affiliated to chemoautotrophic species groups, suggesting that chemosynthesis might be one of the key adaptations employed by mangrove crabs to survive in such challenging environment, as their presence is associated to challenging environments (Dubilier *et al*., 2008).

Mangrove ecosystems harbour diverse and unique microbial communities that play various and important roles, including nutrient cycling, and the regulation of the chemical environment, as well as various environmental activities (Alongi *et al.*, 1993; Holguin *et al*., 2001; Flores-Mireles *et al.*, 2007; Kannan and Vincent, 2011; Gomes *et al*., 2011; Andreote *et al.*, 2012). Specifically, bacteria are reported to have a significant role in formation of detritus in the mangrove ecosystem, and many of them are extremely important in controlling the chemical environment of the mangrove sediments (Alongi *et al.*, 1993). In aquatic ecosystems, it is generally suggested that major differences in the taxonomic composition of bacterial assemblages between freshwater and marine ecosystems exist (Bouvier and del Giorgio, 2002; Kemp and Aller, 2004). Such systematic differences are often correlated with a salinity gradient (Jiang *et al*., 2007). For example, rivers and lakes are generally dominated by Beta-Proteobacteria, whereas Alpha-Proteobacteria are abundant in the marine environment (Glockner *et al*., 1999). The degree of bacterial diversity in estuaries is expected to be high due to a combination of the mixing of seawater and freshwater (Crump *et al.,* 2004; Bharathkumar *et al*., 2008), with salinity acting as a physiological driver/barrier which influences the distribution, zonation and abundance of bacterial taxa within estuaries (Tang *et al.,* 2012). The current study aims to investigate potential effects of different salinity regimes on the overall survival of the crab and on the symbiont bacteria associated with the fiddler crab *Uca urvillei* collected from Mngazana Estuary, a South African subtropical estuarine system.

Materials and methods

Study site: In order to investigate the effects of salinity on mangrove crabs and the associated bacterial symbionts, samples used in this study were collected from Mngazana Estuary (31˚41'29"S, 29˚25'24"E), a mangrove estuary located on the east coast of South Africa (Figure 2.1). The estuary permanently maintains its connection with the sea and is approximately 5.3km in length. The Mngazana River flows through 275 km^2 of catchment before discharging into the Indian Ocean, and the area receives rain throughout the year, with maximum rainfall occurring during spring and summer from October to March. The average rainfall is approximately 1.034 ± 25.1 mm-year^{-1} (Rajkaran and Adams, 2010; Deyzel, 2013). In addition, the area receives two shallow tributaries, also known as creeks. The water temperatures range from a mean of 18.6 ± 1.1 °C in winter to 25 ± 1.8 °C in summer, and salinity levels vary from $0 - 36$‰ in winter and $2 - 37$‰ in summer, with discrepancies along different regions within the system (Deyzel 2013). The estuary is, however, generally marine-dominated with high salinity levels ranging from 25 to 37 ppt (Grant, 2007).

Amongst southern African estuaries, the Mngazana Estuary is ranked 15th in the top 40 in terms of biodiversity importance (Turpie *et al.* 2004), supporting a rich diversity of both invertebrate and fish species (Whitfield and Baliwe 2013). It supports the 3rd largest mangrove forest in South Africa, with three main mangrove tree species: *Rhizophora mucronata* (red mangrove), *Avicennia marina* (white mangrove) and *Bruguiera gymnorrhizza* (black mangrove) (Rajkaran *et al.* 2004).

The Mngazana Estuary is located at the edge of its sub-tropical distribution, lying approximately 25km north of the proposed boundary between the warm temperate and sub-tropical biogeographic provinces (Grant, 2007). It represents one of the southernmost mangrove systems for the African continent and in the world (MacNae, 1963; Emmerson,

1990). According to James *et al.* (2008), systems in transitional areas between biogeographic zones are likely to be particularly vulnerable and, with the onset of predicted global climate change, these regions will even be further compounded. This makes the system an ideal choice to test the possible exacerbation of physical stressors on the metabolism of species occupying the latitudinal edge of the mangrove distribution.

Sampling methods Adult males of *Uca urvillei* (Figure 2.2) were hand-collected in November 2013, from the intertidal zone of various sites within the Mngazana Estuary, during low tide. Animals were transported to the laboratory in plastic containers with mud, leaves and estuarine water, also collected from the Mngazana Estuary. Ice packs were used to prevent overheating and keep the animals cool during transportation.
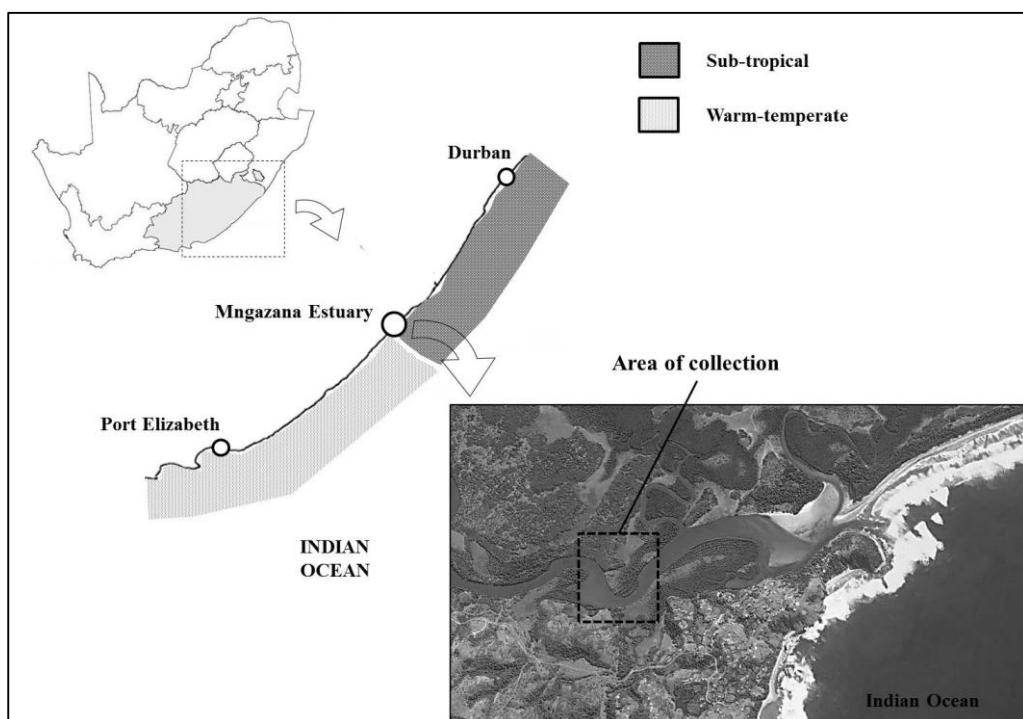
Figure 2.1: The position of Mngazana Estuary along the South African coast in relation to the biogeographic zones (satellite image adapted from Fusi, 2014)

Figure 2.2: An adult male *Uca urvillei* (photo by Bruce Mostert, 2013)

Salinity exposure experiments. All the experiments for the study were conducted in the laboratory at the Department of Ichthyology and Fisheries Sciences (DIFS), Rhodes University, Grahamstown. A total of 144 crabs were used for the experiment. The crabs ranged in size from 2.0 to 3.4 cm carapace width and had weight of 4.4 to 13.7 g. The crabs were acclimated for 48 hours at 20 ± 2°C and a constant 12 hours dark and 12 hours light photoperiod, roughly approximating the natural environment. On day 3, each individual crab was put in a plastic container (aquarium), exposed to one of three experimental salinity regimes (5 ‰, 20 ‰ and 35 ‰) (Figure 2.3). Each treatment and time interval consisted of 6 replicates (Table 2.1). Each aquarium contained a 5 cm layer of sediment and 1.5 litres of water with one of the salinity treatments. Under these conditions, the animals were free to enter or leave the water, but the sediment was saturated with the desired salinity and, ensured that at some stage crabs would have passively or actively experienced such salinity. The 5‰ salinity treatment was selected because during flooding events the salinity of the Mngazana Estuary generally decreases to approximately 2‰ (Deyzel, 2013). The 35‰ is the salinity experienced by these animals in the field, while 20‰ is the intermediate salinity. The desired salinities were achieved by mixing deionised water with filtered (0.2 µm pore-size) sea water (Gillikin *et al.,* 2004; Miranda *et al.,* 2010). Half of the crabs were inoculated via the haemolymph with 0.1

mL of a broad spectrum antibiotic, chloramphenicol (Zymo Research), with a dilution rate of 1:1000, was used to create a control treatment at each salinity. The crabs were injected once a week to minimise stress levels, especially considering that they were kept and monitored in captivity over a long time exposure. Continuous aeration was provided in each aquarium/container using air pumps with rubber tubing fitted with air stones. Salinity was measured daily using a hand-held Atago refractometer. Ammonium was measured using Tetra Test NH3/NH4$^+$, Tetra (GmbH), Germany. This was done four times throughout the experiment to monitor possible contamination of the aquaria by excretion. Crabs were fed cat pellets and the water was changed every two days. The experiment ran for 21 days. The first two time intervals, 3 and 7 days, represented "short term" and the last two represented, 14 and 21 days, "long term" exposures (Table 2.1). Mortality was monitored daily and it was defined as the inability of the crabs to respond to external stimuli.

Table 2.1: Experimental scheme of the salinity experiments. Time exposure (days) of the *Uca urvillei* adult males to different salinity regimes. For each treatment, there were 6 replicates. Animals injected with an antibiotic are marked as A, those without antibiotic are indicated by NA.

Time intervals (days)	3	7	14	21
Salinity (‰)				
5A	6	6	6	6
5NA	6	6	6	6
20A	6	6	6	6
20NA	6	6	6	6
35A	6	6	6	6
35NA	6	6	6	6

Figure 2.3: Individual aquaria used in the experimental design with one adult male crab inside. The crabs were able to choose between being in water or out

DNA extraction and Amplification. At the end of each planned time interval (3 to 21 days), animals were freshly weighed, their metabolism lowered on ice for 15 minutes and finally preserved in 95% ethanol and stored at room temperature. Carapace length (CL), carapace width (CW), cheliped length (CL) and propodus width (PW) were measured using plastic callipers prior to their dissection. DNA was extracted from gill tissue using a Wizard Promega DNA extraction kit (Promega Corporation, Madison, USA) following manufacturer's protocol, and Phenol: chloroform (Sambrook and Russel, 2001) methods. Unlike the PowerWater® Sterivex™ DNA Isolation Kit (and some other kits), these extraction kits do not only extract bacterial DNA. Therefore, initially we extracted both crabs and bacterial DNA. To exclude

possible amplification of eukaryotic rDNA, specific primers for domain bacteria were used during polymerase chain reaction (PCR).

PCR was used for amplification of the V2 and V3 regions of the bacterial 16S rDNA gene fragment using 907R and 357F primer set without the GC clamp (Muyzer *et al*., 1993). PCR reaction was performed in 0.2 ml tubes using a 25 µL reaction volume. The reaction mixture contained 2.5 µL 10X buffer, 2.5 µL 2.5 Mm MgCl$_2$, 2.5 µL 0.8 Mm dNTPs, 0.5 µL 0.2mM each primer, 0.2 µL 1 U *Taq* polymerase (FIREPol DNA polymerase, Solis BioDyne, Estonia), 11.3 µL distilled water and 5 µL of template. Cycling conditions used to amplify the 16S rDNA gene fragment were: 94 ºC for 4 minutes, 65 ºC for 1 minute; followed by 27 cycles of 94 ºC for 0.5 minutes, 5 cycles of 66-55 ºC for 0.5 minutes and 72 ºC for 0.5 minutes; followed by a further 5 cycles of 94 ºC for 0.5 minutes, 55 ºC for 0.5 minutes and 72 ºC for 0.5 minutes. A final extension of 72 ºC for 3 minutes was included. To confirm successful amplification of the 16S rDNA gene, 5 µL of the PCR products were visualised by electrophoresis in 1% agarose gel (final concentration of 0.05 µg/ml) with Tris acetate EDTA (TAE) 1X buffer, stained with 5 µL of ethidium bromide. The bands were visualised under UV light, using an UV transilluminator. 20 µL DNA aliquots were sent for Ion Torrent PGM sequencing to the Central Analytical Facilities (CAF), DNA Sequencing Unit, University of Stellenbosch, South Africa.

Library preparation. The 16S rDNA gene fragment is widely used for standard classification and identification of microbes as it is present in most bacteria at high copies (Rajendhran and Gunasekaran 2010). The gene contains nine highly variable regions (V1-V9); each of these demonstrate considerable sequence diversity among different bacteria. It has been proven however, that no single hyper-region is able to distinguish among all bacteria and it is advised that a combination of two or more regions be used (Charkravorty *et al.*, 2007). The V4-V5 regions of the 16S rDNA gene have high sequence variability and are sensitive enough to

identify diverse groups of bacterial taxa (Charkravorty *et al.*, 2007). The two hyper variable regions, V4 and V5, were, therefore, amplified by the fusion polymerase chain reaction (PCR) method. This method uses fusion primers to attach the Ion A and truncated P1 (trP1) adapters to the amplicons as they are generated in PCR.

The 16S rDNA gene fragments were amplified from extracted DNA using two differently barcoded forward primers, 338f (5´-ACTCCTACGGGAGGCAGCA-3´) and 802r (5´-ACTACCAGGGTATCTAATCCTG-3´). PCR was performed in 50 µL reaction volumes, using 44.0 µL of 2X KAPA HiFi HotStart ReadyMix, 5.0 µL of genomic DNA, and 1.0 µL of a 10 µM primer stock mix. The cycling conditions used to amplify the 16S rDNA were: 94ºC for 3 minutes, followed by 30 cycles of denaturing at 94ºC for 0.5 minutes, annealing at 58ºC for 0.5 minutes and extension at 68ºC for 1 minute. Following amplification, all PCR products were checked for size and specificity by electrophoresis in 2% w/v agarose and gel purified (E-Gel Size Select by Invitrogen). During this time, gene fragments between 400-600 bp were selected. Prior to sequencing, all the purified amplicons were assessed for fragment size distribution and DNA concentration using a Bioanalyzer 2100 (Agilent Technologies, USA). The samples were adjusted to a final concentration of 13 pM.

Template preparation. Emulsion PCR (ePCR) was then carried out using the Ion OneTouch 200 Template Kit v2 DL (Life Technologies) according to the manufacturer's instructions. Templates were enriched using Ion OneTouch ES with unenriched spheres, magnetic beads, wash buffer and melt-off. After enrichment, the template was neutralized and a control was added, a necessary step for Ion Torrent analysis. Enriched templates were sequenced on a 318 micro-chip using the Ion Torrent Personal Genome Machine (Life Technologies, USA). After sequencing, the individual sequence reads were filtered with the PGM software to remove low

quality sequences. All quality filtered data were exported as FastaQ files, split into constituent *.fasta and *.qual files using the Mothur pipeline (Schloss *et al*., 2009).

Sequence analysis. The obtained sequences were analysed using a combination of UPARSE v8 (Kuczynski *et al*., 2012) and QIIME v1.8 (Caporaso *et al*., 2010) software. Briefly, raw forward and reverse reads for each sample were assembled into paired-end reads, considering a minimum overlap of 50 nucleotides and maximum of one mismatch within the region, using the fastq-join algorithm (https://code.google.com/p/ea-utils/wiki/FastqJoin). The paired reads were then quality filtered, the primer sequences were removed and the individual sample files were merged in a single .fasta formatted file. The resulting file was imported in UPARSE where operational taxonomic units (OTUs) of 97% sequence similarity were constructed and chimeras were removed using both de-novo and reference-based detection. For reference chimera detection, the Gold database (Reddy *et al*., 2014) was used. Taxonomy was assigned to the representative sequences of the OTUs in QIIME using UClust (Edgar *et al*., 2010) and searching against the latest version of the Greengenes database (McDonald *et al*., 2012). Finally, an OTU table (i.e., a sample x OTU count matrix with a tab containing the taxonomic % affiliation of each OTU) was created. The OTU table (calculated with FastTree (Price *et al*., 2010) using default parameters and the PyNast-aligned (Caporaso *et al*., 2010) representative sequences as an input) was used as input for all the subsequent analyses regarding alpha- and beta-diversity.

Statistical analysis. The analysis of survival rate was done for time 4 only (21 days), due to the absence of mortality up to time 3 (14 days) and the dependence of data between one date and the others. A two-way permutational analysis of variance (PERMANOVA) was therefore performed on the arcsine transformed percentage of mortality, with Salinity (3 levels) and Antibiotic (2 levels; antibiotics and no antibiotics) as fixed, independent factors. The analysis

was done on a Euclidean similarity matrix, using 9999 permutations of residuals under a reduced model (Anderson *et al.*, 2008).

To estimate the alpha diversity of the samples, a rarefaction analysis was performed in Qiime using the alpha_rarefaction considering the chao 1 index and summarised taxa-scripts to determine whether sampling depth was sufficient to accurately characterise the bacterial community being studied. The non-parametric asymptotic richness estimator, Chao 1 was considered to characterise the bacterial community by extrapolating a species-accumulation curve to predict its asymptote (Chao and Bunge, 2002).S

Due to the extremely high presence of unclassified groups highlighted when exploring resolution finer than Phylum level (family), the analysis of the bacterial community in relation to time, salinity and antibiotic treatments was limited to Phylum level. While the limitation for choosing such broad taxonomic level is acknowledged, it resolved entirely the classification of the full bacterial community. Additionally, given the high diversity of microbial community, such Phylum-approach can be robustly used to compare bacterial diversity (for reviews see Hughes *et al.*, 2001) . The relative contribution of bacteria (in percentages) to the total number of sequence reads at phylum level was arcsine transformed prior to the analysis. In order to test the effects of salinity, treatment (antibiotic/no antibiotic) and time of exposure on the relative contributions of bacteria, a three-way ANOVA, (with Salinity, 3 levels; Treatment, 2 levels; and Time, 3 levels as fixed factors) was performed on a Bray Curtis similarity matrix (Bray and Curtis, 1957) using 9999 permutation (Anderson *et al.*, 2008).

Should the effect of antibiotic treatment result non-significant, the datasets were pooled (ignoring treatment) to perform a two-way ANOVA on the relative contribution of bacteria with Salinity and Time as fixed factors. Analyses were performed as above.

Should the effect of Time and Salinity result non-significant, the datasets were pooled to perform two separate one-way ANOVAs on the relative contribution of bacteria to the total number of sequence of reads at phylum level with Salinity and Time as fixed factors, respectively. Analyses were performed as above.

Principal coordinate (PCO) analyses of the Bray Curtis similarity matrix (Bray and Curtis, 1957) were carried out to further explain the possible associations among the factors.

Results

A total of 144 male crabs, belonging to the species *Uca urvillei*, were exposed to 5, 20 and 35‰ salinities at different time intervals. During the first 14 days of the salinity exposure experiments, the crabs showed normal behavioural responses (e.g. burrowing, regular feeding, response to physical stimulation; data not shown). A 100% survival rate was observed over this period (Figure 2.4). During longer term salinity exposures (up to 21 days), a total of 11 animals out of 36 died, corresponding to a 30.5% of mortality rate. A significant effect of salinity on mortality of *U. urvillei* (PERMANOVA, *F= 7.6563, df= 35, p< 0.01*) was present only after 14 days exposure (Figure 2.4). There was no effect of antibiotic on the mortality rate (PERMANOVA, *F= 0.625, df= 35, p= 0.4398*). The results reported a different mean survival at 5‰ from that of 35‰ (Figure 2.5). Results of a Pairwise comparison tests, reporting significant differences observed within the factor salinity between 5 and 35‰ are shown in figure 2.5. Differences were also observed between 20 and 35 ‰, but not between 5 and 20‰, (Figure 2.5).
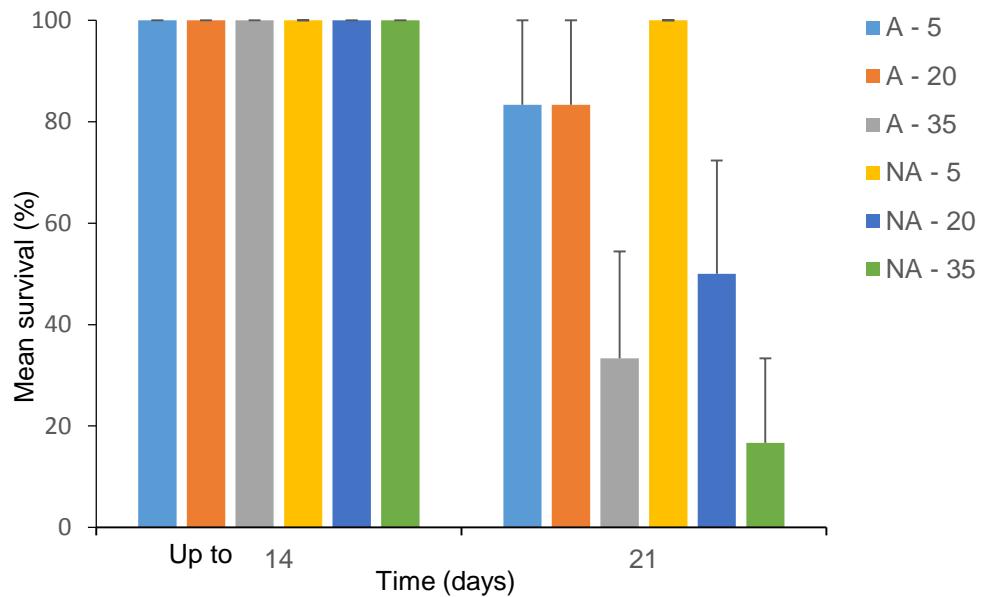
Figure 2.4: Mean survival rate of *U.urvillei* during the first 14 days of the salinity exposure experiment and at the end (day 21). A = antibiotic treatment; NA = no antibiotic treatment; 5-20-35 indicate the three salinity regimes (‰). Error bars report standard errors.
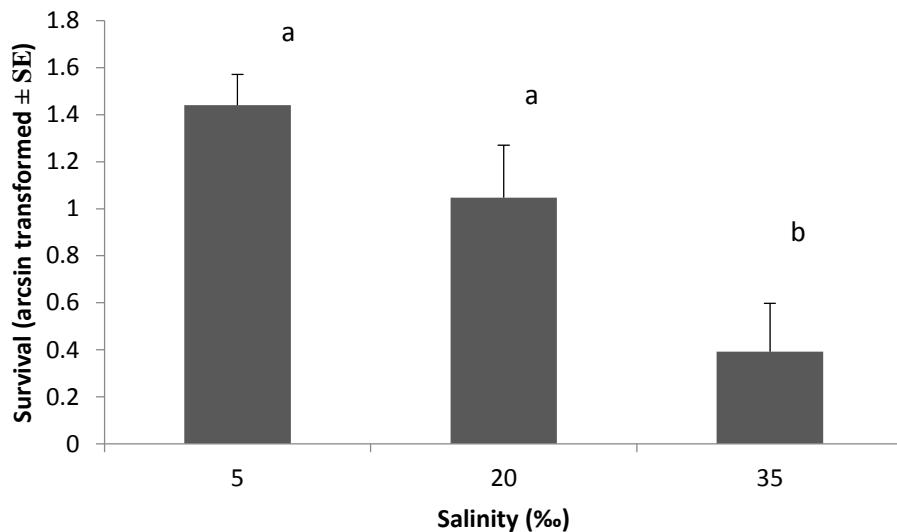
Figure 2.5: Results of the pairwise test of the effect of salinity on the crabs calculated at 21days. Error bars report standard error, a and b indicate homogenous grouping according to the pairwise analysis.

Composition of microbiota by Ion Torrent PGM analysis. Molecular methods were used to explore the structure and provide an overview of the microbial community associated with the gills of *U. urvillei* in response to different salinity regimes and antibiotic treatment over time. The rarefaction curves obtained were constructed from sequenced samples (Figure 2.6). Although the rarefaction curves did not reach full asymptote, and the observed data sequence reads were lower than the Chao1 estimates, the curves had similar patterns and were near enough to the asymptote, indicating with confidence that the dominant bacterial community composition had been sampled. (Figure 2.6). The assigned taxonomies on sequenced 16S rDNAs of the samples are summarised in Table 2, which was resolved up to the level of Family. The Ion Torrent PGM sequencing identified two dominant Phyla, corresponding to Proteobacteria and Actinobacteria. Over any time, salinity and treatment exposure, these two phyla of bacteria were always present. At the Family level, however, a rather diverse array of bacteria was observed, varying in abundance from 1.4% to 4.7%, and including Sphingomonadaceae, Rhodobacteraceae, SC3- 41 as well as Erythrobacteraceae (Table 2.2 and see Appendices A1-A3 for full details of percentage and taxonomic composition).

There was overall variation in bacterial composition among salinity treatments. For instance, animals exposed to 5‰ had more bacterial reads related to Proteobacteria (51.9%) than the crabs exposed to 35‰ (49.0%). Actinobacteria affiliates, on the other hand, were detected in comparatively lower numbers at 5‰ (25.8%) than at 35‰ (36.8%) (Table 2.2 and Figure 2.7 A). Bacteroidetes followed a trend similar to that of Proteobacteria with more bacteria at 5‰ (21.9%) than compared to 35‰ (13.8%). Considering all the time intervals (7, 14 and 21 days), the general trend reported by Ion Torrent PGM sequencing revealed a decreasing presence of Phyllobacteriaceae (a family belonging to the Proteobacteria) from day 7 (27.7%), 14 (19.4%) to day 21 (15.6%) (Table 2.2 and Figure 2.7 B). Weeksellaceae (a family from the Bacteroidetes), on the other hand, were detected in increasing percentages from day 7 (10.4%),

14 (15.6) to 21 (20.2%). Concerning the antibiotic treatment, a peculiar trend compared to those of the time and salinity treatments was observed. The presence of Phyllobacteriaceae was revealed in higher percentages in treated samples (14.7%) and non-treated samples (30.5%), whereas Weeksellaceae was revealed in lower percentages in treated samples (18.9%) and non-treated samples (9.7%) (Table 2.2 and Figure 2.7 C). There was an indication that bacterial community within the gills of *U. urvillei*, whilst relatively diverse, was dominated by a "stable" community of two main taxa, Actinobacteria and Proteobacteria. The patterns were, however, statistically non-significant, as reported below (Table 2.3)

The results of the 3-way ANOVA showed that none of the three factors (individually or in interaction) had a significant effect on the bacterial composition identified by Ion Torrent PGM sequencing (Table 2.3). PCO analyses of the dataset found no differentiation among samples for all the three factors analysed (salinity, time and treatment [Figures 2.8 A, B and C]). In all the three PCOs, the cumulated variance explained by the first two coordinates was very low, 10.6% (Figures 2.8 A, B and C). Due to potential methodological differences in the antibiotic treatments, the dataset for antibiotic and no antibiotic treatments were therefore analysed independently. When separating antibiotic and no antibiotic treatments, the 2-way ANOVA on the antibiotic treated samples reported non-significant differences in the bacterial community driven by Time, Salinity or interaction between the two (Table 2.4). The same results were also found in the 2-way ANOVA of the bacterial community of the non-antibiotic samples (Table 2.5). Differentiation among samples showed little variance explained by the first two coordinates, both for Salinity and Time with antibiotic (Figures 2.9 A and B) and with no antibiotic (Figures 2.10 A and B).

Table 2.2: Taxonomic classification of 16S rRNA V4-V5 regions DNA sequences, obtained from Ion Torrent PGM sequencing, by using the classifier tool of the Ribosomal Database Project. %= the relative percentage contribution of bacteria to the total number of sequence reads.

Classification Factors	Class	%	Order	%	Family	%
Salinity: 5‰	Alphaproteobacteria	50.6	Rhizobiales	45.0	C111	21.4
	Acidimicrobiia	25.5	Acidimicrobiales	25.5	Phylobacteriaceae	26.5
	Flavobacteria	20.7	Flavobacteriales	20.7	Other	17.8
	Saprospirae	1.1	Sphingomonadales	4.1	Weeksellaceae	18.8
35‰	Alphaproteobacteria	48.1	Rhizobiales	35.7	C111	31.6
	Acidimicrobiia	36.5	Acidimicrobiales	36.5	Phylobacteriaceae	18.2
	Flavobacteria	11.1	Flavobacteriales	11.1	Other	16.4
	Saprospirae	2.6	Sphingomonadales	7.9	Weeksellaceae	9.7
Time 1	Alphaproteobacteria	59.3	Rhizobiales	52.9	Phylobacteriaceae	27.7
	Acidimicrobiia	26.2	Acidimicrobiales	26.2	C111	22.4
	Flavobacteria	11.7	Flavobacteriales	11.7	Weeksellaceae	10.4
	Saprospirae	1.6	Sphingomonadales	5.3	Other	24.4
Time 3	Alphaproteobacteria	42.2	Rhizobiales	30.1	Phylobacteriaceae	19.4
	Acidimicrobiia	37.4	Acidimicrobiales	34.4	C111	31.4
	Flavobacteria	17.3	Flavobacteriales	17.3	Weeksellaceae	15.6
	Saprospirae	2.1	Sphingomonadales	6.3	Other	9.3
Time 4	Alphaproteobacteria	37.3	Rhizobiales	26.1	Phylobacteriaceae	15.6
	Acidimicrobiia	34.7	Acidimicrobiales	34.7	C111	30.2
	Flavobacteria	22.3	Flavobacteriales	22.3	Weeksellaceae	20.2
	Saprospirae	2.0	Sphingomonadales	6.8	Other	9.6
Antibiotic	Alphaproteobacteria	47.3	Rhizobiales	38.8	C111	26.2
	Acidimicrobiia	29.3	Acidimicrobiales	29.3	Phylobacteriaceae	14.7
	Flavobacteria	20.3	Flavobacteriales	20.3	Other	23.4
	Saprospirae	1.4	Sphingomonadales	5.8	Weeksellaceae	18.9
Non-antibiotic	Alphaproteobacteria	51.6	Rhizobiales	42.3	C111	26.6
	Acidimicrobiia	32.5	Acidimicrobiales	32.5	Phylobacteriaceae	30.5
	Flavobacteria	11.6	Flavobacteriales	11.6	Other	10.6
	Saprospirae	2.2	Sphingomonadales	6.1	Weeksellaceae	9.7

A

B

C

D

E

F
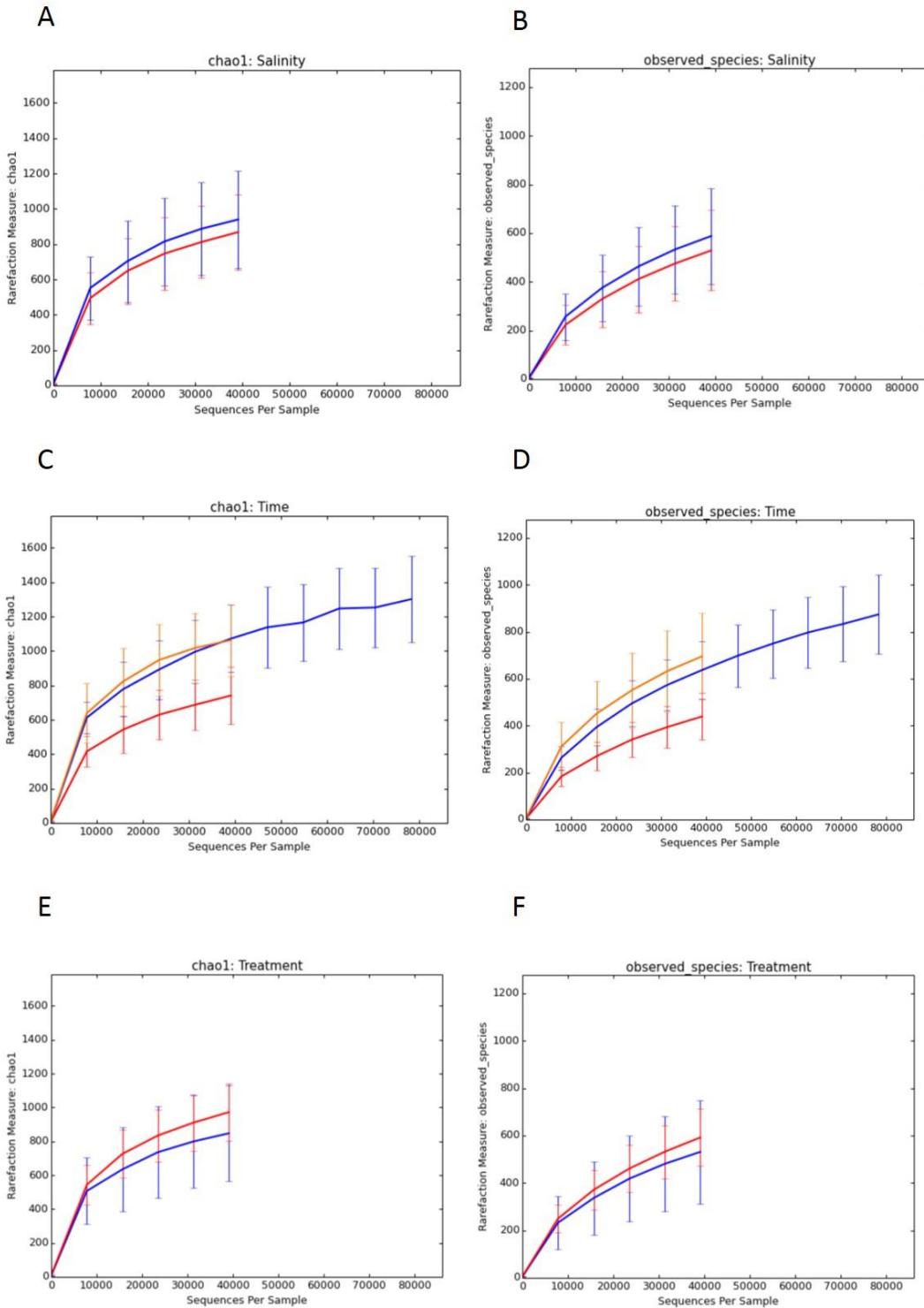
Figure 2.6: Chao1 rarefaction curves of the total number of reads generated by Ion Torrent PGM sequencing versus the total number of 16S rRNA species identified. A-B is the effect of salinity on bacterial composition, the red line is 5‰ while the blue is 35‰. C-D is the effect of salinity over time, the red line is T1, the blue is T3 and the Orange is T4. E-F is the effect of antibiotic on the bacteria, the red line is no antibiotic, blue is with antibiotic.
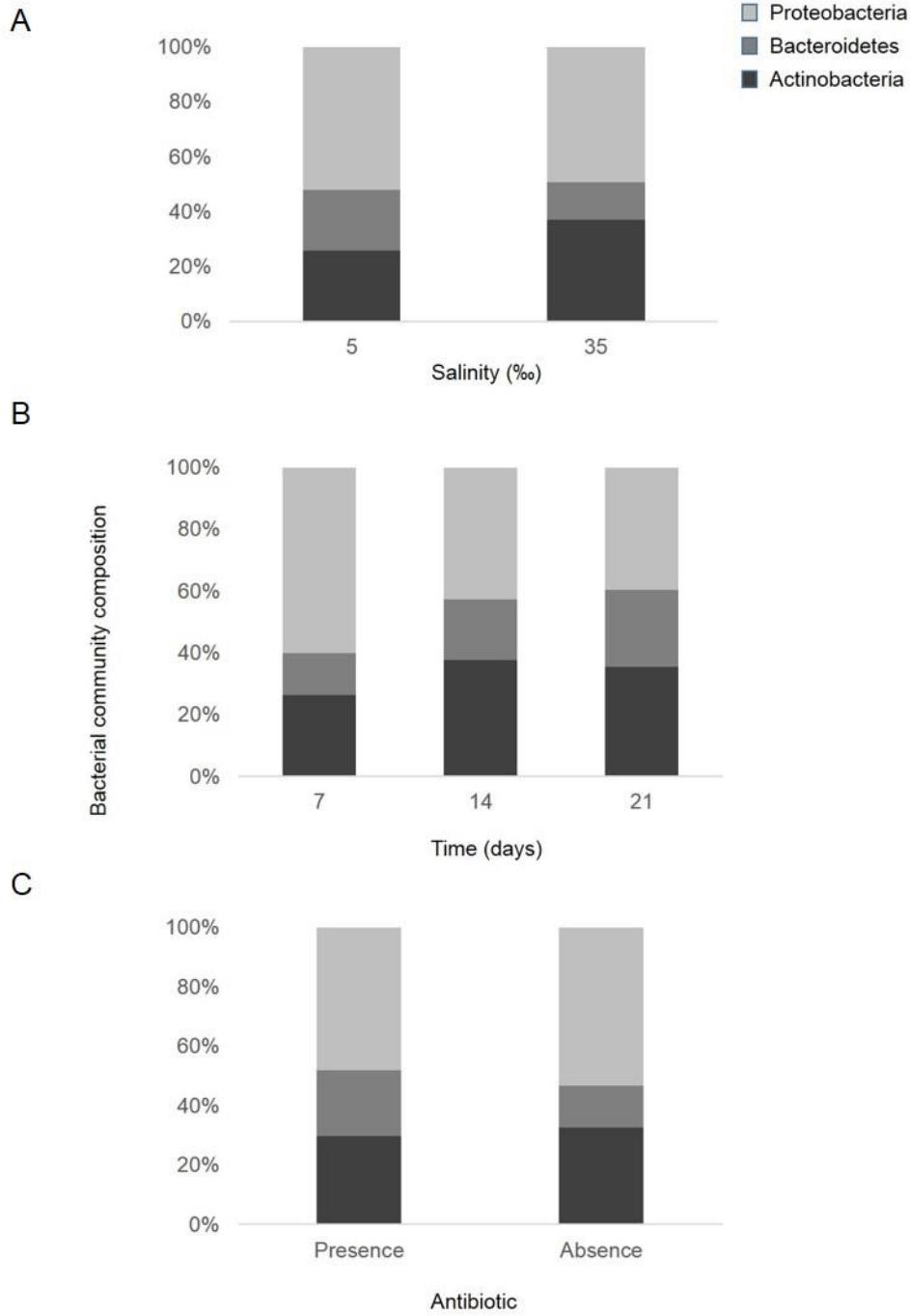
Figure 2.7: Taxonomic classification and percentage community structure of bacterial reads retrieved from the gills *U. urvillei* for: A different salinity levels, B different time intervals and, C with and without antibiotic treatment. These are shown at phylum level.

Table 2.3: Results of the 3-way ANOVA of the effects of salinity, time and treatment on bacterial community composition (at the phylum taxonomic level). df = degrees of freedom; SS = sum of squares; MS = mean sums of squares; F = F-Statistic; p-value = statistical probability; RES = Residuals, Total = total degrees of freedom.

Factors	df	SS	MS	F-ratio	p-value
Treatment (Tr)	1	4943	4943	0.99312	0.446
Salinity (Sa)	1	4953.1	4953.1	0.99516	0.458
Time (Ti)	2	9925.1	4962.6	0.99705	0.474
Tr x Sa	1	4978.8	4978.8	1.0003	0.449
Tr x Ti	2	9946.8	4973.4	0.99923	0.479
Sa x Ti	1	4954.5	4954.5	0.99544	0.463
Tr x Sa x Ti	1	4983.9	4983.9	1.0013	0.462
Res	10	49772	4977.2		
Total	19	94490			

Table 2.4: Results of the 2-way ANOVA of the effects of salinity and time on bacterial community composition (at phylum taxonomic level) of samples treated with antibiotic. df = degrees of freedom; SS = sum of squares MS = mean sums of squares; F = F-Statistic; p-value = statistical probability; RES = Residuals, Total = total degrees of freedom

Factors	df	SS	MS	F- ratio	p-value
Salinity	1	4945.8	4945.8	0.99417	0.455
Time	2	9915.9	4957.9	0.9966	0.481
Salinity ×Time	1	4945.8	4951.8	0.99537	0.458
Res	6	29849	4974.8		
Total	10	49687			

Table 2.5: Results of the 2-way ANOVA of the effects of salinity and time on bacterial community composition (at phylum taxonomic level) of samples not treated with antibiotic. df = degrees of freedom; SS = sum of squares; MS = mean sums of squares; F = F-Statistic; p-value = statistical probability; RES = Residuals, Total = total degrees of freedom.

Factors	df	SS	MS	F-ratio	p-value
Salinity	1	4977.8	4977.8	0.9994	0.439
Time	2	9955.9	4977.9	0.99942	0.469
Salinity×Time	1	4979.5	4979.5	0.99973	0.459
Res	4	19923	4980.8		
Total	8	39836			
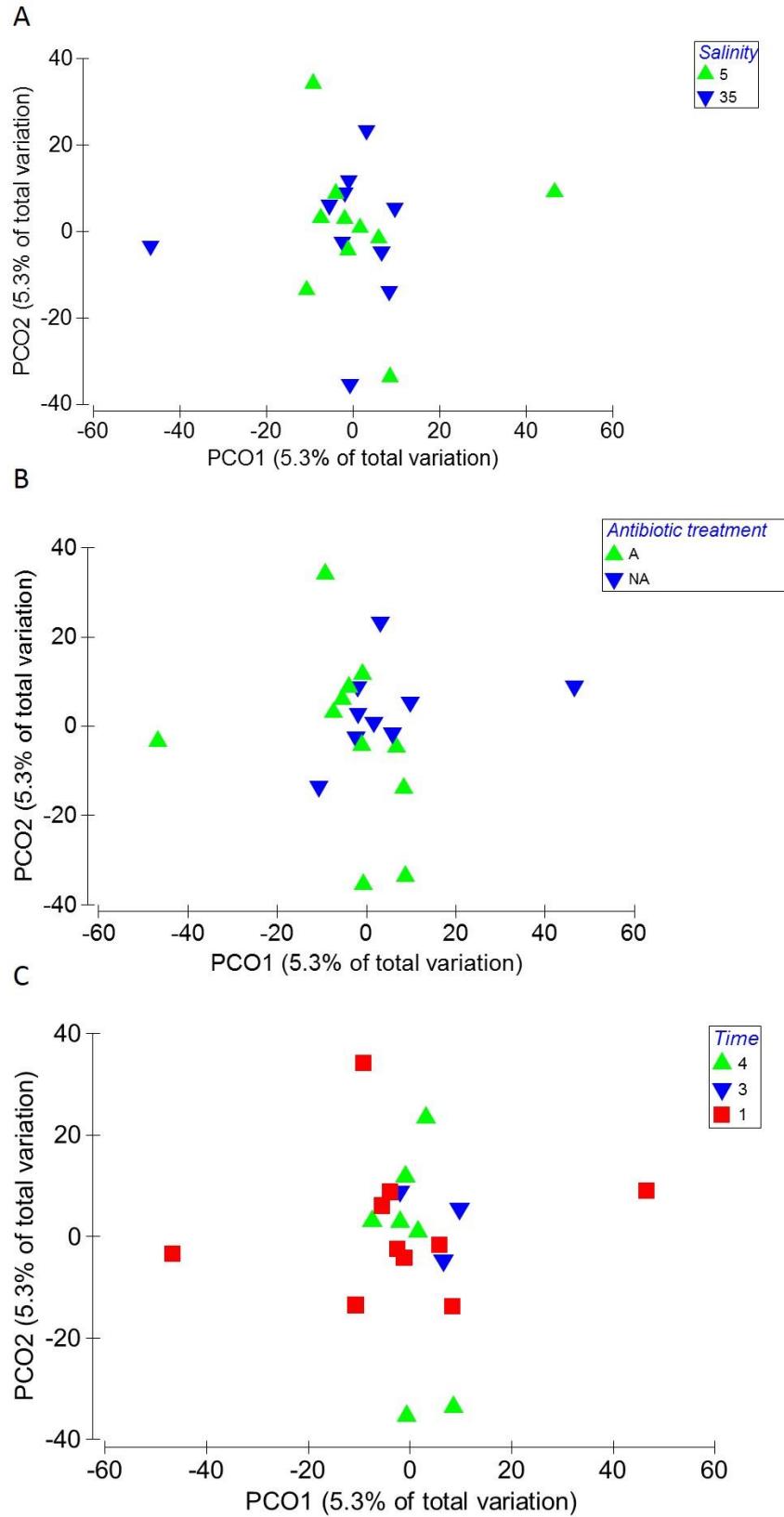
Figure 2.8: Principal Coordinates Analysis (PCO) of bacterial communities associated with the gills of *U. urvillei* grouped by: (A) Salinity, (B) Antibiotic treatment and (C) Time.
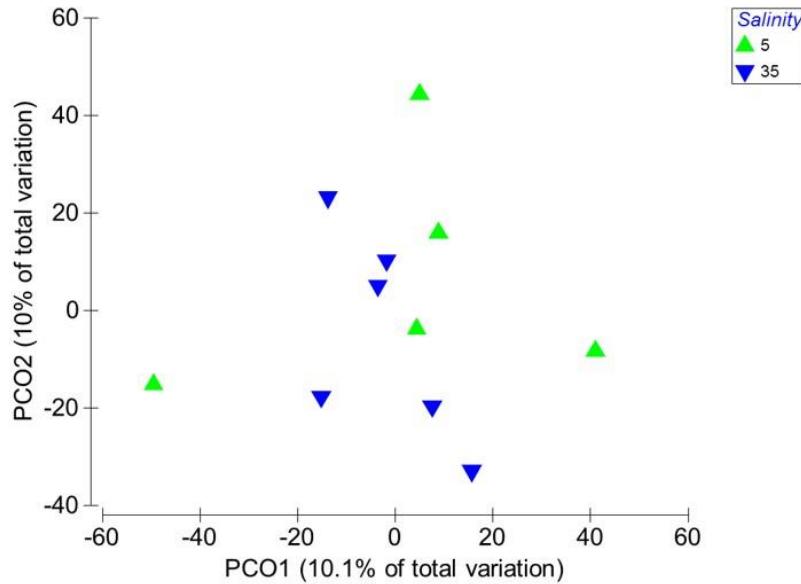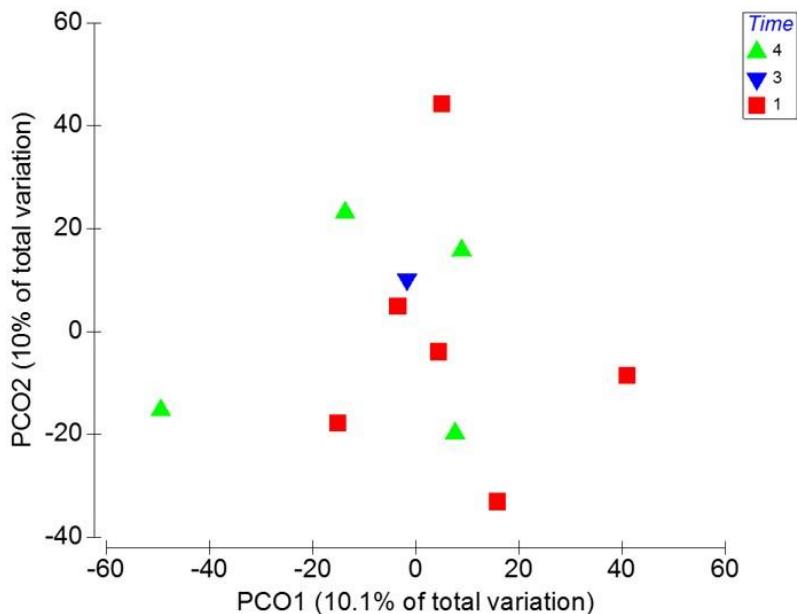
Figure 2.9: Principal Coordinates Analysis (PCO) of bacterial communities associated to the gills of *U. urvillei* grouped by: (A) salinity and (B) time . The graphs represents ordination of samples treated with an antibiotic.
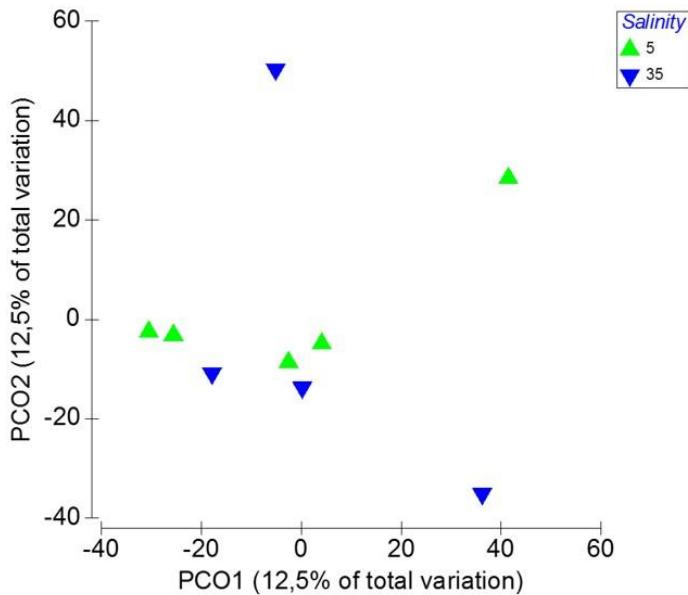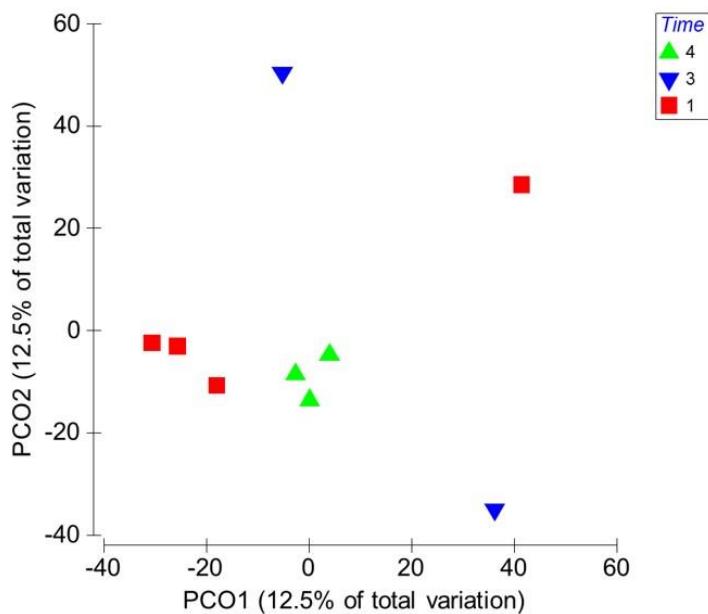
Figure 2.10: Principal Coordinates Analysis (PCO) of bacterial communities associated to the gills of *U. urvillei* grouped by: (A) salinity and (B) time. The graphs represents ordination of samlpes nont treated with an antibiotic.

Discussion

In brachyuran crabs, the gill is an important organ with multiple functions including (but not limited to) respiration, ammonia excretion and ion-regulation (Tsai and Lin, 2012). In bimodal crabs it is of key importance (Burggren and McMahon, 1988), it has to extract oxygen in water and air (Weihrauch *et al*., 2004) and is the first organ to be impacted by changes in the surrounding environment (Henry *et al*., 2012). The gills of *U. urvillei* are suitable habitat for microbes, which are reported to harbour a diverse community of bacterial strains that seem to be closely related to sulphur-oxidizing bacteria (Fusi *et al.*, 2013). Because the majority of environmental microbes are refractory to culture in the laboratory (Su *et al*., 2012; Vincent *et al*., 2013), the conventional culture techniques were not used to demonstrate the bacteria in the gills of this species in the present study.

In the past decades, improved culture-independent molecular profiling methods, such as 16S ribosomal RNA gene sequence analysis, have led to a revolution in the understanding of indigenous microbial communities in all kinds of environments (Liang *et al*., 2007; Dias *et al*., 2010). For this study, therefore, 16S rRNA sequencing approaches were used, particularly Ion Torrent PGM sequencing. This technique allowed one to: (1) detect the high diversity of taxa colonising the gills of the targeted species of study, which includes Firmicutes, Bacteroidetes and Actinobacteria and (2) highlight some shifts and losses of microbial diversity associated with antibiotic treatment and different salinity regimes on a temporal scale.

Antibiotic treatment. Chloramphenicol is a broad spectrum antibiotic that is effective against both gram-negative and gram-positive bacteria (Sorensen *et al*., 2003; Campa-Córdova *et al*., 2006; Huys *et al*., 2007). Chloramphenicol was used in the present study as a control, and it proved to be ineffective and thus showed no differences among all the samples, regardless of time or salinity. The ineffectiveness (inability to kill the bacteria) of chloramphenicol in this

study could be due to the fact that bacteria are constantly evolving organisms, and over the past years they have become resistant to the selective pressures imposed by their natural environment, as well as by human interventions such as antibiotics (Sefton, 2002; Aminov, 2009; Rho *et al.*, 2012). These antimicrobial resistant-bacteria are present in almost all habitats, including those that are apparently antibiotic-free and/or with little to no human influence (Aminov, 2009; Bhullar *et al.*, 2012). Examples of such resistant bacterial strains include *Enterococci* of a large genus, *Enterococcus*, from the Phylum Firmicutes. Over the past decades, these bacteria have become intrinsically resistant to several antibiotics and have the potential for resistance to virtually all clinically available drugs (Sood *et al.*, 2008; Hollenbeck and Rice 2012; Garrido *et al.*, 2014).

Because bacteria generally present a high metabolic diversity compared to other organisms, (Oren, 2009), from which they are thought to deploy protective mechanisms to increase survival, thereby allowing them to be resistant to antibiotics, no single antibiotic is really able to kill all bacterial strains anymore (Oren, 2009). A combination of antibiotics or an antibiotic cocktail is therefore recommended. In medicine, for example, patients with tuberculosis are treated with a cocktail of anti-TB drugs and this has become an essential treatment regimen; today with considerable success (Davies and Davies, 2010). In aquaculture, the regular use of antibiotics has resulted in the development of resistant strains (Karunasagar *et al.*, 1994; Cabello, 2006) and there is a need to move away from antibiotics to more natural treatments. One such treatment is by/through bacteriophages (phages) and this has been successful. Mateus *et al*. (2014) showed that the use of cocktails with two or more phages works even better. They found that using a cocktail of two or three phages was significantly effective against *Vibrio parahaemolyticus* than when used alone.

The lack of an antimicrobial cocktail for use in the present study could explain the statistically "stable" bacterial community reported even after the antibiotic treatment. Unfortunately a

cocktail could not be used in this study, as it could have had detrimental effects on the crabs, and the experiment was to run for a long time as this could have fatal effects on the animals. Several additional explanations could have led to the observed "stability" in bacterial community. For example, even though the water was filtered prior to addition to the individual mesocosms, the mud was not sterilised (due to the instantaneous death of crabs during some preliminary trials) and, therefore, animals could have been able to acquire the bacterial symbionts from the near environment (see Chapter Three of this thesis, where it is shown that gills and eggs harbour similar bacterial profile as that of mud). While a direct effect of salinity on bacteria in the mud was not measured during the study, the target salinity soaked the mud substrate entirely, most likely influencing the bacterial composition of the mud. A possible horizontal transmission from the mud would have, therefore, still been influenced by the experimental salinity. Additionally, the antibiotic, which was inoculated into the haemolymph, the most internal fluid tissue of crabs, may have not reached the gills, which are external organs constantly interacting with the outside environment. While gills might have been a better target organ to administer antibiotics, inoculation via haemolymph still ensured equal antibiotic dosage. It is important to note that less than 1% of the total microbial community from environmental samples are readily cultivable by standard microbiological methods (Li *et al*., 2006) and the bacteria associated with the gills of *U. urvillei* are no exception. Therefore, a susceptibility test, to expose the bacteria directly, according to the Kirby-Bauer disc diffusion method could not be performed (see Najiah *et al*., 2010 for more information on the method).

Salinity treatment. Fiddler crabs are semi-terrestrial crabs of the genus *Uca* found in salt marshes, sandy or muddy beaches and mangroves (Ruwa, 1997). *U. urvillei* inhabits the mangrove intertidal area and is often exposed to extreme environmental variations, including temperature and salinity, over short periods of time. This species is known to tolerate a wide

range of salinity (0-55ppt) (Khanyile, 2012). The current study specifically focussed on lower range of salinities (and its potential effects on the bacterial communities associated with decapods), experienced by the targeted mangrove systems (southernmost African limits) over prolonged flooding events, where salinities drop to freshwater level for about two weeks (Deyzel, 2013). In the present study, therefore, males of the species *Uca urvillei* were exposed to low (5‰), middle (20‰) and seawater (35‰) salinities in order to investigate temporal changes in the bacterial symbionts colonising the gills of the target species. In the laboratory the crabs tolerated all three salinities (5, 20 and 35‰) well for up to 14 days. After 14 days, however, a significantly high mortality rate was reported for crabs exposed to 35‰. After 14 days of the exposure, the mud was almost fully dissolved in water and this condition may have caused mortality events, but only at the 35‰ treatment. Practically, crabs were unable to escape water by simply burrowing in the mud and they were obligated to stay submerged. This obliged underwater condition was, however, similar at all the three treatments. Therefore, one can assume that all crabs experienced "less suitable environments" after 14 days, but even more so at 35‰. Nonetheless, this is an interesting result given that 35‰ is the normal salinity of seawater and represents the "natural" environment for these crabs in the target system. Most *Uca* species occur in dry or muddy substratum in estuarine mangroves (Nobbs, 2003). *U. urvillei* is found in muddy and wetter areas of the estuarine mangroves (Hartnoll, 1973). The results from this study suggest that the crabs avoid water exposure in the wild (more studies are needed to support this), and they show preferences for lower salinities. Fiddler crabs are passive in water (they hide in burrows during high tide) and are very active on land (Ravichandran *et al.*, 2011). This is possible because these crabs have modified gills (lung-like) that are rigid so they do not adhere when surrounded by air, allowing breathing on land (Fusi *et al.,* 2014; Cannicci *et al.*, submitted). Thus, the high mortality levels observed between

day 14 and 21 (Figures 1.4 and 1.5) could be expected, given the poor breathing physiology of this species when submerged (Fusi *et al.*, 2014).

The bacterial community residing in the gills of *U. urvillei* did not change in response to changes in salinity over time. Even though seawater salinity (35‰) had a significant effect on the crab's survival over time, this was not observed in the assemblage of the associated bacterial symbionts. Throughout all three salinity exposures, the bacterial community did not vary, with Proteobacteria and Actinobacteria as dominant taxa. Similarly, Fusi (2014) found two phyla as predominant members of the microbial community using 16S rRNA gene amplification, denaturing gel electrophoresis (DGGE) and pyrosequencing. The author revealed a prevalence of phyla-level taxa, including Cyanobacteria, Firmicutes and Planctomycetes, whereas the current study found a rather less diverse array of bacterial phyla in all the treatments (Figures 2.8A, B and C). Furthermore, Fusi (2014) found more Actinobacterial than Proteobacterial reads, a trend opposite to the one in the current study, where Proteobacteria were always present in higher percentages than Actinobacteria, in all the treatments. Overall, this study revealed a rather less diverse bacterial composition at the phylum level compared to that of Fusi (2014) and a highly diverse structure at the class level (see Appendices A2.1-A2.3). Spatio-temporal variability in bacterial symbiont community composition may be expected due to possible scale dependent biotic and abiotic effects. For example, even though the study by Fusi (2014) was on the same species, the samples had a different geographical origin and were collected at different times. They collected their samples from Gazi Bay in Kenya in 2010, while samples for the current study were collected in 2013, from the Mngazana Estuary, South Africa. Zhang *et al*. (2009) conducted a study in Sanya, a mangrove ecosystem in China, and found that samples collected from the same sites and the same time had similar microbial communities while samples collected from different sites and seasons consisted of quite varied bacterial communities. This is also evident in lakes where different systems present different bacterial

community composition (Yannarell and Triplett, 2004; Jones *et al*., 2009). This suggests that, even though there are major similarities in the composition of bacterial communities worldwide (e.g Glockner *et al*., 2000; Rappe *et al*., 2000; Crump, 2005), different ecosystems might show strong spatio-temporal variations.

 The two dominant phyla of bacteria reported in this study are considered the largest groups of bacteria found virtually in almost all habitats, from freshwater to marine systems (Lee *et al*., 2005; Kersters *et al.*, 2006; Ventura *et al*., 2007). Nonetheless, the Alphaproteobacteria, found abundantly in all samples, mainly predominates marine environments (Morris *et al*., 2002), and this could explain the presence of this class in all treatment conditions, given the typical marine characteristics of this system (Grant, 2007; Deyzel, 2013). Betaproteobacteria was reported in relatively low percentages in all the treatments; this is no surprise since this particular class is often numerically dominant in freshwater systems (Anissi *et al*., 2014).

Finally, the exposure time could be a possible explanation for the lack of statistically different results observed between treatments in the present study. Perhaps longer exposures of the crabs to different salinities could yield different and possibly significant results, but this could not be tested in the present case/instance because the crab's survival rate decreased severely after day 14 (Figures 2.4 and 2.5). Alternatively, the effects of salinity on the bacteria itself could be investigated (by isolating, cultivating and exposing them). Unfortunately, during the time of this study, the bacterial symbionts remained uncultivable (Fusi, personal communication). Overall, the results of this study suggest a high resilience of the bacterial communities associated with gills of mangrove crabs, due to the fact that they are not impacted by short term exposure to different salinities. This way these bacteria can induce a sort of resistance for the host to face climate change related stresses and scenerios. For example, Lau and Lennon (2012) showed that responses of plants to drought stress were governed mostly by rapid

changes in microbial community structure than by plant traits. The results of this study are ecologically important as these bacterial symbionts play a key role, such as nutrient recycling (Holguin *et al*., 2001), essential for the performance and persistence of the crab's population and therefore of the whole functioning of the Mngazana ecosystem.

Chapter 3: Bacterial transmission in symbionts associated with mangrove brachyurans

Introduction

Microbes constitute by far the largest diversity and biomass of all marine organisms (McFall-Ngai, 2002; Kennedy *et al.*, 2010), and are involved in a variety of important symbiotic relationships with invertebrates of almost all phyla (Sharp *et al.*, 2007). Proposed symbiotic functions for marine microbes include: enhancement of chemical defences (Unson et al., 1994), assistance with reproductive processes (Klussmann-Kolb and Brodie, 1999), and with nutrition (DeChaine and Cavanaugh, 2006). Chemosynthetic symbiosis, a nutritional strategy found among an increasing number of marine invertebrates, was discovered approximately 35 years ago at hydrothermal vents on the Galapagos Rift (Dubilier *et al.,* 2008; Martin *et al.* 2008). Prior to this discovery, all life on earth was believed to depend on energy from the sun, through photosynthesis. Vents, however, which are approximately 2100 m deep, are in complete darkness (Vetter, 1985; McMullin *et al.*, 2003; Duperron *et al.,* 2008). Photosynthesis clearly could not be the basis of the vent food chain and an alternative explanation was needed to account for hydrothermal vent community productivity. It was then suggested that chemosynthetic microorganisms serve as primary producers without the aid of sunlight in hydrothermal vents (Cavanaugh, 1983; Doeller *et al.*, 1988; Krueger and Cavanaugh, 1997; Fontanez and Cavanaugh, 2014). In chemosynthetic symbioses, the host provides access to reduced compounds, such as hydrogen sulphide, that the symbionts use (as electron donors with oxygen as an electron acceptor) to drive the formation of fixed carbon from single carbon molecules (either carbon dioxide or methane), thereby providing a source of nutrition for the host (Cavanaugh, *et al.*, 2006; Duperron *et al.*, 2008; Roeselers and Newton, 2012).

The vent communities are perhaps the most dramatic and spectacular examples of the success of symbiosis (Stewart *et al*., 2008), but they prompted a search for these associations in a wide range of habitats. Today, chemosynthetic symbioses are recognised as being universal, occurring in a range of environments, including the easily accessible, sun present habitats (Krueger *et al.,* 1995; Stewart and Cavanaugh, 2006; Dubilier *et al.,* 2008). Undeniably, microbial symbioses enable organisms to exploit otherwise inaccessible habitats such as mangrove swamps, eelgrass beds and deep-sea hydrothermal vents (Roeselers and Newton, 2012; Duperron *et al*., 2009; Rodrigues *et al.*, 2010). Most of these habitats are characterised by low or no oxygen (O_2) content and the presence of free hydrogen sulphide (H_2S) (Julian and Arp, 1992; Joyner *et al.* 2003). Their faunal inhabitants are therefore, exposed to this toxic chemical and cannot escape it, as it freely diffuses into their respiratory surfaces (Joyner *et al*., 2003), reversibly inhibiting cytochrome *c* oxidase and resulting in further inhibition of aerobic respiration (Julian and Arp, 1992). For this reason, organisms dwelling such harsh systems require special mechanisms which assist with the survival in the midst of toxic chemicals. Several strategies have been proposed that organisms use to survive in such challenging environments, including physiological, behavioural and ecological adaptations (Johnson *et al*., 2002; Lee, 2008; Kristensen, 2008; Cannicci et *al.,* 2011; Roeseler and Newton, 2012), as well as chemosynthetic symbiosis (Julian and Arp 1992; Dubilier *et al.* 2008; Smith, 2012, Fusi, 2014). Symbiosis is, therefore, unquestionably an important biological interactive process for both the host and symbiont and, to ensure persistence of such important association, bacteria have to be transmitted or recruited acquired and maintained through and over successive generations (Ewald, 1987; Oh *et al*., 2010; Marsh *et al*., 2014). Acquisition of hosts and transfer across generations can be strictly maternal (vertical), with occasional horizontal transfer (leaky vertical transmission), or entirely environmental (horizontal) (Krueger *et al.,* 1996; Gros *et al.,* 1998; McFall-Ngai, 2002; Nuusbaumer *et al.,* 2006; Schmitt *et al.*, 2008; Bright and

Bulgheresi, 2010; Vrijenhoek, 2010). Any failure to transfer the symbionts may result in significantly reduced fitness, often leading to sterility and/or mortality for both the host and the symbiont (Hosokawa *et al.*, 2013).

Vertical transmission (also termed transovarial transmission) is a parent to offspring flow, in which gametes (most commonly oocytes) carry symbionts either intra- or extracellular to the succeeding generation (Giere and Langheld, 1987; Sharp *et al.*, 2007). This way, symbionts participate directly in the process of embryogenesis of the host as well as postembryonic development (Cary and Giovannoni, 1993; Bandi *et al*., 1999; McFall-Ngai, 1998; 2002; Vizoso *et al*., 2005; Vrijenhoek, 2010). In many vertical transmissions, there is no aposymbiotic phase (before the symbiont is acquired) and so the association is permanent and continues from one generation to the next one. Vertical transmission is documented in many animal phyla including bivalves, bryozoans, porifera and arthropods (Cary and Giovannoni, 1993; Peek *et al.*, 1998; Oliver *et al.*, 2003; Russell and Moran, 2005; Bright and Bulgheresi, 2010). In sponges, several species, including *Tethya citrina* (Gaino *et al.,* 1987) and *Corticium candelabrum* (de Caralt *et al.,* 2007), are reported to vertically transmit their associated bacterial symbionts. Sharp *et al.* (2007) localised bacterial symbionts within various stages of developing embryos of the tropical Pacific sponge *Corticium sp.* (for more examples, see Endow and Ohta, 1990; Usher *et al.*, 2001; Enticknap *et al.,* 2006; Schmitt *et al.*, 2007; 2008; Webster *et al.,* 2010). In most chemoautotrophic symbioses from oligochaetes and bivalves, vertical transmission is thought to be a common mode of transmission (Krueger *et al.,* 1996; Decker *et al.,* 2013). For example, vesicomyid clams, *Calyptogena soyoae* seem to transmit the symbionts vertically between generations via the eggs (Peek *et al*., 1998). Females of the oligochaete worm *Inanidrilus leukodermatus* on the other hand, appear to infect the offspring

by smearing symbionts onto the eggs as they pass through symbiont-containing genital pads of the mother as they leave her body into the sediment (Giere *et al.,* 1991).

In horizontal transmission, also termed environmental transmission, both aposymbiotic and symbiotic phases exist, and the bacterial symbionts are not originally present in eggs (Krueger *et al*., 1996). Symbionts, therefore, do not interact directly with host cells during embryogenesis; instead, upon hatching, juveniles must acquire specific bacteria anew from the environment and this process occurs newly at each generation (Peek *et al*., 1998; McFall-Ngai, 2002; Stewart and Cavanaugh, 2005; Bright and Bulgheresi, 2010; Vrijenhoek, 2010). In the case of marine associations, the environmental medium is generally the ambient seawater or sediment (McFall-Ngai and Ruby, 2000) and one of the most common entry sites for acquisition of symbiotic bacteria for animals is the oral opening (Bright and Bulgheresi, 2010). Horizontal transmission includes two types: direct and indirect transmission (McFall-Ngai, 1998). Direct transmission occurs when juveniles acquire the symbionts from the environment with the help of adult individuals in the population. In most terrestrial organisms, such as ruminants and termites, adults feed the newly hatched juveniles with faeces containing microbes (McFall-Ngai, 2002; Bright and Bulgheresi, 2010). In contrast, juveniles of marine invertebrates acquire their symbionts indirectly from broader environments without assistance from adults. "Adults may, however, be passively responsible for "seeding" the environment" with microbes in the first place (McFall-Ngai, 1998; Bright *et al.,* 2014). For example, the bobtail sepiolid squid, *Euprymna scolopes*, is suggested to have the potential to expel its horizontally transmitted bacterial symbiont, *Vibrio fischeri*, into the surrounding environment (Ruby and Lee, 1998; McFall-Ngai, 1999). With the use of transmission electron microscopy and fluorescence *in situ* hybridization (FISH), it has been shown that larvae of obligate vestimentiferans are aposymbiotic and the infection process occurs at settlement (Nuusbaumer

et al., 2006). Moreover, a study by Won *et al.* (2003), shows that horizontal transmission is the primary mode of acquisition of symbionts by deep-sea mussels of the genus *Bathymodiolus*.

Of the two proposed transmission strategies, vertical transmission (transfer of bacterial symbionts from host to the offspring via the reproductive tissue) ensures that all offspring will immediately and safely host the symbionts required for growth and consequently, survival (Vrijenhoek, 2010; Funkhouser and Bordenstein, 2013). Through vertical transmission, the host also avoids the passive acquisition of "unwanted" bacterial strains from the surrounding environment (Lipsitch *et al.*, 1996). With assurance however, arises the problem of carrying symbionts that might not be particularly suited for the habitat the host will eventually occupy, making larvae further susceptible to environmental pathogens (Vrijenhoek, 2010). This becomes a potential problem for organisms with a bi-phasic life style or that inhabit highly variable environments such as estuaries. Moreover, with vertical transmission, the organism (symbiont) is "stuck" and has no opportunity to escape from the consequences of any sudden, extreme disturbance and resulting deleterious impacts it might have on its partner (Douglas, 2010). Additionally, vertically transmitted symbionts are disconnected from their free-living counterparts and it is suggested that in each and every transmission they undergo a population bottleneck, resulting in reduced genetic diversity within individual hosts and reduced symbiont effective population size (Mira and Moran, 2002; Wernegreen, 2002; Woolfit and Bromham, 2003; Douglas, 2010).

With horizontal transmission, on the other hand, hosts can generally form associations with a broad range of symbionts, allowing the host to select and adopt bacterial strains from the surrounding environment that are optimally adapted to the local habitat (Douglas, 1998). Isolating and identifying the 'wanted' symbionts from the environment is, however, one major challenges associated with horizontal transmission (Nyholm and McFall-Ngai, 2004). The acquisition may, although rarely, include bacterial strains or taxa from which they derive little

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or no benefit, or, even worse, acquire pathogenic taxa. Horizontal transmission, therefore, may result in a high diversity of bacterial symbionts and, can, in rare cases result in increased competition within the individual host (Sachs and Wilcox, 2006; Sachs *et al.*, 2011). This is a rather paradoxical outcome as the host might adopt pathogenic symbionts that exploit the host itself by providing less nutrition or, most importantly, dispersing juveniles might fail to be infected altogether, making them further susceptible to pathogens (Herre *et al.,* 1999; Vrijenhoek, 2010).

Both vertical and horizontal transmissions pose serious risks for the host, and they both, to some extent, do not ensure the 'safe' and efficient transfer of symbionts (Russell and Moran, 2005). This is why a third type of transmission (named leaky vertical transmission or mixed modes of transmission) is thought to be the most likely transmission strategy, employed by more species than initially thought, (Davidson and Stahl, 2008; Schmitt *et al.*, 2008; Stewart *et al.*, 2008; Webster *et al.*, 2010; Olson *et al.,* 2014) throughout aquatic systems. Deep-sea clams of the family Vesicomyidae are suggested to transfer their associated intracellular chemosynthetic bacteria predominantly vertical, coupled with occasional lateral symbiont acquisition (Stewart *et al*., 2008). In a broader sense, the microbiota associated with the leech, *Hirudo verbana*, are first transmitted vertically during the first stages of development and thereafter horizontal transmission becomes evident (Rio *et al.*, 2009; Ott *et al*., 2014). Furthermore, a Caribbean sponge, *Ircinia felix*, is suggested to undergo a mixture of vertical and horizontal transmission (Schmitt *et al.*, 2007; 2008). Ebert (2013) suggested that partners with mixed modes of transmission have the best of both worlds where, either vertical transmission predominates with some environmental symbiont acquisition, or with environmental acquisition coupled with vertical transmission (Bright and Bulgheresi, 2010; Vrijenhoek, 2010).

Symbiotic interactions undoubtedly pose several challenges on the ontogeny of the individual host (especially animals), as the array of symbionts require special organs to invade, which consequently influences animal development, (McFall-Ngai and Ruby, 1991, McFall-Ngai, 1999, 2002; Visick and Ruby, 2006; McFall-Ngai *et al.*, 2013). Visick and McFall-Ngai (2000) reported that, whichever mode of transmission is employed, during embryogenesis, the imprint of the influence of bacteria can be seen in the formation of tissues that are destined to interact with coevolved microbial species. The modes by which symbionts are transmitted to offspring, therefore, have the potential to greatly affect the development, biology and therefore demography and evolution of the microbes and the associated hosts (Vrijenhoek, 2010).

Understanding whether bacteria are vertically transmitted, exclusively acquired from the environment or by integration of these two processes, becomes important in the case of aquatic animals experiencing natural environmental variability (Nyholm *et al.*, 2000). In marine coastal environments, estuaries are known to be susceptible to a high degree of environmental variability, including changes in salinity, temperature, pH, oxygen and food availability (Tine *et al.*, 2011). Investigating whether the environment affects the modes of bacterial transmission (the case of leaky and vertical transmission, respectively) or not, or is directly implicated in such process (i.e. horizontal transmission), could enhance our understanding on the consequences of environmental change on natural populations.

With the use of DNA-based methods, this study aims at investigating the modes of bacterial transmission in the model species for the entire thesis, *U. urvillei*. It is hypothesised that: (1) if eggs and gills have similar bacterial symbionts but different from that of the environment (mud and water), this could indicate a vertical mode of bacterial transmission, (2) if eggs and gills and environment, this could indicate a horizontal or a mixed model of bacterial transmission.

Materials and methods

Sample collection and DNA extraction. Mud, water, gravid and non-gravid females were collected from the Mngazana Estuary in November 2013. Mud samples were collected from the top 15 cm and brought to the laboratory, along with water samples in properly labelled, sterilised and sealed 100 ml plastic tubes on ice. Once in the laboratory, the animals were rinsed, fixed in 95% ethanol, and morphometric measurements taken. DNA was isolated from eggs, and gill tissue using a Promega DNA extraction kit. Mud and water were kept in -80 ℃ for further analysis and their DNA extraction was carried out using a Power Soil Sterivex DNA Isolation Kit (MO BIO Laboratories, Carlsbad) and a Power Water Sterivex DNA Isolation Kit (MO BIO Laboratories, Carlsbad), respectively, with no modifications. For full details used for laboratory analyses refer to Materials and Methods of chapter 2 of this thesis.

Data Analysis. To estimate the alpha diversity of the sample, a rarefaction analysis was performed in Qiime, using the alpha rarefaction and summarised taxa scripts. The bacterial composition (at Phylum level) was calculated per sample and reported in percentage. Data were divided in four categories, depending on the origin of the sample: mud, water, female's gills and eggs. Principal coordinate (PCO) analyses were done to visualise possible separation among categories. To test for differences in the bacterial composition among categories, one-way Permutational ANOVAs (PERMANOVA) were performed on the overall dataset as well as on the most represented phyla, with Category (3 levels) as an independent, fixed factor. While possible problems of interdependence of data could limit the value of the Phylum related analysis, sacrificial replication was not possible due to the limited amount of replicates available for each category. Additionally, a Bonferroni correction was included for this analysis. Analyses were performed on a Bray Curtis similarity matrix (Bray and Curtis, 1957) based on arcsine transformed data (to meet the assumption of ANOVA when data are

proportions), using 9999 permutations (Anderson *et al.*, 2008). The data contained a high proportion of zeros and, therefore, a dummy variable, with a value of 0.0001, was added to the similarity matrix to moderate spurious similarities where no phyla were recorded in two compared samples (Clarke and Gorley, 2006). Analyses were, however, also performed without the addition of a dummy variable, yielding the same results. For each of the analysis, pairwise comparison tests followed whenever the factor Category resulted significant, to further identify which Category yielded different bacterial composition in each of the analyses.

Results

Rarefaction analysis is a method used by biologists to compensate for sample-size differences in the measurement of richness (Foote, 1992). It is an approach used to determine whether all the species in an "ecosystem" have been observed and is based on the construction of rarefaction curves (Moyer *et al*., 1998). A curve approximately reaching an asymptote indicates that few or no species would be collected if sampling effort is further increased, and should it not level out, but rather sharply rise near its end then it provides an indication that many new species could be recorded by additional sampling (Chiarucci *et al*., 2008). Rarefaction curves of the sequenced samples were constructed using the Qiime pipeline. Gills, eggs and mud all had levelled off indicating that most of the bacteria species had been sampled and considered (Figure 3.1). The water sequences on the other hand did not level out towards the asymptote, and therefore, no further analyses were done for this category. Rarefaction curves and tables, along with graphs on taxonomic affiliation at all levels and replicates representing all categories are reported in Appendices 3.1. PCO analysis of the three remaining categories resulted in the grouping of gill and mud samples (hence only visualisation of female samples in Figure 3.2), while egg samples differed from the two grouped categories, the cumulated variance explained by the first two coordinates was very low, 12.6% (Figure 3.2).

The outcome of the PERMANOVA showed significant differences among categories (mud, eggs and gills) (Table 3.1). Overall, pairwise comparisons, indicated that the bacterial composition between eggs and mud differed significantly; eggs and gills also differed significantly, whereas bacterial composition between mud and gills did not differ significantly (Table 3.1, Figure 3.3). Similar results followed from the individual analyses on the most common Phyla were considered. Significant differences (even after a Bonferroni correction which lowered the acceptance p-value to 0.002) within the phylum Firmicutes were found

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between eggs and mud, and between gills and mud, while no significant differences were found between eggs and gill (Table 3.2). These last results could be attributed to that both the categories (gills and eggs) had no Firmicutes detected (0% relative contribution to the total number of sequence reads), (Figure 3.3 and Appendices 3.1). For the two phyla, Actinobacteria and Proteobacteria, eggs significantly differed from both the mud and gills, while gills contained similar bacterial composition as that of mud in both phyla (Table 3.2, Figures 3.4 & 3.5).
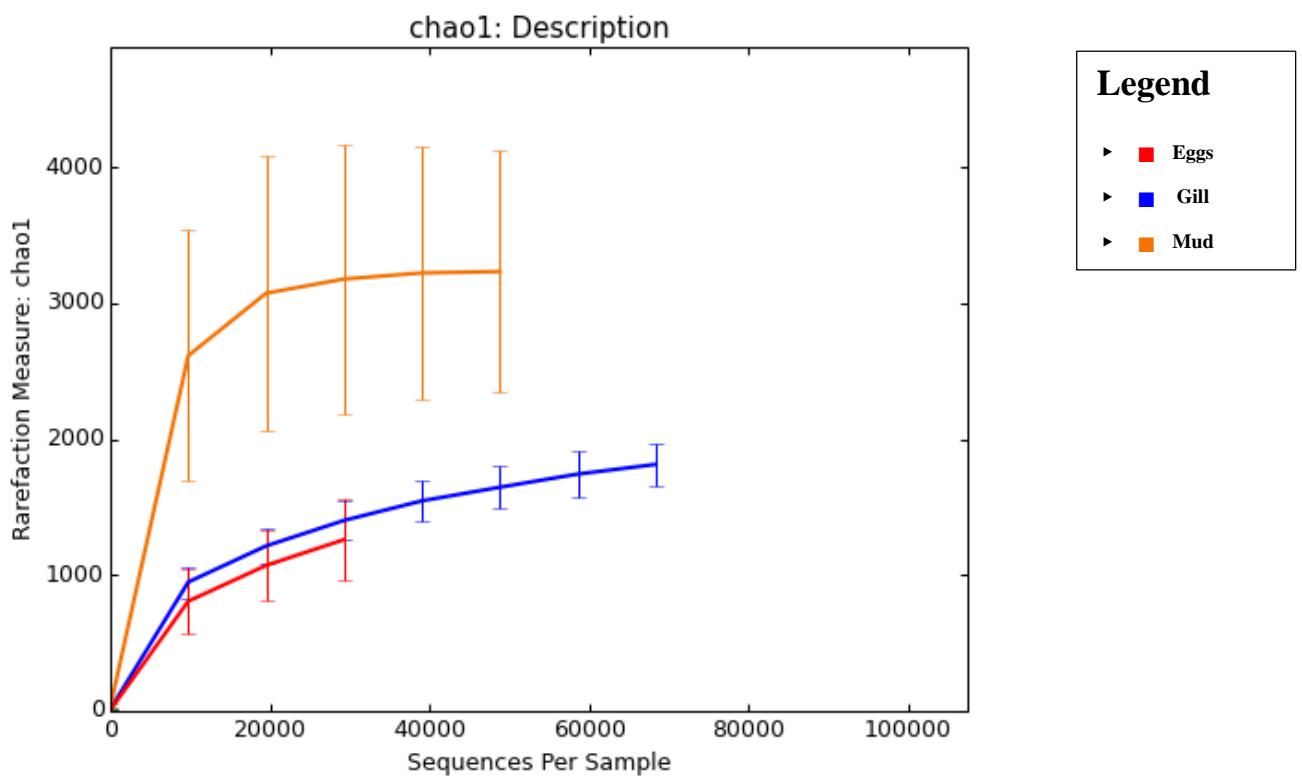
Figure 3.1: Chao1 rarefaction curves of the total number of reads generated by Ion Torrent PGM sequencing versus the total number of 16S rRNA species identified.
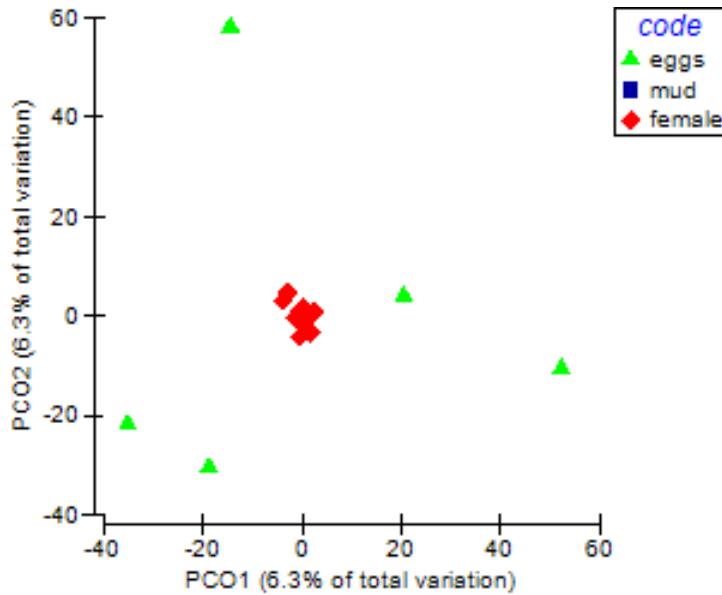
Figure 3.2: Principal Coordinates Analysis (PCO) of all the three categories investigated (mud, females and eggs). Mud data points are hidden behind the female symbols.

Table 3.1: One-way PERMANOVA on the effect of category on the bacterial composition. Category: Eggs, Mud and Gills. df = degrees of freedom; SS = sum of squares; MS = mean sums of squares; F = F-Statistic; RES = Residuals; Total = total degrees of freedom.

Factors	df	SS	MS	F- ratio	p-value
Category (Ca)	2	2206.2	1103.1	8.6551	0.0017
Res	14	1784.3	127.45		
Total	16	3990.6			
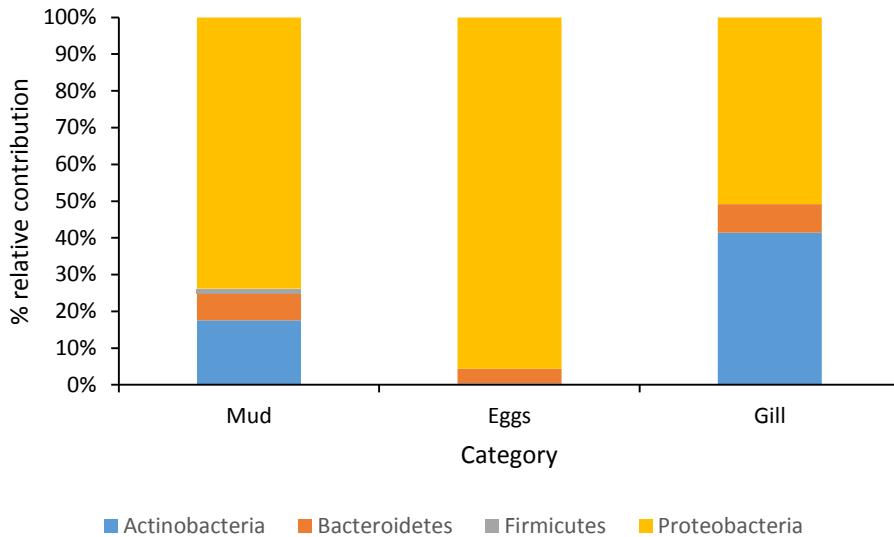
Figure 3.3: Relative contribution (in percentage) of the bacterial composition to the total number of sequence reads for the four major dominant phyla associated with mud, eggs and gills collected from specimen of *U. urvillei* and sediment at Mngazana.

Table 3.2: PERMANOVA on the bacterial composition of the four phyla analysed. Category: Female, Eggs and Mud (see material and methods). d.f: degrees of freedom; SS: Sum of Square; MS: Mean sums of Square; RES: Residuals; Total = total degrees of freedom.

Bacterial composition	d.f	SS	MS	F	p-value
Actinobacteria					
Category	2	0.5364	0.2682	9.112	0.0031
RES	14	0.4121	0.0291	-	-
Bacteroidetes					
Category	2	0.0003	0.0015	0.840	0.8171
RES	14	0.1001	0.0071	-	-
Firmicutes					
Category	2	0.0003	0.0005	27.97	0.0001
RES	14	0.0	0.0005	-	-
Proteobacteria					
Category	2	1.4589	0.7295	0.0013	0.0006
RES	14	0.8735	0.0624	-	-
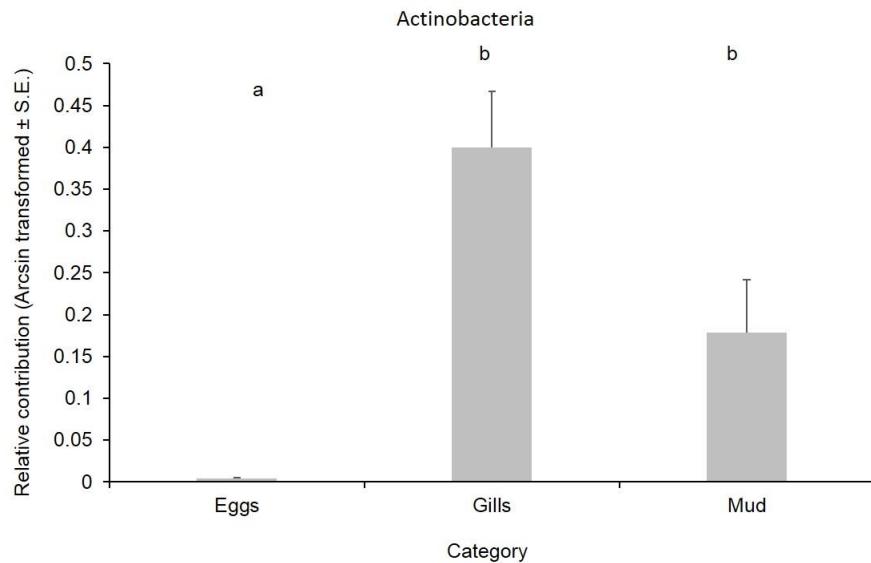
Figure 3.4: Relative contribution (arcsin transformed) of the bacterial composition to the total number of sequence reads for the phylum Actinobacteria associated with eggs, gills and mud. Vertical bars report Standard Error; letters on top of the bars represent the groupings based on the pairwise analyses for the significant effect of factor Category. Egg/mud,$p<0.03$, egg/gill<0.001, gill/mud$p>0.05$.
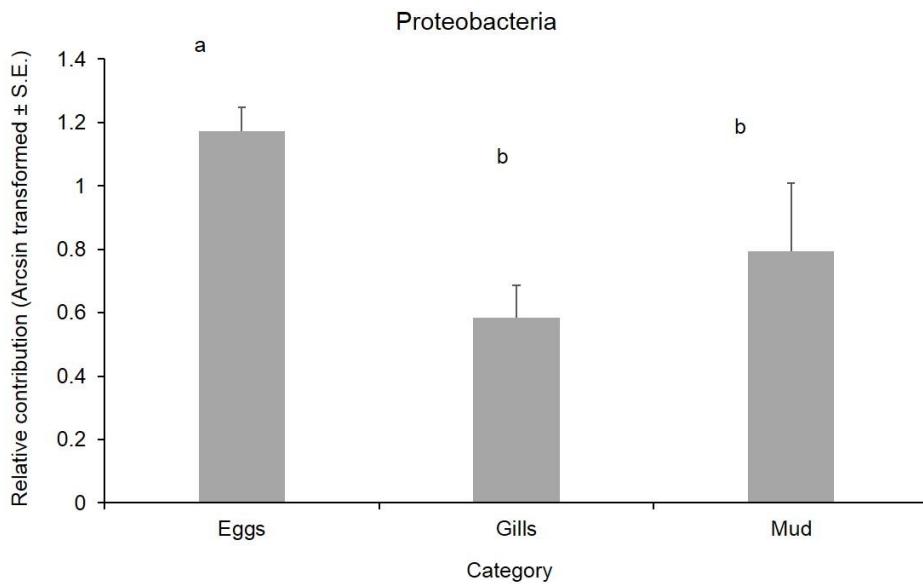
Figure 3.5: Relative contribution (arcsin transformed) of the bacterial composition to the total number of sequence reads for the phylum Proteobacteria associated with eggs and gills of the same individual and mud. Vertical bars report Standard Error; letters on top of the bars represent the groupings based on the pairwise analyses for the significant effect of the factor Category.

Discussion

Interactions between a large animal (host) and its symbiotic microbes is an important foundation for many terrestrial and marine ecosystems (Douglas, 1994). In forming the associations, the host acquires the microbial symbiont's intrinsic metabolism and thereby gains a novel capability, essential for its survival and expansion into new habitats, whereas the microbes, in return, are generally provided with shelter and refuge from predators (Weis *et al.*, 2001). Irrespective of the mechanism of transmission (vertical, horizontal or mixed), both the partners involved in symbiotic associations are vital for the survival and persistence of several ecosystems. The goal of this study was to investigate the composition of the bacterial community among the mother (gills), the eggs of *Uca urvillei*, and mud collected from the Mngazana Estuary, South Africa. The dominant phyla associated with all the categories included: Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes. The analysed organs and samples (gills of females, eggs and mud) revealed the presence of all major phyla, with the exception of Firmicutes, which were present only in mud samples.

The phylum Proteobacteria, was found in relatively high proportions in all the samples, and it consisted primarily of ecologically-important subclasses including Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria (see Appendices A2.1-A2.3). The Proteobacteria group falls within the three major branches of sulphate-reducing bacteria (Becker *et al.*, 2009; Gruber-Vodicka *et al.*, 2011; Marshall and Morris, 2012; Tourna *et al.*, 2014). In eggs, Proteobacteria showed the highest values, with a generally significantly different bacterial composition than gills of females and the environment (mud), which, on the

other hand, presented a similar composition. This is no surprise since the gills are constantly in contact with the outside environment (soil and water) and Proteobacteria is considered one of the abundant groups in soil microbes (Janssen, 2006; Nair *et al*., 2013).

The phylum Actinobacteria showed similar results to those of Proteobacteria, with comparable values for the gills of females and environment, which differed from the eggs. Actinobacteria were present in eggs, but in lower proportions compared to the other two categories. The low levels of Actinobacteria in eggs and high values found in gills and mud suggests that this group is probably mostly acquired from the environment. By comparison, *Acromyrmex* leaf-cutting ants that are known to carry *Pseudonocardia* Actinobacteria exosysymbionts, emerge from their eclose symbiont-free, but exhibit visible Actinobacteria within 14 days after being out of the eclose (Marsh *et al.,* 2014). Moreover, *Siphamia versicolor*, a cardinalfish, does not contain symbiotic bacteria for about 8 days from their release from the male's mouths, after which the bacterial population increased rapidly, suggesting therefore, horizontal transmission (Dunlap *et al*., 2012).

The phylum Bacteroidetes is amongst the abundant bacterial phyla in marine systems with members that are hypothesised to play an important role in the degradation of polysaccharides and recycling of organic matter (O'Sullivan *et al*., 2006). In the present study, the phylum Bacteroidetes was present in all the samples, regardless of the category. Because this group is present "throughout" the samples, and can be found in virtually all habitats (Wexler, 2007), it is then difficult to exclude either of the three proposed ways of transmissions. The phylum Firmicutes, on the other hand, was not detected in egg and gill samples, but was present in the environment (mud). This result confirms the findings reported in Chapter 2 of this thesis, where this phylum was also not detected in the gills of adult crabs. The association of bacteria belonging to the phylum Firmicutes with mangrove sediments is common worldwide

(Bharathkumar *et al*., 2008; Zhang *et al*., 2009; Ghosh *et al*., 2010; Soares Júnior *et al.,* 2013; Vincent *et al.*, 2013).

Generally, in aquatic systems, bacterial symbionts transmitted horizontally are often acquired from the water rather than mud (McFall-Ngai, 1998). These two (sediment and water) are considered among the important realms in aquatic systems (Wang et al., 2012), containing fairly different microorganisms, with sediment containing relatively more microbes in terms of taxon and biomass than the surrounding water (Jiang *et al*., 2006; Zinger *et al*., 2011). In the present study, water samples were therefore also sequenced to further disentangle the environmental source of bacteria. Unfortunately, the rarefaction analysis for the water samples indicated that not all the taxonomic richness in the category was accounted for, suggesting that additional sampling is needed, making these samples not useful for further analysis.

In aquatic systems, many crustaceans pass through a complex life cycle comprising an embryonic, a pelagic larval, and a juvenile-adult phase, instead of direct development from egg to an adult-like juvenile (McEdward, 2000). The metabolic needs of each life cycle phase differs accordingly (Anger, 2006). For example, developing eggs of intertidal crabs stay attached to the abdominal pleopods of females until hatching, and rely therefore on the mothers for nutrition (Taylor, 2001). Once hatched, larvae are transported rapidly away from adult habitats, and become more independent (although still carrying the long-term maternal provisions; Christy and Salmon, 1984; Christy, 1989). Given the lack of Actinobacteria in the eggs and their presence in the adults, the present study suggests that this group of bacteria is acquired from the surrounding environment. To add, an analysis of bacteria associated with larvae would further help clarifying the exact point of transmission of Actinobacteria in *U. urvillei*.

From the present results, it is difficult to determine the mode of transmission employed by *U. urvillei*. These results are preliminary and regrettably, the questions asked at the beginning of

the study cannot be entirely answered as of yet. This can, however, be the treated as the first step towards understanding the modes at which *U. urvillei* maintains its bacterial symbionts through successive generations and, ultimately, further understand the functioning of mangrove systems. Also, while acknowledging the extremely broad taxonomic resolution applied to this study, it is still reasonable to assume that *U. urvillei* employs the mixed mode strategy; where Proteobacteria are potentially transmitted vertically, while further research could highlight the horizontal transmission of Bacteroidetes and even Actinobacteria. In a radically broad and most speculative sense, this mechanism exists for human infants, where a group of bacteria, including *Staphylococcus epidermidis* and *Escherichia coli*, were isolated from umbilical cord blood obtained from healthy neonates (Jiménez *et al*., 2008), suggesting that an infant incorporates an initial microbiome before birth and receives abundant supplementation of maternal microbes afterwards through birth and breastfeeding (Funkhouer and Bordenstein, 2013; Rodríguez *et al*., 2015). Further manipulative studies (coupled with available data from the current research) are required, and the addition of an analysis of the bacterial profile present in the reproductive tissue (gonads), will help clarifying the mode of transmission of symbiotic bacteria in this species. In addition to this, investigating bacterial profiles associated with early larval phases of this species could yield an entire egg-to-adult outline of associated symbionts.

Chapter 4: General discussion and concluding remarks

Marine coastal ecosystems occupy one of the most hostile environments where abiotic conditions such as temperatures, pH, salinity, currents and wind patterns tend to vary abruptly and extremely (Boero, 1994; Julius *et al.*, 2005). Mangrove estuaries are no exception (Krauss and Ball, 2013), where high and rapid fluctuations in abiotic parameters, particularly salinity and temperature, dominate (Laprise and Dodson, 1994). Microorganisms constitute the largest diversity and biomass of all mangrove biota (Holguin *et al.*, 2001; Lakashmipriya and Swakumar, 2012) and due to their phenotypic plasticity, these organisms are able to survive in such harsh environments (Sousa *et al.*, 2011). They have the ability to adapt rapidly, meaning that they can shift their metabolic capabilities, host range, functions and community dynamics in response to changing environmental conditions (Webster and Bourner, 2012). With phenotypic plasticity, the individual's ability to cope with environmental changes and to potentially adapt to new niches are improved (Prada *et al.*, 2008), and this plasticity can be particularly important in the case of (bacterial) symbionts, because of the consequent effect on the host biology and overall persistence (Lau and Lennon, 2012). For a long time, microbial ecologists have relied on traditional culture-dependent techniques, such as petri-dish analysis, to examine and analyse bacterial communities. Nowadays, culture-independent methods that rely on direct amplification and analysis of 16S rRNA gene sequences are rapidly replacing these culture-dependent techniques (Dunbar *et al.*, 1999). The use of Next Generation Sequencing techniques such as Roche 454 pyrosequencing, Solexa/Illumina, Ion Torrent: proton/PGM and SOLiD sequencing, allows sequencing of DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing (Mardis, 2008; Grada and Weinbrecht, 2013; Knief, 2014). These techniques help scientists to capture the diversity of microbial communities at high taxonomic resolution from various habitats on Earth (Schuster, 2008;

Metzker, 2010; Grada and Weinbrecht, 2013; Shyr and Liu, 2013). For example, Yergeau *et al.* (2012) used the Ion Torrent sequencing to evaluate the possible ecological impact of oil sands mining on microbial community structure in soil from the Athabasca River and its tributaries. These molecular methods have also been used to study the potential responses of microbial to environmental changes (Castro *et al.*, 2010; Zhang *et al.*, 2013). For example, through the use of NGS methods, Lau and Lennon (2012) showed that plant fitness to environmental stress (drought) were mostly governed by rapid changes in microbial community structure rather than by evolutionary changes of plant traits. In the present study, Ion Torrent PGM sequencing technique was successfully used to examine possible effects of different salinity levels on chemoautotrophic bacterial symbionts associated with one species of mangrove crabs, *Uca urvillei*.

The purpose of the current study was to document temporal variability of chemoautotrophic bacterial symbionts associated with *U. urvillei* in response to short term changes in salinity, and to investigate the modes at which these symbionts are passed on to the next generation. It was shown that the bacterial community is not affected by short term changes in salinity. All the dominant phyla revealed by sequencing persisted throughout the different salinity levels, regardless of treatment (different salinities and the application of an antibiotic). The biology of crabs, on other hand, was affected significantly by salinity, particularly when this was reflecting environmental conditions (35‰). This could have serious physiological consequences for all the organisms involved, but more particularly for the host, *U. urvillei*. In symbiotic associations, co-adaption is the "best" bet if two different species are to persist against the changing climate (Lau *et al.*, 2010). Failing which, hosts might have to acquire new symbionts and develop new associations, in order to survive in new or extremely changed environments (Peek *et al.*, 1998; Hofmann and Todgham, 2010; Reusch, 2014). For example, stony corals are often associated with dinoflagellates of the genus *Symbiodinium*, and, to cope

with increasing temperatures, corals changed the dominant symbiont type to Clade D, a well-known thermally tolerant *Symbiodinium*, that has increased its thermal tolerance by 1-1.5 ℃ (Berkelsman and van Oppen, 2006).

The study at hand could not explicitly determine the modes at which the bacteria are passed on to the offspring; no evidence was found to support any of the three modes proposed at the beginning of the study. It is, nonetheless, possible that, for this particular species of fiddler crab, the mixed mode strategy is how the symbionts are transferred, at least for some bacterial strains. If this is the case, the targeted species in this study should have a better chance of adapting to local changes in climate, as the larvae have a potential to couple the maternally transmitted symbionts with optimal bacterial symbionts available from the surrounding environment should environment conditions instantaneously change upon settlement. Such "flexible", bidirectional strategy of transmission would allow for a wider and more "generic" bacterial requirements and would increase the chances of sourcing beneficial bacteria, depending on the environmental forcing (van de Bosch *et al*., 2010). Overall, this study recommends that, including long term exposures of *U. urvillei* to different salinities and possibly incorporate or synergise with other environmental parameters, and examining additional reproductive tissues, may increase our understanding of how this species is likely to respond to any local change that the mangrove systems might face in the near future, particularly the ones located at transition zones (from sub-tropical to warm temperate) like the Mngazana Estuary. Populations at the edge of biogeographic boundaries, such as the southernmost limit of mangroves for Mngazana, are exposed to increased risks to drastic and extreme environmental changes (James *et al*., 2008). This exposure may pose further threats to the persistence of organisms and overall ecological communities. Hence, it is crucial to highlight the benefits of any phenotypic flexibility/adaptability (Stillman and Armstrong,

2015), like the wide tolerance of symbiont bacteria to salinity and the possible mixed transmission, as suggested by this study.

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Appendices

A 2.1. List of bacterial composition from Phylum to Family level for Salinity treatment (5 and 35‰)

Taxonomy	count	Total%	5‰	35‰
Unassigned;Other;Other;Other;Other	0	0.3%	0.3%	0.4%
k__Bacteria;Other;Other;Other;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Acidobacteria;c__Solibacteres;o__Solibacterales;f__PAUC26f	0	0.0%	0.0%	0.0%
k__Bacteria;p__Acidobacteria;c__Sva0725;o__Sva0725;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	1	26.5%	21.4%	31.6%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__JdFBGBact	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Microthrixaceae	0	0.1%	0.0%	0.1%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__SC3-41	0	4.4%	4.0%	4.7%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__koll13	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other	0	0.1%	0.1%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinosynnemataceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0	0.2%	0.2%	0.1%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Nitriliruptoria;o__Nitriliruptorales;f__Nitriliruptoraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__OPB41;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;Other;Other;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__BME43;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__VC21_Bac22	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae	0	0.0%	0.1%	0.0%
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;Other	0	0.1%	0.0%	0.1%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__	0	1.0%	1.2%	0.7%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae	0	0.2%	0.3%	0.1%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	0	0.4%	0.4%	0.4%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	0	14.2%	18.8%	9.7%
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__NS11-12	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae	0	0.0%	0.0%	0.1%
k__Bacteria;p__Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae]	0	0.0%	0.0%	0.0%

k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__	0	0.4%	0.6%	0.2%
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae	0	1.4%	0.4%	2.4%
k__Bacteria;p__Caldithrix;c__Caldithrixae;o__Caldithrixales;f__BA059	0	0.0%	0.0%	0.0%
k__Bacteria;p__Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Chlorobi;c__;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Chlorobi;c__OPB56;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Cyanobacteria;c__ML635J-21;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Fibrobacteres;c__TG3;o__TG3-2;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__JTB215	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae]	0	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0	0.0%	0.0%	0.0%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__GN02;c__BD1-5;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Gemmatimonadetes;c__Gemm-2;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Gemmatimonadetes;c__Gemm-4;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Gemmatimonadetes;c__Gemm-5;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;f__Arctic95B-10	0	0.0%	0.0%	0.0%
k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;Other;Other;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other;Other	0	0.2%	0.2%	0.2%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Kiloniellaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Kordiimonadales;f__Kordiimonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	0	17.1%	17.8%	16.4%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	0	0.8%	0.7%	1.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0	0.1%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	0	22.4%	26.5%	18.2%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	0	2.7%	1.2%	4.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__	0	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__	0	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other	0	0.1%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	0	4.5%	2.4%	6.6%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	0	1.5%	1.7%	1.3%

Taxon				
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	0	0.1%	0.3%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__GMD14H09;f__	0	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Cystobacterineae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae	0	0.1%	0.1%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__OM27	0	0.1%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__NB1-i	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__PB19;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Spirobacillales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae]	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae	0	0.1%	0.3%	0.0%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__211ds20	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__HTCC2188	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__J115	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__OM60	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae]	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__	0	0.0%	0.0%	0.0%

k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__Ectothiorhodospiraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Francisellaceae	0	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoraceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae	0	0.2%	0.1%	0.3%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Thiotrichaceae	0	0.1%	0.3%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;Other	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	0	0.0%	0.1%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales];f__[Marinicellaceae]	0	0.0%	0.0%	0.0%
k__Bacteria;p__Spirochaetes;c__MVP-15;o__PL-11B10;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__M2PT2-76;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	0	0.0%	0.0%	0.0%
k__Bacteria;p__TM6;c__SJA-4;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__TM7;c__;o__;f__	0	0.0%	0.0%	0.0%
k__Bacteria;p__TM7;c__TM7-3;o__;f__	0	0.0%	0.0%	0.0%

A2.2. List of bacterial ttransmission from Phylum to Family level exposed to different salinities at different time intervals (Time1, 2 and 3).

Taxonomy	count	Total	T1	T3	T4
Unassigned;Other;Other;Other;Other	0	0.3%	0.2%	0.3%	0.6%
k__Bacteria;Other;Other;Other;Other	0	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__	0	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__PAUC26f	0	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Sva0725;o__Sva0725;f__	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	1	28.0%	22.4%	31.4%	30.2%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__JdFBGBact	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Microthrixaceae	0	0.1%	0.0%	0.0%	0.1%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__SC3-41	0	4.7%	3.8%	6.0%	4.4%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__koll13	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other	0	0.1%	0.0%	0.0%	0.1%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinosynnemataceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0	0.2%	0.0%	0.2%	0.3%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Nitriliruptoria;o__Nitriliruptorales;f__Nitriliruptoraceae	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__OPB41;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;Other;Other;Other	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__BME43;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__VC21_Bac22	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae	0	0.1%	0.0%	0.0%	0.1%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0	0.0%	0.0%	0.0%	0.1%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;Other	0	0.1%	0.0%	0.0%	0.2%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__	0	1.0%	1.2%	1.6%	0.3%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae	0	0.2%	0.0%	0.0%	0.6%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	0	0.4%	0.1%	0.1%	1.1%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	0	15.4%	10.4%	15.6%	20.2%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__NS11-12	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae	0	0.1%	0.0%	0.0%	0.1%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae]	0	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__	0	0.5%	0.1%	0.1%	1.2%

Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0	0.0%	0.0%	0.0%	0.1%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae	0	1.4%	1.5%	1.9%	0.7%
Caldithrix;c__Caldithrixae;o__Caldithrixales;f__BA059	0	0.0%	0.0%	0.0%	0.0%
Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae	0	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__OPB56;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Cyanobacteria;c__ML635J-21;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Fibrobacteres;c__TG3;o__TG3-2;f__	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;Other	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__JTB215	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae]	0	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0	0.0%	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__	0	0.0%	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0	0.0%	0.0%	0.0%	0.0%
GN02;c__BD1-5;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-2;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-4;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-5;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;f__Arctic95B-10	0	0.0%	0.0%	0.0%	0.0%
Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;Other;Other;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;Other;Other	0	0.2%	0.2%	0.2%	0.2%
Proteobacteria;c__Alphaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Kiloniellaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kordiimonadales;f__Kordiimonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	0	14.4%	24.4%	9.3%	9.6%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	0	0.9%	0.8%	1.3%	0.7%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0	0.1%	0.0%	0.0%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	1	20.9%	27.7%	19.4%	15.6%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	0	3.4%	0.9%	5.5%	3.9%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other	0	0.1%	0.1%	0.1%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	0	4.7%	4.0%	5.6%	4.4%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	0	1.4%	1.2%	0.6%	2.4%
Proteobacteria;c__Betaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	0	0.1%	0.0%	0.0%	0.4%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae	0	0.0%	0.0%	0.0%	0.0%

Taxonomy					
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__GMD14H09;f__	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Cystobacterineae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae	0	0.1%	0.0%	0.0%	0.2%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__OM27	0	0.1%	0.0%	0.1%	0.1%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__NB1-i	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__PB19;f__	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Deltaproteobacteria;o__Spirobacillales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae]	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae	0	0.1%	0.2%	0.1%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__211ds20	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__HTCC2188	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__J115	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__OM60	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae]	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__Ectothiorhodospiraceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Francisellaceae	0	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoracaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae	0	0.1%	0.3%	0.0%	0.1%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Thiotrichaceae	0	0.2%	0.0%	0.0%	0.5%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;Other	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	0	0.0%	0.1%	0.0%	0.0%

Taxonomy					
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	0	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales];f__[Marinicellaceae]	0	0.0%	0.0%	0.0%	0.0%
Spirochaetes;c__MVP-15;o__PL-11B10;f__	0	0.0%	0.0%	0.0%	0.0%
Spirochaetes;c__Spirochaetes;o__M2PT2-76;f__	0	0.0%	0.0%	0.0%	0.0%
Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	0	0.0%	0.0%	0.0%	0.0%
TM6;c__SJA-4;o__;f__	0	0.0%	0.0%	0.0%	0.0%
TM7;c__;o__;f__	0	0.0%	0.0%	0.0%	0.0%
TM7;c__TM7-3;o__;f__	0	0.0%	0.0%	0.0%	0.0%

A2.3. List of bacterial ttransmission from Phylum to Family level exposed to different salinities at different time intervals (Time1, 2 and 3).

Taxonomy	count	Total	YES	NO
Unassigned;Other;Other;Other;Other	0	0.3%	0.4%	0.2%
k__Bacteria;Other;Other;Other;Other	0	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__	0	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__PAUC26f	0	0.0%	0.0%	0.0%
Acidobacteria;c__Sva0725;o__Sva0725;f__	0	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other	0	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	1	26.4%	26.2%	26.6%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__JdFBGBact	0	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Microthrixaceae	0	0.1%	0.1%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__SC3-41	0	4.4%	3.0%	5.8%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__koll13	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other	0	0.1%	0.1%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinosynnemataceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0	0.2%	0.2%	0.1%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__Nitriliruptoria;o__Nitriliruptorales;f__Nitriliruptoraceae	0	0.0%	0.0%	0.0%
Actinobacteria;c__OPB41;o__;f__	0	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	0	0.0%	0.0%	0.0%
Bacteroidetes;Other;Other;Other	0	0.0%	0.0%	0.0%
Bacteroidetes;c__BME43;o__;f__	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__VC21_Bac22	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae	0	0.0%	0.1%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;Other	0	0.1%	0.0%	0.1%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__	0	1.0%	0.6%	1.4%

Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae	0	0.2%	0.3%	0.1%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	0	0.4%	0.5%	0.3%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	0	14.3%	18.9%	9.7%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	0	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__NS11-12	0	0.0%	0.0%	0.0%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae	0	0.0%	0.1%	0.0%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae]	0	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__	0	0.5%	0.2%	0.7%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae	0	1.4%	1.2%	1.5%
Caldithrix;c__Caldithrixae;o__Caldithrixales;f__BA059	0	0.0%	0.0%	0.0%
Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Parachlamydiaceae	0	0.0%	0.0%	0.0%
Chlorobi;c__;o__;f__	0	0.0%	0.0%	0.0%
Chlorobi;c__OPB56;o__;f__	0	0.0%	0.0%	0.0%
Cyanobacteria;c__ML635J-21;o__;f__	0	0.0%	0.0%	0.0%
Fibrobacteres;c__TG3;o__TG3-2;f__	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;Other	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__JTB215	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae]	0	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__	0	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0	0.0%	0.0%	0.0%
GN02;c__BD1-5;o__;f__	0	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-2;o__;f__	0	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-4;o__;f__	0	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-5;o__;f__	0	0.0%	0.0%	0.0%
Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;f__Arctic95B-10	0	0.0%	0.0%	0.0%
Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae	0	0.0%	0.0%	0.0%
Proteobacteria;Other;Other;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__;o__;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;Other;Other	0	0.2%	0.2%	0.2%
Proteobacteria;c__Alphaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__	0	0.0%	0.0%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__Kiloniellaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kordiimonadales;f__Kordiimonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	0	17.0%	23.4%	10.6%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	0	0.8%	0.6%	1.1%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0	0.1%	0.1%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	0	22.6%	14.7%	30.5%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	0	2.6%	2.4%	2.9%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__	0	0.0%	0.1%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__	0	0.0%	0.1%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other	0	0.1%	0.1%	0.1%

Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	0	4.4%	5.1%	3.7%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	0	1.5%	0.7%	2.4%
Proteobacteria;c__Betaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	0	0.1%	0.0%	0.3%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Nitrosomonadales;f__Nitrosomonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__GMD14H09;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Cystobacterineae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae	0	0.1%	0.1%	0.1%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__OM27	0	0.1%	0.1%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__NB1-i	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__PB19;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Spirobacillales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae]	0	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae	0	0.2%	0.0%	0.3%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;Other;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__211ds20	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae	0	0.0%	0.1%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__HTCC2188	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__J115	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__OM60	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae]	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__Ectothiorhodospiraceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Francisellaceae	0	0.0%	0.1%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoracaceae	0	0.0%	0.0%	0.0%

Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae	0	0.2%	0.0%	0.4%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Thiotrichaceae	0	0.2%	0.0%	0.3%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;Other	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	0	0.0%	0.0%	0.1%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	0	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales];f__[Marinicellaceae]	0	0.0%	0.0%	0.0%
Spirochaetes;c__MVP-15;o__PL-11B10;f__	0	0.0%	0.0%	0.0%
Spirochaetes;c__Spirochaetes;o__M2PT2-76;f__	0	0.0%	0.0%	0.0%
Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	0	0.0%	0.0%	0.0%
TM6;c__SJA-4;o__;f__	0	0.0%	0.0%	0.0%
TM7;c__;o__;f__	0	0.0%	0.0%	0.0%
TM7;c__TM7-3;o__;f__	0	0.0%	0.0%	0.0%

A 3.1 List of bacterial composition from Phylum to Family level. F= female (gills), E= eggs, M= mud and W= water.

Taxonomy	Tot	F6E6	W2	M2	W3	M3	F1	F2	F3	F4	F5	F6	F7	F8	F8E8	F4E4	F9	F1E1	F9E9	W1	F10
Unassigned;Other;Other;Other;Other	2.1%	1.6%	0.2%	0.4%	0.1%	1.3%	2.5%	1.8%	5.1%	1.2%	1.5%	9.3%	2.4%	4.5%	0.8%	1.1%	2.0%	1.1%	2.2%	0.3%	1.9%
Acidobacteria;Other;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__AT-s2-57;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Acidobacteria-5;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Acidobacteriia;o__Acidobacteriales;f__Acidobacteriaceae	0.1%	0.0%	1.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__DA052;o__E29;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__OS-K;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__RB25;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;Other	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__	0.3%	0.0%	0.0%	0.0%	5.7%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__PAUC26f	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Solibacteres;o__Solibacterales;f__Solibacteraceae	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__Sva0725;o__Sva0725;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__TM1;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__iii1-8;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Acidobacteria;c__iii1-8;o__DS-18;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;Other;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.1%	0.2%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%

Taxon																					
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__	0.6%	0.0%	0.0%	3.2%	1.9%	6.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__AKIW874	0.0%	0.0%	0.0%	0.1%	0.0%	0.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__C111	16.1%	0.3%	1.3%	3.7%	0.3%	9.5%	38.8%	54.5%	45.6%	38.7%	7.2%	26.2%	22.8%	47.5%	0.1%	0.0%	12.1%	0.0%	0.1%	0.0%	13.5%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__EB1017	0.0%	0.0%	0.0%	0.1%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__JdFBGBact	0.1%	0.2%	0.0%	0.0%	0.0%	0.1%	0.1%	0.2%	0.1%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%	0.2%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__Microthrixaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__SC3-41	3.6%	0.1%	0.0%	0.0%	0.0%	0.0%	12.1%	17.6%	7.1%	3.4%	8.3%	4.7%	5.1%	2.4%	0.0%	0.2%	10.5%	0.2%	0.0%	0.0%	0.7%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__TK06	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__ZA3409c	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__koll13	0.1%	0.0%	0.0%	0.6%	0.0%	1.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__wb1_P06	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;Other	0.1%	0.0%	0.1%	0.1%	0.7%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__	0.1%	0.0%	0.7%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinomycetaceae	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Actinosynnemataceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Brevibacteriaceae	0.0%	0.0%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Cellulomonadaceae	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Corynebacteriaceae	0.7%	0.0%	13.7%	0.2%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermabacteraceae	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermacoccaceae	0.2%	0.0%	0.0%	0.0%	3.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%

Taxonomy																								
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dietziaceae	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Geodermatophilaceae	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Gordoniaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Intrasporangiaceae	0.3%	0.0%	3.1%	0.3%	0.9%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Kineosporiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae	0.1%	0.0%	0.6%	0.2%	0.6%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae	0.9%	0.0%	2.1%	0.2%	15.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micromonosporaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Mycobacteriaceae	0.0%	0.0%	0.0%	0.2%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae	0.0%	0.0%	0.0%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardioidaceae	0.7%	0.0%	12.2%	0.3%	1.1%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae	0.0%	0.0%	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	0.0%	0.0%	0.3%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptomycetaceae	0.1%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.8%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Streptosporangiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Thermomonosporaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__MB-A2-108;o__0319-7L14;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Nitriliruptoria;o__Euzebyales;f__Euzebyaceae	0.0%	0.0%	0.0%	0.2%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Nitriliruptoria;o__Nitriliruptorales;f__Nitriliruptoraceae	0.2%	0.0%	0.0%	1.2%	0.0%	2.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Actinobacteria;c__OPB41;o__;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Rubrobacteria;o__Rubrobacterales;f__Rubrobacteraceae	0.2%	0.0%	2.6%	0.0%	1.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%
Actinobacteria;c__Thermoleophilia;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Gaiellales;f__Gaiellaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__	0.0%	0.0%	0.2%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria;c__Thermoleophilia;o__Solirubrobacterales;f__Solirubrobacteraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;Other;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__BME43;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0.0%	0.0%	0.0%	0.2%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Marinilabiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae	0.0%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__SB-1	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__VC21_Bac22	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cyclobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	0.0%	1.0%	0.4%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Flammeovirgaceae	0.1%	0.0%	0.0%	0.3%	0.0%	2.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Cytophagia;o__Cytophagales;f__[Amoebophilaceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxon																					
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Cryomorphaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae	0.7%	2.5%	0.9%	0.9%	0.1%	7.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	0.0%	0.3%	1.5%	0.7%	0.0%
Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae]	3.7%	0.1%	0.0%	0.0%	0.3%	0.1%	3.1%	0.5%	15.7%	0.6%	0.5%	30.5%	5.3%	13.6%	0.0%	0.0%	1.9%	0.0%	0.1%	0.0%	1.3%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__NS11-12	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__Rhodothermaceae	0.1%	0.0%	0.0%	0.2%	0.0%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Rhodothermi];o__[Rhodothermales];f__[Balneolaceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.2%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae	0.0%	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%
Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Saprospiraceae	1.0%	1.3%	0.0%	0.1%	0.0%	0.7%	0.3%	0.0%	0.0%	0.4%	1.1%	0.0%	0.1%	0.0%	0.8%	3.9%	0.0%	0.9%	9.4%	0.0%	0.2%
Caldithrix;c__Caldithrixae;o__Caldithrixales;f__BA059	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Caldithrix;c__Caldithrixae;o__Caldithrixales;f__Caldithrixaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chlamydiae;c__Chlamydiia;o__Chlamydiales;f__Criblamydiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__Ignavibacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chlorobi;c__Ignavibacteria;o__Ignavibacteriales;f__IheB3-7	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy																					
Chlorobi;c__OPB56;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__Anaerolineae;o__Ardenscatenales;f__Ardenscatenaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%
Chloroflexi;c__Anaerolineae;o__OPB11;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__Anaerolineae;o__SHA-20;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__Anaerolineae;o__SJA-15;f__	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__C0119;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__Dehalococcoidetes;o__Dehalococcoidales;f__Dehalococcoidaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__Ellin6529;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi;c__SAR202;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cyanobacteria;c__ML635J-21;o__;f__	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Elusimicrobia;c__Elusimicrobia;o__IIb;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Elusimicrobia;c__Endomicrobia;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;Other	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Alicyclobacillaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Listeriaceae	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.9%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae	0.2%	0.0%	1.4%	0.0%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Bacillales;f__[Exiguobacteraceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxon																						
Firmicutes;c__Bacilli;o__Haloplasmatales;f__Haloplasmataceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Lactobacillales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Lactobacillales;f__Aerococcaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae	0.5%	0.0%	10.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;Other	0.0%	0.0%	0.0%	0.2%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Caldicoprobacteraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0.1%	0.0%	0.5%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Eubacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0.1%	0.0%	0.5%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.9%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Peptostreptococcaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0.0%	0.0%	0.5%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Acidaminobacteraceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae]	0.8%	0.0%	11.9%	0.4%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.4%	0.0%
Firmicutes;c__Clostridia;o__Halanaerobiales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Leptotrichiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

GN02;c__3BR-5F;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
GN02;c__BB34;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
GN02;c__BD1-5;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.2%	0.2%	0.0%	0.0%
GN04;Other;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
GN04;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
GN04;c__GN15;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-1;o__;f__	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-2;o__;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-3;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-4;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-5;o__;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemm-6;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;o__Ellin5290;f__	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Ellin5301	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Gemmatimonadetes;c__Gemmatimonadetes;o__N1423WL;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
H-178;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
LCP-89;c__SAW1_B44;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy																						
Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Lentisphaerae;c__[Lentisphaeria];o__Lentisphaerales;f__Arctic95B-10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Lentisphaerae;c__[Lentisphaeria];o__Z20;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Nitrospirae;c__Nitrospira;o__Nitrospirales;f__[Thermodesulfovibrionaceae]	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
OP8;c__OP8_2;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Planctomycetes;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Planctomycetes;c__C6;o__d113;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Planctomycetes;c__Phycisphaerae;o__Phycisphaerales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Planctomycetes;c__Phycisphaerae;o__Phycisphaerales;f__Phycisphaeraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;Other;Other;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;Other;Other	0.3%	0.1%	0.0%	0.1%	0.0%	0.3%	0.3%	0.3%	0.2%	0.7%	0.2%	0.1%	0.1%	0.1%	0.0%	0.1%	1.0%	0.1%	0.1%	0.0%	2.3%
Proteobacteria;c__Alphaproteobacteria;o__;f__	0.2%	0.0%	0.0%	0.7%	0.4%	2.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__BD7-3;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae	7.4%	0.1%	1.1%	50.8%	21.1%	7.2%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	66.5%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Ellin329;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kiloniellales;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kordiimonadales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Kordiimonadales;f__Kordiimonadaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other	4.6%	0.7%	0.0%	0.3%	0.0%	0.4%	0.8%	0.9%	1.1%	15.8%	1.1%	0.4%	0.6%	0.3%	0.4%	0.5%	8.0%	0.5%	0.5%	1.6%	58.5%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__	1.5%	0.1%	0.0%	0.3%	1.0%	0.9%	6.1%	4.9%	3.7%	1.1%	1.8%	1.5%	3.0%	0.6%	0.0%	0.1%	3.4%	0.1%	0.1%	0.2%	1.1%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bartonellaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Beijerinckiaceae	0.0%	0.0%	0.0%	0.2%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae	0.1%	0.0%	0.0%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.8%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae	0.4%	0.0%	0.6%	1.8%	0.3%	3.8%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.6%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae	0.6%	0.0%	0.6%	7.5%	2.1%	1.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylocystaceae	0.2%	0.0%	4.5%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae	34.5%	87.5%	0.0%	0.5%	0.0%	1.6%	10.5%	15.0%	19.0%	33.3%	73.3%	23.9%	53.1%	25.8%	95.0%	74.2%	23.9%	79.3%	65.6%	1.0%	6.8%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0.5%	0.0%	0.0%	5.5%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.7%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhodobiaceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Xanthobacteraceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;Other	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.1%	0.0%	1.5%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae	2.0%	0.6%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	1.5%	18.0%	0.0%	7.9%	11.6%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae	3.5%	3.4%	5.8%	3.9%	19.8%	10.8%	0.8%	0.8%	0.4%	1.7%	0.2%	1.0%	0.8%	0.2%	0.7%	0.9%	1.7%	4.6%	5.2%	2.0%	5.7%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__	0.4%	0.0%	0.0%	2.1%	0.5%	4.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae	0.1%	0.0%	1.2%	0.2%	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae	0.3%	0.0%	1.0%	1.2%	0.7%	1.4%	0.3%	0.2%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.1%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__	0.1%	0.2%	0.6%	0.0%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.5%	0.2%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__Rickettsiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxon																					
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other	0.3%	0.0%	1.6%	0.2%	0.4%	0.5%	0.9%	0.1%	0.1%	0.1%	0.2%	0.1%	0.2%	0.2%	0.0%	0.0%	1.0%	0.0%	0.1%	0.0%	0.2%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae	2.4%	0.2%	1.6%	0.4%	1.4%	1.3%	1.7%	0.4%	0.4%	1.7%	0.7%	0.4%	1.5%	3.3%	0.1%	0.2%	30.6%	0.2%	1.1%	0.0%	1.1%
Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae	3.2%	0.1%	6.1%	4.2%	10.0%	1.9%	20.4%	2.3%	1.2%	0.3%	2.9%	1.3%	4.5%	1.4%	0.0%	0.0%	1.8%	0.2%	0.0%	0.0%	4.7%
Proteobacteria;c__Betaproteobacteria;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other	0.1%	0.0%	0.0%	1.0%	0.0%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Burkholderiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae	0.2%	0.0%	0.6%	0.6%	0.2%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.5%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Neisseriales;f__Neisseriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__Rhodocyclales;f__Rhodocyclaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Betaproteobacteria;o__SC-I-84;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bdellovibrionaceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfarculales;f__Desulfarculaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobacteraceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfobacterales;f__Desulfobulbaceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Taxonomy																						
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Desulfuromonadaceae	0.0%	0.0%	0.0%	0.1%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Desulfuromonadales;f__Pelobacteraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__GMD14H09;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__MBNT15;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__	0.0%	0.0%	0.0%	0.1%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Cystobacterineae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Haliangiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__OM27	0.0%	0.0%	0.0%	0.3%	0.3%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__JTB38	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__NB1-j;f__NB1-i	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales;f__Syntrophobacteraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Deltaproteobacteria;o__[Entotheonellales];f__[Entotheonellaceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Campylobacteraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Epsilonproteobacteria;o__Campylobacterales;f__Helicobacteraceae	0.0%	0.0%	0.0%	0.2%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;Other;Other	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Proteobacteria;c__Gammaproteobacteria;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__211ds20	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae	0.1%	0.0%	1.6%	0.0%	0.2%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Colwelliaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__HTCC2188	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__OM60	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__[Chromatiaceae]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Cardiobacteriales;f__Cardiobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__	0.1%	0.0%	0.0%	0.4%	0.0%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__Chromatiaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Chromatiales;f__Ectothiorhodospiraceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__HTCC2188;f__HTCC2089	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Coxiellaceae	0.0%	0.0%	0.2%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Francisellaceae	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%

Taxonomy																					
Proteobacteria;c__Gammaproteobacteria;o__Legionellales;f__Legionellaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Methylococcales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;Other	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Alcanivoracaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oceanospirillaceae	0.0%	0.0%	0.3%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Oleiphilaceae	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Saccharospirillaceae	0.1%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.6%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae	0.3%	0.0%	0.8%	0.1%	2.8%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	0.5%	0.0%	2.0%	0.1%	0.1%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.4%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Thiohalorhabdales;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Piscirickettsiaceae	0.3%	0.0%	0.0%	0.4%	0.0%	2.2%	1.0%	0.0%	0.0%	0.0%	0.7%	0.4%	0.1%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Thiotrichales;f__Thiotrichaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	0.0%	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae	0.0%	0.0%	0.4%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	0.0%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%

Taxonomy																								
Proteobacteria;c__Gammaproteobacteria;o__[Marinicellales];f__[Marinicellaceae]	0.0%	0.0%	0.0%	0.1%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__TA18;o__CV90;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__TA18;o__PHOS-HD29;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Proteobacteria;c__Zetaproteobacteria;o__Mariprofundales;f__Mariprofundaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
SAR406;c__AB16;o__Arctic96B-7;f__A714017	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
SR1;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Spirochaetes;c__[Brachyspirae];o__[Brachyspirales];f__A0-023	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM6;c__SJA-4;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM6;c__SJA-4;o__YJF2-48;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM7;c__;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM7;c__SC3;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM7;c__TM7-1;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TM7;c__TM7-3;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Tenericutes;c__Mollicutes;o__;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales];f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
WS3;c__PRR-12;o__LD1-PA13;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
WS3;c__PRR-12;o__Sediment-1;f__	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%