The microbiota of sea turtles

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Abstract

The symbiotic relationship between the microbes inhabiting complex organisms and their hosts is vital for all metazoan species. Without the intricate interplay between host, microbes and their metabolites, multicellular life would not be possible. Investigations into the role that the microbiota plays in non-human animals are uncommon, and those in reptiles are even rarer. To address this knowledge gap, I characterised the microbiota of sea turtles, investigated the microbiotal influence on their physiology, and explored factors that affect the microbiota in these animals.

First, to determine factors important in shaping microbial community composition in vertebrates, I performed a review of microbiota data in 203 vertebrate species. I then compiled this data along with some fundamental natural history data such as diet and geographic location. Using a phylogenetic correction, I discovered that phylogeny is the most important factor influencing microbiota composition in vertebrates.

Using samples collected from free-living turtles, I determined the microbiotas of all seven of the world's extant sea turtle species. I found that in all species there was a predominance of the bacterial phyla Proteobacteria. I also found that there is a phylogenetic signal driving microbiotas in sea turtles, and that closely related species have similar microbial compositions.

To determine how a species with no parental care after oviposition acquires its microbiota, I investigated this phenomenon in the green sea turtle (*Chelonia mydas*). I showed that mother turtles have a complex microbial community in developing eggs that closely resembles that of hatchling turtles and I inferred that there is a transgenerational 'priming' of the developing egg with microbiota. This investigation shows that neonatal turtles acquire a proportion of their microbiota prior to oviposition. Here I propose that in species where post-partum opportunities for microbial transfer are limited, transfer of the microbiota from the mother to the embryo during development in the uterus or oviducts plays a key role.

To examine the role that geographic location has on sea turtle microbiotas I compared samples from loggerhead turtles (*Caretta caretta*) from Australia, to the USA, and from flatback turtles (*Natator depressus*) from Crab Island, Queensland, to animals from Port Hedland in Western Australia. In both groups, I found significant differences in microbial composition between the sampling sites. Possible explanations for these differences may include genetics and environmental degradation.

I also explored how the microbiota may be affecting blood parameters in sea turtles, as a measure of health. I did this by comparing microbiota composition to haematology and biochemistry analytes in flatback turtles at Crab Island to those from flatback turtles at Port Hedland. I found a correlation for packed cell volume, heterophils, uric acid, calcium, total protein, albumin, globulin, and potassium. A number of operational taxonomic units (OTUs) were identified to be contributing to this correlation. Additionally, a correlation was seen in heterophil/lymphocyte ratio, and basophils, associated with microbiota composition in Port Hedland animals, but no specific OTUs could be identified to be significantly contributing to these observations.

In summary, this thesis represents the largest investigation into the microbiota of sea turtles completed to date, and in fact one of the most complete of any vertebrate clade. I have shown that phylogenetic history is important in determining microbiota composition in vertebrates, as well as sea turtles. Additionally, I have found that mother sea turtles pass their microbiota to their offspring using a novel method, that geography markedly affects microbiota composition, and that the microbiota of sea turtles has an effect on health indices in this species

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Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes five unpublished publications. The core theme of the thesis is microbiota of sea turtles. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Richard Reina.

(The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.)

In the case of chapters 2-6 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
2	Host phylogeny is the most important factor in degerming microbiota composition	Not yet submitted	70% design, data collection data analysis, and writing of manuscript.	1. Matthew McGee[ac] 15% 2. Chris Greeningc 2.5% 3. Marcel Klaassenc 2.5% 4. Richard D. Reina[c] 10%	N
3	The microbiota of nesting sea turtles	Not yet submitted	75% design, data collection data analysis, and writing of manuscript.	1. Rob Moore[bc] 10% 2. Thi Thu Hao Van[b] 2.5% 3. Marcel Klassen[b] 2.5% 4. Richard D. Reina[ac] 10%	N
4	Transgenerational transmission of the microbiota from mother to egg	Revised version resubmitted to: *Proceedings of the Royal Society B*	75% design, data collection data analysis, and writing of manuscript.	1. Rob Moore[bc] 10% 2. Thi Thu Hao Van[b] 2.5% 3. Marcel Klassen[b] 2.5% 4. Richard D. Reina[ac] 10%	N
5	The gut microbiota of sea turtles differs between geographically distinct populations	Submitted to: *Endangered Species Research*	75% design, data collection data analysis, and writing of manuscript	1. Rob Moore[bc] 10% 2. Thi Thu Hao Van[b] 2.5% 3. Marcel Klassen[b] 2.5% 4. Richard D. Reina[ac] 10%	N
6	The effect of the microbiota on blood parameters in nesting flatback turtles (*Natator depressus*)	Submitted to: *Scientific Reports*	75% design, data collection data analysis, and writing of manuscript	1. Rob Moore[bc] 10% 2. Thi Thu Hao Van[b] 2.5% 3. Marcel Klassen[b] 2.5% 4. Richard D. Reina[ac] 10%	N

[a] *Aided with study design*
[b] *Aided with data analysis*
[c] *Proof read and contributed to manuscript*

I have not renumbered sections of submitted papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 15/9/19

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 15/9/19

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Chapter 1. General Introduction

1.1 The microbiota in health and disease

Residing on and within every metazoan species is a diverse and complex metropolis of microorganisms (viruses, bacteria, fungi and protozoans) known collectively as the microbiota, and the occupation of higher organisms by these prokaryotic and eukaryotic colonists has been a key factor in driving evolution and radiation of life on Earth (Lee and Mazmanian 2010; McFall-Ngai *et al.* 2013). Indeed, this multigenomic microcosm has become so integral to animal homeostasis that it can no longer be considered separate from the individual. Thus, it has been proposed that animals represent a vastly intricate biological 'super organism' in which a proportion of the physiological function is derived from microbial activity (Dethlefsen *et al.* 2007).

Microorganisms associated with disease in humans have been known since the late 17th century (Leuwenhoek 1693). However, it has only been in recent times that the focus has shifted from their pathogenic potential to the mutualistic benefit of host-microbiota relationships. This fundamental change in thinking has been most profound in human health, where a more holistic approach to the entire 'human biomass' has led to a better understanding of fundamental biological processes (Lee and Mazmanian 2010). There are varied estimates for the number of microbes within the human body. Initial assessments believed that microbes may number as many as 10^{14} cells (Savage 1977), and contribute in excess of two orders of magnitude more genes to the hologenome (the collective genomes of the host and its microbiota) (Turnbaugh *et al.* 2007). However, this number has recently been revised so that the number of bacteria in the body is actually of the same order as the number of human cells, and their total mass is about 0.2kg (Sender *et al.* 2016). The vast majority of these microbes reside within the gastrointestinal tract (Savage 1977), with areas of regional specificity within the host, and microbial habitat preference (Dethlefsen *et al.* 2007; Cho and Blaser 2012; Hollister *et al.*

2014). This microbiotal community is malleable and is highly influenced by a range of factors such as genetics, illness, diet, medicines, and hygiene. No matter whether or not the microbial insult is intrinsic or extrinsic, alterations to the composition have been implicated in predisposing individuals to disease or various systemic or specific organ disorders (Pflughoeft and Versalovic 2012). Dysbiosis (microbial imbalance) is a common consequence of disease in humans and animals and may be further exacerbated by factors such as stress and diet (Hamdi *et al.* 2011; Costa and Weese 2012; Hooda *et al.* 2012; Isaacson and Kim 2012).

The human intestinal bacterial metagenome (the collective bacterial genetic material recovered from a sample) contributes to the production of primary metabolites, and conversion of small molecules into secondary metabolites, that modulate human health in many ways (Sharon *et al.* 2014). This realisation of how the microbiota regulates human health is in its infancy, and we are only beginning to scratch the surface of the depth of the human-microbe relationship. For example, in concert with human metabolites, microbial compounds influence nutrient and xenobiotic metabolism (Maurice *et al.* 2013) to aid in protection against pathogens (Clarke *et al.* 2010), regulate the enteric nervous system (Soret *et al.* 2010), support the immune system (Brestoff and Artis 2013), protect against colorectal cancer (Nicholson *et al.* 2012), affect complex neurological behaviour (Hsiao *et al.* 2013), and influence lipid and cholesterol levels in the serum (Delzenne and Williams 2002). Specific diseases that have been correlated to disturbances in human microbial metabolism include cardiovascular disease, neoplasia, cystic fibrosis, obesity, diabetes, autism and other behavioural disorders, and inflammatory conditions such as irritable bowel disease, asthma, Crohn’s disease and psoriasis (Turnbaugh *et al.* 2007; Cho and Blaser 2012; de Vos and de Vos 2012; Moloney *et al.* 2014; Sharon *et al.* 2014; Shreiner *et al.* 2015).

Like humans, the gastrointestinal tract of animals is populated by vast numbers of microorganisms that confer myriad beneficial properties to their host and these complex interactions are an exciting and emerging area of evolutionary biology. With the exception of laboratory animals that have served as models for human health, historically, the importance of microflora to non-human species has predominately focused on their role during digestion, particularly of complex carbohydrates in herbivorous animals (Hume 1989). However, these investigations have begun to broaden with the realisation of the greater role that they play in the health and ecology of all species (McFall-Ngai *et al.* 2013). Specific investigations into the role that the microbiota plays in wild animal health are rare and have started to emerge only recently, but laboratory-based studies indicate that also non-human metazoans have a similar reliance on the microbiota for health and homeostasis. This is highlighted by a phenomenon in the crucian carp (*Carassius auratus*), in which intermittent fasting has been shown to positively influence the immune system and growth by enriching the gut microbiota (Li *et al.* 2018). Fasted fish had a relative increase in the bacterial genera *Bacteroides, Akkermansia,* and *Erysipelotrichaceae,* resulting in improved activity of superoxide dismutase (important for antioxidant defence) and lysozyme, an antimicrobial enzyme responsible for degradation of bacterial cell walls (Li *et al.* 2018). Distinct gut bacterial communities have been observed in crucian carps affected by “red-operculum” disease (Li *et al.* 2017). The bacterial communities of diseased fish are less diverse and stable than those of healthy individuals, indicating that a normal microbiota plays an important role in protecting the host against certain pathogens (Li *et al.* 2017).

Chronic inflammation and enteric infections have been observed in mice with Na^+/H^+ exchange (NHE) inhibition (Larmonier *et al.* 2013). A loss of NHE activity leads to changes in gut microbial ecology (dysbiosis), which precipitates the development of distal colitis (Larmonier *et al.* 2013). Similarly, altered gastrointestinal microbiota is strongly associated with chronic colitis in rhesus macaques (*Macaca mulatta*) (McKenna *et al.* 2008). Further, it has long been known that the gut influences bone health through the absorption of calcium, the key mineral for bone structure (McCabe *et al.* 2016), but in addition, a variety of animal models (zebra fish, rodents and chickens) have shown that modulation of the gut microbiota can affect bone density and strength. It is thought that the microbiota is able to do this by regulating osteoblast and osteoclast activity, but the signalling pathways remain a mystery (McCabe *et al.* 2016). Altered gut microbiota has been implicated as a cause of "light turkey syndrome", in which affected birds do not reach body weights near their genetic potential (Danzeisen *et al.* 2013). Conversely, disturbances in the microbiota of mice, induced by dietary factors, result in marked weight gain (Turnbaugh *et al.* 2008). Additionally, microbiota transplant from obese mice to lean animals promotes greater fat deposition (Turnbaugh *et al.* 2008). Collectively, these human and animal studies show that the growing assortment of microbiota investigations across a diverse array of metazoans, supports the idea of the great importance of microbiota in the functioning of animals. However, there are areas of significant knowledge gaps, specifically in a range of taxa, including reptiles.

1.3 Acquisition of the microbiota

All animals must acquire their microbial symbionts in some way. Microbiota acquisition studies are rare in vertebrate species, but we know that how animals procure their microbiota is dependent on life history traits and can also be influenced by environmental circumstances. Cutaneous mucus feeding of fry by parent fish is important in shaping the microbial structure of young discus fish (*Symphysodon aequifasciata*) (Sylvain and Derome 2017). The gastrointestinal microbiota of developing chicken embryos partially resembles that of the hen, suggesting at least some transmission via the reproductive tract (Ding *et al.* 2017).

Investigations into gastrointestinal microbial structure of neonatal lizards indicate that a proportion of the microbiota is acquired from their mothers (Kohl *et al.* 2017). In both human and domestic cow neonates, the acquisition of commensal bacteria occurs rapidly after birth as organisms from the mother and surrounding environment invade the naïve gastrointestinal tract. During vaginal delivery, contact with the mother's vaginal and intestinal flora is important for initial colonisation of the infant's intestine (Torrazza and Neu 2011; Taschuk and Griebel 2012). Additional beneficial bacteria are provided to the young in the colostrum and milk of both species (Taschuk and Griebel 2012; Urbaniak *et al.* 2016). During caesarean delivery, this contact does not occur and the intestine is invaded by less diverse, non-maternally derived environmental species (Torrazza and Neu 2011; Taschuk and Griebel 2012) and it has been suggested that this may have lasting effects on the intestinal bacterial composition into later life, with important ramifications for immune system development (Torrazza and Neu 2011; Taschuk and Griebel 2012). In humans, this failure of passive transfer of flora is believed to contribute to a range of autoimmune diseases such as asthma, hyperallergic condition, and food allergies (Torrazza and Neu 2011). Thus, while it is clear that acquisition of the microbiota is a key developmental step for vertebrate neonates, the process by which this occurs is poorly understood and requires investigation, particularly in species in which amniotic eggs are laid, and maternal care is absent.

1.4 The environment and the microbiota

The composition of the microbiota is determined by the same ecological principles that drive diversity and niche occupancy within macro ecosystems. That is, colonist assemblages are established by dispersal, diversification, local abundance, ecological drift, and habitat quality (Costello *et al.* 2012). These are further influenced by individual bacterial traits such as dispersal efficiency and mutation rates, as well as host genetics (Costello *et al.* 2012). Thus, the final microbial community of an animal is determined by a complex interplay of local and external factors, with microorganisms competing fiercely to establish stable populations within the gastro-intestinal environment.

Disturbances in habitat quality appear to have species-specific effects on microbial populations of animals. Black howler monkeys (*Alouatta pigra*) inhabiting suboptimal environments with low floral diversity, consumed less varied diets and had a corresponding decrease in gut microbial diversity than their conspecifics in more ideal environments, with high floral diversity. Further, genetic analysis of microbes isolated from these animals revealed a reduction in genes associated with butyrate production and hydrogen metabolism, which may have implications for host health, due to decreased resilience to microbial disturbance and pathogen invasion (Amato *et al.* 2013). Conversely, a study involving multiple primate species demonstrated that habitat perturbation did not influence faecal microbiotas and that differences observed were explained by differences in digestive physiology and dietary preferences (McCord *et al.* 2014). However, in this same study, differences were noted between the same species in populations from different countries, suggesting that geography and social group membership influence microbial communities in some primates (McCord *et al.* 2014).

In biological terms, fitness of animals is measured by reproductive success (Bunger *et al.* 2005) and this definition is further delineated by species-specific traits relating to reproductive strategy. More specifically, fitness can be measured in absolute values of offspring produced, size of neonates, and number of birthing episodes during reproductive periods. These can be quantified over a single reproductive event, or over the entire period of fecundity for a given individual (Bunger *et al.* 2005). However, the fitness of animals has to be traded-off against the metabolic costs of normal homeostasis. For example, immune surveillance in vertebrates carries a considerable metabolic cost (Lochmiller and Deerenberg 2000), and is downregulated in preference to reproduction (French *et al.* 2007). Despite a functional immune system having a substantial physiological burden, its absence also carries a metabolic risk, as it means that animals have a greater susceptibility to infection and the consequent energetic cost of that infection. In addition to the increased metabolic requirements of mounting an immune response, infectious agents parasitise nutrients from the host and this combination may result in decreased reproductive output in preference to keeping the animal alive (Scott 1988). Given the importance of the microbiota to a range of physiological functions, any process that results in dysbiosis (microbial imbalance) may have far-reaching effects on fitness of animals.

1.5 The microbiota of reptiles

As mentioned earlier, investigations into the microbiota of non-human species are limited, and while this gap is closing, there is a paucity of available information for reptiles, including sea turtles (Hong *et al.* 2011; Keenan *et al.* 2013; Gaillard 2014; Colston *et al.* 2015; Hong *et al.* 2015; Yuan *et al.* 2015; Abdelrhman *et al.* 2016; Price *et al.* 2017). Specific questions, such as how herptiles acquire their microbiotas, remain a mystery. Understanding these fundamental physiological processes in reptiles is important as this taxon occupies an important branch of the evolutionary tree, and in order to fully understand how the microbiota has driven evolution, we need a complete dataset from all metazoans. Birth and conspecific interactions are not responsible for microbial colonisation in a social species, the green iguana (*Iguana iguana*) (Wehrle 2013), while diet, captivity, and feeding regime all influence the microbiota of reptiles (Costello *et al.* 2010; Keenan *et al.* 2013; Kohl *et al.* 2017), and fermenting bacteria are important for digestion in herbivorous species (Mackie *et al.* 2004). Beyond these preliminary investigations there is a paucity of information regarding the influence of microbiota on fitness of reptiles and how it affects their functional ecology.

1.6 Study species

In the investigations outlined in this thesis I chose to study microbial communities in sea turtles. Sea turtles are an interesting species to study microbiotas in due to the vagaries of their natural history. They periodically undergo significant physiological stress associated with migration and breeding (Hays *et al.* 2002), they have not developed any form of live birth or parental care (Rafferty *et al.* 2013), and for most species they have a widespread, yet discontinuous distribution (Bowen and Karl 2007). These factors make sea turtles ideal candidates to explore the evolutionary role of microbiotas in vertebrates.

There are seven extant species of sea turtle, the green turtle (*Chelonia mydas*), loggerhead turtle (*Caretta caretta*), hawksbill turtle (*Eretmochelys imbricata*), flatback turtle (*Natator depressus*), olive ridley turtle (*Lepidochelys olivacea*), Kemp's ridley turtle (*Lepidochelys kempii*), and the leatherback turtle (*Dermochelys coriacea*). With the exception of the flatback turtle, all are listed as 'Threatened' by the IUCN with various levels of risk, while the flatback turtles is listed as 'Data Deficient', but is also likely to be threatened (IUCN 2019). Marine turtles are perfectly evolved for a completely aquatic life, however, like almost all chelonians they are still tied to the terrestrial environment for oviposition (Pritchard 1997). The majority of sea turtle species are scattered unevenly throughout all three tropical oceans, with the exception of the flatback and Kemp's ridley turtles, which have relatively restricted distributions (Pritchard 1997). Additionally, the leatherback turtle, is a more cold adapted species, and may be found occupying waters at higher latitudes than the other species (Pritchard 1997). Sea turtle diets vary remarkably over life stage, and among species, and may range from herbivory, to omnivory and specialised carnivorous diets (Jones and Seminoff 2013). Sea turtles are unique among the Reptilia, in that they are the only members that undergo long-distance migrations, rivalling those of other vertebrate species (Plotkin 2003). How the microbiota contributes to the extraordinary physiological adaptations of sea turtles has been poorly studied, and as a result, I first wanted to explore what differences, if any, exist in microbial populations between sea turtle species.

In order to better understand the evolutionary mechanisms by which microbial acquisition occurs in vertebrates, I investigated how hatchling sea turtles acquire their microbiota. I chose sea turtles because they are an oviparous species, with no parental care and thus the pathways for transmission are much less diverse than in viviparous species or those that raise their young in some way. Thus, I was interested to understand how the microbiota is acquired by these newly-hatched reptiles in the absence of viviparity or any contact between neonate and parent. The important role of microbiota in so many aspects of vertebrate biology suggests that its acquisition by these animals should not occur via some haphazard inoculation from the environment, and there should be some degree of inheritance.

The role of environment at a variety of spatial scales (geographical distance and isolation) is interesting to investigate as a potential driver of microbiota differences between populations of the same species of sea turtles. To further explore these factors, I compared sea turtles of the same species, but from different populations isolated from one another, to determine if this influenced gut microbiota composition.

Finally, given the role that microbiota plays in animal health, I wanted to determine how microbial communities influenced haematology and biochemistry results in different populations of sea turtles as a measure of wellness.

Sea turtles are among the most imperiled species on Earth and therefore a deep understanding of their physiology is paramount to their conservation. The role that microbiota plays in the health, behaviour and physiology of humans and animals is undeniable, and its categorisation in marine turtles is the next important step in understanding how these secretive species interact with their environment and its challenges.

1.7 Microbiota analysis and statistics

The advent of high-throughput DNA sequencing has improved our understanding of the presence and abundance of microbes based on their genetic profiling, and importantly, is independent of traditional culture methods (Pace 1997). This is has been an important technological advancement, as the proportion of cells which can be cultured is estimated to be between 0.1% and 10% of the total population, and there is little information concerning how closely this reflects the actual community composition of samples (Ranjard *et al.* 2000). Therefore, methodologies which examine the presence of genetic material are likely to give a more accurate estimate of community composition (Ranjard *et al.* 2000). Briefly, the procedure for the production of microbial sequencing data relies on barcoding of amplicon sequences obtained from extraction of microbial DNA (McMurdie and Holmes 2013). DNA can be extracted manually, or using commercially available kits or robots, and the variety of methods are vast, and an in-depth review of these is beyond the scope of this chapter. However, once DNA has been extracted, typically the small subunit ribosomal RNA (16S rRNA) gene is the target for barcoding, and there are large reference databases available for their interpretation (DeSantis *et al.* 2006; Pruesse *et al.* 2007; Cole *et al.* 2009). After sequence reads have been successfully barcoded, organisms can be clustered based on similarity to define Operational

Taxonomic Units (OTUs), using a range of pipelines including QIIME, mothur, and PANGEA (McMurdie and Holmes 2013). The resulting data matrix is then ready for statistical analysis.

Differences in microbiota variation are generally assessed by alpha and beta diversity. Alpha diversity quantifies diversity within individual samples and can be compared across sample groups (Knight *et al.* 2018). Common alpha diversity measures include Chao1 (an estimate of species diversity), which is sensitive to the number of sequences per sample, and Shannon index (an estimate of species richness and evenness), which is less so (Knight *et al.* 2018). Visualisation of alpha diversity is typically done using boxplots, and for comparisons between samples, standard parametric and non-parametric tests may be utilized based on their normal distribution (Knight *et al.* 2018). Beta diversity compares feature dissimilarity between each pair of samples, generating a distance matrix between all pairs of samples (Knight *et al.* 2018). Metrics that are commonly used for beta diversity include quantitative measures (Bray-Curtis, Canberra and weighted UniFrac), which use feature abundance in calculations, whereas qualitative metrics (binary-Jaccard and unweighted UniFrac) only consider patterns of presence or absence of OTUs (Knight *et al.* 2018). The strength of UniFrac over Bray-Curtis or Jaccard is that it considers phylogenetic relationships of the species present in the microbiota. Thus, samples with different OTUs from the same genus will be more similar by UniFrac that those with OTUs from different genera. The weakness is that UniFrac is more sensitive to low abundance OTUs and those that are very phylogenetically distant. The choice of distance matrix depends on how much the analyst personally feels that phylogenetic relationships versus sensitively matter in the dataset. There is currently no consensus on whether or not specific distance matrices are more accurate for beta diversity measures, and in fact, there is some evidence to suggest that there is a high degree of similarity and complement among the different techniques (Parks and Beiko 2013). For visualization of beta diversity, ordination techniques such as principal coordinate analysis (PCoA), are commonly used, and statistical tests used to determine diversity clustering between groups include ANOSIM and PERMANOVA (Knight *et al.* 2018). Throughout this thesis I have attempted to keep all analyses consistent, except for circumstances where a chapter has been submitted for publication, and the reviewers have asked for a specific analysis.

1.8 Study aims and thesis structure

The general aim of the studies described in my thesis was to categorise the microbiota of sea turtles. In order to achieve this, I designed experiments that would allow me to explore the evolutionary biology of the microbiota, to understand factors that influence the microbiota, and to investigate physiological effects of the microbiota on the host. By undertaking this investigation, it is hoped that we will gain a better understanding of how marine turtles interact with their environment and determine whether or not microbiota can act as an indicator of ecological health. Further, this study may add to our knowledge of metazoan evolution by exploring the mechanisms by which microbes affect vertebrate physiology in novel species. In order to address these aims I designed a series of experiments and observations (Chapters 2-6) to address a range of general objectives and research aims regarding the microbiota of sea turtles (Figure 1.1).

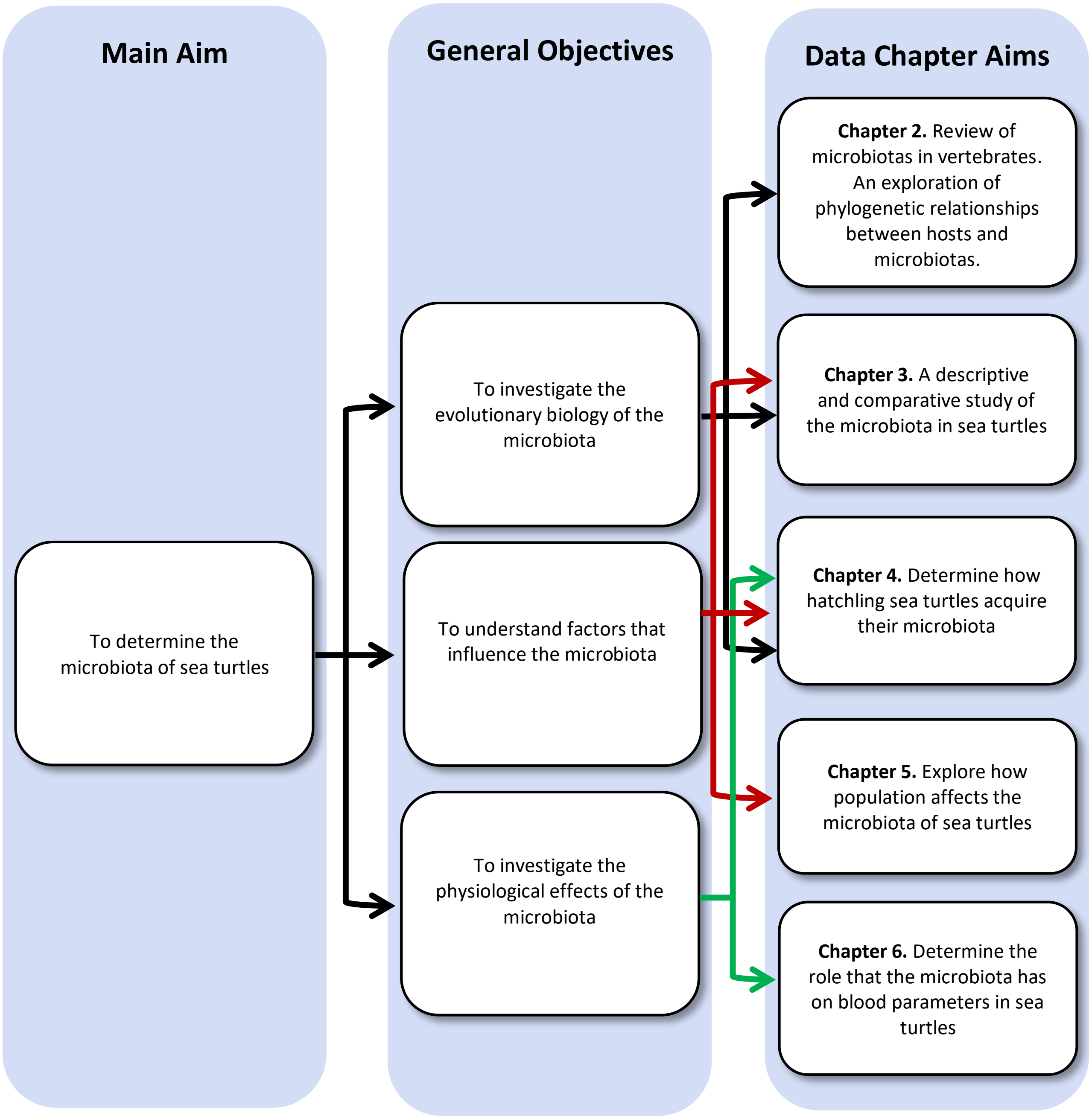

Figure 1.1 Research aims and general structure for thesis

This thesis comprises a General Introduction (this chapter), five data chapters and a General Discussion. At the time of submission, 3 chapters had been submitted for publication as noted in the Thesis Declaration.

Chapter 2 is a review and meta-analysis of microbiota composition in vertebrates. The aim of this investigation was to explore what factors influence microbial community structure in animals and to determine if similarities exist based on phylogenetic history, dietary preference, or environmental factors. This study provided important information on the evolution of microbiotas in vertebrates.

Chapter 3 is a descriptive study that investigated the normal microbiota of nesting sea turtles. I obtained samples from all seven extant species of sea turtle. This chapter comprises a comprehensive dataset on the microbiota of sea turtles.

The study described in Chapter 4 explores how hatchling sea turtles acquire their microbiota. The data provided in this chapter raises some important questions about the evolutionary biology of microbial acquisition in vertebrates.

In Chapter 5 I investigated whether sea turtles of the same species, but originating from different populations, shared similar microbiotas. The findings of this investigation contribute to our understanding on how malleable the microbiota is in vertebrates.

In Chapter 6 I investigated the role that microbial composition plays in sea turtle haematology and biochemistry and discuss the implications of these results for the health of sea turtles.

Chapter 7 is a general discussion and integration of the results from all of the data chapters. The general trends and differences found across the various experiments are discussed. The physiological and evolutionary implications of the findings are summarised. Finally, I outline some of the limitations of my experiments and detail avenues for future research. This chapter includes a conclusion for the thesis.

1.9 References

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Chapter 2. Host Phylogeny is the most important factor in determining microbiota composition

2.1 Introduction

The occupation of higher organisms by prokaryotic and eukaryotic colonists is thought to be a key factor driving evolution and radiation of life on Earth (Lee and Mazmanian 2010; McFall-Ngai *et al.* 2013). The most abundant and diverse populations of these microbes reside within the gastrointestinal tract (Savage 1977). Gastrointestinal microbial community composition varies between anatomical site, due to colonist preferences to a number of host factors (e.g. gut pH, temperature, etc.) (Dethlefsen *et al.* 2007; Cho and Blaser 2012; Hollister *et al.* 2014). Also among individuals composition of the microbiota varies, individual microbiota being highly influenced by external factors such as diet, medicines, and hygiene (Voreades *et al.* 2014). Yet, despite this individual variability, each species maintains a core microbiota that is fundamental to that animal's existence (Henderson *et al.* 2015). Under normal circumstances, the microbiota is critical to homeostasis of the host by contributing to the production of primary metabolites, and conversion of small molecules into secondary metabolites, that modulate health in many ways (Sharon *et al.* 2014). Further, the gastrointestinal microbiota is critical to the metabolism of food items, with altered microbial populations implicated in decreased digestive efficiency (Amato *et al.* 2013). Additionally, alterations to microbiota composition have been linked to predisposing individuals to disease or various systemic or specific organ disorders (Pflughoeft and Versalovic 2012).

The complex interactions between microbiota and host are an exciting and emerging area of evolutionary biology (McFall-Ngai *et al.* 2013) and are underpinned by the necessity of animal hosts to provide their symbiotic microbes with sustenance. The procurement of nutrients is an essential task undertaken by all life forms, and animals have evolved a diverse array of anatomical and physiological adaptations to utilise available resources. The morphological and biochemical function of animals is ultimately driven by the chemistry of their dietary preferences, and their gastrointestinal microbial communities (Karasov *et al.* 2011).

Historically, studies into the microbial communities of vertebrates have focused on the role that diet plays in convergence of the microbiota across various vertebrate taxa (Ley *et al.* 2008a; Ley *et al.* 2008b; Muegge *et al.* 2011; Delsuc *et al.* 2014). However, these investigations have largely been descriptive in nature and did not consider the integral role that phylogenetic history plays in diet, anatomy and physiology. Moreover, given the fundamental relationship between microbes and hosts, it seems likely that their evolutionary journeys are intrinsically linked, and that animals and their microbiotas should be considered in entirety, as a single 'super organism', rather than as separate entities.

While some researchers have indicated that phylogeny may play a role in convergence of the composition of gut microbiotas in some species (Muegge *et al.* 2011), there have been few detailed investigations reporting the effect of phylogenetic history on the microbial communities of vertebrates. Most recently, evolutionary trends in host physiology have been demonstrated to be more important than dietary niche in determining primate gastrointestinal microbiota (Amato *et al.* 2018). To further investigate the role that evolutionary factors have in determining the microbiota in vertebrates, I compiled microbial community data with phylogenetic history from 203 vertebrate species. In addition, I explored whether the rate of evolution of microbiotas differed among vertebrate species. To my knowledge, this is the most comprehensive cross-taxa study performed to date, incorporating the largest data set, and integrating vertebrate classes not included in previous analyses.

2.2 Methods

2.2.1 Data collation

I performed a systematic literature search, as detailed in the Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) (Moher *et al.* 2009). I conducted the literature search between June 2016 and June 2018 using the online database *Web of Science*.

The terms used for this search were ‘microbiota AND animals’, ‘microbiota AND wildlife’, ‘microbiota’, ‘microbiota AND composition’, and ‘microbiota AND vertebrate’. I also searched the Internet via *Google Scholar* using the same terms. The online database search yielded 1,323 potentially eligible peer-reviewed, published papers. These articles were then screened for suitability in my analysis, and if considered suitable as described below, relevant data were extracted and included in the meta-analysis. As a result, a total of 110 studies were included in my data set.

2.2.2 Inclusion and exclusion criteria

Studies were included in the data set only if they fulfilled the following criteria: (*i*) individual species investigated were clearly identified; (*ii*) there was no manipulation of diet that might have altered natural microbial composition; (*iii*) microbial communities were reported to at least the level of phylum; (*iv*) data was collected from adult animals, to limit any confounds due to ontogenetic changes in diet and microbiota; (*v*) identification of microbial communities was performed using next generation sequencing, (*vi*) except for common domestic species, animals were sourced from wild populations. I included these domestic animals because they are physiologically and morphologically distinct from their wild counter-parts, and may provide valuable insight into recent human-induced evolutionary trends. For these studies I only included descriptive analyses but not manipulative studies.

2.2.3 Data extraction

After compiling the list of usable studies, I identified the relative abundance that each bacterial phylum comprised of the total microbial community composition for each host species and imported them into a spreadsheet (Microsoft Excel for Mac 2017, version 15.32). The major bacterial phyla for all investigations included Firmicutes, Proteobacteria, Fusobacteria, Bacteroidetes, Actinobacteria, Tenericutes, Cyanobacteria, Planctomycetes, Synergistetes, Chloroflexi, Spirochaetes, Acidobacteria, Verrucomicrobia, Deferribacteres, TM7, Lentisphareae, Elusimicrobia, Armatimonadetes, Deinococcus-Thermus, and Fibrobacteres. Unidentified phyla, or those that did not fit into these major categories were listed as ‘Other’ (Figure 2.1, Table 2.1). For studies that reported microbial composition in graphical form only, I extracted values using the software Graphclick (Arizona-Software, 2008). I then obtained the phylogenetic history for each of the species in my study and constructed a phylogenetic tree using the online database “Interactive Tree of Life”, https://itol.embl.de. In total, data was obtained for 203 different species comprised of seven vertebrate classes (Figure 2.1, Table 2.1).

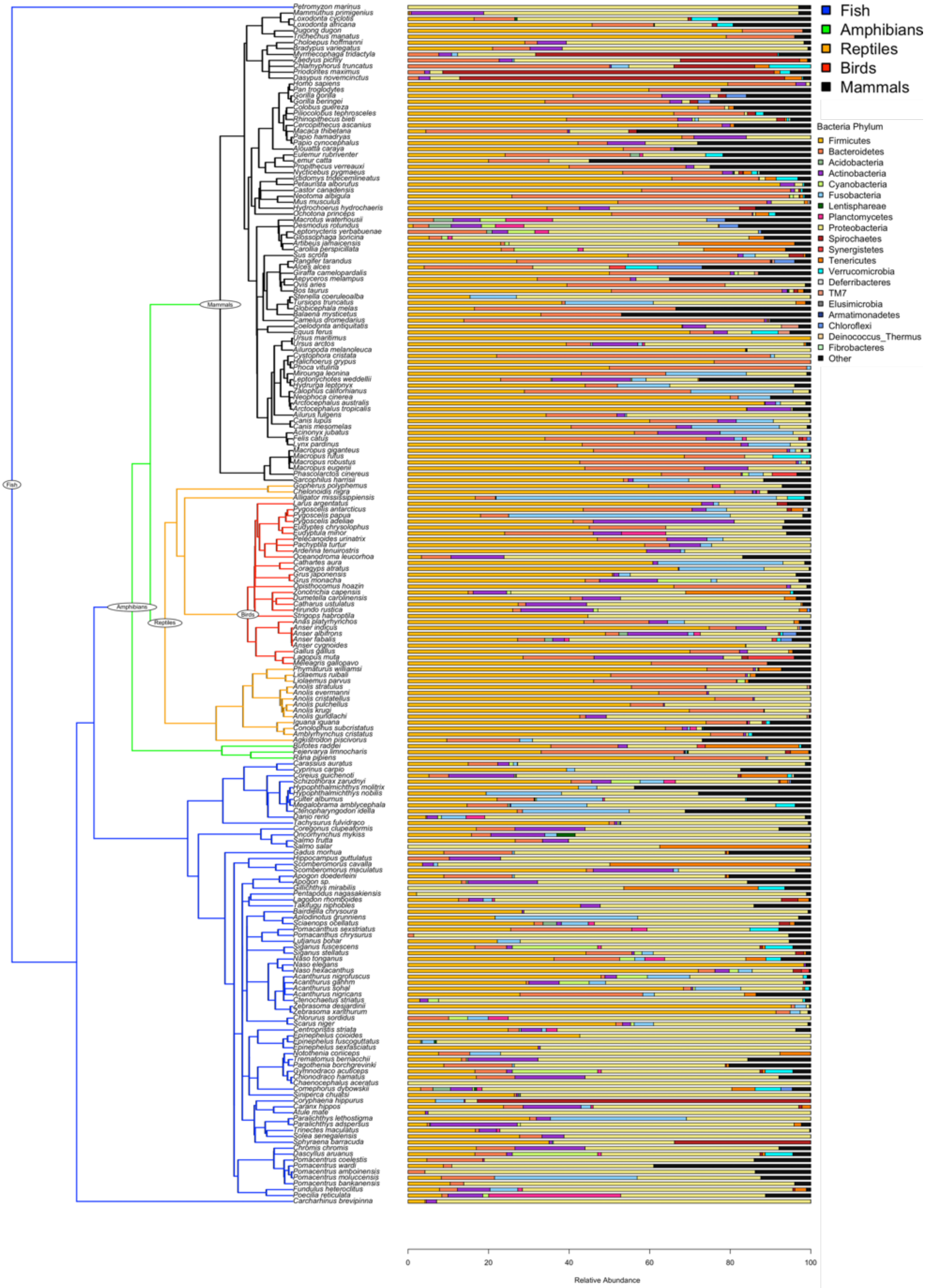

Figure 2.1 Phylogenetic tree representing all vertebrate species used in this study and their microbiota compositions. For a high-resolution image click here.

Table 2.1 Variation in microbiota composition across … vertebrate species using 21 bacterial phyla groups. Values show the relative abundance of the total comprised by each bacterial phylum. Abbreviations, Fir = Firmicutes; Bac = Bacteroidetes; Aci = Acidobacteria; Act = Actinobacteria; Cya = Cyanobacteria; Fus = Fusobacteria; Len = Lentisphareae; Pla = Planctomycetes; Pro = Proteobacteria; Spi = Spirochaetes; Syn = Synergistetes; Ten = Tenericutes; Ver = Verrucomicrobia; Def = Deferribacteres; Elu = Elusimicrobia; Chl = Chloroflexi; Dei = Deinococcus-Thermus; Fib = Fibrobacteres; Oth = Other.

Species	**Fir**	**Bac**	**Aci**	**Act**	**Cya**	**Fus**	**Len**	**Pla**	**Pro**	**Spi**	**Syn**	**Ten**	**Ver**	**Def**	**TM7**	**Elu**	**Chl**	**Dei**	**Fib**	**Oth**	**Reference**
Fish																					
Carcharhinus brevipinna	4.22	0.32	0	2.56	0	0	0	0	92.9	0	0	0	0	0	0	0	0	0	0	0	Givens *et al.* (2015)
Petromyzon marinus	0	0	0	0	0	0	0	0	97	0	0	0	0	0	0	0	0	0	0	3	Tetlock *et al.* (2012)
Comephorus dybowskii	3.13	3.05	4.36	5.44	0.49	0	0.58	1.32	62.03	0	0	5.77	6.43	0	0	0	2.8	0	0	4.6	Bel'kova *et al.* (2015)
Chionodraco hamatus	16.96	9.59	0	17.4	0	0	0	0	48.16	0	0	0	0	0	0	0	0	0	0	7.89	Eichmiller *et al.* (2016)
Aplodinotus grunniens	21.63	0	0	0	0	35.4	0	0	39.92	0	0	0	0	0	0	0	0	0	0	3.05	Eichmiller *et al.* (2016)
Bairdiella chrysoura	28.32	0	0	0.45	0	0	0	0	70.54	0	0	0	0	0	0	0	0	0	0	0.69	Eichmiller *et al.* (2016)
Carassius auratus	14.94	7.34	0	2.8	1.07	0.97	0	0.1	71.42	0	0	0.12	0.19	0	0	0	0	0	0	1.05	Eichmiller *et al.* (2016)
Carassius auratus	27.22	1.85	0.23	0.81	0.32	23.86	0	0.00	40.21	0	0	0.48	0.92	0	0	0	0.13	0	0	3.48	Liu *et al.* (2016)
Cyprinus carpio	26.22	10.72	2.17	8.31	2.06	5.77	0	1.92	34.23	0	0	0	1.81	0	0	0	0.78	0	0	6.03	Liu *et al.* (2016)
Cyprinus carpio	39.38	0	0	0	0	2.01	0	0	41.2	0	0	0	0	0	0	0	0	0	0	17.41	Eichmiller *et al.* (2016)
Hypophthalmichthys nobilis	19.4	0	0	0	0	18.72	0	0	34	0	0	0	0	0	0	0	0	0	0	27.88	Eichmiller *et al.* (2016)
Hypophthalmichthys nobilis	14.56	7.33	1.21	4.41	3.16	6.78	0	0.99	52.73	0	0	0.41	1.03	0	0	0	0.73	0	0	6.67	Liu *et al.* (2016)
Centropristis striata	24.86	2.72	0.49	5.12	0	1.12	0	2.71	59.24	0	0	0	0	0	0	0	0	0	0	3.74	Eichmiller et al. (2016)
Schizothorax zarudnyi	40.5	5.1	0	4.8	7.2	5.9	0	2.9	25.6	0.4	0	1.9	0	0	0.4	0	0.4	0	0	4.9	Ghanbari *et al.* (2016)
Caranx hippos	23.71	4.89	0	14.36	0	2.44	0	0.56	51.04	0.83	0	2.17	0	0	0	0	0	0	0	0	Givens *et al.* (2015)
Coryphaena hippurus	6.83	0	0	0	0	7.09	0	0.26	3.03	82.79	0	0	0	0	0	0	0	0	0	0	Givens *et al.* (2015)
Sciaenops ocellatus	31.29	2.24	3.36	1.2	0	6.66	0	1.53	45.88	2.6	0	1.25	0	0	0	0	0	0	0	3.99	Givens *et al.* (2015)
Scomberomorus maculatus	44.27	1.72	0	19.9	0	1.25	0	0	29.08	0	0	0	0	0	0	0	0	0	0	3.78	Givens *et al.* (2015)
Sphyraena barracuda	35.03	0	0	0.81	0	0.25	0	0	30.06	33.85	0	0	0	0	0	0	0	0	0	0	Givens *et al.* (2015)
Lagodon rhomboides	12.56	2.54	0	3.57	0	2.23	0	0.59	71.2	4.12	0	2.72	0	0	0	0	0	0	0	0.47	Givens *et al.* (2015)
Hypophthalmichthys molitrix	42.31	0	0	0	0	4.58	0	0	9.32	0	0	0	0	0	0	0	0	0	0	43.79	Givens *et al.* (2015)
Hypophthalmichthys molitrix	26.91	9.24	1.13	6.33	5.1	17.21	0	4.20	23.69	0	0	0.00	2.37	0	0	0	0.95	0	0	2.87	Liu *et al.* (2016)

Trinectes maculatus	16.73	0.93	0	4.32	0	0	0	0.83	76.93	0	0	0	0	0	0	0	0	0	0	0.26	Givens *et al.* (2015)
Fundulus heteroclitus	7.81	4.46	0	8.09	0	6.89	0	1.16	67.12	0.53	0	2.85	0	0	0	0	0	0	0	1.09	Givens *et al.* (2015)
Scomberomorus cavalla	3.6	0	0	2.73	0	1.04	0	0	42.74	0	0	49.89	0	0	0	0	0	0	0	0	Givens *et al.* (2015)
Epinephelus fuscoguttatus	3.12	0	0.33	0.06	0	3	0.55	0	92.94	0	0	0	0	0	0	0	0	0	0	0	Hennersdorf *et al.* (2016)
Atule mate	4.3	0	0	0.68	0	0	0	0	95.02	0	0	0	0	0	0	0	0	0	0	0	Hennersdorf *et al.* (2016)
Epinephelus sexfasciatus	32.29	0	0	0.54	0	0	0	0	67.17	0	0	0	0	0	0	0	0	0	0	0	Hennersdorf *et al.* (2016)
Siniperca chuatsi	26.4	0.53	0	0.41	0.22	0.29	0	0.06	71.92	0	0	0	0.05	0	0	0	0	0	0	0.12	Li *et al.* (2014)
Tachysurus fulvidraco	49.91	1.38	0	0.65	0.45	0.32	0	0.12	46.54	0	0	0	0.15	0	0	0	0	0	0	0.48	Li *et al.* (2014)
Megalobrama amblycephala	14.67	10.11	0.51	0.24	0	18.9	0	0	46.89	0	0	0	4.82	0	0	0	0	0	0	3.86	Li *et al.* (2014)
Megalobrama amblycephala	13.66	5.80	1.47	5.22	3.55	15.81	0	1.27	44.24	0	0	0	1.21	0	0	0	0.59	0	0	7.17	Liu *et al.* (2016)
Coreius guichenoti	5.2	4.9	0	16.3	0.33	0.18	0	0	55	0.79	0	11.5	1.11	0	0	0	0	0.53	0	4.16	Li *et al.* (2016)
Culter	22.12	9.19	0.11	0.13	0.34	16.45	0	0.45	34.99	0	0	0	0.32	0	0	0	0	0	0	15.9	Liu *et al.* (2016)
Naso hexacanthus	72.07	4.14	0	3.53	2.29	3.42	0	0	10.15	2.09	1.94	0	0	0	0	0	0	0	0	0.37	Miyake *et al.* (2015)
Acanthurus nigrofuscus	47.92	0.98	0	2.86	7.67	10.53	0	0	28.14	0	0	0	1.02	0	0	0	0	0	0	0.88	Miyake *et al.* (2015)
Acanthurus sohal	68.39	1.92	0	0.41	0.57	11.35	0	0	15.41	0	0	0.46	1.14	0	0	0	0	0	0	0.35	Miyake *et al.* (2015)
Ctenochaetus striatus	2.94	0	0	2.02	2.63	0	0	0	90.46	0	0	0	0.57	0	0	0	0	0	0	1.38	Miyake *et al.* (2015)
Naso elegans	98.3	0	0	0.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.18	Miyake *et al.* (2015)
Zebrasoma desjardinii	95.12	0.91	0	0	0	2.83	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0.34	Miyake *et al.* (2015)
Zebrasoma xanthurum	91.36	3.49	0	0	0	2.51	0	0	1.94	0	0	0	0	0	0	0	0	0	0	0.7	Miyake *et al.* (2015)
Chlorurus sordidus	0	10.1	0	0	4.71	5.09	0	4.99	75.11	0	0	0	0	0	0	0	0	0	0	0	Miyake *et al.* (2015)
Scarus niger	51.59	1.68	0	2	0.89	4.8	0	0	38.33	0	0	0	0	0	0	0	0	0	0	0.71	Miyake *et al.* (2015)
Acanthurus gahhm	29.35	0.66	0	7.51	7.45	4.08	0	0	49.09	0.57	0	0	0	0	0	0	0	0	0	1.29	Miyake *et al.* (2015)
Siganus stellatus	44.18	11.4	0	0.59	1.91	2.8	0	0	35.32	2.05	0	0.61	0	0	0	0	0	0	0	1.14	Miyake *et al.* (2015)
Ctenopharyngodon idella	27.1	1.33	0	0	0	26.5	0	0	13.9	0	0	0	0	0	0	0	0	0	0	31.17	Ni *et al.* (2014)
Siganus fuscescens	16.61	7.87	0	1.41	21.2	0	0	0.91	39.36	0.63	0	0.69	6.88	0	0	0	0	0	0	4.44	Nielsen *et al.* (2017)
Apogon doederleini	8.9	17	0	0	0	0.49	0	0	52.37	0.3	0	0.62	0.01	0.07	0	0	0	0	0	20.24	Parris *et al.* (2016)
Apogon sp.	13.28	1.21	0.43	17.25	0	0	0	0	52.08	0	0	0	0	0	0	0	0	0	0	15.75	Parris *et al.* (2016)
Chromis chromis	16.96	9.59	0	17.4	0	0	0	0	48.16	0	0	0	0	0	0	0	0	0	0	7.89	Parris *et al.* (2016)
Pentapodus nagasakiensis	2.13	0	0	0	0	0	0	0	96.79	0	0	0	0	0	0	0	0	0	0	1.08	Parris *et al.* (2016)
Pomacanthus chrysurus	0	1.43	0	0	0	0	0	0	93.1	0	0	0	0	0	0	0	0	0	0	5.47	Parris *et al.* (2016)

Dascyllus aruanus	16.61	7.87	0	1.41	21.2	0	0	0.91	39.36	0.63	0	0.69	6.88	0	0	0	0	0	0	4.44	Parris *et al.* (2016)
Pomacentrus amboinensis	0	4.21	0	0	0	0	0	0	81.98	0	0	0	0	0	0	0	0	0	0	13.81	Parris *et al.* (2016)
Pomacentrus bankanensis	10.48	3.37	0	0	0	0	0	0	82.15	0	0	0	0	0	0	0	0	0	0	4	Parris *et al.* (2016)
Pomacentrus coelestis	4.69	13.88	0	0	0	0	0	0.36	67	0	0	0	0	0	0	0	0	0	0	14.07	Parris *et al.* (2016)
Pomacentrus moluccensis	8.27	13.22	0	0	0	35.4	0	0	30.69	0	0	0	0	0	0	0	0	0	0	12.42	Parris *et al.* (2016)
Pomacentrus wardi	8.78	2.1	0	0	0	0	0	0	50.12	0	0	0	0	0	0	0	0	0	0	39	Parris *et al.* (2016)
Paralichthys adspersus	4.75	0.6	0.22	21.56	0.78	0	0	0	67.96	0	0	1.8	0	0	0	0	0	0	0	2.33	Ramirez and Romero (2017)
Paralichthys adspersus	23.00	9.00	0.00	4.00	0	0	0	0.00	64.00	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Salas-Leiva *et al.* (2017)
Paralichthys lethostigma	30.21	1.67	0	3.49	0	33.76	0	0	30.87	0	0	0	0	0	0	0	0	0	0	0	Ramirez and Romero (2017)
Coregonus clupeaformis	16.96	9.59	0	17.4	0	0	0	0	48.16	0	0	0	0	0	0	0	0	0	0	7.89	Sevellec *et al.* (2014)
Gymnodraco acuticeps	16.61	7.87	0	1.41	21.2	0	0	0.91	39.36	0.63	0	0.69	6.88	0	0	0	0	0	0	4.44	Song *et al.* (2016)
Pagothenia borchgrevinki	8.9	17	0	0	0	0.49	0	0	52.37	0.3	0	0.62	0.01	0.07	0	0	0	0	0	20.24	Song *et al.* (2016)
Trematomus bernacchii	13.28	1.21	0.43	17.35	0	0	0	0	52.08	0	0	0	0	0	0	0	0	0	0	15.65	Song *et al.* (2016)
Notothenia coriiceps	7.59	7.76	0	0	0	7.62	0	0	69.37	0	0	7.66	0	0	0	0	0	0	0	0	Song *et al.* (2016)
Gadus morhua	8.9	17	0	0	0	0.49	0	0	52.37	0.3	0	0.62	0.01	0.07	0	0	0	0	0	20.24	Star *et al.* (2013)
Poecilia reticulata	8.4	1.53	0	8.64	1.47	0	0	32.78	35.93	0	0	0	0	0	0	0	0	0	0	11.25	Sullam *et al.* (2015)
Chaenocephalus aceratus	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Salmo trutta	26.6	6.8	0	6.45	0	0	0	0	60.15	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Gillichthys mirabilis	0	0	0	0	0	0	0	0	53.58	0	0	33.36	6.6	0	0	0	0	0	0	6.46	Sullam *et al.* (2015)
Epinephelus coioides	42.66	0	0	0	0	0	0	0	57.34	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Oncorhynchus mykiss	15.75	4.83	0	13.38	0	2.98	4.56	0	58.5	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Hippocampus guttulatus	0	10.26	0	12.73	0	0	0	0	77.01	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Takifugu niphobles	42.85	0	0	4.87	0	0	0	0	38.15	0	0	0	0	0	0	0	0	0	0	14.13	Sullam *et al.* (2015)
Danio rerio	4.4	0.29	0	2.67	0.94	6.11	0	4.63	79.58	0	0	0	0.15	0	0	0	0	0	0	1.23	Sullam *et al.* (2015)
Lutjanus bohar	22.18	0	0	0	0	5.67	0	0	66.81	0	0	0	0	0	0	0	0	0	0	5.34	Sullam *et al.* (2015)
Solea senegalensis	27.71	5.57	0	5.45	0	0	0	0	61.27	0	0	0	0	0	0	0	0	0	0	0	Sullam *et al.* (2015)
Acanthurus nigricans	27.79	30.51	0	0	0	2.86	0	0	22.38	0	0	2.89	0	0	0	0	0	0	0	13.57	Sullam *et al.* (2015)
Naso tonganus	36.25	16.3	0	0	3.75	2.49	0	4.92	20.15	0	0	4.99	3.78	0	0	0	0	0	0	7.37	Sullam *et al.* (2015)
Pomacanthus sexstriatus	25.5	30.05	0	0	0	0	0	3.78	25.54	0	0	0	7.17	0	0	0	0	0	0	7.96	Sullam *et al.* (2015)
Salmo salar	0	0	0	0	0	0	0	0	62.5	0	0	37	0	0	0	0	0	0	0	0.5	Llewellyn *et al.* (2016)

Salmo salar	10.93	5.36	1.00	2.05	0	0	0	3.22	32.54	0	0	0.00	3.29	0	0	0	0	0	0	41.62	Webster *et al.* (2018)
Salmo salar	4.71	6.71	0.00	1.15	0	0	0	0.75	19.04	0	0	32.36	0.56	0	0	0	0	0	0	34.72	Webster *et al.* (2018)
Salmo salar	6.99	4.77	0.00	3.02	0	0	0	3.96	37.49	0	0	0.69	4.41	0	0	0	0	0	0	38.67	Webster *et al.* (2018)
Salmo salar	6.25	10.02	0.98	1.65	0	0	0	4.38	41.93	0	0	2.84	6.48	0	0	0	0	0	0	25.46	Webster *et al.* (2018)
Amphibians																					
Fejervarya limnocharis	33.06	35.44	0.01	0.19	0.16	0.39	0.32	0.03	24.08	1.28	0	4.52	0.17	0	0	0	0	0.01	0	0.34	Chang *et al.* (2016)
Rana pipiens	66.1	22.8	0	0.08	0	0.32	0	0	10.4	0	0	0	0.03	0	0	0	0	0	0	0.27	Kohl *et al.* (2013)
Bufotes raddei	35.46	16.7	0	2.3	0	0	0	0	17.32	0	2.02	23.28	0.6	0	0	0	0	0	0	2.32	Zhang *et al.* (2016)
Reptiles																					
Agkistrodon piscivorus	9.63	18.1	0	0	0	3.18	0	0	42.16	0	0	0	0	0	0	0	0	0	0	26.93	Colston *et al.* (2015)
Chelonoidis nigra	81.1	4.4	0.1	0.8	0.1	0	0	0.8	2	0	0.2	0	0.1	0	0	0	0	0	0	10.4	Hong *et al.* (2015)
Amblyrhynchus cristatus	75.1	8.2	0	0.6	0	0	0	0	0.6	0	0	0	1	0	0	0	0	0	0	14.5	Hong *et al.* (2015)
Conolophus subcristatus	63.9	4.2	0	1.3	0	0.6	0	1.6	1.4	0	0.1	0.1	0.2	0	0	0	0	0	0	26.6	Hong *et al.* (2015)
Alligator mississippiensis	16.7	5	0	0.08	0.1	69.6	0	0	2.7	0	0	0	4.3	0	0	0	0	0	0	1.52	Keenan *et al.* (2013)
Phymaturus williamsi	74.24	11.49	0	0.55	0.58	0	0	0	0	0.26	0	5.61	0	0	0	0	0	0	0	7.27	Kohl *et al.* (2017a)
Liolaemus parvus	46.09	35.44	0	0.18	2	0	0	0	0	0	0	0.8	0	0.16	0	0	0	0	0	15.33	Kohl *et al.* (2017a)
Liolaemus ruibali	50.34	33.46	0	0.5	0.67	0	0	0	0	0	0	1.38	0	0.07	0	0	0	0	0	13.58	Kohl *et al.* (2017a)
Anolis cristatellus	76.18	9.43	0	0.38	0	0	0	0	14.01	0	0	0	0	0	0	0	0	0	0	0	Ren *et al.* (2016b)
Anolis evermanni	62.24	11.93	0	0.46	0	0	0	0	25.25	0	0	0	0	0	0.12	0	0	0	0	0	Ren *et al.* (2016b)
Anolis gundlachi	42.65	1.46	0	5.11	0	0	0	0	49.85	0	0	0	0	0	0.5	0	0	0	0	0.43	Ren *et al.* (2016b)
Anolis krugi	69.85	18.49	0	0.14	0	0	0	0	11.21	0	0	0	0	0	0.31	0	0	0	0	0	Ren *et al.* (2016b)
Anolis pulchellus	55.21	8.19	0	0.28	0	0	0	0	36.29	0	0	0	0	0	0.03	0	0	0	0	0	Ren *et al.* (2016b)
Anolis stratulus	55.47	18.29	0	0.29	0	0	0	0	25.05	0	0	0	0	0	0.6	0	0	0	0	0.3	Ren *et al.* (2016b)
Iguana iguana	74	10.1	0	0.1	0	0	0.1	0.5	3.1	0.6	0.4	0	1	0	0	0	0	0	0	10.1	Wehrle (2013)
Gopherus polyphemus	59.7	15.9	0	0	0	0	0	1.9	15.4	0	0	0	0	0	0	0	0	0	0	7.1	Yuan *et al.* (2015)
Birds																					
Pygoscelis adeliae	41	5	0	35	0	0	0	0	12.5	0	0	0	0	0	0	0	0	0	0	6.5	Banks *et al.* (2009)

Pygoscelis adeliae	13.96	0.00	0.00	0.00	0	59.48	0	0.00	26.56	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Yew *et al.* (2017)
Pygoscelis antarcticus	43.51	27.02	0	3.48	0	5.08	0	0	15.02	1.1	0	2.93	0	1.16	0	0	0	0	0	0.7	Barbosa *et al.* (2016)
Pygoscelis antarcticus	10.43	2.51	0.00	0.68	0	79.214	0	0.00	5.57	0	0	3.60	0.00	0	0	0	0	0	0	0.00	Yew *et al.* (2017)
Pygoscelis papua	18	7	0	0	0	55	0	0	18	0	0	0	0	0	0	0	0	0	0	2	Dewar *et al.* (2013)
Eudyptes chrysolophus	45	19	0	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0	0	7	Dewar *et al.* (2013)
Eudyptula minor	24	22	0	7	0	0	0	11	30	0	0	0	0	0	0	0	0	0	0	6	Dewar *et al.* (2013)
Ardenna tenuirostris	59.21	0	0	8.45	0	1.04	0	0	31.3	0	0	0	0	0	0	0	0	0	0	0	Dewar *et al.* (2014)
Pachyptila turtur	58.76	6.15	0	7.71	0	2.76	0	0	24.62	0	0	0	0	0	0	0	0	0	0	0	Dewar *et al.* (2014)
Pelecanoides urinatrix	47.01	17.27	0	9.95	0	3.97	0	0	21.8	0	0	0	0	0	0	0	0	0	0	0	Dewar *et al.* (2014)
Anas platyrhynchos	43.67	16.16	0	4.6	0	4.2	0	0	27.2	0	0	1.3	0	0	0	0	0	0	0	2.87	Ganz *et al.* (2017)
Anas platyrhynchos	0.44	0.17	0.00	0.05	0	0.04	0	0.00	0.27	0	0	0.01	0.00	0	0	0	0	0	0	0.02	Hird *et al.* (2018)
Opisthocomus hoazin	66	28	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	1	Godoy-Vitorino *et al.* (2012)
Zonotrichia capensis	14.93	1.25	0	8.31	1.03	0	0	0	43.41	0	0	26.02	0.75	0	0	0	0	0	0	4.3	Herkrath (2015)
Hirundo rustica	26	2	0	18	1.13	0	0	0	50	0	0	1.96	0	0	0	0	0.7	0	0	0.21	Kreisinger *et al.* (2015)
Dumetella carolinensis	40.29	3.01	0	9.47	0	0	0	0	40.69	0	0	2.93	0	0	0	0	0	0	0	3.61	Lewis *et al.* (2016)
Catharus ustulatus	27.34	1.9	0	15.1	0	0	0	0	53.23	0	0	0.44	0	0	0	0	0	0	0	1.99	Lewis *et al.* (2016)
Larus argentatus	72.89	0	0	2.95	0	1.28	0	0	14.51	2.56	0	0.1	0	0	0	0	0	0	0	5.71	Merkeviciene *et al.* (2017)
Oceanodroma leucorhoa	3.33	7.33	0	13.18	0	0	0	0	59.23	0	0	0	0	0	0	0	0	0	0	16.93	Pearce (2016)
Cathartes aura	60.8	0	0	1.2	0	31.1	0	0	5.4	0	0	0	0	0	0	0	0	0	0	1.5	Roggenbuck *et al.* (2014a)
Coragyps atratus	67.1	0	0	0.2	0	21.1	0	0	11.2	0	0	0	0	0	0	0	0	0	0	0.4	Roggenbuck *et al.* (2014a)
Lagopus muta	28.57	17.61	0.02	32.08	0.04	0	0	0	4.53	1.54	11.5	0.03	0	0	0	0	0	0	0	4.08	Ushida *et al.* (2016)
Strigops habroptila	44.67	0	0	0	0	0	0	0	55.33	0	0	0	0	0	0	0	0	0	0	0	Waite *et al.* (2012)
Anser indicus	74.78	6.65	0	7.49	0	0	0	0	7.84	0	0	0	0	0	0.5	0	0.5	0	0	2.24	Wang *et al.* (2016a)
Gallus gallus	70	12.3	0	2	0	0	0	0	10.2	0	0	1	0	0	0	0	0	0	0	4.5	Wei *et al.* (2013)
Meleagris gallopavo	60.4	28.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.8	Wei *et al.* (2013)
Anser cygnoides	83.8	0.1	0	0	0	0	0	0	15.8	0	0	0	0	0	0	0	0	0	0	0.3	Wang *et al.* (2016b)
Anser cygnoides	91.69	0.13	0.00	1.68	0	0.24	0	0.00	4.75	0	0	0.00	0.00	0	0	0	0	0	0	1.51	Wu *et al.* (2018)
Anser cygnoides	68.34	0.73	0.00	3.26	0	0.47	0	0.41	24.46	0	0	0.00	0.00	0	0	0	0.72	0	0	1.61	Wu *et al.* (2018)
Grus japonensis	50.72	0.33	0	1.29	0	2.83	0	0	41.19	0	0	0	0	0	0	0	0	0	0	3.64	Xie *et al.* (2016)
Anser albifrons	49.02	3.35	2.15	14.95	0	1.5	0	1.6	19.4	0	0.45	0	0.65	0	0	0	3.35	0	0	3.58	Yang *et al.* (2016)

Anser fabalis	27.23	6.7	1.98	2.9	0	0	0	1.18	50.4	0	1.17	0	1	0	0	0	2.8	0	0	4.64	Yang *et al.* (2016)
Grus monacha	44	3.55	0	14.4	13.3	1.4	0	0	20.4	0	0	0	0	0	0	0	0	0	0	2.95	Zhao *et al.* (2017)
<u>Mammals</u>																					
Lynx pardinus	43.25	39.43	0	1.78	0	10.45	0	0	4.27	0	0	0	0	0	0	0	0	0	0	0.82	Alcaide *et al.* (2012)
Alouatta caraya	53.44	11.78	0	0.84	0	0	0	0	0.16	0	0.11	0	0.11	0	0	0	0	0	0	33.56	Amato *et al.* (2013)
Pan troglodytes	59.8	17.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22.3	Angelakis *et al.* (2016a)
Pan troglodytes	53.31	24.76	0	2.51	0	0.21	0.12	0.11	1.1	0.98	0.2	2	1.43	0	0.07	0	0.06	0	0.44	12.69	Degnan *et al.* (2012)
Papio cynocephalus	42.2	7.3	0	9.4	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	28.1	Angelakis *et al.* (2016b)
Papio cynocephalus	48.8	7.2	0	17.2	0	0	0	0	4.1	0.7	0	0	0	0	0	0	0	0	0	22	Ren *et al.* (2016a)
Leptonychotes weddellii	23	12.5	0.3	19.5	0.3	3.5	0	0	12.9	0	0	0.3	0	0	0	0	0	0	0	27.7	Banks *et al.* (2014)
Phascolarctos cinereus	62.91	19.79	0	0.3	1.59	3.61	0	0	2.22	0	6.13	0	0	0	0	0	0	0	0	3.45	Barker *et al.* (2013)
Felis catus	34	40	0	7	0	2	0	1	13	1	1	0	0	0	0	0	1	0	0	0	Barry *et al.* (2012)
Camelus dromedarius	13.86	74.64	0	0.59	0	0	0	0	0.26	0	0.28	0	0	0.28	0	0	0	0	0	10.09	Bhatt *et al.* (2013)
Camelus dromedarius	31	51	0	0	0	0	0	0	4.8	3.5	0	0.95	2.7	0	0	0	0	0	3.1	2.95	Gharechahi *et al.* (2015)
Tursiops truncatus	38.2	0.8	0	0	0	21.9	0	0	35.4	0	0	2.4	0	0	0	0	0	0	0	1.3	Bik *et al.* (2016)
Zalophus californianus	28.9	41.3	0	0	0	25.4	0	0	4	0	0	0	0	0	0	0	0	0	0	0.4	Bik *et al.* (2016)
Ictidomys tridecemlineatus	65.5	21.6	0	0.1	0	0	0	0	1.5	0	0	2.7	5.4	0	0	0	0	0	0	3.2	Carey *et al.* (2013)
Desmodus rotundus	1.25	3.92	5.53	7.54	3.47	0	0	7.06	48.26	0	0	0.22	0	0	0	0	4.78	0	0	17.97	Carrillo-Araujo *et al.* (2015)
Macrotus waterhousii	0	6.35	4.83	6.76	6.31	0	0	11.66	38.26	0	0	0	0	0	0	0	4.59	0	0	21.24	Carrillo-Araujo *et al.* (2015)
Artibeus jamaicensis	23.01	1	0	0	1.06	0	0	0	42.15	0	0	28.78	0	0	0	0	0	0	0	4	Carrillo-Araujo *et al.* (2015)
Carollia perspicillata	23.14	3.21	0	0	15.84	0	0	1.32	29.88	0	0	20.35	0	0	0	0	0	0	0	6.26	Carrillo-Araujo *et al.* (2015)
Glossophaga soricina	5.22	3.16	0	0	1.49	0	0	1.1	73.66	0	0	3.78	0	0	0	0	0	0	0	11.59	Carrillo-Araujo *et al.* (2015)
Leptonycteris yerbabuenae	0	19.53	1.35	3.9	6.71	0	0	3.42	52.09	0	0	0	0	0	0	0	0.71	0	0	12.29	Carrillo-Araujo *et al.* (2015)
Aepyceros melampus	32.1	23.1	0	1.4	0	0	0	0	8.3	0	0	0	0	0	0	0	0	0	0	35.1	Cersosimo *et al.* (2015)
Sarcophilus harrisii	53.5	1.2	0	1.2	0	13.8	0	0	18.6	0	0	0	0	0	0	0	0	0	0	11.7	Cheng *et al.* (2015)
Equus ferus	70	5.9	0	3.4	0	0	0	0	6	0	0	0	6.6	0	2.9	0	0	0	0	5.2	Costa and Weese (2012)
Dasypus novemcinctus	0	2.67	0	2.75	0	0.03	0	0	7.36	80.7	0	4.3	0.33	0	0	0	0	0	0	1.86	Delsuc *et al.* (2014)
Chlamyphorus truncatus	0	50.09	0	0.38	0	4.33	0	0	11.22	20.05	0	3.6	10.33	0	0	0	0	0	0	0	Delsuc *et al.* (2014)
Priodontes maximus	0	4.12	0	1.31	0	0	0	0	3.19	82.28	0	1.47	2.55	0	0	0	0	0	0	5.08	Delsuc *et al.* (2014)

Zaedyus pichiy	0	22.63	0	3.27	0	0.45	0	0	41.26	29.62	0	1.95	0	0	0	0	0	0	0	0.82	Delsuc *et al.* (2014)
Myrmecophaga tridactyla	0	7.66	0	3.17	0	1.48	0	0	19.37	60.09	0	0	0.33	0	0	0	0	0	0	7.9	Delsuc *et al.* (2014)
Bradypus variegatus	21	9.3	0	8	0	0	0	0	61	0	0	0	0	0	0	0	0	0	0	0.7	Dill-McFarland *et al.* (2016)
Choloepus hoffmanni	29	3.1	0	7.2	0	0	0	0	59	0	0	0	0	0	0	0	0	0	0	1.7	Dill-McFarland *et al.* (2016)
Loxodonta africana	45.7	15.3	0	0	0	0	0.2	0	14.3	1.2	0	0	4	0	0	0	0	0	0	19.3	Finch (2013)
Loxodonta cyclotis	16.4	10.1	0	0	0	0	0.6	0	42.4	0.9	0	0	6.7	0	0	0	0	0	0	22.9	Finch (2013)
Propithecus verreauxi	40	29	0	2	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	25	Fogel (2015)
Lemur catta	20	15	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	55	Fogel (2015)
Hydrochoerus hydrochaeris	34.5	8.1	0	7.4	0	0	0	0	32.3	4	0	0	0	0	0	0	0	0	0	13.7	García-Amado *et al.* (2012)
Ursus maritimus	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Glad *et al.* (2010)
Bos taurus	50.49	42.39	0	1.27	0.07	0.02	0.35	0	1.15	1.02	0.02	1.43	0.02	0	0	0.02	0.13	0	0.01	1.6	Godoy-Vitorino *et al.* (2012)
Stenella coeruleoalba	15.4	0	0	0	0	11.5	0	0	73	0	0	0	0	0	0	0	0	0	0	0.1	Godoy-Vitorino *et al.* (2017)
Gorilla gorilla	41	22	0	12	0	0	0	0	2	2	0	0	0	0	0	0	5	0	0	16	Gomez *et al.* (2015)
Gorilla beringei	34	31	0	3	0	0	0	0	2	2	0	0	0	0	0	0	3	0	0	25	Gomez *et al.* (2016)
Castor canadensis	58	37	0	1	0	0	0	0	1	0.5	0	0	0.5	0	0	0	0	0	0	2	Gruninger *et al.* (2016)
Macropus giganteus	46.05	48.05	0	0.66	0	1.45	0	0	1.37	0.45	0	0.02	0	0	0	0	0	0	1.54	0.41	Gulino *et al.* (2013)
Macropus robustus	42.64	53.76	0	0.54	0	0.67	0	0	1.29	0.36	0	0.06	0	0	0	0	0	0	0.26	0.42	Gulino *et al.* (2013)
Macropus rufus	68.6	15	0	0	0	0	0	0	7	0	0	0	9.4	0	0	0	0	0	0	0	Gulino *et al.* (2013)
Sus scrofa	54.6	27.3	0	1.3	0	3	0	0	8.4	3.6	0	0.5	0	0	0	0	0	0	0	1.3	Isaacson and Kim (2012)
Alces alces	4	27	0	0	0	0	0	0	19	0	4	0	8	0	0	0	11	0	0	27	Ishaq and Wright (2012)
Balaena mysticetus	33	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	June *et al.* (2016)
Globicephala melas	16.5	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33.5	June *et al.* (2016)
Neotoma albigula	25.8	68.9	0	0.06	0.94	0	0	0	1.4	0.2	0	0.8	0.4	0	0	0	0	0	0	1.5	Kohl *et al.* (2015)
Ochotona princeps	50.55	34.88	0	0.25	0	0	0	0	0.61	0	0	3.28	1.68	0	0	0	0	0	0	8.75	Kohl *et al.* (2017b)
Ailurus fulgens	34.28	17.58	0	1.99	0	0.41	0	0	45.25	0	0	0	0	0	0	0	0	0	0	0.49	Kong *et al.* (2014)
Ovis aries	39.46	39.27	0	0	0	0	0	0	19.91	0	0	0	0	0	0	0	0	0	0	1.36	Lopes *et al.* (2015)
Petaurista alborufus	92.4	0	0	3.6	0	0	0	0	2	0	0	0	0.4	0	0	0	0	0	0	1.6	Lu *et al.* (2012)
Mammuthus primigenius	0.3	0.5	0	18	0	0	0	0	78.2	0	0	0	0	0	0	0	0	0	0	3	Mardanov *et al.* (2012)
Coelodonta antiquitatis	68	0.2	0	5.7	0	0	0	0	18.8	0	0	0	0	0	4.3	0	0	0	0	3	Mardanov *et al.* (2012)
Mus musculus	52.1	37	0	1.1	0	0	0	0	8.2	0	0	0.9	0	0.4	0	0	0	0	0	0.3	Maurice *et al.* (2015)

Arctocephalus australis	88.5	0.17	0	2.6	0	1.2	0	0	6.3	0	0	0	0	0	0	0	0	0	0	1.23	Medeiros *et al.* (2016)
Arctocephalus tropicalis	84	0.2	0	10.9	0	0	0	0	0.4	0	0	0	0	0	0	0	0	0	0	4.5	Medeiros *et al.* (2016)
Canis mesomelas	40.5	26.1	0	3.8	0	21.8	0	0	6.9	0	0	0	0	0	0	0	0	0	0	0.9	Menke *et al.* (2014)
Acinonyx jubatus	56.2	5.8	0	15.5	0	18.1	0	0	4.2	0	0	0	0	0	0	0	0	0	0	0.2	Menke *et al.* (2014)
Cystophora cristata	22	68	0	0	0	1	0	0	9	0	0	0	0	0	0	0	0	0	0	0	Nelson *et al.* (2015)
Hydrurga leptonyx	44	8	0	0	0	13	0	0	31	0	0	0	0	0	0	0	0	0	0	4	Nelson *et al.* (2015)
Halichoerus grypus	76	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nelson *et al.* (2015)
Mirounga leonina	43	21	0	0	0	20	0	0	15	0	0	0	0	0	0	0	0	0	0	1	Nelson *et al.* (2015)
Neophoca cinerea	80	2	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	10	Nelson *et al.* (2015)
Neophoca cinerea	76.39	9.44	0.00	2.78	0	0.56	0	0.00	10.83	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Delport *et al.* (2016)
Phoca vitulina	50	49	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Nelson *et al.* (2015)
Dugong dugon	83	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Nelson *et al.* (2015)
Trichechus manatus	79	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	Nelson *et al.* (2015)
Macropus eugenii	43.9	29.7	0	10.8	0	0	0	0	15.5	0	0	0	0	0	0	0	0	0	0	0.1	Pope *et al.* (2010)
Rangifer tarandus	27	62.94	0	0.02	0	0	0	0.19	0.16	0.06	0	0	0	0	0.63	0	5	0	0	4	Pope *et al.* (2012)
Eulemur rubriventer	24.1	31.1	2.3	0	0	0	0	0.8	15.6	0	0	0	4.3	0	0	0	0	0	0	21.8	Raulo (2015)
Giraffa camelopardalis	50	30	0	1	1	0	0	0	4	0	0	0	0	0	1	0	0	0	0	13	Roggenbuck *et al.* (2014b)
Ursus arctos	39.29	6.05	0.48	11.21	0.03	1.12	0	0.56	39.56	0	0	0.51	0.2	0	0.02	0	0	0	0	0.97	Sommer *et al.* (2016)
Macaca thibetana	4.43	35.2	0	0.48	0	0	0	0	14.67	2.03	0	0	0.1	0	0	0	0	0	0	43.09	Sun *et al.* (2016)
Homo sapiens	79.4	16.9	0	2.5	0	0	0	0	1	0	0	0	0.1	0	0	0	0	0	0	0.1	Tap *et al.* (2009)
Papio hamadryas	68	3	0	13	0	0	0	0	16	0	0	0	0	0	0	0	0	0	0	0	Tung *et al.* (2015)
Ailuropoda melanoleuca	1.5	83.8	0.1	0.1	0.1	0.1	0	0	0	15.8	0	0	0	0	0	0	0	0	0	0	Zhu *et al.* (2011)
Ailuropoda melanoleuca	95.50	0.00	0.00	0.00	1	0	0	0.00	3.50	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Wu *et al.* (2017)
Ailuropoda melanoleuca	78.72	0.00	0.00	0.91	1.21	0	0	0.00	19.16	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Wu *et al.* (2017)
Ailuropoda melanoleuca	87.21	0.00	0.00	1.20	1.34	0	0	0.00	10.25	0	0	0.00	0.00	0	0	0	0	0	0	0.00	Wu *et al.* (2017)
Nycticebus pygmaeus	9.44	41.19	0	10.98	0	0.26	0	0	30.43	0.5	0	0	1.33	0	0	0	0	0	0	5.87	Xu *et al.* (2013)
Rhinopithecus bieti	39.36	27.6	0	3.61	0.66	0.87	0	0	19.41	2.01	0	0.48	0.53	0	0	0	0	0	0.43	5.04	Xu *et al.* (2015)
Cercopithecus ascanius	67	11	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	19	Yildirim *et al.* (2010)
Colobus guereza	72	6.6	0	0	0	0	0	0	1	0	0	1.3	0	0	0	0	0	0	0	19.1	Yildirim *et al.* (2010)
Piliocolobus tephrosceles	65.98	17.54	0	0	0.01	0	0	0	0.73	2.01	0	0.08	1.95	0	0.01	0.01	0	0	0	11.68	Yildirim *et al.* (2010)

Canis lupus	60	16.9	0	4.6	0	9.2	0	0	9.2	0	0	0	0	0	0	0	0	0	0	0.1	Zhang and Chen (2010)

2.2.4 Host species characterisations

Host species were arranged into the following sets of mutually exclusive groups based on diet and natural history: non-ray-finned fish (n=2), ray-finned fish (n=74) or tetrapods (n=127); ectotherms (n=95) or endotherms (n=108); herbivores (n=66), omnivores (n=37), carnivores (n=96), frugivores (n=1) or nectivores (n=3). Habitat preference was divided into freshwater (n=28), marine (n=85), and terrestrial (n=90). Animals were divided based on climactic preference as determined by latitude: polar (n=20), boreal (n=2), temperate (n=67), subtropical (n=35), and tropical (n=79) (Table 2.3). Finally, animals were divided based on biomes, a list of these including their definitions and the number of species assigned to each is provided below (Table 2.2). All biological and ecological data were obtained from the online databases; FishBase (www.fishbase.org), Animal Diversity Web (www.animaldiversity.org), International Union for Conservation of Nature Red List of Threatened Species (www.iucnredlidt.org), and Encyclopedia of life (www.eol.org). Biological or ecological information not available from these online databases was sourced from peer-reviewed publications (Table 2.3).

Table 2.2 Definitions of biomes used in this study, including the number of species (n) assigned to each biome.

Biome	Definition	n
Lentic	Still, fresh water	16
Lotic	Rapidly moving fresh water	8
Estuary	Tidal mouth of a river	1
Reef	A ridge of rock or coral, just below the surface of the sea	34
Littoral	The area on the shore of the sea	33
Oceanic	The area of the ocean lying beyond the continental shelf	16
Marsh	Low-lying land that remains waterlogged at all times	2
Tundra	Flat, treeless Arctic region where the subsoil is permanently frozen	5
Agricultural	Area developed by humans for farming	9
Grassland	An open area of country covered by grass	11
Forest	A large area covered mainly by trees and undergrowth	29
Rainforest	A forest characterised by high rainfall	12
Scrub Forest	An area where the vegetation is dominated by shrubs and grasses	7
Savanna	A grassy plain in tropical and subtropical areas with few trees	9
Urban	Relating to a town or city	4
Mountain	A large landform that rises above the surrounding land	5
Desert	An area with an annual rainfall of <25cm	1
Wetland	Saturated land consisting of marshes and swamps	1

Table 2.3 Ecological and biological data for species used in this investigation.

Species	Phylogeny	Thermoregulation	Digestive Physiology	Habitat	Biome	Climactic preference	Reference
Fish							
Carcharhinus brevipinna	Non-ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019)
Petromyzon marinus	Non-ray-finned fish	Ectotherm	Carnivore	Freshwater	Lotic	Temperate	Froese and Pauly (2019)
Comephorus dybowskii	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Boreal	Froese and Pauly (2019); Miyasaka *et al.* (2006)
Aplodinotus grunniens	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lotic	Subtropical	Froese and Pauly (2019)
Schizothorax zarudnyi	Ray-finned fish	Ectotherm	Herbivore	Freshwater	Lentic	Subtropical	Froese and Pauly (2019); Ghanbari *et al.* (2016); Gharaei (2012)
Ctenopharyngodon idella	Ray-finned fish	Ectotherm	Herbivore	Freshwater	Lentic	Subtropical	Froese and Pauly (2019)
Carassius auratus	Ray-finned fish	Ectotherm	Omnivore	Freshwater	Lentic	Subtropical	Froese and Pauly (2019)
Cyprinus carpio	Ray-finned fish	Ectotherm	Omnivore	Freshwater	Lentic	Subtropical	Froese and Pauly (2019)
Hypophthalmichthys molitrix	Ray-finned fish	Ectotherm	Omnivore	Freshwater	Lotic	Subtropical	Froese and Pauly (2019)
Coreius guichenoti	Ray-finned fish	Ectotherm	Omnivore	Freshwater	Lotic	Subtropical	Froese and Pauly (2019); Liu *et al.* (2013); Parr *et al.* (2019)
Hypophthalmichthys nobilis	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lotic	Temperate	Froese and Pauly (2019)
Siniperca chuatsi	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lotic	Temperate	Froese and Pauly (2019)
Tachysurus fulvidraco	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Temperate	Froese and Pauly (2019)
Culter alburnus	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Temperate	Froese and Pauly (2019); Chiu *et al.* (2012)
Coregonus clupeaformis	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Temperate	Froese and Pauly (2019)
Megalobrama amblycephala	Ray-finned fish	Ectotherm	Herbivore	Freshwater	Lentic	Temperate	Froese and Pauly (2019); Gao *et al.* (2012)
Danio rerio	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Tropical	Froese and Pauly (2019)
Poecilia reticulata	Ray-finned fish	Ectotherm	Omnivore	Freshwater	Lotic	Tropical	Froese and Pauly (2019)
Chionodraco hamatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Polar	Froese and Pauly (2019); Parr *et al.* (2019)
Gymnodraco acuticeps	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Polar	Froese and Pauly (2019)
Pagothenia borchgrevinki	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Polar	Froese and Pauly (2019); Myers *et al.* (2019)
Trematomus bernacchii	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Polar	Froese and Pauly (2019)
Chaenocephalus aceratus	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Polar	Froese and Pauly (2019)
Notothenia coriiceps	Ray-finned fish	Ectotherm	Omnivore	Marine	Oceanic	Polar	Froese and Pauly (2019); Iken *et al.* (1997)
Bairdiella chrysoura	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019)
Caranx hippos	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Subtropical	Froese and Pauly (2019)

Coryphaena hippurus	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Subtropical	Froese and Pauly (2019)
Sciaenops ocellatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019)
Scomberomorus maculatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019)
Sphyraena barracuda	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Subtropical	Froese and Pauly (2019)
Epinephelus fuscoguttatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019)
Apogon doederleini	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019); Parris *et al.* (2016)
Apogon sp.	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019); Parris *et al.* (2016)
Chromis chromis	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019)
Paralichthys adspersus	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019); IUCN (2019)
Paralichthys lethostigma	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019)
Gillichthys mirabilis	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019); Parr *et al.* (2019)
Epinephelus coioides	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Subtropical	Froese and Pauly (2019); Myers *et al.* (2019)
Oncorhynchus mykiss	Ray-finned fish	Ectotherm	Carnivore	Marine	Oceanic	Subtropical	Froese and Pauly (2019)
Salmo trutta	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lentic	Subtropical	Froese and Pauly (2019)
Lagodon rhomboides	Ray-finned fish	Ectotherm	Herbivore	Marine	Littoral	Subtropical	Froese and Pauly (2019)
Trinectes maculatus	Ray-finned fish	Ectotherm	Omnivore	Marine	Littoral	Subtropical	Froese and Pauly (2019); Myers *et al.* (2019)
Centropristis striata	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Temperate	Froese and Pauly (2019); IUCN (2019)
Salmo salar	Ray-finned fish	Ectotherm	Carnivore	Freshwater	Lotic	Temperate	Froese and Pauly (2019)
Gadus morhua	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Temperate	Froese and Pauly (2019)
Hippocampus guttulatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Temperate	Froese and Pauly (2019)
Takifugu niphobles	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Temperate	Froese and Pauly (2019); Wallace *et al.* (2011)
Fundulus heteroclitus	Ray-finned fish	Ectotherm	Omnivore	Marine	Marsh	Temperate	Froese and Pauly (2019); James-Pirri *et al.* (2001)
Scomberomorus cavalla	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Atule mate	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Epinephelus sexfasciatus	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Naso hexacanthus	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pentapodus nagasakiensis	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacanthus chrysurus	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019); James-Pirri *et al.* (2001)
Lutjanus bohar	Ray-finned fish	Ectotherm	Carnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Solea senegalensis	Ray-finned fish	Ectotherm	Carnivore	Marine	Littoral	Tropical	Froese and Pauly (2019)
Acanthurus nigrofuscus	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Acanthurus sohal	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)

Ctenochaetus striatus	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Naso elegans	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Zebrasoma desjardinii	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019); IUCN (2019)
Zebrasoma xanthurum	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Chlorurus sordidus	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Scarus niger	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Siganus fuscescens	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Acanthurus nigricans	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Naso tonganus	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacanthus sexstriatus	Ray-finned fish	Ectotherm	Herbivore	Marine	Reef	Tropical	Froese and Pauly (2019); Wallace *et al.* (2011)
Acanthurus gahhm	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019); Miyake *et al.* (2015)
Siganus stellatus	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019); Miyake *et al.* (2015)
Dascyllus aruanus	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacentrus amboinensis	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacentrus bankanensis	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacentrus coelestis	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacentrus moluccensis	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Pomacentrus wardi	Ray-finned fish	Ectotherm	Omnivore	Marine	Reef	Tropical	Froese and Pauly (2019)
Amphibians							
Fejervarya limnocharis	Tetrapod	Ectotherm	Carnivore	Freshwater	Forest	Temperate	Wallace *et al.* (2011); IUCN (2019); Solé and Rödder (2009); Parr *et al.* (2019)
Bufotes raddei	Tetrapod	Ectotherm	Carnivore	Freshwater	Forest	Temperate	Wallace *et al.* (2011); IUCN (2019); Solé and Rödder (2009); Parr *et al.* (2019)
Rana pipiens	Tetrapod	Ectotherm	Carnivore	Freshwater	Wetland	Temperate	Wallace *et al.* (2011); IUCN (2019); Solé and Rödder (2009); Parr *et al.* (2019)
Reptiles							
Alligator mississippiensis	Tetrapod	Ectotherm	Carnivore	Freshwater	Lentic	Tropical	Myers *et al.* (2019)
Anolis cristatellus	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Anolis evermanni	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Parr *et al.* (2019)
Anolis gundlachi	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Parr *et al.* (2019)

Anolis krugi	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Parr *et al.* (2019); Ren *et al.* (2016b)
Anolis pulchellus	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Parr *et al.* (2019); Ren *et al.* (2016b)
Anolis stratulus	Tetrapod	Ectotherm	Carnivore	Terrestrial	Forest	Tropical	Parr *et al.* (2019); Myers *et al.* (2019)
Agkistrodon piscivorus	Tetrapod	Ectotherm	Carnivore	Terrestrial	Lentic	Temperate	Parr *et al.* (2019); Myers *et al.* (2019)
Phymaturus williamsi	Tetrapod	Ectotherm	Herbivore	Terrestrial	Mountain	Temperate	IUCN (2019); Kohl *et al.* (2017a)
Iguana iguana	Tetrapod	Ectotherm	Herbivore	Terrestrial	Rainforest	Tropical	Myers *et al.* (2019)
Gopherus polyphemus	Tetrapod	Ectotherm	Herbivore	Terrestrial	Scrub Forest	Temperate	Myers *et al.* (2019)
Amblyrhynchus cristatus	Tetrapod	Ectotherm	Herbivore	Marine	Littoral	Tropical	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Chelonoidis nigra	Tetrapod	Ectotherm	Herbivore	Terrestrial	Grassland	Tropical	Myers *et al.* (2019)
Conolophus subcristatus	Tetrapod	Ectotherm	Herbivore	Terrestrial	Scrub Forest	Tropical	Parr *et al.* (2019); Myers *et al.* (2019)
Liolaemus parvus	Tetrapod	Ectotherm	Omnivore	Terrestrial	Mountain	Temperate	IUCN (2019); Kohl *et al.* (2017a)
Liolaemus ruibali	Tetrapod	Ectotherm	Omnivore	Terrestrial	Mountain	Temperate	IUCN (2019); Kohl *et al.* (2017a)
Birds							
Ardenna tenuirostris	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Temperate	Myers *et al.* (2019); IUCN (2019); Parr *et al.* (2019)
Pachyptila turtur	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Temperate	Parr *et al.* (2019)
Pygoscelis adeliae	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	Parr *et al.* (2019)
Pygoscelis antarcticus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	Myers *et al.* (2019); Parr *et al.* (2019)
Pygoscelis papua	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	Myers *et al.* (2019); Parr *et al.* (2019)
Eudyptes chrysolophus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	Myers *et al.* (2019); Parr *et al.* (2019)
Eudyptula minor	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Pelecanoides urinatrix	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Larus argentatus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Hirundo rustica	Tetrapod	Endotherm	Carnivore	Terrestrial	Agricultural	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Cathartes aura	Tetrapod	Endotherm	Carnivore	Terrestrial	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Coragyps atratus	Tetrapod	Endotherm	Carnivore	Terrestrial	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Oceanodroma leucorhoa	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Anser albifrons	Tetrapod	Endotherm	Herbivore	Freshwater	Lentic	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Anser fabalis	Tetrapod	Endotherm	Herbivore	Freshwater	Lentic	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Opisthocomus hoazin	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019); Godoy-Vitorino *et al.* (2012)
Lagopus muta	Tetrapod	Endotherm	Herbivore	Terrestrial	Tundra	Temperate	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)

Strigops habroptila	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Anser cygnoides	Tetrapod	Endotherm	Herbivore	Freshwater	Lentic	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Anas platyrhynchos	Tetrapod	Endotherm	Omnivore	Freshwater	Estuary	Temperate	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Zonotrichia capensis	Tetrapod	Endotherm	Omnivore	Terrestrial	Agricultural	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Dumetella carolinensis	Tetrapod	Endotherm	Omnivore	Terrestrial	Forest	Subtropical	Myers *et al.* (2019); Parr *et al.* (2019)
Catharus ustulatus	Tetrapod	Endotherm	Omnivore	Terrestrial	Forrest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Anser indicus	Tetrapod	Endotherm	Omnivore	Freshwater	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Gallus gallus	Tetrapod	Endotherm	Omnivore	Terrestrial	Agricultural	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Meleagris gallopavo	Tetrapod	Endotherm	Omnivore	Terrestrial	Forest	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Grus japonensis	Tetrapod	Endotherm	Omnivore	Terrestrial	Marsh	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Grus monacha	Tetrapod	Endotherm	Omnivore	Terrestrial	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Mammals							
Tursiops truncatus	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Zalophus californianus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Stenella coeruleoalba	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Globicephala melas	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Polar	IUCN (2019); Myers *et al.* (2019)
Arctocephalus australis	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Subtropical	IUCN (2019); Myers *et al.* (2019)
Leptonychotes weddellii	Tetrapod	Endotherm	Carnivore	Marine	Oceanic	Polar	IUCN (2019); Myers *et al.* (2019)
Ursus maritimus	Tetrapod	Endotherm	Carnivore	Marine	Tundra	Polar	IUCN (2019); Myers *et al.* (2019)
Balaena mysticetus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Cystophora cristata	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Hydrurga leptonyx	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Arctocephalus tropicalis	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Halichoerus grypus	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Mirounga leonina	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	IUCN (2019); Myers *et al.* (2019)
Neophoca cinerea	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Polar	IUCN (2019); Myers *et al.* (2019)
Felis catus	Tetrapod	Endotherm	Carnivore	Terrestrial	Urban	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Dasypus novemcinctus	Tetrapod	Endotherm	Carnivore	Terrestrial	Scrub Forest	Subtropical	Myers *et al.* (2019); Parr *et al.* (2019)
Canis mesomelas	Tetrapod	Endotherm	Carnivore	Terrestrial	Savanna	Subtropical	Myers *et al.* (2019); Parr *et al.* (2019)
Acinonyx jubatus	Tetrapod	Endotherm	Carnivore	Terrestrial	Savanna	Subtropical	IUCN (2019); Myers *et al.* (2019)
Canis lupus	Tetrapod	Endotherm	Carnivore	Terrestrial	Urban	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)

Desmodus rotundus	Tetrapod	Endotherm	Carnivore	Terrestrial	Scrub Forest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Chlamyphorus truncatus	Tetrapod	Endotherm	Carnivore	Terrestrial	Scrub Forest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Priodontes maximus	Tetrapod	Endotherm	Carnivore	Terrestrial	Savanna	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Zaedyus pichiy	Tetrapod	Endotherm	Carnivore	Terrestrial	Savanna	Tropical	IUCN (2019); Myers *et al.* (2019)
Myrmecophaga tridactyla	Tetrapod	Endotherm	Carnivore	Terrestrial	Savanna	Tropical	IUCN (2019); Myers *et al.* (2019)
Phoca vitulina	Tetrapod	Endotherm	Carnivore	Marine	Littoral	Temperate	IUCN (2019); Myers *et al.* (2019)
Lynx pardinus	Tetrapod	Endotherm	Carnivore	Terrestrial	Scrub Forest	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Sarcophilus harrisii	Tetrapod	Endotherm	Carnivore	Terrestrial	Forest	Temperate	IUCN (2019); Myers *et al.* (2019)
Macrotus waterhousii	Tetrapod	Endotherm	Carnivore	Terrestrial	Forest	Tropical	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Artibeus jamaicensis	Tetrapod	Endotherm	Frugivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Dugong dugon	Tetrapod	Endotherm	Herbivore	Marine	Littoral	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Trichechus manatus	Tetrapod	Endotherm	Herbivore	Marine	Littoral	Tropical	IUCN (2019); Myers *et al.* (2019)
Ochotona princeps	Tetrapod	Endotherm	Herbivore	Terrestrial	Mountain	Temperate	IUCN (2019); Myers *et al.* (2019)
Rhinopithecus bieti	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Camelus dromedarius	Tetrapod	Endotherm	Herbivore	Terrestrial	Agricultural	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Propithecus verreauxi	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Macropus rufus	Tetrapod	Endotherm	Herbivore	Terrestrial	Grassland	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Phascolarctos cinereus	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Subtropical	IUCN (2019); Myers *et al.* (2019)
Aepyceros melampus	Tetrapod	Endotherm	Herbivore	Terrestrial	Savanna	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Equus ferus	Tetrapod	Endotherm	Herbivore	Terrestrial	Agricultural	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Loxodonta africana	Tetrapod	Endotherm	Herbivore	Terrestrial	Savanna	Tropical	Myers *et al.* (2019); Parr *et al.* (2019)
Bos taurus	Tetrapod	Endotherm	Herbivore	Terrestrial	Agricultural	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Castor canadensis	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Macropus giganteus	Tetrapod	Endotherm	Herbivore	Terrestrial	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Macropus robustus	Tetrapod	Endotherm	Herbivore	Terrestrial	Grassland	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Neotoma albigula	Tetrapod	Endotherm	Herbivore	Terrestrial	Desert	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Ovis aries	Tetrapod	Endotherm	Herbivore	Terrestrial	Agricultural	Temperate	IUCN (2019); Myers *et al.* (2019)
Mus musculus	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Temperate	Myers *et al.* (2019); Parr *et al.* (2019)
Giraffa camelopardalis	Tetrapod	Endotherm	Herbivore	Terrestrial	Scrub Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Alouatta caraya	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Bradypus variegatus	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Choloepus hoffmanni	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)

Hydrochoerus hydrochaeris	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Gorilla beringei	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Alces alces	Tetrapod	Endotherm	Herbivore	Terrestrial	Tundra	Temperate	IUCN (2019); Myers *et al.* (2019)
Coelodonta antiquitatis	Tetrapod	Endotherm	Herbivore	Terrestrial	Grassland	Subpolar	Myers *et al.* (2019); Parr *et al.* (2019)
Mammuthus primigenius	Tetrapod	Endotherm	Herbivore	Terrestrial	Tundra	Polar	Myers *et al.* (2019); Parr *et al.* (2019)
Rangifer tarandus	Tetrapod	Endotherm	Herbivore	Terrestrial	Tundra	Polar	IUCN (2019); Myers *et al.* (2019)
Eulemur rubriventer	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Ailurus fulgens	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Temperate	IUCN (2019); Myers *et al.* (2019)
Petaurista alborufus	Tetrapod	Endotherm	Herbivore	Terrestrial	Mountain	Subtropical	Myers *et al.* (2019); Parr *et al.* (2019); IUCN (2019)
Macropus eugenii	Tetrapod	Endotherm	Herbivore	Terrestrial	Savanna	Subtropical	IUCN (2019); Myers *et al.* (2019)
Ailuropoda melanoleuca	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Temperate	IUCN (2019); Myers *et al.* (2019)
Loxodonta cyclotis	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Gorilla gorilla	Tetrapod	Endotherm	Herbivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Cercopithecus ascanius	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Colobus guereza	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Piliocolobus tephrosceles	Tetrapod	Endotherm	Herbivore	Terrestrial	Rainforest	Tropical	IUCN (2019)
Carollia perspicillata	Tetrapod	Endotherm	Nectivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Glossophaga soricina	Tetrapod	Endotherm	Nectivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Leptonycteris yerbabuenae	Tetrapod	Endotherm	Nectivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Lemur catta	Tetrapod	Endotherm	Omnivore	Terrestrial	Forest	Tropical	IUCN (2019); Myers *et al.* (2019)
Papio hamadryas	Tetrapod	Endotherm	Omnivore	Terrestrial	Savanna	Tropical	IUCN (2019); Myers *et al.* (2019)
Papio cynocephalus	Tetrapod	Endotherm	Omnivore	Terrestrial	Urban	Tropical	IUCN (2019); Myers *et al.* (2019)
Sus scrofa	Tetrapod	Endotherm	Omnivore	Terrestrial	Agricultural	Temperate	IUCN (2019); Myers *et al.* (2019)
Ursus arctos	Tetrapod	Endotherm	Omnivore	Terrestrial	Forest	Temperate	IUCN (2019); Myers *et al.* (2019)
Homo sapiens	Tetrapod	Endotherm	Omnivore	Terrestrial	Urban	Temperate	IUCN (2019); Myers *et al.* (2019)
Macaca thibetana	Tetrapod	Endotherm	Omnivore	Terrestrial	Grassland	Temperate	Parr *et al.* (2019)
Pan troglodytes	Tetrapod	Endotherm	Omnivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Nycticebus pygmaeus	Tetrapod	Endotherm	Omnivore	Terrestrial	Rainforest	Tropical	IUCN (2019); Myers *et al.* (2019)
Ictidomys tridecemlineatus	Tetrapod	Endotherm	Omnivore	Terrestrial	Grassland	Temperate	IUCN (2019); Parr *et al.* (2019)

2.2.5 Statistical analysis

Statistical analysis was conducted in R (2.15.1, R Core Development Team 2010), using the packages ‘ape’, ‘geiger’, ‘phytools’ and ‘brms’. In order to explore the relationships between phylogeny, microbiota composition, habitat preference and physiology we constructed a linear model under a Brownian motion model of evolution (Revell 2012). Using this method, I assumed that changes to microbiota composition were independent from time, and that variation in microbiota compositions increased proportionally with time. I used the aforementioned groups as factors in the model. To account for the effects that variation in methodology between laboratories may have in obtaining sequence reads, I sourced repeat data for a number of species (Table 2.2), and constructed the model so that it would randomly select from all of the included information. This process was repeated over 1000 permutations and did not alter my results. In addition, I used the aforementioned R packages to examine differences in the rate of evolution of microbial composition between my assigned groups. This was achieved using a Bayesian Markov chain Monte Carlo (MCMC) approach to identify the phylogenetic location of a shift in the evolutionary rate through time (Revell 2012).

2.3 Results

For all species examined, microbiotas were dominated by three major bacterial phyla, namely Firmicutes, Bacteroidetes, and Proteobacteria, with differences between groups in their proportions (Figure 2.1, Table 2.1). Most other phyla were less frequently identified, and typically had a relative abundance of <5% (Figure 2.1, Table 2.1). In fish, the predominate bacterial phyla observed was Proteobacteria, with an average relative abundance of 49.93%. This was followed by Firmicutes (21.74%), Other (7.53%), Bacteroidetes (4.95%), Fusobacteria (4.34%), Actinobacteria (3.9%), Tenericutes (2.39%), and Spirochaetes (1.53%), with all remaining phyla having a relative abundance of <1.5% (Figure 2.1, Table 2.1). In amphibians the predominate phyla were Firmicutes (44.87%), Bacteroidetes (24.98%), Proteobacteria (17.27%), and Tenericutes (9.27%), with the remaining phyla having a relative abundance of <1% (Figure 2.1, Table 2.1). Similarly, reptile microbiotas were dominated by Firmicutes (57.03%), Proteobacteria (14.31%), and Bacteroidetes (13.38%), followed by Other (8.38%) and Fusobacteria (4.59%), with the remaining phyla having a relative abundance of <0.7% (Figure 2.1, Table 2.1). A slightly different pattern was seen in birds, with a predominance of Firmicutes (44.75%) and Proteobacteria (23.57%), and then a more even spread of the minor phyla including Fusobacteria (8.2%), Bacteroidetes (7.46%), Actinobacteria (6.86%), and Other (3.05%), with all remaining phyla having a relative abundance of <1.5% (Figure 2.1, Table 2.1). Lastly, in mammals, the microbiota in general comprised of Firmicutes (43.19%), Bacteroidetes (20.32%), Proteobacteria (15.05%), Other (9.13%), Spirochaetes (3.47%), Actinobacteria (3.07%) and Fusobacteria (2.3%), with all remaining phyla having a relative abundance of <1.0% (Figure 2.1, Table 2.1).

Using a multivariate model that simultaneously took into consideration phylogenetic history, microbiota composition, and a variety of physiological and environmental factors including thermoregulation, digestive physiology, habitat preference, biome, and climactic preference, I found that phylogeny ($f=0.1285$, $r^2=0.0013$, $p=0.001$) was the most important factor determining the microbiota of vertebrates, and not diet as previously proposed ($f=3.201$, $r^2=0.0607$, $p=0.494$). Additionally, I discovered that climactic preference ($f=6.0235$, $r^2=0.1326$, $p=0.048$) may be exerting some effect on microbial communities of vertebrates, but it did not appear that thermoregulatory strategy (ectothermy vs endothermy) played any role in the development of microbiota of vertebrates ($f=0.0273$, $r^2=0.0001$, $p=0.124$), nor did habitat ($f=5.301$, $r^2=0.141$, $p=0.358$) and biome ($f=6.013$, $r^2=0.113$, $p=0.48$). I determined that there were significant differences in the rate with which microbiotas changed over time between various subgroups within my dataset. For example, tetrapod microbiotas have evolved at 20.67

times the rate of non-ray finned fish, and 1.53 times the rate of ray finned fish (p=0.001) (Table 2.4). I also found that ectotherm microbiotas have evolved 2.09 times faster than endotherm microbiotas (p=0.001) (Table 2.4). On comparison of digestive strategies, microbiotas of omnivores had the fastest rate of evolution, evolving 1.45 times faster than those of non-omnivores (p=0.001) (Table 2.4). Animals originating from polar regions had microbiotas that have evolved 2.5 times faster than animals at all other latitudes (p=0.001) (Table 2.4). Within biomes, microbiotas of animals with a habitat preference for tundra, evolved 5.06 times faster than microbiotas of animals from any other habitat (p=0.001) (Table 2.4).

Table 2.4 Comparison of evolutionary rates of microbiota composition between selected groups in this study.

Factor	Ratio	p	Group	Rate
Phylogeny	20.67	0.001	Non-ray-finned fish	0.47
			Ray-finned fish	6.48
Tetrapod	1.53	0.001	False	6.32
			True	9.65
Digestive Physiology	4.37	0.001	Carnivore	6.84
			Frugivore	2.58
			Herbivore	9.40
			Nectivore	3.62
			Omnivore	11.26
Omnivore	1.45	0.001	False	7.79
			True	11.26
Climactic Preference	26.11	0.001	Boreal	0.70
			Polar	18.33
			Subpolar	1.42
			Subtropical	4.84
			Temperate	10.11
			Tropical	6.21
Polar	2.50	0.001	False	7.32
			True	18.33
Thermoregulation	2.09	0.001	Ectotherm	5.31
			Endotherm	11.12
Biome	128.76	0.001	Agricultural	2.18
			Desert	5.20
			Estuary	4.67
			Forest	6.96
			Grassland	10.86
			Lentic	13.55
			Littoral	6.75
			Lotic	2.49
			Marsh	1.73
			Mountain	1.50
			Oceanic	9.02
			Rainforest	2.61
			Reef	8.02
			Savanna	7.42
			Scrub Forest	2.77
			Tundra	35.44
			Urban	7.43
			Wetland	0.28
Tundra	5.06	0.001	False	7.00
			True	35.44

2.4 Discussion

My results indicate that vertebrates have co-evolved with their microbial inhabitants, and that microbiota community composition within a species is largely evolutionarily determined, regardless of diet or other natural history factors. The results of this investigation contrast with previous studies that infer convergence of microbiotas based on diet (Delsuc *et al.* 2014). Further, we propose that the influence of diet on the microbiota is likely to be at the individual organism level, rather than at a broader taxonomic level. The effects of diet may be more profound if investigations focused at lower taxonomic levels with respect to microbiotas.

The findings of this analysis provide some novel insights into understanding the microbiota of vertebrates. Under most circumstances, animals with similar dietary preferences have evolved gastrointestinal tracts with analogous morphological characteristics (Furness *et al.* 2015). However, this does not mean that their microbial communities are analogous. For example, both the Tasmanian devil (*Sarcophilus harrisii*) and the cheetah (*Acinonyx jubatus*), are strict carnivores (IUCN 2019), with a similar simple gastrointestinal tract morphology. However, investigations into their microbial communities show significant differences such as the higher proportion of Proteobacteria and undescribed phyla in the Tasmanian devil compared to the cheetah, and the higher proportion of the phyla Bacteroides, Actinobacteria, and Fusobacteria in the cheetah (Figure 2.1). These dissimilarities can be explained by the approximately 104 million years of evolution that separate these species (Kirsch *et al.* 1997), and not because of any minor differences in their diets. Conversely, the Wuchang bream (*Megalobrama amblycephala*), a herbivorous freshwater species (Gao *et al.* 2012), and the topmouth culter (*Culter alburnus*), a carnivorous freshwater species (Froese and Pauly 2019), are closely related fish with different dietary preferences but remarkably similar gastrointestinal microbiota compositions (Figure 2.1, Table 2.1).

Although there has been some suggestion that phylogeny may influence gut microbiota (Muegge *et al.* 2011; Nishida and Ochman 2017), my novel approach of integrating phylogenetic comparative methods in my analysis categorically links phylogeny and microbiota across multiple vertebrate classes for the first time. I found no indication that rate of gut microbiota divergence differed among the various vertebrate classes, suggesting that the microbiota of vertebrates is an inherited trait that co-evolves with the host. In a similar manner that specific selection pressures result in morphological or behavioural adaptations, such selection pressures may also result in selection for specific microbial community compositions. Thus, the myriad of form and function evident in modern vertebrates is likely a result of bidirectional evolution between host and microbe.

How microbes and hosts have become co-adapted, and how their mutualistic existence drives their co-dependent evolutionary paths remains unknown. However, it is likely to be multifactorial in nature with a complex interplay of host anatomy, immune function, and diet, in combination with microbial factors such as production of beneficial metabolic by-products, and immune evasion (Amato 2013). Understanding these intricate relationships, and how they have shaped the vertebrate-microbe symbiosis is an important biological frontier to conquer. An interdisciplinary approach to future microbiota investigations that incorporates wild animal ecological data such as diet, social structure, behaviour, and health, is fundamental to comprehending the true extent of the co-evolution of host and microbe, and determining factors that address both the fitness of the gut microbiota and the fitness of the host (Amato 2013).

In addition to the effect of host phylogeny on the gastrointestinal microbiota of vertebrates, a second novel finding in this study was the comparatively fast rate of microbiota evolution in animals from polar and tundra regions. There is conflicting evidence on the effects that latitude has on evolutionary rates of vertebrates, with some reports suggesting that genetic diversity is greatest at lower latitudes (Gillman *et al.* 2009; Adams and Hadly 2012; Pyron and Wiens

2013), while others finding limited effect of latitude in some vertebrate classes (Rabosky *et al.* 2015; Rolland *et al.* 2016). Although these investigations have focused on host genetics with no consideration given to their symbionts, similar patterns are seen in environmental microbial communities (Fuhrman 2009). An important distinction between gastrointestinal microbiotas, and environmental microbiotas is that the host acts as a vessel in which evolution can occur independent of external factors. Generally speaking, vertebrates inhabiting polar or tundra regions tend to be endothermic because there are fewer opportunities to reach a preferred body temperature using behavioural means, they possess an insulative layer of fat, feathers, or fur, and they often have a lower surface area to volume ratio (Blix 2016). Thus, they provide an ideal environment for microbial evolution despite the inhospitable conditions in which their hosts live. In this investigation I was not able to determine the factors driving this rapid microbial change and this is an area of microbiota research that warrants further study.

My discovery that microbiota evolutionary rate is fastest in tetrapods, and also endotherms, fits with other, larger investigations into vertebrate evolution in which similar patterns have been reported (Kumar and Hedges 1998; Gillooly *et al.* 2005). These findings further highlight the interconnected nature of microbes and their hosts, and the need to consider them as a single ‘superorganism’ in which their evolutionary history is intrinsically linked. Why the microbiota of omnivores has evolved at a faster rate than those of other digestive physiologies was not able to be determined in this investigation. However, omnivory confers a significant teleological advantage over more specialised feeding strategies in that these species may be better adapted to conditions with varying resource availability (Denny *et al.* 2018). Therefore, under certain conditions, evolution of specialists may be supressed while there is a concurrent rapid radiation of generalists to take advantage of unoccupied niches.

The results of this investigation indicate that specific bacterial phyla are particularly adapted to life within the vertebrate gastrointestinal tract. Of the more than 50 phyla described to date

(Yarza *et al.* 2014), community composition is dominated by relatively few groups, that are common to all vertebrate taxa (Figure 2.1). Interestingly, vertebrate microbiotas differ significantly from invertebrate microbiotas (Newton *et al.* 2013), and future research should focus on the evolutionary and physiological mechanisms by which such marked differences occur across metazoan guts.

Naturally, my analysis was constrained to the available microbiota data. In general, microbiota studies focus on charismatic species that are easy to capture, resulting in unequal representation of taxa in the phylogenetic history. To build upon my discovery of the connection between vertebrate phylogeny and microbiota, I encourage study on a broader range of vertebrate taxa to fill some of these gaps. In particular, future investigations should focus on less charismatic species, that are nonetheless important from an evolutionary perspective, such as Chondrichthyes (rays and sharks), Amphibia (amphibians), Reptilia (reptiles), Palaeognathae (ratites), Psittaciformes (parrots), Strisores (nightjars), Columbaves (pigeons and relatives), Mirandornithes (flamingos and grebes), Accipitriformes (hawks and relatives), Afroaves (owls, kingfishers, woodpeckers, etc.), Monotremata (echidna and platypus), Eulipotyphla (moles, shrews, hedgehogs, etc.), Lagomorphs (rabbits), and Pholidota (pangolins). In addition, the role that biome plays in shaping microbiota communities also warrants further study, and where practically possible, animals should be sampled from a diverse range of biomes and climatic preferences.

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Chapter 3. The gut microbiota of sea turtles and its relationship to their phylogenetic history

3.1 Introduction

Residing on and within most metazoan species is a diverse and complex metropolis of microorganisms (viruses, bacteria, fungi and protozoans) known collectively as the microbiota. The occupation of higher organisms by prokaryotic and eukaryotic colonists has been a key factor in driving evolution and radiation of life on Earth (Lee and Mazmanian 2010; McFall-Ngai *et al.* 2013). Hosts and their microbiotas form a mutualistic symbiosis so intertwined that the amalgamation of these distinct organisms is often referred to as a holobiome, and their combined genetic material collectively defined as the hologenome (Shapira 2016; Theis *et al.* 2016). This multigenomic microcosm has become so integral to animal homeostasis that it can no longer be considered separate from the individual. Thus, it has been proposed that animals represent a vastly intricate biological 'super organism' in which a proportion of the physiological function is derived from microbial activity (Dethlefsen *et al.* 2007). The microbiota has been shown to be a heritable trait in a range of vertebrates (Dominguez-Bello *et al.* 2010; Ding *et al.* 2017; Kohl *et al.* 2017; Sylvain and Derome 2017; Trevelline *et al.* 2018), and there is evidence to suggest that its composition and function is largely influenced by host phylogeny (Carrillo-Araujo *et al.* 2015; Amato *et al.* 2018), with co-evolution of the host and microbiota a critical process in shaping metazoan life (Shapira 2016; Theis *et al.* 2016). Ultimately, it appears that a bidirectional interaction of host physiology and gut microbiota over evolutionary time is responsible for determining host dietary niche and adaptation (Amato *et al.* 2018). Understanding these relationships using a multi-disciplinary approach by combining microbiological techniques with phylogenetic analyses, are fundamental to exploring the origins of complex, multicellular organisms. A good way to do this is by investigating closely related species, with clearly defined phylogenies, where analysis is not confounded by vast expanses of evolutionary periods with multiple missing links. Therefore, sea turtles represent an ideal species to study in this context as all extant species, including their phylogeny, are well described (Pritchard 1997), and they originate from an ancient lineage that has gone through intermittent periods of slow and intermediate evolution and diversification (Duchene *et al.* 2012), meaning that any described relationships are robust and profound. Exploring the bidirectional interplay of evolutionary forces acting on the host-microbiota relationship is an important step in comprehending the origins of metazoan physiology.

The major site for microbial inhabitation in animals is the gastrointestinal tract, where immense numbers of microorganisms confer myriad beneficial properties to their host. These complex interactions are an exciting and emerging area of evolutionary biology. Historically, the importance of microflora to non-human species has predominately focused on their role during digestion, particularly of complex carbohydrates in herbivorous animals. However, these investigations have begun to broaden with the realisation of the greater role that they play in the health and ecology of all species (McFall-Ngai *et al.* 2013).

Investigations into the microbiota of reptiles, including sea turtles, are limited (Hong *et al.* 2011; Keenan *et al.* 2013; Gaillard 2014; Colston *et al.* 2015; Hong *et al.* 2015b; Yuan *et al.* 2015; Abdelrhman *et al.* 2016; Price *et al.* 2017) and beyond these preliminary investigations there is a paucity of information regarding the influence that microbiota has on fitness of reptiles and how it affects their ecology. Irrespective of the potential host-phylogenetic signal in microbiota composition at a broad taxonomic level, it has been shown that at a more individual level, diet, captivity, geography, and feeding regime all influence the microbiota (Costello *et al.* 2010; Keenan *et al.* 2013; Kohl *et al.* 2017), and that fermenting bacteria are important for digestion in herbivorous species (Mackie *et al.* 2004).

Sea turtles are among the most imperilled species on Earth and therefore a deeper understanding of their physiology is important to their conservation. The role that microbiota plays in the health, behaviour and physiology of humans and animals is undeniable, and its categorisation in marine turtles is the next important step in understanding how these secretive species adapt to a changing environment. There are seven extant species of sea turtle, the leatherback turtle (*Dermochelys coriacea*) (Figure 3.1), green turtle (*Chelonia mydas*) (Figure 3.2), flatback turtle (*Natator depressus*) (Figure 3.3), hawksbill turtle (*Eretmochelys imbricata*) (Figure 3.4), loggerhead turtle (*Caretta caretta*) (Figure 3.5), olive ridley turtle (*Lepidochelys olivacea*) (Figure 3.6), and the Kemp's ridley turtle (*Lepidochelys kempii*) (Figure 3.7). With the exception of the flatback turtle, all are listed as 'Threatened' by the IUCN with various levels of risk, while the flatback turtle is listed as 'Data Deficient', but is also likely to be threatened (IUCN 2019). Marine turtles are highly evolved for a completely aquatic life, but, like almost all reptiles they are still tied to the terrestrial environment for oviposition (Pritchard 1997). The majority of sea turtle species are scattered unevenly throughout all three tropical oceans, with the exception of the flatback and Kemp's ridley turtles, which have relatively restricted distributions (Pritchard 1997). Additionally, the leatherback turtle, is a more cold adapted species, and may be found occupying waters at higher latitudes than the other species (Pritchard 1997). Sea turtle diets vary remarkably over life stage, and among species (Jones and Seminoff 2013). For example, the leatherback feeds primarily on gelatinous zooplankton for its entire existence, while the green turtle is predominately omnivorous during the oceanic phase of its life, but then undergoes an ontogenic dietary shift to herbivory as it transitions to neritic habitats later in life (Jones and Seminoff 2013), resulting in a discernible shift in the bacterial communities during this phase of their life (Price *et al.* 2017). Sea turtles are unique among the Reptilia, in that they are the only members that undergo long-distance migrations, rivalling those of other vertebrate species (Plotkin 2003), and during this time they typically do not forage (Hays *et al.* 2002), which may have an effect on their microbiota (Keenan *et al.*

2013; Dewar *et al.* 2014; Xia *et al.* 2014; Remely *et al.* 2015; Sommer *et al.* 2016; Beli *et al.* 2018).

How the microbiota has contributed to the evolutionary history of sea turtles, and the extraordinary physiological adaptations of these species has been little studied, and as a result, I attempted to address this deficit by examining the microbial populations of the world's sea turtle species. In this investigation I present the most comprehensive data on the microbiota of sea turtles that has been compiled to date. Furthermore, this is one of the most complete microbiota studies to have been conducted in any taxa, as I was able to obtain samples from an entire clade of the evolutionary tree and I was able to explore some of the phylogenetic relationships that exist between sea turtles and their microbiotas. The aims of this investigation were to categorise the microbiota composition of the world's sea turtle species, to explore any relationships between sea turtle phylogeny and microbiota composition, and to examine how sea turtle microbiotas have changed over time.

Figure 3.1 Leatherback turtle (*Dermochelys coriacea*)

Figure 3.2 Green turtle (*Chelonia mydas*)

Figure 3.3 Flatback turtle (*Natator depressus*)

Figure 3.4 Hawksbill turtle (*Eretmochelys imbricata*)

Figure 3.5 Loggerhead turtle (*Caretta caretta*)

Figure 3.6 Olive ridley turtle (*Lepidochelys olivacea*)

Figure 3.7 Kemp’s ridley turtle (*Lepidochelys kempii*)

3.2 Methods

3.2.1 Ethics statement

This study was approved by the Biological Sciences Animal Ethics Committee of Monash University (approval 14694). Green sea turtles and loggerhead turtles were sampled under the permit WITK17730216 from the Queensland Department of Environment and Heritage Protection, Australia. Hawksbill turtles and flatback turtles were sampled under the permit 01-000121-1 from the Department of Parks and Wildlife Western Australia. Olive ridley turtles were sampled under the permit 60298 from the Parks and Wildlife Commission Northern Territory, Australia. Leatherback turtles were sampled under the permit MTP-18-205A from the Florida Fish and Wildlife Conservation Commission, USA and samples were imported into Australia under the CITES permit PWS2018-AU-000738 and quarantine permit 0003027044. Kemp's ridley turtles were sampled under the permit 00663/19 from the Secretaría de Medio Ambiente y Recursos Naturales, Mexico, exported under the CITES permit MX100214, and imported into Australia under the CITES permit PWS2019-AU-000981 and quarantine permit 0003027044.

3.2.2 Study populations

All samples were collected from adult female turtles as they nested. Flatback turtles (n=17) were sampled from Port Hedland, Western Australia (20.3107° S, 118.5878° E) in November 2016. Green sea turtles (n=18), were sampled from Heron Island in the Great Barrier Reef (23.4423° S, 151.9148° E) in January 2017. Loggerhead turtles (n=20) were sampled from Mon Repos, Queensland (24.8059° S, 152.4416° E) in January 2017. Hawksbill turtles (n=20) were sampled from Rosemary Island, Western Australia (20.2846° S, 116.3540° E) in October 2017. Olive ridley turtles (n=10) were sampled from Tiwi Islands, Northern Territory (11.6969° S, 130.8779° E) in April 2018. Leatherback turtles (n=18) were sample from Juno

Beach, Florida, USA (26.5224° N, 80.315° E) in May 2018. Kemp's ridley turtles (n=20) were sampled from Playa Rancho Nuevo, Tamaulipas, Mexico (23.11° N, 97.46° W) in June 2019.

3.2.3 Sample collection

For leatherback turtles I waited until the female was covering the nest and then we dug a channel behind the turtle so that a cloacal swab could be inserted while the animal was still in ventral recumbency. For all other species, we waited until they had finished laying and were returning to the ocean and the turtle was then flipped into dorsal recumbency. In all turtles an equine uterine swab (Minitube, Smythesdale, Victoria, Australia) was inserted into the cloaca so that it entered the distal colon. These swabs were housed in a sterile sheath, the entire apparatus was inserted into the cloaca and the swab tip was extruded when correct placement of the sheath had been achieved. The swab tip was then retracted back into the sheath prior to extraction. Turtles were then permitted to return to the ocean and then the tip of the swab was cut using a sterile wire cutter, placed into a sterile Eppendorf tube and sealed. Total sample collection time was approximately 10 minutes. Swabs were stored at -80°C until analysis could take place after approximately 2 weeks.

3.2.4 DNA extraction

DNA was extracted manually. In each Eppendorf tube, 500µL of extraction buffer (20mM EDTA, 0.1M Tris, 1% CTAB, 56mM NaCl, pH 8) was added so that swabs were completely covered. We then added 20µL of Proteinase K (QIAGEN Proteinase K (10ml) to each vial, along with 60µL of 10% SDS. The mixture was then incubated at 55°C overnight. The next day, 50µL of 5M NaCl and 500µL of Phenol was added, and the tubes shaken until an emulsion was formed. They were then incubated at room temperature for 10 minutes, with intermittent mixing. The tubes were then centrifuged at 10,000 RPM for 10 minutes and the supernatant removed and added to a new tube containing 250µL Phenol and 250µL Chlorophorm:Isoamyl-Alcohol (24:1). The tubes were again centrifuged at 10,000 RPM for 10 minutes and the resultant supernatant added to a new tube containing 500µL of Chlorophorm:Isoamyl-Alcohol. Once again, the tubes were centrifuged at 10,000 RPM for 10 minutes. The supernatant was then added to a new tube containing 3M Sodium Acetate at a volume equal to 10% of the extraction solution. We then added 1ml of ice-cold 99% ethanol to each test tube and then placed them into a freezer at -20°C for 1 hour. The tubes were then centrifuged at 4°C at 12,000 RPM for 10 minutes. The fluid in the test tube was then removed with a glass pipette and 1ml of ice-cold 70% alcohol was added. The tubes were centrifuged a final time at 4°C at 12,000 RPM for 5 minutes. After centrifugation the alcohol was removed and the lids left off the tubes to allow the DNA pellet to dry. Once dried, 25µL of 1 x TE was added to each tube and the extracted DNA was stored at -20°C until amplicon sequencing could take place.

3.2.5 16s gene amplicon sequencing

The V3-V4 region of 16S rRNA genes were amplified with forward primer 5' ACTCCTACGGGAGGCAGCAG 3' and reverse primer 5' GGACTACHVGGGTWTCTAAT 3' using Q5 high fidelity polymerase (New England Biolabs). Sequencing was performed on an Illumina MiSeq system (2 x 300 bp) by the method of Fadrosh *et al.* (2014).

3.2.6 Data processing

Sequence data was analysed using QIIME version 1.9.1 (Caporaso *et al.* 2010) using default parameters and a Phred quality threshold of > 20. The UCLUST algorithm (Edgar 2010) was used to pick OTUs at 97% sequence identity and a Biome table was produced. Potentially chimeric sequences were identified using Pintail (Ashelford *et al.* 2005). Blast was used to assign taxonomy against the Greengenes database (DeSantis *et al.* 2006) and QIIME version 1.9.1 defaults. Additional assignment of taxonomy was performed using a command line version of BLASTN (Altschul *et al.* 1997) against the NCBI 16S Microbial database.

3.2.7 Statistics and data analysis

Initial exploration of the Biome table data was performed using the online Calypso software (http://cgenome.net/wiki/index.php/Calypso) (Zakrzewski *et al.* 2017). Data was further analysed in R, utilising the package 'phyloseq'. Alpha diversity was explored using Observed OTUs, Shannon index and Chao1 estimates. Alpha diversity was tested for normality using the Shapiro-Wilks test, with all metrics being non-normally distributed (Observed: $W=0.95$, $p<0.001$; Chao1: $W=0.96$, $p=0.001$; Shannon: $W=0.9$, $p<0.001$), and so comparisons between groups were first made using the Kruskal-Wallis test, and then paired comparisons between groups were made using the pairwise Wilcoxon rank sum test with (Holm) p-values that were adjusted for multiple comparisons. Beta diversity was investigated using principle co-ordinate analysis (PCoA) (Bray-Curtis) and Adonis tests to compare all species, and then pairwise comparisons were made between all combinations of species with Holm correction of p-values for multiple comparisons.

To test phylogenetic signal related to microbial composition in sea turtles, I first constructed a phylogenetic tree, with branch lengths, using the online database "Interactive Tree of Life", https://itol.embl.de. I then compiled the microbial data for all species into a Microsoft Excel spread sheet, and imported both the tree and microbiota data into R, and used the package 'phytools' to explore the effects of phylogeny on microbiota composition (Revell 2012). I was able to create a phylogenetic matrix (Table 1), and use Moran's I, and Abouheif's C_{mean} to determine if specific bacterial phyla had a phylogenetic signal and whether or not there was a phylogenetic signal in alpha diversity. Where a significant result was found, this was further confirmed using Blomberg's *K*-statistics, and Pagel's λ for the individual bacterial phylum (Diniz-Filho *et al.* 2012). Finally, I was able to use my data to reconstruct probable microbiota compositions of extinct sea turtle species at each of the nodes in my phylogenetic tree. For all statistical analyses significance was accepted if $p>0.05$.

Table 3.1 Phylogenetic matrix for the world's sea turtle species. It can be seen that the leatherback turtle (*Dermochelys coriacea*) is the only extant species with no living close relatives. For a graphical representation of sea turtle phylogeny refer to Figure 3.8.

Species	Kemp's ridley	Olive ridley	Loggerhead	Hawksbill	Flatback	Green	**Leatherback**
Kemp's ridley	1	0.9	0.68	0.56	0.3	0.3	0
Olive ridley	0.9	1	0.68	0.56	0.3	0.3	0
Loggerhead	0.68	0.68	1	0.56	0.3	0.3	0
Hawksbill	0.56	0.56	0.56	1	0.3	0.3	0
Flatback	0.3	0.3	0.3	0.3	1	0.5	0
Green	0.3	0.3	0.3	0.3	0.5	1	0
Leatherback	0	0	0	0	0	0	1

3.3 Results

Sequencing results were obtained in all but three animals, in which failure of DNA extraction meant that results were not obtained for these individuals (Table 3.2).

Table 3.2 Summary of sequencing results

Species	Number Sampled	Number successfully sequenced	Total Sequences	Average sequences per sample
Leatherback	18	17	693,932	4,0819
Green	18	17	612,452	3,6026
Flatback	17	17	147,317	8,665
Loggerhead	20	20	555,204	32,659
Hawksbill	20	20	27,986	1,646
Olive ridley	10	9	152,007	16,889
Kemp’s ridley	20	20	212,631	12,507

The taxonomic summary of microbial components from all samples yielded a total of 20 bacterial phyla, 36 classes, 63 orders, 122 families, 202 genera, and 362 OTUs (Figure 3.8 and Supplementary Table 1). The predominant bacterial phyla were Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes (Figure 3.8 and Supplementary Table 1), while the phyla Euryarchaeota, Deferribacteres, and Cyannobacteria were only seen in Kemp’s ridley turtles. The prevalence of the phylum SR1 was greatest in flatback and green turtles, intermediate in hawksbill and leatherbacks, and lowest in loggerhead and ridley turtles (Figure 3.8).

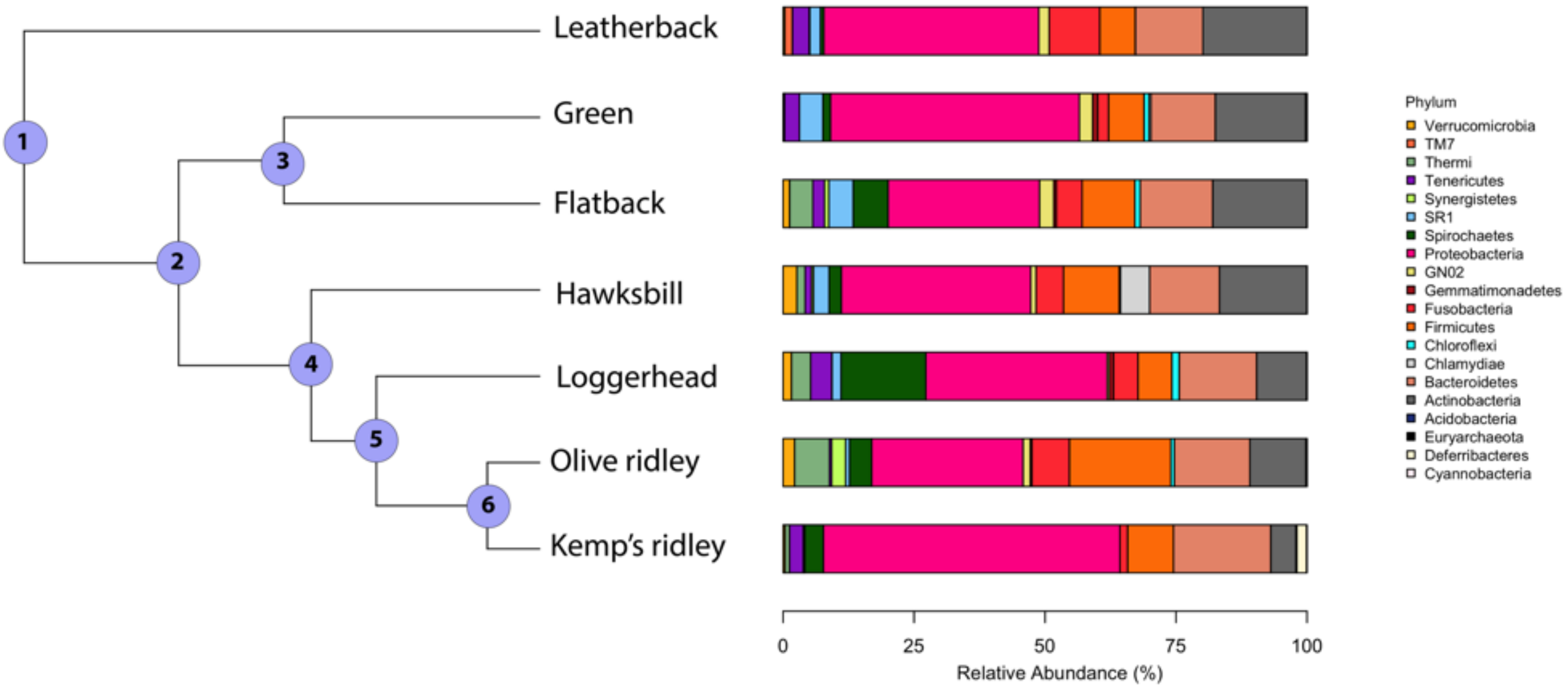

Figure 3.8 Relative abundance of bacterial phyla in each sea turtle species together with their phylogenetic tree. Numbered circles depict nodes of the sea turtle evolutionary tree and are representative of extinct common ancestors.

Analysis of alpha diversity revealed that there were significant differences between Observed OTUs (χ^2=45.83, df=6, p<0.001) (Figure 3.9 and Table 3.3), and in species richness between samples as measured by Chao1 (χ^2=43.28, df=6, p<0.001) (Figure 3.9 and Table 3.4), but not in Shannon diversity (χ^2=7.48, df=6, p=0.28) (Figure 3.9 and Table 3.5). These observations were consistent when all samples were analysed in entirety, and also for pairwise comparisons (Tables 3.3-3.5).

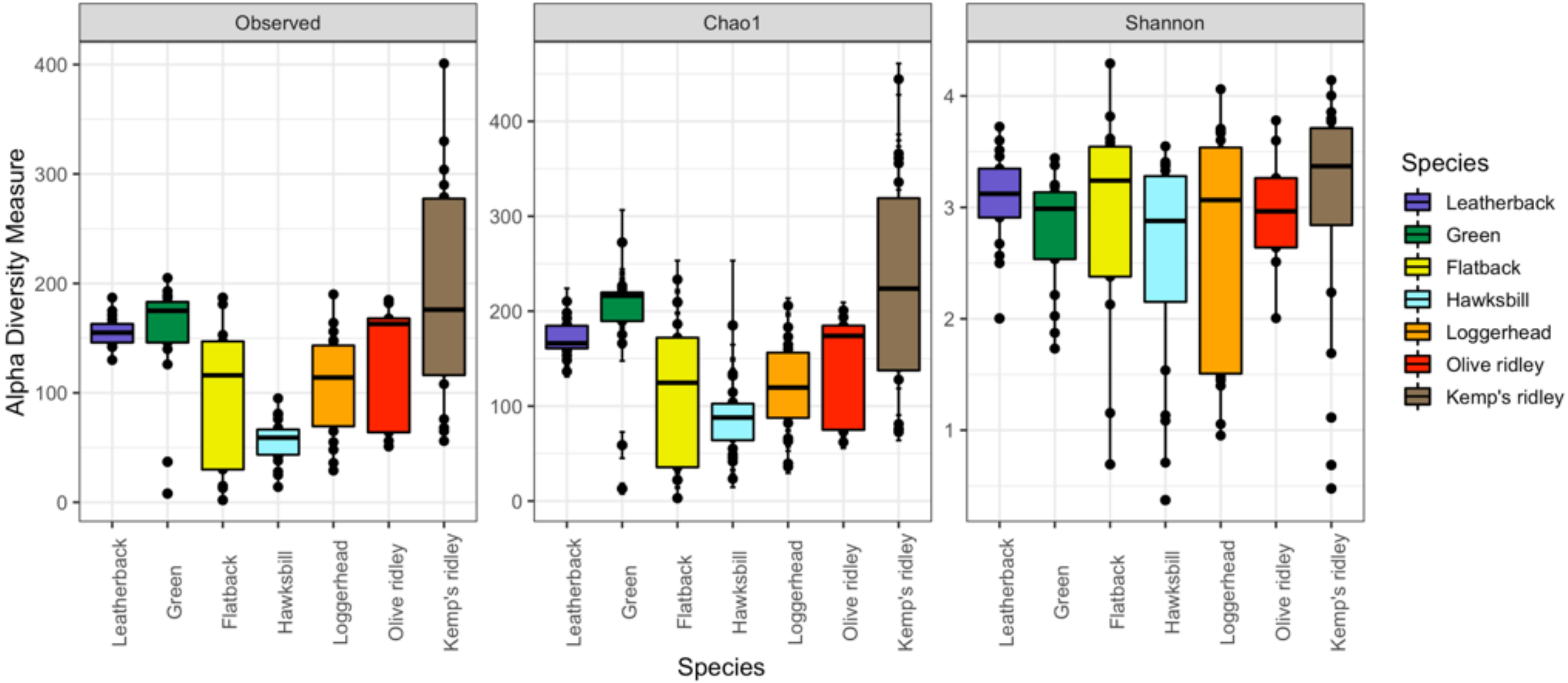

Figure 3.9 Alpha diversity measures across all species. Individual points and brackets represent the richness estimate and the theoretical standard error range associated with that estimate, respectively. Within each panel, the samples are organized into species, and a boxplot is overlaid on top of this. The mean microbial diversity estimate using Shannon's diversity index did not differ significantly among all samples (p=0.28). However, there were significant differences between samples as measured by Observed (p<0.001) and Chao1 (p<0.001).

Table 3.3 Pairwise Wilcoxon rank sum test comparisons between species for Observed OTUs. Numbers represent corrected (Holm) p-values for multiple comparisons. Significant values are indicated by bold text.

Species	Flatback	Green	Hawksbill	Kemp's ridley	Leatherback	Loggerhead
Green	0.08	-	-	-	-	-
Hawksbill	0.82	**<0.01**	-	-	-	-
Kemp's ridley	0.08	1.00	**<0.01**	-	-	-
Leatherback	0.06	0.82	**<0.01**	1.00	-	-
Loggerhead	1.00	**0.02**	**0.01**	0.06	**0.01**	-
Olive ridley	1.00	1.00	0.08	0.96	1.00	1.00

Table 3.4 Pairwise Wilcoxon rank sum test comparisons between species for Chao1. Numbers represent corrected (Holm) p-values for multiple comparisons. Significant values are indicated by bold text.

Species	Flatback	Green	Hawksbill	Kemp's ridley	Leatherback	Loggerhead
Green	**0.03**	-	-	-	-	-
Hawksbill	1.00	**<0.01**	-	-	-	-
Kemp's ridley	**0.03**	1.00	**<0.01**	-	-	-
Leatherback	0.21	**0.03**	**<0.01**	0.52	-	-
Loggerhead	1.00	**<0.01**	0.29	**0.02**	**<0.01**	-
Olive ridley	1.00	0.21	0.31	0.23	1.00	1.00

Table 3.5 Pairwise Wilcoxon rank sum test comparisons between species for Shannon index. Numbers represent corrected (Holm) p-values for multiple comparisons. No significance was observed between Shannon index for any inter-species comparisons.

Species	Flatback	Green	Hawksbill	Kemp's ridley	Leatherback	Loggerhead
Green	1.00	-	-	-	-	-
Hawksbill	1.00	1.00	-	-	-	-
Kemp's ridley	1.00	0.81	0.81	-	-	-
Leatherback	1.00	1.00	1.00	1.00	-	-
Loggerhead	1.00	1.00	1.00	1.00	1.00	-
Olive ridley	1.00	1.00	1.00	1.00	1.00	1.00

For beta diversity, we noticed clustering patterns for the more ancient species, leatherback and green, as well as the most modern species, Kemp's ridley (Figure 3.10). For the remaining species there was overlap of microbiota compositions (Figure 3.10). However, Adonis analysis of the PCoA plot revealed that there was significant differences between all species when analysed together (df=6, SS_T=15.42, MS = 2.57, f.model=7.72, R^2=0.29, p=0.001), and similarly, when pairwise comparisons were made between all combinations of species, significant differences in microbiota compositions existed for all combinations (Table 3.6).

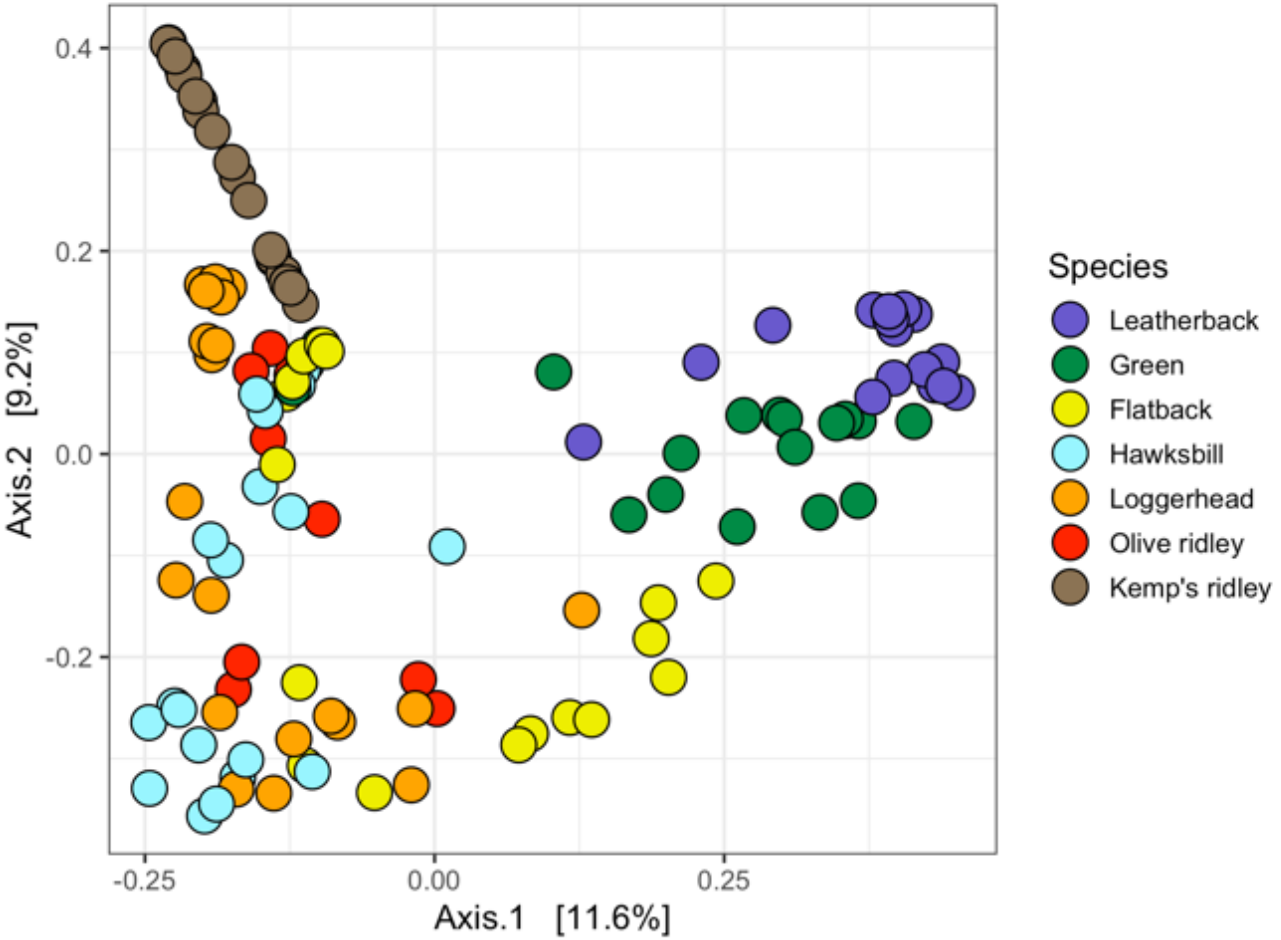

Figure 3.10 Principle co-ordinate analysis (PCoA) of Bray-Curtis distance showing functional microbial diversity across all species. Significant differences for microbiota composition existed between all species (R^2=0.29, p=0.001). For pairwise comparisons between species see Table 3.6.

Table 3.6 Pairwise Adonis comparisons of beta diversity for all combinations of microbiota composition in sea turtles. Both the p, and adjusted (Holm) p-values for multiple comparisons are reported. All pairwise comparisons were significantly different. For microbial composition of species and how they relate to other species refer to Figure 3.10.

Pairs	df	SS_T	F.model	R^2	p	p.adjusted
Leatherback vs Olive ridley	1.00	2.59	10.36	0.30	0.001	0.021
Leatherback vs Green	1.00	2.13	8.41	0.21	0.001	0.021
Leatherback vs Hawksbill	1.00	3.89	13.37	0.28	0.001	0.021
Leatherback vs Loggerhead	1.00	3.80	13.21	0.27	0.001	0.021
Leatherback vs Flatback	1.00	2.91	10.29	0.24	0.001	0.021
Leatherback vs Kemp's ridley	1.00	4.25	14.62	0.29	0.001	0.021
Olive ridley vs Green	1.00	1.98	6.15	0.20	0.001	0.021
Olive ridley vs Hawksbill	1.00	1.56	4.31	0.14	0.001	0.021
Olive ridley vs Loggerhead	1.00	1.02	2.83	0.09	0.006	0.021
Olive ridley vs Flatback	1.00	1.39	3.85	0.14	0.001	0.021
Olive ridley vs Kemp's ridley	1.00	2.10	5.78	0.18	0.001	0.021
Green vs Hawksbill	1.00	2.92	8.59	0.20	0.001	0.021
Green vs Loggerhead	1.00	2.79	8.28	0.19	0.001	0.021
Green vs Flatback	1.00	1.69	5.02	0.14	0.001	0.021
Green vs Kemp's ridley	1.00	3.32	9.77	0.22	0.001	0.021
Hawksbill vs Loggerhead	1.00	1.89	5.20	0.12	0.001	0.021
Hawksbill vs Flatback	1.00	1.76	4.80	0.12	0.001	0.021
Hawksbill vs Kemp's ridley	1.00	3.02	8.22	0.18	0.001	0.021
Loggerhead vs Flatback	1.00	1.79	4.92	0.12	0.001	0.021
Loggerhead vs Kemp's ridley	1.00	3.07	8.40	0.18	0.001	0.021
Flatback vs Kemp's ridley	1.00	2.82	7.68	0.18	0.001	0.021

Analysis of sea turtle phylogeny showed that modern sea turtles form a monophyletic group where leatherback turtles are the only extant species with no closely-related living relatives (Figure 3.8 and Table 3.1). I did not discover a phylogenetic signal for any alpha diversity measure for any of the species in my investigation (Table 3.7 and Figures 3.11 and 3.12). However, when microbiota composition was analysed in concert with evolutionary history, I found that the bacterial phyla SR1, GN02 and Actinobacteria had a phylogenetic signal for both Moran's *I* and Abouheif's C_{mean} calculations (Table 3.8 and Figures 3.13 and 3.14).

Table 3.7 Moran's I and Abouheif's C_{mean} calculations for alpha diversity and their correlation to sea turtle phylogeny. The observed value of I (Obs), is the expected value under the null hypothesis of no correlation. Positive values indicate that the data is spatially clustered in some way. Other values represented in this table include the standard-deviation of the observed I (Std.Obs), and the alternative hypothesis (alter) which has been set to "greater" meaning that the p-value is estimated as a number of random values equal to, or greater than the observed, +1. No significance was detected for any diversity measure.

Diversity Measure	**Moran's *I***				**Abouheif's C_{mean}**			
	Obs	Std.Obs	Alter	p	Obs	Std.Obs	Alter	p
Observed	-0.22	-0.30	greater	0.56	-0.22	-0.25	greater	0.53
Chao1	-0.29	-0.61	greater	0.68	-0.29	-0.57	greater	0.661
Shannon	-0.14	0.12	greater	0.40	-0.14	0.08	greater	0.42

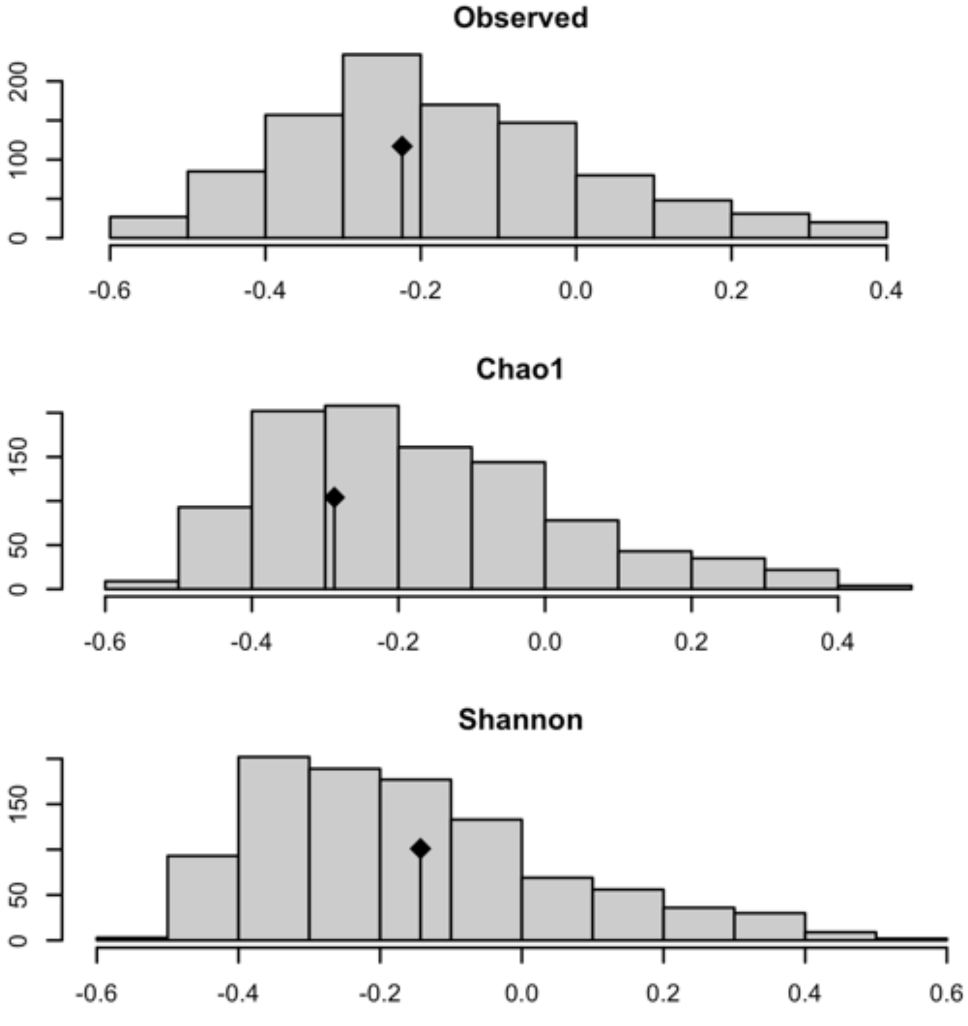

Figure 3.11 Graphical representation of Moran's *I* calculations for alpha diversity. Bars represent phylogenetic tree branches, the diamond represents the observed value, and the y-scale is frequency.

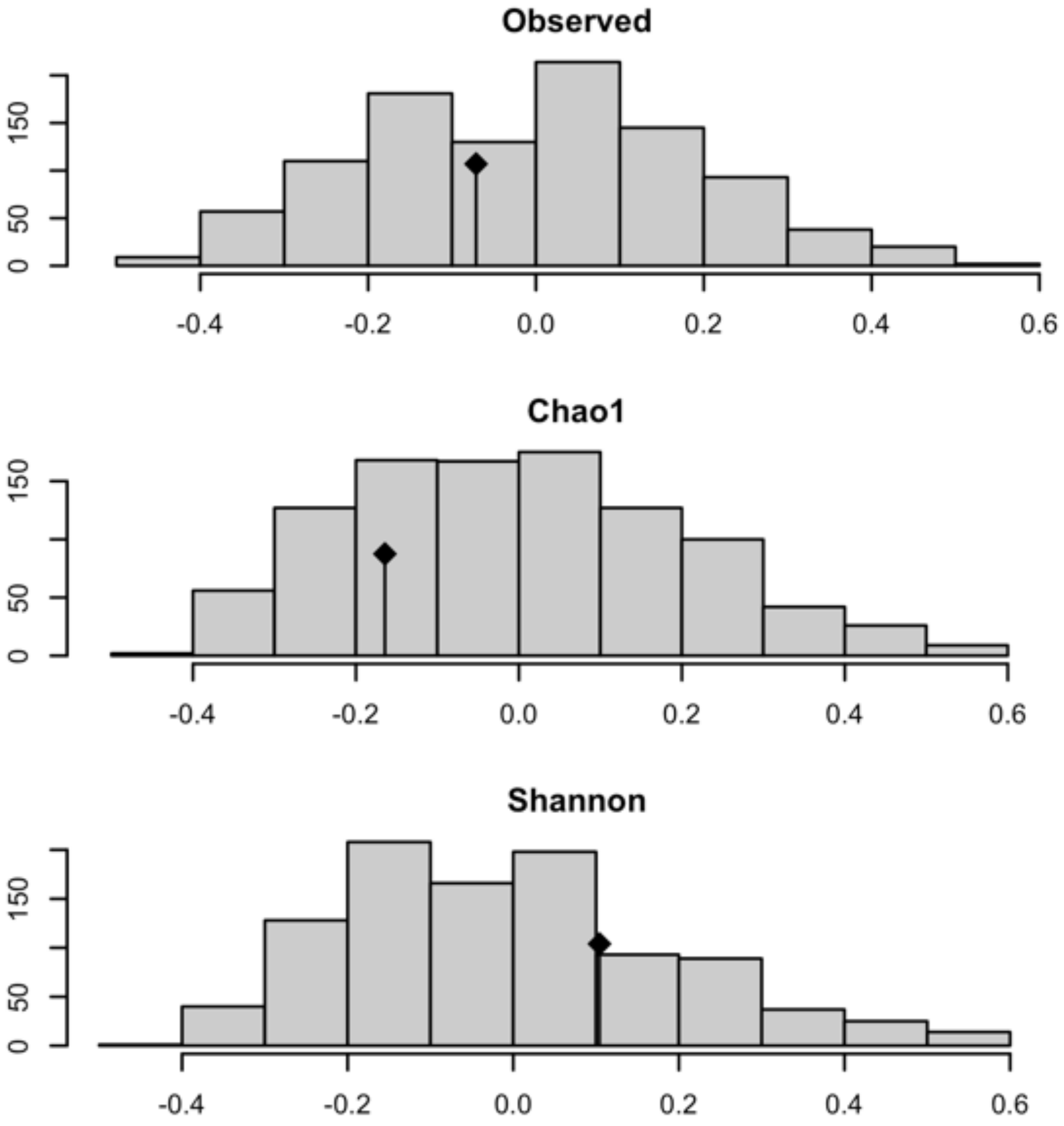

Figure 3.12 Graphical representation of Abouheif's C_{mean} calculations for alpha diversity. Bars represent phylogenetic tree branches, the diamond represents the observed value, and the y-scale is frequency.

Table 3.8 Moran's I and Abouheif's C_{mean} calculations for bacterial phyla and their correlation to sea turtle phylogeny. Significant values are indicated by bold text.

	Moran's *I*				**Abouheif's C_{mean}**			
Phylum	Obs	Std.Obs	Alter	p	Obs	Std.Obs	Alter	p
Verrucomicrobia	-0.20	-0.17	greater	0.48	-0.03	-0.16	greater	0.51
TM7	-0.07	1.72	greater	0.13	0.42	2.46	greater	0.06
Thermi	-0.31	-0.70	greater	0.71	-0.16	-0.80	greater	0.76
Tenericutes	-0.31	-0.74	greater	0.77	-0.19	-0.96	greater	0.79
Synergistetes	-0.29	-1.11	greater	0.87	-0.22	-1.19	greater	0.95
SR1	0.46	2.92	greater	**0.01**	0.52	2.56	greater	**0.01**
Spirochaetes	-0.21	-0.33	greater	0.60	-0.07	-0.44	greater	0.62
Proteobacteria	-0.49	-1.64	greater	0.99	-0.39	-1.98	greater	1.00
GN02	0.36	2.54	greater	**0.01**	0.42	2.21	greater	**0.02**
Gemmatimonadetes	-0.18	-0.05	greater	0.48	0.01	-0.04	greater	0.47
Fusobacteria	-0.28	-0.57	greater	0.70	0.03	0.18	greater	0.42
Firmicutes	-0.20	-0.30	greater	0.59	-0.12	-0.64	greater	0.69
Chloroflexi	-0.22	-0.26	greater	0.53	-0.02	-0.20	greater	0.55
Chlamydiae	-0.18	-0.42	greater	0.52	-0.02	-0.13	greater	0.30
Bacteroidetes	0.09	1.84	greater	0.05	0.15	0.80	greater	0.22
Actinobacteria	0.35	2.54	greater	**0.03**	0.46	2.24	greater	**0.02**
Acidobacteria	-0.41	-1.23	greater	0.94	-0.24	-1.15	greater	0.87
Euryarchaeota	-0.18	-0.32	greater	0.42	-0.13	-0.75	greater	1.00
Deferribacteres	-0.18	-0.32	greater	0.42	-0.13	-0.75	greater	0.87
Cyannobacteria	-0.18	-0.32	greater	0.42	-0.13	-0.75	greater	1.00

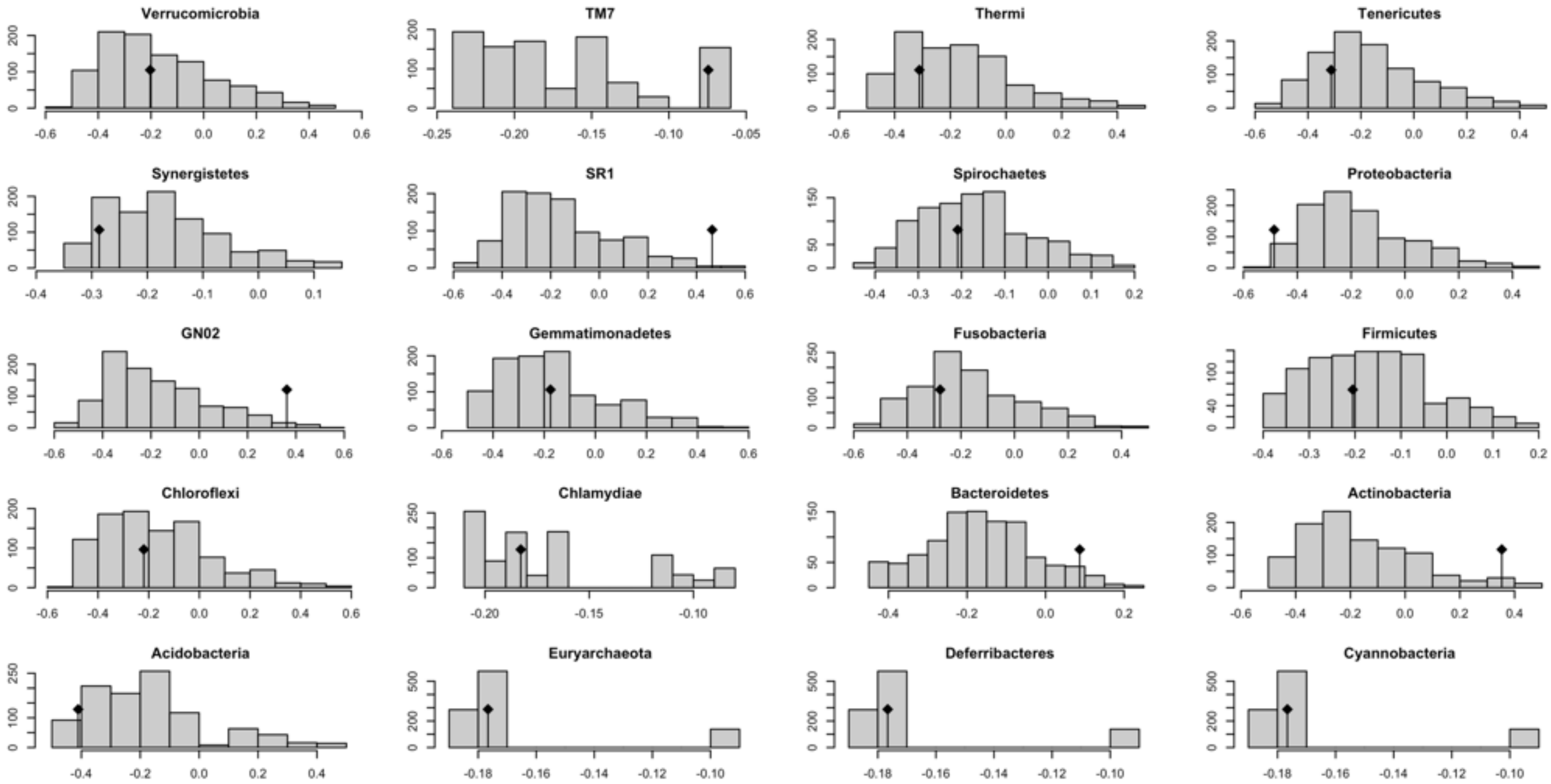

Figure 3.13 Graphical representation of Moran’s *I* calculations for microbial composition. Bars represent phylogenetic tree branches, the diamond represents the observed value, and the y-scale is frequency.

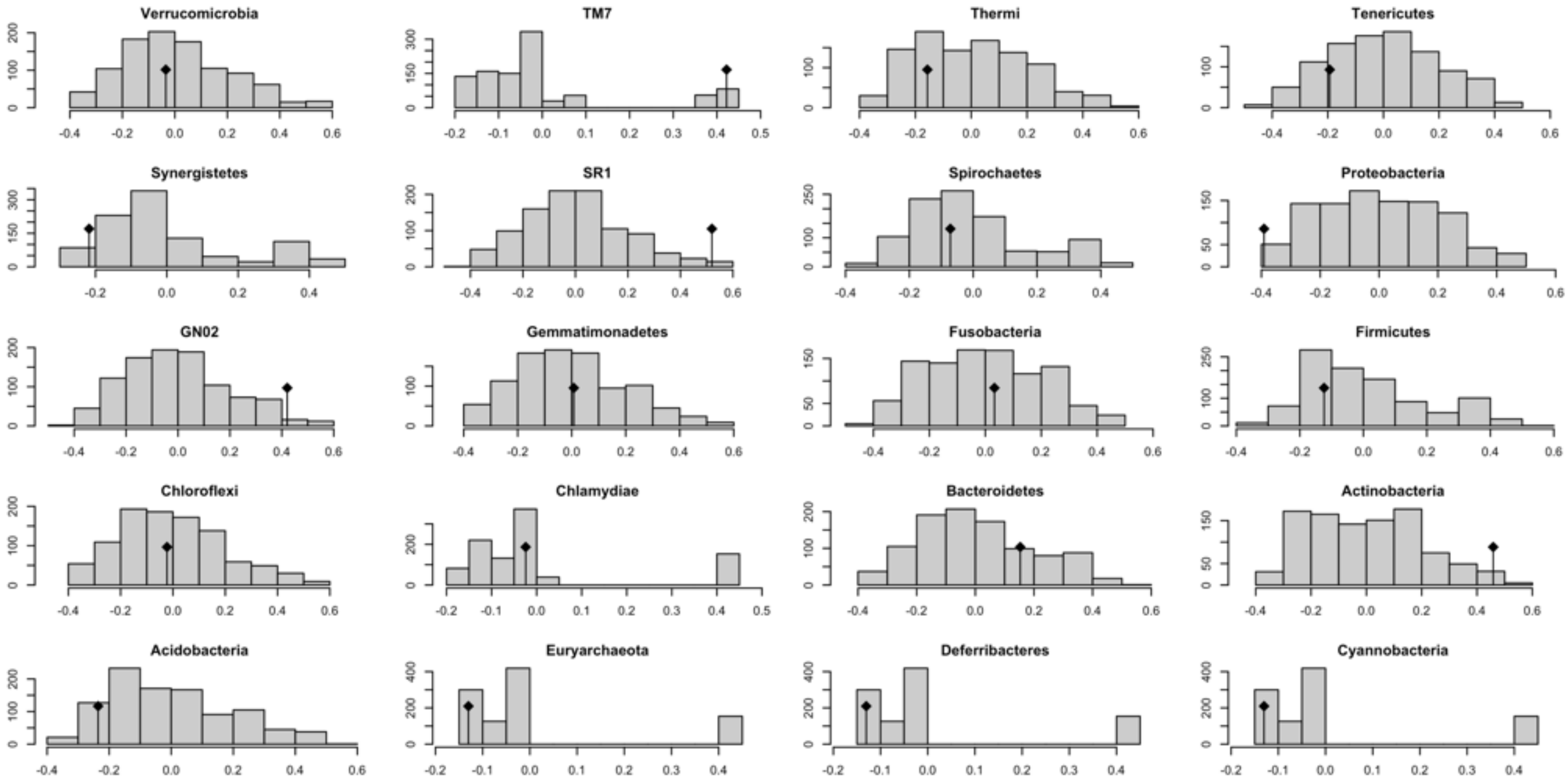

Figure 3.14 Graphical representation of Abouheif's C_{mean} calculations for microbial composition. Bars represent phylogenetic tree branches, the diamond represents the observed value, and the y-scale is frequency.

However, when these phyla were further examined using Pagels λ and Blomberg's K-statistic, it was discovered that only SR1 (λ=1.06, LogL=-11.67, LogL0=-13.20, p=0.08; K=1.28, p=0.02) was correlated to sea turtle phylogeny (GN02: λ=0.62, LogL=-9.38, LogL0=-9.67, p=0.44; K=0.69, p=0.28; Actinobacteria: λ=0.69, LogL=-21.0, LogL0=-21.36, p=0.4; K=0.9, p=0.14). The relative abundance of SR1 was greatest in green and flatback turtles, and lowest in olive ridley turtles (Figure 3.8). Finally, I reconstructed extinct sea turtle microbiotas (numbered nodes (1-6) on the phylogenetic tree (Figure 3.8)), to examine how these have changed over the course of sea turtle evolution. I discovered that most bacterial phyla have been relatively stable over time, with the exception of Actinobacteria which decreased as sea turtles evolved, and Spirochaetes, which went through a period of increase between nodes 1-5, but then decreased again from nodes 5-6. The phyla Thermi, showed a steady increase across nodes as time progressed (Figure 3.15). The results of this analysis show that sea turtle microbiotas have changed very little over the course of over 100 million years of evolution, despite the phenotypic changes that have occurred in their hosts, and this may be an indication that certain combinations of microbes are fundamental to specific aspects of all sea turtle physiology, regardless of differences in natural history between species.

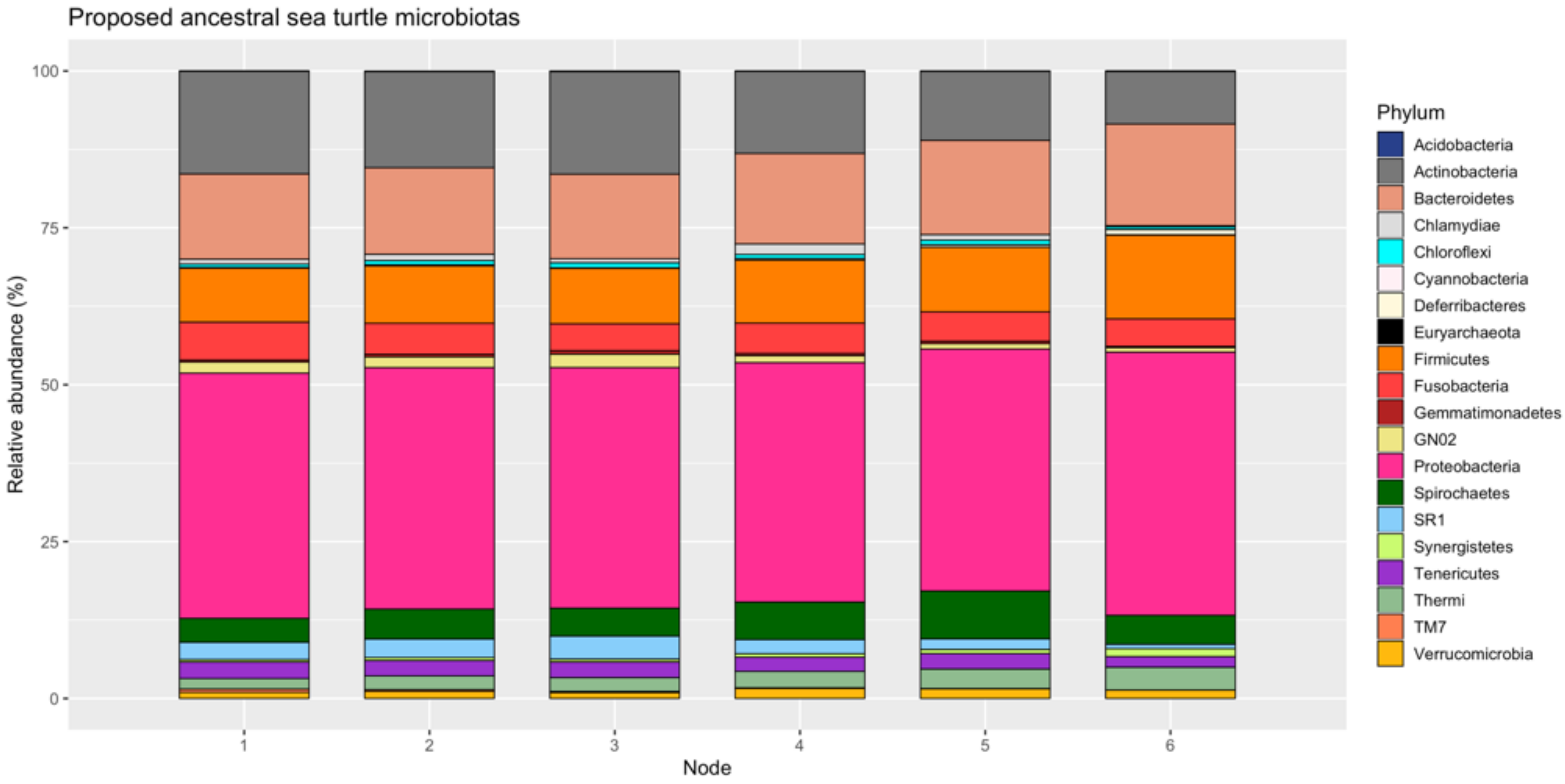

Figure 3.15 Proposed microbiotas for extinct sea turtle species. Nodes represent numbered nodes on the sea turtle phylogenetic tree.

3.4 Discussion

In this investigation I present the most comprehensive data on the microbiota composition of sea turtles to date, and demonstrate a clear link between microbiota composition and sea turtle phylogeny. Furthermore, this is one of the only studies in wild animals to have obtained samples from all extant species of an entire clade of the evolutionary tree. This makes my analysis unique, and enables me to perform a detailed evaluation of the phylogenetic signal that exists between host and microbiota. I showed that microbiota composition differs among all sea turtle species, but in all species, the predominant bacterial phylum was Proteobacteria. This is consistent with the results of an investigation into the microbiota of juvenile green turtles from Florida, in which Proteobacteria was also the major phylum dominating samples (Price *et al.* 2017). In contrast, the microbiota of juvenile green turtles from coastal areas of Brazil are co-dominated by the phyla Bacteroidetes and Firmicutes, with the investigators speculating that Proteobacteria increased in abundance in response to anthropogenic factors (Campos *et al.* 2018). Similarly, in a study conducted on green turtles from the Great Barrier Reef, Firmicutes was the most common phyla isolated from healthy individuals, but in sick turtles Proteobacteria was the dominate phylum (Ahasan *et al.* 2017). There are also reports of Firmicutes dominating the microbiota of stranded loggerhead turtles (Abdelrhman *et al.* 2016; Arizza *et al.* 2019), but these investigations are confounded by the fact that samples were collected from sick individuals, and in many cases there were delays from when the turtle was rescued to when samples were collected. Given that both captivity (Nelson *et al.* 2013; Kohl *et al.* 2014; Clayton *et al.* 2016; Delport *et al.* 2016; Ahasan *et al.* 2018) and health (Pflughoeft and Versalovic 2012) have both been shown to affect the microbiota of individuals, these results should be interpreted with caution as they are unlikely to represent the normal gut microbiota. In my investigation, all turtles sampled were wild and apparently healthy, and some had been sourced from extremely remote locations with no human habitation, so the likelihood of anthropogenic or other factors influencing my results is low. Furthermore, given that Proteobacteria was overwhelmingly the predominate phylum across all sea turtle species, and the strong phylogenetic influence that microbiota appears to have on sea turtles, I believe that in nesting animals, any microbiota in which Proteobacteria is not the most abundant phylum represents an abnormal gut flora.

In comparison to other taxa, there are considerably few studies on the microbiota of wild reptiles, and the relative abundance of specific bacterial phyla in herpetofauna appears to vary greatly. For example, Firmicutes was the dominant phylum identified in anoles (*Anolis* sp.) (Ren *et al.* 2016a), Galápagos tortoises (*Chelonoidis nigra*) (Hong *et al.* 2015a), gopher tortoises (*Gopherus polyphemus*) (Yuan *et al.* 2015), green iguanas (*Iguana iguana*) (Wehrle 2013), Galápagos land iguanas (*Conolophus subcristatus*) (Hong *et al.* 2015a), marine iguanas (*Amblyrhynchus cristatus*) (Hong *et al.* 2015a), and the montane iguana species *Liolaemus parvus*, *Liolaemus ruibali*, and *Phymaturus williamsi* (Kohl *et al.* 2017). Such findings have led some researchers to believe that reptile microbiotas resemble that of mammals (Costello *et al.* 2010; Hong *et al.* 2011), but in a wide-ranging investigation into squamate microbiotas, in which individuals representing 22 squamate families were sampled, Proteobacteria was the predominate phylum, and supports a hypothesis that the reptile gut microbiota is similar to that of birds (Colston 2017). However, in the single reptile study conducted in the species most closely related to birds, the archosaurian American alligator (*Alligator mississippiensis*), gut microbiota was overwhelmingly dominated by the phylum Fusobacteria (Keenan *et al.* 2013). Furthermore, the results of my investigation are similar to those seen in fish, in which the predominate phyla are Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria (Ghanbari *et al.* 2015). These discrepancies highlight the difficulties associated with making any assumptions on microbial assembly between taxa based solely on compositional data without incorporating any phylogenetic techniques in the analysis.

Unlike their microbiotas, the natural histories of sea turtles differ widely among the seven extant species. Leatherbacks are largely oceanic-pelagic throughout their life-history, and have the most specialised diet, feeding almost entirely on an array of dense gelatinous zooplankton (Jones and Seminoff 2013). Their large size means that they are more cold-adapted than other species, allowing them to traverse through the boreal waters that act as a barrier to other warm-water adapted turtles (Bowen and Karl 2007). Green turtles have an oceanic-neritic developmental pattern (Bolten 2003), are found in tropical and temperate waters worldwide (Bowen and Karl 2007), and consume a variety of seagrass, marine algae, and invertebrates (Jones and Seminoff 2013). Like green turtles, loggerheads also have an oceanic-neritic developmental pattern and prefer temperate to tropical waters (Bolten 2003). However, unlike green turtles, they are largely carnivorous, feeding on a wide array of prey items including Hydrozoa, Bivalvia, Cephalopodia, Porifera, Scyphoza, Bryozoa, Gastropoda, Polychaeta, Maxillopoda, Malacostraca, Insecta, Holothuroidea, Echinoidea, Anthozoa, Actinopterygii, and occasional plant material (Jones and Seminoff 2013). Evidence suggests that hawksbills also have an oceanic-neritic developmental pattern (Bolten 2003), with a preference for tropical waters (Bolten 2003). Although primarily carnivorous, the composition of prey items varies among populations of hawksbills, with some having a preference for sponges, while others feed predominately on corals (Jones and Seminoff 2013). The flatback turtle has a completely neritic life history (Bolten 2003), residing within the tropical waters of the Australian continental shelf (Limpus 2008). Detailed investigations into their diet are lacking, but it is presumed that they are carnivorous (Jones and Seminoff 2013). The olive ridley turtle is predominately oceanic for the duration of its life (Bolten 2003), and is found in both temperate and tropical waters, but most feeding probably occurs in warm water and they are likely omnivorous (Bowen and Karl 2007; Jones and Seminoff 2013). Finally, Kemp's ridleys have an oceanic-neritic developmental pattern (Bolten 2003), have a preference for tropical waters, and as adults are primarily carnivorous ingesting a range of molluscs, fish, jellyfish and gastropods (Jones and Seminoff 2013). Given the vast array of niche occupancy, and dietary preferences of sea turtles, some with marked overlap between species, it is likely that the similarities seen in relative abundance of the major bacterial phyla are driven by their shared evolutionary history. The similarities seen between gut microbiotas in nesting female sea turtles is remarkable, since there is nearly 100 million years of evolution separating the most ancient species, the leatherback, and the most modern species, the Kemp's ridley (Duchene *et al.* 2012). Such conservation of community similarity may be an indication that specific combinations of bacterial phyla are fundamental to sea turtle physiology, but this remains to be seen in any taxa.

Based on the results of this study, it appears that gut microbial composition and sea turtle phylogeny are intrinsically linked, and it is likely that a process of co-evolution exists between host and microbial community composition. Microbes have been identified as a key driver of vertebrate evolution (McFall-Ngai *et al.* 2013), and more recent research has focused on the role that phylogeny may play in convergence of microbiotas in some species (Muegge *et al.* 2011). Investigations in primates suggest that evolutionary trends in host physiology are more important than dietary niche in determining gastrointestinal microbiota (Amato *et al.* 2018), which supported by this research. For example, previous investigations into sea turtle microbiotas have speculated that the high proportion of Firmicutes found in some green turtle samples may be due to this phylum's ability to break down plant-derived polysaccharides (Ahasan *et al.* 2017). However, I found that green turtles, along with loggerheads, had the lowest relative abundance of Firmicutes of all the sea turtle species. Moreover, there are many examples in vertebrates, both terrestrial and aquatic, and from disparate branches of the evolutionary tree, where Firmicutes are lower in abundance than other phyla in herbivorous species (Ishaq and Wright 2012; Pope *et al.* 2012; Sullam *et al.* 2012; Fogel 2015; Givens *et* *al.* 2015; Miyake *et al.* 2015; Raulo 2015; Dill-McFarland *et al.* 2016; Nielsen *et al.* 2017). Therefore, caution should be applied when making assumptions on functionality of the microbiota within a species in the absence of specific functional testing, especially where phylogeny has not been considered as a component of the analysis. I showed that the bacterial phylum SR1 was strongly linked to sea turtle phylogeny. The candidate phylum SR1 (Absconditabacteria), includes ubiquitous organisms found in marine and terrestrial high-temperature environments, fresh-water lakes, subsurface aquifers, and animals (Davis *et al.* 2009; Ghotra 2014). There are no cultured representatives of SR1, with all current knowledge on their presence and diversity obtained from genomic sequencing (Hug *et al.* 2016; Solden *et al.* 2016). They have a predilection for sulphur-rich and oxygen-limited environments suggesting a potential microaerophilic, sulphur-based metabolism, and in general, environmental and animal-derived SR1 species appear to cluster differently (Campbell *et al.* 2013; Ghotra 2014). SR1 is routinely found in a range of vertebrates, but is most commonly associated with H_2S-related malodour and periodontal disease in humans (Griffen *et al.* 2012; Takeshita *et al.* 2012). How SR1 is involved in sea turtle gastrointestinal physiology remains unknown at this stage. The role of the microbiota in shaping vertebrate phylogeny should be an important focus for future investigations, and an effort must be made to sample as diverse an array of species as possible, spanning multiple clades of the evolutionary tree, so that these relationships can be further explored.

Using results obtained from extant turtles, I reconstructed possible microbiotas of extinct sea turtle species. This analysis showed that the composition of sea turtle microbiotas did not change greatly over time, with only moderately minor fluctuations in the relative abundance of specific phyla responsible for observed differences. Conversely, the human microbiota has diverged rapidly from our closest relatives (Moeller *et al.* 2014), and among populations of humans, continues to rapidly evolve (Davenport *et al.* 2017). Sea turtles are among the most ancient species on Earth, with the leatherback existing in its current form for more than 100 million years (Duchene *et al.* 2012), while modern humans first appeared around 350,000 to 260,000 years ago (Schlebusch *et al.* 2017). These seemingly marked differences in rate of evolution of microbiotas, may be reflective of differences in rates of evolution between taxa, and warrants further investigation.

A limitation of my study was that I was restricted to sampling nesting females. This was due to the fact that living males are difficult to obtain, and this can only be done in-water. Importantly, it is likely that all of the animals sampled in this investigation had not eaten for an extended period, as sea turtles may undergo long periods of fasting, particularly during breeding and migration (Hays *et al.* 2002; Jessop *et al.* 2004). Periods of inappetence have been shown to affect the microbiotas of a range of vertebrates including humans (Remely *et al.* 2015), fish (Xia *et al.* 2014), bears (Sommer *et al.* 2016), alligators (Keenan *et al.* 2013), mice (Beli *et al.* 2018), penguins (Dewar *et al.* 2014), and the Burmese python (Costello *et al.* 2010). How this may have influenced my results in unknown at this stage, and it may explain the differences in my results and those of other green turtles captured on the Great Barrier Reef (Ahasan *et al.* 2017), and future investigations should focus on obtaining samples from a range of age classes, sexes, and physiological states. Although I was not able to determine if fasting in nesting turtles affected microbial diversity, some authors propose that fasting samples represent the core microbial OTUs, with other OTUs fluctuating in number in response to post-prandial physiological changes (Costello *et al.* 2010; Keenan *et al.* 2013). If, as hypothesised, diet has little effect on this core microbiota, then this strengthens the results of my phylogenetic analysis as my results have not been confounded by transient microbial species.

This investigation represents the most comprehensive microbiota study to have been conducted in sea turtles to date, but most importantly, I was able to obtain samples from an entire clade of the evolutionary tree which allowed me to perform a comprehensive phylogenetic analysis on my results. While other investigations have sampled multiple species in the single study (Gulino *et al.* 2013; Givens *et al.* 2015; Miyake *et al.* 2015; Eichmiller *et al.* 2016; Ren *et al.* 2016b), the breadth of taxonomic coverage in these analyses was not as comprehensive as mine from a phylogenetic perspective. Understanding the structure of microbial populations, and the complexities of the host-microbiome relationship is the next critical step in managing threatened species populations. Furthermore, only by unravelling these mysteries can we truly understand the origins of life, and the forces that have shaped the diversity in form and function we see today.

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Chapter 4. Acquisition of the microbiota in an oviparous vertebrate without parental care

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4.1 Abstract

The symbiotic relationship between gastrointestinal microbes and their hosts is vital for most metazoan species. How an animal acquires this essential microbiota is dependent on its life history traits and is mostly thought to occur through parent-offspring contact. Here we studied the green sea turtle (*Chelonia mydas*) to determine how the microbiota is acquired in a species with no parental care after oviposition. We show that mother turtles have a complex microbial community in both their blood and developing eggs that closely resembles that of hatchling turtles. Our investigation also showed that neonatal turtles do not acquire their microbiota from their incubation environment. We propose that in species such as the green sea turtle, where post-partum opportunities for microbial transfer are limited, transfer of the microbiota from the mother to the embryo during development in the uterus or oviducts plays a key role.

Key Words: *Microbiota, inheritance, transgenerational, bacteremia, green turtle, parental care*

4.2 Introduction

The vertebrate host-microbiota relationship has evolved into an essential symbiosis, with many of the host's physiological processes closely linked with microbial activity. Acquisition of the microbiota is the critical first step in developing the host-microbiota relationship. For those species dependent on parent-offspring transfer of microbiota, a failure to do so may have lasting effects on the intestinal bacterial composition into later life, with important ramifications for immune system development and other negative fitness consequences (Torrazza and Neu 2011; Taschuk and Griebel 2012). In non-human vertebrates, the microbiota is commonly acquired through vertical transmission (i.e. from parent to offspring), and may occur either during, or post-partum. An example of post-partum acquisition in vertebrates is the cutaneous mucus feeding of fry by parent discus fish (*Symphysodon aequifasciata*), which acts to inoculate the offspring with skin microbes (Sylvain and Derome 2017). Direct transmission from mother to embryo via the reproductive tract as one of the possible avenues has been inferred from similarities in the gastrointestinal microbiome of developing chicken embryos and that of the hen (Ding *et al.* 2017), as well as the demonstration of *in ovo* bacterial populations in eggs collected from wild birds, and eastern fence lizards (*Sceloporus undulatus*) (Trevelline *et al.* 2018). In addition, investigations into gastrointestinal microbial structure of neonatal lizards indicate that a proportion of the microbiota is acquired from their mothers (Kohl *et al.* 2017). Although recent discoveries suggest that human babies acquire a proportion of their microbiome via the placenta (Gilbert 2014), the majority of its acquisition occurs rapidly after birth as organisms from the mother and surrounding environment invade the naïve gastrointestinal tract (Dominguez-Bello *et al.* 2010). This is likely to be true for many vertebrates, because the typically close association of mother and offspring mean that many opportunities and pathways exist for transmission of the microbiota.

While there are multiple opportunities for viviparous vertebrate animals to transmit microbiota to their offspring during birth or through subsequent parental care, oviparous vertebrates (fish, amphibians, most reptiles and birds) have fewer options. However, other than a very few species of megapodes, birds provide extensive parental care to both precocial and altricial offspring (Cockburn 2006) and so can potentially transmit microbiota through close physical contact or provision of food. Similarly, some oviparous reptile species interact with their neonates, providing similar opportunities for inoculation (Gans 1996). However, oviparous species with no parental care form a large proportion of the 10,000+ reptilian species (Uetz *et al.* 2018) and include the Order Testudines (turtles and tortoises), which unlike other reptilian Orders, have never evolved live birth or parental care (Rafferty *et al.* 2013). Thus, the question arises: in the absence of viviparity or any contact between neonate and parent, how is the microbiota acquired by these newly-hatched reptiles? The important role of microbiota in so many aspects of vertebrate biology suggests that its acquisition by these animals should not occur via some haphazard inoculation from the environment. The absence of parental care in the Testudines, their wide distribution and super-precocial neonates make them an ideal group to answer these interesting and important questions. Therefore, we investigated how neonate sea turtles acquire their microbiota and the extent to which the hatchling sea turtle microbiome resembles that of the mother. By understanding the microbiota of species within this clade, and the processes by which microbial communities become established, we will be able to gain a broader comprehension of vertebrate evolution and function, particularly in the consequences of the fascinating array of reproductive modes found in the group.

4.3 Methods

4.3.1 Ethics statement

This study was approved by the Biological Sciences Animal Ethics Committee of Monash University (approval 14694). Adult green sea turtles (*Chelonia mydas*) and eggs were sampled and collected under permit WITK17730216 from the Queensland Department of Environment and Heritage Protection and were housed under permit 10008208 from the Victorian Department of Environment, Land, Water and Planning.

4.3.2 Study population

Nesting green sea turtles (n=18), and eggs (n=60), were sampled from Heron Island (HI) in the Great Barrier Reef, Queensland, Australia (23.4423° S, 151.9148° E) in January 2017.

4.3.3 Sample collection

During the nesting season over the Austral summer (late October to late March), female green turtles come ashore to dig a nest and lay eggs, at which time we were able to collect eggs and samples from nesting females. To collect a cloacal swab and blood sample, we waited until females had finished nesting and were returning to the ocean. For blood collection, an area of skin was prepared using alcohol wipes and 10ml of blood was collected from the dorsal cervical sinus using an 18G needle attached to a 10ml syringe. A portion of this blood was used to soak a sterile cotton-tipped swab by removing the needle from the hub and squirting the contents onto the swab tip, which was also cut and placed into a sterile Eppendorf tube. Blood was collected from 3 adult turtles for microbiota analysis. Latex gloves were worn during this procedure and were changed between individuals. The turtle was then flipped into dorsal recumbency, and an equine uterine swab (Minitube, Smythesdale, Victoria, Australia) was inserted into the cloaca so that it entered the distal colon. These swabs were housed in a sterile sheath, the entire apparatus was inserted into the cloaca and the swab tip was not extruded until correct placement of the sheath had been achieved. The swab tip was then retracted back into the sheath prior to extraction. The tip of the swab was cut using a sterile wire cutter, placed into a sterile Eppendorf tube and sealed. Turtles were then permitted to return to the ocean.

We collected eggs from the same three females that we collected the blood from, by placing a sterile plastic bag under the tail just as the turtle began to lay. Once the turtle had finished laying, the bag containing the eggs was sealed using a zip lock, removed, and she was allowed to fill in the nest as normal.

The surfaces of 15 eggs from each of the three clutches were immediately swabbed and the swab tips placed into sterile Eppendorf tubes. Five eggs from each clutch were then opened using a sterile scalpel blade and the contents macerated by using a sterile cotton-tipped swab, and the tips placed into sterile Eppendorf tubes. All eggs were handled using sterile gloves that were changed between each treatment group and a new scalpel blade was used to open each egg. Egg surface samples were pooled during DNA extraction.

From the remaining eggs in each clutch, 10 eggs were selected to be incubated in the laboratory, while the rest of the eggs were deposited into pre-dug nests on Heron Island. We did not count how many eggs were placed into each hand-dug nest but it was approximately 50-80 eggs. To enable later retrieval of neonates from these clutches, nests were marked with flagging tape external to the nest, as well as within the nest chamber. The remaining eggs were transported to Monash University by air, using hypoxia to delay embryonic development (Rafferty *et al.* 2013), and to protect them from movement-induced mortality (Williamson *et al.* 2017). Once at Monash University, eggs were artificially incubated using standard techniques (Rafferty and Reina 2014), in commercially available landscaping sand. Eggs were placed into the sand so that the top third of the surface of the egg was unburied. After hatching (n=7), a cloacal swab was obtained from each hatchling using a sterile paediatric nasopharyngeal swab (Transwab® Pernasal Amies Plain, MWE Medical Wire, Wiltshire, England) and 0.1ml of blood was collected from the dorsal cervical sinus by preparing the skin with an alcohol wipe and by using a sterile insulin syringe. The collected blood was used to soak a second sterile swab. Swabs were cut and placed into Eppendorf tubes. Latex gloves were used for all of these procedures and changed between individuals. Only 7 laboratory hatchlings were able to be obtained for sampling as an incubation error resulted in the death of the other eggs. After sample collection hatchlings were returned to Heron Island and released.

In March 2017 we returned to Heron Island to obtain samples from naturally incubated eggs (n=44). Nests were dug up and samples were collected from emerging hatchlings in exactly the same manner as those from laboratory animals.

Finally, to determine if incubation environment played a role in microbial composition we obtained pre- and post-incubation sand samples from Heron Island (natural incubation) and landscape sand (artificial incubation) for DNA extraction and microbial analysis. Due to the constraints of working in the field environment with wild animals there was not opportunity for collecting negative control swabs for subsequent extraction and analysis.

4.3.4 DNA extraction

DNA was extracted manually using the phenol-chloroform method (Green *et al.* 2012). In each Eppendorf tube, 500µL of extraction buffer (20mM ethylenediaminetetraacetic acid (EDTA), 0.1M Tris, 1% cetrimonium bromide (CTAB), 56mM NaCl, pH 8) was added so that swabs were completely covered. We then added 20µL of Proteinase K (QIAGEN Proteinase K (10ml) to each vial, along with 60µL of 10% sodium dodecyl sulphate (SDS). The mixture was then incubated at 55°C overnight. The next day, 50µL of 5M NaCl and 500µL of Phenol was added, and the tubes shaken until an emulsion was formed. They were then incubated at room temperature for 10 minutes, with intermittent mixing. The tubes were then centrifuged at 10,000 RPM for 10 minutes and the supernatant removed and added to a new tube containing 250µL Phenol and 250µL Chlorophorm:Isoamyl-Alcohol (24:1). The tubes were again centrifuged at 10,000 RPM for 10 minutes and the resultant supernatant added to a new tube containing 500µL of Chlorophorm:Isoamyl-Alcohol. Once again, the tubes were centrifuged at 10,000 RPM for 10 minutes. The supernatant was then added to a new tube containing 3M Sodium Acetate at a volume equal to 10% of the extraction solution. We then added 1ml of ice-cold 99% ethanol to each test tube and then placed them into a freezer at -20°C for 1 hour. The tubes were then centrifuged at 4°C at 12,000 RPM for 10 minutes. The fluid in the test tube was then removed with a glass pipette and 1ml of ice-cold 70% alcohol was added. The tubes were centrifuged a final time at 4°C at 12,000 RPM for 5 minutes. After centrifugation the alcohol was removed and the lids left off the tubes to allow the DNA pellet to dry. Once dried, 25µL of 1 x Tris-EDTA (TE) was added to each tube and the extracted DNA was stored at -20°C until amplicon sequencing could take place.

4.3.4 16S rRNA gene amplicon sequencing

The V3-V4 region of 16S rRNA genes were amplified with forward primer 5' ACTCCTACGGGAGGCAGCAG 3' and reverse primer 5' GGACTACHVGGGTWTCTAAT 3' using Q5 high fidelity polymerase (New England Biolabs) using the barcoding strategy of Fadrosh *et al.* (2014). Sequencing was performed on an Illumina MiSeq system (2 x 300 bp).

4.3.5 Data processing

Sequence data was analysed using QIIME version 1.9.1 (Caporaso *et al.* 2010) using default parameters and a Phred quality threshold of > 20. The UCLUST algorithm (Edgar 2010) was used to pick OTUs at 97% sequence identity and a Biome table was produced. Potentially chimeric sequences were identified using Pintail (Ashelford *et al.* 2005). Blast was used to assign taxonomy against the Greengenes database (DeSantis *et al.* 2006) and QIIME version 1.9.1 defaults. Additional assignment of taxonomy was performed using a command line version of BLASTN (Altschul *et al.* 1997) against the NCBI 16S Microbial database.

4.3.6 Statistics and data analysis

Data was not rarefied prior to statistical analysis as this has been shown to be inaccurate for assessment of microbiota samples as it results in a high rate of false positives in tests for species that are differentially abundant across sample classes (23). Initial exploration of the Biome table data was performed using the online Calypso software (http://cgenome.net/wiki/index.php/Calypso) (24). Data was further analysed with the free software program R, utilising the package ‘phyloseq’. Simple data visualisation was achieved using barcharts. Alpha diversity was explored using Observed OTUs, Shannon index and Chao1 estimates. Alpha diversity was tested for normality using the Shapiro-Wilks test, with all metrics, Observed ($W=0.94$, $p<0.001$), Chao1 ($W=0.94$, $p<0.001$), and Shannon ($W=0.96$, $p=0.001$) being non-normally distributed, and so comparisons between groups were first made using the Kruskal-Wallis test, and then paired comparisons between groups were made using the pairwise Wilcoxon rank sum test with adjusted (Holm) p-values. For alpha diversity comparisons between mothers and their biological offspring, statistical analysis was not possible because for many groups there were only a single observation and thus there was no variance. Beta diversity was investigated using principle co-ordinate analysis (PCoA) (weighted and unweighted UniFrac) and Adonis tests to compare all species, and then pairwise comparisons were made between all combinations of species with Holm correction of p-values. Venn diagrams were used to detect core microbiota communities which best characterised each sample group as well as shared OTUs.

4.4 Results

Sequencing results are summarised in Table 4.1, but we did not obtain sequence results for all samples due to failure of DNA extraction in some cases.

Table 4.1 Summary of sequencing results

Sample type	**Number Sampled**	**Number successfully sequenced**	**Total Sequences**	**Average sequences per sample**
Adult gut	18	17	612,452	36,026.6
HI hatchling gut	44	44	1,727,660	39,265
Lab hatchling gut	7	7	134,673	19,239
Adult blood	3	2	5,093	2,546.5
HI hatchling blood	44	43	144,365	3,357.3
Lab hatchling blood	7	7	37,402	5,343.1
Egg surface	15 (pooled by mother)	3	23,595	7,865
Egg contents	15	15	58,937	3929.1
Sand	11	11	157,703	14,336.6

The taxonomic summary of microbial components from all samples yielded a total of 14 bacterial phyla, 24 classes, 56 orders, 119 families, 208 genera, and 431 OTUs (Appendix 1). The predominant bacterial phyla were Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Figure. 4.1 and Appendix 1).

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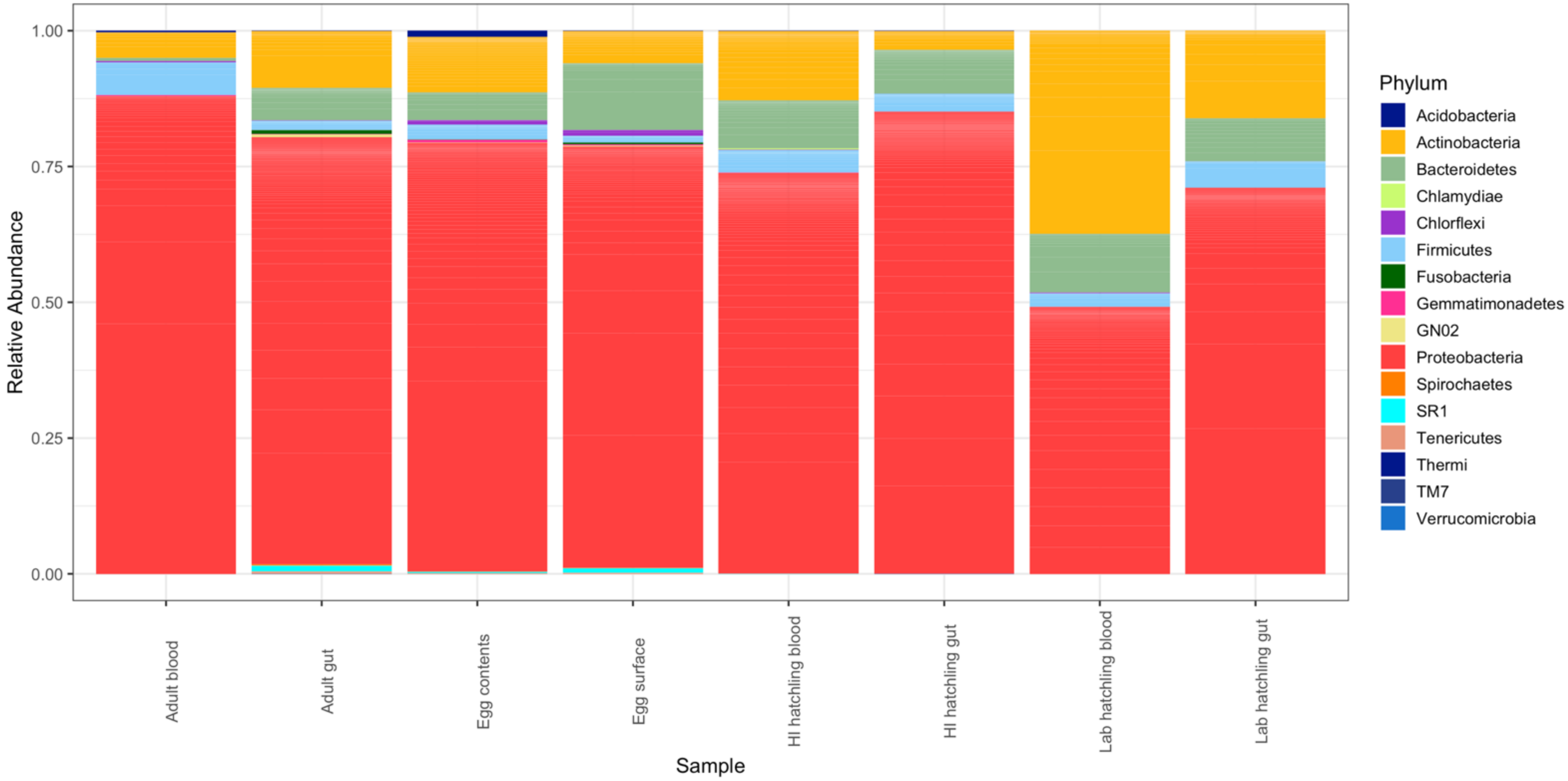

Figure 4.1 Relative abundance of the dominant bacterial phyla across all samples.

The predominant bacterial classes were Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, and Actinobacteria, (Supplementary Table 1), the predominant bacterial orders were Xanthomonadales, Flavobacteriales, Pseudomonadales, and Burkholderiales (Supplementary Table 1), the predominant bacterial families were Enterobacteriaceae, Burkholderiaceae, Pseudomonadaceae and Xanthomonadaceae (Supplementary Table 1) and the predominant bacterial genera were *Klebsiella*, unclassified Pseudomonadaceae, *Bacillus*, and *Ralstonia* (Supplementary Table 1). The mean microbial diversity estimate using Shannon's diversity index did not differ significantly among all samples (χ^2=6.33, df=8, p=0.61). However, there were significant differences between Observed OTUs (χ^2=85.87, df=8, p<0.001) and in species richness between samples as measured by Chao1 (χ^2=90.34, df=8, p<0.001) (Figure 4.2). These observations were consistent when all samples were analysed in entirety, and also for pairwise comparisons (Tables 4.2 - 4.4).

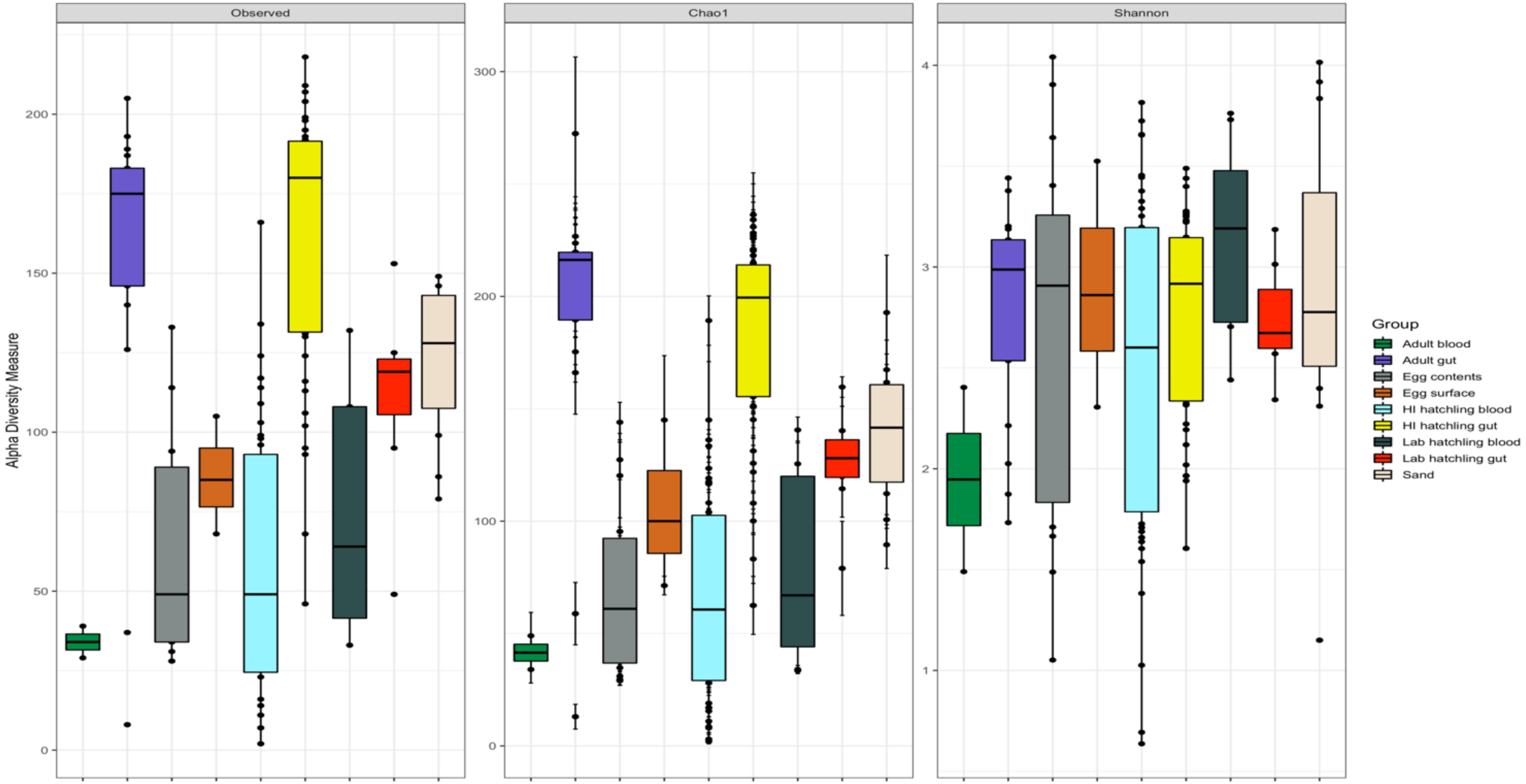

Figure 4.2 Alpha diversity estimates across all samples. Individual points and brackets represent the richness estimate and the theoretical standard error range associated with that estimate, respectively. Within each panel, the samples are organized into goups, and a boxplot is overlaid on top of this. Significant differences were seen between Observed ($p<0.001$) and Chao1 ($p<0.001$) estimates between groups, but no differences in Shannon diversity between groups ($p=0.46$).

Table 4.2 Pairwise Wilcoxon rank sum test comparisons between groups for Observed OTUs. Numbers represent corrected (Holm) p-values. Significant values are indicated by bold text.

	Adult blood	Adult gut	Egg contents	Egg surface	HI hatchling blood	HI hatchling gut	Lab hatchling blood	Lab hatchling gut
Adult gut	1.00	-	-	-	-	-	-	-
Egg contents	1.00	**<0.01**	-	-	-	-	-	-
Egg surface	1.00	0.73	1.00	-	-	-	-	-
HI hatchling blood	1.00	**<0.01**	1.00	1.00	-	-	-	-
HI hatchling gut	0.39	1.00	**<0.01**	0.30	**<0.01**	-	-	-
Lab hatchling blood	1.00	0.10	1.00	1.00	1.00	**0.02**	-	-
Lab hatchling gut	0.94	0.14	0.16	1.00	0.07	0.17	1.00	-
Sand	0.72	0.14	**0.01**	0.98	**<0.01**	0.14	0.39	1.00

Table 4.3 Pairwise Wilcoxon rank sum test comparisons between groups for Chao1. Numbers represent corrected (Holm) p-values. Significant values are indicated by bold text.

	Adult blood	Adult gut	Egg contents	Egg surface	HI hatchling blood	HI hatchling gut	Lab hatchling blood	Lab hatchling gut
Adult gut	0.80	-	-	-	-	-	-	-
Egg contents	1.00	**<0.01**	-	-	-	-	-	-
Egg surface	1.00	0.73	1.00	-	-	-	-	-
HI hatchling blood	1.00	**<0.01**	1.00	1.00	-	-	-	-
HI hatchling gut	0.05	1.00	**<0.01**	0.19	**<0.01**	-	-	-
Lab hatchling blood	1.00	**0.03**	1.00	1.00	1.00	**<0.01**	-	-
Lab hatchling gut	0.89	0.06	0.09	1.00	**0.04**	**0.04**	1.00	-
Sand	0.49	**0.04**	**<0.01**	1.00	**<0.01**	0.09	0.31	1.00

Table 4.4 Pairwise Wilcoxon rank sum test comparisons between species for Shannon index. Numbers represent corrected (Holm) p-values. No significance was observed between Shannon index for any inter-group comparisons.

	Adult blood	Adult gut	Egg contents	Egg surface	HI hatchling blood	HI hatchling gut	Lab hatchling blood	Lab hatchling gut
Adult gut	1.00	-	-	-	-	-	-	-
Egg contents	1.00	1.00	-	-	-	-	-	-
Egg surface	1.00	1.00	1.00	-	-	-	-	-
HI hatchling blood	1.00	1.00	1.00	1.00	-	-	-	-
Hi hatchling gut	1.00	1.00	1.00	1.00	1.00	-	-	-
Lab hatchling blood	1.00	1.00	1.00	1.00	1.00	1.00	-	-
Lab hatchling gut	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-
Sand	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

We observed clustering patterns across sample groups on the PCoA plot for both weighted UniFrac (Figure 4.3a), and unweighted UniFrac (Figure 4.3b), with some overlapping of microbiota compositions, particularly on the weighted plot. Observed differences between sample groups were significant for both the weighted UniFrac (df=8, SS_T=5.60, MS = 0.70, f.model=8.64, R^2=0.33, *p*=0.001) and unweighted UniFrac (df=8, SS_T=12.80, MS = 1.60, f.model=10.38, R^2=0.38, *p*=0.001) ordinations (Figures 4.3a and 4.3b). Most importantly, we discovered that each group (adult gut, hatchling gut, adult blood, hatchling blood, egg contents, egg surface, and sand) had a distinct microbial community, and similarly, when pairwise comparisons were made between all combinations of species, significant differences in microbiota compositions existed between most environmental samples and gut and blood combinations (Tables 4.5 and 4.6). The only exception was when comparisons were made between adult blood and sand samples (weighted: df=1, SS_T=0.25, f.model=4.88, R^2=0.31, *p*=0.1; unweighted: df=1, SS_T=0.54, f.model=6.11, R^2=0.36, *p*=0.09) (Tables 4.5 and 4.6), but due to the low sample size of mother's blood (n=2), there is low statistical power in the analysis. When analysed in concert with all samples, hatchling blood microbiotas clustered together regardless of their incubation environment (natural vs artificial sand), and similarly, hatchling gut microbiotas closely resembled each other independent of incubation environment. Furthermore, these clusters were different to adult and environmental samples (Figures 4.3a and 4.3b).

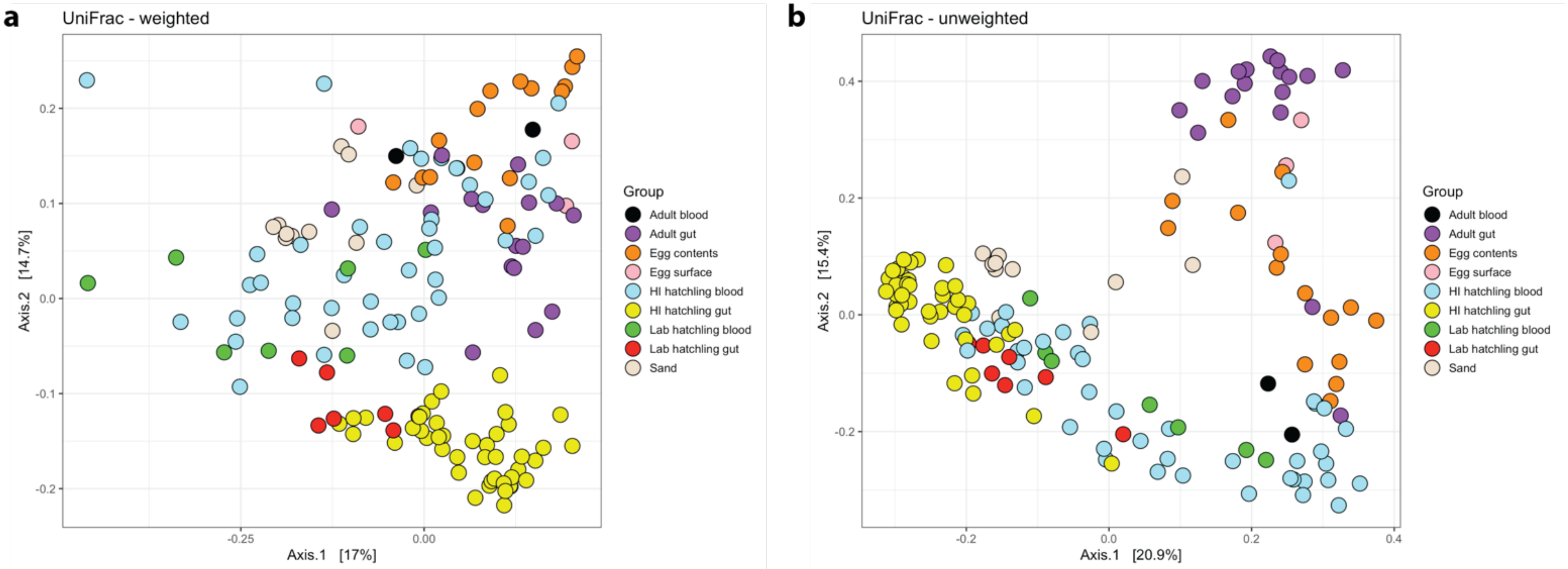

Figure 4.3 Principle co-ordinate analysis (PCoA) showing functional microbial diversity across all samples, with sample groups being identified using different colours. **(a)** Weighted UniFrac showed significant differences for microbiota composition between samples groups (R^2=0.33, *p*=0.001). **(b)** Similarly, unweighted UniFrac ordination showed significant differences between sample groups (R^2=0.38, *p*=0.001).

Table 4.5 Pairwise Adonis comparisons of beta diversity (weighted UniFrac) for all combinations of microbiota composition. Both the p, and adjusted (Holm) p-values are reported. Significant values are indicated by bold text.

Pairs	Df	SS_T	F.model	R^2	p	p.adjusted
HI hatchling gut vs HI hatchling blood	1.00	1.37	15.16	0.15	0.00	**0.04**
HI hatchling gut vs Adult gut	1.00	1.18	20.32	0.26	0.00	**0.04**
HI hatchling gut vs Sand	1.00	1.34	27.57	0.35	0.00	**0.04**
HI hatchling gut vs Lab hatchling gut	1.00	0.33	7.06	0.13	0.00	**0.04**
HI hatchling gut vs Egg contents	1.00	1.34	24.62	0.31	0.00	**0.04**
HI hatchling gut vs Lab hatchling blood	1.00	0.79	14.84	0.24	0.00	**0.04**
HI hatchling gut vs Egg surface	1.00	0.40	7.83	0.15	0.00	**0.04**
HI hatchling gut vs Adult blood	1.00	0.29	5.72	0.12	0.00	0.05
HI hatchling blood vs Adult gut	1.00	1.14	9.68	0.15	0.00	**0.04**
HI hatchling blood vs Sand	1.00	0.78	6.77	0.12	0.00	**0.04**
HI hatchling blood vs Lab hatchling gut	1.00	0.40	3.32	0.07	0.00	0.06
HI hatchling blood vs Egg contents	1.00	0.62	5.29	0.09	0.00	**0.04**
HI hatchling blood vs Lab hatchling blood	1.00	0.40	3.13	0.06	0.01	0.08
HI hatchling blood vs Egg surface	1.00	0.30	2.33	0.05	0.04	0.27
HI hatchling blood vs Adult blood	1.00	0.14	1.09	0.03	0.32	0.80
Adult gut vs Sand	1.00	1.06	15.85	0.38	0.00	**0.04**
Adult gut vs Lab hatchling gut	1.00	0.58	8.74	0.28	0.00	**0.04**
Adult gut vs Egg contents	1.00	0.57	7.54	0.20	0.00	**0.04**
Adult gut vs Lab hatchling blood	1.00	0.82	10.11	0.31	0.00	**0.04**
Adult gut vs Egg surface	1.00	0.09	1.16	0.06	0.26	0.80
Adult gut vs Adult blood	1.00	0.29	3.54	0.17	0.01	0.11
Sand vs Lab hatchling gut	1.00	0.53	13.35	0.45	0.00	**0.04**
Sand vs Egg contents	1.00	0.86	14.52	0.38	0.00	**0.04**
Sand vs Lab hatchling blood	1.00	0.63	10.62	0.40	0.00	**0.04**
Sand vs Egg surface	1.00	0.40	7.71	0.39	0.00	**0.04**
Sand vs Adult blood	1.00	0.25	4.88	0.31	0.01	0.10
Lab hatchling gut vs Egg contents	1.00	0.74	12.81	0.39	0.00	**0.04**
Lab hatchling gut vs Lab hatchling blood	1.00	0.24	4.17	0.26	0.00	**0.04**
Lab hatchling gut vs Egg surface	1.00	0.36	8.13	0.50	0.01	0.10
Lab hatchling gut vs Adult blood	1.00	0.28	6.86	0.49	0.03	0.22
Egg contents vs Lab hatchling blood	1.00	0.80	10.82	0.35	0.00	**0.04**
Egg contents vs Egg surface	1.00	0.14	1.89	0.11	0.10	0.49
Egg contents vs Adult blood	1.00	0.08	1.06	0.07	0.39	0.80
Lab hatchling blood vs Egg surface	1.00	0.38	4.46	0.36	0.01	0.11
Lab hatchling blood vs Adult blood	1.00	0.29	3.40	0.33	0.06	0.37
Egg surface vs Adult blood	1.00	0.18	1.91	0.39	0.20	0.80

Table 4.6 Pairwise Adonis comparisons of beta diversity (unweighted UniFrac) for all combinations of microbiota composition. Both the p, and adjusted (Holm) p-values are reported. Significant values are indicated by bold text.

Pairs	Df	SS_T	F.**m**odel	R^2	p	p.adjusted
HI hatchling gut vs HI hatchling blood	1.00	3.21	19.44	0.19	0.00	**0.04**
HI hatchling gut vs Adult gut	1.00	4.24	44.36	0.43	0.00	**0.04**
HI hatchling gut vs Sand	1.00	1.32	16.81	0.24	0.00	**0.04**
HI hatchling gut vs Lab hatchling gut	1.00	0.76	9.74	0.17	0.00	**0.04**
HI hatchling gut vs Egg contents	1.00	3.31	30.62	0.35	0.00	**0.04**
HI hatchling gut vs Lab hatchling blood	1.00	1.24	14.56	0.23	0.00	**0.04**
HI hatchling gut vs Egg surface	1.00	1.04	12.82	0.23	0.00	**0.04**
HI hatchling gut vs Adult blood	1.00	0.79	9.88	0.19	0.00	**0.04**
HI hatchling blood vs Adult gut	1.00	3.59	16.11	0.22	0.00	**0.04**
HI hatchling blood vs Sand	1.00	1.51	6.84	0.12	0.00	**0.04**
HI hatchling blood vs Lab hatchling gut	1.00	1.09	4.69	0.09	0.00	**0.04**
HI hatchling blood vs Egg contents	1.00	1.57	6.52	0.11	0.00	**0.04**
HI hatchling blood vs Lab hatchling blood	1.00	0.63	2.63	0.05	0.01	0.09
HI hatchling blood vs Egg surface	1.00	0.73	2.92	0.06	0.01	0.07
HI hatchling blood vs Adult blood	1.00	0.37	1.46	0.03	0.11	0.30
Adult gut vs Sand	1.00	2.10	17.76	0.41	0.00	**0.04**
Adult gut vs Lab hatchling gut	1.00	2.01	16.28	0.43	0.00	**0.04**
Adult gut vs Egg contents	1.00	1.53	9.12	0.23	0.00	**0.04**
Adult gut vs Lab hatchling blood	1.00	1.80	12.89	0.37	0.00	**0.04**
Adult gut vs Egg surface	1.00	0.35	2.47	0.12	0.04	0.19
Adult gut vs Adult blood	1.00	0.83	5.79	0.25	0.01	0.10
Sand vs Lab hatchling gut	1.00	0.93	12.00	0.43	0.00	**0.04**
Sand vs Egg contents	1.00	1.32	8.88	0.27	0.00	**0.04**
Sand vs Lab hatchling blood	1.00	0.73	7.25	0.31	0.00	**0.04**
Sand vs Egg surface	1.00	0.59	6.60	0.35	0.00	0.06
Sand vs Adult blood	1.00	0.54	6.11	0.36	0.01	0.09
Lab hatchling gut vs Egg contents	1.00	1.50	9.32	0.32	0.00	**0.04**
Lab hatchling gut vs Lab hatchling blood	1.00	0.32	3.04	0.20	0.01	0.07
Lab hatchling gut vs Egg surface	1.00	0.89	9.87	0.55	0.00	0.05
Lab hatchling gut vs Adult blood	1.00	0.63	7.17	0.51	0.03	0.17
Egg contents vs Lab hatchling blood	1.00	0.88	4.91	0.20	0.00	**0.04**
Egg contents vs Egg surface	1.00	0.22	1.16	0.07	0.27	0.30
Egg contents vs Adult blood	1.00	0.31	1.59	0.10	0.02	0.13
Lab hatchling blood vs Egg surface	1.00	0.63	4.66	0.37	0.01	0.07
Lab hatchling blood vs Adult blood	1.00	0.37	2.67	0.28	0.04	0.19
Egg surface vs Adult blood	1.00	0.38	2.52	0.46	0.10	0.30

However, closer examination of individual hatchlings, with their biological mothers, showed significant differences between the adult and her hatchlings incubated in different conditions (Figure 4.4 – 4.6, Tables 4.7 – 4.9) (green16 weighted: df=2, SS_T=0.22, MS = 0.11, f.model=2.58, R^2=0.28, p=0.01; green16 unweighted: df=2, SS_T=0.57, MS = 0.28, f.model=4.53, R^2=0.41, p=0.01; green17 weighted: df=2, SS_T=0.32, MS = 0.16, f.model=4.65, R^2=0.51, p=0.009; green17 unweighted: df=2, SS_T=0.54, MS = 0.27, f.model=2.81, R^2=0.38, p=0.029; green18 weighted: df=2, SS_T=0.46, MS = 0.23, f.model=6.7, R^2=0.38, p=0.01; green18 unweighted: df=2, SS_T=1.25, MS = 0.63, f.model=15.7, R^2=0.59, p=0.01).

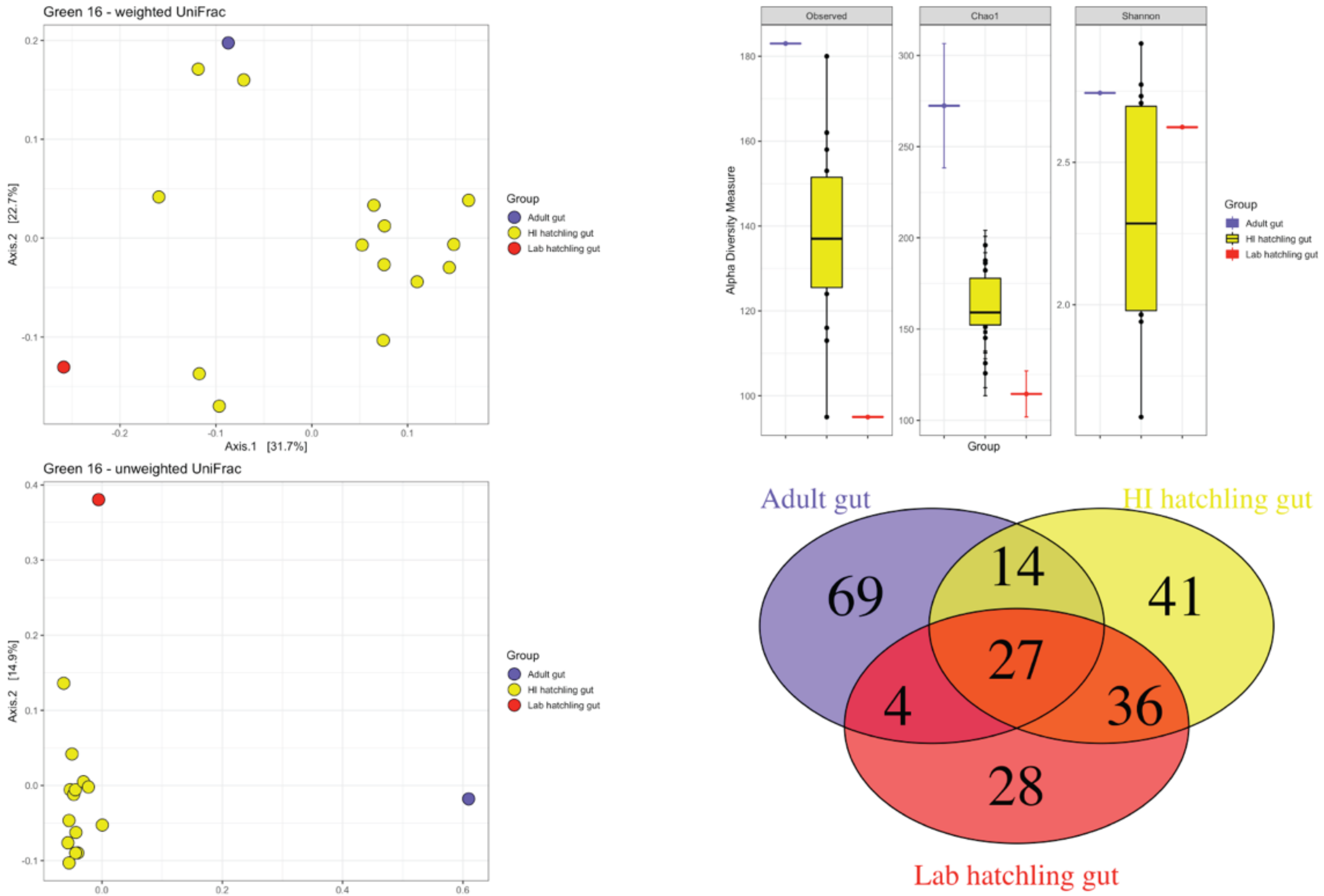

Figure 4.4 Summary of gut microbiota composition between adult turtle Green 16 and her biological offspring. Venn diagram represents shared OTUs.

Table 4.7 Shared and unique OTUs between gut samples of adult Green 16 and her biological offspring.

Taxa	Type	Details	Adult gut abundance	HI hatchling gut abundance	Lab hatchling gut abundance	Adult gut occurrence	HI hatchling gut occurrence	Lab hatchling gut occurrence
p__Proteobacteria__f__Pasteurellaceae_142268	unique	Adult gut	4.56	0	0	1	0	0
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	unique	Adult gut	3.66	0	0	1	0	0
p__Actinobacteria__o__03197L14_108564	unique	Adult gut	3.6	0	0	1	0	0
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	unique	Adult gut	0.01	0.214	0	1	0.27	0
p__Actinobacteria__g__Propionibacterium_427206	unique	Adult gut	2.49	0	0	1	0	0
p__Proteobacteria__g__Kaistia_437081	unique	Adult gut	2.06	0.002	0	1	0.2	0
p__Proteobacteria__g__Arcobacter_587211	unique	Adult gut	1.13	0	0	1	0	0
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	unique	Adult gut	0.64	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_12392	unique	Adult gut	0.59	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_396392	unique	Adult gut	0.55	0	0	1	0	0
p__SR1__c___571035	unique	Adult gut	0.53	0	0	1	0	0
p__Proteobacteria__f__Burkholderiaceae_432832	unique	Adult gut	0.52	0	0	1	0	0
p__Bacteroidetes__f__Porphyromonadaceae_463755	unique	Adult gut	0.5	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_253237	unique	Adult gut	0.48	0	0	1	0	0
p__Actinobacteria__g__Actinomyces_119009	unique	Adult gut	0.4	0	0	1	0	0
p__Proteobacteria__g__Pseudomonas_89597	unique	Adult gut	0.35	0	0	1	0	0
p__Proteobacteria__o__Myxococcales_668245	unique	Adult gut	0.28	0	0	1	0	0
p__Firmicutes__o__Clostridiales_226726	unique	Adult gut	0.27	0	0	1	0	0
p__Proteobacteria__g__Bosea_424613	unique	Adult gut	0.18	0.005	0	1	0.2	0
p__Proteobacteria__g__Acinetobacter_78571	unique	Adult gut	0.21	0.001	0	1	0.07	0
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	unique	Adult gut	0.22	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_572518	unique	Adult gut	0.21	0	0	1	0	0
p__Proteobacteria__f__Alcaligenaceae_536615	unique	Adult gut	0.2	0	0	1	0	0
p__Actinobacteria__g__Arthrobacter_694831	unique	Adult gut	0.18	0	0	1	0	0

p__Proteobacteria__f__Acetobacteraceae_509759	unique	Adult gut	0.17	0	0	1	0	0
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	unique	Adult gut	0.17	0	0	1	0	0
p__Firmicutes__g__Coprococcus_628747	unique	Adult gut	0.15	0	0	1	0	0
p__Proteobacteria__g__Pseudomonas_165065	unique	Adult gut	0.13	0	0	1	0	0
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	unique	Adult gut	0.13	0	0	1	0	0
p__Proteobacteria__f__SUP05_285720	unique	Adult gut	0.13	0	0	1	0	0
p__Tenericutes__g__Bulleidia_166205	unique	Adult gut	0.12	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_174851	unique	Adult gut	0.12	0	0	1	0	0
p__Tenericutes__g__Mycoplasma_551498	unique	Adult gut	0.09	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_90130	unique	Adult gut	0.09	0	0	1	0	0
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_585025	unique	Adult gut	0.09	0	0	1	0	0
p__Proteobacteria__f__Sinobacteraceae_155918	unique	Adult gut	0.01	0.005	0	1	0.27	0
p__Proteobacteria__f__Alcaligenaceae_720941	unique	Adult gut	0.08	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_448949	unique	Adult gut	0.07	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_589139	unique	Adult gut	0.07	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_398453	unique	Adult gut	0.07	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_732564	unique	Adult gut	0.06	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_63291	unique	Adult gut	0.06	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_384138	unique	Adult gut	0.06	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_729660	unique	Adult gut	0.06	0	0	1	0	0
p__Proteobacteria__g__Achromobacter_324477	unique	Adult gut	0.05	0	0	1	0	0
p__GN02__c__VC12cl04_595855	unique	Adult gut	0.05	0	0	1	0	0
p__GN02__c__VC12cl04_222792	unique	Adult gut	0.05	0	0	1	0	0
p__Firmicutes__o__Clostridiales_701726	unique	Adult gut	0.05	0	0	1	0	0
p__Firmicutes__o__Clostridiales_66573	unique	Adult gut	0.05	0	0	1	0	0
p__Proteobacteria__g__Arcobacter_130909	unique	Adult gut	0.04	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_733417	unique	Adult gut	0.03	0	0	1	0	0

p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Pelistega__s__Pelistegaeuropaea_183483	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Achromobacter_375749	unique	Adult gut	0.03	0	0	1	0	0
p__Bacteroidetes__g__Dysgonomonas_475976	unique	Adult gut	0.03	0	0	1	0	0
p__SR1__c___526194	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_444197	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__f__Rhodospirillaceae_362274	unique	Adult gut	0.01	0.001	0	1	0.07	0
p__Proteobacteria__f__Pasteurellaceae_88612	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__f__Enterobacteriaceae_696135	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__f__Alcaligenaceae_545115	unique	Adult gut	0.02	0	0	1	0	0
p__Fusobacteria__g__Streptobacillus__s__Streptobacillusmoniliformis_287396	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__o__Chromatiales_537609	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_366866	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__f__Campylobacteraceae_360616	unique	Adult gut	0.01	0	0	1	0	0
p__Fusobacteria__g__Streptobacillus_621876	unique	Adult gut	0.01	0	0	1	0	0
p__Firmicutes__f__Planococcaceae_668479	unique	Adult gut	0.01	0	0	1	0	0
p__Bacteroidetes__g__Tenacibaculum_428560	unique	Adult gut	0.01	0	0	1	0	0
p__Actinobacteria__g__Actinomyces__s__Actinomyceseuropaeus_648304	unique	Adult gut	0.01	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_555372	pan	Adult gut, HI hatchling gut	1.7	0.303	0	1	0.73	0
p__Firmicutes__g__Bacillus_535651	pan	Adult gut, HI hatchling gut	2.65	0.185	0	1	0.53	0
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	pan	Adult gut, HI hatchling gut	0.01	0.317	0	1	0.93	0
p__Proteobacteria__g__Pseudomonas_174392	pan	Adult gut, HI hatchling gut	0.01	0.096	0	1	0.87	0
p__Proteobacteria__g__Achromobacter_155903	pan	Adult gut, HI hatchling gut	0.01	0.075	0	1	1	0
p__Proteobacteria__g__Pseudomonas_161575	pan	Adult gut, HI hatchling gut	0.02	0.073	0	1	0.8	0
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	pan	Adult gut, HI hatchling gut	0.08	0.045	0	1	0.87	0
p__Proteobacteria__g__Pseudomonas_10820	pan	Adult gut, HI hatchling gut	0.01	0.043	0	1	0.4	0

p__Proteobacteria__o__Rhizobiales_602739	pan	Adult gut, HI hatchling gut	0.34	0.01	0	1	0.6	0
p__Firmicutes__g__Bacillus_161559	pan	Adult gut, HI hatchling gut	0.08	0.015	0	1	0.73	0
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_629547	pan	Adult gut, HI hatchling gut	0.01	0.017	0	1	0.47	0
p__Proteobacteria__f__Rhodospirillaceae_441088	pan	Adult gut, HI hatchling gut	0.01	0.013	0	1	0.8	0
p__Proteobacteria__g__Steroidobacter_168803	pan	Adult gut, HI hatchling gut	0.01	0.007	0	1	0.4	0
p__Actinobacteria__f__Solirubrobacteraceae_1327	pan	Adult gut, HI hatchling gut	0.01	0.006	0	1	0.53	0
p__Proteobacteria__g__Pseudomonas_639712	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.37	9.528	3.68	1	1	1
p__Proteobacteria__f__Pseudomonadaceae_79058	core	Adult gut, HI hatchling gut, Lab hatchling gut	14.6	7.563	5.87	1	1	1
p__Proteobacteria__g__Agrobacterium_442913	core	Adult gut, HI hatchling gut, Lab hatchling gut	16.79	4.706	2.9	1	1	1
p__Proteobacteria__g__Achromobacter_19649	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.45	4.725	10.45	1	1	1
p__Proteobacteria__g__Klebsiella_558424	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.02	3.809	0.12	1	0.87	1
p__Proteobacteria__g__Brucella_305522	core	Adult gut, HI hatchling gut, Lab hatchling gut	20.75	1.597	2.49	1	1	1
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	2.191	0.09	1	1	1
p__Actinobacteria__g__Arthrobacter_706755	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.07	1.729	4.73	1	0.93	1
p__Actinobacteria__g__Microbacterium__s__Microbacterium esteraromaticum_75491	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.568	11.7	1	1	1

p__Proteobacteria__g__Aquamicrobium_748021	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.17	0.619	0.87	1	1	1
p__Proteobacteria__g__Pseudomonas_34989	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.05	0.546	0.8	1	0.93	1
p__Proteobacteria__g__Brevundimonas__s__Brevundimonas diminuta_54795	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.54	0.385	0.1	1	1	1
p__Proteobacteria__g__Pseudomonas_159349	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.05	0.385	0.01	1	1	1
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.1	0.29	0.04	1	1	1
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.02	0.263	0.02	1	1	1
p__Proteobacteria__g__Shinella_393155	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.24	0.163	0.01	1	1	1
p__Proteobacteria__g__Lysobacter_318152	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.055	1.55	1	0.8	1
p__Actinobacteria__g__Leucobacter_697145	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.091	0.7	1	0.87	1
p__Proteobacteria__g__Achromobacter_468982	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.113	0.01	1	1	1
p__Proteobacteria__g__Shinella_386729	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.12	0.041	0.05	1	0.93	1
p__Proteobacteria__g__Devosia_700503	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.02	0.037	0.03	1	0.8	1

p__Proteobacteria__g__Nitratireductor_5045	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.03	0.026	0.15	1	0.87	1
p__Proteobacteria__g__Pseudomonas_40331	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.31	0.008	0.02	1	0.4	1
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.06	0.019	0.06	1	0.73	1
p__Proteobacteria__g__Agrobacterium_477436	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.12	0.017	0.01	1	0.6	1
p__Proteobacteria__g__Stenoxybacter_687490	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.05	0.019	0.04	1	0.67	1
p__Actinobacteria__g__Streptomyces_420197	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.03	0.014	0.01	1	0.6	1
p__Proteobacteria__g__Stenoxybacter_751919	pan	Adult gut, Lab hatchling gut	12.82	0	0.01	1	0	1
p__Proteobacteria__f__Rhodobacteraceae_649099	pan	Adult gut, Lab hatchling gut	0.41	0.009	0.05	1	0.27	1
p__Actinobacteria__g__Arthrobacter__s__Arthrobacterureafaciens_610242	pan	Adult gut, Lab hatchling gut	0.24	0.007	0.16	1	0.33	1
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	pan	Adult gut, Lab hatchling gut	0.01	0	0.01	1	0	1
p__Proteobacteria__f__Enterobacteriaceae_641353	unique	HI hatchling gut	0	21.767	0	0	1	0
p__Proteobacteria__g__Serratia__s__Serratiamarcescens_432257	unique	HI hatchling gut	0	6.261	0	0	0.4	0
p__Proteobacteria__f__Brucellaceae_698093	unique	HI hatchling gut	0	0.327	0	0	0.8	0
p__Proteobacteria__g__Stenotrophomonas_729526	unique	HI hatchling gut	0	0.316	0	0	0.8	0
p__Proteobacteria__f__Pseudomonadaceae_99078	unique	HI hatchling gut	0	0.265	0	0	0.8	0
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	unique	HI hatchling gut	0	0.247	0	0	0.93	0

p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	unique	HI hatchling gut	0	0.208	0	0	0.73	0
p__Firmicutes__g__Paenibacillus_212495	unique	HI hatchling gut	0	0.195	0	0	1	0
p__Firmicutes__g__Oceanobacillus_357784	unique	HI hatchling gut	0	0.193	0	0	1	0
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	unique	HI hatchling gut	0	0.175	0	0	0.87	0
p__Proteobacteria__g__Pseudomonas_132221	unique	HI hatchling gut	0	0.144	0	0	0.67	0
p__Proteobacteria__g__Stenotrophomonas_505642	unique	HI hatchling gut	0	0.105	0	0	0.93	0
p__Proteobacteria__f__Enterobacteriaceae_297365	unique	HI hatchling gut	0	0.099	0	0	0.67	0
p__Proteobacteria__g__Stenotrophomonas_657476	unique	HI hatchling gut	0	0.096	0	0	0.47	0
p__Proteobacteria__g__Pseudomonas_391544	unique	HI hatchling gut	0	0.092	0	0	0.8	0
p__Proteobacteria__g__Stenotrophomonas_531558	unique	HI hatchling gut	0	0.091	0	0	0.8	0
p__Proteobacteria__f__Acetobacteraceae_614109	unique	HI hatchling gut	0	0.089	0	0	0.8	0
p__Proteobacteria__g__Stenotrophomonas_502872	unique	HI hatchling gut	0	0.083	0	0	0.93	0
p__Proteobacteria__g__Enterobacter_572676	unique	HI hatchling gut	0	0.082	0	0	0.73	0
p__Bacteroidetes__f__Flavobacteriaceae_555335	unique	HI hatchling gut	0	0.074	0	0	0.4	0
p__Proteobacteria__g__Pseudomonas_572655	unique	HI hatchling gut	0	0.06	0	0	0.6	0
p__Proteobacteria__f__Alcaligenaceae_503155	unique	HI hatchling gut	0	0.054	0	0	0.8	0
p__Proteobacteria__g__Achromobacter_678564	unique	HI hatchling gut	0	0.049	0	0	0.73	0
p__Proteobacteria__g__Pseudomonas_403884	unique	HI hatchling gut	0	0.045	0	0	0.6	0
p__Proteobacteria__g__Pseudomonas_110207	unique	HI hatchling gut	0	0.045	0	0	0.73	0
p__Proteobacteria__g__Pseudomonas_511660	unique	HI hatchling gut	0	0.045	0	0	0.4	0
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	unique	HI hatchling gut	0	0.045	0	0	0.67	0
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	unique	HI hatchling gut	0	0.041	0	0	0.67	0
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	unique	HI hatchling gut	0	0.034	0	0	0.73	0
p__Proteobacteria__g__Pseudomonas_235588	unique	HI hatchling gut	0	0.032	0	0	0.6	0

p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	unique	HI hatchling gut	0	0.029	0	0	0.6	0
p__Proteobacteria__g__Achromobacter_376840	unique	HI hatchling gut	0	0.029	0	0	0.67	0
p__Actinobacteria__g__Leucobacter_418594	unique	HI hatchling gut	0	0.021	0	0	0.4	0
p__Proteobacteria__g__Pseudomonas_34101	unique	HI hatchling gut	0	0.02	0	0	0.6	0
p__Firmicutes__g__Bacillus_207834	unique	HI hatchling gut	0	0.011	0	0	0.53	0
p__Actinobacteria__f__Nocardioidaceae_554632	unique	HI hatchling gut	0	0.011	0	0	0.53	0
p__Proteobacteria__f__Rhodospirillaceae_494802	unique	HI hatchling gut	0	0.009	0	0	0.47	0
p__Proteobacteria__g__Paracoccus_110077	unique	HI hatchling gut	0	0.006	0	0	0.47	0
p__Proteobacteria__f__Xanthomonadaceae_702358	unique	HI hatchling gut	0	0.006	0	0	0.4	0
p__Gemmatimonadetes__o__Gemmatimonadales_757385	unique	HI hatchling gut	0	0.006	0	0	0.53	0
p__Firmicutes__g__Bacillus__s__Bacillusbarbaricus_663170	unique	HI hatchling gut	0	0.006	0	0	0.4	0
p__Proteobacteria__g__Stenotrophomonas_727055	pan	HI hatchling gut, Lab hatchling gut	0	13.815	34.43	0	1	1
p__Bacteroidetes__f__Flavobacteriaceae_374120	pan	HI hatchling gut, Lab hatchling gut	0	2.849	0.11	0	1	1
p__Bacteroidetes__g__Sphingobacterium_298499	pan	HI hatchling gut, Lab hatchling gut	0	2.003	0.08	0	0.93	1
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	pan	HI hatchling gut, Lab hatchling gut	0	1.646	1.04	0	1	1
p__Proteobacteria__f__Alcaligenaceae_541229	pan	HI hatchling gut, Lab hatchling gut	0	1.261	0.02	0	0.6	1
p__Firmicutes__g__Lysinibacillus_81925	pan	HI hatchling gut, Lab hatchling gut	0	1.055	1.02	0	0.93	1
p__Bacteroidetes__g__Sphingobacterium_176945	pan	HI hatchling gut, Lab hatchling gut	0	1.063	0.05	0	0.67	1
p__Bacteroidetes__g__Sphingobacterium_246851	pan	HI hatchling gut, Lab hatchling gut	0	0.44	1.29	0	0.53	1
p__Bacteroidetes__g__Sphingobacterium_204117	pan	HI hatchling gut, Lab hatchling gut	0	0.188	2.33	0	0.73	1

p__Proteobacteria__f__Enterobacteriaceae_171036	pan	HI hatchling gut, Lab hatchling gut	0	0.321	0.24	0	0.8	1
p__Proteobacteria__g__Stenotrophomonas_247119	pan	HI hatchling gut, Lab hatchling gut	0	0.277	0.01	0	0.93	1
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	pan	HI hatchling gut, Lab hatchling gut	0	0.265	0.05	0	0.87	1
p__Proteobacteria__g__Pseudomonas_142124	pan	HI hatchling gut, Lab hatchling gut	0	0.258	0.04	0	0.93	1
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	pan	HI hatchling gut, Lab hatchling gut	0	0.155	0.01	0	0.87	1
p__Bacteroidetes__g__Sphingobacterium_250053	pan	HI hatchling gut, Lab hatchling gut	0	0.142	0.05	0	0.93	1
p__Actinobacteria__g__Microbacterium_545806	pan	HI hatchling gut, Lab hatchling gut	0	0.037	1.19	0	0.53	1
p__Firmicutes__g__Paenibacillus_556209	pan	HI hatchling gut, Lab hatchling gut	0	0.073	0.2	0	0.87	1
p__Proteobacteria__g__Stenotrophomonas_168758	pan	HI hatchling gut, Lab hatchling gut	0	0.079	0.01	0	0.93	1
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	pan	HI hatchling gut, Lab hatchling gut	0	0.061	0.27	0	1	1
p__Proteobacteria__f__Alcaligenaceae_126597	pan	HI hatchling gut, Lab hatchling gut	0	0.077	0.01	0	0.93	1
p__Proteobacteria__g__Pseudomonas_466613	pan	HI hatchling gut, Lab hatchling gut	0	0.075	0.02	0	0.8	1
p__Proteobacteria__g__Pseudomonas_711667	pan	HI hatchling gut, Lab hatchling gut	0	0.075	0.01	0	0.8	1
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	pan	HI hatchling gut, Lab hatchling gut	0	0.061	0.1	0	0.93	1
p__Proteobacteria__g__Sphingopyxis_102703	pan	HI hatchling gut, Lab hatchling gut	0	0.037	0.38	0	0.73	1
p__Proteobacteria__g__Rheinheimera__s__Rheinheimeraaquimaris_486023	pan	HI hatchling gut, Lab hatchling gut	0	0.051	0.09	0	0.6	1

p__Proteobacteria__g__Stenotrophomonas_695630	pan	HI hatchling gut, Lab hatchling gut	0	0.049	0.06	0	0.87	1
p__Proteobacteria__g__Alcanivorax_427659	pan	HI hatchling gut, Lab hatchling gut	0	0.034	0.2	0	0.73	1
p__Proteobacteria__g__Ralstonia_478818	pan	HI hatchling gut, Lab hatchling gut	0	0.019	0.33	0	0.93	1
p__Bacteroidetes__g__Chitinophaga_80088	pan	HI hatchling gut, Lab hatchling gut	0	0.033	0.12	0	0.6	1
p__Actinobacteria__f__Nocardioidaceae_632222	pan	HI hatchling gut, Lab hatchling gut	0	0.017	0.35	0	0.8	1
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	pan	HI hatchling gut, Lab hatchling gut	0	0.006	0.3	0	0.4	1
p__Actinobacteria__g__Aeromicrobium_619093	pan	HI hatchling gut, Lab hatchling gut	0	0.019	0.06	0	0.73	1
p__Proteobacteria__o__Sphingomonadales_709703	pan	HI hatchling gut, Lab hatchling gut	0	0.011	0.09	0	0.6	1
p__Proteobacteria__f__Sphingomonadaceae_363679	pan	HI hatchling gut, Lab hatchling gut	0	0.01	0.09	0	0.8	1
p__Proteobacteria__g__Devosia_497251	pan	HI hatchling gut, Lab hatchling gut	0	0.012	0.03	0	0.67	1
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	pan	HI hatchling gut, Lab hatchling gut	0	0.006	0.09	0	0.47	1
p__Proteobacteria__g__Thermomonas_132820	unique	Lab hatchling gut	0	0.302	0.09	0	0.2	1
p__Bacteroidetes__g__Sphingobacterium_512955	unique	Lab hatchling gut	0	0.025	3.95	0	0.33	1
p__Bacteroidetes__o__Sphingobacteriales_152865	unique	Lab hatchling gut	0	0.12	0.48	0	0.13	1
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	unique	Lab hatchling gut	0	0.001	1.09	0	0.07	1
p__Proteobacteria__g__Pseudomonas_704131	unique	Lab hatchling gut	0	0.001	0.7	0	0.07	1
p__Proteobacteria__g__Delftia_587073	unique	Lab hatchling gut	0	0.008	0.35	0	0.27	1
p__Actinobacteria__g__Corynebacterium_68777	unique	Lab hatchling gut	0	0	0.43	0	0	1
p__Actinobacteria__g__Brevibacterium__s__Brevibacteriumaureum_674805	unique	Lab hatchling gut	0	0	0.42	0	0	1

p__Actinobacteria__g__Cellulosimicrobium_382216	unique	Lab hatchling gut	0	0.009	0.14	0	0.33	1
p__Firmicutes__g__Staphylococcus_171970	unique	Lab hatchling gut	0	0.003	0.12	0	0.27	1
p__Proteobacteria__g__Methylobacterium_99391	unique	Lab hatchling gut	0	0	0.12	0	0	1
p__Bacteroidetes__c__Flavobacteria_105335	unique	Lab hatchling gut	0	0	0.12	0	0	1
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasmendocina_126983	unique	Lab hatchling gut	0	0.003	0.05	0	0.2	1
p__Proteobacteria__f__Phyllobacteriaceae_589505	unique	Lab hatchling gut	0	0.006	0.01	0	0.33	1
p__Firmicutes__o__Clostridiales_748404	unique	Lab hatchling gut	0	0.005	0.01	0	0.07	1
p__Actinobacteria__f__Microbacteriaceae_486696	unique	Lab hatchling gut	0	0.001	0.07	0	0.07	1
p__Actinobacteria__f__Nocardioidaceae_115549	unique	Lab hatchling gut	0	0.001	0.05	0	0.13	1
p__Proteobacteria__f__Moraxellaceae_414919	unique	Lab hatchling gut	0	0.001	0.06	0	0.07	1
p__Actinobacteria__f__AKIW874_693966	unique	Lab hatchling gut	0	0	0.06	0	0	1
p__Proteobacteria__g__Xenophilus_188340	unique	Lab hatchling gut	0	0.002	0.02	0	0.2	1
p__Actinobacteria__g__Gordonia_255893	unique	Lab hatchling gut	0	0	0.05	0	0	1
p__Proteobacteria__g__Devosia_350266	unique	Lab hatchling gut	0	0.001	0.01	0	0.13	1
p__Actinobacteria__g__Dietzia__s__Dietziamaris_276529	unique	Lab hatchling gut	0	0	0.03	0	0	1
p__Actinobacteria__g__Dietzia_70231	unique	Lab hatchling gut	0	0.001	0.01	0	0.13	1
p__Spirochaetes__g__Brachyspira_69417	unique	Lab hatchling gut	0	0	0.02	0	0	1
p__Fusobacteria__g__Cetobacterium__s__Cetobacteriumsomerae_541959	unique	Lab hatchling gut	0	0	0.02	0	0	1
p__Firmicutes__f__Planococcaceae_152858	unique	Lab hatchling gut	0	0.001	0.01	0	0.07	1
p__Actinobacteria__g__Tropheryma_445164	unique	Lab hatchling gut	0	0	0.02	0	0	1

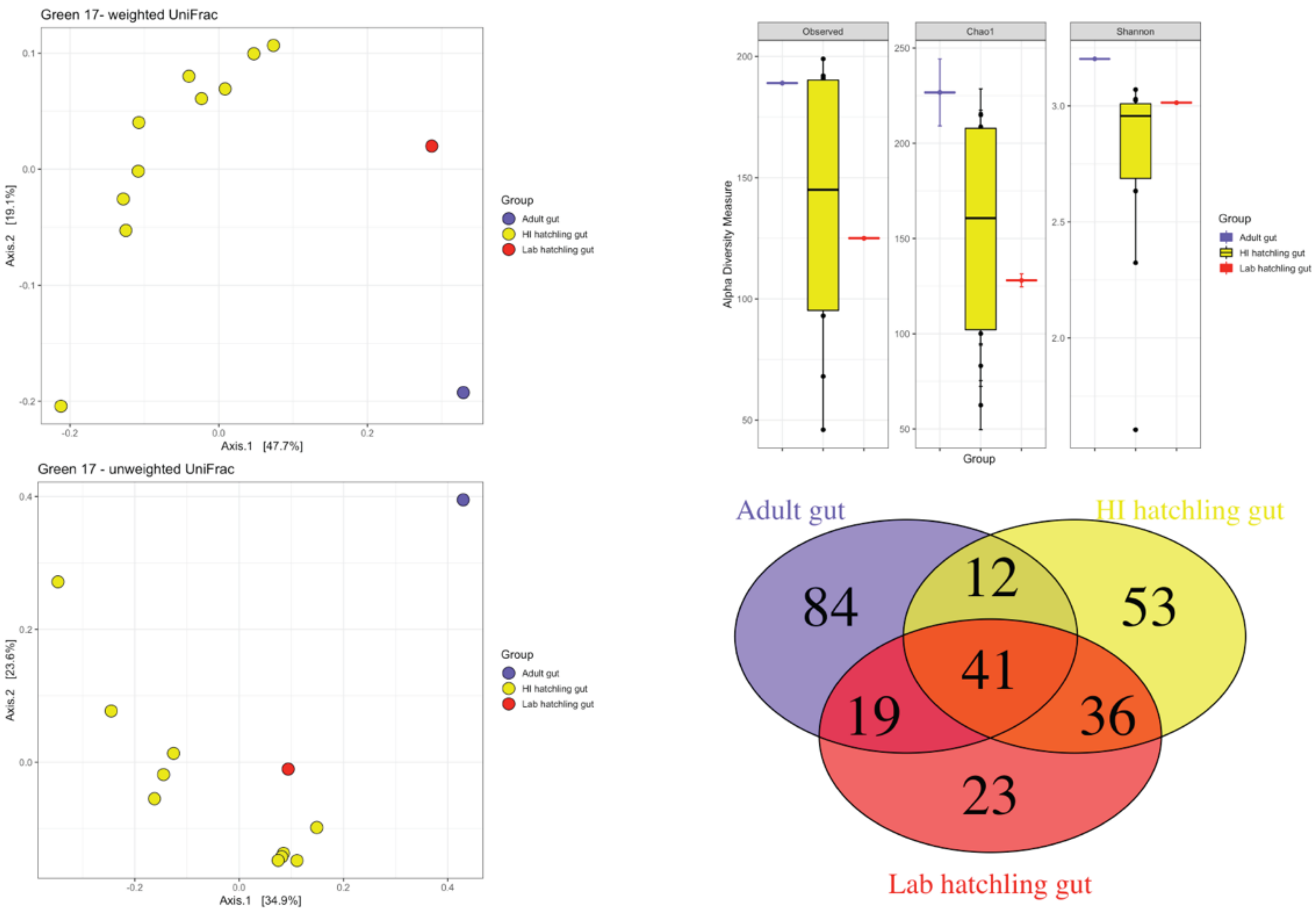

Figure 4.5 Summary of gut microbiota composition between adult turtle Green 17 and her biological offspring. Venn diagram represents shared OTUs.

Table 4.8 Shared and unique OTUs between gut samples of adult Green 17 and her biological offspring.

Taxa	Type	Details	Adult gut abundance	HI hatchling gut abundance	Lab hatchling gut abundance	Adult gut occurrence	Lab hatchling gut occurrence	HI hatchling gut occurrence
p__Proteobacteria__g__Stenoxybacter_751919	unique	Adult gut	9.02	0.001	0	1	0	0.1
p__Actinobacteria__g__Dermacoccus_337626	unique	Adult gut	8.08	0	0	1	0	0
p__Actinobacteria__o__03197L14_108564	unique	Adult gut	5.85	0	0	1	0	0
p__Gemmatimonadetes__o__Gemmatimonadales_757385	unique	Adult gut	1.14	0.004	0	1	0	0.2
p__Bacteroidetes__f__Flavobacteriaceae_12392	unique	Adult gut	1.06	0	0	1	0	0
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	unique	Adult gut	0.01	0.101	0	1	0	0.3
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	unique	Adult gut	1	0	0	1	0	0
p__Proteobacteria__g__Arcobacter_587211	unique	Adult gut	0.99	0	0	1	0	0
p__Proteobacteria__f__Pasteurellaceae_142268	unique	Adult gut	0.89	0	0	1	0	0
p__GN02__c__VC12cl04_595855	unique	Adult gut	0.79	0	0	1	0	0
p__Firmicutes__g__Bacillus__s__Bacillussp.NRRLB14911_432592	unique	Adult gut	0.21	0.046	0	1	0	0.2
p__Actinobacteria__o__koll13_76572	unique	Adult gut	0.56	0.005	0	1	0	0.2
p__Actinobacteria__g__Streptomyces_420197	unique	Adult gut	0.53	0.004	0	1	0	0.2
p__Actinobacteria__g__Arcanobacterium_246345	unique	Adult gut	0.56	0	0	1	0	0
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	unique	Adult gut	0.54	0	0	1	0	0
p__Actinobacteria__o__MC47_21675	unique	Adult gut	0.41	0.011	0	1	0	0.2
p__Proteobacteria__f__Rhodospirillaceae_622262	unique	Adult gut	0.51	0	0	1	0	0
p__Proteobacteria__f__Sinobacteraceae_232273	unique	Adult gut	0.49	0.001	0	1	0	0.1
p__Proteobacteria__f__SUP05_285720	unique	Adult gut	0.49	0	0	1	0	0
p__Proteobacteria__g__Amaricoccus_603022	unique	Adult gut	0.19	0.028	0	1	0	0.2
p__Proteobacteria__f__Sinobacteraceae_668199	unique	Adult gut	0.4	0.004	0	1	0	0.3
p__Proteobacteria__g__Acinetobacter_78571	unique	Adult gut	0.02	0.041	0	1	0	0.1
p__Actinobacteria__o__Solirubrobacterales_711193	unique	Adult gut	0.41	0.001	0	1	0	0.1

p__Actinobacteria__o__Actinomycetales_409160	unique	Adult gut	0.41	0.001	0	1	0	0.1
p__Chloroflexi__c__SOGA31_541293	unique	Adult gut	0.4	0	0	1	0	0
p__Actinobacteria__o__MC47_160960	unique	Adult gut	0.4	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_379313	unique	Adult gut	0.38	0	0	1	0	0
p__Proteobacteria__g__Rhodoplanes_273401	unique	Adult gut	0.28	0.009	0	1	0	0.1
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	unique	Adult gut	0.34	0	0	1	0	0
p__Proteobacteria__o__Rhodocyclales_496609	unique	Adult gut	0.29	0.002	0	1	0	0.2
p__Actinobacteria__o__Solirubrobacterales_238455	unique	Adult gut	0.26	0.003	0	1	0	0.3
p__SR1__c___571035	unique	Adult gut	0.28	0	0	1	0	0
p__Proteobacteria__f__Rhodospirillaceae_362274	unique	Adult gut	0.19	0.006	0	1	0	0.1
p__Chloroflexi__c__SOGA31_42333	unique	Adult gut	0.2	0.003	0	1	0	0.2
p__Proteobacteria__g__Pedomicrobium_452291	unique	Adult gut	0.2	0.001	0	1	0	0.1
p__Proteobacteria__f__Rhodospirillaceae_237338	unique	Adult gut	0.09	0.011	0	1	0	0.2
p__Proteobacteria__g__Stenoxybacter_396392	unique	Adult gut	0.19	0	0	1	0	0
p__Actinobacteria__o__Acidimicrobiales_244996	unique	Adult gut	0.19	0	0	1	0	0
p__Proteobacteria__f__Xanthomonadaceae_702358	unique	Adult gut	0.09	0.007	0	1	0	0.2
p__Proteobacteria__f__Rhodospirillaceae_494802	unique	Adult gut	0.14	0.001	0	1	0	0.1
p__Actinobacteria__g__Nocardioides_128926	unique	Adult gut	0.13	0.002	0	1	0	0.1
p__Proteobacteria__g__Pseudomonas_89597	unique	Adult gut	0.14	0	0	1	0	0
p__Actinobacteria__f__Solirubrobacteraceae_502654	unique	Adult gut	0.14	0	0	1	0	0
p__Firmicutes__g__Streptococcus_269499	unique	Adult gut	0.09	0.005	0	1	0	0.2
p__Actinobacteria__f__CL50029_390474	unique	Adult gut	0.13	0	0	1	0	0
p__Acidobacteria__o__Acidobacteriales_743933	unique	Adult gut	0.13	0	0	1	0	0
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	unique	Adult gut	0.11	0	0	1	0	0
p__Actinobacteria__g__Arthrobacter_694831	unique	Adult gut	0.09	0	0	1	0	0
p__Proteobacteria__f__Rhodospirillaceae_213569	unique	Adult gut	0.06	0.002	0	1	0	0.2
p__Actinobacteria__g__Mycobacterium_393834	unique	Adult gut	0.07	0.001	0	1	0	0.1

p__Actinobacteria__f__Micromonosporaceae_38158	unique	Adult gut	0.08	0	0	1	0	0
p__Proteobacteria__f__Campylobacteraceae_360616	unique	Adult gut	0.07	0	0	1	0	0
p__Firmicutes__o__Clostridiales_226726	unique	Adult gut	0.07	0	0	1	0	0
p__Firmicutes__g__Bacillus__s__Bacillusbarbaricus_663170	unique	Adult gut	0.07	0	0	1	0	0
p__Tenericutes__g__Mycoplasma_551498	unique	Adult gut	0.06	0	0	1	0	0
p__Proteobacteria__g__Pseudomonas_466613	unique	Adult gut	0.02	0.004	0	1	0	0.2
p__Bacteroidetes__g__Bacteroides_689514	unique	Adult gut	0.06	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_448949	unique	Adult gut	0.04	0	0	1	0	0
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	unique	Adult gut	0.04	0	0	1	0	0
p__Firmicutes__o__Clostridiales_701726	unique	Adult gut	0.04	0	0	1	0	0
p__Firmicutes__g__Bacillus_207834	unique	Adult gut	0.03	0.001	0	1	0	0.1
p__Proteobacteria__g__Sulfurimonas_444197	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_253237	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Pseudomonas_165065	unique	Adult gut	0.03	0	0	1	0	0
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	unique	Adult gut	0.01	0.002	0	1	0	0.1
p__Bacteroidetes__o__Bacteroidales_330844	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_51163	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_174851	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__g__Arcobacter_130909	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__g__Achromobacter_324477	unique	Adult gut	0.02	0	0	1	0	0
p__Proteobacteria__f__Burkholderiaceae_432832	unique	Adult gut	0.02	0	0	1	0	0
p__SR1__c___3865	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__o__Myxococcales_668245	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_317953	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__g__Pseudoruegeria_390046	unique	Adult gut	0.01	0	0	1	0	0
p__Proteobacteria__f__Alcaligenaceae_536615	unique	Adult gut	0.01	0	0	1	0	0

p__Fusobacteria__g__Streptobacillus__s__Streptobacillusmoniliformis_287396	unique	Adult gut	0.01	0	0	1	0	0
p__Bacteroidetes__o__Bacteroidales_711203	unique	Adult gut	0.01	0	0	1	0	0
p__Bacteroidetes__g__Pontibacter__s__Pontibacterkorlensis_406748	unique	Adult gut	0.01	0	0	1	0	0
p__Actinobacteria__o__03197L14_629656	unique	Adult gut	0.01	0	0	1	0	0
p__Actinobacteria__g__Propionibacterium_427206	unique	Adult gut	0.01	0	0	1	0	0
p__Actinobacteria__g__Actinotalea_764221	unique	Adult gut	0.01	0	0	1	0	0
p__Actinobacteria__g__Actinomyces_119009	unique	Adult gut	0.01	0	0	1	0	0
p__Firmicutes__g__Bacillus_535651	pan	Adult gut, HI hatchling gut	0.63	0.74	0	1	0	0.5
p__Bacteroidetes__g__Wautersiella_232450	pan	Adult gut, HI hatchling gut	0.01	0.349	0	1	0	1
p__Firmicutes__g__Lysinibacillus_81925	pan	Adult gut, HI hatchling gut	0.05	0.15	0	1	0	0.9
p__Actinobacteria__f__Solirubrobacteraceae_1327	pan	Adult gut, HI hatchling gut	0.86	0.019	0	1	0	0.4
p__Proteobacteria__g__Steroidobacter_168803	pan	Adult gut, HI hatchling gut	0.33	0.045	0	1	0	0.5
p__Proteobacteria__f__Rhodospirillaceae_441088	pan	Adult gut, HI hatchling gut	0.58	0.009	0	1	0	0.5
p__Firmicutes__g__Bacillus_161559	pan	Adult gut, HI hatchling gut	0.54	0.006	0	1	0	0.6
p__Proteobacteria__g__Pseudomonas_40331	pan	Adult gut, HI hatchling gut	0.12	0.046	0	1	0	0.8
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	pan	Adult gut, HI hatchling gut	0.11	0.031	0	1	0	0.5
p__Proteobacteria__g__Xenophilus_188340	pan	Adult gut, HI hatchling gut	0.19	0.013	0	1	0	0.6
p__Firmicutes__g__Oceanobacillus_357784	pan	Adult gut, HI hatchling gut	0.07	0.018	0	1	0	0.4
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_629547	pan	Adult gut, HI hatchling gut	0.01	0.015	0	1	0	0.4
p__Proteobacteria__g__Klebsiella_558424	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.15	27.744	6.71	1	1	1
p__Proteobacteria__f__Pseudomonadaceae_79058	core	Adult gut, HI hatchling gut, Lab hatchling gut	30.54	6.943	13.06	1	1	1
p__Proteobacteria__g__Achromobacter_19649	core	Adult gut, HI hatchling gut, Lab hatchling gut	3.49	3.63	8.7	1	1	1

p__Proteobacteria__g__Stenotrophomonas_727055	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.1	2.423	12.96	1	1	1
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.19	2.902	0.37	1	1	1
p__Actinobacteria__g__Microbacterium__s__Microbacterium esteraromaticum_75491	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.37	0.673	13.25	1	1	1
p__Proteobacteria__g__Aquamicrobium_748021	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.1	1.838	1.53	1	1	1
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.15	1.515	0.1	1	1	1
p__Proteobacteria__g__Brucella_305522	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.61	0.581	8.58	1	1	0.9
p__Actinobacteria__g__Leucobacter_697145	core	Adult gut, HI hatchling gut, Lab hatchling gut	4.55	0.373	1.46	1	1	1
p__Proteobacteria__g__Pseudomonas_639712	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.14	0.932	0.09	1	1	0.9
p__Proteobacteria__g__Pseudomonas_34989	core	Adult gut, HI hatchling gut, Lab hatchling gut	4.21	0.227	0.58	1	1	1
p__Proteobacteria__g__Agrobacterium_442913	core	Adult gut, HI hatchling gut, Lab hatchling gut	1.89	0.346	1.62	1	1	0.9
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.08	0.322	0.1	1	1	0.9
p__Proteobacteria__g__Brevundimonas__s__Brevundimonas diminuta_54795	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.11	0.256	0.71	1	1	0.9

p__Actinobacteria__g__Arthrobacter_706755	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.49	0.171	0.29	1	1	0.9
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.09	0.222	0.03	1	1	0.7
p__Actinobacteria__g__Aeromicrobium_619093	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.81	0.075	0.17	1	1	0.8
p__Proteobacteria__g__Shinella_393155	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.28	0.133	0.08	1	1	0.8
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasmendocina_126983	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.03	0.1	0.54	1	1	0.5
p__Proteobacteria__g__Devosia_497251	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.24	0.079	0.39	1	1	0.7
p__Proteobacteria__f__Bradyrhizobiaceae_116562	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.14	0.122	0.02	1	1	0.4
p__Proteobacteria__f__Sphingomonadaceae_363679	core	Adult gut, HI hatchling gut, Lab hatchling gut	1.17	0.012	0.08	1	1	0.5
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.07	0.119	0.03	1	1	0.9
p__Proteobacteria__f__Phyllobacteriaceae_589505	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.03	0.113	0.02	1	1	0.8
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.02	0.104	0.07	1	1	1
p__Proteobacteria__g__Devosia_700503	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.44	0.046	0.11	1	1	0.5

p__Proteobacteria__o__Sphingomonadales_709703	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.08	0.079	0.05	1	1	0.7
p__Proteobacteria__g__Sphingopyxis_102703	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.1	0.068	0.08	1	1	0.7
p__Firmicutes__g__Paenibacillus_556209	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.04	0.071	0.04	1	1	0.6
p__Actinobacteria__f__Nocardioidaceae_632222	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.47	0.012	0.15	1	1	0.6
p__Actinobacteria__f__Nocardioidaceae_554632	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.069	0.04	1	1	0.7
p__Proteobacteria__g__Pseudomonas_161575	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.07	0.041	0.07	1	1	0.5
p__Actinobacteria__g__Cellulosimicrobium_382216	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.03	0.017	0.35	1	1	0.5
p__Proteobacteria__g__Pseudomonas_159349	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.05	0.047	0.02	1	1	0.6
p__Proteobacteria__g__Nitratireductor_5045	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.12	0.012	0.26	1	1	0.5
p__Proteobacteria__g__Achromobacter_468982	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.05	0.033	0.06	1	1	0.5
p__Proteobacteria__g__Achromobacter_155903	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.01	0.032	0.02	1	1	0.5
p__Actinobacteria__g__Dietzia_70231	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.02	0.007	0.12	1	1	0.4

p__Proteobacteria__o__Rhizobiales_602739	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.04	0.006	0.1	1	1	0.4
p__Proteobacteria__g__Stenoxybacter_687490	core	Adult gut, HI hatchling gut, Lab hatchling gut	0.07	0.007	0.02	1	1	0.4
p__Bacteroidetes__g__Chryseobacterium_308134	pan	Adult gut, Lab hatchling gut	0.01	0.006	5.16	1	1	0.3
p__Chloroflexi__c__SOGA31_314194	pan	Adult gut, Lab hatchling gut	1.26	0.002	0.04	1	1	0.1
p__Actinobacteria__g__Arthrobacter__s__Arthrobacterureafaciens_610242	pan	Adult gut, Lab hatchling gut	0.7	0.001	0.1	1	1	0.1
p__Proteobacteria__g__Bosea_424613	pan	Adult gut, Lab hatchling gut	0.38	0.002	0.11	1	1	0.2
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	pan	Adult gut, Lab hatchling gut	0.31	0.008	0.08	1	1	0.2
p__Actinobacteria__g__Iamia_160105	pan	Adult gut, Lab hatchling gut	0.37	0	0.02	1	1	0
p__Proteobacteria__g__Kaistobacter_682533	pan	Adult gut, Lab hatchling gut	0.3	0.002	0.01	1	1	0.1
p__Proteobacteria__g__Ralstonia_478818	pan	Adult gut, Lab hatchling gut	0.15	0.005	0.02	1	1	0.3
p__Proteobacteria__g__Devosia_350266	pan	Adult gut, Lab hatchling gut	0.18	0.001	0.01	1	1	0.1
p__Actinobacteria__g__Janibacter_703951	pan	Adult gut, Lab hatchling gut	0.11	0.005	0.02	1	1	0.3
p__Proteobacteria__f__Sinobacteraceae_155918	pan	Adult gut, Lab hatchling gut	0.11	0.005	0.01	1	1	0.3
p__Actinobacteria__g__Pimelobacter_394756	pan	Adult gut, Lab hatchling gut	0.04	0.009	0.02	1	1	0.3
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	pan	Adult gut, Lab hatchling gut	0.02	0	0.11	1	1	0

p__Proteobacteria__g__Achromobacter_376840	pan	Adult gut, Lab hatchling gut	0.01	0.009	0.02	1	1	0.2
p__Actinobacteria__g__Microbacterium_545806	pan	Adult gut, Lab hatchling gut	0.01	0.002	0.09	1	1	0.2
p__Actinobacteria__f__Nocardioidaceae_115549	pan	Adult gut, Lab hatchling gut	0.01	0.008	0.03	1	1	0.2
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	pan	Adult gut, Lab hatchling gut	0.05	0.004	0.02	1	1	0.2
p__Firmicutes__g__Staphylococcus_171970	pan	Adult gut, Lab hatchling gut	0.03	0	0.01	1	1	0
p__Actinobacteria__f__AKIW874_693966	pan	Adult gut, Lab hatchling gut	0.01	0	0.02	1	1	0
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	unique	HI hatchling gut	0	4.731	0	0	0	0.9
p__Bacteroidetes__g__Sphingobacterium_176945	unique	HI hatchling gut	0	3.379	0	0	0	1
p__Proteobacteria__f__Enterobacteriaceae_641353	unique	HI hatchling gut	0	2.755	0	0	0	1
p__Proteobacteria__g__Acinetobacter_168646	unique	HI hatchling gut	0	1.802	0	0	0	0.9
p__Firmicutes__g__Paenibacillus_212495	unique	HI hatchling gut	0	1.571	0	0	0	0.7
p__Bacteroidetes__g__Myroides_398565	unique	HI hatchling gut	0	1.521	0	0	0	0.9
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	unique	HI hatchling gut	0	1.309	0	0	0	0.9
p__Proteobacteria__f__Alcaligenaceae_662472	unique	HI hatchling gut	0	0.677	0	0	0	0.9
p__Bacteroidetes__g__Myroides__s__Myroidesodoratimimus_489867	unique	HI hatchling gut	0	0.649	0	0	0	0.9
p__Bacteroidetes__g__Myroides__s__Myroidesodoratimimus_295565	unique	HI hatchling gut	0	0.46	0	0	0	0.9
p__Firmicutes__o__Clostridiales_748404	unique	HI hatchling gut	0	0.421	0	0	0	0.4
p__Bacteroidetes__f__Flexibacteraceae_122867	unique	HI hatchling gut	0	0.414	0	0	0	0.8
p__Firmicutes__g__Enterococcus_396129	unique	HI hatchling gut	0	0.401	0	0	0	0.8
p__Bacteroidetes__f__Cryomorphaceae_690566	unique	HI hatchling gut	0	0.3	0	0	0	0.9
p__Proteobacteria__f__Enterobacteriaceae_172645	unique	HI hatchling gut	0	0.299	0	0	0	0.7

p__Proteobacteria__f__Enterobacteriaceae_245606	unique	HI hatchling gut	0	0.229	0	0	0	0.6
p__Actinobacteria__g__Tessaracoccus_287453	unique	HI hatchling gut	0	0.227	0	0	0	0.7
p__Proteobacteria__f__Rhodospirillaceae_424659	unique	HI hatchling gut	0	0.182	0	0	0	0.9
p__Bacteroidetes__o__Sphingobacteriales_571041	unique	HI hatchling gut	0	0.163	0	0	0	0.8
p__Proteobacteria__g__Devosia_749390	unique	HI hatchling gut	0	0.142	0	0	0	0.7
p__TM7__o__EW055_125519	unique	HI hatchling gut	0	0.117	0	0	0	0.4
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	unique	HI hatchling gut	0	0.089	0	0	0	0.6
p__Proteobacteria__g__Rhodobacter_747540	unique	HI hatchling gut	0	0.087	0	0	0	0.6
p__Proteobacteria__g__Pseudomonas_132221	unique	HI hatchling gut	0	0.082	0	0	0	0.7
p__Firmicutes__g__Bacillus_245239	unique	HI hatchling gut	0	0.076	0	0	0	0.7
p__Proteobacteria__f__Enterobacteriaceae_231744	unique	HI hatchling gut	0	0.075	0	0	0	0.7
p__Proteobacteria__g__Pseudomonas_235588	unique	HI hatchling gut	0	0.072	0	0	0	0.6
p__Proteobacteria__g__Paracoccus__s__Paracoccussolventivorans_641772	unique	HI hatchling gut	0	0.068	0	0	0	0.6
p__Proteobacteria__g__Klebsiella_430511	unique	HI hatchling gut	0	0.064	0	0	0	0.7
p__Proteobacteria__g__Klebsiella_664333	unique	HI hatchling gut	0	0.057	0	0	0	0.7
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	unique	HI hatchling gut	0	0.054	0	0	0	0.7
p__Proteobacteria__g__Enterobacter_572676	unique	HI hatchling gut	0	0.051	0	0	0	0.7
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	unique	HI hatchling gut	0	0.048	0	0	0	0.8
p__Proteobacteria__g__Paracoccus_110077	unique	HI hatchling gut	0	0.036	0	0	0	0.4
p__Bacteroidetes__g__Dyadobacter_763044	unique	HI hatchling gut	0	0.036	0	0	0	0.4
p__Proteobacteria__f__Alcaligenaceae_503155	unique	HI hatchling gut	0	0.035	0	0	0	0.7
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	unique	HI hatchling gut	0	0.033	0	0	0	0.5
p__Proteobacteria__f__Enterobacteriaceae_84333	unique	HI hatchling gut	0	0.033	0	0	0	0.7
p__Proteobacteria__g__Diaphorobacter_687258	unique	HI hatchling gut	0	0.031	0	0	0	0.5

p__Proteobacteria__g__Erwinia_25313	unique	HI hatchling gut	0	0.029	0	0	0	0.6
p__Proteobacteria__g__Achromobacter_678564	unique	HI hatchling gut	0	0.029	0	0	0	0.5
p__Proteobacteria__g__Klebsiella_347396	unique	HI hatchling gut	0	0.027	0	0	0	0.5
p__Firmicutes__g__Tissierella_366131	unique	HI hatchling gut	0	0.026	0	0	0	0.6
p__Proteobacteria__g__Shinella_386729	unique	HI hatchling gut	0	0.017	0	0	0	0.4
p__Proteobacteria__g__Pseudomonas_308359	unique	HI hatchling gut	0	0.017	0	0	0	0.7
p__Proteobacteria__f__Xanthomonadaceae_258602	unique	HI hatchling gut	0	0.017	0	0	0	0.5
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	unique	HI hatchling gut	0	0.015	0	0	0	0.5
p__Proteobacteria__g__Pseudomonas_391544	unique	HI hatchling gut	0	0.012	0	0	0	0.5
p__Proteobacteria__o__Rhizobiales_675457	unique	HI hatchling gut	0	0.009	0	0	0	0.5
p__Proteobacteria__f__Acetobacteraceae_614109	unique	HI hatchling gut	0	0.008	0	0	0	0.5
p__Proteobacteria__g__Pseudomonas_110207	unique	HI hatchling gut	0	0.007	0	0	0	0.4
p__Proteobacteria__g__Pseudomonas_403884	unique	HI hatchling gut	0	0.005	0	0	0	0.4
p__Proteobacteria__f__Enterobacteriaceae_297365	unique	HI hatchling gut	0	0.004	0	0	0	0.4
p__Proteobacteria__f__Enterobacteriaceae_171036	pan	HI hatchling gut, Lab hatchling gut	0	7.178	0.02	0	1	1
p__Proteobacteria__f__Brucellaceae_698093	pan	HI hatchling gut, Lab hatchling gut	0	4.042	0.06	0	1	1
p__Proteobacteria__g__Thermomonas_132820	pan	HI hatchling gut, Lab hatchling gut	0	3.841	0.3	0	1	1
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	pan	HI hatchling gut, Lab hatchling gut	0	2.449	0.02	0	1	1
p__Proteobacteria__g__Stenotrophomonas_657476	pan	HI hatchling gut, Lab hatchling gut	0	0.605	0.04	0	1	0.8
p__Bacteroidetes__f__Flavobacteriaceae_374120	pan	HI hatchling gut, Lab hatchling gut	0	0.264	2.84	0	1	0.9
p__Bacteroidetes__g__Sphingobacterium_298499	pan	HI hatchling gut, Lab hatchling gut	0	0.162	3.16	0	1	0.8

p__Proteobacteria__f__Alcaligenaceae_541229	pan	HI hatchling gut, Lab hatchling gut	0	0.368	0.02	0	1	0.9
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	pan	HI hatchling gut, Lab hatchling gut	0	0.121	2.45	0	1	0.6
p__Bacteroidetes__g__Sphingobacterium_250053	pan	HI hatchling gut, Lab hatchling gut	0	0.155	0.8	0	1	0.7
p__Proteobacteria__g__Lysobacter_318152	pan	HI hatchling gut, Lab hatchling gut	0	0.146	0.81	0	1	0.8
p__Bacteroidetes__f__Flavobacteriaceae_555335	pan	HI hatchling gut, Lab hatchling gut	0	0.2	0.04	0	1	0.8
p__Proteobacteria__g__Pseudomonas_10820	pan	HI hatchling gut, Lab hatchling gut	0	0.192	0.07	0	1	0.8
p__Proteobacteria__f__Enterobacteriaceae_452665	pan	HI hatchling gut, Lab hatchling gut	0	0.165	0.01	0	1	0.8
p__Proteobacteria__g__Pseudomonas_174392	pan	HI hatchling gut, Lab hatchling gut	0	0.16	0.04	0	1	0.7
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	pan	HI hatchling gut, Lab hatchling gut	0	0.138	0.01	0	1	0.8
p__Proteobacteria__g__Pseudomonas_572655	pan	HI hatchling gut, Lab hatchling gut	0	0.127	0.06	0	1	0.6
p__Bacteroidetes__g__Sphingobacterium_204117	pan	HI hatchling gut, Lab hatchling gut	0	0.053	0.69	0	1	0.4
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	pan	HI hatchling gut, Lab hatchling gut	0	0.086	0.04	0	1	0.6
p__Proteobacteria__f__Enterobacteriaceae_410502	pan	HI hatchling gut, Lab hatchling gut	0	0.077	0.04	0	1	0.7
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	pan	HI hatchling gut, Lab hatchling gut	0	0.047	0.29	0	1	0.6
p__Proteobacteria__f__Pseudomonadaceae_99078	pan	HI hatchling gut, Lab hatchling gut	0	0.073	0.02	0	1	0.5
p__Proteobacteria__g__Klebsiella_117934	pan	HI hatchling gut, Lab hatchling gut	0	0.053	0.03	0	1	0.8

p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	pan	HI hatchling gut, Lab hatchling gut	0	0.055	0.01	0	1	0.6
p__Proteobacteria__g__Stenotrophomonas_729526	pan	HI hatchling gut, Lab hatchling gut	0	0.049	0.02	0	1	0.5
p__Proteobacteria__f__Acetobacteraceae_741892	pan	HI hatchling gut, Lab hatchling gut	0	0.047	0.03	0	1	0.7
p__Proteobacteria__g__Stenotrophomonas_695630	pan	HI hatchling gut, Lab hatchling gut	0	0.043	0.04	0	1	0.9
p__Proteobacteria__g__Stenotrophomonas_247119	pan	HI hatchling gut, Lab hatchling gut	0	0.038	0.09	0	1	0.6
p__Proteobacteria__g__Delftia_587073	pan	HI hatchling gut, Lab hatchling gut	0	0.036	0.05	0	1	0.5
p__Actinobacteria__g__Rhodococcus_413286	pan	HI hatchling gut, Lab hatchling gut	0	0.032	0.02	0	1	0.7
p__Proteobacteria__g__Pseudomonas_281622	pan	HI hatchling gut, Lab hatchling gut	0	0.025	0.01	0	1	0.4
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	pan	HI hatchling gut, Lab hatchling gut	0	0.005	0.19	0	1	0.4
p__Proteobacteria__g__Pseudomonas_711667	pan	HI hatchling gut, Lab hatchling gut	0	0.015	0.07	0	1	0.4
p__Proteobacteria__g__Stenotrophomonas_531558	pan	HI hatchling gut, Lab hatchling gut	0	0.019	0.02	0	1	0.5
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	pan	HI hatchling gut, Lab hatchling gut	0	0.017	0.03	0	1	0.4
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	pan	HI hatchling gut, Lab hatchling gut	0	0.007	0.01	0	1	0.4
p__Bacteroidetes__g__Chryseobacterium_171211	unique	Lab hatchling gut	0	0	3.19	0	1	0
p__Bacteroidetes__g__Sphingobacterium_246851	unique	Lab hatchling gut	0	0.001	2.36	0	1	0.1
p__Bacteroidetes__g__Sphingobacterium_512955	unique	Lab hatchling gut	0	0.005	1.16	0	1	0.2
p__Proteobacteria__g__Alcanivorax_427659	unique	Lab hatchling gut	0	0.056	0.14	0	1	0.3
p__Actinobacteria__g__Dietzia__s__Dietziamaris_276529	unique	Lab hatchling gut	0	0	0.54	0	1	0

p__Bacteroidetes__o__Sphingobacteriales_152865	unique	Lab hatchling gut	0	0.001	0.32	0	1	0.1
p__Proteobacteria__f__Rhodobacteraceae_649099	unique	Lab hatchling gut	0	0.02	0.09	0	1	0.3
p__Proteobacteria__g__Stenotrophomonas_505642	unique	Lab hatchling gut	0	0.018	0.06	0	1	0.3
p__Proteobacteria__g__Pseudomonas_704131	unique	Lab hatchling gut	0	0.014	0.05	0	1	0.1
p__Bacteroidetes__f__Sphingobacteriaceae_540060	unique	Lab hatchling gut	0	0.002	0.17	0	1	0.1
p__Proteobacteria__f__Alcaligenaceae_126597	unique	Lab hatchling gut	0	0.014	0.04	0	1	0.3
p__Proteobacteria__g__Stenotrophomonas_168758	unique	Lab hatchling gut	0	0.009	0.03	0	1	0.3
p__Proteobacteria__g__Agrobacterium_477436	unique	Lab hatchling gut	0	0.003	0.08	0	1	0.1
p__Actinobacteria__g__Brevibacterium__s__Brevibacteriumaureum_674805	unique	Lab hatchling gut	0	0	0.11	0	1	0
p__Proteobacteria__g__Stenotrophomonas_502872	unique	Lab hatchling gut	0	0.005	0.05	0	1	0.3
p__Bacteroidetes__c__Flavobacteria_105335	unique	Lab hatchling gut	0	0	0.09	0	1	0
p__Actinobacteria__g__Leucobacter_418594	unique	Lab hatchling gut	0	0.007	0.02	0	1	0.3
p__Proteobacteria__g__Pseudomonas_142124	unique	Lab hatchling gut	0	0.004	0.04	0	1	0.3
p__Proteobacteria__g__Rheinheimera__s__Rheinheimeraaquimaris_486023	unique	Lab hatchling gut	0	0.003	0.02	0	1	0.2
p__Proteobacteria__g__Shewanella__s__Shewanellaalgae_485511	unique	Lab hatchling gut	0	0	0.02	0	1	0
p__Bacteroidetes__g__Chitinophaga_80088	unique	Lab hatchling gut	0	0	0.02	0	1	0
p__Actinobacteria__f__Microbacteriaceae_486696	unique	Lab hatchling gut	0	0	0.02	0	1	0
p__Actinobacteria__g__Sporichthya_500877	unique	Lab hatchling gut	0	0	0.01	0	1	0

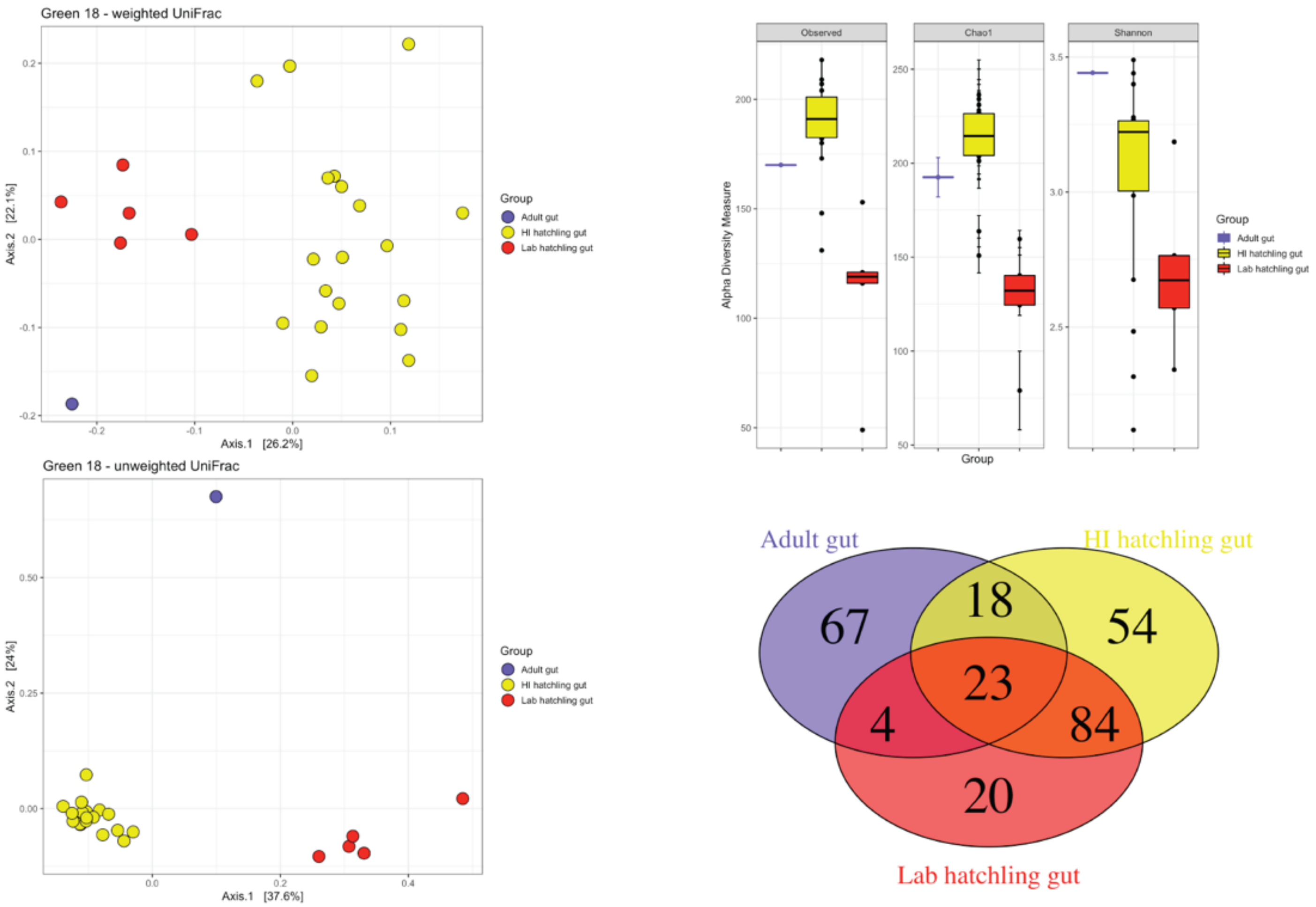

Figure 4.6 Summary of gut microbiota composition between adult turtle Green 18 and her biological offspring. Venn diagram represents shared OTUs.

Table 4.9 Shared and unique OTUs between gut samples of adult Green 18 and her biological offspring.

Taxa	Type	Details	Adult gut abundance	HI hatchling gut abundance	Lab hatchling gut abundance	Adult gut occurrence	HI hatchling gut occurrence	Lab hatchling gut occurrence
p__Proteobacteria__f__SUP05_285720	unique	Adult gut	12.65	0	0	1	0	0
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	unique	Adult gut	10.97	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_751919	unique	Adult gut	10.86	0.001	0	1	0.05	0
p__Proteobacteria__g__Arcobacter_587211	unique	Adult gut	6.43	0	0	1	0	0
p__Actinobacteria__g__Arcanobacterium_246345	unique	Adult gut	4.5	0	0	1	0	0
p__Proteobacteria__o__Myxococcales_668245	unique	Adult gut	3.82	0	0	1	0	0
p__Actinobacteria__o__03197L14_108564	unique	Adult gut	3.01	0	0	1	0	0
p__Firmicutes__g__Coprococcus_312354	unique	Adult gut	2.98	0	0	1	0	0
p__Proteobacteria__g__Arcobacter_130909	unique	Adult gut	2.69	0	0	1	0	0
p__Firmicutes__g__Coprococcus_517519	unique	Adult gut	1.98	0	0	1	0	0
p__Proteobacteria__g__Pseudoruegeria_390046	unique	Adult gut	1.82	0	0	1	0	0
p__Proteobacteria__f__Rhodobacteraceae_729919	unique	Adult gut	1.69	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_379313	unique	Adult gut	1.69	0	0	1	0	0
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	unique	Adult gut	1.65	0	0	1	0	0
p__Proteobacteria__f__Campylobacteraceae_360616	unique	Adult gut	1.57	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_444197	unique	Adult gut	1.51	0	0	1	0	0
p__Bacteroidetes__f__Porphyromonadaceae_463755	unique	Adult gut	1.23	0	0	1	0	0
p__Bacteroidetes__g__Bacteroides_689514	unique	Adult gut	1.06	0	0	1	0	0
p__Firmicutes__g__Coprococcus_628747	unique	Adult gut	0.79	0	0	1	0	0
p__Firmicutes__o__Clostridiales_226726	unique	Adult gut	0.77	0	0	1	0	0
p__Firmicutes__o__Clostridiales_701726	unique	Adult gut	0.74	0	0	1	0	0
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	unique	Adult gut	0.64	0	0	1	0	0

p__Proteobacteria__f__Pasteurellaceae_142268	unique	Adult gut	0.62	0	0	1	0	0
p__Bacteroidetes__o__Bacteroidales_330844	unique	Adult gut	0.61	0	0	1	0	0
p__Bacteroidetes__g__Dysgonomonas_475976	unique	Adult gut	0.58	0	0	1	0	0
p__Firmicutes__o__Clostridiales_66573	unique	Adult gut	0.54	0	0	1	0	0
p__Actinobacteria__g__Propionibacterium_427206	unique	Adult gut	0.54	0	0	1	0	0
p__Proteobacteria__g__Desulfovibrio__s__Desulfovibriodesulfuricans_35517	unique	Adult gut	0.5	0	0	1	0	0
p__SR1__c___571035	unique	Adult gut	0.47	0	0	1	0	0
p__Actinobacteria__g__Dermacoccus_337626	unique	Adult gut	0.46	0	0	1	0	0
p__Actinobacteria__g__Arthrobacter_694831	unique	Adult gut	0.4	0	0	1	0	0
p__Spirochaetes__g__Treponema_30617	unique	Adult gut	0.35	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_729660	unique	Adult gut	0.35	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_317953	unique	Adult gut	0.34	0	0	1	0	0
p__GN02__c__VC12cl04_595855	unique	Adult gut	0.34	0	0	1	0	0
p__Bacteroidetes__o__Bacteroidales_711203	unique	Adult gut	0.34	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_412977	unique	Adult gut	0.3	0	0	1	0	0
p__Proteobacteria__f__Rhodobacteraceae_445008	unique	Adult gut	0.27	0.001	0	1	0.05	0
p__Bacteroidetes__g__Tenacibaculum_428560	unique	Adult gut	0.29	0	0	1	0	0
p__Tenericutes__g__Bulleidia_166205	unique	Adult gut	0.24	0	0	1	0	0
p__Proteobacteria__o__Chromatiales_537609	unique	Adult gut	0.21	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_733417	unique	Adult gut	0.19	0	0	1	0	0
p__Proteobacteria__g__Kingella_306832	unique	Adult gut	0.19	0	0	1	0	0
p__Proteobacteria__f__Rhodospirillaceae_362274	unique	Adult gut	0.01	0.009	0	1	0.26	0
p__SR1__c___526194	unique	Adult gut	0.17	0	0	1	0	0
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	unique	Adult gut	0.14	0.002	0	1	0.05	0
p__Proteobacteria__f__Rhodospirillaceae_237338	unique	Adult gut	0.01	0.007	0	1	0.32	0
p__GN02__c__VC12cl04_222792	unique	Adult gut	0.15	0	0	1	0	0
p__Bacteroidetes__f__Flavobacteriaceae_12392	unique	Adult gut	0.15	0	0	1	0	0

p__Proteobacteria__f__Burkholderiaceae_432832	unique	Adult gut	0.14	0	0	1	0	0
p__Proteobacteria__g__Pedomicrobium_452291	unique	Adult gut	0.01	0.007	0	1	0.32	0
p__Actinobacteria__f__CL50029_390474	unique	Adult gut	0.01	0.006	0	1	0.37	0
p__Actinobacteria__g__Actinomyces_119009	unique	Adult gut	0.12	0	0	1	0	0
p__Firmicutes__g__Streptococcus_269499	unique	Adult gut	0.01	0.005	0	1	0.16	0
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	unique	Adult gut	0.1	0	0	1	0	0
p__Firmicutes__g__Clostridium_482667	unique	Adult gut	0.09	0.001	0	1	0.05	0
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	unique	Adult gut	0.06	0.002	0	1	0.05	0
p__Firmicutes__o__Clostridiales_70341	unique	Adult gut	0.09	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_174851	unique	Adult gut	0.08	0	0	1	0	0
p__Proteobacteria__g__Achromobacter_324477	unique	Adult gut	0.08	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_651794	unique	Adult gut	0.05	0	0	1	0	0
p__Firmicutes__o__Clostridiales_635308	unique	Adult gut	0.05	0	0	1	0	0
p__Firmicutes__g__Eubacterium_622811	unique	Adult gut	0.05	0	0	1	0	0
p__Proteobacteria__g__Sulfurimonas_448949	unique	Adult gut	0.04	0	0	1	0	0
p__Proteobacteria__g__Stenoxybacter_589139	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__g__Arcobacter_167287	unique	Adult gut	0.03	0	0	1	0	0
p__Proteobacteria__f__SUP05_293055	unique	Adult gut	0.03	0	0	1	0	0
p__Bacteroidetes__g__Wautersiella_232450	pan	Adult gut&HI hatchling gut	0.01	0.371	0.004	1	0.89	0.2
p__Firmicutes__g__Clostridium_110727	pan	Adult gut&HI hatchling gut	0.3	0.189	0	1	0.47	0
p__Actinobacteria__g__Janibacter_703951	pan	Adult gut&HI hatchling gut	0.01	0.198	0.018	1	0.79	0.2
p__Proteobacteria__o__Rhizobiales_675457	pan	Adult gut&HI hatchling gut	0.01	0.161	0	1	0.84	0
p__Proteobacteria__g__Mesorhizobium_4131	pan	Adult gut&HI hatchling gut	0.04	0.117	0	1	0.89	0
p__Proteobacteria__o__Sphingomonadales_709703	pan	Adult gut&HI hatchling gut	0.02	0.1	0.002	1	0.95	0.2
p__Proteobacteria__f__Rhodospirillaceae_494802	pan	Adult gut&HI hatchling gut	0.02	0.058	0	1	0.68	0
p__Actinobacteria__g__Streptomyces_420197	pan	Adult gut&HI hatchling gut	0.01	0.045	0	1	0.79	0

p__Proteobacteria__g__Steroidobacter_168803	pan	Adult gut&HI hatchling gut	0.01	0.039	0	1	0.84	0
p__Proteobacteria__f__Rhodospirillaceae_441088	pan	Adult gut&HI hatchling gut	0.01	0.038	0.002	1	0.68	0.2
p__Actinobacteria__o__koll13_76572	pan	Adult gut&HI hatchling gut	0.02	0.028	0.002	1	0.58	0.2
p__Gemmatimonadetes__o__Gemmatimonadales_757385	pan	Adult gut&HI hatchling gut	0.02	0.028	0	1	0.58	0
p__Chloroflexi__c__SOGA31_314194	pan	Adult gut&HI hatchling gut	0.01	0.026	0	1	0.53	0
p__Proteobacteria__f__Sinobacteraceae_668199	pan	Adult gut&HI hatchling gut	0.01	0.022	0	1	0.53	0
p__Actinobacteria__f__Solirubrobacteraceae_1327	pan	Adult gut&HI hatchling gut	0.02	0.021	0	1	0.63	0
p__Actinobacteria__g__Mycobacterium_393834	pan	Adult gut&HI hatchling gut	0.01	0.015	0.002	1	0.58	0.2
p__Proteobacteria__f__Sphingomonadaceae_363679	pan	Adult gut&HI hatchling gut	0.01	0.006	0.002	1	0.42	0.2
p__Proteobacteria__f__Rhodospirillaceae_622262	pan	Adult gut&HI hatchling gut	0.01	0.006	0	1	0.42	0
p__Proteobacteria__g__Pseudomonas_639712	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	9.377	1.858	1	1	1
p__Proteobacteria__g__Achromobacter_19649	core	Adult gut&HI hatchling gut&Lab hatchling gut	1.72	3.986	7.064	1	1	1
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	core	Adult gut&HI hatchling gut&Lab hatchling gut	1.81	5.126	0.028	1	1	0.4
p__Proteobacteria__g__Aquamicrobium_748021	core	Adult gut&HI hatchling gut&Lab hatchling gut	1.17	4.768	0.78	1	1	1
p__Proteobacteria__f__Enterobacteriaceae_641353	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.02	2.446	0.28	1	1	1
p__Proteobacteria__g__Brucella_305522	core	Adult gut&HI hatchling gut&Lab hatchling gut	4.37	1.706	2.016	1	1	1
p__Proteobacteria__g__Agrobacterium_442913	core	Adult gut&HI hatchling gut&Lab hatchling gut	1.15	0.397	4.236	1	0.95	1

p__Actinobacteria__g__Leucobacter_697145	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.94	0.516	2.282	1	1	1
p__Proteobacteria__g__Shinella_393155	core	Adult gut&HI hatchling gut&Lab hatchling gut	1.12	0.301	0.234	1	0.95	1
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.225	0.308	1	0.95	0.8
p__Proteobacteria__f__Phyllobacteriaceae_589505	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.119	0.012	1	1	0.6
p__Proteobacteria__g__Devosia_700503	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.078	0.026	1	0.89	0.6
p__Proteobacteria__g__Sphingopyxis_102703	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.06	0.065	0.012	1	1	0.6
p__Proteobacteria__g__Nitratireductor_5045	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.03	0.057	0.03	1	0.95	0.6
p__Proteobacteria__g__Xenophilus_188340	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.06	0.054	0.006	1	0.58	0.4
p__Proteobacteria__o__Rhizobiales_602739	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.05	0.024	1	0.63	0.6
p__Firmicutes__g__Bacillus_161559	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.042	0.004	1	0.63	0.4
p__Actinobacteria__f__Nocardioidaceae_632222	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.021	0.024	1	0.79	0.8
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.09	0.014	0.028	1	0.68	0.8

p__Proteobacteria__g__Shinella_386729	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.017	0.02	1	0.47	0.4
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.016	0.018	1	0.58	0.6
p__Proteobacteria__g__Stenoxybacter_687490	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.09	0.008	0.008	1	0.58	0.6
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	core	Adult gut&HI hatchling gut&Lab hatchling gut	0.01	0.008	0.018	1	0.63	0.4
p__Actinobacteria__g__Corynebacterium_68777	pan	Adult gut&Lab hatchling gut	0.01	0	3.454	1	0	1
p__Actinobacteria__g__Dietzia__s__Dietziamaris_276529	pan	Adult gut&Lab hatchling gut	1.37	0.001	0.322	1	0.11	0.8
p__Bacteroidetes__g__Chryseobacterium_308134	pan	Adult gut&Lab hatchling gut	0.08	0.014	0.1	1	0.26	0.4
p__Proteobacteria__g__Alcanivorax_427659	pan	Adult gut&Lab hatchling gut	0.02	0.007	0.004	1	0.26	0.4
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	unique	HI hatchling gut	0	4.22	0	0	1	0
p__Bacteroidetes__g__Sphingobacterium_176945	unique	HI hatchling gut	0	2.919	0	0	1	0
p__Firmicutes__g__Paenibacillus_212495	unique	HI hatchling gut	0	1.111	0	0	1	0
p__Proteobacteria__f__Rhodospirillaceae_424659	unique	HI hatchling gut	0	0.566	0	0	1	0
p__Actinobacteria__g__Tessaracoccus_287453	unique	HI hatchling gut	0	0.473	0	0	0.89	0
p__Proteobacteria__g__Devosia_749390	unique	HI hatchling gut	0	0.458	0	0	1	0
p__Bacteroidetes__g__Myroides_398565	unique	HI hatchling gut	0	0.311	0	0	0.53	0
p__Bacteroidetes__f__Cryomorphaceae_690566	unique	HI hatchling gut	0	0.293	0	0	1	0
p__Bacteroidetes__f__Flavobacteriaceae_555335	unique	HI hatchling gut	0	0.278	0	0	0.95	0
p__Bacteroidetes__o__Sphingobacteriales_571041	unique	HI hatchling gut	0	0.267	0	0	0.74	0

p__Proteobacteria__c__Alphaproteobacteria_462900	unique	HI hatchling gut	0	0.255	0	0	0.74	0
p__Proteobacteria__g__Rhodobacter_747540	unique	HI hatchling gut	0	0.247	0	0	0.53	0
p__Bacteroidetes__f__Sphingobacteriaceae_540060	unique	HI hatchling gut	0	0.225	0	0	0.53	0
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	unique	HI hatchling gut	0	0.198	0	0	0.89	0
p__Bacteroidetes__g__Dyadobacter_763044	unique	HI hatchling gut	0	0.189	0	0	0.79	0
p__Proteobacteria__f__Enterobacteriaceae_231744	unique	HI hatchling gut	0	0.174	0.01	0	0.95	0.2
p__Actinobacteria__g__Gordonia_255893	unique	HI hatchling gut	0	0.175	0.002	0	0.89	0.2
p__Proteobacteria__g__Pseudomonas_511660	unique	HI hatchling gut	0	0.161	0	0	0.84	0
p__Proteobacteria__g__Diaphorobacter_687258	unique	HI hatchling gut	0	0.16	0.002	0	0.79	0.2
p__Proteobacteria__g__Pseudomonas_731148	unique	HI hatchling gut	0	0.151	0.01	0	0.89	0.2
p__Firmicutes__g__Tissierella_366131	unique	HI hatchling gut	0	0.151	0	0	0.53	0
p__Proteobacteria__f__Enterobacteriaceae_84333	unique	HI hatchling gut	0	0.136	0	0	0.89	0
p__Firmicutes__g__Clostridium_436602	unique	HI hatchling gut	0	0.134	0	0	0.53	0
p__Proteobacteria__g__Pseudomonas_308359	unique	HI hatchling gut	0	0.126	0.004	0	1	0.2
p__Bacteroidetes__f__Flexibacteraceae_122867	unique	HI hatchling gut	0	0.124	0	0	0.58	0
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	unique	HI hatchling gut	0	0.123	0	0	0.95	0
p__Firmicutes__g__Clostridium__s__Clostridiumsubterminale_449559	unique	HI hatchling gut	0	0.114	0	0	0.47	0
p__Firmicutes__o__Clostridiales_748404	unique	HI hatchling gut	0	0.109	0	0	0.47	0
p__Actinobacteria__f__Nocardioidaceae_554632	unique	HI hatchling gut	0	0.107	0.006	0	1	0.2
p__Firmicutes__g__Clostridium_356361	unique	HI hatchling gut	0	0.092	0	0	0.53	0
p__Firmicutes__g__Bacillus_245239	unique	HI hatchling gut	0	0.092	0	0	0.74	0
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	unique	HI hatchling gut	0	0.078	0	0	0.89	0
p__Proteobacteria__g__Paracoccus__s__Paracoccussolventivorans_641772	unique	HI hatchling gut	0	0.076	0	0	0.42	0
p__Proteobacteria__g__Alcaligenes_538514	unique	HI hatchling gut	0	0.069	0	0	0.84	0

p__Proteobacteria__g__Acinetobacter_168646	unique	HI hatchling gut	0	0.065	0	0	0.79	0
p__Proteobacteria__g__Pseudomonas_159349	unique	HI hatchling gut	0	0.051	0.004	0	0.58	0.2
p__Proteobacteria__g__Agrobacterium_477436	unique	HI hatchling gut	0	0.05	0.002	0	0.58	0.2
p__Proteobacteria__g__Pseudomonas_34101	unique	HI hatchling gut	0	0.046	0.012	0	0.79	0.2
p__Bacteroidetes__g__Chitinophaga_80088	unique	HI hatchling gut	0	0.038	0	0	0.79	0
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	unique	HI hatchling gut	0	0.03	0.002	0	0.84	0.2
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	unique	HI hatchling gut	0	0.027	0	0	0.47	0
p__Proteobacteria__f__Acetobacteraceae_614109	unique	HI hatchling gut	0	0.026	0	0	0.53	0
p__Firmicutes__g__Oceanobacillus_357784	unique	HI hatchling gut	0	0.02	0	0	0.42	0
p__Proteobacteria__g__Pseudomonas_391544	unique	HI hatchling gut	0	0.019	0	0	0.53	0
p__Firmicutes__f__Bacillaceae_664374	unique	HI hatchling gut	0	0.018	0	0	0.47	0
p__Proteobacteria__f__Bradyrhizobiaceae_116562	unique	HI hatchling gut	0	0.018	0	0	0.63	0
p__Proteobacteria__g__Rhodoplanes_273401	unique	HI hatchling gut	0	0.016	0	0	0.53	0
p__Proteobacteria__c__Gammaproteobacteria_417606	unique	HI hatchling gut	0	0.014	0.002	0	0.68	0.2
p__Actinobacteria__g__Nocardioides_128926	unique	HI hatchling gut	0	0.013	0	0	0.47	0
p__Proteobacteria__f__Sinobacteraceae_155918	unique	HI hatchling gut	0	0.012	0	0	0.53	0
p__Proteobacteria__g__Enterobacter_572676	unique	HI hatchling gut	0	0.009	0	0	0.53	0
p__Proteobacteria__g__Kaistia_437081	unique	HI hatchling gut	0	0.008	0	0	0.47	0
p__Proteobacteria__f__Xanthomonadaceae_702358	unique	HI hatchling gut	0	0.008	0	0	0.47	0
p__Proteobacteria__g__Kaistobacter_682533	unique	HI hatchling gut	0	0.006	0	0	0.42	0
p__Proteobacteria__g__Klebsiella_558424	pan	HI hatchling gut&Lab hatchling gut	0	17.301	29.408	0	1	1
p__Proteobacteria__f__Pseudomonadaceae_79058	pan	HI hatchling gut&Lab hatchling gut	0	5.552	3.734	0	1	1
p__Proteobacteria__g__Stenotrophomonas_727055	pan	HI hatchling gut&Lab hatchling gut	0	2.079	15.78	0	1	1

p__Proteobacteria__f__Brucellaceae_698093	pan	HI hatchling gut&Lab hatchling gut	0	4.749	0.684	0	1	1
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	pan	HI hatchling gut&Lab hatchling gut	0	4.457	0.318	0	1	1
p__Proteobacteria__g__Thermomonas_132820	pan	HI hatchling gut&Lab hatchling gut	0	4.105	0.378	0	1	1
p__Actinobacteria__g__Microbacterium__s__Microbacterium esteraromaticum_75491	pan	HI hatchling gut&Lab hatchling gut	0	0.826	5.438	0	1	1
p__Bacteroidetes__g__Sphingobacterium_298499	pan	HI hatchling gut&Lab hatchling gut	0	1.367	0.134	0	0.95	0.8
p__Proteobacteria__f__Alcaligenaceae_541229	pan	HI hatchling gut&Lab hatchling gut	0	1.123	0.024	0	0.84	0.6
p__Proteobacteria__g__Pseudomonas_10820	pan	HI hatchling gut&Lab hatchling gut	0	0.655	0.318	0	0.95	0.8
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	pan	HI hatchling gut&Lab hatchling gut	0	0.536	0.632	0	1	1
p__Actinobacteria__g__Arthrobacter_706755	pan	HI hatchling gut&Lab hatchling gut	0	0.524	0.64	0	1	0.8
p__Proteobacteria__g__Pseudomonas_132221	pan	HI hatchling gut&Lab hatchling gut	0	0.62	0.054	0	1	0.6
p__Firmicutes__g__Lysinibacillus_81925	pan	HI hatchling gut&Lab hatchling gut	0	0.23	1.37	0	1	1
p__Firmicutes__g__Bacillus_535651	pan	HI hatchling gut&Lab hatchling gut	0	0.134	1.718	0	0.53	0.4
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	pan	HI hatchling gut&Lab hatchling gut	0	0.495	0.05	0	0.89	0.8
p__Bacteroidetes__o__Sphingobacteriales_152865	pan	HI hatchling gut&Lab hatchling gut	0	0.165	1.246	0	0.89	1
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	pan	HI hatchling gut&Lab hatchling gut	0	0.431	0.058	0	0.53	0.4
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	pan	HI hatchling gut&Lab hatchling gut	0	0.442	0.008	0	0.95	0.4

p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	pan	HI hatchling gut&Lab hatchling gut	0	0.262	0.362	0	1	0.8
p__Actinobacteria__g__Leucobacter_418594	pan	HI hatchling gut&Lab hatchling gut	0	0.309	0.17	0	0.58	0.8
p__Proteobacteria__f__Enterobacteriaceae_452665	pan	HI hatchling gut&Lab hatchling gut	0	0.252	0.336	0	1	0.8
p__Bacteroidetes__f__Flavobacteriaceae_374120	pan	HI hatchling gut&Lab hatchling gut	0	0.094	0.856	0	0.68	1
p__Bacteroidetes__g__Sphingobacterium_250053	pan	HI hatchling gut&Lab hatchling gut	0	0.275	0.078	0	0.95	1
p__Proteobacteria__g__Lysobacter_318152	pan	HI hatchling gut&Lab hatchling gut	0	0.218	0.262	0	1	1
p__Proteobacteria__f__Enterobacteriaceae_171036	pan	HI hatchling gut&Lab hatchling gut	0	0.223	0.078	0	1	0.8
p__Proteobacteria__g__Brevundimonas__s__Brevundimonas diminuta_54795	pan	HI hatchling gut&Lab hatchling gut	0	0.134	0.392	0	0.95	1
p__Proteobacteria__g__Stenotrophomonas_729526	pan	HI hatchling gut&Lab hatchling gut	0	0.074	0.564	0	0.95	0.8
p__Proteobacteria__g__Erwinia_25313	pan	HI hatchling gut&Lab hatchling gut	0	0.193	0.052	0	0.95	0.8
p__Proteobacteria__g__Pseudomonas_34989	pan	HI hatchling gut&Lab hatchling gut	0	0.135	0.262	0	0.95	0.8
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	pan	HI hatchling gut&Lab hatchling gut	0	0.138	0.24	0	0.95	0.8
p__Proteobacteria__g__Pseudomonas_174392	pan	HI hatchling gut&Lab hatchling gut	0	0.175	0.074	0	1	0.8
p__Proteobacteria__f__Enterobacteriaceae_410502	pan	HI hatchling gut&Lab hatchling gut	0	0.136	0.196	0	1	0.8
p__Actinobacteria__g__Cellulosimicrobium_382216	pan	HI hatchling gut&Lab hatchling gut	0	0.182	0.01	0	1	0.6
p__Proteobacteria__f__Acetobacteraceae_741892	pan	HI hatchling gut&Lab hatchling gut	0	0.148	0.128	0	1	0.8

p__Proteobacteria__g__Pseudomonas_572655	pan	HI hatchling gut&Lab hatchling gut	0	0.169	0.032	0	1	0.8
p__Proteobacteria__g__Achromobacter_678564	pan	HI hatchling gut&Lab hatchling gut	0	0.176	0.008	0	0.95	0.6
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	pan	HI hatchling gut&Lab hatchling gut	0	0.025	0.566	0	0.47	0.8
p__Firmicutes__g__Paenibacillus_556209	pan	HI hatchling gut&Lab hatchling gut	0	0.094	0.226	0	1	1
p__Proteobacteria__f__Pseudomonadaceae_99078	pan	HI hatchling gut&Lab hatchling gut	0	0.091	0.204	0	0.89	0.8
p__Proteobacteria__g__Klebsiella_117934	pan	HI hatchling gut&Lab hatchling gut	0	0.127	0.06	0	0.89	1
p__Proteobacteria__f__Alcaligenaceae_503155	pan	HI hatchling gut&Lab hatchling gut	0	0.141	0.006	0	0.89	0.4
p__Proteobacteria__g__Delftia_587073	pan	HI hatchling gut&Lab hatchling gut	0	0.041	0.386	0	0.58	1
p__Proteobacteria__g__Achromobacter_468982	pan	HI hatchling gut&Lab hatchling gut	0	0.124	0.068	0	0.95	0.6
p__Proteobacteria__g__Stenotrophomonas_247119	pan	HI hatchling gut&Lab hatchling gut	0	0.095	0.16	0	0.95	0.8
p__Proteobacteria__g__Defluvibacter_651784	pan	HI hatchling gut&Lab hatchling gut	0	0.125	0.028	0	1	0.8
p__Proteobacteria__g__Klebsiella_664333	pan	HI hatchling gut&Lab hatchling gut	0	0.124	0.028	0	0.95	0.6
p__Proteobacteria__f__Xanthomonadaceae_258602	pan	HI hatchling gut&Lab hatchling gut	0	0.122	0.006	0	1	0.4
p__Proteobacteria__g__Pseudomonas_466613	pan	HI hatchling gut&Lab hatchling gut	0	0.105	0.046	0	0.84	0.6
p__Proteobacteria__g__Pseudomonas_110207	pan	HI hatchling gut&Lab hatchling gut	0	0.11	0.022	0	0.84	0.4
p__Proteobacteria__g__Klebsiella_430511	pan	HI hatchling gut&Lab hatchling gut	0	0.108	0.02	0	0.95	0.6

p__Proteobacteria__g__Pseudomonas_235588	pan	HI hatchling gut&Lab hatchling gut	0	0.103	0.036	0	1	0.6
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	pan	HI hatchling gut&Lab hatchling gut	0	0.11	0.008	0	0.89	0.4
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	pan	HI hatchling gut&Lab hatchling gut	0	0.102	0.036	0	0.95	0.6
p__Proteobacteria__g__Klebsiella_347396	pan	HI hatchling gut&Lab hatchling gut	0	0.109	0.004	0	0.89	0.4
p__Actinobacteria__g__Rhodococcus_413286	pan	HI hatchling gut&Lab hatchling gut	0	0.106	0.016	0	0.95	0.8
p__Proteobacteria__g__Paracoccus_110077	pan	HI hatchling gut&Lab hatchling gut	0	0.075	0.112	0	0.84	1
p__Proteobacteria__g__Pseudomonas_142124	pan	HI hatchling gut&Lab hatchling gut	0	0.073	0.1	0	0.95	1
p__Actinobacteria__g__Aeromicrobium_619093	pan	HI hatchling gut&Lab hatchling gut	0	0.089	0.022	0	0.95	0.8
p__Proteobacteria__g__Stenotrophomonas_695630	pan	HI hatchling gut&Lab hatchling gut	0	0.024	0.264	0	0.79	1
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	pan	HI hatchling gut&Lab hatchling gut	0	0.028	0.234	0	0.68	0.8
p__Proteobacteria__g__Pseudomonas_281622	pan	HI hatchling gut&Lab hatchling gut	0	0.072	0.056	0	0.89	0.8
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	pan	HI hatchling gut&Lab hatchling gut	0	0.08	0.016	0	0.79	0.6
p__Proteobacteria__g__Pseudomonas_161575	pan	HI hatchling gut&Lab hatchling gut	0	0.067	0.052	0	0.89	1
p__Proteobacteria__g__Achromobacter_155903	pan	HI hatchling gut&Lab hatchling gut	0	0.054	0.046	0	0.89	0.8
p__Proteobacteria__g__Stenotrophomonas_505642	pan	HI hatchling gut&Lab hatchling gut	0	0.027	0.138	0	0.79	0.8
p__Proteobacteria__g__Pseudomonas_403884	pan	HI hatchling gut&Lab hatchling gut	0	0.052	0.046	0	0.63	0.6

p__Proteobacteria__f__Alcaligenaceae_126597	pan	HI hatchling gut&Lab hatchling gut	0	0.026	0.136	0	0.89	0.8
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_629547	pan	HI hatchling gut&Lab hatchling gut	0	0.054	0.03	0	0.89	1
p__Proteobacteria__g__Stenotrophomonas_531558	pan	HI hatchling gut&Lab hatchling gut	0	0.018	0.12	0	0.74	0.8
p__Proteobacteria__g__Devosia_497251	pan	HI hatchling gut&Lab hatchling gut	0	0.028	0.07	0	0.89	1
p__Proteobacteria__g__Ralstonia_478818	pan	HI hatchling gut&Lab hatchling gut	0	0.042	0.008	0	0.84	0.6
p__Proteobacteria__g__Stenotrophomonas_168758	pan	HI hatchling gut&Lab hatchling gut	0	0.026	0.064	0	0.74	0.8
p__Actinobacteria__f__Nocardioidaceae_115549	pan	HI hatchling gut&Lab hatchling gut	0	0.026	0.064	0	0.58	0.8
p__Proteobacteria__g__Pseudomonas_40331	pan	HI hatchling gut&Lab hatchling gut	0	0.041	0.006	0	0.84	0.4
p__Proteobacteria__g__Pseudomonas_711667	pan	HI hatchling gut&Lab hatchling gut	0	0.025	0.064	0	0.79	0.8
p__Proteobacteria__g__Achromobacter_376840	pan	HI hatchling gut&Lab hatchling gut	0	0.033	0.034	0	0.89	0.8
p__Actinobacteria__g__Dietzia_70231	pan	HI hatchling gut&Lab hatchling gut	0	0.037	0.012	0	0.63	0.6
p__Proteobacteria__g__Stenotrophomonas_657476	pan	HI hatchling gut&Lab hatchling gut	0	0.032	0.006	0	0.47	0.4
p__Proteobacteria__g__Bosea_424613	pan	HI hatchling gut&Lab hatchling gut	0	0.028	0.014	0	0.74	0.8
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	pan	HI hatchling gut&Lab hatchling gut	0	0.028	0.004	0	0.68	0.4
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	pan	HI hatchling gut&Lab hatchling gut	0	0.026	0.006	0	0.68	0.4
p__Proteobacteria__g__Stenotrophomonas_502872	pan	HI hatchling gut&Lab hatchling gut	0	0.014	0.03	0	0.74	0.8

p__Actinobacteria__g__Pimelobacter_394756	pan	HI hatchling gut&Lab hatchling gut	0	0.016	0.006	0	0.58	0.4
p__Actinobacteria__g__Brevibacterium__s__Brevibacteriumaureum_674805	unique	Lab hatchling gut	0	0.171	3.108	0	0.16	1
p__Bacteroidetes__g__Sphingobacterium_246851	unique	Lab hatchling gut	0	0.001	1.462	0	0.05	1
p__Proteobacteria__g__Shewanella__s__Shewanellaalgae_485511	unique	Lab hatchling gut	0	0	0.696	0	0	1
p__Proteobacteria__g__Trabulsiella__s__Kluyveraascorbata_152515	unique	Lab hatchling gut	0	0.126	0.006	0	0.32	0.6
p__Bacteroidetes__g__Sphingobacterium_204117	unique	Lab hatchling gut	0	0.003	0.344	0	0.11	1
p__Firmicutes__g__Staphylococcus_171970	unique	Lab hatchling gut	0	0.002	0.304	0	0.21	1
p__Bacteroidetes__c__Flavobacteria_105335	unique	Lab hatchling gut	0	0.008	0.252	0	0.11	1
p__Bacteroidetes__g__Sphingobacterium_512955	unique	Lab hatchling gut	0	0.005	0.194	0	0.11	1
p__Proteobacteria__g__Pseudomonas_704131	unique	Lab hatchling gut	0	0	0.12	0	0	0.8
p__Actinobacteria__g__Arthrobacter__s__Arthrobacterureafaciens_610242	unique	Lab hatchling gut	0	0.005	0.098	0	0.26	0.8
p__Actinobacteria__g__Microbacterium_545806	unique	Lab hatchling gut	0	0.001	0.066	0	0.11	0.8
p__Proteobacteria__f__Enterobacteriaceae_172645	unique	Lab hatchling gut	0	0.003	0.056	0	0.11	0.8
p__Bacteroidetes__g__Chryseobacterium_171211	unique	Lab hatchling gut	0	0	0.058	0	0	0.4
p__Proteobacteria__f__Enterobacteriaceae_245606	unique	Lab hatchling gut	0	0.002	0.048	0	0.11	0.8
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	unique	Lab hatchling gut	0	0.001	0.04	0	0.11	0.8
p__Actinobacteria__f__Microbacteriaceae_486696	unique	Lab hatchling gut	0	0.003	0.014	0	0.16	0.8
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	unique	Lab hatchling gut	0	0.004	0.008	0	0.26	0.4
p__Proteobacteria__f__Rhodobacteraceae_649099	unique	Lab hatchling gut	0	0.003	0.004	0	0.32	0.4
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	unique	Lab hatchling gut	0	0.003	0.004	0	0.21	0.4
p__Actinobacteria__g__Leucobacter_145505	unique	Lab hatchling gut	0	0.001	0.004	0	0.11	0.4

Analysis of community relatedness revealed that adult turtles and hatchlings shared 42 (21.2%) of their OTUs (Figure 4.7, Table 4.10). We also found that hatchlings shared between 36-50 (15.4-21%) of their OTUs with their biological mother (Figure 4.4 - 4.6, Table 4.7 – 4.9). Adult gut and adult blood shared 21(16%) OTUs (Figure 4.7, Table 4.11), while hatchling gut and hatchling blood shared 45 (31.3%) OTUs (Figure 4.7, Table 4.13). Adult blood and egg contents shared 37 (44%) of their OTUs (Figure 4.7, Table 4.12). Hatchling gut and egg contents shared 32 (18.1%) of their OTUs (Figure 4.7, Table 4.14). Hatchling blood and egg contents shared 26 (28.3%) of their OTUs (Figure 4.7, Table 4.15). PCoA analysis of egg contents revealed no significant differences in microbiota composition between females for weighted UniFrac ordination (df=2, SS_T=0.20, MS = 0.10, f.model=1.59, R^2=0.21, *p*=0.14), but significance for unweighted UniFrac ordination (df=2, SS_T=0.58, MS = 0.29, f.model=1.57, R^2=0.21, *p*=0.032) (Figure 4.8). Lastly, analysis of sand and hatchlings showed significant differences for PCoA plots on both the weighted UniFrac (df=3, SS_T=1.74, MS = 0.58, f.model=12.89, R^2=0.40, *p*=0.001) and unweighted UniFrac ordinations (df=3, SS_T=2.21, MS = 0.74, f.model=9.6, R^2=0.34, *p*=0.001), and that all hatchlings and sand samples shared 53 (9.8%) OTUs (Figure 4.9).

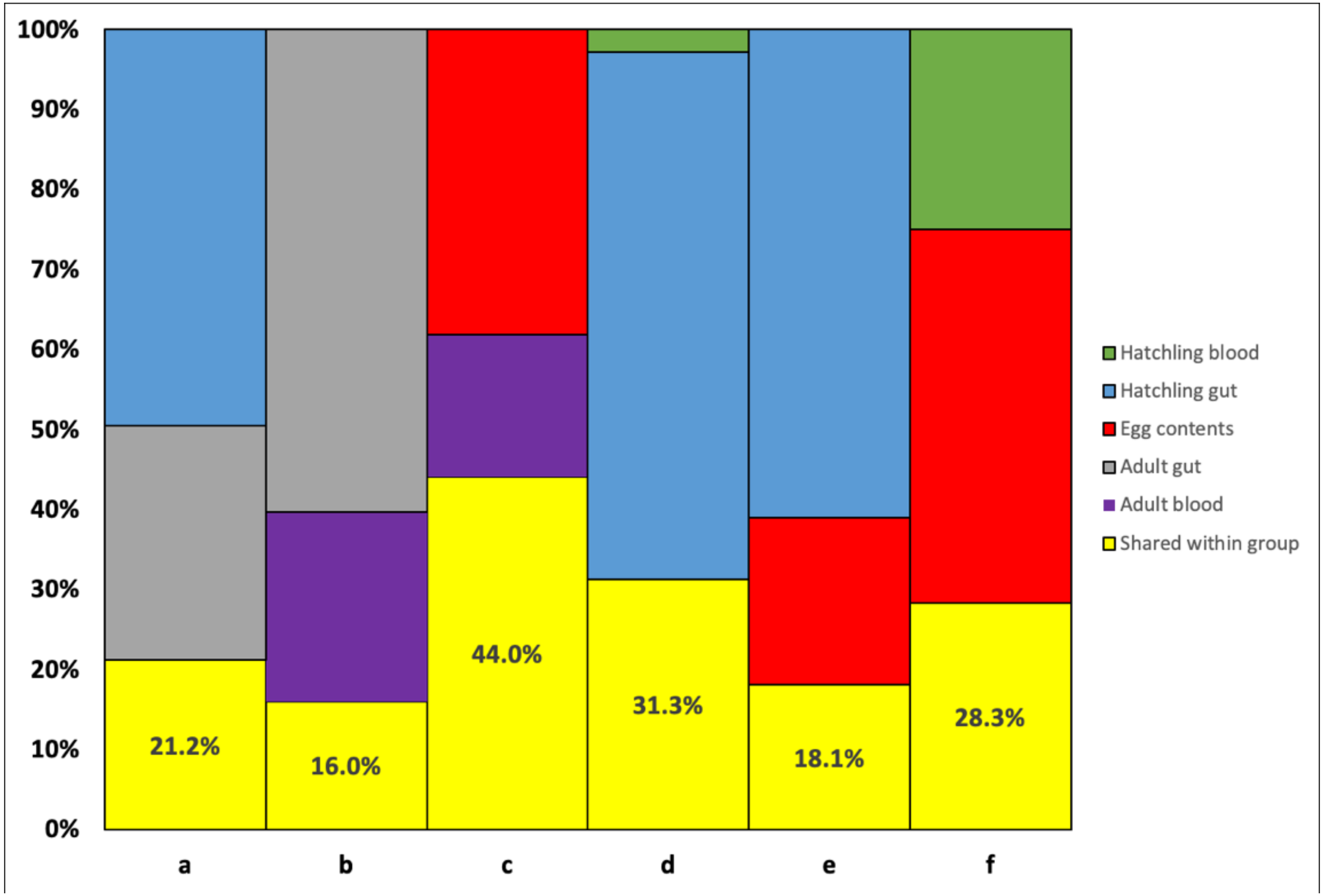

Figure 4.7 Representation of shared OTUs between sample groups. **(a)** Number of shared OTUs between adults and hatchlings. **(b)** number of shared OTUs between adult blood and adult gut. **(c)** number of shared OTUs between adult blood and egg contents. **(d)** number of shared OTUs between hatchling blood and hatchling gut. **(e)** number of shared OTUs between hatchling gut and egg contents. **(f)** number of shared OTUs between hatchling blood and egg contents.

Table 4.10 Shared and unique OTUs between adult gut and hatchling gut samples.

Taxa	Type	Details	Adult gut abundance	Hatchling gut abundance	Adult gut occurrence	Hatchling gut occurrence
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Adult gut	18.41	0.001	0.94	0.06
p__Proteobacteria__f__SUP05_285720	Unique	Adult gut	5.472	0	0.94	0
p__Proteobacteria__f__Pasteurellaceae_142268	Unique	Adult gut	4.802	0	0.94	0
p__Actinobacteria__o__03197L14_108564	Unique	Adult gut	5.696	0	0.94	0
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Adult gut	3.948	0	0.76	0
p__Chloroflexi__c__SOGA31_314194	Unique	Adult gut	0.082	0.012	0.41	0.31
p__Bacteroidetes__f__Flavobacteriaceae_729660	Unique	Adult gut	0.406	0	0.53	0
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	Unique	Adult gut	0.006	0	0.47	0.02
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	Unique	Adult gut	2.183	0	0.94	0
p__Bacteroidetes__f__Flavobacteriaceae_379313	Unique	Adult gut	1.6	0	0.47	0
p__Bacteroidetes__f__Flavobacteriaceae_12392	Unique	Adult gut	1.412	0	0.94	0
p__Proteobacteria__g__Arcobacter_587211	Unique	Adult gut	1.469	0	0.88	0
p__SR1__c___571035	Unique	Adult gut	0.974	0	0.76	0
p__Proteobacteria__f__Sinobacteraceae_155918	Unique	Adult gut	0.031	0.007	0.41	0.35
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Adult gut	0.717	0	0.65	0
p__Proteobacteria__o__Myxococcales_668245	Unique	Adult gut	1.339	0	0.65	0
p__Proteobacteria__f__Sinobacteraceae_668199	Unique	Adult gut	0.035	0.01	0.47	0.35
p__Actinobacteria__g__Dermacoccus_337626	Unique	Adult gut	0.646	0	0.53	0
p__Actinobacteria__o__koll13_76572	Unique	Adult gut	0.048	0.013	0.59	0.35
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	Unique	Adult gut	0.834	0	0.88	0
p__Proteobacteria__f__Rhodospirillaceae_622262	Unique	Adult gut	0.042	0.003	0.65	0.25
p__Actinobacteria__g__Propionibacterium_427206	Unique	Adult gut	0.69	0	0.88	0
p__Actinobacteria__g__Arcanobacterium_246345	Unique	Adult gut	0.695	0	0.47	0
p__Proteobacteria__g__Sulfurimonas_444197	Unique	Adult gut	0.49	0	0.76	0
p__Fusobacteria__g__Streptobacillus__s__Streptobacillusmoniliformis_287396	Unique	Adult gut	0.52	0	0.59	0

p__GN02__c__VC12cl04_595855	Unique	Adult gut	0.451	0	0.53	0
p__Proteobacteria__g__Arcobacter_130909	Unique	Adult gut	0.43	0	0.82	0
p__Tenericutes__g__Mycoplasma_551498	Unique	Adult gut	0.183	0	0.76	0
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	Unique	Adult gut	0.417	0	0.71	0
p__Proteobacteria__g__Sulfurimonas_448949	Unique	Adult gut	0.389	0	0.88	0
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Adult gut	0.066	0.002	0.41	0.12
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Adult gut	0.249	0	0.71	0
p__Proteobacteria__g__Stenoxybacter_253237	Unique	Adult gut	0.431	0	0.71	0
p__Proteobacteria__f__Rhodobacteraceae_729919	Unique	Adult gut	0.41	0	0.53	0
p__Proteobacteria__f__Rhodospirillaceae_362274	Unique	Adult gut	0.021	0.005	0.59	0.14
p__Firmicutes__g__Coprococcus_628747	Unique	Adult gut	0.32	0	0.53	0
p__Bacteroidetes__g__Bacteroides_689514	Unique	Adult gut	0.106	0	0.59	0
p__Proteobacteria__f__Campylobacteraceae_360616	Unique	Adult gut	0.222	0	0.76	0
p__SR1__c___526194	Unique	Adult gut	0.134	0	0.53	0
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	Unique	Adult gut	0.24	0	0.71	0
p__Firmicutes__g__Coprococcus_312354	Unique	Adult gut	0.263	0	0.41	0
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Adult gut	0.087	0	0.71	0
p__Firmicutes__o__Clostridiales_226726	Unique	Adult gut	0.134	0	0.76	0
p__Proteobacteria__g__Stenoxybacter_396392	Unique	Adult gut	0.271	0	0.71	0.04
p__Firmicutes__o__Clostridiales_701726	Unique	Adult gut	0.258	0	0.88	0
p__Actinobacteria__g__Arthrobacter_694831	Unique	Adult gut	0.133	0	0.82	0
p__Actinobacteria__g__Actinomyces_119009	Unique	Adult gut	0.105	0	0.59	0
p__Proteobacteria__f__Alcaligenaceae_536615	Unique	Adult gut	0.234	0	0.65	0
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_585025	Unique	Adult gut	0.165	0	0.71	0
p__Proteobacteria__g__Stenoxybacter_174851	Unique	Adult gut	0.226	0	0.82	0
p__Proteobacteria__f__Rhodobacteraceae_445008	Unique	Adult gut	0.175	0	0.47	0.02
p__Proteobacteria__g__Stenoxybacter_572518	Unique	Adult gut	0.199	0	0.76	0
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	Unique	Adult gut	0.133	0.001	0.65	0.02
p__Proteobacteria__g__Stenoxybacter_317953	Unique	Adult gut	0.179	0	0.82	0

p__Bacteroidetes__g__Tenacibaculum_428560	Unique	Adult gut	0.057	0	0.71	0
p__Spirochaetes__g__Treponema_30617	Unique	Adult gut	0.146	0	0.53	0
p__Proteobacteria__g__Moraxella_610605	Unique	Adult gut	0.034	0	0.41	0
p__Tenericutes__g__Bulleidia_166205	Unique	Adult gut	0.141	0	0.65	0
p__Proteobacteria__g__Pseudomonas_165065	Unique	Adult gut	0.137	0	0.59	0
p__Proteobacteria__o__Chromatiales_537609	Unique	Adult gut	0.134	0	0.82	0
p__Proteobacteria__g__Klebsiella_558424	Core	Adult gut, Hatchling gut	0.843	16.023	0.47	0.96
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Adult gut, Hatchling gut	0.554	7.789	0.47	1
p__Proteobacteria__g__Pseudomonas_639712	Core	Adult gut, Hatchling gut	7.649	6.735	0.82	0.98
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Adult gut, Hatchling gut	4.486	6.391	0.53	1
p__Proteobacteria__g__Achromobacter_19649	Core	Adult gut, Hatchling gut	3.192	4.655	0.88	1
p__Proteobacteria__g__Aquamicrobium_748021	Core	Adult gut, Hatchling gut	0.606	2.442	0.82	1
p__Proteobacteria__g__Agrobacterium_442913	Core	Adult gut, Hatchling gut	2.127	2.104	0.94	0.96
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Adult gut, Hatchling gut	1.492	2.566	0.82	0.94
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Core	Adult gut, Hatchling gut	0.088	1.629	0.65	1
p__Proteobacteria__f__Brucellaceae_698093	Core	Adult gut, Hatchling gut	0.712	2.726	0.41	0.92
p__Proteobacteria__g__Brucella_305522	Core	Adult gut, Hatchling gut	3.461	1.634	0.94	0.98
p__Proteobacteria__g__Lysobacter_318152	Core	Adult gut, Hatchling gut	1.121	0.198	0.47	0.9
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	Core	Adult gut, Hatchling gut	0.015	1.831	0.41	0.63
p__Actinobacteria__g__Leucobacter_697145	Core	Adult gut, Hatchling gut	0.706	0.558	0.65	0.96
p__Firmicutes__g__Bacillus_161559	Core	Adult gut, Hatchling gut	0.1	0.022	0.65	0.61
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Core	Adult gut, Hatchling gut	0.026	0.018	0.47	0.47
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Adult gut, Hatchling gut	0.199	0.268	0.65	0.96
p__Proteobacteria__f__Sphingomonadaceae_363679	Core	Adult gut, Hatchling gut	0.086	0.011	0.53	0.55
p__Proteobacteria__f__Rhodospirillaceae_441088	Core	Adult gut, Hatchling gut	0.044	0.02	0.53	0.61
p__Proteobacteria__g__Shinella_393155	Core	Adult gut, Hatchling gut	0.422	0.211	0.76	0.94
p__Proteobacteria__g__Pseudomonas_34989	Core	Adult gut, Hatchling gut	0.29	0.308	0.47	0.94
p__Proteobacteria__g__Nitratireductor_5045	Core	Adult gut, Hatchling gut	0.292	0.042	0.71	0.8
p__Proteobacteria__g__Steroidobacter_168803	Core	Adult gut, Hatchling gut	0.038	0.025	0.47	0.53

p__Gemmatimonadetes__o__Gemmatimonadales_757385	Core	Adult gut, Hatchling gut	0.088	0.013	0.53	0.41
p__Actinobacteria__f__Solirubrobacteraceae_1327	Core	Adult gut, Hatchling gut	0.059	0.013	0.59	0.47
p__Actinobacteria__g__Streptomyces_420197	Core	Adult gut, Hatchling gut	0.118	0.022	0.59	0.53
p__Proteobacteria__g__Sphingopyxis_102703	Core	Adult gut, Hatchling gut	0.071	0.059	0.65	0.82
p__Proteobacteria__f__Phyllobacteriaceae_589505	Core	Adult gut, Hatchling gut	0.064	0.07	0.47	0.73
p__Actinobacteria__f__Nocardioidaceae_632222	Core	Adult gut, Hatchling gut	0.084	0.027	0.41	0.76
p__Proteobacteria__f__Rhodospirillaceae_494802	Core	Adult gut, Hatchling gut	0.029	0.024	0.53	0.41
p__Proteobacteria__o__Sphingomonadales_709703	Core	Adult gut, Hatchling gut	0.012	0.059	0.47	0.73
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Core	Adult gut, Hatchling gut	0.041	0.019	0.47	0.67
p__Proteobacteria__g__Devosia_700503	Core	Adult gut, Hatchling gut	0.031	0.054	0.41	0.76
p__Proteobacteria__g__Achromobacter_468982	Core	Adult gut, Hatchling gut	0.121	0.094	0.59	0.84
p__Proteobacteria__o__Rhizobiales_602739	Core	Adult gut, Hatchling gut	0.029	0.027	0.41	0.57
p__Proteobacteria__g__Pseudomonas_466613	Core	Adult gut, Hatchling gut	0.115	0.067	0.53	0.67
p__Proteobacteria__g__Stenoxybacter_687490	Core	Adult gut, Hatchling gut	0.212	0.012	0.82	0.59
p__Proteobacteria__g__Shinella_386729	Core	Adult gut, Hatchling gut	0.025	0.025	0.65	0.59
p__Proteobacteria__g__Pseudomonas_161575	Core	Adult gut, Hatchling gut	0.041	0.061	0.47	0.78
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	Core	Adult gut, Hatchling gut	0.039	0.056	0.41	0.76
p__Proteobacteria__g__Achromobacter_155903	Core	Adult gut, Hatchling gut	0.038	0.054	0.65	0.82
p__Proteobacteria__g__Pseudomonas_40331	Core	Adult gut, Hatchling gut	0.074	0.028	0.47	0.65
p__Proteobacteria__g__Ralstonia_478818	Unique	Hatchling gut	0.028	0.03	0.18	0.75
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Unique	Hatchling gut	0.029	0.327	0.35	0.8
p__Proteobacteria__f__Enterobacteriaceae_641353	Unique	Hatchling gut	0.002	7.881	0.12	0.96
p__Proteobacteria__g__Thermomonas_132820	Unique	Hatchling gut	0.099	2.416	0.06	0.76
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	Unique	Hatchling gut	0.329	2.677	0.18	1
p__Proteobacteria__f__Enterobacteriaceae_171036	Unique	Hatchling gut	0	1.598	0	0.92
p__Bacteroidetes__g__Sphingobacterium_176945	Unique	Hatchling gut	0	2.064	0	0.78
p__Proteobacteria__g__Acinetobacter_168646	Unique	Hatchling gut	0	0.377	0	0.47
p__Bacteroidetes__g__Sphingobacterium_298499	Unique	Hatchling gut	0	1.207	0	0.9
p__Bacteroidetes__f__Flavobacteriaceae_374120	Unique	Hatchling gut	0.084	1.066	0.18	0.86

p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	Unique	Hatchling gut	0.006	0.42	0.18	0.94
p__Actinobacteria__g__Arthrobacter_706755	Unique	Hatchling gut	0.034	0.898	0.24	0.94
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	Unique	Hatchling gut	0.022	0.907	0.29	0.86
p__Firmicutes__g__Bacillus_535651	Unique	Hatchling gut	0.46	0.418	0.29	0.49
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	Unique	Hatchling gut	0.023	0.993	0.06	0.43
p__Firmicutes__g__Paenibacillus_212495	Unique	Hatchling gut	0	0.779	0	0.8
p__Proteobacteria__f__Alcaligenaceae_541229	Unique	Hatchling gut	0.062	0.865	0.12	0.76
p__Bacteroidetes__o__Sphingobacteriales_152865	Unique	Hatchling gut	0.078	0.235	0.12	0.53
p__Firmicutes__g__Bacillus_245239	Unique	Hatchling gut	0	0.049	0	0.41
p__Actinobacteria__g__Aeromicrobium_619093	Unique	Hatchling gut	0.052	0.06	0.29	0.84
p__Proteobacteria__g__Delftia_587073	Unique	Hatchling gut	0.014	0.07	0.12	0.53
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Unique	Hatchling gut	0.009	0.032	0.12	0.43
p__Bacteroidetes__f__Cryomorphaceae_690566	Unique	Hatchling gut	0	0.168	0	0.55
p__Firmicutes__g__Lysinibacillus_81925	Unique	Hatchling gut	0.004	0.58	0.12	0.94
p__Actinobacteria__g__Janibacter_703951	Unique	Hatchling gut	0.007	0.077	0.12	0.45
p__Bacteroidetes__g__Wautersiella_232450	Unique	Hatchling gut	0.001	0.233	0.12	0.63
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	Unique	Hatchling gut	0.032	0.288	0.29	0.82
p__Proteobacteria__g__Pseudomonas_159349	Unique	Hatchling gut	1.185	0.142	0.35	0.69
p__Bacteroidetes__g__Sphingobacterium_250053	Unique	Hatchling gut	0.001	0.199	0.06	0.9
p__Proteobacteria__g__Devosia_497251	Unique	Hatchling gut	0.019	0.045	0.18	0.8
p__Bacteroidetes__f__Flavobacteriaceae_555335	Unique	Hatchling gut	0.102	0.165	0.18	0.65
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	Unique	Hatchling gut	0.016	0.141	0.29	0.86
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Hatchling gut	0.001	0.058	0.06	0.71
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Hatchling gut	0.01	0.235	0.24	0.41
p__Proteobacteria__g__Pseudomonas_10820	Unique	Hatchling gut	0.002	0.327	0.12	0.73
p__Proteobacteria__f__Rhodospirillaceae_424659	Unique	Hatchling gut	0	0.247	0	0.55
p__Actinobacteria__g__Tessaracoccus_287453	Unique	Hatchling gut	0	0.221	0	0.49
p__Bacteroidetes__g__Sphingobacterium_204117	Unique	Hatchling gut	0.181	0.16	0.18	0.47
p__Proteobacteria__g__Pseudomonas_132221	Unique	Hatchling gut	0	0.295	0	0.76

p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Unique	Hatchling gut	0.004	0.149	0.24	0.86
p__Proteobacteria__g__Devosia_749390	Unique	Hatchling gut	0.001	0.2	0.06	0.61
p__Bacteroidetes__f__Flexibacteraceae_122867	Unique	Hatchling gut	0	0.151	0	0.45
p__Firmicutes__g__Paenibacillus_556209	Unique	Hatchling gut	0.006	0.097	0.18	0.88
p__Proteobacteria__g__Alcanivorax_427659	Unique	Hatchling gut	0.046	0.031	0.29	0.45
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	Unique	Hatchling gut	0.001	0.221	0.06	0.84
p__Proteobacteria__g__Stenotrophomonas_657476	Unique	Hatchling gut	0.001	0.16	0.06	0.53
p__Proteobacteria__g__Stenotrophomonas_729526	Unique	Hatchling gut	0	0.186	0	0.78
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	Unique	Hatchling gut	0.001	0.144	0.06	0.82
p__Proteobacteria__g__Stenotrophomonas_247119	Unique	Hatchling gut	0.088	0.142	0.12	0.86
p__Actinobacteria__g__Pimelobacter_394756	Unique	Hatchling gut	0.002	0.01	0.06	0.43
p__Proteobacteria__g__Bosea_424613	Unique	Hatchling gut	0.075	0.016	0.35	0.47
p__Proteobacteria__f__Enterobacteriaceae_452665	Unique	Hatchling gut	0	0.16	0	0.69
p__Actinobacteria__g__Leucobacter_418594	Unique	Hatchling gut	0	0.14	0	0.49
p__Bacteroidetes__o__Sphingobacteriales_571041	Unique	Hatchling gut	0	0.131	0	0.43
p__Proteobacteria__f__Pseudomonadaceae_99078	Unique	Hatchling gut	0.001	0.146	0.06	0.76
p__Proteobacteria__g__Pseudomonas_174392	Unique	Hatchling gut	0.009	0.133	0.24	0.86
p__Proteobacteria__g__Pseudomonas_142124	Unique	Hatchling gut	0.075	0.115	0.18	0.82
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	Unique	Hatchling gut	0	0.11	0	0.71
p__Actinobacteria__g__Rhodococcus_413286	Unique	Hatchling gut	0	0.048	0	0.67
p__Firmicutes__g__Oceanobacillus_357784	Unique	Hatchling gut	0.005	0.068	0.18	0.53
p__Proteobacteria__g__Pseudomonas_572655	Unique	Hatchling gut	0	0.11	0	0.76
p__Proteobacteria__g__Diaphorobacter_687258	Unique	Hatchling gut	0.006	0.067	0.06	0.45
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Hatchling gut	0.002	0.085	0.12	0.67
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.001	0.019	0.06	0.45
p__Proteobacteria__g__Achromobacter_678564	Unique	Hatchling gut	0.017	0.086	0.24	0.73
p__Proteobacteria__f__Enterobacteriaceae_410502	Unique	Hatchling gut	0	0.088	0	0.67
p__Proteobacteria__f__Alcaligenaceae_503155	Unique	Hatchling gut	0.026	0.076	0.24	0.75
p__Proteobacteria__g__Erwinia_25313	Unique	Hatchling gut	0	0.083	0	0.55

p__Proteobacteria__f__Enterobacteriaceae_231744	Unique	Hatchling gut	0.001	0.082	0.06	0.57
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	Unique	Hatchling gut	0	0.081	0	0.45
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0	0.69
p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0.018	0.073	0.06	0.45
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0.001	0.02	0.06	0.41
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0.025	0.051	0.35	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.047	0.026	0.24	0.47
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0.001	0.067	0.06	0.59
p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0.002	0.065	0.12	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0.025	0.058	0.18	0.43
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0	0.06	0	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0	0.064	0	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0.005	0.063	0.24	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0.011	0.059	0.12	0.73
p__Bacteroidetes__g__Chitinophaga_80088	Unique	Hatchling gut	0	0.027	0	0.51
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0.011	0.031	0.12	0.65
p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0.004	0.058	0.18	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0.008	0.033	0.12	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0.001	0.058	0.06	0.57
p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.032	0.024	0.24	0.45
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0.013	0.049	0.12	0.78
p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0	0.052	0	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0	0.53
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0	0.048	0	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0	0.049	0	0.71
p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0.005	0.038	0.12	0.55

p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	Unique	Hatchling gut	0.001	0.047	0.06	0.45
p__Proteobacteria__g__Klebsiella_347396	Unique	Hatchling gut	0	0.047	0	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	Unique	Hatchling gut	0.014	0.041	0.12	0.61
p__Proteobacteria__g__Stenotrophomonas_168758	Unique	Hatchling gut	0.005	0.042	0.18	0.73
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	Unique	Hatchling gut	0.025	0.034	0.18	0.65
p__Bacteroidetes__f__Cryomorphaceae_690566	Unique	Hatchling gut	0	0.168	0	0.55
p__Firmicutes__g__Lysinibacillus_81925	Unique	Hatchling gut	0.004	0.58	0.12	0.94
p__Actinobacteria__g__Janibacter_703951	Unique	Hatchling gut	0.007	0.077	0.12	0.45
p__Proteobacteria__g__Devosia_497251	Unique	Hatchling gut	0.019	0.045	0.18	0.8
p__Bacteroidetes__g__Wautersiella_232450	Unique	Hatchling gut	0.001	0.233	0.12	0.63
p__Proteobacteria__g__Pseudomonas_159349	Unique	Hatchling gut	1.185	0.142	0.35	0.69
p__Bacteroidetes__g__Sphingobacterium_250053	Unique	Hatchling gut	0.001	0.199	0.06	0.9
p__Bacteroidetes__f__Flavobacteriaceae_555335	Unique	Hatchling gut	0.102	0.165	0.18	0.65
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Hatchling gut	0.001	0.058	0.06	0.71
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Hatchling gut	0.01	0.235	0.24	0.41
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	Unique	Hatchling gut	0.016	0.141	0.29	0.86
p__Firmicutes__g__Paenibacillus_556209	Unique	Hatchling gut	0.006	0.097	0.18	0.88
p__Actinobacteria__g__Pimelobacter_394756	Unique	Hatchling gut	0.002	0.01	0.06	0.43
p__Proteobacteria__g__Pseudomonas_10820	Unique	Hatchling gut	0.002	0.327	0.12	0.73
p__Proteobacteria__f__Rhodospirillaceae_424659	Unique	Hatchling gut	0	0.247	0	0.55
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Unique	Hatchling gut	0.004	0.149	0.24	0.86
p__Bacteroidetes__g__Sphingobacterium_204117	Unique	Hatchling gut	0.181	0.16	0.18	0.47
p__Actinobacteria__g__Tessaracoccus_287453	Unique	Hatchling gut	0	0.221	0	0.49
p__Proteobacteria__g__Pseudomonas_132221	Unique	Hatchling gut	0	0.295	0	0.76
p__Proteobacteria__g__Devosia_749390	Unique	Hatchling gut	0.001	0.2	0.06	0.61
p__Bacteroidetes__f__Flexibacteraceae_122867	Unique	Hatchling gut	0	0.151	0	0.45
p__Proteobacteria__g__Alcanivorax_427659	Unique	Hatchling gut	0.046	0.031	0.29	0.45
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	Unique	Hatchling gut	0.001	0.221	0.06	0.84
p__Proteobacteria__g__Bosea_424613	Unique	Hatchling gut	0.075	0.016	0.35	0.47

p__Proteobacteria__g__Stenotrophomonas_657476	Unique	Hatchling gut	0.001	0.16	0.06	0.53
p__Proteobacteria__g__Stenotrophomonas_729526	Unique	Hatchling gut	0	0.186	0	0.78
p__Proteobacteria__g__Stenotrophomonas_247119	Unique	Hatchling gut	0.088	0.142	0.12	0.86
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	Unique	Hatchling gut	0.001	0.144	0.06	0.82
p__Proteobacteria__f__Enterobacteriaceae_452665	Unique	Hatchling gut	0	0.16	0	0.69
p__Actinobacteria__g__Leucobacter_418594	Unique	Hatchling gut	0	0.14	0	0.49
p__Bacteroidetes__o__Sphingobacteriales_571041	Unique	Hatchling gut	0	0.131	0	0.43
p__Proteobacteria__f__Pseudomonadaceae_99078	Unique	Hatchling gut	0.001	0.146	0.06	0.76
p__Proteobacteria__g__Pseudomonas_174392	Unique	Hatchling gut	0.009	0.133	0.24	0.86
p__Proteobacteria__g__Pseudomonas_142124	Unique	Hatchling gut	0.075	0.115	0.18	0.82
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	Unique	Hatchling gut	0	0.11	0	0.71
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0.001	0.02	0.06	0.41
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.001	0.019	0.06	0.45
p__Actinobacteria__g__Rhodococcus_413286	Unique	Hatchling gut	0	0.048	0	0.67
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Hatchling gut	0.002	0.085	0.12	0.67
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.001	0.019	0.06	0.45
p__Proteobacteria__g__Achromobacter_678564	Unique	Hatchling gut	0.017	0.086	0.24	0.73
p__Proteobacteria__f__Enterobacteriaceae_410502	Unique	Hatchling gut	0	0.088	0	0.67
p__Proteobacteria__f__Alcaligenaceae_503155	Unique	Hatchling gut	0.026	0.076	0.24	0.75
p__Proteobacteria__g__Erwinia_25313	Unique	Hatchling gut	0	0.083	0	0.55
p__Proteobacteria__f__Enterobacteriaceae_231744	Unique	Hatchling gut	0.001	0.082	0.06	0.57
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	Unique	Hatchling gut	0	0.081	0	0.45
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0	0.69
p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0.018	0.073	0.06	0.45
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0.001	0.02	0.06	0.41
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0.025	0.051	0.35	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.047	0.026	0.24	0.47
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0.001	0.067	0.06	0.59

p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0.002	0.065	0.12	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0.025	0.058	0.18	0.43
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0	0.06	0	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0	0.064	0	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0.005	0.063	0.24	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0.011	0.059	0.12	0.73
p__Bacteroidetes__g__Chitinophaga_80088	Unique	Hatchling gut	0	0.027	0	0.51
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0.011	0.031	0.12	0.65
p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0.004	0.058	0.18	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0.008	0.033	0.12	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0.001	0.058	0.06	0.57
p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.032	0.024	0.24	0.45
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0.013	0.049	0.12	0.78
p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0	0.052	0	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0	0.53
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0	0.048	0	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0	0.049	0	0.71
p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0.005	0.038	0.12	0.55
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	Unique	Hatchling gut	0.001	0.047	0.06	0.45
p__Proteobacteria__g__Klebsiella_347396	Unique	Hatchling gut	0	0.047	0	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	Unique	Hatchling gut	0.014	0.041	0.12	0.61
p__Proteobacteria__g__Stenotrophomonas_168758	Unique	Hatchling gut	0.005	0.042	0.18	0.73
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	Unique	Hatchling gut	0.025	0.034	0.18	0.65
p__Bacteroidetes__f__Cryomorphaceae_690566	Unique	Hatchling gut	0	0.168	0	0.55
p__Firmicutes__g__Lysinibacillus_81925	Unique	Hatchling gut	0.004	0.58	0.12	0.94
p__Actinobacteria__g__Janibacter_703951	Unique	Hatchling gut	0.007	0.077	0.12	0.45
p__Proteobacteria__g__Devosia_497251	Unique	Hatchling gut	0.019	0.045	0.18	0.8

p__Bacteroidetes__g__Wautersiella_232450	Unique	Hatchling gut	0.001	0.233	0.12	0.63
p__Proteobacteria__g__Pseudomonas_159349	Unique	Hatchling gut	1.185	0.142	0.35	0.69
p__Bacteroidetes__g__Sphingobacterium_250053	Unique	Hatchling gut	0.001	0.199	0.06	0.9
p__Bacteroidetes__f__Flavobacteriaceae_555335	Unique	Hatchling gut	0.102	0.165	0.18	0.65
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Hatchling gut	0.001	0.058	0.06	0.71
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Hatchling gut	0.01	0.235	0.24	0.41
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	Unique	Hatchling gut	0.016	0.141	0.29	0.86
p__Firmicutes__g__Paenibacillus_556209	Unique	Hatchling gut	0.006	0.097	0.18	0.88
p__Actinobacteria__g__Pimelobacter_394756	Unique	Hatchling gut	0.002	0.01	0.06	0.43
p__Proteobacteria__g__Pseudomonas_10820	Unique	Hatchling gut	0.002	0.327	0.12	0.73
p__Proteobacteria__f__Rhodospirillaceae_424659	Unique	Hatchling gut	0	0.247	0	0.55
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Unique	Hatchling gut	0.004	0.149	0.24	0.86
p__Bacteroidetes__g__Sphingobacterium_204117	Unique	Hatchling gut	0.181	0.16	0.18	0.47
p__Actinobacteria__g__Tessaracoccus_287453	Unique	Hatchling gut	0	0.221	0	0.49
p__Proteobacteria__g__Pseudomonas_132221	Unique	Hatchling gut	0	0.295	0	0.76
p__Proteobacteria__g__Devosia_749390	Unique	Hatchling gut	0.001	0.2	0.06	0.61
p__Bacteroidetes__f__Flexibacteraceae_122867	Unique	Hatchling gut	0	0.151	0	0.45
p__Proteobacteria__g__Alcanivorax_427659	Unique	Hatchling gut	0.046	0.031	0.29	0.45
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	Unique	Hatchling gut	0.001	0.221	0.06	0.84
p__Proteobacteria__g__Bosea_424613	Unique	Hatchling gut	0.075	0.016	0.35	0.47
p__Proteobacteria__g__Stenotrophomonas_657476	Unique	Hatchling gut	0.001	0.16	0.06	0.53
p__Proteobacteria__g__Stenotrophomonas_729526	Unique	Hatchling gut	0	0.186	0	0.78
p__Proteobacteria__g__Stenotrophomonas_247119	Unique	Hatchling gut	0.088	0.142	0.12	0.86
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	Unique	Hatchling gut	0.001	0.144	0.06	0.82
p__Proteobacteria__f__Enterobacteriaceae_452665	Unique	Hatchling gut	0	0.16	0	0.69
p__Actinobacteria__g__Leucobacter_418594	Unique	Hatchling gut	0	0.14	0	0.49
p__Bacteroidetes__o__Sphingobacteriales_571041	Unique	Hatchling gut	0	0.131	0	0.43
p__Proteobacteria__f__Pseudomonadaceae_99078	Unique	Hatchling gut	0.001	0.146	0.06	0.76
p__Proteobacteria__g__Pseudomonas_174392	Unique	Hatchling gut	0.009	0.133	0.24	0.86

p__Proteobacteria__g__Pseudomonas_142124	Unique	Hatchling gut	0.075	0.115	0.18	0.82
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	Unique	Hatchling gut	0	0.11	0	0.71
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0.001	0.02	0.06	0.41
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.001	0.019	0.06	0.45
p__Actinobacteria__g__Rhodococcus_413286	Unique	Hatchling gut	0	0.048	0	0.67
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Hatchling gut	0.002	0.085	0.12	0.67
p__Proteobacteria__g__Diaphorobacter_687258	Unique	Hatchling gut	0.006	0.067	0.06	0.45
p__Firmicutes__g__Oceanobacillus_357784	Unique	Hatchling gut	0.005	0.068	0.18	0.53
p__Proteobacteria__g__Pseudomonas_572655	Unique	Hatchling gut	0	0.11	0	0.76
p__Proteobacteria__g__Achromobacter_678564	Unique	Hatchling gut	0.017	0.086	0.24	0.73
p__Proteobacteria__f__Enterobacteriaceae_410502	Unique	Hatchling gut	0	0.088	0	0.67
p__Proteobacteria__f__Alcaligenaceae_503155	Unique	Hatchling gut	0.026	0.076	0.24	0.75
p__Bacteroidetes__g__Chitinophaga_80088	Unique	Hatchling gut	0	0.027	0	0.51
p__Proteobacteria__g__Erwinia_25313	Unique	Hatchling gut	0	0.083	0	0.55
p__Proteobacteria__f__Enterobacteriaceae_231744	Unique	Hatchling gut	0.001	0.082	0.06	0.57
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	Unique	Hatchling gut	0	0.081	0	0.45
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0	0.69
p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0.018	0.073	0.06	0.45
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0.025	0.051	0.35	0.49
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.047	0.026	0.24	0.47
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0.001	0.067	0.06	0.59
p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0.002	0.065	0.12	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0.025	0.058	0.18	0.43
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0	0.06	0	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0	0.064	0	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0.005	0.063	0.24	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0.011	0.059	0.12	0.73

p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.032	0.024	0.24	0.45
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0.011	0.031	0.12	0.65
p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0.004	0.058	0.18	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0.008	0.033	0.12	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0.001	0.058	0.06	0.57
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0.013	0.049	0.12	0.78
p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0	0.052	0	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0	0.53
p__Proteobacteria__g__Pseudomonas_711667	Unique	Hatchling gut	0	0.042	0	0.73
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0	0.048	0	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0	0.049	0	0.71
p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0.005	0.038	0.12	0.55
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0	0.69
p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0.018	0.073	0.06	0.45
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0.025	0.051	0.35	0.49
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.047	0.026	0.24	0.47
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0.001	0.067	0.06	0.59
p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0.002	0.065	0.12	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0.025	0.058	0.18	0.43
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0	0.06	0	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0	0.064	0	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0.005	0.063	0.24	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0.011	0.059	0.12	0.73
p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.032	0.024	0.24	0.45
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0.011	0.031	0.12	0.65

p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0.004	0.058	0.18	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0.008	0.033	0.12	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0.001	0.058	0.06	0.57
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0.013	0.049	0.12	0.78
p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0	0.052	0	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0	0.53
p__Proteobacteria__g__Pseudomonas_711667	Unique	Hatchling gut	0	0.042	0	0.73
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0	0.048	0	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0	0.049	0	0.71
p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0.005	0.038	0.12	0.55

Table 4.11 Shared and unique OTUs between adult gut and adult blood samples.

Taxa	Type	Details	Adult gut abundance	Adult blood abundance	Adult gut occurrence	Adult blood occurrence
p__Proteobacteria__g__Ralstonia_478818	Unique	Adult blood	36.355	0.028	1	0.18
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Unique	Adult blood	6.795	0.029	1	0.35
p__Proteobacteria__f__Enterobacteriaceae_171036	Unique	Adult blood	1.305	0	1	0
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Unique	Adult blood	2.365	0.005	1	0.18
p__Actinobacteria__g__Arthrobacter_706755	Unique	Adult blood	0.76	0.034	0.5	0.24
p__Firmicutes__g__Bacillus_535651	Unique	Adult blood	1.515	0.46	0.5	0.29
p__Proteobacteria__f__Moraxellaceae_414919	Unique	Adult blood	1.44	0.033	1	0.06
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	Unique	Adult blood	0.085	0.005	0.5	0.18
p__Actinobacteria__f__Corynebacteriaceae_361263	Unique	Adult blood	0.03	0	0.5	0
p__Firmicutes__g__Streptococcus_269499	Unique	Adult blood	5.24	0.01	1	0.18
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Unique	Adult blood	1.04	0.007	0.5	0.18
p__Bacteroidetes__g__Chryseobacterium_308134	Unique	Adult blood	0.845	1.293	0.5	0.29
p__Proteobacteria__g__Delftia_587073	Unique	Adult blood	0.845	0.014	1	0.12
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Unique	Adult blood	0.79	0.009	1	0.12
p__Actinobacteria__g__Janibacter_703951	Unique	Adult blood	0.535	0.007	0.5	0.12
p__Firmicutes__g__Staphylococcus_171970	Unique	Adult blood	0.8	0.005	0.5	0.18
p__Proteobacteria__g__Acinetobacter_78571	Unique	Adult blood	2.965	0.021	1	0.24
p__Proteobacteria__f__Rhodospirillaceae_213569	Unique	Adult blood	0.225	0.009	0.5	0.29
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Adult blood	0.015	0.001	0.5	0.06
p__Proteobacteria__g__Pedomicrobium_452291	Unique	Adult blood	0.42	0.021	0.5	0.24
p__Proteobacteria__g__Rheinheimera__s__Rheinheimeraaquimaris_486023	Unique	Adult blood	0.03	0	0.5	0
p__Proteobacteria__g__Devosia_749390	Unique	Adult blood	0.03	0.001	0.5	0.06
p__Acidobacteria__o__Acidobacteriales_743933	Unique	Adult blood	0.45	0.009	0.5	0.18
p__Proteobacteria__f__Sinobacteraceae_232273	Unique	Adult blood	0.48	0.032	0.5	0.29
p__Actinobacteria__o__Solirubrobacterales_711193	Unique	Adult blood	0.53	0.026	0.5	0.18

p__Proteobacteria__g__Bosea_424613	Unique	Adult blood	0.755	0.075	0.5	0.35
p__Actinobacteria__f__CL50029_390474	Unique	Adult blood	0.03	0.014	0.5	0.29
p__Firmicutes__g__Enterococcus_396129	Unique	Adult blood	0.335	0.021	0.5	0.06
p__Actinobacteria__o__Actinomycetales_409160	Unique	Adult blood	0.28	0.025	0.5	0.18
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Adult blood	0.015	0.002	0.5	0.12
p__Actinobacteria__g__Dietzia_70231	Unique	Adult blood	0.015	0.001	0.5	0.06
p__Chloroflexi__c__SOGA31_42333	Unique	Adult blood	0.31	0.014	0.5	0.29
p__Proteobacteria__g__Trabulsiella__s__Kluyveraascorbata_152515	Unique	Adult blood	0.03	0.023	0.5	0.12
p__Proteobacteria__g__Klebsiella_558424	Core	Adult blood, Adult gut	0.255	0.843	0.5	0.47
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Adult blood, Adult gut	1.44	0.554	1	0.47
p__Proteobacteria__g__Pseudomonas_639712	Core	Adult blood, Adult gut	0.38	7.649	1	0.82
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Adult blood, Adult gut	1.505	4.486	1	0.53
p__Proteobacteria__g__Achromobacter_19649	Core	Adult blood, Adult gut	0.775	3.192	1	0.88
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Adult blood, Adult gut	1	1.492	1	0.82
p__Proteobacteria__f__SUP05_285720	Core	Adult blood, Adult gut	0.375	5.472	0.5	0.94
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Adult blood, Adult gut	0.255	0.199	0.5	0.65
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	Core	Adult blood, Adult gut	21.545	0.006	0.5	0.47
p__Proteobacteria__f__Rhodospirillaceae_441088	Core	Adult blood, Adult gut	0.995	0.044	1	0.53
p__Proteobacteria__g__Pseudomonas_34989	Core	Adult blood, Adult gut	0.3	0.29	0.5	0.47
p__Proteobacteria__f__Sinobacteraceae_155918	Core	Adult blood, Adult gut	0.395	0.031	0.5	0.41
p__Proteobacteria__f__Sinobacteraceae_668199	Core	Adult blood, Adult gut	0.48	0.035	0.5	0.47
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Core	Adult blood, Adult gut	0.255	0.088	0.5	0.53
p__Actinobacteria__g__Streptomyces_420197	Core	Adult blood, Adult gut	0.03	0.118	0.5	0.59
p__Proteobacteria__f__Rhodospirillaceae_622262	Core	Adult blood, Adult gut	1.545	0.042	0.5	0.65
p__Proteobacteria__o__Rhizobiales_602739	Core	Adult blood, Adult gut	0.62	0.029	0.5	0.41
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Adult gut	0	18.41	0	0.94
p__Proteobacteria__g__Aquamicrobium_748021	Unique	Adult gut	0	0.606	0	0.82
p__Proteobacteria__g__Agrobacterium_442913	Unique	Adult gut	0	2.127	0	0.94
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Unique	Adult gut	0	0.088	0	0.65

p__Proteobacteria__f__Brucellaceae_698093	Unique	Adult gut	0	0.712	0	0.41
p__Proteobacteria__g__Brucella_305522	Unique	Adult gut	0	3.461	0	0.94
p__Proteobacteria__g__Lysobacter_318152	Unique	Adult gut	0	1.121	0	0.47
p__Proteobacteria__f__Pasteurellaceae_142268	Unique	Adult gut	0	4.802	0	0.94
p__Actinobacteria__o__03197L14_108564	Unique	Adult gut	0	5.696	0	0.94
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Adult gut	0	3.948	0	0.76
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	Unique	Adult gut	0	0.015	0	0.41
p__Actinobacteria__g__Leucobacter_697145	Unique	Adult gut	0	0.706	0	0.65
p__Firmicutes__g__Bacillus_161559	Unique	Adult gut	0	0.1	0	0.65
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Unique	Adult gut	0	0.026	0	0.47
p__Chloroflexi__c__SOGA31_314194	Unique	Adult gut	0	0.082	0	0.41
p__Bacteroidetes__f__Flavobacteriaceae_729660	Unique	Adult gut	0	0.406	0	0.53
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	Unique	Adult gut	0	2.183	0	0.94
p__Proteobacteria__f__Sphingomonadaceae_363679	Unique	Adult gut	0	0.086	0	0.53
p__Bacteroidetes__f__Flavobacteriaceae_379313	Unique	Adult gut	0	1.6	0	0.47
p__Bacteroidetes__f__Flavobacteriaceae_12392	Unique	Adult gut	0	1.412	0	0.94
p__Proteobacteria__g__Shinella_393155	Unique	Adult gut	0	0.422	0	0.76
p__Proteobacteria__g__Arcobacter_587211	Unique	Adult gut	0	1.469	0	0.88
p__Proteobacteria__g__Nitratireductor_5045	Unique	Adult gut	0	0.292	0	0.71
p__SR1__c___571035	Unique	Adult gut	0	0.974	0	0.76
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Adult gut	0	0.717	0	0.65
p__Proteobacteria__o__Myxococcales_668245	Unique	Adult gut	0	1.339	0	0.65
p__Proteobacteria__g__Steroidobacter_168803	Unique	Adult gut	0	0.038	0	0.47
p__Actinobacteria__f__Solirubrobacteraceae_1327	Unique	Adult gut	0	0.059	0	0.59
p__Actinobacteria__g__Dermacoccus_337626	Unique	Adult gut	0	0.646	0	0.53
p__Proteobacteria__g__Sphingopyxis_102703	Unique	Adult gut	0	0.071	0	0.65
p__Actinobacteria__o__koll13_76572	Unique	Adult gut	0	0.048	0	0.59
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	Unique	Adult gut	0	0.834	0	0.88
p__Proteobacteria__f__Phyllobacteriaceae_589505	Unique	Adult gut	0	0.064	0	0.47

p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Adult gut	0	0.084	0	0.41
p__Proteobacteria__f__Rhodospirillaceae_494802	Unique	Adult gut	0	0.029	0	0.53
p__Proteobacteria__o__Sphingomonadales_709703	Unique	Adult gut	0	0.012	0	0.47
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Unique	Adult gut	0	0.041	0	0.47
p__Actinobacteria__g__Propionibacterium_427206	Unique	Adult gut	0	0.69	0	0.88
p__Actinobacteria__g__Arcanobacterium_246345	Unique	Adult gut	0	0.695	0	0.47
p__Proteobacteria__g__Sulfurimonas_444197	Unique	Adult gut	0	0.49	0	0.76
p__Fusobacteria__g__Streptobacillus__s__Streptobacillusmoniliformis_287396	Unique	Adult gut	0	0.52	0	0.59
p__GN02__c__VC12cl04_595855	Unique	Adult gut	0	0.451	0	0.53
p__Proteobacteria__g__Arcobacter_130909	Unique	Adult gut	0	0.43	0	0.82
p__Tenericutes__g__Mycoplasma_551498	Unique	Adult gut	0	0.183	0	0.76
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	Unique	Adult gut	0	0.417	0	0.71
p__Proteobacteria__g__Sulfurimonas_448949	Unique	Adult gut	0	0.389	0	0.88
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Adult gut	0	0.066	0	0.41
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Adult gut	0	0.249	0	0.71
p__Proteobacteria__g__Devosia_700503	Unique	Adult gut	0	0.031	0	0.41
p__Proteobacteria__g__Stenoxybacter_253237	Unique	Adult gut	0	0.431	0	0.71
p__Proteobacteria__f__Rhodobacteraceae_729919	Unique	Adult gut	0	0.41	0	0.53
p__Proteobacteria__f__Rhodospirillaceae_362274	Unique	Adult gut	0	0.021	0	0.59
p__Proteobacteria__g__Achromobacter_468982	Unique	Adult gut	0	0.121	0	0.59
p__Firmicutes__g__Coprococcus_628747	Unique	Adult gut	0	0.32	0	0.53
p__Bacteroidetes__g__Bacteroides_689514	Unique	Adult gut	0	0.106	0	0.59
p__Proteobacteria__f__Campylobacteraceae_360616	Unique	Adult gut	0	0.222	0	0.76
p__Proteobacteria__g__Pseudomonas_466613	Unique	Adult gut	0	0.115	0	0.53
p__SR1__c___526194	Unique	Adult gut	0	0.134	0	0.53
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	Unique	Adult gut	0	0.24	0	0.71
p__Firmicutes__g__Coprococcus_312354	Unique	Adult gut	0	0.263	0	0.41
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Adult gut	0	0.087	0	0.71
p__Firmicutes__o__Clostridiales_226726	Unique	Adult gut	0	0.134	0	0.76

p__Proteobacteria__g__Stenoxybacter_396392	Unique	Adult gut	0	0.271	0	0.71
p__Firmicutes__o__Clostridiales_701726	Unique	Adult gut	0	0.258	0	0.88
p__Proteobacteria__g__Stenoxybacter_687490	Unique	Adult gut	0	0.212	0	0.82
p__Proteobacteria__g__Shinella_386729	Unique	Adult gut	0	0.025	0	0.65
p__Actinobacteria__g__Arthrobacter_694831	Unique	Adult gut	0	0.133	0	0.82
p__Actinobacteria__g__Actinomyces_119009	Unique	Adult gut	0	0.105	0	0.59
p__Proteobacteria__f__Alcaligenaceae_536615	Unique	Adult gut	0	0.234	0	0.65
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_585025	Unique	Adult gut	0	0.165	0	0.71
p__Proteobacteria__g__Stenoxybacter_174851	Unique	Adult gut	0	0.226	0	0.82
p__Proteobacteria__g__Pseudomonas_161575	Unique	Adult gut	0	0.041	0	0.47
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	Unique	Adult gut	0	0.039	0	0.41
p__Proteobacteria__f__Rhodobacteraceae_445008	Unique	Adult gut	0	0.175	0	0.47
p__Proteobacteria__g__Stenoxybacter_572518	Unique	Adult gut	0	0.199	0	0.76
p__Proteobacteria__g__Achromobacter_155903	Unique	Adult gut	0	0.038	0	0.65
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	Unique	Adult gut	0	0.133	0	0.65
p__Proteobacteria__g__Stenoxybacter_317953	Unique	Adult gut	0	0.179	0	0.82
p__Bacteroidetes__g__Tenacibaculum_428560	Unique	Adult gut	0	0.057	0	0.71
p__Spirochaetes__g__Treponema_30617	Unique	Adult gut	0	0.146	0	0.53
p__Proteobacteria__g__Pseudomonas_40331	Unique	Adult gut	0	0.074	0	0.47
p__Proteobacteria__g__Moraxella_610605	Unique	Adult gut	0	0.034	0	0.41
p__Tenericutes__g__Bulleidia_166205	Unique	Adult gut	0	0.141	0	0.65
p__Proteobacteria__g__Pseudomonas_165065	Unique	Adult gut	0	0.137	0	0.59
p__Proteobacteria__o__Chromatiales_537609	Unique	Adult gut	0	0.134	0	0.82

Table 4.12 Shared and unique OTUs between adult blood and egg contents samples.

Taxa	**Type**	**Details**	**Adult blood abundance**	**Egg contents abundance**	**Adult blood occurrence**	**Egg contents occurrence**
p__Actinobacteria__g__Arthrobacter_706755	Unique	Adult blood	0.76	0.159	0.5	0.33
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	Unique	Adult blood	0.085	0.055	0.5	0.27
p__Actinobacteria__f__Corynebacteriaceae_361263	Unique	Adult blood	0.03	0.097	0.5	0.27
p__Proteobacteria__g__Pseudomonas_34989	Unique	Adult blood	0.3	0.029	0.5	0.33
p__Firmicutes__g__Staphylococcus_171970	Unique	Adult blood	0.8	0.139	0.5	0.33
p__Proteobacteria__f__Rhodospirillaceae_213569	Unique	Adult blood	0.225	0.939	0.5	0.27
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Adult blood	0.015	0.006	0.5	0.07
p__Proteobacteria__g__Rheinheimera__s__Rheinheimeraaquimaris_486023	Unique	Adult blood	0.03	0.005	0.5	0.07
p__Proteobacteria__g__Devosia_749390	Unique	Adult blood	0.03	0.01	0.5	0.13
p__Proteobacteria__g__Bosea_424613	Unique	Adult blood	0.755	0.146	0.5	0.27
p__Proteobacteria__o__Rhizobiales_602739	Unique	Adult blood	0.62	0.083	0.5	0.13
p__Firmicutes__g__Enterococcus_396129	Unique	Adult blood	0.335	0	0.5	0
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Adult blood	0.015	0.002	0.5	0.07
p__Actinobacteria__g__Dietzia_70231	Unique	Adult blood	0.015	0.043	0.5	0.2
p__Chloroflexi__c__SOGA31_42333	Unique	Adult blood	0.31	0.137	0.5	0.27
p__Proteobacteria__g__Trabulsiella__s__Kluyveraascorbata_152515	Unique	Adult blood	0.03	0	0.5	0
p__Proteobacteria__g__Ralstonia_478818	Core	Adult blood, Egg contents	36.355	30.82	1	0.93
p__Proteobacteria__g__Klebsiella_558424	Core	Adult blood, Egg contents	0.255	0.281	0.5	0.53
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Core	Adult blood, Egg contents	6.795	6.784	1	1
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Adult blood, Egg contents	1.44	0.513	1	0.6
p__Proteobacteria__g__Pseudomonas_639712	Core	Adult blood, Egg contents	0.38	0.271	1	0.47
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Adult blood, Egg contents	1.505	1.015	1	0.87
p__Proteobacteria__g__Achromobacter_19649	Core	Adult blood, Egg contents	0.775	0.732	1	0.8
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Adult blood, Egg contents	1	1.012	1	0.67
p__Proteobacteria__f__SUP05_285720	Core	Adult blood, Egg contents	0.375	3.915	0.5	0.87

p__Proteobacteria__f__Enterobacteriaceae_171036	Core	Adult blood, Egg contents	1.305	0.121	1	0.67
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Core	Adult blood, Egg contents	2.365	1.098	1	0.87
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Adult blood, Egg contents	0.255	0.355	0.5	0.53
p__Firmicutes__g__Bacillus_535651	Core	Adult blood, Egg contents	1.515	0.337	0.5	0.67
p__Proteobacteria__f__Moraxellaceae_414919	Core	Adult blood, Egg contents	1.44	1.641	1	1
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	Core	Adult blood, Egg contents	21.545	0.399	0.5	0.47
p__Firmicutes__g__Streptococcus_269499	Core	Adult blood, Egg contents	5.24	1.663	1	0.87
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Core	Adult blood, Egg contents	1.04	0.449	0.5	0.4
p__Bacteroidetes__g__Chryseobacterium_308134	Core	Adult blood, Egg contents	0.845	0.465	0.5	0.6
p__Proteobacteria__g__Delftia_587073	Core	Adult blood, Egg contents	0.845	0.381	1	0.47
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Core	Adult blood, Egg contents	0.79	0.37	1	0.67
p__Proteobacteria__f__Rhodospirillaceae_441088	Core	Adult blood, Egg contents	0.995	1.091	1	0.47
p__Actinobacteria__g__Janibacter_703951	Core	Adult blood, Egg contents	0.535	0.219	0.5	0.47
p__Proteobacteria__g__Acinetobacter_78571	Core	Adult blood, Egg contents	2.965	0.383	1	0.47
p__Proteobacteria__f__Sinobacteraceae_155918	Core	Adult blood, Egg contents	0.395	1.066	0.5	0.6
p__Proteobacteria__f__Sinobacteraceae_668199	Core	Adult blood, Egg contents	0.48	0.736	0.5	0.47
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Core	Adult blood, Egg contents	0.255	0.484	0.5	0.47
p__Actinobacteria__g__Streptomyces_420197	Core	Adult blood, Egg contents	0.03	0.429	0.5	0.67
p__Proteobacteria__g__Pedomicrobium_452291	Core	Adult blood, Egg contents	0.42	0.469	0.5	0.53
p__Acidobacteria__o__Acidobacteriales_743933	Core	Adult blood, Egg contents	0.45	0.777	0.5	0.47
p__Proteobacteria__f__Rhodospirillaceae_622262	Core	Adult blood, Egg contents	1.545	0.446	0.5	0.53
p__Proteobacteria__f__Sinobacteraceae_232273	Core	Adult blood, Egg contents	0.48	0.424	0.5	0.4
p__Actinobacteria__o__Solirubrobacterales_711193	Core	Adult blood, Egg contents	0.53	0.352	0.5	0.4
p__Actinobacteria__f__CL50029_390474	Core	Adult blood, Egg contents	0.03	0.191	0.5	0.4
p__Actinobacteria__o__Actinomycetales_409160	Core	Adult blood, Egg contents	0.28	0.231	0.5	0.4
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Egg contents	0	6.725	0	1
p__Proteobacteria__g__Aquamicrobium_748021	Unique	Egg contents	0	0.109	0	0.4
p__Proteobacteria__g__Agrobacterium_442913	Unique	Egg contents	0	0.377	0	0.47
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Unique	Egg contents	0	0.359	0	0.4

p__Proteobacteria__g__Brucella_305522	Unique	Egg contents	0	0.331	0	0.6
p__Proteobacteria__f__Pasteurellaceae_142268	Unique	Egg contents	0	2.668	0	0.87
p__Actinobacteria__o__03197L14_108564	Unique	Egg contents	0	1.14	0	0.8
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Egg contents	0	2.407	0	0.73
p__Firmicutes__g__Bacillus_161559	Unique	Egg contents	0	0.259	0	0.47
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Unique	Egg contents	0	0.267	0	0.47
p__Bacteroidetes__f__Flavobacteriaceae_729660	Unique	Egg contents	0	1.848	0	0.73
p__Proteobacteria__f__Sphingomonadaceae_363679	Unique	Egg contents	0	1.281	0	0.6
p__Actinobacteria__g__Aeromicrobium_619093	Unique	Egg contents	0	0.665	0	0.67
p__Bacteroidetes__f__Flavobacteriaceae_12392	Unique	Egg contents	0	0.68	0	0.8
p__Proteobacteria__g__Shinella_393155	Unique	Egg contents	0	0.173	0	0.47
p__Proteobacteria__g__Nitratireductor_5045	Unique	Egg contents	0	0.583	0	0.53
p__SR1__c___571035	Unique	Egg contents	0	0.441	0	0.4
p__Proteobacteria__g__Devosia_497251	Unique	Egg contents	0	0.965	0	0.4
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Egg contents	0	0.437	0	0.4
p__Proteobacteria__g__Steroidobacter_168803	Unique	Egg contents	0	0.678	0	0.4
p__Actinobacteria__g__Mycobacterium_393834	Unique	Egg contents	0	0.155	0	0.47
p__Actinobacteria__f__Solirubrobacteraceae_1327	Unique	Egg contents	0	0.363	0	0.47
p__Proteobacteria__g__Sphingopyxis_102703	Unique	Egg contents	0	0.418	0	0.4
p__Actinobacteria__o__koll13_76572	Unique	Egg contents	0	0.707	0	0.47
p__Proteobacteria__f__Phyllobacteriaceae_589505	Unique	Egg contents	0	0.208	0	0.47
p__Proteobacteria__g__Kaistobacter_682533	Unique	Egg contents	0	0.43	0	0.47
p__Actinobacteria__g__Propionibacterium_427206	Unique	Egg contents	0	0.093	0	0.4
p__Proteobacteria__g__Rhodoplanes_273401	Unique	Egg contents	0	0.436	0	0.4
p__Actinobacteria__g__Nocardioides_128926	Unique	Egg contents	0	0.227	0	0.47
p__Actinobacteria__o__MC47_21675	Unique	Egg contents	0	0.201	0	0.4
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Egg contents	0	0.317	0	0.47
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Egg contents	0	0.177	0	0.4
p__Proteobacteria__g__Devosia_350266	Unique	Egg contents	0	0.271	0	0.4

p__Actinobacteria__o__MC47_160960	Unique	Egg contents	0	0.291	0	0.4
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Egg contents	0	0.183	0	0.4

Table 4.13 Shared and unique OTUs between hatchling gut and hatchling blood samples.

Taxa	Type	Details	Hatchling gut abundance	Hatchling blood abundance	Hatchling gut occurrence	Hatchling blood occurrence
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Unique	Hatchling blood	2.189	0.025	0.74	0.35
p__Proteobacteria__g__Pseudomonas_704131	Unique	Hatchling blood	1.201	0.029	0.42	0.16
p__Proteobacteria__f__Moraxellaceae_414919	Unique	Hatchling blood	0.425	0.002	0.46	0.06
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Unique	Hatchling blood	0.63	0.003	0.56	0.22
p__Actinobacteria__g__Mycobacterium_393834	Unique	Hatchling blood	0.332	0.007	0.4	0.33
p__Proteobacteria__g__Ralstonia_478818	Core	Hatchling blood, Hatchling gut	11.301	0.03	0.92	0.75
p__Proteobacteria__g__Klebsiella_558424	Core	Hatchling blood, Hatchling gut	1.382	16.023	0.62	0.96
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Core	Hatchling blood, Hatchling gut	9.365	0.327	0.74	0.8
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Hatchling blood, Hatchling gut	3.19	7.789	0.66	1
p__Proteobacteria__g__Pseudomonas_639712	Core	Hatchling blood, Hatchling gut	1.059	6.735	0.64	0.98
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Hatchling blood, Hatchling gut	1.183	6.391	0.72	1
p__Proteobacteria__f__Enterobacteriaceae_641353	Core	Hatchling blood, Hatchling gut	0.694	7.881	0.4	0.96
p__Proteobacteria__g__Achromobacter_19649	Core	Hatchling blood, Hatchling gut	2.143	4.655	0.74	1
p__Proteobacteria__g__Thermomonas_132820	Core	Hatchling blood, Hatchling gut	4.905	2.416	0.64	0.76
p__Proteobacteria__g__Aquamicrobium_748021	Core	Hatchling blood, Hatchling gut	2.516	2.442	0.72	1
p__Proteobacteria__g__Agrobacterium_442913	Core	Hatchling blood, Hatchling gut	1.797	2.104	0.6	0.96
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Hatchling blood, Hatchling gut	1.281	2.566	0.62	0.94

p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Core	Hatchling blood, Hatchling gut	2.89	1.629	0.76	1
p__Proteobacteria__f__Brucellaceae_698093	Core	Hatchling blood, Hatchling gut	1.65	2.726	0.68	0.92
p__Proteobacteria__g__Brucella_305522	Core	Hatchling blood, Hatchling gut	1.392	1.634	0.68	0.98
p__Proteobacteria__g__Lysobacter_318152	Core	Hatchling blood, Hatchling gut	3.405	0.198	0.74	0.9
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	Core	Hatchling blood, Hatchling gut	0.773	2.677	0.54	1
p__Proteobacteria__f__Enterobacteriaceae_171036	Core	Hatchling blood, Hatchling gut	1.243	1.598	0.46	0.92
p__Bacteroidetes__g__Sphingobacterium_176945	Core	Hatchling blood, Hatchling gut	0.382	2.064	0.46	0.78
p__Proteobacteria__g__Acinetobacter_168646	Core	Hatchling blood, Hatchling gut	1.851	0.377	0.44	0.47
p__Actinobacteria__g__Leucobacter_697145	Core	Hatchling blood, Hatchling gut	1.076	0.558	0.62	0.96
p__Bacteroidetes__g__Sphingobacterium_298499	Core	Hatchling blood, Hatchling gut	0.649	1.207	0.46	0.9
p__Bacteroidetes__f__Flavobacteriaceae_374120	Core	Hatchling blood, Hatchling gut	0.678	1.066	0.56	0.86
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	Core	Hatchling blood, Hatchling gut	1.309	0.42	0.7	0.94
p__Firmicutes__g__Bacillus_161559	Core	Hatchling blood, Hatchling gut	1.497	0.022	0.46	0.61
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Core	Hatchling blood, Hatchling gut	1.326	0.018	0.42	0.47
p__Actinobacteria__g__Arthrobacter_706755	Core	Hatchling blood, Hatchling gut	0.402	0.898	0.44	0.94
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	Core	Hatchling blood, Hatchling gut	0.45	0.907	0.58	0.86
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Hatchling blood, Hatchling gut	0.844	0.268	0.64	0.96
p__Proteobacteria__f__Alcaligenaceae_541229	Core	Hatchling blood, Hatchling gut	0.194	0.865	0.4	0.76

p__Firmicutes__g__Bacillus_245239	Core	Hatchling blood, Hatchling gut	0.851	0.049	0.4	0.41
p__Proteobacteria__f__Sphingomonadaceae_363679	Core	Hatchling blood, Hatchling gut	0.358	0.011	0.42	0.55
p__Actinobacteria__g__Aeromicrobium_619093	Core	Hatchling blood, Hatchling gut	0.444	0.06	0.48	0.84
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Core	Hatchling blood, Hatchling gut	0.527	0.032	0.5	0.43
p__Proteobacteria__g__Shinella_393155	Core	Hatchling blood, Hatchling gut	0.253	0.211	0.5	0.94
p__Proteobacteria__g__Pseudomonas_34989	Core	Hatchling blood, Hatchling gut	0.228	0.308	0.5	0.94
p__Proteobacteria__g__Nitratireductor_5045	Core	Hatchling blood, Hatchling gut	0.217	0.042	0.48	0.8
p__Bacteroidetes__g__Sphingobacterium_250053	Core	Hatchling blood, Hatchling gut	0.284	0.199	0.4	0.9
p__Actinobacteria__f__Nocardioidaceae_554632	Core	Hatchling blood, Hatchling gut	0.281	0.058	0.4	0.71
p__Proteobacteria__f__Phyllobacteriaceae_589505	Core	Hatchling blood, Hatchling gut	0.171	0.07	0.46	0.73
p__Actinobacteria__f__Nocardioidaceae_632222	Core	Hatchling blood, Hatchling gut	0.212	0.027	0.52	0.76
p__Proteobacteria__o__Sphingomonadales_709703	Core	Hatchling blood, Hatchling gut	0.147	0.059	0.4	0.73
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Core	Hatchling blood, Hatchling gut	0.143	0.149	0.42	0.86
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Core	Hatchling blood, Hatchling gut	0.243	0.019	0.5	0.67
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	Unique	Hatchling gut	0.227	1.831	0.26	0.63
p__Firmicutes__g__Bacillus_535651	Unique	Hatchling gut	0.456	0.418	0.36	0.49
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	Unique	Hatchling gut	0.172	0.993	0.32	0.43
p__Firmicutes__g__Paenibacillus_212495	Unique	Hatchling gut	0.377	0.779	0.18	0.8
p__Bacteroidetes__o__Sphingobacteriales_152865	Unique	Hatchling gut	0.646	0.235	0.2	0.53
p__Proteobacteria__g__Delftia_587073	Unique	Hatchling gut	0.487	0.07	0.38	0.53
p__Proteobacteria__f__Rhodospirillaceae_441088	Unique	Hatchling gut	0.194	0.02	0.32	0.61

p__Bacteroidetes__f__Cryomorphaceae_690566	Unique	Hatchling gut	0.492	0.168	0.32	0.55
p__Firmicutes__g__Lysinibacillus_81925	Unique	Hatchling gut	0.048	0.58	0.2	0.94
p__Actinobacteria__g__Janibacter_703951	Unique	Hatchling gut	0.402	0.077	0.34	0.45
p__Bacteroidetes__g__Wautersiella_232450	Unique	Hatchling gut	0.341	0.233	0.32	0.63
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	Unique	Hatchling gut	0.24	0.288	0.34	0.82
p__Proteobacteria__g__Pseudomonas_159349	Unique	Hatchling gut	0.001	0.142	0.06	0.69
p__Proteobacteria__g__Devosia_497251	Unique	Hatchling gut	0.134	0.045	0.38	0.8
p__Proteobacteria__g__Steroidobacter_168803	Unique	Hatchling gut	0.21	0.025	0.36	0.53
p__Bacteroidetes__f__Flavobacteriaceae_555335	Unique	Hatchling gut	0.213	0.165	0.36	0.65
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Unique	Hatchling gut	0.144	0.013	0.3	0.41
p__Actinobacteria__f__Solirubrobacteraceae_1327	Unique	Hatchling gut	0.149	0.013	0.36	0.47
p__Actinobacteria__g__Streptomyces_420197	Unique	Hatchling gut	0.158	0.022	0.34	0.53
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	Unique	Hatchling gut	0.134	0.141	0.36	0.86
p__Proteobacteria__g__Sphingopyxis_102703	Unique	Hatchling gut	0.141	0.059	0.38	0.82
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Hatchling gut	0.098	0.235	0.26	0.41
p__Proteobacteria__g__Pseudomonas_10820	Unique	Hatchling gut	0	0.327	0.02	0.73
p__Proteobacteria__f__Rhodospirillaceae_424659	Unique	Hatchling gut	0.066	0.247	0.16	0.55
p__Proteobacteria__f__Rhodospirillaceae_494802	Unique	Hatchling gut	0.11	0.024	0.22	0.41
p__Actinobacteria__g__Tessaracoccus_287453	Unique	Hatchling gut	0.084	0.221	0.16	0.49
p__Bacteroidetes__g__Sphingobacterium_204117	Unique	Hatchling gut	0.071	0.16	0.14	0.47
p__Proteobacteria__g__Pseudomonas_132221	Unique	Hatchling gut	0.001	0.295	0.06	0.76
p__Proteobacteria__g__Devosia_749390	Unique	Hatchling gut	0.083	0.2	0.24	0.61
p__Bacteroidetes__f__Flexibacteraceae_122867	Unique	Hatchling gut	0.128	0.151	0.22	0.45
p__Firmicutes__g__Paenibacillus_556209	Unique	Hatchling gut	0.177	0.097	0.38	0.88
p__Proteobacteria__g__Alcanivorax_427659	Unique	Hatchling gut	0.176	0.031	0.24	0.45
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	Unique	Hatchling gut	0.004	0.221	0.1	0.84
p__Proteobacteria__g__Stenotrophomonas_657476	Unique	Hatchling gut	0.044	0.16	0.08	0.53
p__Proteobacteria__g__Stenotrophomonas_729526	Unique	Hatchling gut	0	0.186	0.02	0.78
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	Unique	Hatchling gut	0.029	0.144	0.02	0.82

p__Proteobacteria__g__Stenotrophomonas_247119	Unique	Hatchling gut	0	0.142	0.02	0.86
p__Actinobacteria__g__Pimelobacter_394756	Unique	Hatchling gut	0.142	0.01	0.26	0.43
p__Proteobacteria__g__Bosea_424613	Unique	Hatchling gut	0.055	0.016	0.24	0.47
p__Proteobacteria__f__Enterobacteriaceae_452665	Unique	Hatchling gut	0.003	0.16	0.12	0.69
p__Actinobacteria__g__Leucobacter_418594	Unique	Hatchling gut	0.024	0.14	0.18	0.49
p__Bacteroidetes__o__Sphingobacteriales_571041	Unique	Hatchling gut	0.029	0.131	0.16	0.43
p__Proteobacteria__g__Devosia_700503	Unique	Hatchling gut	0.089	0.054	0.38	0.76
p__Proteobacteria__f__Pseudomonadaceae_99078	Unique	Hatchling gut	0.005	0.146	0.06	0.76
p__Proteobacteria__g__Pseudomonas_174392	Unique	Hatchling gut	0.012	0.133	0.14	0.86
p__Proteobacteria__g__Pseudomonas_142124	Unique	Hatchling gut	0	0.115	0.04	0.82
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	Unique	Hatchling gut	0.029	0.11	0.02	0.71
p__Proteobacteria__g__Achromobacter_468982	Unique	Hatchling gut	0	0.094	0	0.84
p__Proteobacteria__o__Rhizobiales_602739	Unique	Hatchling gut	0.035	0.027	0.18	0.57
p__Actinobacteria__g__Rhodococcus_413286	Unique	Hatchling gut	0.07	0.048	0.34	0.67
p__Firmicutes__g__Oceanobacillus_357784	Unique	Hatchling gut	0.019	0.068	0.12	0.53
p__Proteobacteria__g__Pseudomonas_572655	Unique	Hatchling gut	0	0.11	0	0.76
p__Proteobacteria__g__Diaphorobacter_687258	Unique	Hatchling gut	0.028	0.067	0.14	0.45
p__Proteobacteria__g__Pseudomonas_466613	Unique	Hatchling gut	0.001	0.067	0.08	0.67
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Hatchling gut	0.017	0.085	0.2	0.67
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.069	0.019	0.26	0.45
p__Proteobacteria__g__Achromobacter_678564	Unique	Hatchling gut	0	0.086	0	0.73
p__Proteobacteria__f__Enterobacteriaceae_410502	Unique	Hatchling gut	0	0.088	0	0.67
p__Proteobacteria__f__Alcaligenaceae_503155	Unique	Hatchling gut	0.001	0.076	0.02	0.75
p__Proteobacteria__g__Stenoxybacter_687490	Unique	Hatchling gut	0	0.012	0.02	0.59
p__Proteobacteria__g__Erwinia_25313	Unique	Hatchling gut	0	0.083	0	0.55
p__Proteobacteria__f__Enterobacteriaceae_231744	Unique	Hatchling gut	0	0.082	0	0.57
p__Proteobacteria__g__Shinella_386729	Unique	Hatchling gut	0.033	0.025	0.24	0.59
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	Unique	Hatchling gut	0	0.081	0.02	0.45
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0.02	0.69

p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0	0.073	0	0.45
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0.058	0.02	0.26	0.41
p__Proteobacteria__g__Pseudomonas_161575	Unique	Hatchling gut	0	0.061	0	0.78
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0.014	0.051	0.22	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	Unique	Hatchling gut	0.001	0.056	0.04	0.76
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.025	0.026	0.12	0.47
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0.002	0.067	0.06	0.59
p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0.001	0.065	0.06	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0	0.058	0	0.43
p__Proteobacteria__g__Achromobacter_155903	Unique	Hatchling gut	0	0.054	0.02	0.82
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0.006	0.06	0.12	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0.001	0.064	0.06	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0	0.063	0.02	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0	0.059	0.02	0.73
p__Bacteroidetes__g__Chitinophaga_80088	Unique	Hatchling gut	0.037	0.027	0.12	0.51
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0.026	0.031	0.08	0.65
p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0	0.058	0.02	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0.024	0.033	0.16	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0	0.058	0	0.57
p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.013	0.024	0.1	0.45
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0	0.049	0	0.78
p__Proteobacteria__g__Pseudomonas_40331	Unique	Hatchling gut	0	0.028	0.02	0.65
p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0.001	0.052	0.04	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0.02	0.53
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0.002	0.048	0.1	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0.001	0.049	0.02	0.71

p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0.011	0.038	0.14	0.55
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	Unique	Hatchling gut	0	0.047	0	0.45
p__Proteobacteria__g__Klebsiella_347396	Unique	Hatchling gut	0	0.047	0	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	Unique	Hatchling gut	0	0.041	0	0.61
p__Proteobacteria__g__Stenotrophomonas_168758	Unique	Hatchling gut	0	0.042	0.02	0.73
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	Unique	Hatchling gut	0.002	0.034	0.06	0.65

Table 4.14 Shared and unique OTUs between hatchling gut and egg content samples.

Taxa	Type	Details	Hatchling gut abundance	Egg contents abundance	Hatchling gut occurrence	Egg contents occurrence
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Egg contents	6.725	0.001	1	0.06
p__Proteobacteria__f__SUP05_285720	Unique	Egg contents	3.915	0	0.87	0
p__Proteobacteria__f__Pasteurellaceae_142268	Unique	Egg contents	2.668	0	0.87	0
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Unique	Egg contents	1.098	0.025	0.87	0.35
p__Actinobacteria__o__03197L14_108564	Unique	Egg contents	1.14	0	0.8	0
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Egg contents	2.407	0	0.73	0
p__Bacteroidetes__f__Flavobacteriaceae_729660	Unique	Egg contents	1.848	0	0.73	0
p__Proteobacteria__f__Moraxellaceae_414919	Unique	Egg contents	1.641	0.002	1	0.06
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	Unique	Egg contents	0.399	0	0.47	0.02
p__Firmicutes__g__Streptococcus_269499	Unique	Egg contents	1.663	0.003	0.87	0.1
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Unique	Egg contents	0.449	0.003	0.4	0.22
p__Bacteroidetes__g__Chryseobacterium_308134	Unique	Egg contents	0.465	0.117	0.6	0.22
p__Bacteroidetes__f__Flavobacteriaceae_12392	Unique	Egg contents	0.68	0	0.8	0
p__Proteobacteria__g__Acinetobacter_78571	Unique	Egg contents	0.383	0.008	0.47	0.04
p__SR1__c___571035	Unique	Egg contents	0.441	0	0.4	0
p__Proteobacteria__f__Sinobacteraceae_155918	Unique	Egg contents	1.066	0.007	0.6	0.35
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Egg contents	0.437	0	0.4	0
p__Proteobacteria__f__Sinobacteraceae_668199	Unique	Egg contents	0.736	0.01	0.47	0.35
p__Actinobacteria__g__Mycobacterium_393834	Unique	Egg contents	0.155	0.007	0.47	0.33
p__Actinobacteria__o__koll13_76572	Unique	Egg contents	0.707	0.013	0.47	0.35
p__Proteobacteria__g__Pedomicrobium_452291	Unique	Egg contents	0.469	0.003	0.53	0.2
p__Acidobacteria__o__Acidobacteriales_743933	Unique	Egg contents	0.777	0.002	0.47	0.08
p__Proteobacteria__g__Kaistobacter_682533	Unique	Egg contents	0.43	0.004	0.47	0.29
p__Proteobacteria__f__Rhodospirillaceae_622262	Unique	Egg contents	0.446	0.003	0.53	0.25
p__Actinobacteria__g__Propionibacterium_427206	Unique	Egg contents	0.093	0	0.4	0
p__Proteobacteria__f__Sinobacteraceae_232273	Unique	Egg contents	0.424	0.004	0.4	0.24

p__Actinobacteria__o__Solirubrobacterales_711193	Unique	Egg contents	0.352	0.003	0.4	0.18
p__Proteobacteria__g__Rhodoplanes_273401	Unique	Egg contents	0.436	0.008	0.4	0.25
p__Actinobacteria__g__Nocardioides_128926	Unique	Egg contents	0.227	0.006	0.47	0.29
p__Actinobacteria__o__MC47_21675	Unique	Egg contents	0.201	0.003	0.4	0.12
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Egg contents	0.317	0.002	0.47	0.12
p__Actinobacteria__f__CL50029_390474	Unique	Egg contents	0.191	0.003	0.4	0.16
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Egg contents	0.177	0	0.4	0
p__Proteobacteria__g__Devosia_350266	Unique	Egg contents	0.271	0.002	0.4	0.18
p__Actinobacteria__o__MC47_160960	Unique	Egg contents	0.291	0	0.4	0
p__Actinobacteria__o__Actinomycetales_409160	Unique	Egg contents	0.231	0.004	0.4	0.08
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Egg contents	0.183	0	0.4	0
p__Proteobacteria__g__Ralstonia_478818	Core	Egg contents, Hatchling gut	30.82	0.03	0.93	0.75
p__Proteobacteria__g__Klebsiella_558424	Core	Egg contents, Hatchling gut	0.281	16.023	0.53	0.96
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Core	Egg contents, Hatchling gut	6.784	0.327	1	0.8
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Egg contents, Hatchling gut	0.513	7.789	0.6	1
p__Proteobacteria__g__Pseudomonas_639712	Core	Egg contents, Hatchling gut	0.271	6.735	0.47	0.98
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Egg contents, Hatchling gut	1.015	6.391	0.87	1
p__Proteobacteria__g__Achromobacter_19649	Core	Egg contents, Hatchling gut	0.732	4.655	0.8	1
p__Proteobacteria__g__Aquamicrobium_748021	Core	Egg contents, Hatchling gut	0.109	2.442	0.4	1
p__Proteobacteria__g__Agrobacterium_442913	Core	Egg contents, Hatchling gut	0.377	2.104	0.47	0.96
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Egg contents, Hatchling gut	1.012	2.566	0.67	0.94
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Core	Egg contents, Hatchling gut	0.359	1.629	0.4	1
p__Proteobacteria__g__Brucella_305522	Core	Egg contents, Hatchling gut	0.331	1.634	0.6	0.98
p__Proteobacteria__f__Enterobacteriaceae_171036	Core	Egg contents, Hatchling gut	0.121	1.598	0.67	0.92
p__Firmicutes__g__Bacillus_161559	Core	Egg contents, Hatchling gut	0.259	0.022	0.47	0.61
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Core	Egg contents, Hatchling gut	0.267	0.018	0.47	0.47
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Egg contents, Hatchling gut	0.355	0.268	0.53	0.96
p__Firmicutes__g__Bacillus_535651	Core	Egg contents, Hatchling gut	0.337	0.418	0.67	0.49
p__Proteobacteria__f__Sphingomonadaceae_363679	Core	Egg contents, Hatchling gut	1.281	0.011	0.6	0.55

p__Actinobacteria__g__Aeromicrobium_619093	Core	Egg contents, Hatchling gut	0.665	0.06	0.67	0.84
p__Proteobacteria__g__Delftia_587073	Core	Egg contents, Hatchling gut	0.381	0.07	0.47	0.53
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Core	Egg contents, Hatchling gut	0.37	0.032	0.67	0.43
p__Proteobacteria__f__Rhodospirillaceae_441088	Core	Egg contents, Hatchling gut	1.091	0.02	0.47	0.61
p__Proteobacteria__g__Shinella_393155	Core	Egg contents, Hatchling gut	0.173	0.211	0.47	0.94
p__Actinobacteria__g__Janibacter_703951	Core	Egg contents, Hatchling gut	0.219	0.077	0.47	0.45
p__Proteobacteria__g__Nitratireductor_5045	Core	Egg contents, Hatchling gut	0.583	0.042	0.53	0.8
p__Proteobacteria__g__Devosia_497251	Core	Egg contents, Hatchling gut	0.965	0.045	0.4	0.8
p__Proteobacteria__g__Steroidobacter_168803	Core	Egg contents, Hatchling gut	0.678	0.025	0.4	0.53
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Core	Egg contents, Hatchling gut	0.484	0.013	0.47	0.41
p__Actinobacteria__f__Solirubrobacteraceae_1327	Core	Egg contents, Hatchling gut	0.363	0.013	0.47	0.47
p__Actinobacteria__g__Streptomyces_420197	Core	Egg contents, Hatchling gut	0.429	0.022	0.67	0.53
p__Proteobacteria__g__Sphingopyxis_102703	Core	Egg contents, Hatchling gut	0.418	0.059	0.4	0.82
p__Proteobacteria__f__Phyllobacteriaceae_589505	Core	Egg contents, Hatchling gut	0.208	0.07	0.47	0.73
p__Proteobacteria__f__Enterobacteriaceae_641353	Unique	Hatchling gut	0.002	7.881	0.07	0.96
p__Proteobacteria__g__Thermomonas_132820	Unique	Hatchling gut	0.021	2.416	0.13	0.76
p__Proteobacteria__f__Brucellaceae_698093	Unique	Hatchling gut	0.001	2.726	0.07	0.92
p__Proteobacteria__g__Lysobacter_318152	Unique	Hatchling gut	0.157	0.198	0.13	0.9
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	Unique	Hatchling gut	0.025	2.677	0.13	1
p__Bacteroidetes__g__Sphingobacterium_176945	Unique	Hatchling gut	0.003	2.064	0.13	0.78
p__Proteobacteria__g__Acinetobacter_168646	Unique	Hatchling gut	0.001	0.377	0.07	0.47
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	Unique	Hatchling gut	0.125	1.831	0.2	0.63
p__Actinobacteria__g__Leucobacter_697145	Unique	Hatchling gut	0.166	0.558	0.27	0.96
p__Bacteroidetes__g__Sphingobacterium_298499	Unique	Hatchling gut	0	1.207	0	0.9
p__Bacteroidetes__f__Flavobacteriaceae_374120	Unique	Hatchling gut	0.064	1.066	0.07	0.86
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	Unique	Hatchling gut	0.042	0.42	0.27	0.94
p__Actinobacteria__g__Arthrobacter_706755	Unique	Hatchling gut	0.159	0.898	0.33	0.94
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	Unique	Hatchling gut	0	0.907	0	0.86
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	Unique	Hatchling gut	0	0.993	0	0.43

p__Firmicutes__g__Paenibacillus_212495	Unique	Hatchling gut	0.012	0.779	0.07	0.8
p__Proteobacteria__f__Alcaligenaceae_541229	Unique	Hatchling gut	0	0.865	0	0.76
p__Bacteroidetes__o__Sphingobacteriales_152865	Unique	Hatchling gut	0	0.235	0	0.53
p__Firmicutes__g__Bacillus_245239	Unique	Hatchling gut	0	0.049	0	0.41
p__Proteobacteria__g__Pseudomonas_34989	Unique	Hatchling gut	0.029	0.308	0.33	0.94
p__Bacteroidetes__f__Cryomorphaceae_690566	Unique	Hatchling gut	0.003	0.168	0.07	0.55
p__Firmicutes__g__Lysinibacillus_81925	Unique	Hatchling gut	0.035	0.58	0.13	0.94
p__Bacteroidetes__g__Wautersiella_232450	Unique	Hatchling gut	0	0.233	0	0.63
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	Unique	Hatchling gut	0.052	0.288	0.33	0.82
p__Proteobacteria__g__Pseudomonas_159349	Unique	Hatchling gut	0.001	0.142	0.07	0.69
p__Bacteroidetes__g__Sphingobacterium_250053	Unique	Hatchling gut	0	0.199	0	0.9
p__Bacteroidetes__f__Flavobacteriaceae_555335	Unique	Hatchling gut	0	0.165	0	0.65
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	Unique	Hatchling gut	0.221	0.141	0.2	0.86
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Hatchling gut	0.006	0.058	0.07	0.71
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Hatchling gut	0	0.235	0	0.41
p__Proteobacteria__g__Pseudomonas_10820	Unique	Hatchling gut	0	0.327	0	0.73
p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Hatchling gut	0.159	0.027	0.33	0.76
p__Proteobacteria__f__Rhodospirillaceae_424659	Unique	Hatchling gut	0	0.247	0	0.55
p__Proteobacteria__f__Rhodospirillaceae_494802	Unique	Hatchling gut	0.523	0.024	0.33	0.41
p__Proteobacteria__o__Sphingomonadales_709703	Unique	Hatchling gut	0.22	0.059	0.33	0.73
p__Actinobacteria__g__Tessaracoccus_287453	Unique	Hatchling gut	0	0.221	0	0.49
p__Bacteroidetes__g__Sphingobacterium_204117	Unique	Hatchling gut	0.023	0.16	0.27	0.47
p__Proteobacteria__g__Pseudomonas_132221	Unique	Hatchling gut	0.002	0.295	0.07	0.76
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Unique	Hatchling gut	0	0.149	0	0.86
p__Proteobacteria__g__Devosia_749390	Unique	Hatchling gut	0.01	0.2	0.13	0.61
p__Bacteroidetes__f__Flexibacteraceae_122867	Unique	Hatchling gut	0	0.151	0	0.45
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Unique	Hatchling gut	0.012	0.019	0.13	0.67
p__Firmicutes__g__Paenibacillus_556209	Unique	Hatchling gut	0	0.097	0	0.88
p__Proteobacteria__g__Alcanivorax_427659	Unique	Hatchling gut	0.024	0.031	0.07	0.45

p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	Unique	Hatchling gut	0.001	0.221	0.07	0.84
p__Proteobacteria__g__Stenotrophomonas_657476	Unique	Hatchling gut	0	0.16	0	0.53
p__Proteobacteria__g__Stenotrophomonas_729526	Unique	Hatchling gut	0	0.186	0	0.78
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	Unique	Hatchling gut	0	0.144	0	0.82
p__Proteobacteria__g__Stenotrophomonas_247119	Unique	Hatchling gut	0	0.142	0	0.86
p__Actinobacteria__g__Pimelobacter_394756	Unique	Hatchling gut	0.067	0.01	0.27	0.43
p__Proteobacteria__g__Bosea_424613	Unique	Hatchling gut	0.146	0.016	0.27	0.47
p__Proteobacteria__f__Enterobacteriaceae_452665	Unique	Hatchling gut	0.005	0.16	0.13	0.69
p__Actinobacteria__g__Leucobacter_418594	Unique	Hatchling gut	0	0.14	0	0.49
p__Bacteroidetes__o__Sphingobacteriales_571041	Unique	Hatchling gut	0.003	0.131	0.07	0.43
p__Proteobacteria__g__Devosia_700503	Unique	Hatchling gut	0.004	0.054	0.07	0.76
p__Proteobacteria__f__Pseudomonadaceae_99078	Unique	Hatchling gut	0	0.146	0	0.76
p__Proteobacteria__g__Pseudomonas_174392	Unique	Hatchling gut	0	0.133	0	0.86
p__Proteobacteria__g__Pseudomonas_142124	Unique	Hatchling gut	0	0.115	0	0.82
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	Unique	Hatchling gut	0	0.11	0	0.71
p__Proteobacteria__g__Achromobacter_468982	Unique	Hatchling gut	0	0.094	0	0.84
p__Proteobacteria__o__Rhizobiales_602739	Unique	Hatchling gut	0.083	0.027	0.13	0.57
p__Actinobacteria__g__Rhodococcus_413286	Unique	Hatchling gut	0	0.048	0	0.67
p__Firmicutes__g__Oceanobacillus_357784	Unique	Hatchling gut	0.084	0.068	0.2	0.53
p__Proteobacteria__g__Pseudomonas_572655	Unique	Hatchling gut	0	0.11	0	0.76
p__Proteobacteria__g__Diaphorobacter_687258	Unique	Hatchling gut	0.036	0.067	0.2	0.45
p__Proteobacteria__g__Pseudomonas_466613	Unique	Hatchling gut	0	0.067	0	0.67
p__Actinobacteria__g__Cellulosimicrobium_382216	Unique	Hatchling gut	0.002	0.085	0.07	0.67
p__Actinobacteria__g__Dietzia_70231	Unique	Hatchling gut	0.043	0.019	0.2	0.45
p__Proteobacteria__g__Achromobacter_678564	Unique	Hatchling gut	0	0.086	0	0.73
p__Proteobacteria__f__Enterobacteriaceae_410502	Unique	Hatchling gut	0	0.088	0	0.67
p__Proteobacteria__f__Alcaligenaceae_503155	Unique	Hatchling gut	0	0.076	0	0.75
p__Proteobacteria__g__Stenoxybacter_687490	Unique	Hatchling gut	0.003	0.012	0.07	0.59
p__Proteobacteria__g__Erwinia_25313	Unique	Hatchling gut	0	0.083	0	0.55

p__Proteobacteria__f__Enterobacteriaceae_231744	Unique	Hatchling gut	0	0.082	0	0.57
p__Proteobacteria__g__Shinella_386729	Unique	Hatchling gut	0.031	0.025	0.13	0.59
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	Unique	Hatchling gut	0.002	0.081	0.07	0.45
p__Proteobacteria__f__Acetobacteraceae_741892	Unique	Hatchling gut	0	0.081	0	0.69
p__Proteobacteria__g__Pseudomonas_511660	Unique	Hatchling gut	0	0.073	0	0.45
p__Actinobacteria__f__Nocardioidaceae_115549	Unique	Hatchling gut	0	0.02	0	0.41
p__Proteobacteria__g__Pseudomonas_161575	Unique	Hatchling gut	0	0.061	0	0.78
p__Proteobacteria__g__Defluvibacter_651784	Unique	Hatchling gut	0	0.051	0	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	Unique	Hatchling gut	0	0.071	0	0.69
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	Unique	Hatchling gut	0	0.056	0	0.76
p__Proteobacteria__g__Agrobacterium_477436	Unique	Hatchling gut	0.009	0.026	0.07	0.47
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	Unique	Hatchling gut	0	0.067	0	0.59
p__Proteobacteria__g__Klebsiella_117934	Unique	Hatchling gut	0	0.069	0	0.69
p__Proteobacteria__g__Pseudomonas_235588	Unique	Hatchling gut	0	0.065	0	0.73
p__Proteobacteria__g__Pseudomonas_731148	Unique	Hatchling gut	0	0.058	0	0.43
p__Proteobacteria__g__Achromobacter_155903	Unique	Hatchling gut	0	0.054	0	0.82
p__Proteobacteria__g__Stenotrophomonas_695630	Unique	Hatchling gut	0	0.06	0	0.86
p__Proteobacteria__g__Klebsiella_664333	Unique	Hatchling gut	0	0.064	0	0.57
p__Proteobacteria__o__Rhizobiales_675457	Unique	Hatchling gut	0	0.063	0	0.47
p__Proteobacteria__g__Stenotrophomonas_505642	Unique	Hatchling gut	0	0.059	0	0.73
p__Bacteroidetes__g__Chitinophaga_80088	Unique	Hatchling gut	0	0.027	0	0.51
p__Proteobacteria__g__Klebsiella_430511	Unique	Hatchling gut	0	0.061	0	0.57
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	Unique	Hatchling gut	0	0.031	0	0.65
p__Proteobacteria__g__Pseudomonas_110207	Unique	Hatchling gut	0	0.058	0	0.65
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	Unique	Hatchling gut	0	0.033	0	0.55
p__Proteobacteria__f__Enterobacteriaceae_84333	Unique	Hatchling gut	0	0.058	0	0.57
p__Proteobacteria__g__Xenophilus_188340	Unique	Hatchling gut	0.021	0.024	0.13	0.45
p__Proteobacteria__f__Alcaligenaceae_126597	Unique	Hatchling gut	0	0.049	0	0.78
p__Proteobacteria__g__Pseudomonas_40331	Unique	Hatchling gut	0.002	0.028	0.07	0.65

p__Proteobacteria__g__Pseudomonas_308359	Unique	Hatchling gut	0	0.052	0	0.55
p__Proteobacteria__f__Xanthomonadaceae_258602	Unique	Hatchling gut	0	0.051	0	0.53
p__Proteobacteria__g__Paracoccus_110077	Unique	Hatchling gut	0	0.048	0	0.63
p__Proteobacteria__g__Stenotrophomonas_531558	Unique	Hatchling gut	0	0.049	0	0.71
p__Proteobacteria__g__Enterobacter_572676	Unique	Hatchling gut	0	0.038	0	0.55
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	Unique	Hatchling gut	0	0.047	0	0.45
p__Proteobacteria__g__Klebsiella_347396	Unique	Hatchling gut	0	0.047	0	0.49
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	Unique	Hatchling gut	0.002	0.041	0.07	0.61
p__Proteobacteria__g__Stenotrophomonas_168758	Unique	Hatchling gut	0.001	0.042	0.07	0.73
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	Unique	Hatchling gut	0	0.034	0	0.65

Table 4.15 Shared and unique OTUs between hatchling blood and egg content samples.

Taxa	**Type**	**Details**	**Egg contents abundance**	**Hatchling blood abundance**	**Egg contents occurrence**	**Hatchling blood occurrence**
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Egg contents	6.725	0.664	1	0.14
p__Proteobacteria__f__SUP05_285720	Unique	Egg contents	3.915	0.033	0.87	0.04
p__Proteobacteria__f__Pasteurellaceae_142268	Unique	Egg contents	2.668	0.281	0.87	0.06
p__Actinobacteria__o__03197L14_108564	Unique	Egg contents	1.14	0.171	0.8	0.08
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Egg contents	2.407	0.098	0.73	0.04
p__Firmicutes__g__Bacillus_535651	Unique	Egg contents	0.337	0.456	0.67	0.36
p__Bacteroidetes__f__Flavobacteriaceae_729660	Unique	Egg contents	1.848	0.063	0.73	0.08
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	Unique	Egg contents	0.399	0.006	0.47	0.1
p__Firmicutes__g__Streptococcus_269499	Unique	Egg contents	1.663	0.136	0.87	0.3
p__Bacteroidetes__g__Chryseobacterium_308134	Unique	Egg contents	0.465	0.053	0.6	0.14
p__Bacteroidetes__f__Flavobacteriaceae_12392	Unique	Egg contents	0.68	0.016	0.8	0.02
p__Proteobacteria__g__Delftia_587073	Unique	Egg contents	0.381	0.487	0.47	0.38
p__Proteobacteria__f__Rhodospirillaceae_441088	Unique	Egg contents	1.091	0.194	0.47	0.32
p__Actinobacteria__g__Janibacter_703951	Unique	Egg contents	0.219	0.402	0.47	0.34
p__Proteobacteria__g__Acinetobacter_78571	Unique	Egg contents	0.383	0.286	0.47	0.2
p__SR1__c___571035	Unique	Egg contents	0.441	0.025	0.4	0.02
p__Proteobacteria__f__Sinobacteraceae_155918	Unique	Egg contents	1.066	0.132	0.6	0.3
p__Proteobacteria__g__Devosia_497251	Unique	Egg contents	0.965	0.134	0.4	0.38
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Egg contents	0.437	0.003	0.4	0.02
p__Proteobacteria__g__Steroidobacter_168803	Unique	Egg contents	0.678	0.21	0.4	0.36
p__Proteobacteria__f__Sinobacteraceae_668199	Unique	Egg contents	0.736	0.145	0.47	0.34
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Unique	Egg contents	0.484	0.144	0.47	0.3
p__Actinobacteria__f__Solirubrobacteraceae_1327	Unique	Egg contents	0.363	0.149	0.47	0.36
p__Actinobacteria__g__Streptomyces_420197	Unique	Egg contents	0.429	0.158	0.67	0.34
p__Proteobacteria__g__Sphingopyxis_102703	Unique	Egg contents	0.418	0.141	0.4	0.38

p__Actinobacteria__o__koll13_76572	Unique	Egg contents	0.707	0.066	0.47	0.24
p__Proteobacteria__g__Pedomicrobium_452291	Unique	Egg contents	0.469	0.125	0.53	0.24
p__Acidobacteria__o__Acidobacteriales_743933	Unique	Egg contents	0.777	0.029	0.47	0.12
p__Proteobacteria__g__Kaistobacter_682533	Unique	Egg contents	0.43	0.13	0.47	0.38
p__Proteobacteria__f__Rhodospirillaceae_622262	Unique	Egg contents	0.446	0.055	0.53	0.2
p__Actinobacteria__g__Propionibacterium_427206	Unique	Egg contents	0.093	0.002	0.4	0.02
p__Proteobacteria__f__Sinobacteraceae_232273	Unique	Egg contents	0.424	0.054	0.4	0.22
p__Actinobacteria__o__Solirubrobacterales_711193	Unique	Egg contents	0.352	0.042	0.4	0.14
p__Proteobacteria__g__Rhodoplanes_273401	Unique	Egg contents	0.436	0.046	0.4	0.22
p__Actinobacteria__g__Nocardioides_128926	Unique	Egg contents	0.227	0.1	0.47	0.3
p__Actinobacteria__o__MC47_21675	Unique	Egg contents	0.201	0.076	0.4	0.1
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Egg contents	0.317	0.028	0.47	0.1
p__Actinobacteria__f__CL50029_390474	Unique	Egg contents	0.191	0.083	0.4	0.2
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Egg contents	0.177	0.017	0.4	0.02
p__Proteobacteria__g__Devosia_350266	Unique	Egg contents	0.271	0.031	0.4	0.12
p__Actinobacteria__o__MC47_160960	Unique	Egg contents	0.291	0.007	0.4	0.02
p__Actinobacteria__o__Actinomycetales_409160	Unique	Egg contents	0.231	0.015	0.4	0.14
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Egg contents	0.183	0	0.4	0.02
p__Proteobacteria__g__Ralstonia_478818	Core	Egg contents, Hatchling blood	30.82	11.301	0.93	0.92
p__Proteobacteria__g__Klebsiella_558424	Core	Egg contents, Hatchling blood	0.281	1.382	0.53	0.62
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Core	Egg contents, Hatchling blood	6.784	9.365	1	0.74
p__Proteobacteria__g__Stenotrophomonas_727055	Core	Egg contents, Hatchling blood	0.513	3.19	0.6	0.66
p__Proteobacteria__g__Pseudomonas_639712	Core	Egg contents, Hatchling blood	0.271	1.059	0.47	0.64
p__Proteobacteria__f__Pseudomonadaceae_79058	Core	Egg contents, Hatchling blood	1.015	1.183	0.87	0.72
p__Proteobacteria__g__Achromobacter_19649	Core	Egg contents, Hatchling blood	0.732	2.143	0.8	0.74
p__Proteobacteria__g__Aquamicrobium_748021	Core	Egg contents, Hatchling blood	0.109	2.516	0.4	0.72
p__Proteobacteria__g__Agrobacterium_442913	Core	Egg contents, Hatchling blood	0.377	1.797	0.47	0.6
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Egg contents, Hatchling blood	1.012	1.281	0.67	0.62
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Core	Egg contents, Hatchling blood	0.359	2.89	0.4	0.76

p__Proteobacteria__g__Brucella_305522	Core	Egg contents, Hatchling blood	0.331	1.392	0.6	0.68
p__Proteobacteria__f__Enterobacteriaceae_171036	Core	Egg contents, Hatchling blood	0.121	1.243	0.67	0.46
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Core	Egg contents, Hatchling blood	1.098	2.189	0.87	0.74
p__Firmicutes__g__Bacillus_161559	Core	Egg contents, Hatchling blood	0.259	1.497	0.47	0.46
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Core	Egg contents, Hatchling blood	0.267	1.326	0.47	0.42
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Core	Egg contents, Hatchling blood	0.355	0.844	0.53	0.64
p__Proteobacteria__f__Moraxellaceae_414919	Core	Egg contents, Hatchling blood	1.641	0.425	1	0.46
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Core	Egg contents, Hatchling blood	0.449	0.63	0.4	0.56
p__Proteobacteria__f__Sphingomonadaceae_363679	Core	Egg contents, Hatchling blood	1.281	0.358	0.6	0.42
p__Actinobacteria__g__Aeromicrobium_619093	Core	Egg contents, Hatchling blood	0.665	0.444	0.67	0.48
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Core	Egg contents, Hatchling blood	0.37	0.527	0.67	0.5
p__Proteobacteria__g__Shinella_393155	Core	Egg contents, Hatchling blood	0.173	0.253	0.47	0.5
p__Proteobacteria__g__Nitratireductor_5045	Core	Egg contents, Hatchling blood	0.583	0.217	0.53	0.48
p__Actinobacteria__g__Mycobacterium_393834	Core	Egg contents, Hatchling blood	0.155	0.332	0.47	0.4
p__Proteobacteria__f__Phyllobacteriaceae_589505	Core	Egg contents, Hatchling blood	0.208	0.171	0.47	0.46
p__Proteobacteria__f__Enterobacteriaceae_641353	Unique	Hatchling blood	0.002	0.694	0.07	0.4
p__Proteobacteria__g__Thermomonas_132820	Unique	Hatchling blood	0.021	4.905	0.13	0.64
p__Proteobacteria__f__Brucellaceae_698093	Unique	Hatchling blood	0.001	1.65	0.07	0.68
p__Proteobacteria__g__Lysobacter_318152	Unique	Hatchling blood	0.157	3.405	0.13	0.74
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	Unique	Hatchling blood	0.025	0.773	0.13	0.54
p__Bacteroidetes__g__Sphingobacterium_176945	Unique	Hatchling blood	0.003	0.382	0.13	0.46
p__Proteobacteria__g__Acinetobacter_168646	Unique	Hatchling blood	0.001	1.851	0.07	0.44
p__Actinobacteria__g__Leucobacter_697145	Unique	Hatchling blood	0.166	1.076	0.27	0.62
p__Bacteroidetes__g__Sphingobacterium_298499	Unique	Hatchling blood	0	0.649	0	0.46
p__Bacteroidetes__f__Flavobacteriaceae_374120	Unique	Hatchling blood	0.064	0.678	0.07	0.56
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	Unique	Hatchling blood	0.042	1.309	0.27	0.7
p__Actinobacteria__g__Arthrobacter_706755	Unique	Hatchling blood	0.159	0.402	0.33	0.44
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	Unique	Hatchling blood	0	0.45	0	0.58
p__Proteobacteria__g__Pseudomonas_704131	Unique	Hatchling blood	0.002	1.201	0.13	0.42

p__Proteobacteria__f__Alcaligenaceae_541229	Unique	Hatchling blood	0	0.194	0	0.4
p__Firmicutes__g__Bacillus_245239	Unique	Hatchling blood	0	0.851	0	0.4
p__Proteobacteria__g__Pseudomonas_34989	Unique	Hatchling blood	0.029	0.228	0.33	0.5
p__Bacteroidetes__g__Sphingobacterium_250053	Unique	Hatchling blood	0	0.284	0	0.4
p__Actinobacteria__f__Nocardioidaceae_554632	Unique	Hatchling blood	0.006	0.281	0.07	0.4
p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Hatchling blood	0.159	0.212	0.33	0.52
p__Proteobacteria__o__Sphingomonadales_709703	Unique	Hatchling blood	0.22	0.147	0.33	0.4
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	Unique	Hatchling blood	0	0.143	0	0.42
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Unique	Hatchling blood	0.012	0.243	0.13	0.5
p__Proteobacteria__g__Stenoxybacter_751919	Unique	Egg contents	6.725	0.664	1	0.14

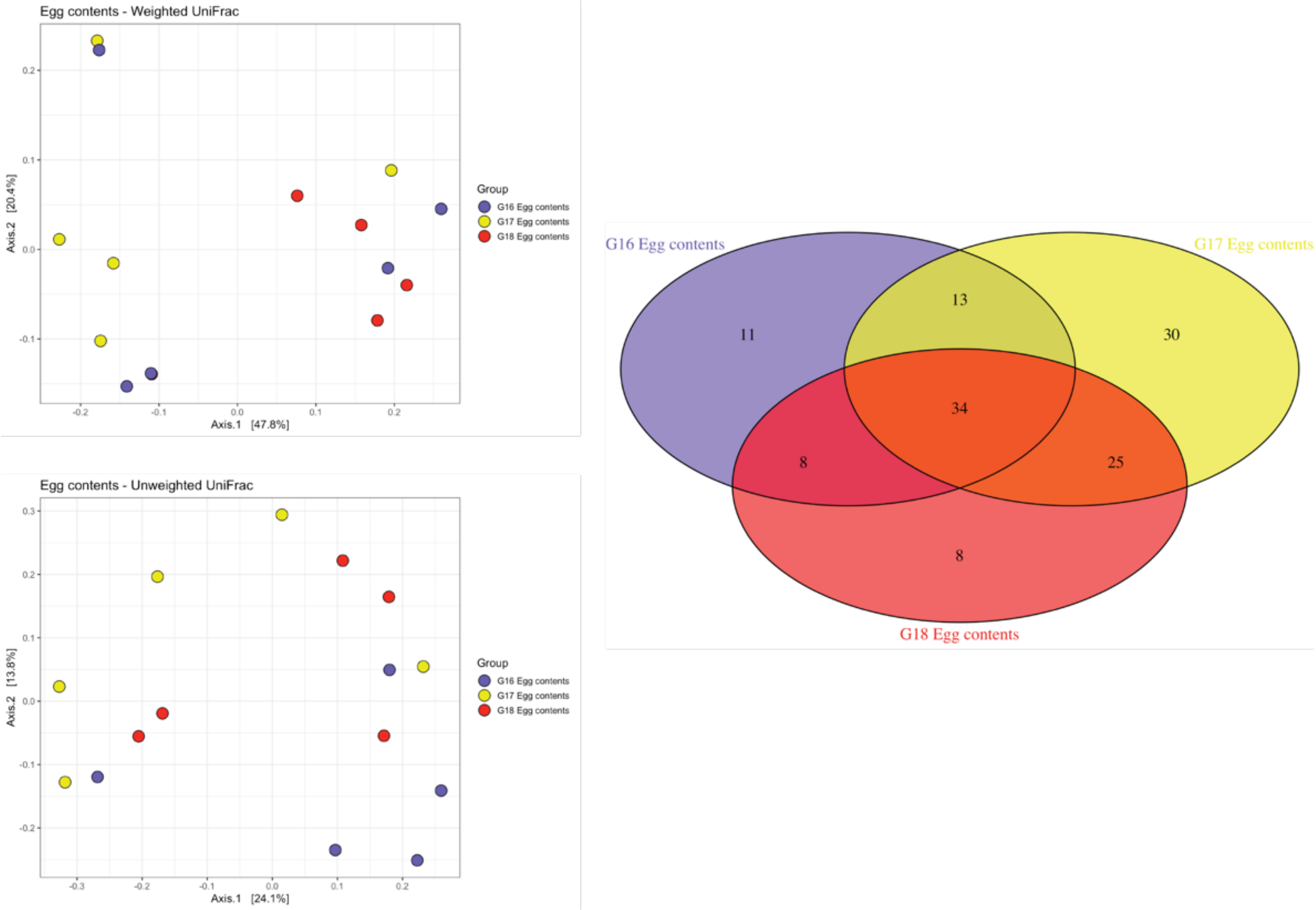

Figure 4.8 Principle co-ordinate analysis (PCoA) of egg contents revealed no significant differences in microbiota composition between females for weighted UniFrac ordination (R^2=0.21, *p*=0.14), but significance for unweighted UniFrac ordination (df=2, SS_T=0.58, MS = 0.29, f.model=1.57, R^2=0.21, *p*=0.032), with all eggs sharing 34 (26.3%) OTUs.

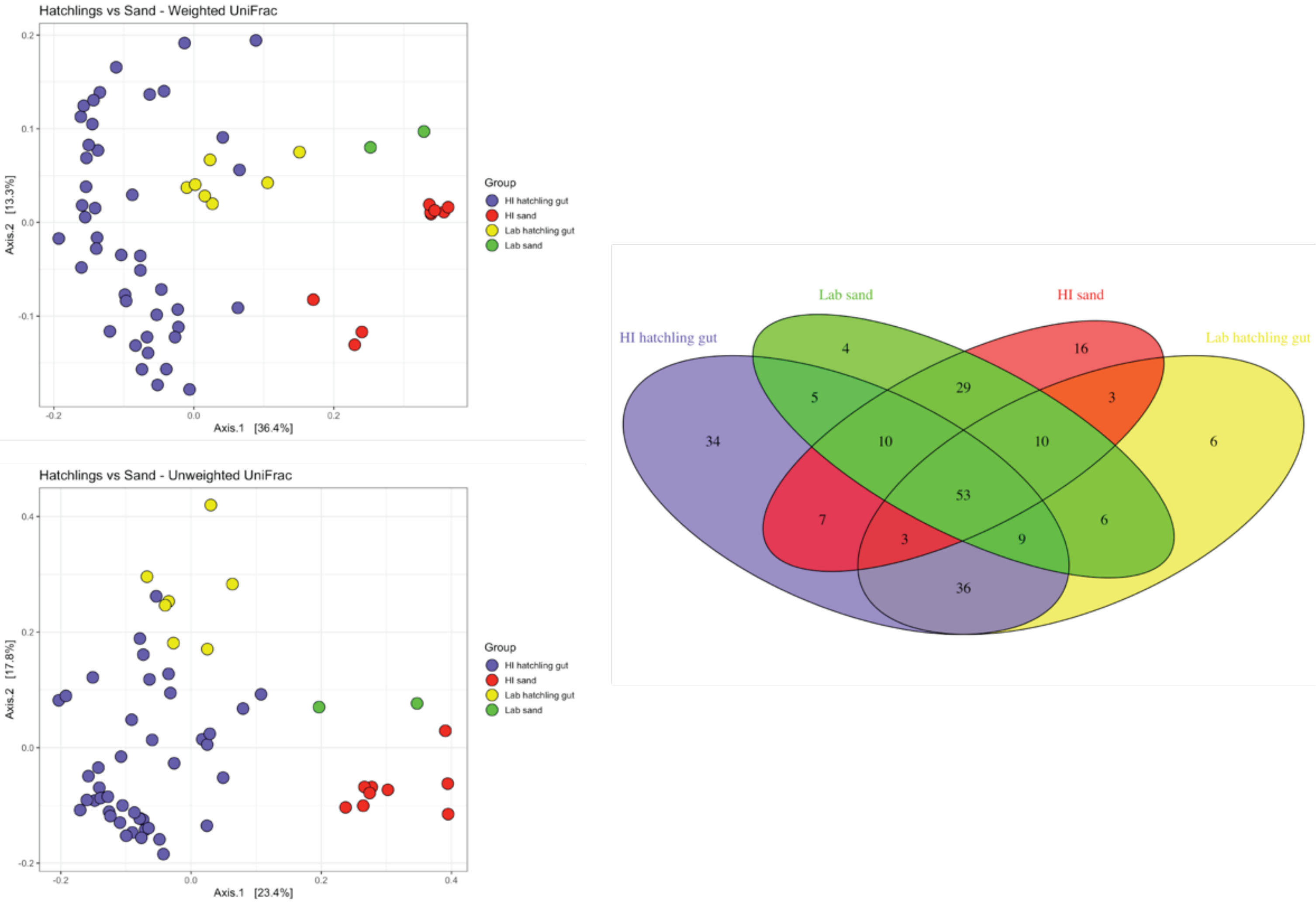

Figure 4.9 Principle co-ordinate analysis (PCoA) of sand and hatchlings showed significant for both weighted UniFrac (R^2=0.40, *p*=0.001) and unweighted UniFrac ordinations (R^2=0.34, *p*=0.001), and that all hatchlings and sand samples shared 53 (9.8%) OTUs.

4.5 Discussion

Our investigation details how the microbiota of hatchling sea turtles compares to that of their mothers. The bacterial communities we detected varied between the different sample types, but there was a significant overlap in the OTUs that were present in each sample type. Most interestingly, the adult blood and the egg contents samples had similar microbiotas. In turn, hatchlings shared between 15-21% of their OTUs with their biological mothers. This is in comparison to neonate lizards which share 34.3% with their mothers (Kohl *et al.* 2017), and 62% shared OTUs between chicks and hens of domestic fowl (Ding *et al.* 2017). Our results indicate that as a group, hatchling turtles emerge from the egg with a microbiota that partially resembles that of nesting green turtles in its bacterial composition. Given our discovery of a closely related microbial population in both the blood and eggs of adult turtles, it is possible that mother turtles transfer a proportion of their microbiota to their offspring from the blood to the egg, presumably prior to formation of the shell. Whether this process occurs in the developing ovarian follicle prior to ovulation, or while the egg is within the oviduct, we were not able to determine. Nevertheless, our discovery of bacteria in the blood and the contents of the egg at oviposition, provides a plausible pathway by which vertical transmission of the microbiome may occur in oviparous animals without parental care. However, despite this discovery, we cannot rule out other possible routes by which vertical microbial transfer may occur in sea turtles. For example, it is plausible that bacteria present in the cloaca or distal colon, might migrate into the oviducts and seed developing eggs. An additional route of transmission could be coating of the egg shells by microbiota as they pass through the cloaca, but this is less likely to be contributing to initial colonisation, as evidenced by our finding of a microbiota in the internal contents of freshly laid eggs, coupled with the time that it takes for bacteria to ingress through the protective shell layer. For example, *Salmonella* has been demonstrated to penetrate turtle eggs, with contamination of the internal contents, but this takes about one hour after initial exposure (Johnson-Delaney 2006), a much longer time than it took for us to process eggs and collect a sample of their contents. It is also possible that a combination of all of these mechanisms is responsible for constructing the hatchling microbiota, with a series of staged colonisation events important for establishing a stable microbial population. A process by which eggs are populated by multiple waves of microbes is supported by our finding of shared OTUs between sand and hatchling samples, as well as a difference in hatchling microbiotas from naturally incubated turtles to those incubated in the laboratory as there is some agreement between hatchling OTUs and a proportion of incubation environment OTUs.

Hatchling turtle microbiota did not exactly match those of adult females, but this is not surprising given the life history traits of these animals. Green sea turtles undergo an ontogenetic dietary shift from hatchlings to adults (Arthur *et al.* 2008), and it has been shown that their microbiota changes accordingly (Price *et al.* 2017). The shared OTUs we found in adults and hatchlings may form the initial microbiota and may also help to shape the development of microbial interactions and ecology in the later life of the host. For example, in human neonates, preliminary colonisation by facultative anaerobes such as *E. coli* and other Enterobacteriaceae results in depletion of initial oxygen supplies and primes the gut for strict anaerobes such as *Bifidobacterium*, *Clostridium*, and *Bacteroides* to subsequently dominate (Matamoros *et al.* 2013). How these interactions play out, and the ecological principles involved, warrants further investigation, not only in sea turtles, but in all metazoans.

An interesting finding in this investigation was the presence of a diverse microbial community in the blood of both adult and hatchling turtles. Under the field conditions in which we collected our samples, we did not obtain negative controls for analysis, and we acknowledge our findings would be strengthened if we had done so, particularly the unexpected finding of bacterial DNA in maternal blood samples. However, bacteria have been cultured from blood samples taken from healthy, free-ranging western ratsnakes (*Pantherophis obsoletus*) (Waugh *et al.* 2017), which lends support to our suggestion that bacteria may be a normal inhabitant of the blood in some reptile species. Furthermore, bacterial infections of the reptilian reproductive tract involving gastrointestinal isolates are frequently reported in the absence of other disease, and it has long been suspected that these pathologies are haematogenous in origin (Chiodini and Sundberg 1981; Chiodini 1982; Mitchell and Shane 2000; Scheelings *et al.* 2011), These pathologies may represent a failure of host immunity, or failure of selection for beneficial bacteria. A distinct microbiota has been described from various components of human blood (Paisse *et al.* 2016), and it has been hypothesised that this may result in seeding of the placenta and transference to the developing human foetus (Aagaard *et al.* 2014). Similar to our results, the human blood microbiome is dominated by Proteobacteria, followed by Actinobacteria and Bacteroidetes (Paisse *et al.* 2016), and it may be that these phyla have a predilection for blood. The presence of a microbial community in the blood in adults was an important finding in this investigation, and it would be instructive to determine whether males and non-breeding females are similarly bacteraemic. Studies into the presence of bacteria in the blood are typically biased towards sick animals or humans, and investigators rarely examine healthy individuals. The identification of bacteraemia in apparently healthy individuals (both adults and hatchlings), is an important point that sets this investigation apart from other microbiota studies. Future investigations into blood microbiota should include extensive negative controls, and focus on a range of chelonian species, of all age classes and reproductive status, to further our understanding of this apparent phenomenon.

A second important discovery in our study was the identification of bacteria within the contents of eggs. Particularly for sea turtle eggs, presence of bacteria are only reported in cases of egg failure (Wyneken *et al.* 1988; Awong-Taylor *et al.* 2008), but never within healthy eggs. Our results show bacteria appear to be a normal component of viable sea turtle eggs, and reveal an important potential conduit for transference of the mother's microbiota to offspring. In other species, such as birds, the presence of microbial communities within eggs is controversial. Some investigators suggest that avian eggs are sterile internally, with microbial invasion considered to be a major risk factor for poor hatching success (Cook *et al.* 2003; Cook *et al.* 2005; Godard *et al.* 2007; Hansen *et al.* 2015; van Veelen *et al.* 2018). However, more recent research has suggested that in some species of birds, microbial colonisation of eggs is important for vertical transmission of the microbiota (Trevelline *et al.* 2018). In the same study, lizard eggs were also shown to harbour a distinct microbial community more diverse than that of birds (Trevelline *et al.* 2018). This difference in microbial presence may be due to critical differences in reproductive strategy. Other than some megapodes (Cockburn 2006), birds incubate their eggs and then provide parental care to their offspring. During this process there is potential for vertical transmission of microbes at multiple stages, but in contrast, most reptile eggs are laid in a nest and then have no further contact with either parent. Faecal contamination of green sea turtle eggs during oviposition is unlikely to play any role in microbiota transfer, as female turtles are inappetant for prolonged periods of time prior to nesting, thus they are not producing any faecal material (Limpus 2008). Therefore, microbial transfer must occur before, or during oviposition using a method independent of faecal contamination. If this did not occur, then the initial establishment of the microbiota would be left to chance from opportunistic environmental species, which would seem a poor strategy. Our results indicate that turtles (and potentially other reptiles) have overcome this obstacle by seeding embryos with microbiota while they are developing in the oviducts. How the developing turtle embryo, with its immature immune system, survives this microbial invasion remains unknown at this stage. However, it is possible that maternally derived antibodies (Schumacher *et al.* 1999), or white blood cells (Brown and Shine 2016), buffer turtle embryos against infection until they are immunologically competent to form a synergy with their microbiota themselves. In addition to discovering that the internal contents of eggs contained a distinct microbiota, we also found that variation in this microbial community did not significantly differ between females. This may mean that sea turtles transfer a core microbiota, consisting of the most critical OTUs, to their offspring, which are important for establishment of a healthy microbiota later in life. Similar mechanisms occur in humans, in which a selective number of OTUs are passed from the mother to the emerging foetus, with subsequent waves of invasion occurring throughout the early phases of life (Gilbert 2014). These preliminary bacterial colonists are critical to the lifelong health of the host, with a failure of this initial transfer implicated in the development of a number of diseases (Mueller *et al.* 2015).

We are confident that the presence of microbiota in blood and egg samples is not due to contamination, because 1) samples were collected using aseptic technique, 2) there is a high degree of similarity between blood and egg content samples, 3) there is a high degree of similarity between all hatchling samples (natural incubation vs artificial incubation) which were sampled at locations more than 2000km apart with different potential contaminants (one in the nesting beach, the other in a laboratory), 4) all samples were collected and processed at different times using different, single-use equipment, and finally, 5) for the DNA extraction, all samples were not extracted in a single batch but over multiple periods in the laboratory, with fresh reagents used for each extraction procedure using a standardised methodology and reagents. In light of all these variables, we think that the chance of identical contaminants confounding our results are infinitesimally small, and if contamination had occurred then we would not expect to see such distinct and diverse microbial communities as identified in these samples, and there would be greater overlap between the groups, especially on the PCoA plot.

We decided not to sterilise eggs prior to opening them in order to facilitate rapid sample collection and as we do not think that it was necessary. A number of involved techniques have been used to sterilise the outer surface of chelonian eggs with varying results, but none of which completely eliminated contaminants (Mitchell *et al.* 2007), and these would not have been practical in the field under the constraints of time and availability of equipment that we were working with. Furthermore, although chemical treatment of the egg surface may have resulted in death of bacterial contaminants, it is not guaranteed that this treatment would have removed bacterial DNA, which still could have been introduced into the egg as it was penetrated by the scalpel blade. Therefore, we considered sterilisation of the egg surface to be an unnecessary procedure in a field setting. Additionally, as would be expected, microbiota composition of egg surfaces was more closely related to adult gut samples than internal contents, indicating that there was little, if any, cross-contamination.

We also investigated environmental sources that might contribute to the gut microbial communities of hatchling sea turtles. Some sand OTUs were found in the gut communities of hatchling turtles, and these differed with incubation environment. Despite some differences existing between hatchlings incubated under different conditions, overall there was still significant similarity in microbial communities between laboratory hatchlings and wild hatchlings. It is possible that environmental OTUs penetrate the shell after eggs have been deposited in the sand, and differences in environmental microbial communities may account for observed differences between hatchlings. We can also not rule out that handling, and transport of eggs to the laboratory did not have any effect on egg microbiotas. If incubation environment plays a role in hatchling microbiota, this is most likely to occur while eggs are developing in the sand and not after they have hatched. This is evidenced by the fact that sand samples were significantly different from each other in their microbiotal composition, and because artificially incubated animals did not have to dig through any sand once hatched. Furthermore, based on the life history traits of hatchling sea turtles, it is unlikely that sand plays a major role in gastrointestinal flora composition once hatchlings have emerged from the egg. Once turtles hatch, they surface from their nest, crawl down to the ocean, and for several days swim continuously and rapidly away from shore (Wyneken and Salmon 1992). During this time, turtles do not feed, and their entire caloric requirements are supplied by the residual internalised yolk sac, for up to 10 days post-hatching (Kraemer and Bennett 1981; Higgins 2002). Therefore, we think it is unlikely that the environment plays any significant role during this time as the turtles are not actively consuming anything while in the nest or during the first few days after emergence. Whether or not the observed differences in microbial populations in natural versus artificially incubated sea turtle hatchlings results in a functional difference to the host was beyond the scope of our investigation. If differences result in deleterious effects to host physiology, then artificial incubation and head starting of sea turtle eggs as a conservation strategy would need to be reassessed.

A limitation of our investigation was the number of animals that were able to be sampled. In total, blood and eggs were sampled from three individual female green turtles. Initially, we planned to sample at least 5 females and their entire clutches, but on the night that we conducted the field work there was relatively low nesting density and females spent up to four hours each selecting an appropriate nesting site. Some dug multiple nest chambers that collapsed, necessitating them to change location and begin the process from the beginning. This type of nesting behaviour is common for sea turtles under conditions of low rainfall, leading to dry substrate (Mortimer 1990), and severely hampered our sampling attempts. Since we had already collected three nests, and factoring in the time it required to return to the laboratory, we could not extend our field work for another night or we would risk death of the eggs that had been collected (Williamson *et al.* 2017). A second complication was the developmental failure of eggs in the laboratory leading to relatively few laboratory hatchlings.

In utero bacterial colonisation of developing embryos occurs to varying degrees in a range of metazoans (Sacchi and Gricolo 1985; Endow and Ohta 1990; Cary and Giovannoni 1993; Moran *et al.* 1993; Gilbert 2014), but it could be argued that it is especially important in species that do not provide parental care, and even more so if those species are also oviparous. Perhaps the offspring of more precocial species derive a higher proportion of their microbiota via this method, although empirical data is needed to determine this. A mechanism for vertical transmission ensures that offspring start life with an initial microbiota, although there may be some risk to the embryo due to microbial competition for nutrients resulting in death of the developing embryo (Cook *et al.* 2005). Our data provides some support for a transgenerational transmission of the microbiota in oviparous species from mother to neonate. Given the finding of a microbiota in the blood of both mother and hatchling turtles, a haematogenous seeding of developing embryos as the mechanism to transfer a proportion of the microbiota warrants further investigation. Importantly, this study is a correlative investigation, and so no categorical statements can be made regarding methodology of transference, but we are hopeful that our results will direct future research. Further study in a range of vertebrates where parental care is minimal will be needed to establish the evolutionary mechanisms by which microbial acquisition occurs, but our results are a useful step in understanding this fascinating adaptation.

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Chapter 5. The gut microbiota of sea turtles differs between geographically-distinct populations

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5.1 Abstract

The microbiota of metazoans is malleable and can be influenced by a variety of factors including diet, environment and genetics. In this study we obtained microbiota samples from sea turtles of the same species at different locations, in order to test whether their microbiotas are species-specific, or more variable. Cloacal swabs were collected from loggerhead turtles originating from Florida, USA and Queensland, Australia, as well as from flatback turtles from Crab Island, Queensland, Australia, and Port Hedland, Western Australia, Australia. We then manually extracted bacterial DNA and used 16s rRNA sequencing to explore microbial communities. Our investigation showed that the microbiota of sea turtles is heavily influenced by geography, with loggerhead turtles originating from the USA and Australia, harbouring significantly different microbial populations in terms of composition. Similarly, we also found that flatback turtles from Crab Island, had significantly less diverse microbiotas, with a predominance of the bacterial phylum Firmicutes, in comparison to their genetically similar counterparts from Port Hedland. Factors that may explain these observed differences between populations include host genetics, differences in foraging habitat quality and differences in migratory distance (and thus durations of inappetence) between foraging and breeding grounds. The mechanisms by which these factors may influence microbial composition of sea turtle gastrointestinal tracts warrants further investigation. The results of this study highlight the importance of interpreting microbiota data of wild animals in the context of geography.

Key Words: *flatback turtle, geography, loggerhead turtle, microbiota*

5.2 Introduction

The composition of the gastrointestinal microbiota is determined by the same ecological principles that drive diversity and niche occupancy within macro ecosystems. That is, colonist assemblages are established by founder populations, dispersal, diversification, local abundance, ecological drift, and habitat quality (Costello *et al.* 2012; Koskella *et al.* 2017). These are further influenced by individual bacterial traits such as dispersal efficiency and mutation rates, as well as host genetics (Costello *et al.* 2012; Coyte *et al.* 2015). Thus, the final microbial community of an individual is determined by a complex interplay of local and external factors, with microorganisms competing fiercely to establish stable populations within the gastrointestinal environment (Costello *et al.* 2012; Coyte *et al.* 2015).

In humans, diet strongly influences the composition of gut microbiota (Voreades *et al.* 2014; Albenberg and Wu 2014; David *et al.* 2014). Transient fluctuations in community structure may become evident in as little as 24 hours after consumption of novel dietary items (David *et al.* 2014). Such plasticity may confer teleological advantages by permitting human dietary and lifestyle diversity (David *et al.* 2014), but it may also be a contributing factor in the development of disease (Albenberg and Wu 2014). Genetics are also a strong driver of microbiota composition in humans, with potential implications for host metabolism (Goodrich *et al.* 2014; Spor *et al.* 2011).

Like humans, the gut microbiota of animals is malleable and may be similarly influenced by factors such as genetics (Zhao *et al.* 2013), and diet (Frese *et al.* 2015; Henderson *et al.* 2015; Nelson *et al.* 2013). Furthermore, disturbances in habitat quality appear to have species-specific effects on microbial populations of animals. Black howler monkeys (*Alouatta pigra*) inhabiting suboptimal environments consumed less diverse diets and had a corresponding decrease in gut microbial diversity. Additionally, genetic analysis of microbes isolated from these animals revealed a reduction in genes associated with butyrate production and hydrogen metabolism, which may have implications for host health, because they are unable to use environmental resources as efficiently as animals from pristine habitats (Amato *et al.* 2013). Conversely, a study involving multiple primate species demonstrated that habitat perturbation did not influence faecal microbiotas and that differences observed were explained by differences in digestive physiology and dietary preferences (McCord *et al.* 2014). However, in this same study, differences were noted between populations of the same species from different countries, suggesting that geography and social group membership influence microbial communities in some primates (McCord *et al.* 2014).

Despite the apparent malleability of the microbiota, investigations in mice have revealed that populations harbor a core microbial community, which is subject to host genetic control (Benson *et al.* 2010; Zhang *et al.* 2009). The relative abundances of taxa within this core microbiota collectively behave as a complex polygenic trait, and may exert its effects on other taxonomic groups and subsequently affect their ability to establish within the gastrointestinal tract (Benson *et al.* 2010). This multifaceted relationship between microbes and their hosts may be one of the key drivers of evolution and account for some of the enormous diversity we see in form and function of metazoans (Fraune and Bosch 2010). However, in order to understand the importance of microbes in driving host evolution, investigations of a wider variety of host taxa are needed. This is particularly important for less studied species, such as many of the Reptilia, which are grossly under-represented in microbiota studies. To address this deficit in the literature we chose to explore the relationships that exist between geography and microbiotas in sea turtles.

There are seven extant species of sea turtles, which may occupy a diverse array of ecological niches, and employ a variety of foraging strategies (Bowen and Karl 2007). The global phylogeography of sea turtles is markedly influenced by habitat preference and thermal tolerance (Bowen and Karl 2007). For example, the more cold-adapted leatherback turtle (*Dermochelys coriacea*), shows the lowest geographic structuring as their large size and pelagic lifestyle permits them to traverse through the boreal waters that act as a barrier to other warm-water adapted turtles (Bowen and Karl 2007). Conversely, tropical species, such as green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), and ridley turtles (*Lepidochelys olivacea* and *L. kempii*) have ancient separations between Atlantic and Indo-Pacific populations caused by the closure of the Isthmus of Panama around 3.5 million years ago (Bowen and Karl 2007). Within sea turtle species, there may be genetically distinct populations living within the same broad geographic regions, as well as genetically distinct populations living in very separate geographic regions. On the other hand, there may also be limited genetic variation of some species living in separate populations, but within the same broad geographic area. For example, loggerhead turtles (*Caretta caretta*), that nest on the east coast of Australia are genetically distinct from those that nest on the west coast of Australia (Limpus 2008b), and both of these populations are distinct from animals that nest in Florida, USA (Bowen and Karl 2007). In contrast, there is very little genetic diversity between flatback turtles (*Natator depressus*), despite there being four distinct nesting populations within Australia, with no inter-population breeding (Limpus 2008a). These phylogeographic traits make sea turtles interesting subjects to study in the context of microbiotas, and to explore the influence of genetic and environmental factors on microbial community structures. The aims of this investigation were to explore if variations in microbiota can be seen between different groups of the same species of sea turtles, and to identify possible explanations for any observed disparities. To do this we investigated how microbiotas differed among populations of loggerhead turtles in Australia and the USA, as well as between different populations of flatback turtles within Australia.

5.3 Methods

5.3.1 Ethics statement

This study was approved by the Biological Sciences Animal Ethics Committee of Monash University (approval 14694). Flatback turtles (*Natator depressus*) and loggerhead turtles (*Caretta caretta*) were sampled in Queensland under permit WITK17730216 from the Queensland Department of Environment and Heritage Protection and permit G12/35326.1 from the Great Barrier Reef Marine Park Authority. Flatback turtles from Western Australia were sampled under permit 01-000121-1 from the Department of Parks and Wildlife. Loggerhead turtles from the USA were sampled under the permit MTP-18-205A from the Florida Fish and Wildlife Conservation Commission and imported into Australia under permit PWS2018-AU-000738.

5.3.2 Study populations

Nesting adult flatback turtles of unknown age were sampled from Crab Island (n=20), Queensland (10.972° S, 142.106° E) in September 2016, and from Port Hedland (n=17), Western Australia (20.3107° S, 118.5878° E) in November 2016. Animals from these two areas are considered to be from genetically separate populations with little gene flow between them (Limpus 2008a). Loggerhead turtles were sampled from Mon Repos Queensland (24.8059° S, 152.4416° E) in January 2017, and from Juno Beach, Florida, USA (26.5224° N, 80.315° E) in May 2018. These two populations of loggerhead turtles represent distinct genetic stocks (IUCN 2019)(IUCN 2019)(IUCN 2019)(IUCN 2019)(IUCN 2019)(IUCN 2019).

5.3.3 Sample collection

If turtles were nesting, we waited until they had finished laying and were returning to the ocean. We then flipped them into dorsal recumbency, and an equine uterine swab (Minitube, Smythesdale, Victoria, Australia) was inserted into the cloaca so that it entered the distal colon. These swabs were housed in a sterile sheath, the entire apparatus was inserted into the cloaca and the swab tip was extruded when correct placement of the sheath had been achieved. The swab tip was then retracted back into the sheath prior to extraction, then turtles were permitted to return to the ocean. The tip of the swab was cut using a sterile wire cutter, placed into a sterile Eppendorf tube and sealed. Total sample collection time was approximately 10 minutes. Swabs were stored at -80°C until analysis could take place approximately 1 week later.

5.3.4 DNA extraction

DNA was extracted manually using the phenol-chloroform method (Green *et al.* 2012). In each Eppendorf tube, 500µL of extraction buffer (20mM ethylenediaminetetraacetic acid (EDTA), 0.1M Tris, 1% cetrimonium bromide (CTAB), 56mM NaCl, pH 8, was added so that swabs were completely covered. We then added 20µL of Proteinase K (Qiagen Proteinase K (10ml) to each vial, along with 60µL of 10% sodium dodecyl sulphate (SDS). The mixture was then incubated at 55°C overnight. The next day, 50µL of 5M NaCl and 500µL of Phenol was added, and the tubes shaken until an emulsion was formed. Tubes were then incubated at room temperature for 10 minutes, with intermittent mixing. The tubes were then centrifuged at 10,000 RPM for 10 minutes and the supernatant removed and added to a new tube containing 250µL Phenol and 250µL Chlorophorm:Isoamyl-Alcohol (24:1). The tubes were again centrifuged at 10,000 RPM for 10 minutes and the resultant supernatant added to a new tube containing 500µL of Chlorophorm:Isoamyl-Alcohol. Once again, the tubes were centrifuged at 10,000 RPM for 10 minutes. The supernatant was then added to a new tube containing 3M Sodium Acetate at a volume equal to 10% of the extraction solution. We then added 1ml of ice-cold 99% ethanol to each test tube and then placed them into a freezer at -20°C for 1 hour. The tubes were then centrifuged at 4°C at 12,000 RPM for 10 minutes. The fluid in the test tube was then removed with a glass pipette and 1ml of ice-cold 70% alcohol was added. The tubes were centrifuged a final time at 4°C at 12,000 RPM for 5 minutes. After centrifugation the alcohol was removed and the lids left off the tubes to allow the DNA pellet to dry. Once dried, 25µL of 1 x Tris-EDTA (TE) was added to each tube and the extracted DNA was stored at -20°C until amplicon sequencing could take place.

5.3.5 16S rRNA gene amplicon sequencing

The V3-V4 region of 16S rRNA genes were amplified with forward primer 5' ACTCCTACGGGAGGCAGCAG 3' and reverse primer 5' GGACTACHVGGGTWTCTAAT 3' using Q5 high fidelity polymerase (New England Biolabs) using the barcoding strategy of Fadrosh *et al.* (2014). Sequencing was performed on an Illumina MiSeq system (2 x 300 bp), and negative controls were included in each sequence run.

5.3.6 Data processing

Sequence data was analysed using QIIME version 1.9.1 (Caporaso *et al.* 2010) using default parameters and a Phred quality threshold of > 20. The UCLUST algorithm (Edgar 2010) was used to pick OTUs at 97% sequence identity and a Biome table was produced. Potentially chimeric sequences were identified using Pintail (Ashelford *et al.* 2005). Blast was used to assign taxonomy against the Greengenes database (DeSantis *et al.* 2006) and QIIME version 1.9.1 defaults. Additional assignment of taxonomy was performed using a command line version of BLASTN (Altschul *et al.* 1997) against the NCBI 16S Microbial database.

5.3.7 Statistics and data analysis

Initial exploration of the Biome table data was performed using the online Calypso software (http://cgenome.net/wiki/index.php/Calypso) (Zakrzewski *et al.* 2017). Data was further analysed with R, utilising package 'phyloseq'. Alpha diversity was explored using Observed OTUs, Shannon index and Chao1 estimates. Alpha diversity was tested for normality using the Shapiro-Wilks test and then parametric and non-parametric methods were adopted to test for significant differences between groups. For both loggerhead and flatback turtles, Observed (loggerhead: W=0.95, p=0.22; flatback: W=0.96, p=0.29) and Chao1 (loggerhead: W=0.97, p=0.64; flatback: W=0.97, p=0.6), were normally distributed, and so comparisons between populations were made using ANOVA, while Shannon diversity was non-normally distributed (loggerhead: W=0.9, p=0.02; flatback: W=0.9, p=0.002) and so comparisons were made using the Kruskal-Wallis test. Beta diversity was investigated using principle coordinate analysis (PCoA) (Bray-Curtis) and Adonis tests. Linear discriminant analysis (LDA) effect size (LEfSe), was performed to determine if differential abundances of OTUs were attributable to location of capture. For all statistical analysis significance was accepted if p<0.05.

5.4 Results

5.4.1 Loggerhead turtles

Sequence reads were obtained from 9 of the loggerheads from Juno Beach and 20 of the loggerhead turtles from Mon Repos (Supplementary Table 1). In Juno Beach loggerheads we identified 264 OTUs, with a total of 68,366 sequence reads, and an average of 7596.2 reads per sample. In Mon Repos loggerheads we identified 309 OTUs, with a total of 708,342 sequence reads, and an average of 35,417.1 reads per sample. For both populations, the predominate phylum was Proteobacteria, with differential abundance in the two populations being observed for other phyla identified (Figure 5.1). In animals from Mon Repos, the next most commonly isolated phyla were Spirochaetes, Bacteroides and Actinobacteria, while in Juno Beach animals Actinobacteria followed by Firmicutes were the most populous phyla (Figure 5.1). Alpha diversity did not differ among loggerheads from the two different populations, with Observed OTUs (df=1, f=4.03, SS_T=12337, p=0.054), Shannon diversity (χ^2=0.64, df=1, p=0.42) and Chao1 (df=1, f=3.6, SS_T=13308, p=0.07) estimates similar between Juno Beach and Mon Repos (Figure 5.2). Beta diversity, as assessed by PCoA, showed that the two populations clustered separately and distinctly from each other (Figure 5.3). These observations were significantly different (df=1, SS_T=1.0, f=2.7, R^2=0.09, p=0.004). Overall, 16 (14.2%) of the OTUs were shared between the two populations (Table 5.1). Eighty-nine (78.8%), OTUs were unique to animals from Mon Repos, in comparison to 10 (7.1%) unique OTUs from animals from Juno Beach (Table 5.1). Linear discriminant analysis revealed 37 OTUs to be responsible for explaining the variance seen between the different populations, with 26 of these being found in Mon Repos loggerheads and 11 being found in Juno Beach loggerheads (Figure 5.4, Table 5.2). Some of the most significant OTUs explaining variation for Mon Repos animals included *Helicobacter helicobactermustelae*_327563 and *Brachyspira* sp._69417 (Figure 5.4, Table 5.2). While for Juno Beach turtles the major OTUs responsible for explaining variation included Pasteurellaceae_142268 and SUP05_293055 (Figure 5.4, Table 5.2).

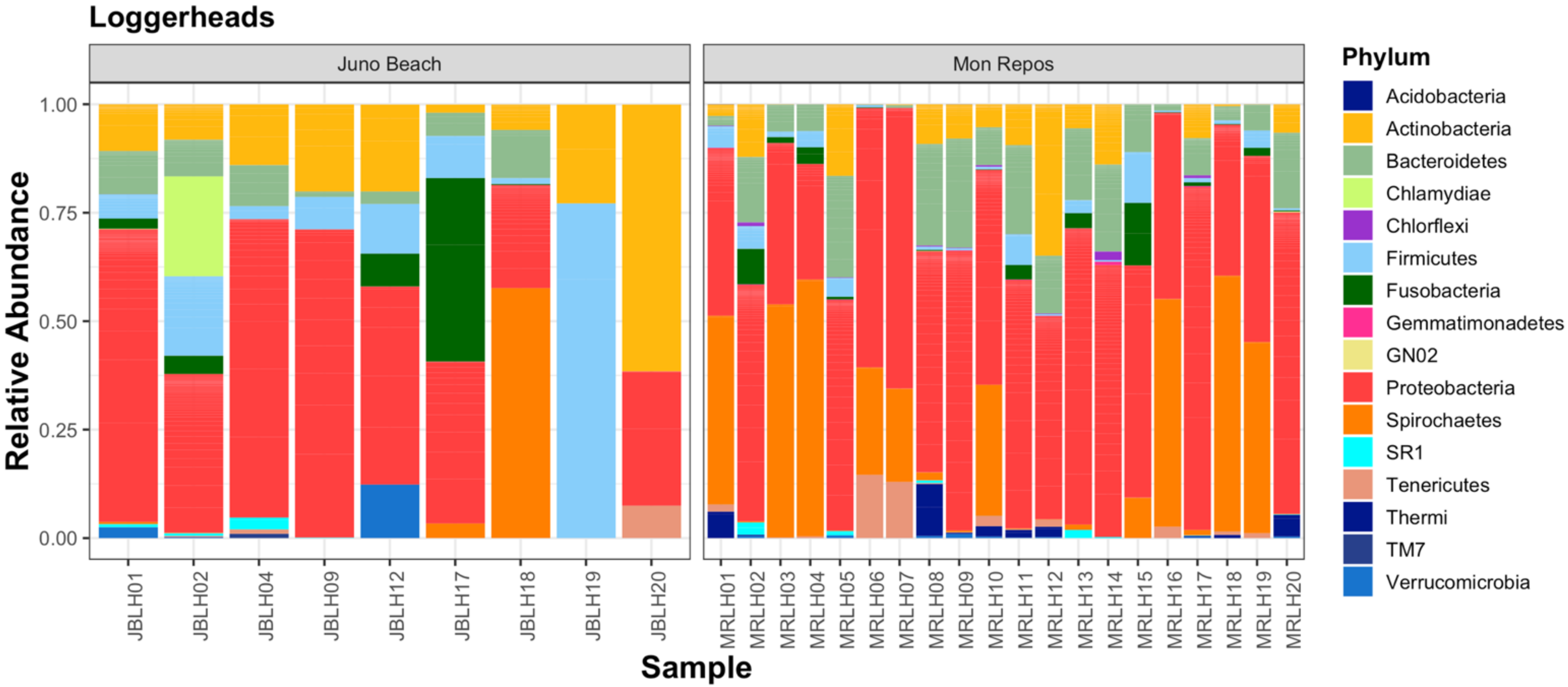

Figure 5.1 Relative abundance of the dominant bacterial phyla in loggerheads.

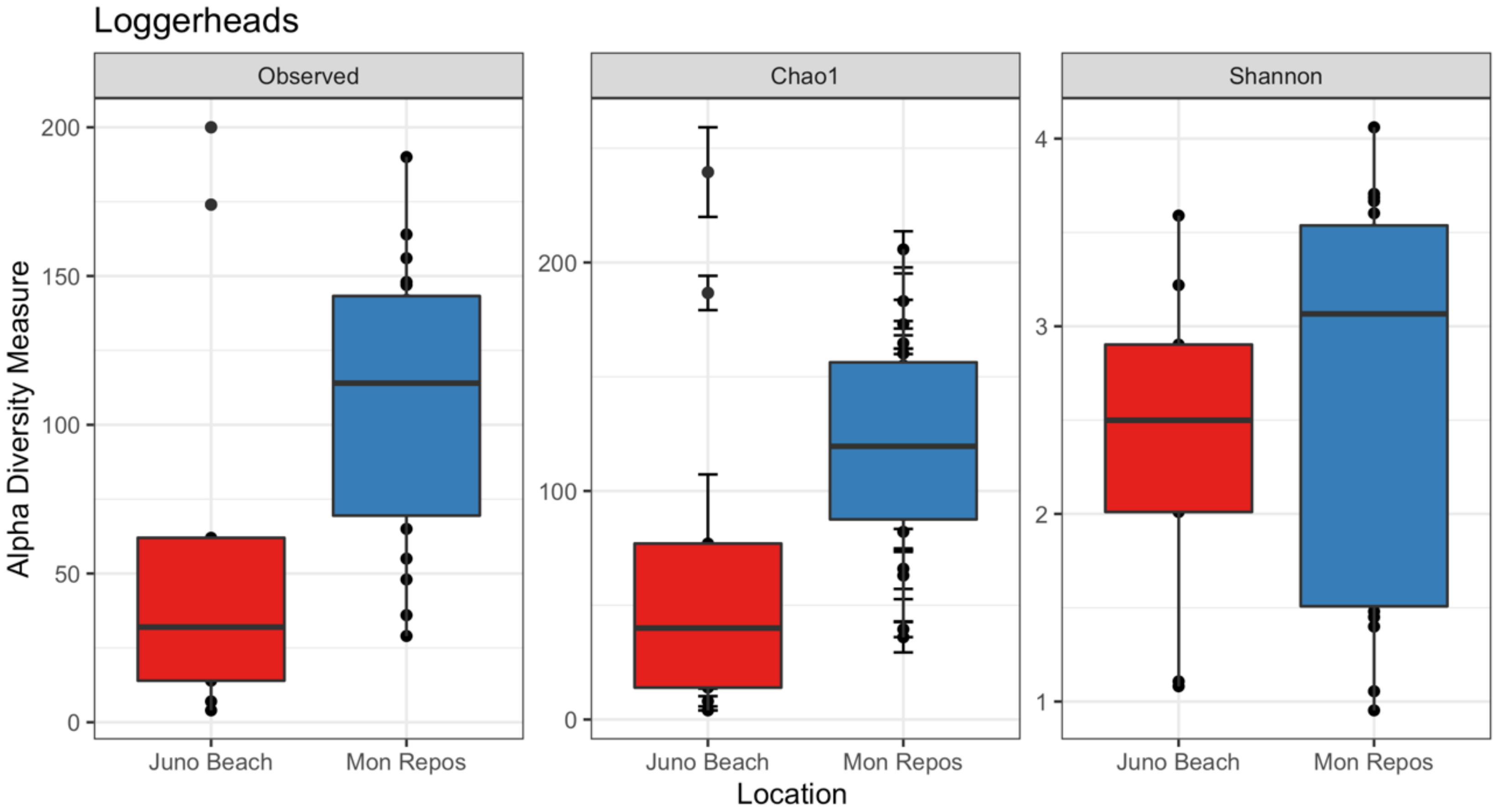

Figure 5.2 Alpha diversity estimates for loggerhead sea turtles. Individual points and brackets represent the richness estimate and the theoretical standard error range associated with that estimate, respectively. Within each panel, the samples are organized into location of capture, and a boxplot is overlaid on top of this for the two groups. Loggerhead turtles showed no differences in alpha diversity for either Observed (p=0.054), Chao1 (p=0.07) or Shannon diversity (p=0.42) between the two populations.

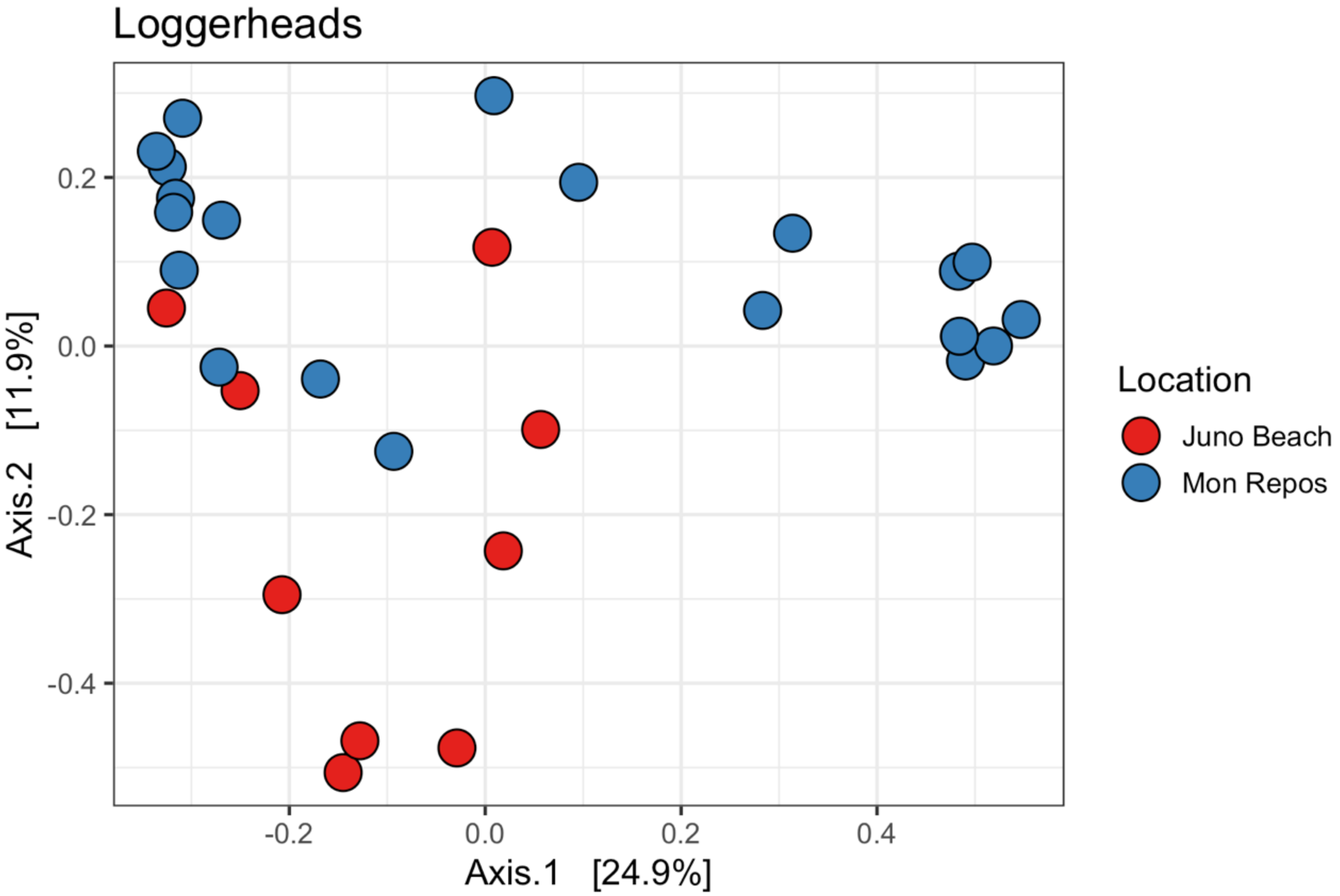

Figure 5.3 Principal coordinate analysis plot of Bray-Curtis distances. **(a)** Comparison of loggerhead turtles from two locations, Mon Repos, Australia, and Juno Beach, USA. Observed differences between the populations were significant (R^2=0.09, p=0.04).

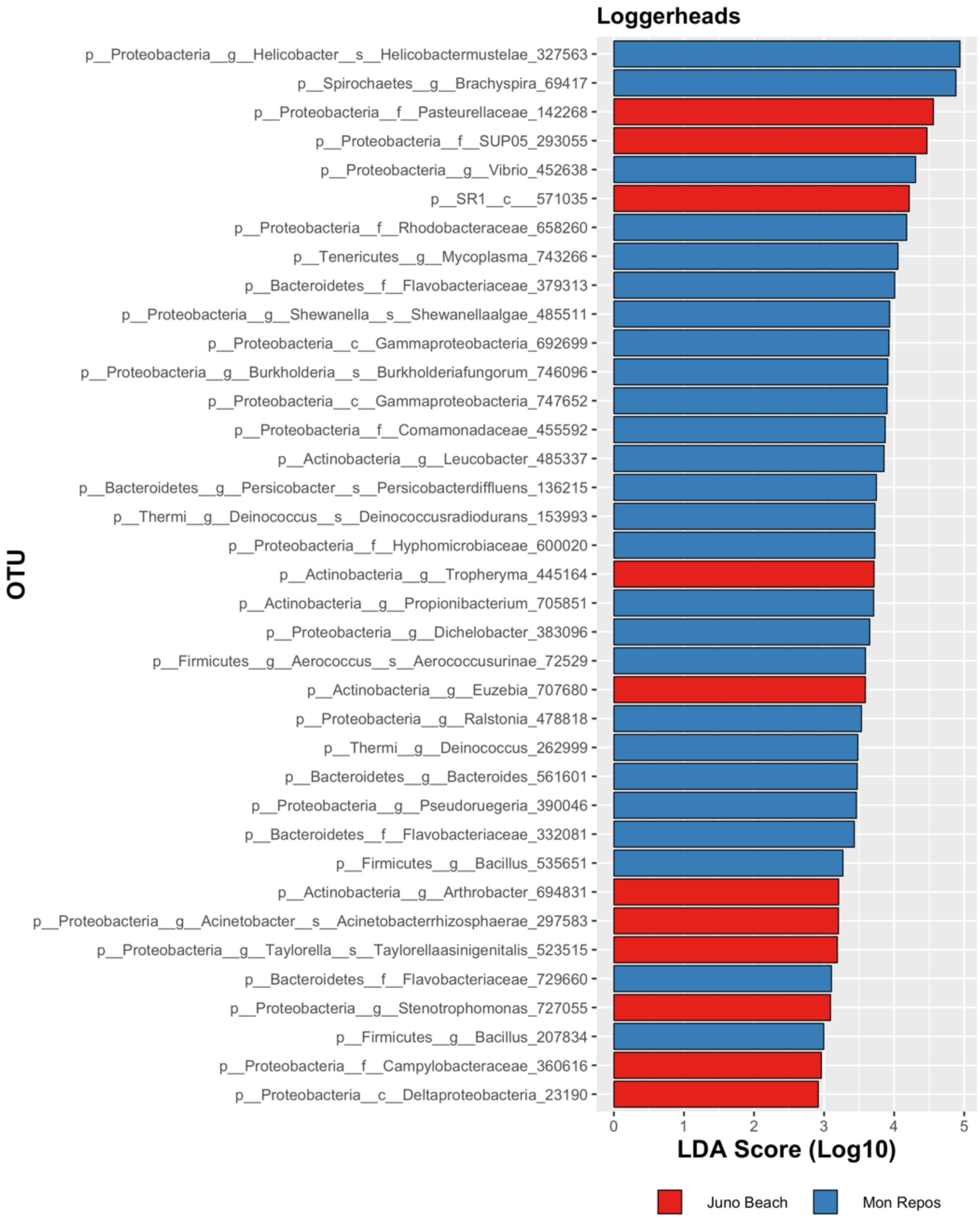

Figure 5.4 LDA Effect Size (LEfSe) algorithm was used on OTU tables to determine taxa that best characterized differences between each location of capture. Comparison of loggerheads from Mon Repos, Australia, with loggerheads from Juno Beach, USA.

Table 5.1 Shared and unique OTUs in samples from loggerhead turtles from Juno Beach, Florida, USA and Mon Repos, Queensland, Australia.

Taxa	Type	Details	Juno Beach abundance	Mon Repos abundance	Juno Beach occurrence	Mon Repos occurrence
p__Firmicutes__g__Bacillus_161559	Unique	Mon Repos	0.016	0.082	0.22	0.5
p__Spirochaetes__g__Brachyspira_69417	Core	Juno Beach, Mon Repos	6.849	20.047	0.44	0.95
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_327563	Unique	Mon Repos	0.974	16.542	0.33	1
p__Firmicutes__g__Bacillus_535651	Unique	Mon Repos	0	0.252	0	0.4
p__Proteobacteria__g__Stenoxybacter_751919	Core	Juno Beach, Mon Repos	8.488	2.23	0.67	0.85
p__Firmicutes__g__Bacillus_207834	Unique	Mon Repos	0	0.115	0	0.4
p__Actinobacteria__o__03197L14_108564	Core	Juno Beach, Mon Repos	2.871	1.576	0.67	0.75
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Unique	Mon Repos	0	1.537	0	0.85
p__Bacteroidetes__f__Flavobacteriaceae_379313	Unique	Mon Repos	1.839	3.544	0.33	0.9
p__Proteobacteria__g__Sulfurimonas_444197	Unique	Mon Repos	0.247	0.362	0.33	0.65
p__Proteobacteria__g__Dichelobacter_361604	Core	Juno Beach, Mon Repos	2.682	1.842	0.44	0.8
p__Proteobacteria__f__Pasteurellaceae_142268	Core	Juno Beach, Mon Repos	5.676	0.288	0.78	0.7
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Core	Juno Beach, Mon Repos	8.251	0.077	0.67	0.45
p__Proteobacteria__g__Vibrio_452638	Unique	Mon Repos	0.038	3.566	0.33	0.95
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	Unique	Mon Repos	0.961	0.642	0.33	0.65
p__Firmicutes__o__Clostridiales_376161	Unique	Mon Repos	0.038	0.556	0.22	0.45
p__Proteobacteria__f__Rhodobacteraceae_658260	Unique	Mon Repos	0.01	2.256	0.22	0.8
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	Unique	Mon Repos	0.012	0.98	0.11	0.5
p__Tenericutes__g__Mycoplasma_743266	Unique	Mon Repos	0.006	1.915	0.11	0.65
p__Proteobacteria__c__Betaproteobacteria_554275	Unique	Mon Repos	0.896	1.738	0.33	0.7
p__Proteobacteria__f__SUP05_293055	Unique	Juno Beach	5.112	0.002	0.56	0.2
p__Actinobacteria__o__03197L14_176541	Core	Juno Beach, Mon Repos	0.748	1.518	0.44	0.8
p__Proteobacteria__f__Comamonadaceae_455592	Unique	Mon Repos	0	1.165	0	0.65
p__Fusobacteria__g__Streptobacillus_719947	Unique	Mon Repos	0.731	0.296	0.33	0.55
p__Proteobacteria__g__Methylarcula__s__Loktanellahongkongensis_46703	Core	Juno Beach, Mon Repos	1.903	0.37	0.67	0.65

p__Proteobacteria__g__Photobacterium__s__Photobacteriumdamselae_224255	Unique	Mon Repos	1.164	1.018	0.22	0.7
p__Fusobacteria__g__Cetobacterium__s__Cetobacteriumsomerae_541959	Unique	Mon Repos	1.719	0.704	0.22	0.45
p__Proteobacteria__g__Pseudoruegeria_390046	Unique	Mon Repos	0.013	0.486	0.22	0.65
p__Proteobacteria__g__Ralstonia_478818	Unique	Mon Repos	0.001	0.738	0.11	0.7
p__Proteobacteria__g__Dichelobacter_383096	Unique	Mon Repos	0.26	1.101	0.33	0.8
p__Proteobacteria__f__SUP05_224259	Core	Juno Beach, Mon Repos	0.789	0.864	0.44	0.75
p__Proteobacteria__g__Shewanella__s__Shewanellaalgae_485511	Unique	Mon Repos	0	1.216	0	0.4
p__Proteobacteria__c__Gammaproteobacteria_692699	Unique	Mon Repos	0	1.196	0	0.65
p__Proteobacteria__f__Rhodobacteraceae_160906	Unique	Mon Repos	0.103	1.078	0.33	0.65
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Core	Juno Beach, Mon Repos	1.388	0.458	0.44	0.55
p__Proteobacteria__c__Gammaproteobacteria_747652	Unique	Mon Repos	0	1.128	0	0.8
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	Unique	Mon Repos	0.036	0.997	0.11	0.7
p__Firmicutes__o__Clostridiales_226726	Unique	Mon Repos	0.811	0.028	0.33	0.45
p__Actinobacteria__g__Leucobacter_697145	Core	Juno Beach, Mon Repos	2.042	0.117	0.56	0.4
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Juno Beach	0.191	0.004	0.44	0.05
p__Bacteroidetes__f__Flavobacteriaceae_332081	Unique	Mon Repos	0.117	0.573	0.22	0.75
p__Actinobacteria__g__Leucobacter_485337	Unique	Mon Repos	0.008	0.998	0.22	0.7
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	Core	Juno Beach, Mon Repos	0.18	0.072	0.44	0.55
p__Actinobacteria__g__Propionibacterium_705851	Unique	Mon Repos	0.048	0.92	0.22	0.65
p__Proteobacteria__g__Moraxella_406471	Unique	Mon Repos	0.203	0.364	0.22	0.6
p__Bacteroidetes__f__Flavobacteriaceae_12392	Core	Juno Beach, Mon Repos	0.42	0.2	0.44	0.5
p__Proteobacteria__g__Amaricoccus_603022	Unique	Mon Repos	1.268	0.094	0.33	0.5
p__Proteobacteria__f__Rhodobacteraceae_649099	Unique	Mon Repos	0.021	0.762	0.11	0.45
p__Proteobacteria__g__Marinobacter_334925	Unique	Mon Repos	0.182	0.682	0.33	0.55
p__Proteobacteria__g__Moraxella_610605	Unique	Mon Repos	0.018	0.71	0.22	0.6
p__Firmicutes__g__Aerococcus__s__Aerococcusurinae_72529	Unique	Mon Repos	0	0.729	0	0.55
p__Bacteroidetes__g__Tenacibaculum__s__Tenacibaculumlutimaris_437286	Unique	Mon Repos	0.15	0.43	0.33	0.6
p__Proteobacteria__g__Vibrio__s__Vibrioichthyoenteri_763288	Unique	Mon Repos	0.261	0.528	0.22	0.55
p__Proteobacteria__o__Sphingomonadales_709703	Core	Juno Beach, Mon Repos	0.933	0.174	0.67	0.6

p__Bacteroidetes__g__Bacteroides_561601	Unique	Mon Repos	0	0.618	0	0.5
p__Bacteroidetes__f__Porphyromonadaceae_612601	Unique	Mon Repos	0.318	0.36	0.33	0.75
p__Thermi__g__Deinococcus_262999	Unique	Mon Repos	0	0.58	0	0.45
p__Proteobacteria__f__Rhodobacteraceae_729919	Unique	Mon Repos	0.019	0.535	0.22	0.45
p__Verrucomicrobia__g__Verrucomicrobium_175087	Unique	Mon Repos	0.287	0.205	0.22	0.65
p__Proteobacteria__g__Arcobacter_167287	Unique	Mon Repos	0.084	0.405	0.33	0.4
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	Core	Juno Beach, Mon Repos	0.689	0.238	0.44	0.45
p__Firmicutes__g__Acidaminococcus_127229	Unique	Juno Beach	1.088	0.034	0.44	0.2
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Unique	Mon Repos	0.138	0.16	0.33	0.45
p__Bacteroidetes__f__Flavobacteriaceae_48296	Unique	Mon Repos	0	0.45	0	0.65
p__Proteobacteria__g__Dichelobacter_140648	Core	Juno Beach, Mon Repos	0.438	0.248	0.44	0.45
p__Tenericutes__g___Unclassified.Erysipelotrichaceae__s__Clostridiuminnocuum_474350	Unique	Juno Beach	0.938	0	0.44	0
p__Actinobacteria__o__Actinomycetales_369980	Unique	Juno Beach	0.01	0	0.44	0
p__Proteobacteria__f__Rhodobacteraceae_328268	Unique	Mon Repos	0	0.4	0	0.5
p__Bacteroidetes__f__Cryomorphaceae_613739	Unique	Mon Repos	0	0.405	0	0.6
p__Bacteroidetes__g__Tenacibaculum_474405	Unique	Mon Repos	0.031	0.108	0.22	0.45
p__Proteobacteria__g__Sulfurimonas_412977	Unique	Mon Repos	0.054	0.337	0.22	0.6
p__Bacteroidetes__g__Tenacibaculum_241145	Unique	Mon Repos	0.239	0.258	0.33	0.65
p__SR1__c___3865	Unique	Mon Repos	0.092	0.238	0.33	0.6
p__Bacteroidetes__f__Flavobacteriaceae_118381	Unique	Juno Beach	0.723	0.004	0.44	0.2
p__Bacteroidetes__f__Saprospiraceae_536374	Unique	Mon Repos	0	0.33	0	0.6
p__Proteobacteria__g__Dichelobacter_255035	Unique	Mon Repos	0.306	0.182	0.33	0.6
p__Actinobacteria__g__Mycobacterium_393834	Unique	Juno Beach	0.404	0.009	0.56	0.25
p__Proteobacteria__f__Enterobacteriaceae_641353	Unique	Juno Beach	0.643	0.006	0.44	0.15
p__Proteobacteria__f__Oceanospirillaceae_500535	Unique	Mon Repos	0.009	0.242	0.11	0.5
p__Bacteroidetes__f__Flavobacteriaceae_174976	Unique	Mon Repos	0	0.23	0	0.55
p__Bacteroidetes__g__Bacteroides_9387	Unique	Mon Repos	0	0.288	0	0.45
p__Proteobacteria__g__Aquimonas_359512	Unique	Mon Repos	0	0.268	0	0.55
p__Actinobacteria__f__AKIW874_693966	Unique	Mon Repos	0.07	0.032	0.33	0.4

p__Proteobacteria__g__Comamonas_41770	Unique	Mon Repos	0.334	0.104	0.33	0.5
p__Proteobacteria__g__Brucella_305522	Unique	Mon Repos	0.018	0.058	0.11	0.5
p__Proteobacteria__g__Aquamicrobium_748021	Unique	Mon Repos	0.258	0.112	0.33	0.45
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Mon Repos	0	0.127	0	0.5
p__Proteobacteria__g__Magnetococcus_659945	Unique	Mon Repos	0	0.237	0	0.45
p__Proteobacteria__f__Phyllobacteriaceae_589505	Unique	Mon Repos	0.001	0.098	0.11	0.55
p__Proteobacteria__g__Desulfovibrio_214348	Unique	Mon Repos	0	0.129	0	0.5
p__Firmicutes__o__Clostridiales_748404	Unique	Mon Repos	0.038	0.216	0.22	0.55
p__Actinobacteria__g__Actinomyces_119009	Unique	Mon Repos	0.08	0.03	0.33	0.4
p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Mon Repos	0.161	0.08	0.33	0.6
p__Bacteroidetes__o__Bacteroidales_63588	Unique	Mon Repos	0	0.094	0	0.5
p__Actinobacteria__o__03197L14_629656	Unique	Mon Repos	0	0.046	0	0.45
p__Chloroflexi__c__SOGA31_314194	Unique	Mon Repos	0.002	0.148	0.11	0.5
p__Actinobacteria__g__Aeromicrobium_619093	Unique	Mon Repos	0.012	0.163	0.22	0.6
p__Proteobacteria__g__Pseudoalteromonas_279501	Unique	Mon Repos	0.001	0.182	0.11	0.5
p__Proteobacteria__g__Kaistobacter_682533	Unique	Mon Repos	0.004	0.108	0.22	0.55
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_567033	Unique	Mon Repos	0.001	0.145	0.11	0.45
p__Actinobacteria__g__Microbacterium__s__Microbacteriumesteraromaticum_75491	Unique	Mon Repos	0.011	0.102	0.22	0.45
p__Spirochaetes__g__Brachyspira_27753	Unique	Mon Repos	0.001	0.112	0.11	0.45
p__Proteobacteria__f__Sphingomonadaceae_363679	Unique	Mon Repos	0.004	0.092	0.11	0.55
p__Proteobacteria__f__Sinobacteraceae_232273	Unique	Mon Repos	0	0.078	0	0.5
p__Gemmatimonadetes__o__Gemmatimonadales_757385	Unique	Mon Repos	0	0.074	0	0.55
p__Proteobacteria__f__Bradyrhizobiaceae_116562	Unique	Mon Repos	0.022	0.052	0.22	0.45
p__Proteobacteria__g__Vibrio__s__Vibriosp.AND4_634776	Unique	Mon Repos	0	0.078	0	0.45
p__Chloroflexi__c__SOGA31_541293	Unique	Mon Repos	0.004	0.046	0.11	0.45
p__Actinobacteria__f__Solirubrobacteraceae_1327	Unique	Mon Repos	0	0.033	0	0.45
p__Proteobacteria__g__Rhodoplanes_273401	Unique	Mon Repos	0	0.051	0	0.45
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	Unique	Mon Repos	0.002	0.054	0.11	0.45
p__Actinobacteria__g__Arthrobacter_706755	Unique	Mon Repos	0.007	0.016	0.11	0.4

p__Actinobacteria__o__Actinomycetales_409160	Unique	Mon Repos	0.002	0.025	0.11	0.5

Table 5.2 LDA scores for loggerhead turtles.

Operational Taxonomic Unit	LDA	Group
p__Proteobacteria__c__Deltaproteobacteria_23190	2.916999583	Juno Beach
p__Proteobacteria__f__Campylobacteraceae_360616	2.960606179	Juno Beach
p__Proteobacteria__g__Stenotrophomonas_727055	3.091680855	Juno Beach
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	3.189530466	Juno Beach
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	3.205064411	Juno Beach
p__Actinobacteria__g__Arthrobacter_694831	3.207738634	Juno Beach
p__Actinobacteria__g__Euzebia_707680	3.589192766	Juno Beach
p__Actinobacteria__g__Tropheryma_445164	3.712906989	Juno Beach
p__SR1__c___571035	4.215251626	Juno Beach
p__Proteobacteria__f__SUP05_293055	4.468737499	Juno Beach
p__Proteobacteria__f__Pasteurellaceae_142268	4.560168599	Juno Beach
p__Firmicutes__g__Bacillus_207834	2.995540961	Mon Repos
p__Bacteroidetes__f__Flavobacteriaceae_729660	3.104781954	Mon Repos
p__Firmicutes__g__Bacillus_535651	3.268476141	Mon Repos
p__Bacteroidetes__f__Flavobacteriaceae_332081	3.431237095	Mon Repos
p__Proteobacteria__g__Pseudoruegeria_390046	3.461540899	Mon Repos
p__Bacteroidetes__g__Bacteroides_561601	3.474046388	Mon Repos
p__Thermi__g__Deinococcus_262999	3.482701373	Mon Repos
p__Proteobacteria__g__Ralstonia_478818	3.533511153	Mon Repos
p__Firmicutes__g__Aerococcus__s__Aerococcusurinae_72529	3.5902764	Mon Repos
p__Proteobacteria__g__Dichelobacter_383096	3.651290537	Mon Repos
p__Actinobacteria__g__Propionibacterium_705851	3.707504335	Mon Repos
p__Proteobacteria__f__Hyphomicrobiaceae_600020	3.72460035	Mon Repos
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	3.726419562	Mon Repos
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	3.746064497	Mon Repos
p__Actinobacteria__g__Leucobacter_485337	3.855644279	Mon Repos
p__Proteobacteria__f__Comamonadaceae_455592	3.871845109	Mon Repos
p__Proteobacteria__c__Gammaproteobacteria_747652	3.898504559	Mon Repos
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	3.910254535	Mon Repos
p__Proteobacteria__c__Gammaproteobacteria_692699	3.925197138	Mon Repos
p__Proteobacteria__g__Shewanella__s__Shewanellaalgae_485511	3.935243721	Mon Repos
p__Bacteroidetes__f__Flavobacteriaceae_379313	4.007989853	Mon Repos
p__Tenericutes__g__Mycoplasma_743266	4.054209512	Mon Repos
p__Proteobacteria__f__Rhodobacteraceae_658260	4.178385269	Mon Repos
p__Proteobacteria__g__Vibrio_452638	4.305943145	Mon Repos
p__Spirochaetes__g__Brachyspira_69417	4.881649027	Mon Repos
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_327563	4.9385515	Mon Repos

5.4.2 Flatbacks turtles

Sequence reads were obtained from 20 of the flatbacks from Crab Island and from 16 of the flatbacks from Port Hedland (Appendix 1). In Crab Island flatbacks we identified 264 OTUs, with a total of 1,484,081 sequence reads, and an average of 74,204 reads per sample. In Port Hedland flatbacks we identified 301 OTUs, with a total of 147,315 sequence reads, and an average of 9207.2 reads per sample. Firmicutes was by far the most predominant phyla in animals from Crab Island, while Proteobacteria was the most commonly isolated phyla in Port Hedland turtles, followed by Actinobacteria, Bacteroidetes and Firmicutes (Figure 5.5). Microbial diversity differed markedly among flatback turtle groups from the two different populations. Shannon diversity estimates revealed that flatback turtles from Port Hedland harbored nearly 2.5 times the number of microbial OTUs as animals from Crab Island (χ^2=14.05, df=1, p <0.001) (Figure 5.6, Table 5.3). However, Observed OTUs (df=1, f=2.38, SS_T=5701, p=0.13), and Chao1 estimates indicated that Port Hedland and Crab Island flatbacks were similar (df=1, f=0.5, SS_T=1577, p=0.49) (Figure 5.6). Principal coordinate analysis of microbiota composition indicated that animals from Port Hedland and Crab Island had distinct and significant differences (df=1, SS_T=2.11, f=5.6, R^2=0.138, p=0.001) in their clustering patterns (Figure 5.7). Overall, only 27 (28.1%) OTUs were shared between the two populations (Table 5.3). Sixty-two (64.6%), OTUs were unique to animals from Port Hedland, in comparison to 7 (7.3%) unique OTUs from animals from Crab Island (Table 5.3). Linear discriminant analysis revealed 60 OTUs to be responsible for explaining the variance seen between the different populations, with 52 of these being found in Port Hedland turtles, and 8 being found in Crab Island turtles (Figure 5.8, Table 5.4). Some of the most significant OTUs explaining variation for Crab Island animals included *Bacillus* sp._161559, *Bacillus* sp._535651, *Bacillis bascillusendophyticus*_410037, and *Bacillus* sp._207834 (Figure 5.8, Table 5.4). While for Port Hedland turtles the major OTUs responsible for explaining variation included SUP05_285720, 03197L14_108564, and *Burkholderia burkholderiafungorum*_746096 (Figure 5.8, Table 5.4).

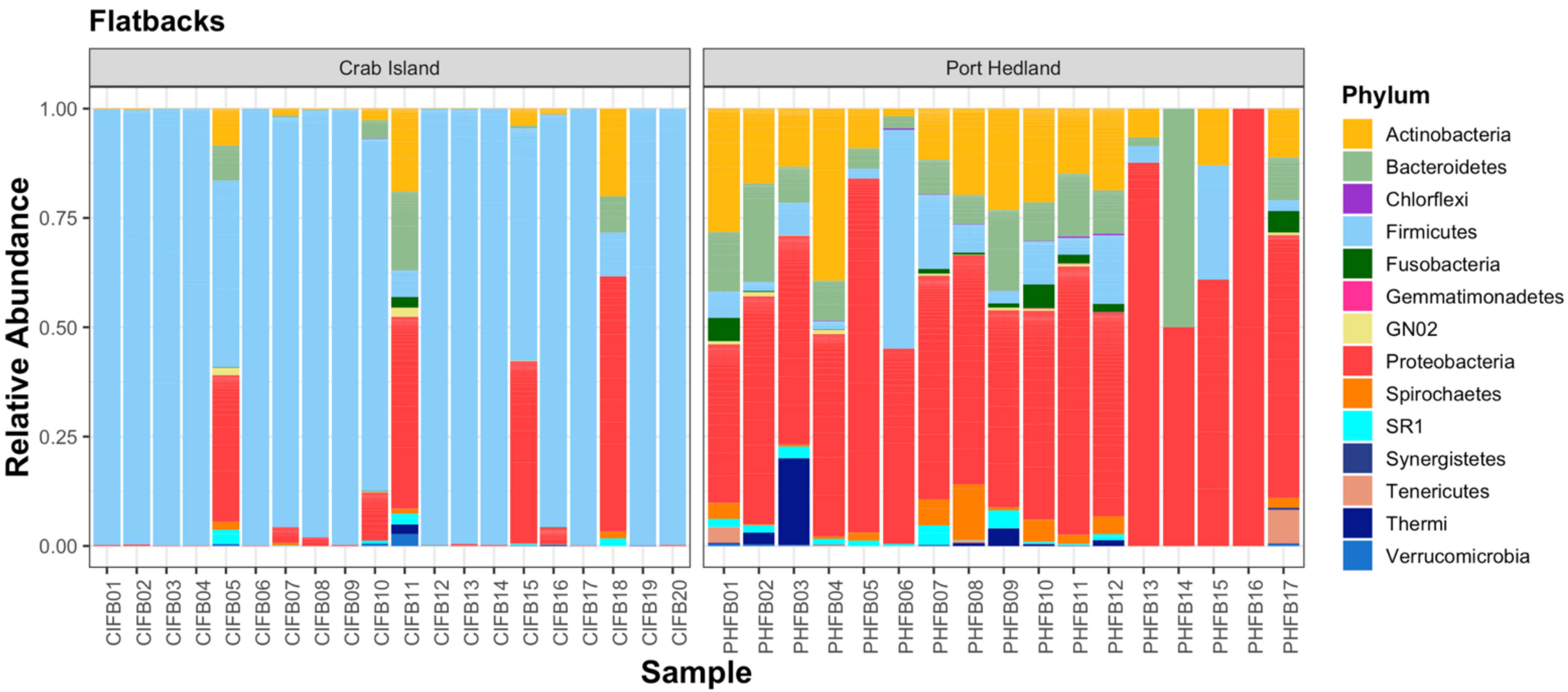

Figure 5.5 Relative abundance of the dominant bacterial phyla in flatbacks.

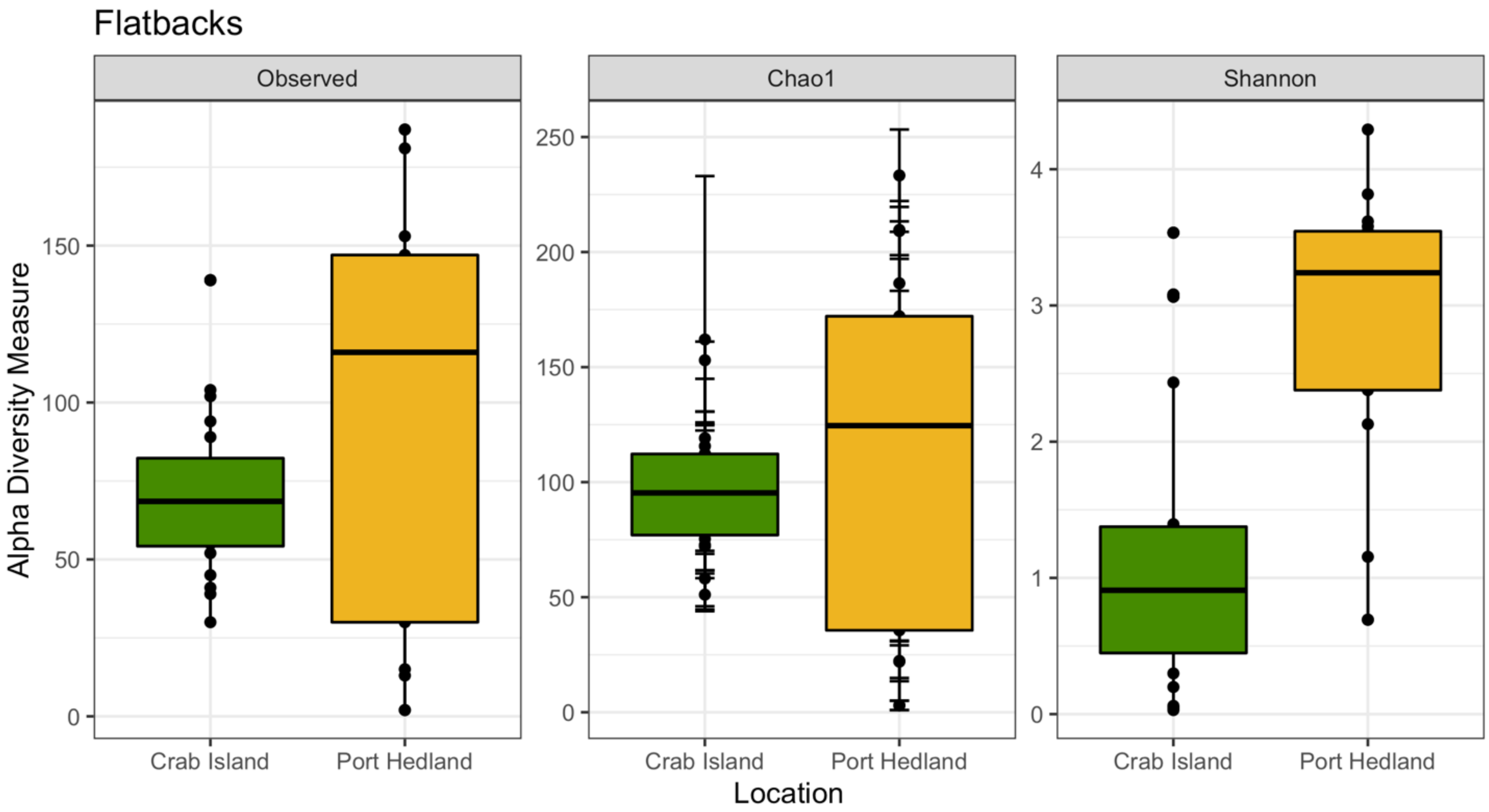

Figure 5.6 Alpha diversity estimates for flatback sea turtles. Individual points and brackets represent the richness estimate and the theoretical standard error range associated with that estimate, respectively. Within each panel, the samples are organized into location of capture, and a boxplot is overlayed on top of this for the two groups. Flatback turtles showed no differences for Observed (p=0.13) or Chao1 (p=0.48) but marked differences in Shannon diversity (p<0.001) between the two populations.

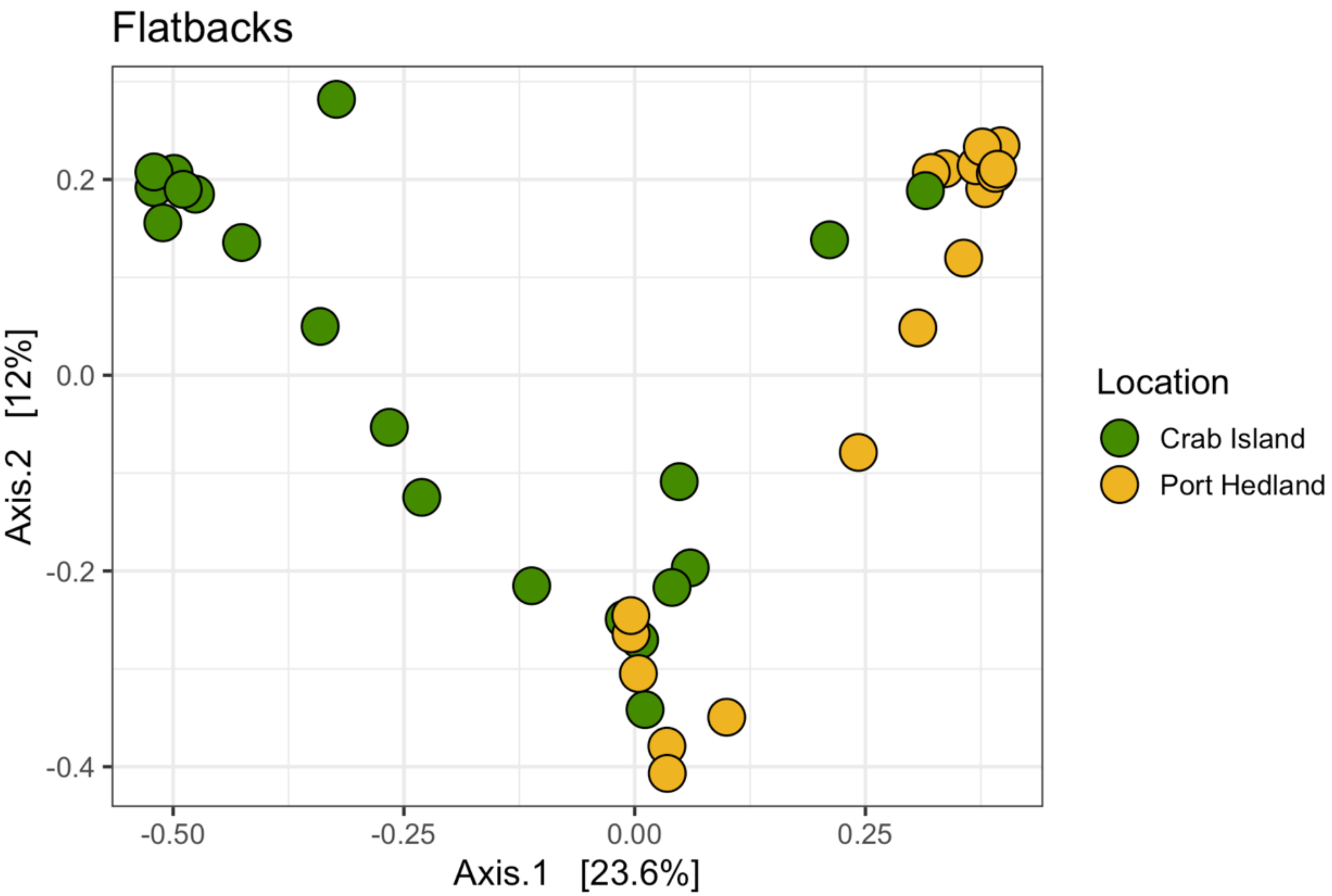

Figure 5.7 Principal coordinate analysis plot of Bray-Curtis distances. Comparison of flatback turtles from two locations, Crab Island, Queensland, and Port Hedland, Western Australia. Each point represents the gut microbiota of an individual turtle. Observed differences between the populations were significant (R^2=0.138, p=0.001).

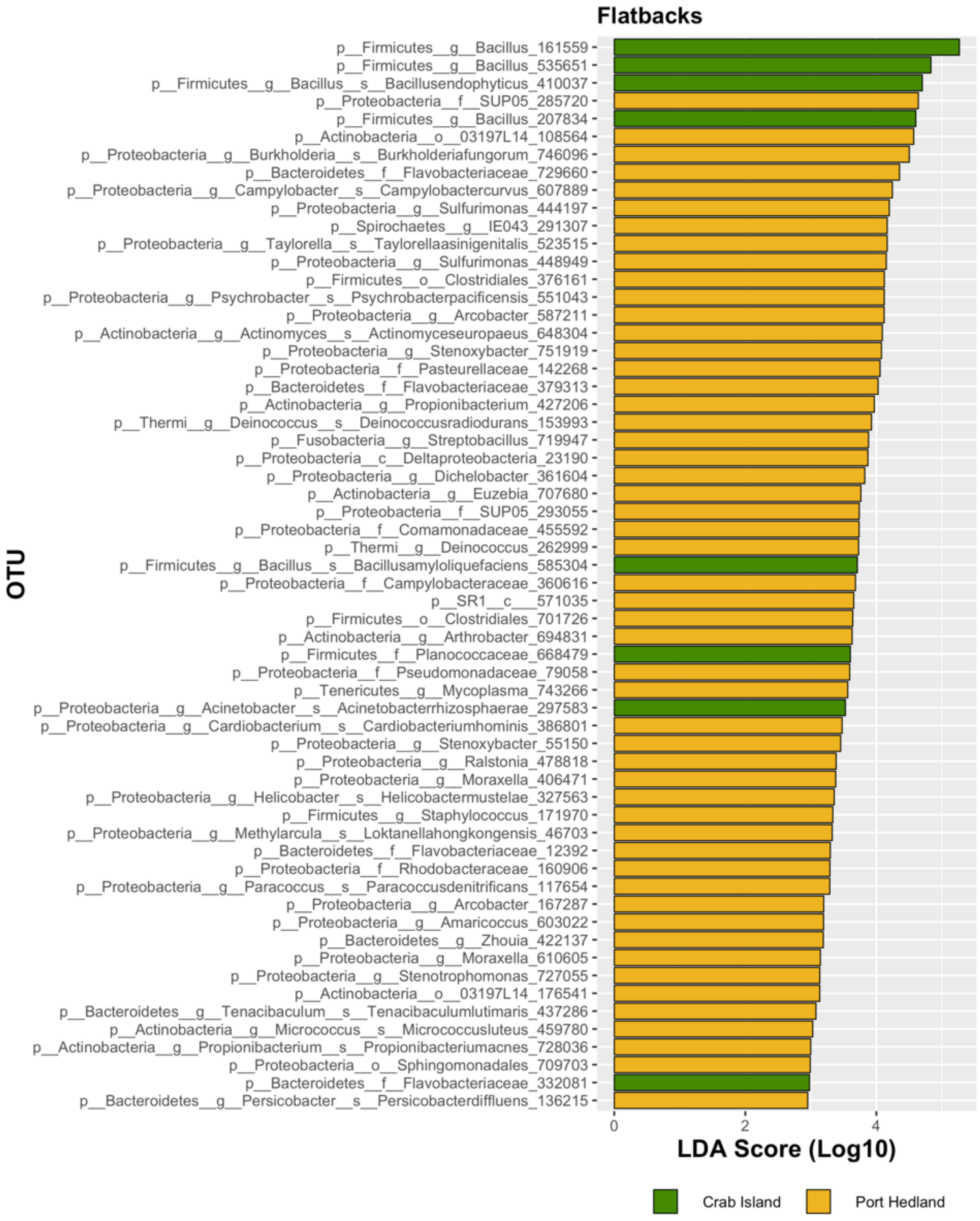

Figure 5.8 LDA Effect Size (LEfSe) algorithm was used on OTU tables to determine taxa that best characterized differences between each location of capture. Comparison of flatbacks from Crab Island, Queensland, with flatbacks from Port Hedland, Western Australia.

Table 5.3 Shared and unique OTUs in samples from flatback turtles from Crab Island, Queensland and Port Hedland, Queensland.

Taxa	Type	Details	Crab Island abundance	Port Hedland abundance	Crab Island occurrence	Port Hedland occurrence
p__Verrucomicrobia__g__Verrucomicrobium_175087	Unique	Port Hedland	0.192	0.062	0.2	0.47
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	Unique	Port Hedland	0.123	1.709	0.1	0.59
p__SR1__c___571035	Unique	Port Hedland	0.169	1.002	0.3	0.71
p__Spirochaetes__g__Treponema_30617	Unique	Port Hedland	0.098	0.44	0.25	0.59
p__Spirochaetes__g__IE043_291307	Core	Crab Island, Port Hedland	0.09	1.876	0.4	0.59
p__Proteobacteria__o__Sphingomonadales_709703	Unique	Port Hedland	0	0.087	0	0.47
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Port Hedland	0	2.571	0	0.59
p__Proteobacteria__g__Sulfurimonas_448949	Unique	Port Hedland	0.016	3.356	0.15	0.65
p__Proteobacteria__g__Sulfurimonas_444197	Core	Crab Island, Port Hedland	1.242	5.128	0.6	0.82
p__Proteobacteria__g__Sulfurimonas_412977	Unique	Port Hedland	0.004	0.021	0.15	0.41
p__Proteobacteria__g__Stenoxybacter_751919	Core	Crab Island, Port Hedland	1.171	3.227	0.85	0.82
p__Proteobacteria__g__Stenoxybacter_55150	Core	Crab Island, Port Hedland	0.208	0.695	0.65	0.76
p__Proteobacteria__g__Stenotrophomonas_727055	Unique	Port Hedland	0.01	0.249	0.2	0.65
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Unique	Port Hedland	0.002	0.335	0.1	0.53
p__Proteobacteria__g__Rhodocyclus__s__Rhodocyclustenuis_740177	Core	Crab Island, Port Hedland	0.259	2.468	0.65	0.65
p__Proteobacteria__g__Ralstonia_478818	Unique	Port Hedland	0.167	0.604	0.3	0.76
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	Unique	Port Hedland	0.081	2.602	0.35	0.65
p__Proteobacteria__g__Pseudoruegeria_390046	Core	Crab Island, Port Hedland	0.476	0.592	0.5	0.65
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Unique	Port Hedland	0.002	0.061	0.1	0.53
p__Proteobacteria__g__Moraxella_610605	Unique	Port Hedland	0.002	0.043	0.15	0.47
p__Proteobacteria__g__Moraxella_406471	Unique	Port Hedland	0.06	0.394	0.25	0.65
p__Proteobacteria__g__Methylarcula__s__Loktanellahongkongensis_46703	Unique	Port Hedland	0.09	0.392	0.1	0.71
p__Proteobacteria__g__Klebsiella_558424	Core	Crab Island, Port Hedland	2.204	0.022	0.7	0.47
p__Proteobacteria__g__Dichelobacter_361604	Core	Crab Island, Port Hedland	0.418	1.762	0.5	0.76
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	Core	Crab Island, Port Hedland	0.223	0.713	0.4	0.76

p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	Core	Crab Island, Port Hedland	0.124	2.802	0.6	0.82
p__Proteobacteria__g__Campylobacter__s__Campylobacterconcisus_141754	Unique	Port Hedland	0.012	0.319	0.2	0.76
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Unique	Port Hedland	0.368	7.435	0.25	0.82
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Unique	Port Hedland	0	0.015	0.05	0.41
p__Proteobacteria__g__Arcobacter_587211	Core	Crab Island, Port Hedland	0.286	2.652	0.55	0.82
p__Proteobacteria__g__Amaricoccus_603022	Unique	Port Hedland	0.001	0.175	0.05	0.59
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Crab Island	0.745	0.269	0.6	0.06
p__Proteobacteria__g__Acinetobacter_78571	Core	Crab Island, Port Hedland	0.456	0.094	0.4	0.53
p__Proteobacteria__g__Achromobacter_19649	Unique	Port Hedland	0.006	0.246	0.15	0.41
p__Proteobacteria__f__SUP05_293055	Unique	Port Hedland	0.001	0.012	0.05	0.59
p__Proteobacteria__f__SUP05_285720	Core	Crab Island, Port Hedland	0.398	8.589	0.6	0.82
p__Proteobacteria__f__Rhodospirillaceae_494802	Unique	Port Hedland	0	0.178	0	0.53
p__Proteobacteria__f__Rhodobacteraceae_658260	Core	Crab Island, Port Hedland	0.188	0.278	0.5	0.47
p__Proteobacteria__f__Rhodobacteraceae_37016	Unique	Port Hedland	0	0.069	0	0.59
p__Proteobacteria__f__Rhodobacteraceae_160906	Unique	Port Hedland	0.015	0.066	0.1	0.53
p__Proteobacteria__f__Pseudomonadaceae_79058	Unique	Port Hedland	0.058	0.766	0.2	0.65
p__Proteobacteria__f__Pasteurellaceae_142268	Core	Crab Island, Port Hedland	0.16	1.805	0.5	0.76
p__Proteobacteria__f__Comamonadaceae_455592	Unique	Port Hedland	0.1	1.109	0.35	0.76
p__Proteobacteria__f__Campylobacteraceae_360616	Unique	Port Hedland	0.117	1.025	0.35	0.76
p__Proteobacteria__c__Deltaproteobacteria_23190	Unique	Port Hedland	0.019	1.015	0.2	0.53
p__GN02__c__VC12cl04_595855	Unique	Port Hedland	0.125	0.174	0.25	0.59
p__GN02__c__VC12cl04_222792	Unique	Port Hedland	0.094	0.17	0.15	0.47
p__Fusobacteria__g__Streptobacillus_719947	Unique	Port Hedland	0.129	1.294	0.2	0.59
p__Firmicutes__o__Clostridiales_70341	Unique	Port Hedland	0.016	0.318	0.25	0.47
p__Firmicutes__o__Clostridiales_701726	Unique	Port Hedland	0.012	0.549	0.2	0.53
p__Firmicutes__o__Clostridiales_635308	Unique	Port Hedland	0.004	0.186	0.1	0.41
p__Firmicutes__o__Clostridiales_376161	Unique	Port Hedland	0.058	2.574	0.25	0.59
p__Firmicutes__o__Clostridiales_226726	Core	Crab Island, Port Hedland	0.258	0.553	0.5	0.65
p__Firmicutes__g__Streptococcus_269499	Unique	Port Hedland	0.004	0.49	0.15	0.76

p__Firmicutes__g__Staphylococcus_171970	Unique	Port Hedland	0.007	0.435	0.15	0.71
p__Firmicutes__g__Clostridium_482667	Unique	Port Hedland	0.005	0.047	0.05	0.59
p__Firmicutes__g__Bacillus__s__Bacillussp.NRRLB14911_432592	Core	Crab Island, Port Hedland	0.382	0.088	0.4	0.53
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	Unique	Crab Island	9.646	0.012	0.7	0.12
p__Firmicutes__g__Bacillus__s__Bacillusamyloliquefaciens_585304	Unique	Crab Island	1.004	0.008	0.6	0.29
p__Firmicutes__g__Bacillus_535651	Unique	Crab Island	12.042	0.022	0.75	0.12
p__Firmicutes__g__Bacillus_207834	Unique	Crab Island	9.716	0.094	0.65	0.24
p__Firmicutes__g__Bacillus_161559	Core	Crab Island, Port Hedland	36.704	0.648	1	0.71
p__Firmicutes__f__Planococcaceae_668479	Unique	Crab Island	0.71	0.001	0.7	0.06
p__Firmicutes__f__Bacillaceae_724791	Unique	Crab Island	2.486	0.012	0.5	0.29
p__Firmicutes__f__Bacillaceae_664374	Core	Crab Island, Port Hedland	2.828	0.197	0.75	0.47
p__Chloroflexi__c__SOGA31_314194	Unique	Port Hedland	0	0.055	0	0.47
p__Bacteroidetes__o__Bacteroidales_711203	Unique	Port Hedland	0.038	0.158	0.2	0.59
p__Bacteroidetes__g__Zhouia_422137	Unique	Port Hedland	0.197	0.26	0.3	0.59
p__Bacteroidetes__g__Tenacibaculum__s__Tenacibaculumlutimaris_437286	Unique	Port Hedland	0.029	0.17	0.15	0.47
p__Bacteroidetes__g__Tenacibaculum_474405	Unique	Port Hedland	0.086	0.228	0.15	0.71
p__Bacteroidetes__g__Tenacibaculum_428560	Unique	Port Hedland	0.092	0.169	0.25	0.59
p__Bacteroidetes__g__Pontibacter__s__Pontibacterkorlensis_406748	Unique	Port Hedland	0	0.382	0	0.65
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	Unique	Port Hedland	0.036	0.089	0.1	0.53
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Port Hedland	0.004	0.538	0.1	0.53
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Port Hedland	0.023	0.631	0.3	0.47
p__Bacteroidetes__f__Flavobacteriaceae_729660	Core	Crab Island, Port Hedland	0.311	4.421	0.4	0.76
p__Bacteroidetes__f__Flavobacteriaceae_379313	Core	Crab Island, Port Hedland	0.82	2.776	0.45	0.71
p__Bacteroidetes__f__Flavobacteriaceae_332081	Unique	Port Hedland	0.242	0.182	0.3	0.71
p__Bacteroidetes__f__Flavobacteriaceae_12392	Core	Crab Island, Port Hedland	0.124	0.373	0.45	0.71
p__Actinobacteria__o__koll13_76572	Unique	Port Hedland	0.004	0.104	0.15	0.59
p__Actinobacteria__o__Actinomycetales_369980	Unique	Port Hedland	0.034	0.438	0.25	0.65
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Port Hedland	0.005	0.125	0.2	0.47
p__Actinobacteria__o__03197L14_629656	Unique	Port Hedland	0	0.178	0.05	0.41

p__Actinobacteria__o__03197L14_176541	Unique	Port Hedland	0.174	0.259	0.2	0.53
p__Actinobacteria__o__03197L14_108564	Core	Crab Island, Port Hedland	0.654	6.544	0.7	0.82
p__Actinobacteria__g__Streptomyces_420197	Unique	Port Hedland	0.376	0.042	0.2	0.41
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Unique	Port Hedland	0.115	0.258	0.25	0.65
p__Actinobacteria__g__Propionibacterium_427206	Core	Crab Island, Port Hedland	0.578	2.177	0.6	0.82
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Unique	Port Hedland	0.086	0.085	0.15	0.59
p__Actinobacteria__g__Euzebia_707680	Core	Crab Island, Port Hedland	0.122	0.968	0.6	0.71
p__Actinobacteria__g__Arthrobacter_694831	Core	Crab Island, Port Hedland	0.106	0.695	0.65	0.71
p__Actinobacteria__g__Aeromicrobium_619093	Unique	Port Hedland	0.01	0.018	0.2	0.41
p__Actinobacteria__g__Actinomyces__s__Actinomyceseuropaeus_648304	Core	Crab Island, Port Hedland	0.118	1.824	0.5	0.65
p__Actinobacteria__g__Actinomyces_119009	Unique	Port Hedland	0.008	0.181	0.25	0.47
p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Port Hedland	0.008	0.072	0.15	0.47
p__Actinobacteria__f__AKIW874_693966	Unique	Port Hedland	0.083	0.138	0.05	0.53

Table 5.4 LDA scores for flatback turtles.

Operational Taxonomic Unit	LDA	Group
p__Bacteroidetes__f__Flavobacteriaceae_332081	2.98	Crab Island
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	3.53	Crab Island
p__Firmicutes__f__Planococcaceae_668479	3.60	Crab Island
p__Firmicutes__g__Bacillus__s__Bacillusamyloliquefaciens_585304	3.71	Crab Island
p__Firmicutes__g__Bacillus_207834	4.60	Crab Island
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	4.70	Crab Island
p__Firmicutes__g__Bacillus_535651	4.83	Crab Island
p__Firmicutes__g__Bacillus_161559	5.27	Crab Island
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	2.95	Port Hedland
p__Proteobacteria__o__Sphingomonadales_709703	2.99	Port Hedland
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	3.00	Port Hedland
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	3.03	Port Hedland
p__Bacteroidetes__g__Tenacibaculum__s__Tenacibaculumlutimaris_437286	3.08	Port Hedland
p__Actinobacteria__o__03197L14_176541	3.14	Port Hedland
p__Proteobacteria__g__Stenotrophomonas_727055	3.14	Port Hedland
p__Proteobacteria__g__Moraxella_610605	3.15	Port Hedland
p__Bacteroidetes__g__Zhouia_422137	3.19	Port Hedland
p__Proteobacteria__g__Amaricoccus_603022	3.19	Port Hedland
p__Proteobacteria__g__Arcobacter_167287	3.20	Port Hedland
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	3.29	Port Hedland
p__Proteobacteria__f__Rhodobacteraceae_160906	3.29	Port Hedland
p__Bacteroidetes__f__Flavobacteriaceae_12392	3.30	Port Hedland
p__Proteobacteria__g__Methylarcula__s__Loktanellahongkongensis_46703	3.33	Port Hedland
p__Firmicutes__g__Staphylococcus_171970	3.34	Port Hedland
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_327563	3.36	Port Hedland
p__Proteobacteria__g__Moraxella_406471	3.38	Port Hedland
p__Proteobacteria__g__Ralstonia_478818	3.39	Port Hedland
p__Proteobacteria__g__Stenoxybacter_55150	3.46	Port Hedland
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	3.48	Port Hedland
p__Tenericutes__g__Mycoplasma_743266	3.56	Port Hedland
p__Proteobacteria__f__Pseudomonadaceae_79058	3.59	Port Hedland
p__Actinobacteria__g__Arthrobacter_694831	3.63	Port Hedland
p__Firmicutes__o__Clostridiales_701726	3.64	Port Hedland
p__SR1__c___571035	3.65	Port Hedland
p__Proteobacteria__f__Campylobacteraceae_360616	3.68	Port Hedland
p__Thermi__g__Deinococcus_262999	3.73	Port Hedland
p__Proteobacteria__f__Comamonadaceae_455592	3.74	Port Hedland
p__Proteobacteria__f__SUP05_293055	3.74	Port Hedland
p__Actinobacteria__g__Euzebia_707680	3.76	Port Hedland
p__Proteobacteria__g__Dichelobacter_361604	3.82	Port Hedland

p__Proteobacteria__c__Deltaproteobacteria_23190	3.87	Port Hedland
p__Fusobacteria__g__Streptobacillus_719947	3.88	Port Hedland
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	3.93	Port Hedland
p__Actinobacteria__g__Propionibacterium_427206	3.97	Port Hedland
p__Bacteroidetes__f__Flavobacteriaceae_379313	4.03	Port Hedland
p__Proteobacteria__f__Pasteurellaceae_142268	4.06	Port Hedland
p__Proteobacteria__g__Stenoxybacter_751919	4.08	Port Hedland
p__Actinobacteria__g__Actinomyces__s__Actinomyceseuropaeus_648304	4.09	Port Hedland
p__Proteobacteria__g__Arcobacter_587211	4.12	Port Hedland
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	4.12	Port Hedland
p__Firmicutes__o__Clostridiales_376161	4.12	Port Hedland
p__Proteobacteria__g__Sulfurimonas_448949	4.15	Port Hedland
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	4.17	Port Hedland
p__Spirochaetes__g__IE043_291307	4.17	Port Hedland
p__Proteobacteria__g__Sulfurimonas_444197	4.20	Port Hedland
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	4.24	Port Hedland
p__Bacteroidetes__f__Flavobacteriaceae_729660	4.35	Port Hedland
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	4.50	Port Hedland
p__Actinobacteria__o__03197L14_108564	4.57	Port Hedland
p__Proteobacteria__f__SUP05_285720	4.64	Port Hedland

5.5 Discussion

Our analysis of the microbiota of wild nesting sea turtles indicates that microbial populations differ significantly among animals of the same species, but originating from different populations. These differences may be apparent in both composition and diversity. This investigation highlights the importance of interpreting microbiota data in concert with environmental factors and potential genetic differences among population. Elucidating the intrinsic and extrinsic causes driving these differences is difficult due to the lack of basic biological data for both individual animals, as well as the different populations of turtles in this investigation. While broadly speaking, dietary, and migratory information exists for some species and populations of sea turtles, these generalisations should be applied with caution to individuals captured from a nesting beach. For example, nesting loggerheads show a high degree of fidelity to nesting beaches and foraging grounds, but females nesting at the one rookery will have migrated from numerous foraging areas (Limpus 2008b). Female loggerheads tagged at Queensland rookeries have been recorded from feeding areas as far away as Indonesia, Papua New Guinea, Solomon Islands, and New Caledonia, but also from local areas including the Northern Territory, Queensland and New South Wales (Limpus *et al.* 1992). We were unable to identify the origin of each turtle that was sampled, and therefore it is difficult to determine exactly what factors may be influencing microbiota community structure in our samples.

In addition to observed differences between sea turtle populations, we also noticed differences between individuals within populations. In humans, the establishment and maintenance of the microbiota is heavily influenced by a range of factors including diet, host genotype, and microbial interactions (Dethlefsen *et al.* 2006). These factors are further influenced by other random, unpredictable events such as colonisation history. Within an individual, microbes are unevenly distributed and they have differences in transmissibility (Dethlefsen *et al.* 2006). This variation is greatly amplified under the paradigm of stochastic niche occupancy, in which microbes must be able to persist and multiply using available resources, in the face of fierce competition from existing colonists (Dethlefsen *et al.* 2006). Such unpredictability accounts for the differences observed in microbiotas between closely related individuals such as inbred mice (Hoy *et al.* 2015), and human identical twins (Turnbaugh *et al.* 2010). Therefore, individual variation within a population of animals, such as the turtles in our investigation, is an expected finding.

The role that diet may be playing in determining microbiota composition in our samples is unknown. Loggerhead turtles are primarily carnivorous (Jones and Seminoff 2013), feeding on a wide range of prey items with considerable dietary plasticity (Frick *et al.* 2009a; Frick *et al.* 2009b; Seney and Musick 2007; Limpus 2008b). Although there are differences in the species of prey items consumed among loggerhead populations, the taxonomic classes of ingested prey is relatively conserved over phylogeographic separations (Jones and Seminoff 2013). The major differences in dietary preferences come from the proportion of benthic or pelagic fauna in the diet, which is predominately affected by life stage of the turtle (Jones and Seminoff 2013). All of the loggerheads in this investigation were adult, and therefore broad dietary preference is likely to be similar for the two populations. In humans, diet markedly affects the microbiota and is principally driven by the volume of fibre consumed (Albenberg and Wu 2014). It remains to be seen whether or not the observed species-level prey differences across loggerhead populations, rather than dietary variations at genus, family or higher taxonomic levels, selects for specific microbial OTUs in sea turtles.

In addition to food items consumed, food acquisition and digestive strategy can play a role in altering microbial communities in animals. For example, the Burmese python (*Python molurus*) is a sit-and-wait predator that consumes enormous, infrequent meals, often punctuated by prolonged periods of starvation (Costello *et al.* 2010). This feast or famine tactic means that the gastrointestinal tract, including the microbiota, undergoes significant change depending on the presence or absence of food in the gut (Costello *et al.* 2010). These changes start to become evident between 12 hours to 3 days after prey ingestion (Costello *et al.* 2010). Similar patterns of fluctuations in microbial community composition are seen in other vertebrates in response to caloric restriction including humans (Remely *et al.* 2015), fish (Xia *et al.* 2014), bears (Sommer *et al.* 2016), alligators (Keenan *et al.* 2013), mice (Beli *et al.* 2018), and penguins (Dewar *et al.* 2014). Like Burmese pythons, sea turtles may undergo long periods of fasting, particularly during breeding and migration (Hays *et al.* 2002; Jessop *et al.* 2004), but how this affects gastrointestinal bacterial community composition is unknown. All animals sampled during this investigation were nesting females, and it is likely that they had been inappetant for an extended period, which may be influencing the microbiota composition of our populations. It is of particular relevance to study microbiotas of female sea turtles during their egg-laying reproductive period because it is the microbiota during this time that is likely to be transferred to their offspring, rather than the microbiota during foraging, non-reproductive periods. Given the rapidity with which the microbiota fluctuates in other vertebrates, it is reasonable to assume that the microbiota of nesting females is not the same as the microbiota of foraging animals. Although we were not able to determine how fasting in nesting turtles affects microbial diversity, some authors propose that fasting samples represent the core microbial OTUs, with other OTUs fluctuating in number in response to post-prandial physiological changes (Keenan *et al.* 2013; Costello *et al.* 2010). If, as hypothesised, diet has little effect on this core microbiota, then a possible explanation for differences observed in our loggerhead samples is differences in host genetics. In humans, host genetic variation has been shown to account for microbiota composition from a number of different body sites (Blekhman *et al.* 2015; Goodrich *et al.* 2014), and this phenomenon may be a significant contributing factor in the differences observed in our loggerhead populations, given their genetic separation

(Bowen and Karl 2007). In contrast it seems reasonable that genetics is less likely to be contributing to observed differences among flatback populations, given the limited genetic variability in this species compared with loggerheads (Dutton *et al.* 2002), and so other factors are more important in shaping microbial communities in flatback populations.

We identified that flatback turtles from Crab Island had much lower microbial diversity than their counterparts from Port Hedland. Such observations in other species have been attributed to habitat degradation, and may result in decreased ability of animals to utilise environmental resources (Barelli *et al.* 2015; Rosenfeld 2017; Amato *et al.* 2013). We did not attempt to assess habitat quality in this investigation, but Crab Island is a remote, uninhabited island in the Endeavour Strait in the Gulf of Carpentaria with little signs of human impact (pers obs). Importantly, it is believed that flatback turtles nesting on Crab Island do not feed in the Gulf of Carpentaria, and so migrate considerable distances from foraging grounds for reproduction (Limpus *et al.* 1993). Thus, in the context of their lives, flatback turtles only spend a relatively short period of time in this area, and therefore any environmental influence is most likely coming from elsewhere. Further work is required to identify the effects that habitat disturbances have on sea turtle microbiotas, and whether or not they can act as sentinels for oceanic health.

The observed increase in relative abundance of Firmicutes in flatback turtles from Crab Island may possibly be associated with increased adiposity (Backhed *et al.* 2004; Cui *et al.* 2013). Bacteria from this phylum are able to increase fat metabolism through modulation of the host genes that regulate fat storage (Turnbaugh *et al.* 2006). Flatback turtles from Western Australia undergo a shorter migration to their natal beaches than Crab Island turtles, as they forage relatively close to their nesting rookeries (Pendoley *et al.* 2014). This might mean that turtles from Crab Island are inappetant for a longer period of time, and therefore will have a greater reliance on stored body fat to fuel their migration. This physiological phenomenon may preferentially select for Firmicutes as an adaptation to their prolonged fast.

The results of this investigation indicate that the microbiota composition of sea turtles differs between populations. Genetics, environment and physiology may all affect microbial populations but may also co-vary, making identification of the most important factors difficult. Our study highlights the importance of interpreting microbiotas of wild animals in context of their location as well as their physiological state.

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Chapter 6. The effect of the microbiota on blood parameters in nesting flatback turtles (*Natator depressus*)

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6.1 Abstract

The microbiota is considered critical for normal vertebrate homeostasis. To date, investigations into the role that the microbiota plays in non-human physiology are rare, and are non-existent in reptiles. To address this knowledge gap, we explored the relationship between differences in microbial communities to see if they accounted for differences in haematology and biochemistry values, in different populations of nesting flatback turtles (*Natator depressus*), from Port Hedland, Western Australia, and Crab Island, Queensland. Blood was collected from the dorsal cervical sinus as well as cloacal swabs from nesting turtles. Blood was analysed using standard techniques applicable to reptiles and for cloacal swabs we manually extracted bacterial DNA and used 16s rRNA sequencing to explore microbial communities. We found that microbiota composition had a correlation with a number of analytes in animals from Port Hedland including packed cell volume, heterophils, uric acid, calcium, total protein, albumin, globulin, and potassium. A number of operational taxonomic units (OTUs) were identified to be contributing to this correlation. Additionally, a correlation was seen in heterophil/lymphocyte ratio, and basophils, associated with microbiota composition in Port Hedland animals, but no specific OTUs could be identified to be significantly contributing to these observations. This study is the first of its kind in reptiles and highlights the importance of the link between microbiota and physiology in this taxon. However, further investigation is required to determine the exact mechanisms by which the microbiota exerts these effects in reptiles.

Key Words: *biochemistry, flatback turtle, haematology, microbiota, Natator depressus*

6.2 Introduction

Diverse and complex communities of microorganisms, known collectively as the microbiota, reside on and within every metazoan species. The occupation of higher organisms by prokaryotic and eukaryotic colonists is thought to be a key factor in driving evolution and radiation of life on Earth (Lee and Mazmanian 2010; McFall-Ngai *et al.* 2013). Primarily based on human research, with a limited number of investigations in other animals, we are only just beginning to understand the complexity of the host-microbiota relationship, and how it contributes to normal physiology and homeostasis (Dethlefsen *et al.* 2007). Importantly, the microbiota primes and directs maturation of the immune system (Mazmanian *et al.* 2005), and contributes to the function of both the innate and adaptive immune systems (Thaiss *et al.* 2016; Lee and Mazmanian 2010). In comparison to other vertebrates, the reptile immune system is relatively poorly understood. Like all jawed vertebrates, reptiles possess both an innate and adaptive immune system (Zimmerman *et al.* 2010) and they also possess many of the anatomical and cellular components that higher vertebrates, such as birds and mammals, use in defending themselves from invading pathogens (Zimmerman *et al.* 2010). Given these similarities, it may be speculated that the microbiota of reptiles has a similar effect on their immune system maturation and function as is seen in other vertebrates.

To date, there have been no attempts to link microbiota composition with health indices in reptiles. In an endeavour to correct this deficit, we explored whether differences in microbiota composition could explain differences in haematology and biochemistry results in two populations of nesting flatback turtles (*Natator depressus*). As an indicator of immune system function, we investigated the relationships between microbial composition and red and white blood cell indices. To assess general organ function we examined how microbiota correlated with a range of biochemical parameters including aspartate aminotransferase, uric acid, creatinine kinase, total protein, glucose, calcium, phosphate, albumin, globulin, potassium, and sodium. We chose these analytes because they are recommended analytes for the assessment of health in chelonians (Heatley and Russell 2019), and are able to be measured by the Vet Scan analyzer (Abaxis, Union City, California, USA). We sampled animals from two spatially separate populations because environmental factors have been shown to influence both blood values (Ferrer and Dobado-Berrios 1998; Whiting *et al.* 2007; Poljičak-Milas *et al.* 2004), and microbiota composition (Eichmiller *et al.* 2016; Amato *et al.* 2013; Ren *et al.* 2017) of wild animals. Thus, sampling animals from different populations was important to elucidate if any consistent correlations exist between blood analytes and microbiota.

Alterations in the microbiota have been shown to affect the fitness of humans and other species (Bahrndorff *et al.* 2016), although the implications for these variations in an ecological and conservation context are poorly understood. Improving our comprehension of the host-microbial relationship, and factors that drive dysbiosis and reduced fitness, are imperatives for modern conservation efforts (Bahrndorff *et al.* 2016). This information may be used as a screening tool to monitor how animals are able to respond, in a physiological sense, to an ever-changing world. This is especially important for sea turtles, which as a group are some of the most imperilled species on Earth (IUCN 2019).

To better understand how microbial composition affects health in sea turtles, we used the flatback turtle (*Natator depressus*), as a model species. Listed as ‘Data Deficient’ by the IUCN (IUCN 2019), we know relatively little about this species but its restricted range and relatively small population size make it a useful study species. It is the single member of the genus *Natator*, and is one of only two marine turtle species that does not have a global distribution (Limpus 2008). All recorded flatback nesting beaches occur in Australia (Limpus *et al.* 1988) and the species feeds widely throughout the waters of the Australian continental shelf (Limpus 2008). Four major management units have been identified for flatback turtle rookeries in Australia (Limpus 2008), with genetic analysis indicating that there is a low level of genetic variability in the species and there is limited gene flow between the rookeries (Dutton *et al.* 2002).

Interpretation of microbial composition and its effects on the health of wild animals are hampered by a lack of controlled studies in species other than humans and laboratory animals. For many species, such as sea turtles, manipulative investigations are not practical due to the constraints of long-term captivity on individuals. Furthermore, captivity may dramatically alter the microbial composition of wild animals (Kohl *et al.* 2014; Clayton *et al.* 2016; Delport *et al.* 2016; Nelson *et al.* 2013), therefore any conclusions drawn from such investigations would also need to be inferred with caution. Given these constraints, interpreting wild animal data using the available literature (i.e. on human and laboratory animals) remains a valid alternative to further our knowledge on microbiome-health relationships in wildlife. In this study, our aims were to determine and compare the microbiota and blood parameters of two flatback populations and to detect measurable relationships between the microbiota and the health of animals. It is hoped that by conducting this research we may gain a better understanding of how microbial populations interact with a reptilian host, and identify areas of potential future research.

6.3 Methods

6.3.1 Ethics statement

This study was approved by the Biological Sciences Animal Ethics Committee of Monash University (approval 14694). Adult flatback turtles (*Natator depressus*) were sampled under permit WITK17730216 from the Queensland Department of Environment and Heritage Protection, and the permit 01-000121-1 from the Department of Parks and Wildlife Western Australia.

6.3.2 Study populations

Nesting flatback turtles were sampled from Crab Island, Queensland, Australia (10.9947° S, 142.1090° E) in September 2016 (n=20), and from Port Hedland, Western Australia, (20.3107° S, 118.5878° E) in November 2016 (n=17).

6.3.3 Sample collection

During the nesting season female flatback turtles came ashore to dig a nest and lay eggs, at which time we were able to collect samples when females had finished nesting and were returning to the ocean. For blood collection, an area of skin was prepared using alcohol wipes and 10ml of blood was collected from the dorsal cervical sinus using an 18G needle attached to a 10ml syringe. The turtle was then flipped into dorsal recumbency, and an equine uterine swab (Minitube, Smythesdale, Victoria, Australia) was inserted into the cloaca so that it entered the distal colon. These swabs were housed in a sterile sheath, the entire apparatus was inserted into the cloaca and the swab tip was extruded when correct placement of the sheath had been achieved. The swab tip was then retracted back into the sheath prior to extraction. The tip of the swab was cut using a sterile wire cutter, placed into a sterile Eppendorf tube and sealed. Turtles were then permitted to return to the ocean. Total sample collection time was approximately 10 minutes.

6.3.4 Analysis of Blood

Immediately after blood had been collected, one milliliter was transferred into a lithium heparin container (BD Microtainer tubes, Becton Dickinson, Franklin Lakes, New Jersey, USA) and the remainder into plain BD Microtainer Tubes (Becton Dickinson). At this same time a drop of fresh blood (without anticoagulant) was placed onto a microscope slide, smeared using the beveled edge of another slide and allowed to air-dry. The blood tubes were then placed into a portable ice pack and taken back to the field laboratory. Once at the field laboratory, the packed cell volume (PCV) was determined using standard centrifugation in microhematocrit tubes and the microscope slides were stained with Romanowsky stain (Rapid Diff, Australian Biostain Pty. Ltd., Traralgon, Victoria, Australia). Leukocyte differential counts were performed manually on blood films, and white cells were classified as heterophils, lymphocytes, eosinophils, basophils, or monocytes (Campbell and Ellis 2007). Heterophil/eosinophil counts were performed manually using a hemocytometer and by staining whole blood with phloxine B (made in-house). The total white blood cell (TWBC) count was calculated by correcting the manual count for the percentage of heterophils and eosinophils present (Dien *et al.* 1994). In addition, we calculated the heterophil/lymphocyte ratio (H:L) as a measure of stress (Davis *et al.* 2008). Both PCV and TWBC counts were determined within 3 h of blood collection. Blood in the plain tube was centrifuged, and the resultant serum removed and stored in a portable freezer (-20° C) in the field for a maximum of three days. The serum was then transported to the lab on dry ice and frozen at -70°C for up to 2 months until analysis. Serum was analyzed using the avian-reptilian rotor on the Vet Scan analyzer (Abaxis, Union City, California, USA).

6.3.5 DNA extraction of cloacal swabs

DNA was extracted manually using the phenol-chloroform method (Green *et al.* 2012). In each Eppendorf tube, 500 µL of extraction buffer (20 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M Tris, 1% cetrimonium bromide, 56 mM NaCl, pH 8) was added so that swabs were completely covered. We then added 20 µL of proteinase K (Qiagen proteinase K (10 ml) to each vial, along with 60 µL of 10% sodium dodecyl sulphate. The mixture was then incubated at 55°C overnight. The next day, 50 µL of 5 M NaCl and 500 µL of phenol was added, and the tubes shaken until an emulsion was formed. They were then incubated at room temperature for

10 minutes, with intermittent mixing. The tubes were then centrifuged at 10,000 RPM for 10 minutes and the supernatant removed and added to a new tube containing 250 µL phenol and 250 µL chloroform:isoamyl-alcohol (24:1). The tubes were again centrifuged at 10,000 RPM for 10 minutes and the resultant supernatant added to a new tube containing 500 µL of chlorophorm:isoamyl-alcohol. Once again, the tubes were centrifuged at 10,000 RPM for 10 minutes. The supernatant was then added to a new tube containing 3 M Sodium Acetate at a volume equal to 10% of the extraction solution. We then added 1 ml of ice-cold 99% ethanol to each test tube and then placed them into a freezer at -20°C for 1 hour. The tubes were then centrifuged at 4°C at 12,000 RPM for 10 minutes. The fluid in the test tube was then removed with a glass pipette and 1 ml of ice-cold 70% alcohol was added. The tubes were centrifuged a final time at 4°C at 12,000 RPM for 5 minutes. After centrifugation the alcohol was removed and the lids left off the tubes to allow the DNA pellet to dry. Once dried, 25 µL of 1 x Tris-EDTA (TE) was added to each tube and the extracted DNA was stored at -20°C until amplicon sequencing could take place.

6.3.6 16S rRNA gene amplicon sequencing

The V3-V4 region of 16S rRNA genes were amplified with forward primer 5' ACTCCTACGGGAGGCAGCAG 3' and reverse primer 5' GGACTACHVGGGTWTCTAAT 3' using Q5 high fidelity polymerase (New England Biolabs) using the barcoding strategy of Fadrosh *et al.* (2014). Sequencing was performed on an Illumina MiSeq system (2 x 300 bp).

6.3.7 Data processing

Sequence data was analysed using QIIME version 1.9.1 (Caporaso *et al.* 2010) using default parameters and a Phred quality threshold of > 20. The UCLUST algorithm (Edgar 2010) was used to pick OTUs at 97% sequence identity and a Biome table was produced. Potentially chimeric sequences were identified using Pintail (Ashelford *et al.* 2005). Blast was used to assign taxonomy against the Greengenes database (DeSantis *et al.* 2006) and QIIME version 1.9.1 defaults. Additional assignment of taxonomy was performed using a command line version of BLASTN (Altschul *et al.* 1997) against the NCBI 16S Microbial database. Although some researchers advocate the use of amplicon sequence variants (ASVs) over operational taxonomic units (OTUs), there is no consensus to suggest that this results in more robust analysis, and there is evidence that both methodologies produce similar results, especially in datasets such as ours (Glassman and Martiny 2018). Therefore, we elected to use OUT analysis to interpret our data.

6.3.7 Statistics and data analysis

For blood results, statistical analysis was performed using the statistical software program R (R Development Core Team 2015). For all data collected, ranges were calculated by Dixon Q test analysis of data with outliers (defined by a D/R ratio greater than 1:3) excluded. Data were assessed for normality with the Shapiro-Wilk test. For normally distributed data, Welch's two sample t-test was conducted to identify whether there was a significant difference in hematologic and biochemical values between populations, and for non-normally distributed data we used the Wilcoxon Rank sum test. Significance was accepted at $p<0.05$.

Initial exploration of the Biome table data was performed using the online Calypso software (http://cgenome.net/wiki/index.php/Calypso) (Zakrzewski *et al.* 2017). Data was further analysed in R, utilising the package 'phyloseq'. Alpha diversity was explored using Observed OTUs, Shannon index and Chao1 estimates. Alpha diversity was tested for normality using the Shapiro-Wilks test and then parametric and non-parametric methods were adopted to test for significant differences between groups. Both Observed OTUs ($W=0.96$, $p=0.29$) and

Chao1(W=0.97, p=0.6), were normally distributed and so comparisons between populations were made using ANOVA, while Shannon diversity was non-normally distributed (W=0.9, p=0.002) and so comparisons were made using the Kruskal-Wallis test. Beta diversity was investigated using principle co-ordinate analysis (PCoA) (Bray-Curtis) and Adonis tests.

To determine if microbiota samples had an effect on haematological and biochemical data we combined individual blood parameter results with the microbiota PCoA coordinates (Vector 1 and Vector 2) for each turtle. We confirmed the suitability of this data for linear model testing by exploring the homogeneity of variances (residuals vs fitted plots and scale-location plots), the normality of residuals (Q-Q plots), and Cook's Distance. Although some relationships had the appearance of being non-monotonic when fitted with a loess smoothed line (Figure 6.5), an examination of the diagnostic plots still supported a best interpretation that the residuals of all model(s) were suitable for linear fitting. OTUs were subjected to a similar screening prior to investigating their relationships with the two vectors (see below). Because the interaction of population and vectors was of interest, linear models included the main effects population and vector, as well as the interaction term site:vector (i.e. each blood parameter was tested as a function of vector 1 + population + vector 1 x population, and this was repeated for vector 2). Where significant interactions of site:vector were evident, we investigated these further using interaction plots to examine how each blood parameter depended on locality. Moreover, for transparency of data, any relationship that included a significant main effect (regardless of whether there was an interaction) has also been presented as an interaction plot, so that the reader can visually compare slopes of lines. Identifying relationships with synthetic axes is not meaningful, if we do not know what the axes represent. Normally, in a PCA, this would be explored using loadings. However, because PCoA loadings are not equivalent to PCA loadings, and the interpretation of PCoA loadings is mathematically not straightforward (Postma *et al.* 2011; Gower and Harding 1988), we have used a simple Pearson correlation value to establish likely associations between vectors and OTUs. This is important for interpreting which OTUs contribute to which vectors, and in which direction. Similar to how thresholds are used to cut-off loading interpretations (Stevens 1992), we opted to use a correlation threshold of 0.4, where values weaker than 0.4 were excluded. This is a stricter threshold than the often used 0.3 threshold. However, the large number of OTUs meant that we deemed it preferable to use a threshold that excluded weak associations, even where they were significant (i.e. many of the correlation values <0.4 and >-0.4 were significant, but their inclusion risked over-interpretation). We think that in this case a simple, conservative and transparent method that is understandable to most biologists is preferable to a mathematically complex one.

6.4 Results

6.4.1 Blood results

We found significant differences between the two populations for PCV (t=4.02, df=32.96, p<0.01), heterophils (t=-3.01, df=34.09, p=0.01), heterophil/lymphocyte ratio (H:L) (W=90, p=0.02), basophils (W=241, p<0.01uric acid (t=-6.28, df=27.85, p<0.01), total protein (W=292.5, p<0.01), globulin (W=278, p<0.01), albumin (W=81, p<0.01) and potassium (W=283, p<0.01) (Table 6.1).

Table 6.1 Comparison of hematology and serum biochemistry results for free-ranging nesting flatback turtles (*Natator depressus*) captured at Crab Island, September 2016, and Port Hedland, October 2016. Analytes were tested for normality using the Shapiro-Wilk test and then parametric and non-parametric methods were used to test for differences between locality.

	Crab Island (*n*=20)		Port Hedland (*n*=17)					
Parameter	Range	Mean ± SE	Range	Mean ± SE	p	t	df	W
PCV (%)	24.0-38.0	30.9 ± 4.1	29.0-43.0	36.5 ± 4.4	<0.01	4.03	32.96	N/A
TWBC (10^3/μL)	3.8-13	7.9 ± 3.3	3.0-11.1	6.5 ± 2.6	0.29	N/A	N/A	135
Heterophils (10^3/μL)	2.2-8.5	4.4 ± 1.7	1.0-5.6	2.9 ± 1.2	0.01	-3.01	34.09	N/A
Lymphocytes (10^3/μL)	0.6-5.7	2.6 ± 1.6	0.8-6.0	2.7 ± 1.7	0.80	N/A	N/A	179
H:L	0.8-4.8	2.2 ± 1.2	0.6-3.0	1.4 ± 0.7	0.02	N/A	N/A	90
Monocytes (10^3/μL)	0.0-0.7	0.2 ± 0.2	0.0-0.7	0.2 ± 0.2	0.94	N/A	N/A	167
Eosinophils (10^3/μL)	0.2-1.8	0.6 ± 0.4	0.0-1.4	0.1 ± 0.1	0.64	N/A	N/A	154
Basophils (10^3/μL)	0.0 – 0.4	0.1 ± 0.1	0.0-0.4	0.1 ± 0.1	0.03	N/A	N/A	241
Aspartate aminotransferase (U/L)	94.0-493.0	184.2 ± 100.8	92.0-317.0	185.8 ± 77.3	0.90	N/A	N/A	174.5
Creatinine kinase (U/L)	182.0-1464.0	527.7 ± 360.6	111.1-1040.0	455.1-302.9	0.40	N/A	N/A	142
Uric acid (μmol/L)	24.0-111.0	59.0 ± 24.5	5.0-51.0	20.4-11.5	<0.01	-6.28	27.85	N/A
Glucose (mmol/L)	3.5-6.3	4.7 ± 0.8	3.5-5.6	4.4 ± 0.5	0.11	-1.64	33.4	N/A
Calcium (mmol/L)	0.9-5.0	2.9 ± 0.8	0.5-5.0	2.7 ± 1.4	0.87	N/A	N/A	164
Phosphorus (mmol/L)	2.4-4.5	3.0 ± 0.5	2.1-7.0	3.3 ± 1.2	0.88	N/A	N/A	175.5
Total protein (g/L)	26.0-47.0	34.8 ± 6.3	33.0-78.0	46.0 ± 9.9	<0.01	N/A	N/A	292.5
Albumin (g/L)	10.0-25.0	15.4 ± 4.4	13.0-37.0	21.8 ± 5.1	<0.01	N/A	N/A	281
Globulin (g/L)	15.0-26.0	19.3 ± 3.4	19.0-40.0	24.3 ± 5.4	<0.01	N/A	N/A	278
Potassium (mmol/L)	5.0-8.2	6.4 ± 0.7	6.2-12.0	7.9 ± 1.9	<0.01	N/A	N/A	283
Sodium (mmol/L)	143.0-159.0	149.7 ± 4.5	142.0-160.0	149.2 ± 5.1	0.68	-0.3	32.26	N/A

6.4.2 Microbiota composition

16S rRNA gene sequence reads were obtained from 19 animals from Crab Island and from 11 animals from Port Hedland. In total, we identified 492 operational taxonomic units (OTUs) (Supplementary Table 1). Within the microbiota, Firmicutes was by far the most predominate phylum in animals from Crab Island, while Proteobacteria was the most commonly identified phylum in Port Hedland turtles, followed by Actinobacteria, Bacteroidetes and Firmicutes (Figure 6.1). Microbial diversity differed markedly among flatback turtle groups, with animals from Crab Island harboring a less diverse microbiota, as estimated by Shannon diversity (χ^2=14.05, df=1, p<0.01). However, no differences existed for Observed OTUs (df=1, f=2.38, SS_T=5701, p=0.13), or Chao1 estimates (df=1, f=0.5, SS_T=1577, p=0.49), between the two populations (Figure 6.2). Principal co-ordinate analysis of microbiota composition indicated that animals from Port Hedland clustered closely together, with similar overall composition, while animals from Crab Island had a more diverse pattern (Figure 6.3). The observed differences between populations were statistically significant (df=1, SS_T=2.11, f=5.6, R^2=0.138, p<0.01). Overall, 59 (37.1%) of the OTUs were shared between the two populations (Table 6.2). Ninety (56.6%), OTUs were unique to animals from Port Hedland, in comparison to 10 (6.3%) unique OTUs from animals from Crab Island (Table 6.2).

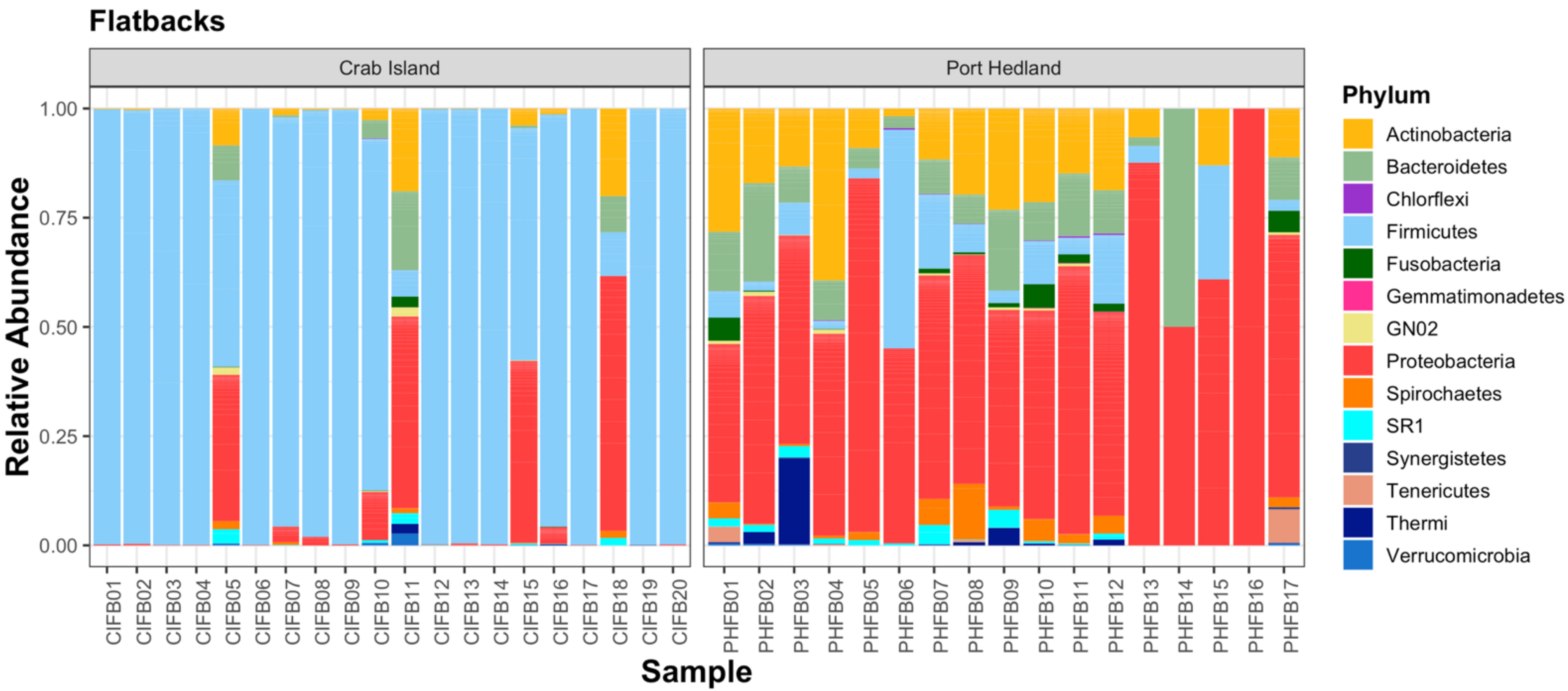


Figure 6.1 Relative abundance of the dominant bacterial phyla in flatback turtles from Crab Island, Queensland, and Port Hedland, Western Australia.

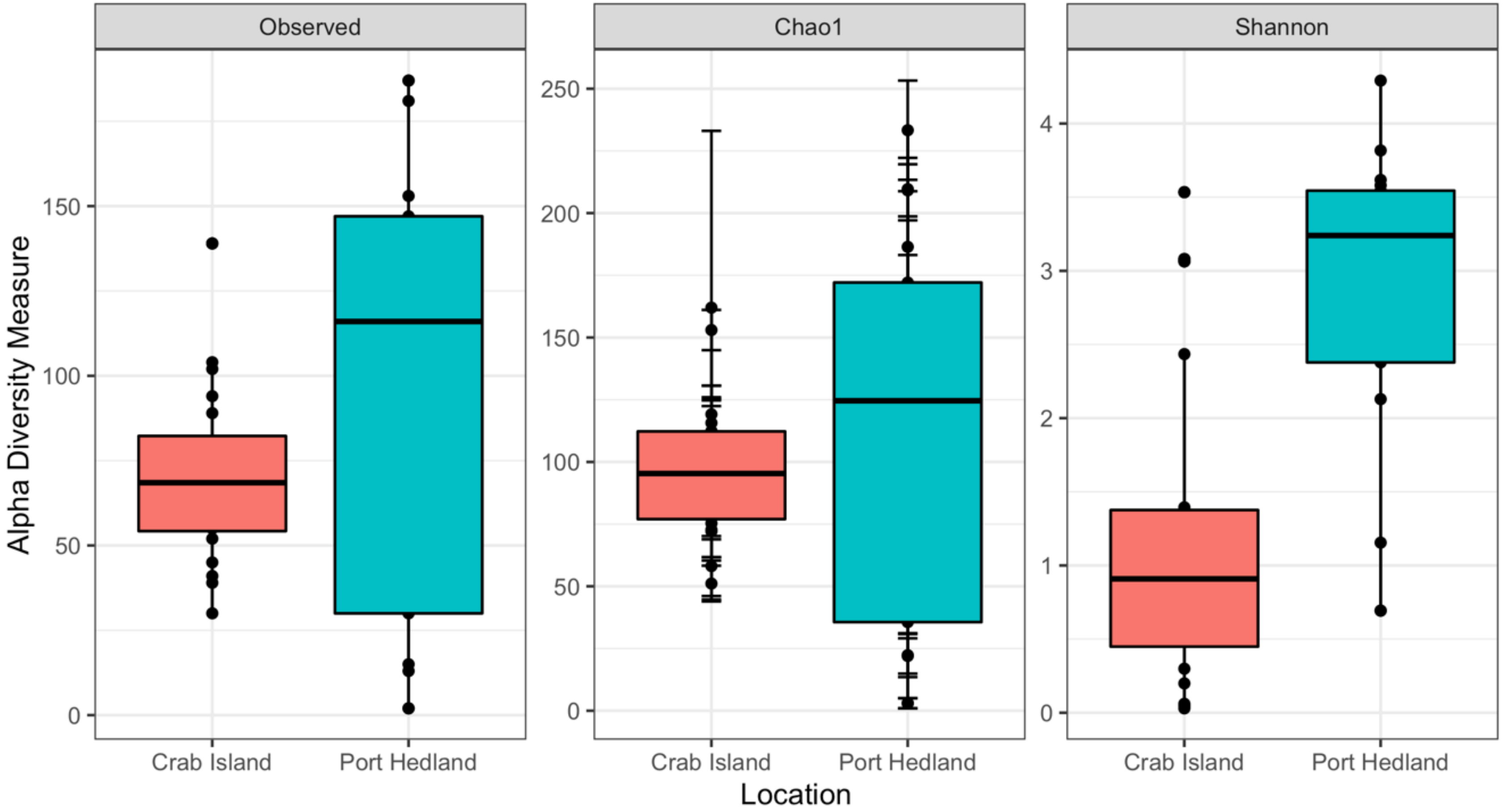


Figure 6.2 Alpha diversity estimates for flatback turtles from Crab Island, Queensland and Port Hedland, Western Australia. Individual points and brackets represent the richness estimate and the theoretical standard error range associated with that estimate, respectively. Within each panel, the samples are organized into location of capture, and a boxplot is overlaid on top of this for the two groups. No differences existed for Observed OTUs (p=0.13) or Chao1 estimates (p=0.49) between populations, however significant differences existed for Shannon diversity between the two populations with animals from Crab Island showing much lower microbial diversity (p<0.01).

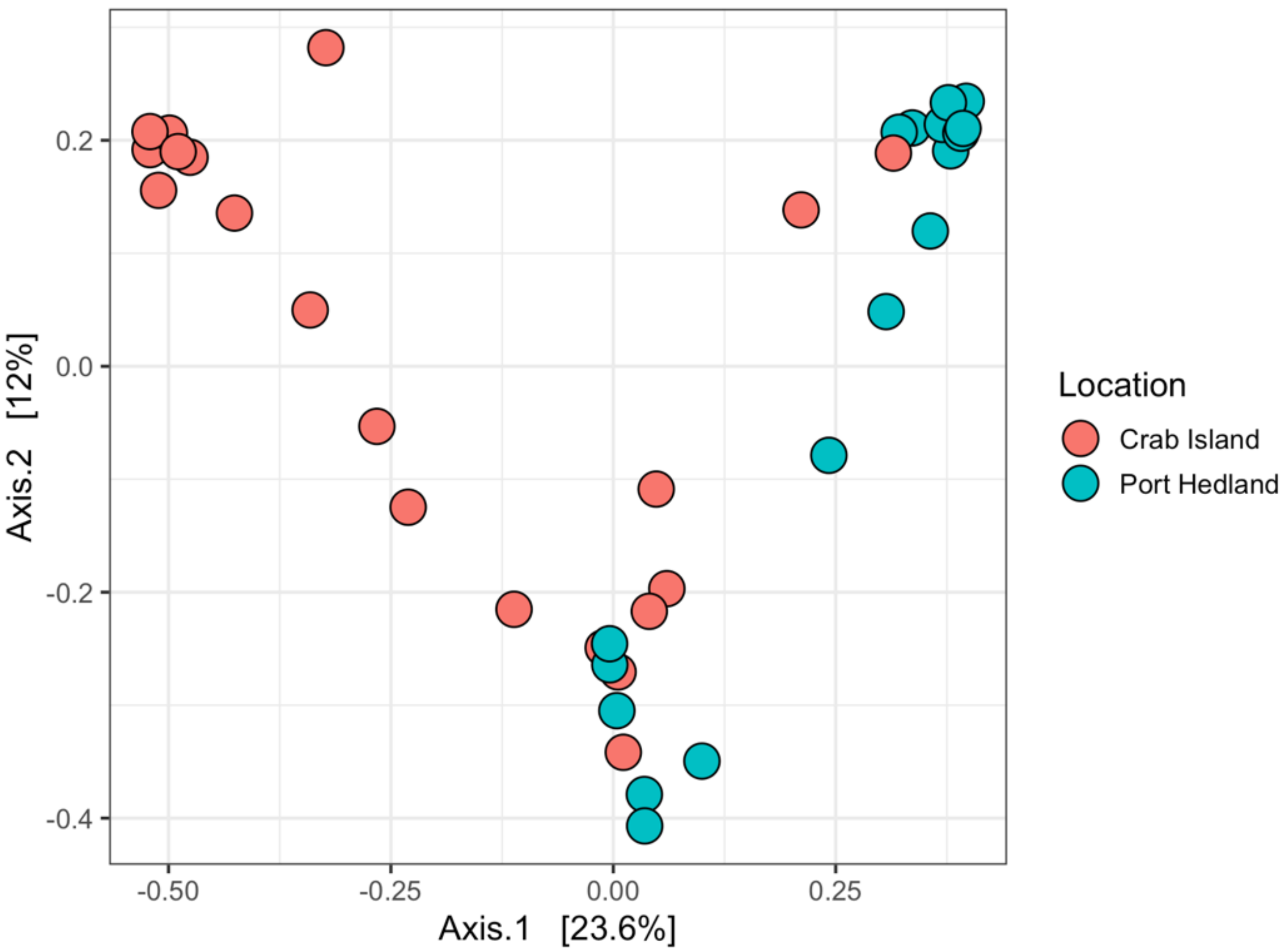


Figure 6.3 Principal coordinate analysis plot of Bray-Curtis distances for gut microbiota in flatback turtles from two locations, Crab Island, and Port Hedland. Each point represents the gut microbiota of an individual turtle. Observed differences between the two populations were significant (Adonis R^2=0.138, p<0.01).

Table 6.2 Shared and unique OTUs in samples from flatback turtles from Crab Island, Queensland and Port Hedland, Queensland.

Taxa	Type	Details	Crab Island abundance	Port Hedland abundance	Crab Island occurrence	Port Hedland occurrence
p__Verrucomicrobia__g__Verrucomicrobium_175087	Unique	Port Hedland	0.192	0.062	0.2	0.47
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	Unique	Port Hedland	0.123	1.709	0.1	0.59
p__SR1__c___571035	Unique	Port Hedland	0.169	1.002	0.3	0.71
p__Spirochaetes__g__Treponema_30617	Unique	Port Hedland	0.098	0.44	0.25	0.59
p__Spirochaetes__g__IE043_291307	Core	Crab Island, Port Hedland	0.09	1.876	0.4	0.59
p__Proteobacteria__o__Sphingomonadales_709703	Unique	Port Hedland	0	0.087	0	0.47
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	Unique	Port Hedland	0	2.571	0	0.59
p__Proteobacteria__g__Sulfurimonas_448949	Unique	Port Hedland	0.016	3.356	0.15	0.65
p__Proteobacteria__g__Sulfurimonas_444197	Core	Crab Island, Port Hedland	1.242	5.128	0.6	0.82
p__Proteobacteria__g__Sulfurimonas_412977	Unique	Port Hedland	0.004	0.021	0.15	0.41
p__Proteobacteria__g__Stenoxybacter_751919	Core	Crab Island, Port Hedland	1.171	3.227	0.85	0.82
p__Proteobacteria__g__Stenoxybacter_55150	Core	Crab Island, Port Hedland	0.208	0.695	0.65	0.76
p__Proteobacteria__g__Stenotrophomonas_727055	Unique	Port Hedland	0.01	0.249	0.2	0.65
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	Unique	Port Hedland	0.002	0.335	0.1	0.53
p__Proteobacteria__g__Rhodocyclus__s__Rhodocyclustenuis_740177	Core	Crab Island, Port Hedland	0.259	2.468	0.65	0.65
p__Proteobacteria__g__Ralstonia_478818	Unique	Port Hedland	0.167	0.604	0.3	0.76
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	Unique	Port Hedland	0.081	2.602	0.35	0.65
p__Proteobacteria__g__Pseudoruegeria_390046	Core	Crab Island, Port Hedland	0.476	0.592	0.5	0.65
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	Unique	Port Hedland	0.002	0.061	0.1	0.53
p__Proteobacteria__g__Moraxella_610605	Unique	Port Hedland	0.002	0.043	0.15	0.47
p__Proteobacteria__g__Moraxella_406471	Unique	Port Hedland	0.06	0.394	0.25	0.65
p__Proteobacteria__g__Methylarcula__s__Loktanellahongkongensis_46703	Unique	Port Hedland	0.09	0.392	0.1	0.71
p__Proteobacteria__g__Klebsiella_558424	Core	Crab Island, Port Hedland	2.204	0.022	0.7	0.47
p__Proteobacteria__g__Dichelobacter_361604	Core	Crab Island, Port Hedland	0.418	1.762	0.5	0.76
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	Core	Crab Island, Port Hedland	0.223	0.713	0.4	0.76

p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	Core	Crab Island, Port Hedland	0.124	2.802	0.6	0.82
p__Proteobacteria__g__Campylobacter__s__Campylobacterconcisus_141754	Unique	Port Hedland	0.012	0.319	0.2	0.76
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	Unique	Port Hedland	0.368	7.435	0.25	0.82
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	Unique	Port Hedland	0	0.015	0.05	0.41
p__Proteobacteria__g__Arcobacter_587211	Core	Crab Island, Port Hedland	0.286	2.652	0.55	0.82
p__Proteobacteria__g__Amaricoccus_603022	Unique	Port Hedland	0.001	0.175	0.05	0.59
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	Unique	Crab Island	0.745	0.269	0.6	0.06
p__Proteobacteria__g__Acinetobacter_78571	Core	Crab Island, Port Hedland	0.456	0.094	0.4	0.53
p__Proteobacteria__g__Achromobacter_19649	Unique	Port Hedland	0.006	0.246	0.15	0.41
p__Proteobacteria__f__SUP05_293055	Unique	Port Hedland	0.001	0.012	0.05	0.59
p__Proteobacteria__f__SUP05_285720	Core	Crab Island, Port Hedland	0.398	8.589	0.6	0.82
p__Proteobacteria__f__Rhodospirillaceae_494802	Unique	Port Hedland	0	0.178	0	0.53
p__Proteobacteria__f__Rhodobacteraceae_658260	Core	Crab Island, Port Hedland	0.188	0.278	0.5	0.47
p__Proteobacteria__f__Rhodobacteraceae_37016	Unique	Port Hedland	0	0.069	0	0.59
p__Proteobacteria__f__Rhodobacteraceae_160906	Unique	Port Hedland	0.015	0.066	0.1	0.53
p__Proteobacteria__f__Pseudomonadaceae_79058	Unique	Port Hedland	0.058	0.766	0.2	0.65
p__Proteobacteria__f__Pasteurellaceae_142268	Core	Crab Island, Port Hedland	0.16	1.805	0.5	0.76
p__Proteobacteria__f__Comamonadaceae_455592	Unique	Port Hedland	0.1	1.109	0.35	0.76
p__Proteobacteria__f__Campylobacteraceae_360616	Unique	Port Hedland	0.117	1.025	0.35	0.76
p__Proteobacteria__c__Deltaproteobacteria_23190	Unique	Port Hedland	0.019	1.015	0.2	0.53
p__GN02__c__VC12cl04_595855	Unique	Port Hedland	0.125	0.174	0.25	0.59
p__GN02__c__VC12cl04_222792	Unique	Port Hedland	0.094	0.17	0.15	0.47
p__Fusobacteria__g__Streptobacillus_719947	Unique	Port Hedland	0.129	1.294	0.2	0.59
p__Firmicutes__o__Clostridiales_70341	Unique	Port Hedland	0.016	0.318	0.25	0.47
p__Firmicutes__o__Clostridiales_701726	Unique	Port Hedland	0.012	0.549	0.2	0.53
p__Firmicutes__o__Clostridiales_635308	Unique	Port Hedland	0.004	0.186	0.1	0.41
p__Firmicutes__o__Clostridiales_376161	Unique	Port Hedland	0.058	2.574	0.25	0.59
p__Firmicutes__o__Clostridiales_226726	Core	Crab Island, Port Hedland	0.258	0.553	0.5	0.65
p__Firmicutes__g__Streptococcus_269499	Unique	Port Hedland	0.004	0.49	0.15	0.76

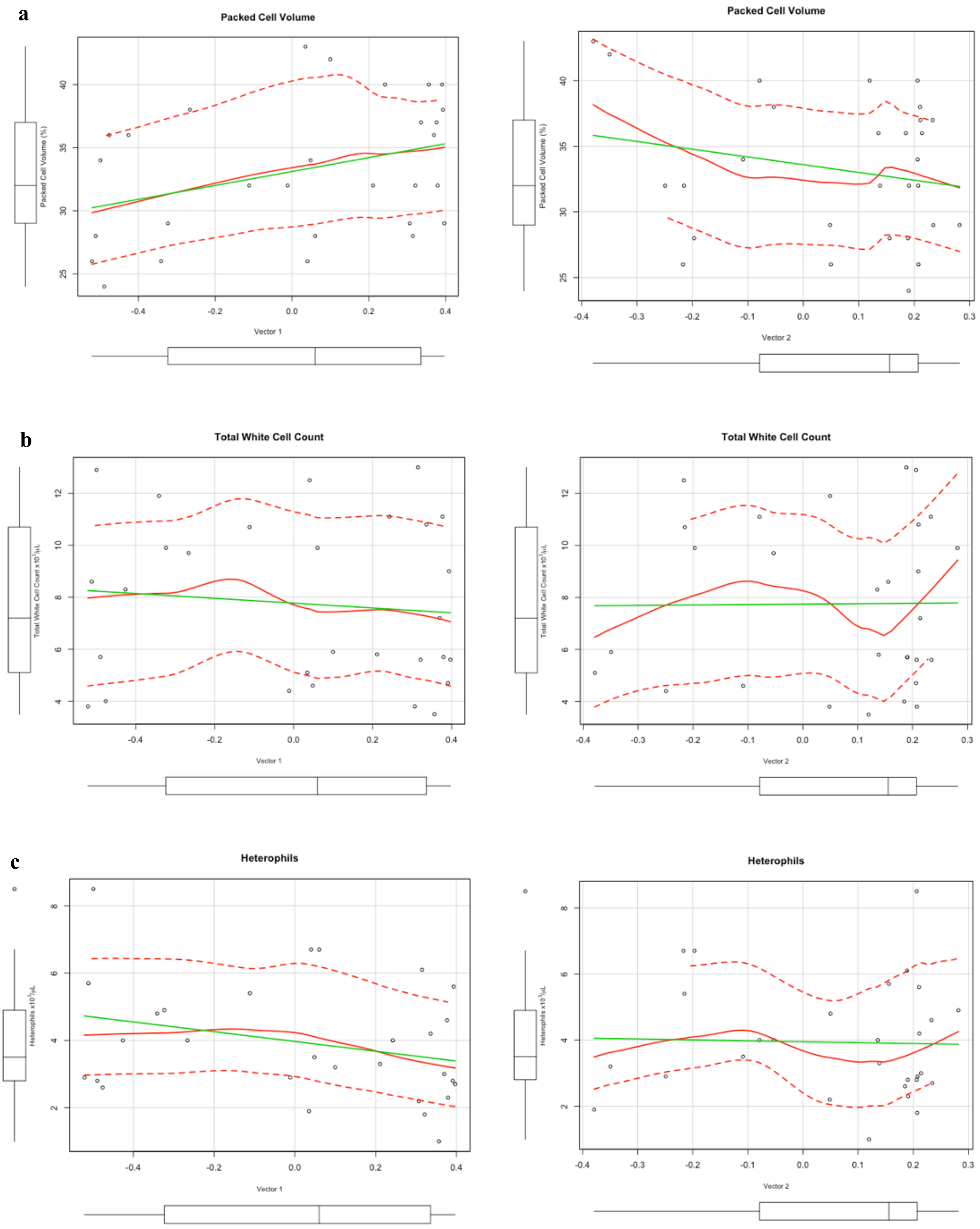
p__Firmicutes__g__Staphylococcus_171970	Unique	Port Hedland	0.007	0.435	0.15	0.71
p__Firmicutes__g__Clostridium_482667	Unique	Port Hedland	0.005	0.047	0.05	0.59
p__Firmicutes__g__Bacillus__s__Bacillussp.NRRLB14911_432592	Core	Crab Island, Port Hedland	0.382	0.088	0.4	0.53
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	Unique	Crab Island	9.646	0.012	0.7	0.12
p__Firmicutes__g__Bacillus__s__Bacillusamyloliquefaciens_585304	Unique	Crab Island	1.004	0.008	0.6	0.29
p__Firmicutes__g__Bacillus_535651	Unique	Crab Island	12.042	0.022	0.75	0.12
p__Firmicutes__g__Bacillus_207834	Unique	Crab Island	9.716	0.094	0.65	0.24
p__Firmicutes__g__Bacillus_161559	Core	Crab Island, Port Hedland	36.704	0.648	1	0.71
p__Firmicutes__f__Planococcaceae_668479	Unique	Crab Island	0.71	0.001	0.7	0.06
p__Firmicutes__f__Bacillaceae_724791	Unique	Crab Island	2.486	0.012	0.5	0.29
p__Firmicutes__f__Bacillaceae_664374	Core	Crab Island, Port Hedland	2.828	0.197	0.75	0.47
p__Chloroflexi__c__SOGA31_314194	Unique	Port Hedland	0	0.055	0	0.47
p__Bacteroidetes__o__Bacteroidales_711203	Unique	Port Hedland	0.038	0.158	0.2	0.59
p__Bacteroidetes__g__Zhouia_422137	Unique	Port Hedland	0.197	0.26	0.3	0.59
p__Bacteroidetes__g__Tenacibaculum__s__Tenacibaculumlutimaris_437286	Unique	Port Hedland	0.029	0.17	0.15	0.47
p__Bacteroidetes__g__Tenacibaculum_474405	Unique	Port Hedland	0.086	0.228	0.15	0.71
p__Bacteroidetes__g__Tenacibaculum_428560	Unique	Port Hedland	0.092	0.169	0.25	0.59
p__Bacteroidetes__g__Pontibacter__s__Pontibacterkorlensis_406748	Unique	Port Hedland	0	0.382	0	0.65
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	Unique	Port Hedland	0.036	0.089	0.1	0.53
p__Bacteroidetes__g__Dysgonomonas_475976	Unique	Port Hedland	0.004	0.538	0.1	0.53
p__Bacteroidetes__f__Porphyromonadaceae_463755	Unique	Port Hedland	0.023	0.631	0.3	0.47
p__Bacteroidetes__f__Flavobacteriaceae_729660	Core	Crab Island, Port Hedland	0.311	4.421	0.4	0.76
p__Bacteroidetes__f__Flavobacteriaceae_379313	Core	Crab Island, Port Hedland	0.82	2.776	0.45	0.71
p__Bacteroidetes__f__Flavobacteriaceae_332081	Unique	Port Hedland	0.242	0.182	0.3	0.71
p__Bacteroidetes__f__Flavobacteriaceae_12392	Core	Crab Island, Port Hedland	0.124	0.373	0.45	0.71
p__Actinobacteria__o__koll13_76572	Unique	Port Hedland	0.004	0.104	0.15	0.59
p__Actinobacteria__o__Actinomycetales_369980	Unique	Port Hedland	0.034	0.438	0.25	0.65
p__Actinobacteria__o__Acidimicrobiales_244996	Unique	Port Hedland	0.005	0.125	0.2	0.47
p__Actinobacteria__o__03197L14_629656	Unique	Port Hedland	0	0.178	0.05	0.41

p__Actinobacteria__o__03197L14_176541	Unique	Port Hedland	0.174	0.259	0.2	0.53
p__Actinobacteria__o__03197L14_108564	Core	Crab Island, Port Hedland	0.654	6.544	0.7	0.82
p__Actinobacteria__g__Streptomyces_420197	Unique	Port Hedland	0.376	0.042	0.2	0.41
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	Unique	Port Hedland	0.115	0.258	0.25	0.65
p__Actinobacteria__g__Propionibacterium_427206	Core	Crab Island, Port Hedland	0.578	2.177	0.6	0.82
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	Unique	Port Hedland	0.086	0.085	0.15	0.59
p__Actinobacteria__g__Euzebia_707680	Core	Crab Island, Port Hedland	0.122	0.968	0.6	0.71
p__Actinobacteria__g__Arthrobacter_694831	Core	Crab Island, Port Hedland	0.106	0.695	0.65	0.71
p__Actinobacteria__g__Aeromicrobium_619093	Unique	Port Hedland	0.01	0.018	0.2	0.41
p__Actinobacteria__g__Actinomyces__s__Actinomyceseuropaeus_648304	Core	Crab Island, Port Hedland	0.118	1.824	0.5	0.65
p__Actinobacteria__g__Actinomyces_119009	Unique	Port Hedland	0.008	0.181	0.25	0.47
p__Actinobacteria__f__Nocardioidaceae_632222	Unique	Port Hedland	0.008	0.072	0.15	0.47
p__Actinobacteria__f__AKIW874_693966	Unique	Port Hedland	0.083	0.138	0.05	0.53

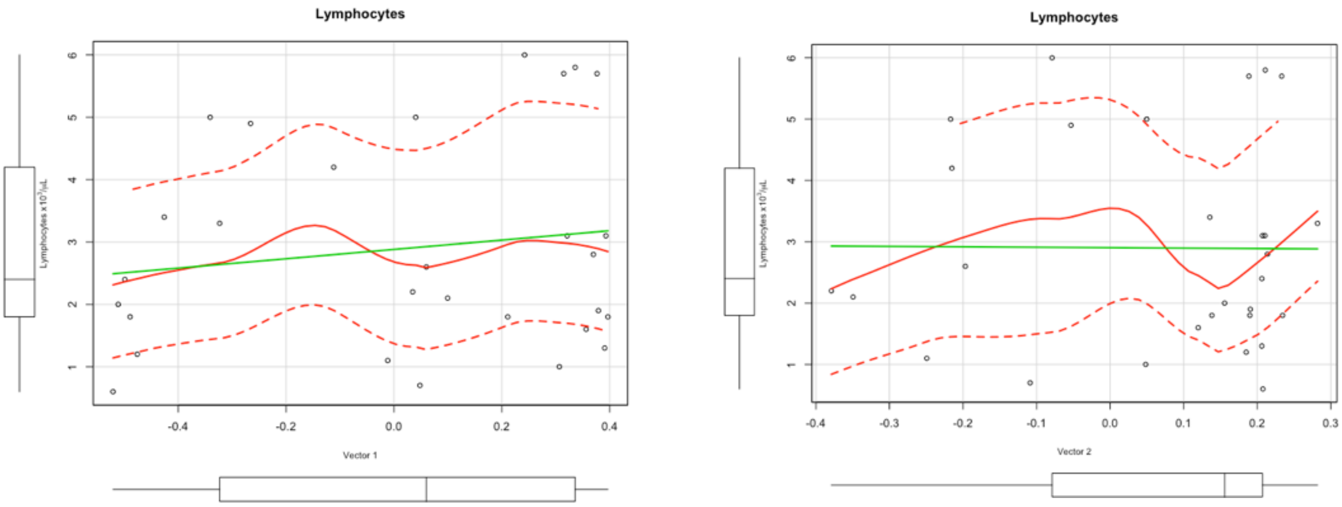
6.4.3 *Correlation of blood and microbiota results*

No correlation was found between microbiota composition and TWBC (V1 p=0.09, V2=0.19), lymphocytes (V1 p=0.32, V2 p=0.88), monocytes (V1=0.2, V2=0.8), eosinophils (V1 p=0.51, V2 p=0.68), aspartate aminotransferase (V1 p=0.59, V2 p=0.98), creatinine kinase (V1 p= 0.54, V2 p=0.63), glucose (V1 p=0.36, V2 p=0.27), phosphate (V1 p=0.12, V2 p=0.18), and sodium (V1 p=0.99, V2 p=0.94) (Figure 6.4, Table 6.3).

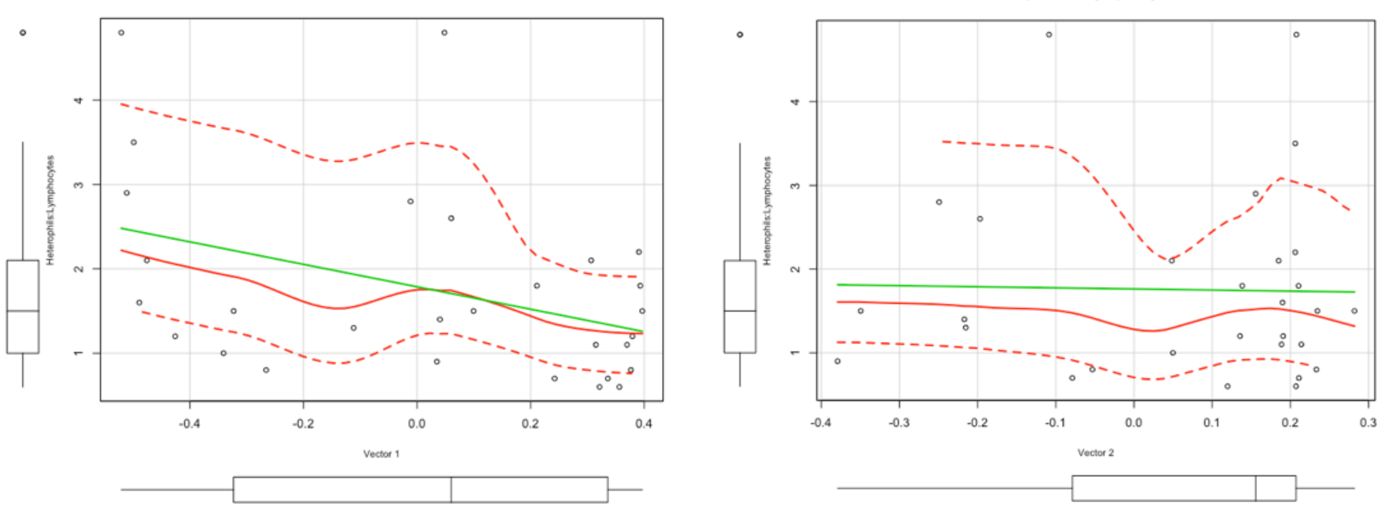
A correlation was discovered between microbiota composition and a number of analytes in animals from Port Hedland. These included PCV (V1 p<0.01, V2 p<0.01), heterophils (V1 p=0.02, V2 p=0.01), uric acid (V1 p=0.01, V2 p<0.01), calcium (V1 p=0.01, V2 p=0.5), total protein (V1 p<0.01 V2 p<0.01), albumin (V1 p<0.01, V2 p<0.00), globulin (V1 p=0.01, V2 p<0.01), and potassium (V1 p<0.01, V2 p<0.01) (Figure 6.5, Table 6.3). For these interactions, a decrease in the analyte was associated with a decrease in the OTUs 668479 (p_Firmicutes, f_*Planococcaceae*), 161559 (p_Firmicutes, g_*Bacillus*), and 746934 (p_Firmicutes, *Paenibacillus paenibacilluschondroitinus*) (Table 6.4), while an increase in analyte values was associated with an increase in 104 different OTUs, comprising all identified phyla except of Acidobacteria, Chlamydiae, Synergistetes, and TM7 (Table 6.4). Although a correlation was seen in the heterophil/lymphocyte ratio (V1 p=0.42, V2 p=0.02), and basophils (V1 p=0.13, V2 p=0.02), in Port Hedland animals, no OTUs could be identified to be significantly contributing to these observations based on our correlation standards of >0.4 and <-0.4 (Figure 6.5, Table 6.4).


a
Packed Cell Volume
Packed Cell Volume (%)
25
30
35
40
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Packed Cell Volume
Packed Cell Volume (%)
25
30
35
40
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2
b
Total White Cell Count
Total White Cell Count x10³/μL
4
6
8
10
12
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Total White Cell Count
Total White Cell Count x10³/μL
4
6
8
10
12
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2
c
Heterophils
Heterophils x10³/μL
2
4
6
8
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Heterophils
Heterophils x10³/μL
2
4
6
8
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

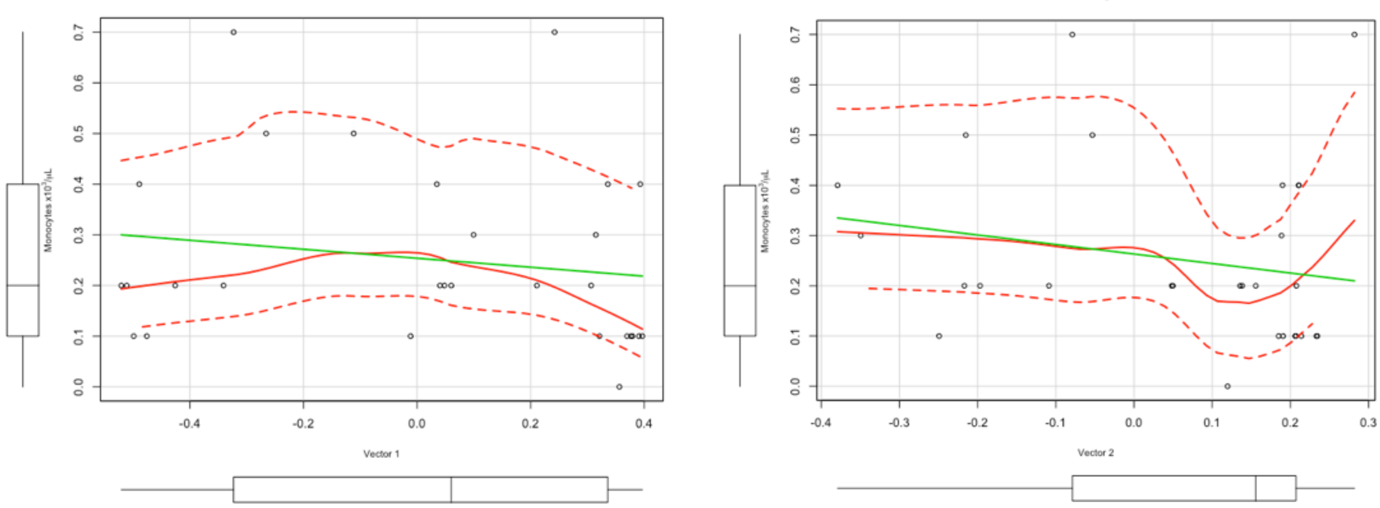
d


Lymphocytes
Lymphocytes x10³/µL
Vector 1
-0.4
-0.2
0.0
0.2
0.4
1
2
3
4
5
6
Lymphocytes
Lymphocytes x10³/µL
Vector 2
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

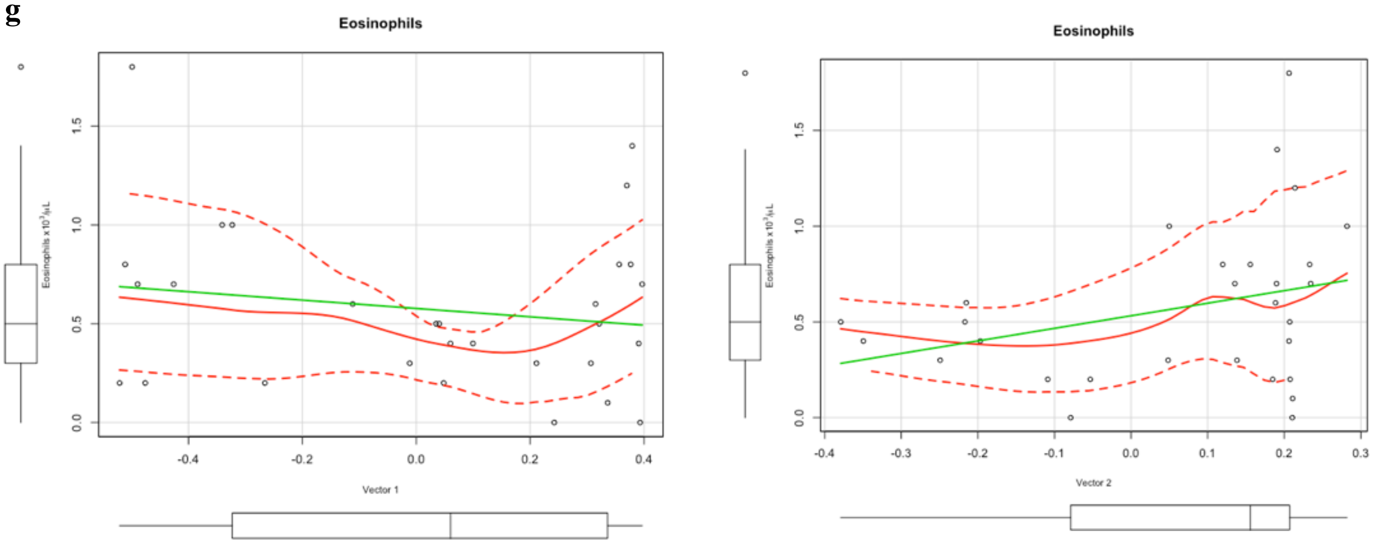
e


Heterophil and Lymphocyte Ratio
Heterophils:Lymphocytes
Vector 1
-0.4
-0.2
0.0
0.2
0.4
1
2
3
4
Heterophil and Lymphocyte Ratio
Heterophils:Lymphocytes
Vector 2
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

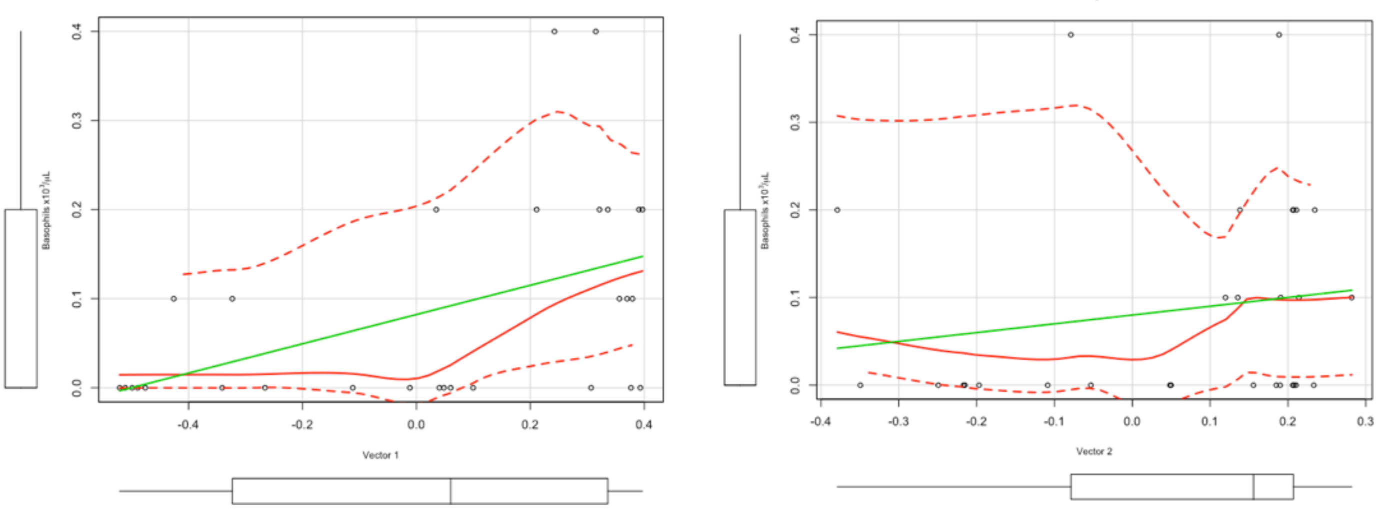
f


Monocytes
Monocytes x10³/µL
Vector 1
-0.4
-0.2
0.0
0.2
0.4
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Monocytes
Monocytes x10³/µL
Vector 2
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

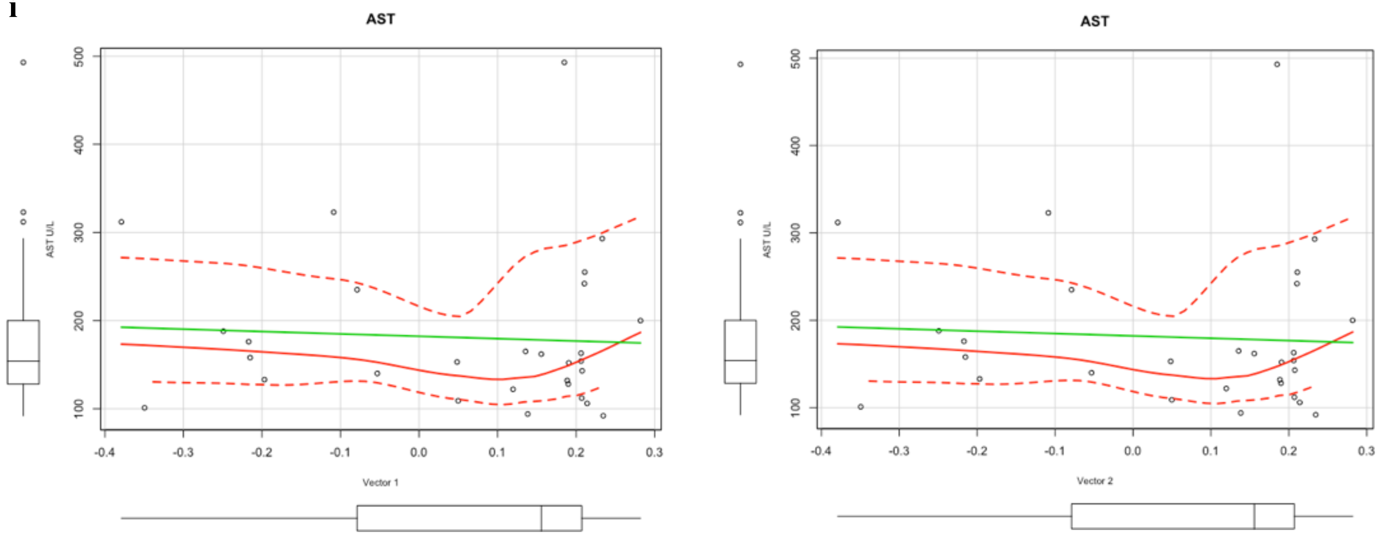
g


Eosinophils
Eosinophils x10³/µL
0.0
0.5
1.0
1.5
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Eosinophils
Eosinophils x10³/µL
0.0
0.5
1.0
1.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

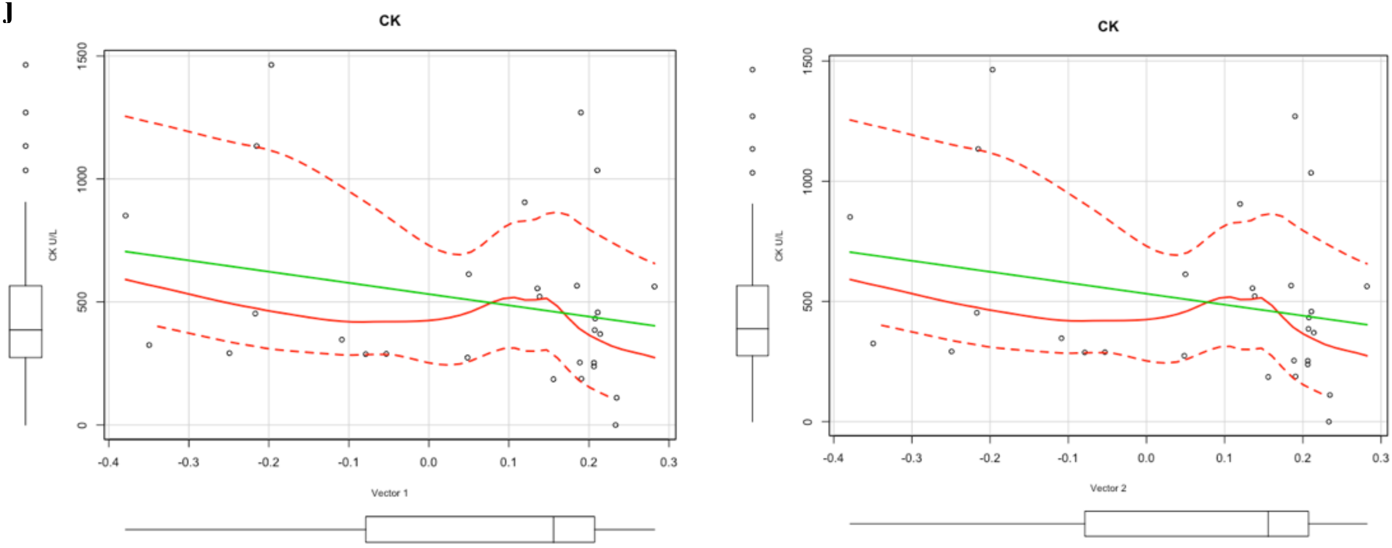
h


Basophils
Basophils x10³/µL
0.0
0.1
0.2
0.3
0.4
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Basophils
Basophils x10³/µL
0.0
0.1
0.2
0.3
0.4
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

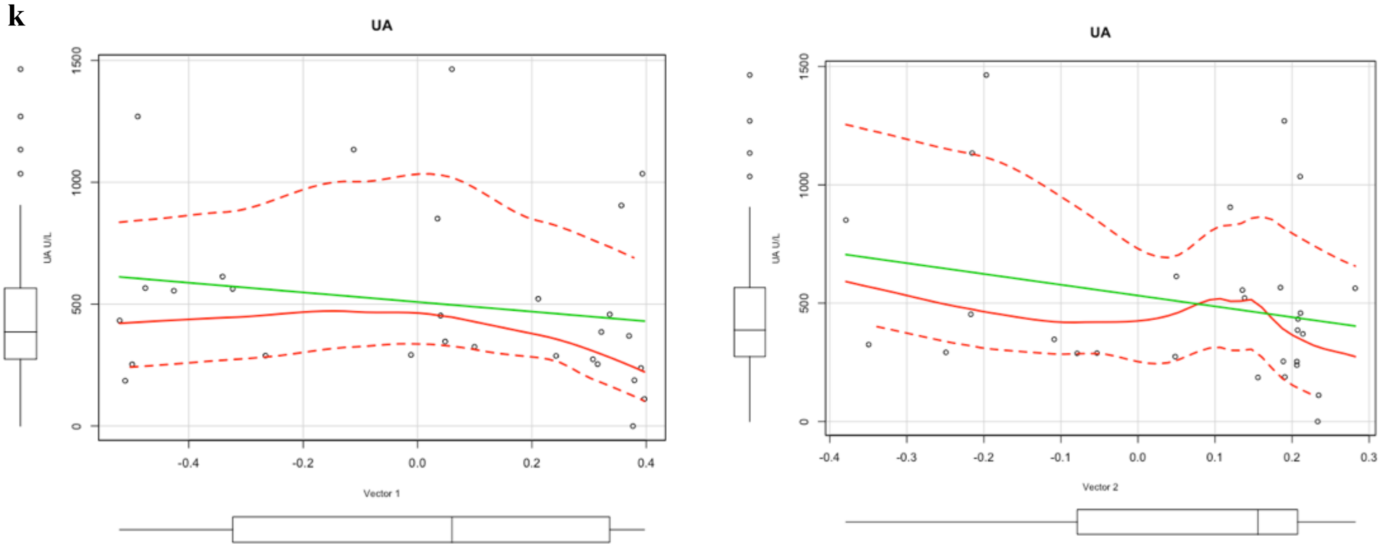
i


AST
AST U/L
100
200
300
400
500
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 1
AST
AST U/L
100
200
300
400
500
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

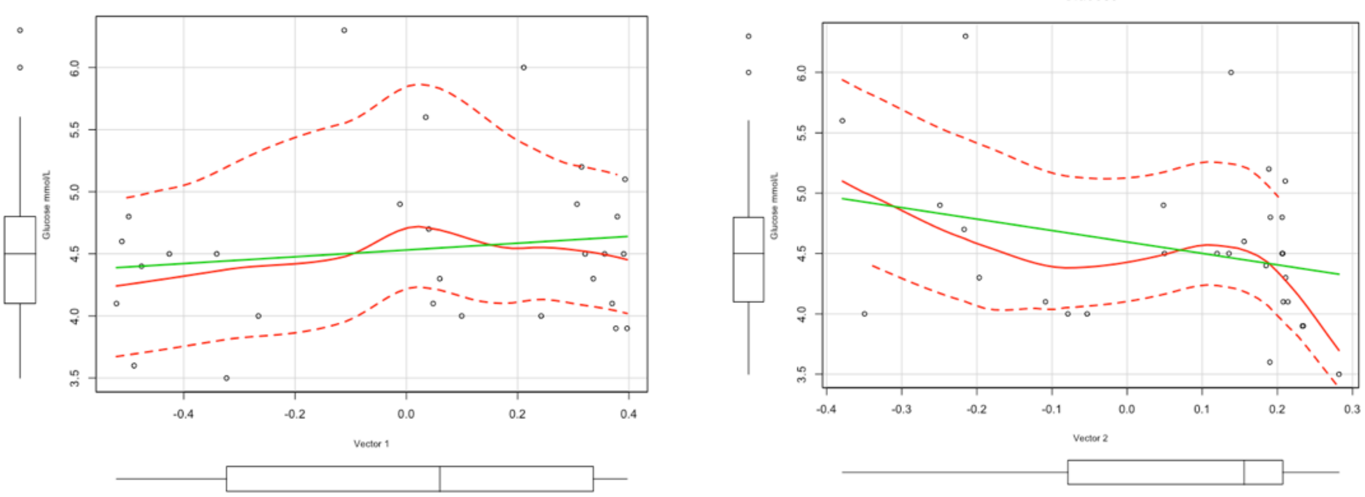
j


CK
1500
1000
500
0
CK U/L
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 1
CK
1500
1000
500
0
CK U/L
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

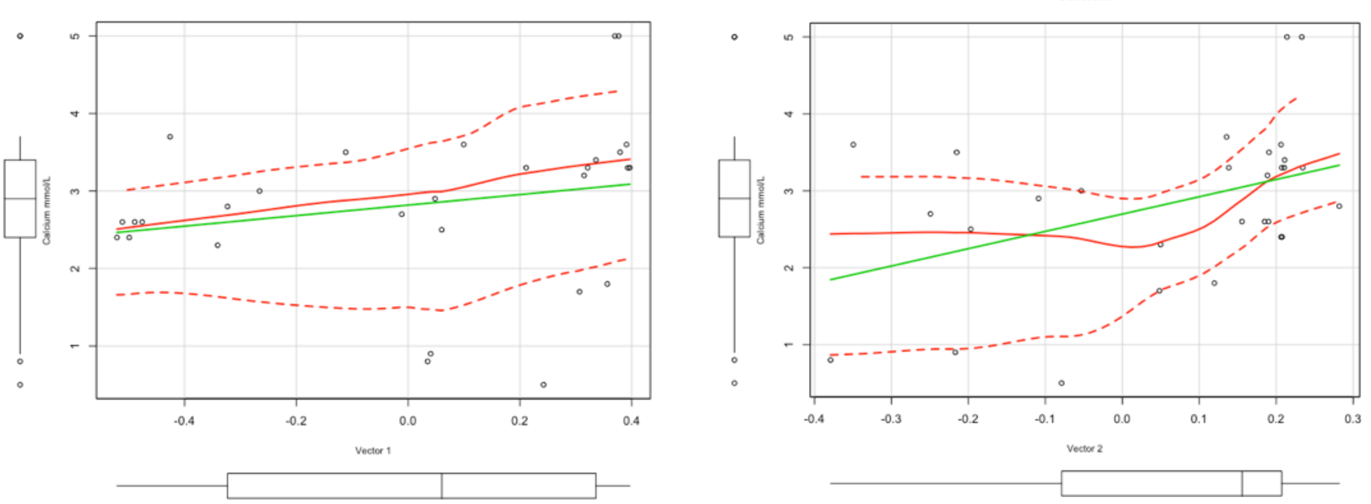
k


UA
1500
1000
500
0
UA U/L
-0.4
-0.2
0.0
0.2
0.4
Vector 1
UA
1500
1000
500
0
UA U/L
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

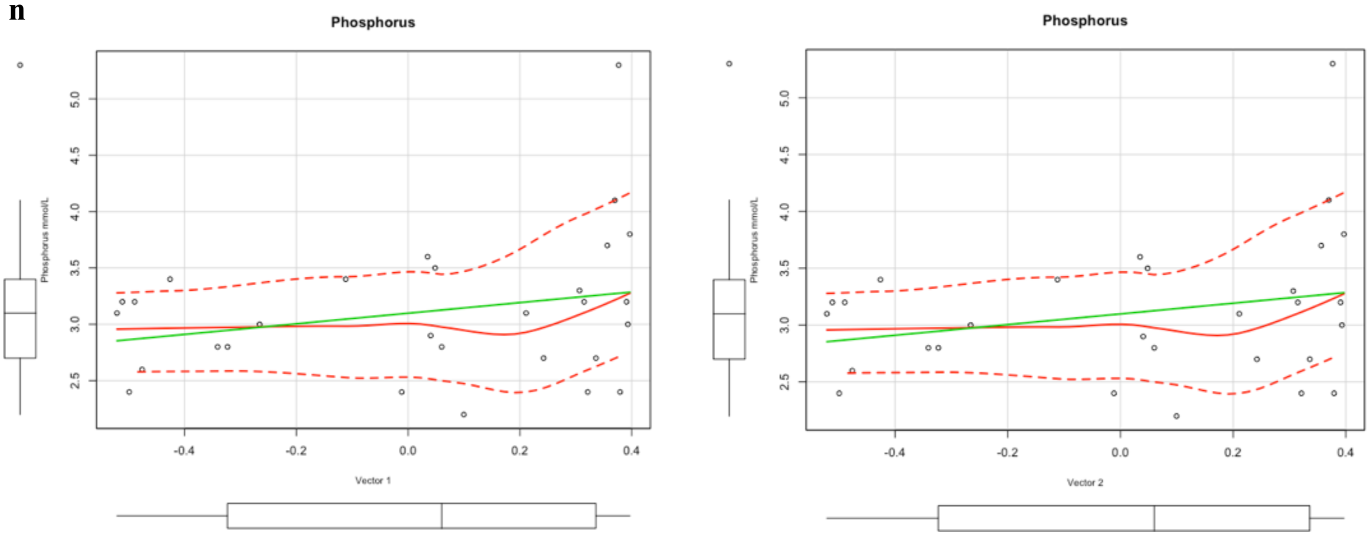
l


Glucose
6.0
5.5
5.0
4.5
4.0
3.5
Glucose mmol/L
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Glucose
6.0
5.5
5.0
4.5
4.0
3.5
Glucose mmol/L
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

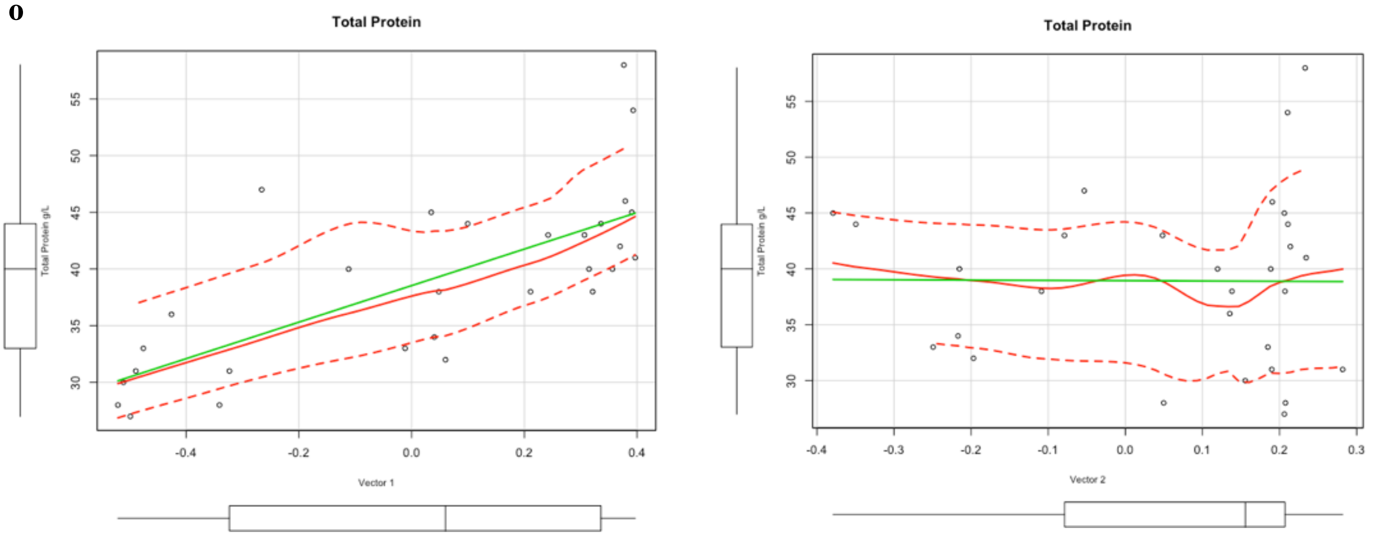
m


Calcium
Calcium
Calcium mmol/L
Calcium mmol/L
Vector 1
Vector 2
5
4
3
2
1
-0.4
-0.2
0.0
0.2
0.4
-0.3
-0.1
0.1
0.3

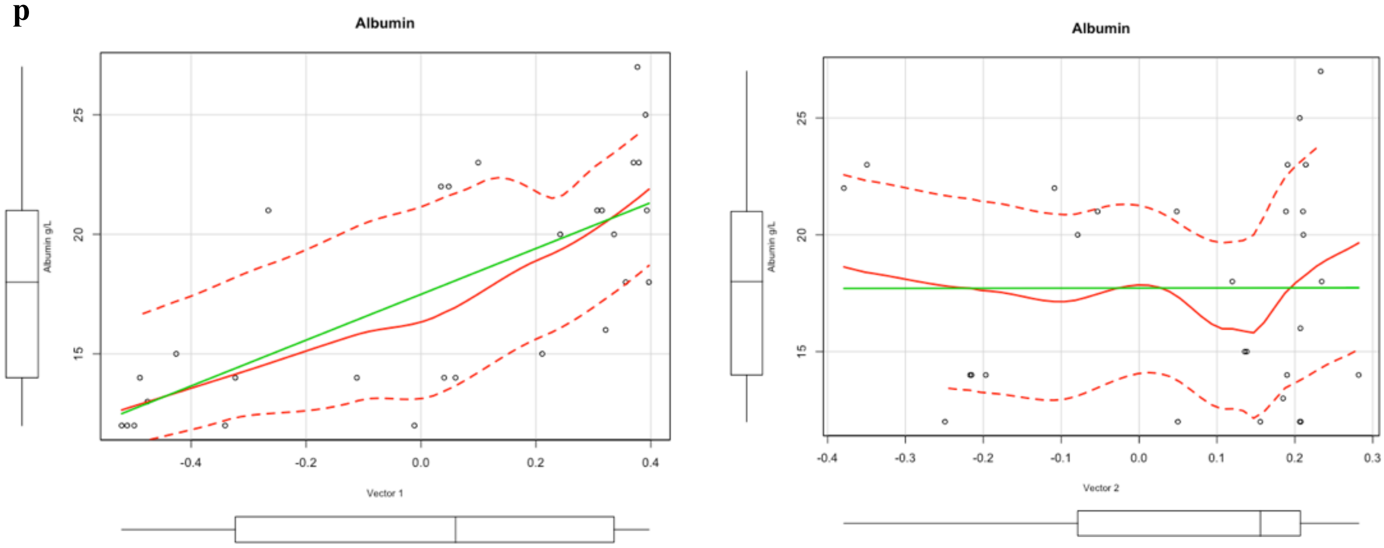
n


Phosphorus
Phosphorus
Phosphorus mmol/L
Phosphorus mmol/L
Vector 1
Vector 2
5.0
4.5
4.0
3.5
3.0
2.5
-0.4
-0.2
0.0
0.2
0.4

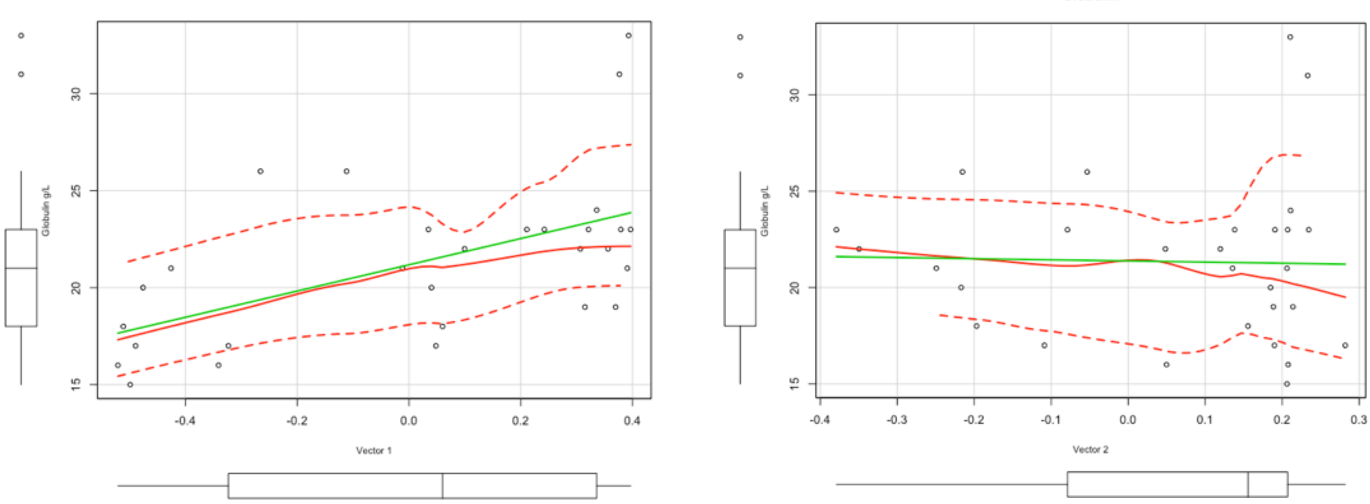
o


Total Protein
Total Protein
Total Protein g/L
Total Protein g/L
Vector 1
Vector 2
55
50
45
40
35
30
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4

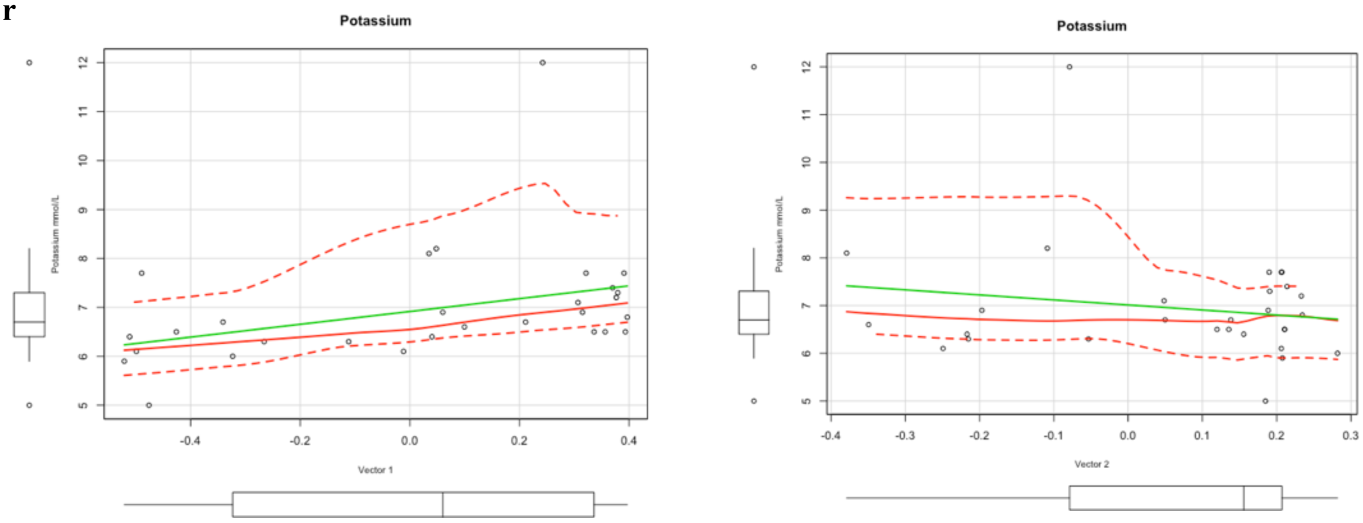
p


Albumin
Albumin g/L
25
20
15
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Albumin
Albumin g/L
25
20
15
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

q


Globulin
Globulin g/L
30
25
20
15
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Globulin
Globulin g/L
30
25
20
15
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

r


Potassium
Potassium mmol/L
12
11
10
9
8
7
6
5
-0.4
-0.2
0.0
0.2
0.4
Vector 1
Potassium
Potassium mmol/L
12
11
10
9
8
7
6
5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Vector 2

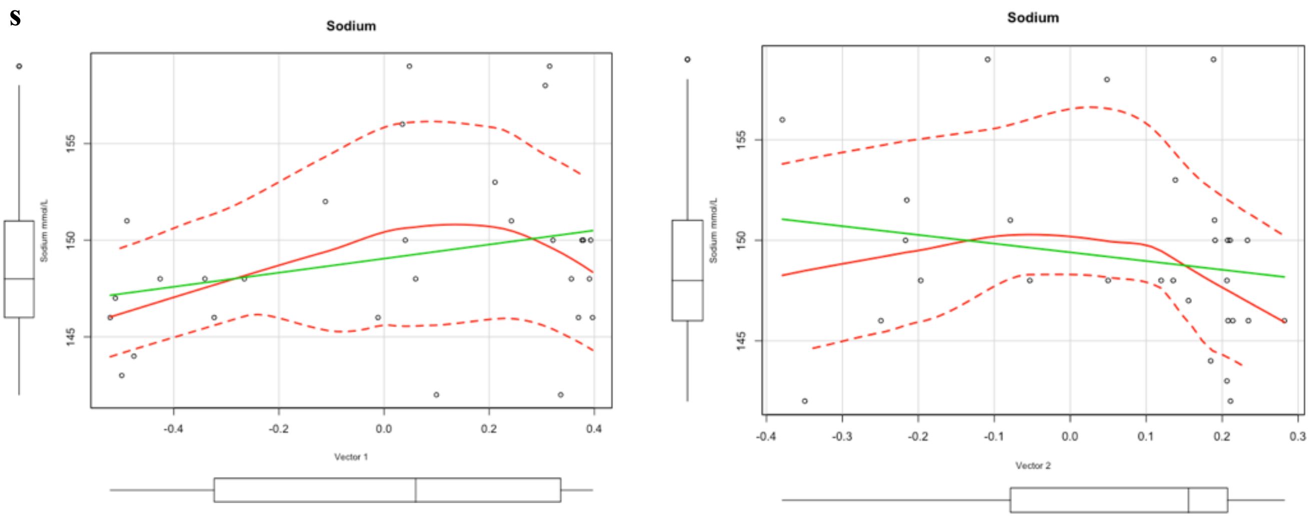
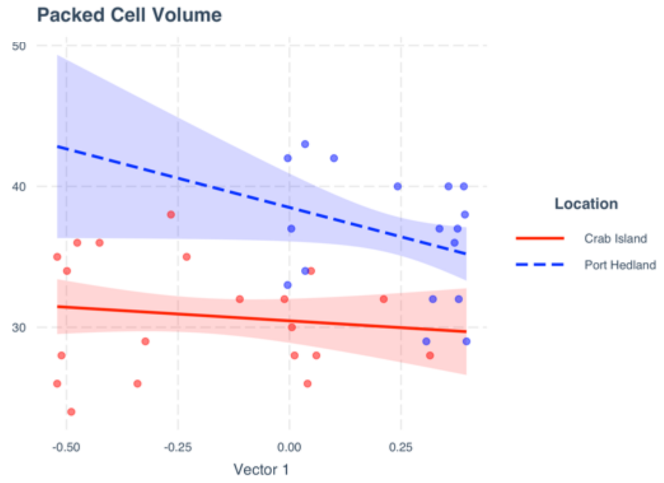
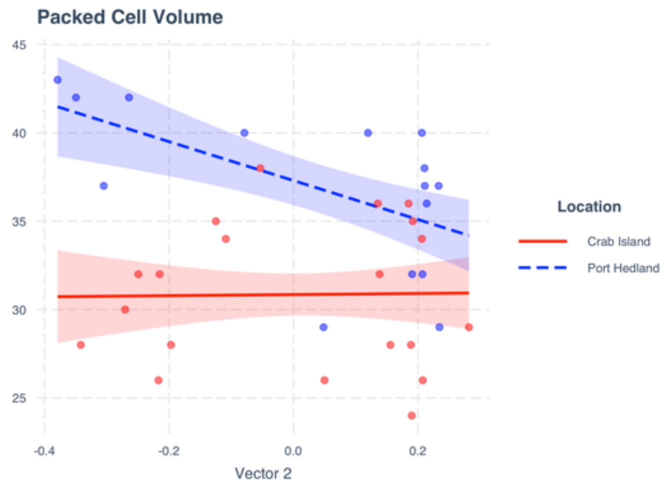


Figure 6.4 Graphical representations of correlations between microbiota composition and blood analytes. Scatter plots indicating significant relationships between microbiota composition according to location include; **(a)** packed cell volume, **(c)** heterophils, **(h)** basophils, **(k)** uric acid, **(o)** total protein, **(p)** albumin, **(q)** globulins and **(r)** potassium numbers.

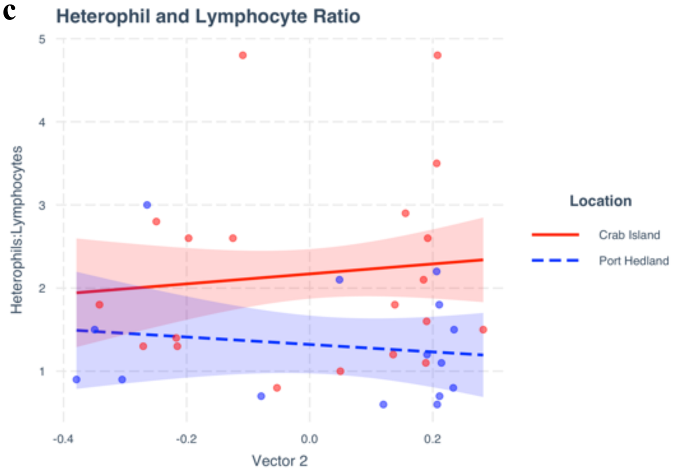
a


Packed Cell Volume
50
40
30
Packed Cell Volume (%)
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland

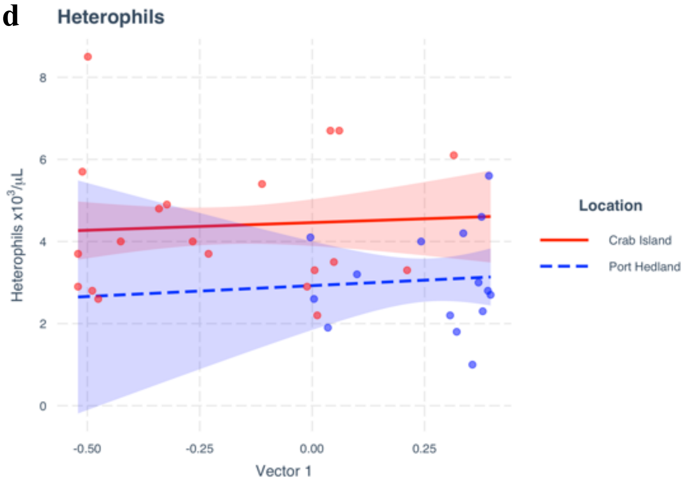
b


Packed Cell Volume
45
40
35
30
25
Packed Cell Volume (%)
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

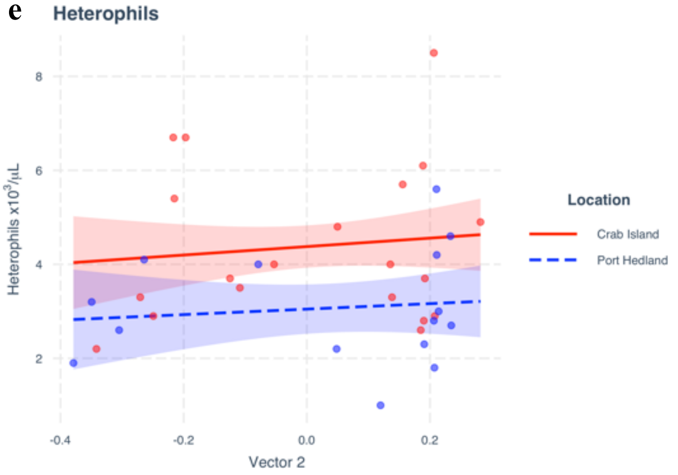
c


Heterophil and Lymphocyte Ratio
5
4
3
2
1
Heterophils:Lymphocytes
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

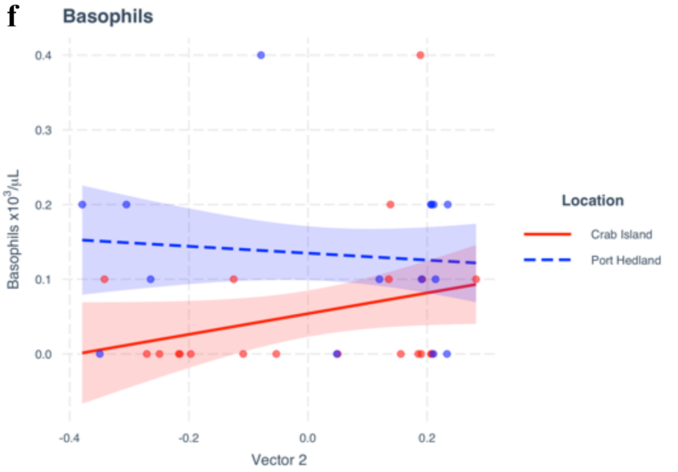
d


Heterophils
8
6
4
2
0
Heterophils x10^3/μL
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland

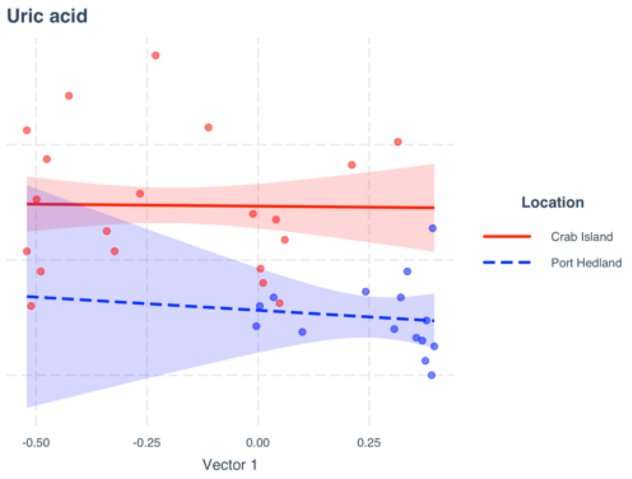
e


Heterophils
8
6
4
2
Heterophils x10^3/μL
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

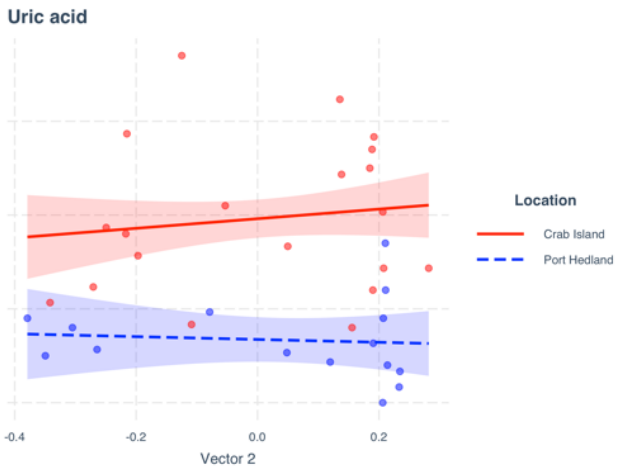
f


Basophils
0.4
0.3
0.2
0.1
0.0
Basophils x10^3/μL
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

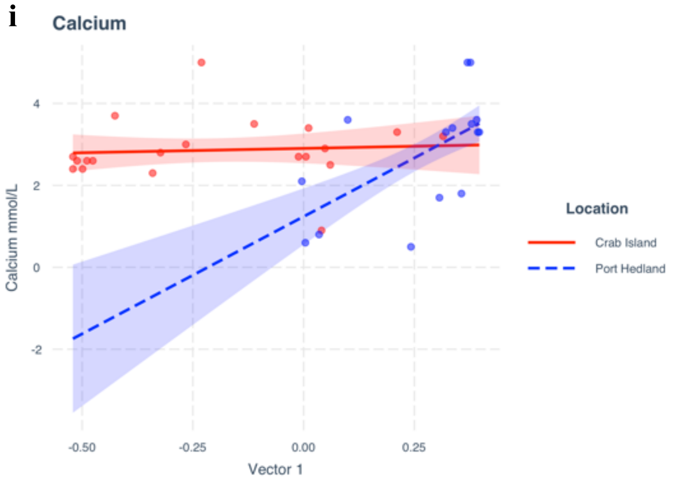
g


Uric acid
Uric acid U/L
80
40
0
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland

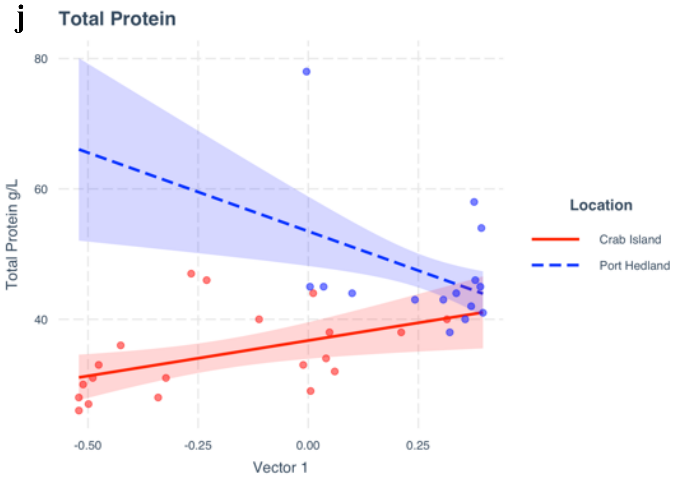
h


Uric acid
Uric acid U/L
90
60
30
0
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

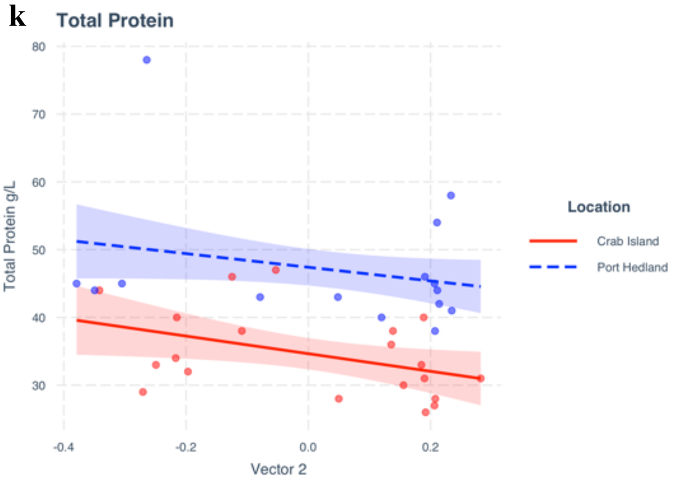
i


Calcium
Calcium mmol/L
4
2
0
-2
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland

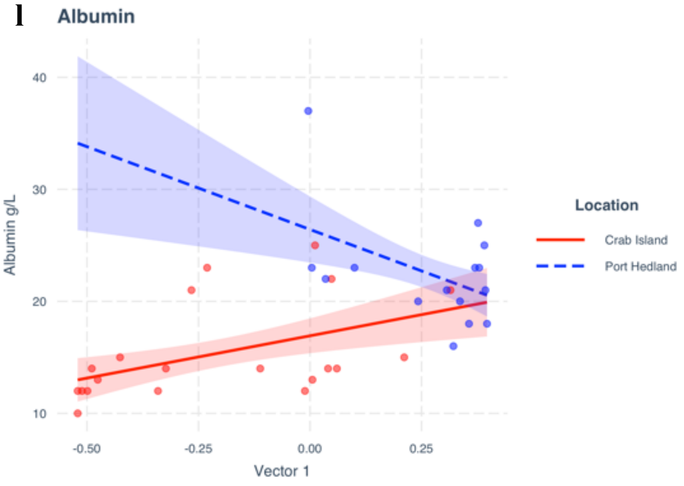
j

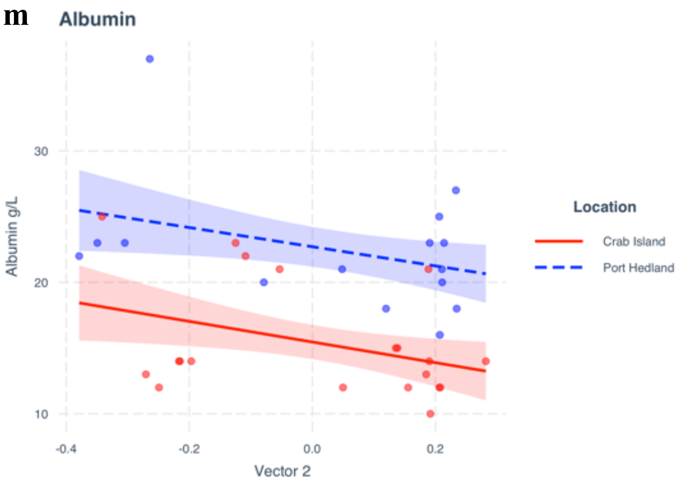

Total Protein
Total Protein g/L
80
60
40
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland

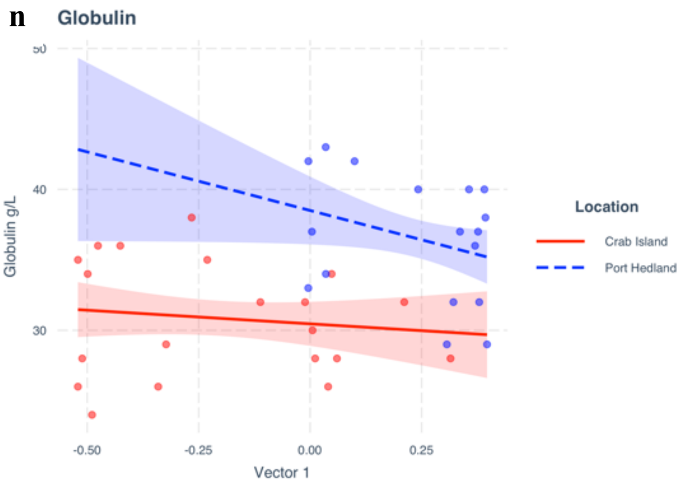
k

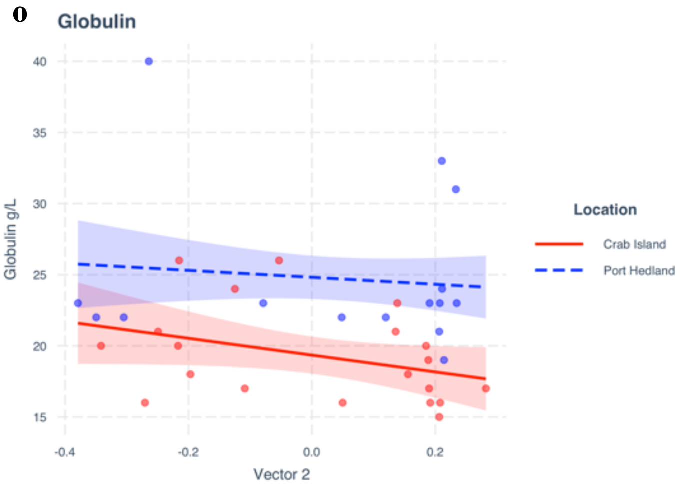

Total Protein
Total Protein g/L
80
70
60
50
40
30
-0.4
-0.2
0.0
0.2
Vector 2
Location
Crab Island
Port Hedland

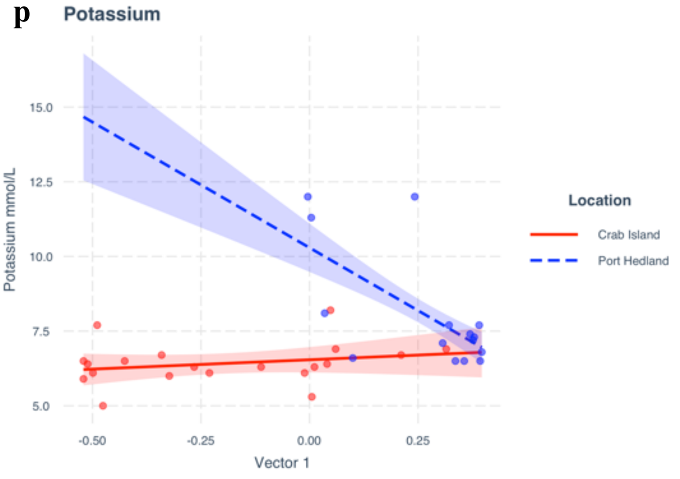
l


Albumin
Albumin g/L
40
30
20
10
-0.50
-0.25
0.00
0.25
Vector 1
Location
Crab Island
Port Hedland









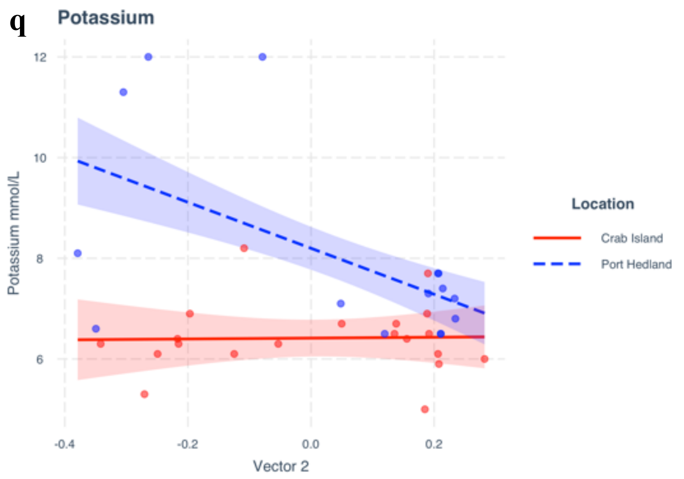


Figure 6.5 Interaction plots exploring the role that microbiota composition based on location has on (a-b) PCV, (c) heterophil/lymphocyte ratio, (d-e) heterophils, (f) basophils, (g-h) uric acid, (i) calcium, (j-k) total protein, (l-m) albumin, (n-o) globulin, and (p-q) potassium numbers. Note that all linear models where either a significant main effect or interaction term have been presented for completeness. Dots represent actual values and lines represent predicted values based on the model. Values less than zero are not biologically possible for the analytes measured in this investigation. Vectors represents the PCoA coordinates.

Table 6.3 Correlation matrix for OTUs and PCoA vectors. OTUs were considered to have a positive correlation if values were > 0.4 and a negative correlation if values were < -0.4.

Taxonomy	V1	V2
p__Actinobacteria__o__03197L14_108564	0.78	-0.05
p__Actinobacteria__g__Euzebia_707680	0.71	-0.04
p__Proteobacteria__f__Pasteurellaceae_142268	0.71	-0.03
p__Proteobacteria__f__SUP05_285720	0.71	-0.1
p__Proteobacteria__g__Arcobacter_587211	0.7	0.01
p__Proteobacteria__f__Campylobacteraceae_360616	0.69	0.05
p__Actinobacteria__g__Actinomyces__s__Actinomyceseuropaeus_648304	0.67	0.05
p__Actinobacteria__o__Actinomycetales_369980	0.67	0
p__Proteobacteria__g__Campylobacter__s__Campylobactercurvus_607889	0.67	0.06
p__Proteobacteria__g__Campylobacter__s__Campylobacterconcisus_141754	0.64	0.08
p__Proteobacteria__g__Moraxella_406471	0.64	-0.04
p__Proteobacteria__g__Sulfurimonas_448949	0.64	0.11
p__Proteobacteria__g__Stenoxybacter_751919	0.63	-0.03
p__Actinobacteria__o__koll13_76572	0.62	0.01
p__Bacteroidetes__g__Tenacibaculum_428560	0.62	-0.03
p__Proteobacteria__g__Sulfurimonas_444197	0.62	-0.04
p__Bacteroidetes__f__Flavobacteriaceae_332081	0.61	-0.12
p__Firmicutes__g__Streptococcus_269499	0.61	-0.05
p__Fusobacteria__g__Streptobacillus_719947	0.61	0.1
p__Proteobacteria__g__Stenoxybacter_55150	0.61	-0.03
p__Bacteroidetes__f__Flavobacteriaceae_12392	0.6	-0.07
p__Proteobacteria__g__Dichelobacter_361604	0.6	0.04
p__Proteobacteria__g__Massilia__s__Massiliatimonae_223880	0.6	-0.11
p__Firmicutes__o__Clostridiales_701726	0.59	0.05
p__Proteobacteria__f__SUP05_293055	0.59	-0.16
p__Proteobacteria__g__Ralstonia_478818	0.59	0.02
p__Thermi__g__Deinococcus__s__Deinococcusradiodurans_153993	0.59	0.11
p__Actinobacteria__g__Arthrobacter_694831	0.58	-0.15
p__Actinobacteria__g__Propionibacterium_427206	0.57	-0.12
p__Proteobacteria__g__Methylarcula__s__Loktanellahongkongensis_46703	0.57	-0.06
p__SR1__c___571035	0.57	-0.11
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_551043	0.56	-0.02
p__Bacteroidetes__f__Flavobacteriaceae_729660	0.55	-0.09
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_386801	0.55	-0.1
p__Proteobacteria__g__Stenoxybacter_174851	0.55	-0.1
p__Actinobacteria__g__Micrococcus__s__Micrococcusluteus_459780	0.54	-0.07
p__Bacteroidetes__f__Flavobacteriaceae_379313	0.54	-0.15
p__Proteobacteria__f__Comamonadaceae_455592	0.54	-0.16
p__Proteobacteria__g__Pseudoruegeria_390046	0.54	-0.03
p__Proteobacteria__f__Moraxellaceae_414919	0.53	-0.07
p__Proteobacteria__g__Amaricoccus_603022	0.53	-0.1
p__Proteobacteria__g__Burkholderia__s__Burkholderiafungorum_746096	0.53	-0.07
p__Actinobacteria__f__Nocardioidaceae_554632	0.52	-0.04
p__Bacteroidetes__o__Bacteroidales_711203	0.52	-0.01
p__Firmicutes__o__Clostridiales_226726	0.52	0.08
p__Proteobacteria__f__Rhodobacteraceae_37016	0.52	-0.06
p__Firmicutes__g__Staphylococcus_171970	0.51	-0.14
p__GN02__c__VC12cl04_222792	0.51	0.07
p__GN02__c__VC12cl04_595855	0.51	0.01
p__Proteobacteria__g__Kingella_306832	0.51	-0.08
p__Proteobacteria__g__Xenophilus_188340	0.5	-0.04
p__Proteobacteria__o__Chromatiales_485544	0.5	-0.08
p__Spirochaetes__g__Treponema_30617	0.5	0.01
p__Actinobacteria__f__AKIW874_693966	0.49	-0.06

p__Bacteroidetes__g__Dysgonomonas_475976	0.49	0.13
p__Bacteroidetes__g__Persicobacter__s__Persicobacterdiffluens_136215	0.49	0.03
p__Bacteroidetes__g__Pontibacter__s__Pontibacterkorlensis_406748	0.49	-0.02
p__Firmicutes__o__Clostridiales_70341	0.49	0.1
p__Proteobacteria__f__Rhodospirillaceae_494802	0.49	-0.05
p__Proteobacteria__g__Arcobacter_167287	0.49	-0.04
p__Proteobacteria__g__Pseudomonas_34989	0.49	-0.09
p__Tenericutes__g__Mycoplasma_743266	0.49	-0.12
p__Proteobacteria__f__Pasteurellaceae_63291	0.48	-0.08
p__Proteobacteria__g__Stenotrophomonas_727055	0.48	-0.09
p__Bacteroidetes__g__Tenacibaculum__s__Tenacibaculumlutimaris_437286	0.47	0.09
p__Proteobacteria__g__Haemophilus__s__Haemophilusparainfluenzae_49885	0.47	-0.1
p__Proteobacteria__o__Chromatiales_511266	0.47	0.03
p__Actinobacteria__o__03197L14_176541	0.46	0.12
p__Bacteroidetes__g__Zhouia_422137	0.46	-0.07
p__Firmicutes__o__Clostridiales_635308	0.46	0.14
p__Proteobacteria__f__Rhodospirillaceae_362274	0.45	-0.03
p__Proteobacteria__g__Kaistobacter_682533	0.45	0.12
p__Proteobacteria__f__Pasteurellaceae_732564	0.44	0
p__Proteobacteria__g__Moraxella_610605	0.44	-0.07
p__Proteobacteria__g__Taylorella__s__Taylorellaasinigenitalis_523515	0.44	-0.03
p__Spirochaetes__g__IE043_291307	0.44	0.1
p__Bacteroidetes__g__Tenacibaculum_474405	0.43	-0.07
p__Chloroflexi__c__SOGA31_541293	0.43	-0.01
p__Fusobacteria__g__Streptobacillus_621876	0.43	-0.08
p__Proteobacteria__f__Bradyrhizobiaceae_116562	0.43	-0.08
p__Proteobacteria__f__Rhodospirillaceae_622262	0.43	-0.02
p__Proteobacteria__g__Psychrobacter__s__Psychrobacterpacificensis_585025	0.43	-0.06
p__Proteobacteria__c__Deltaproteobacteria_23190	0.42	0.14
p__Proteobacteria__f__Pasteurellaceae_88612	0.42	-0.03
p__Proteobacteria__g__Rhodocyclus__s__Rhodocyclustenuis_740177	0.42	0.07
p__Proteobacteria__g__Sphingomonas__s__Sphingomonasazotifigens_533454	0.42	0.04
p__Proteobacteria__g__Steroidobacter_168803	0.42	-0.01
p__Proteobacteria__g__Sulfurimonas_412977	0.42	0.21
p__Proteobacteria__o__Sphingomonadales_709703	0.42	0.01
p__Actinobacteria__o__MC47_21675	0.41	-0.03
p__Proteobacteria__f__Oceanospirillaceae_500535	0.41	0.08
p__Proteobacteria__g__Brevundimonas__s__Brevundimonasdiminuta_54795	0.41	-0.2
p__Proteobacteria__g__Paracoccus__s__Paracoccusdenitrificans_117654	0.41	-0.09
p__Thermi__g__Deinococcus_262999	0.41	-0.07
p__Actinobacteria__f__Solirubrobacteraceae_1327	0.4	0.04
p__Actinobacteria__g__Propionibacterium__s__Propionibacteriumacnes_728036	0.4	-0.14
p__Actinobacteria__o__Solirubrobacterales_238455	0.4	-0.07
p__Bacteroidetes__g__Chryseobacterium_308134	0.4	-0.03
p__Proteobacteria__c__Betaproteobacteria_554275	0.4	0.16
p__Proteobacteria__f__Rhodospirillaceae_237338	0.4	0.04
p__Proteobacteria__f__Rhodospirillaceae_424659	0.4	0.01
p__Proteobacteria__g__Dichelobacter_255035	0.4	-0.1
p__Proteobacteria__g__Stenoxybacter_253237	0.4	-0.04
p__Verrucomicrobia__g__Verrucomicrobium_175087	0.4	0.02
p__Actinobacteria__o__Acidimicrobiales_244996	0.39	-0.01
p__Bacteroidetes__g__Bacteroides_689514	0.39	-0.11
p__Firmicutes__o__Clostridiales_376161	0.39	0.25
p__Proteobacteria__f__Rhodobacteraceae_160906	0.39	0.04
p__Proteobacteria__f__Rhodospirillaceae_213569	0.39	0.05
p__Proteobacteria__g__Devosia_497251	0.39	0.02
p__Proteobacteria__g__Vibrio_452638	0.38	-0.06
p__SR1__c___3865	0.38	0.19
p__Actinobacteria__g__Actinomyces_119009	0.37	0.09
p__Fusobacteria__g__Sneathia__s__Leptotrichiaamnionii_364034	0.37	-0.02

p__Proteobacteria__f__Pseudomonadaceae_79058	0.37	-0.11
p__Proteobacteria__f__Rhodobacteraceae_658260	0.37	0.02
p__Proteobacteria__f__Rhodocyclaceae_371305	0.37	0.06
p__Actinobacteria__g__Leucobacter_697145	0.36	0.04
p__Actinobacteria__o__Solirubrobacterales_711193	0.36	-0.09
p__Firmicutes__g__Clostridium_482667	0.36	-0.19
p__Firmicutes__g__Coprococcus_312354	0.36	-0.02
p__Proteobacteria__f__Pasteurellaceae_171803	0.36	0.04
p__Proteobacteria__f__Pasteurellaceae_626259	0.36	-0.02
p__Proteobacteria__f__Sinobacteraceae_155918	0.36	-0.07
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_327563	0.36	-0.03
p__Actinobacteria__f__CL50029_390474	0.35	-0.03
p__Actinobacteria__o__MC47_160960	0.35	0.14
p__Bacteroidetes__f__Flavobacteriaceae_118381	0.35	0.02
p__Bacteroidetes__f__Flavobacteriaceae_174976	0.35	0.1
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p__Proteobacteria__g__Sphingopyxis_102703	0.35	0.01
p__Actinobacteria__g__Aeromicrobium_619093	0.34	0.11
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p__Proteobacteria__f__Pasteurellaceae_648872	0.34	0.01
p__Proteobacteria__g__Pedomicrobium_452291	0.34	0.01
p__Proteobacteria__g__Stenoxybacter_572518	0.34	0.01
p__Tenericutes__g__Mycoplasma_551498	0.34	0.01
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p__Chloroflexi__c__SOGA31_314194	0.33	-0.07
p__Proteobacteria__g__Delftia_587073	0.33	0.03
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p__Actinobacteria__f__Nocardioidaceae_632222	0.32	0.21
p__Proteobacteria__f__Rhodospirillaceae_441088	0.32	0.18
p__Proteobacteria__g__Devosia_700503	0.32	0.02
p__Proteobacteria__o__Myxococcales_668245	0.32	0.18
p__Proteobacteria__f__Alcaligenaceae_536615	0.31	0.12
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p__Proteobacteria__g__Agrobacterium_442913	0.31	-0.06
p__Proteobacteria__g__Erwinia_12320	0.31	-0.09
p__Actinobacteria__f__Micromonosporaceae_38158	0.3	0.11
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p__Proteobacteria__g__Desulfovibrio_214348	0.3	-0.14
p__Proteobacteria__g__Devosia_749390	0.3	-0.05
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p__Proteobacteria__g__Stenoxybacter_733417	0.3	-0.13
p__Actinobacteria__f__Microbacteriaceae_486696	0.29	-0.07
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p__Proteobacteria__o__Rhodocyclales_496609	0.28	0.02
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p__Proteobacteria__g__Dichelobacter_383096	0.27	-0.01
p__Proteobacteria__g__Stenoxybacter_317953	0.27	0.15
p__Proteobacteria__o__Chromatiales_253749	0.27	0.07
p__Actinobacteria__g__Corynebacterium_68777	0.26	-0.06
p__Firmicutes__g__Bacillus__s__Bacillussp.NRRLB14911_432592	0.26	0.13
p__Gemmatimonadetes__o__Gemmatimonadales_757385	0.26	-0.04
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_720429	0.26	0.05
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_729784	0.26	0.13
p__Proteobacteria__g__Devosia_350266	0.26	0.06
p__Proteobacteria__g__Dichelobacter_140648	0.26	0.01
p__Proteobacteria__g__Thauera__s__Thauerasp.MZ1T_194738	0.26	-0.11
p__Spirochaetes__g__Brachyspira_69417	0.26	0.09
p__Actinobacteria__g__Friedmanniella_135197	0.25	0.07
p__Actinobacteria__g__Sporichthya_500877	0.24	0.02
p__Actinobacteria__o__03197L14_125940	0.24	0.05
p__Proteobacteria__f__Acetobacteraceae_509759	0.24	0.02
p__Proteobacteria__f__Enterobacteriaceae_696135	0.24	-0.09
p__Proteobacteria__f__Pasteurellaceae_384138	0.24	0.02
p__Proteobacteria__g__Diaphorobacter_687258	0.24	0.04
p__Actinobacteria__g__Streptomyces_420197	0.23	0.19
p__Actinobacteria__o__Actinomycetales_409160	0.23	0.01
p__Bacteroidetes__f__Cryomorphaceae_613739	0.23	-0.02
p__Bacteroidetes__g__Bacteroides_706790	0.23	-0.02
p__Bacteroidetes__g__Parabacteroides_72081	0.23	-0.17
p__Proteobacteria__f__Pasteurellaceae_142293	0.23	-0.02
p__Proteobacteria__f__Pasteurellaceae_25667	0.23	-0.02
p__Proteobacteria__f__Rhodobacteraceae_729919	0.23	-0.17
p__Proteobacteria__g__Alcanivorax_427659	0.23	-0.02
p__Proteobacteria__o__Chromatiales_429245	0.23	0.02
p__Proteobacteria__o__Xanthomonadales_411751	0.23	-0.02
p__Actinobacteria__g__Janibacter_703951	0.22	0.1
p__Proteobacteria__g__Achromobacter_19649	0.22	-0.08
p__Proteobacteria__g__Rhodoplanes_273401	0.22	0.09
p__Bacteroidetes__f__Porphyromonadaceae_329193	0.21	-0.01
p__Bacteroidetes__g__Aureispira_253433	0.21	-0.13
p__Bacteroidetes__g__Prevotella_144636	0.21	-0.01
p__Proteobacteria__g__Stenoxybacter_90130	0.21	-0.01
p__Actinobacteria__g__Leucobacter_485337	0.2	0.04
p__Actinobacteria__g__Dietzia__s__Dietziamaris_276529	0.19	0.05
p__Proteobacteria__g__Acinetobacter_78571	0.19	0.06
p__Actinobacteria__g__Gordonia_255893	0.17	-0.13
p__Bacteroidetes__g__Sphingobacterium_204117	0.17	-0.23
p__Firmicutes__g__Enterococcus_396129	0.17	-0.01
p__Fusobacteria__g__Propionigenium__s__Propionigeniummaris_617140	0.17	-0.06
p__Proteobacteria__o__Chromatiales_537609	0.17	-0.13
p__Firmicutes__f__Lachnospiraceae_765062	0.16	-0.17
p__Proteobacteria__g__Aquamicrobium_748021	0.16	-0.04
p__Proteobacteria__g__Lysobacter_318152	0.16	0.15
p__Actinobacteria__f__Kineosporiaceae_705398	0.15	0.06
p__Actinobacteria__g__Nocardioides_128926	0.15	0.1
p__Bacteroidetes__o__Bacteroidales_611944	0.15	-0.14
p__Proteobacteria__g__Pseudoalteromonas_279501	0.15	0.06
p__Proteobacteria__g__Pseudomonas_40331	0.15	0.07
p__Proteobacteria__g__Vibrio__s__Vibrioichthyoenteri_763288	0.15	-0.02
p__Bacteroidetes__f__Flavobacteriaceae_48296	0.14	-0.08
p__Firmicutes__g__Paenibacillus_556209	0.14	-0.03
p__Synergistetes__f__Synergistaceae_760316	0.14	-0.25
p__Actinobacteria__f__Nocardioidaceae_115549	0.13	0.09

p__Bacteroidetes__f__Saprospiraceae_536374	0.13	0.09
p__Bacteroidetes__g__Sejongia_520682	0.13	0.16
p__Firmicutes__g__Bacillus__s__Bacillusbarbaricus_663170	0.13	-0.03
p__Proteobacteria__f__Burkholderiaceae_432832	0.13	0.09
p__Proteobacteria__g__Aquimonas_359512	0.13	-0.12
p__Proteobacteria__g__Rhodobacter_747540	0.13	0.09
p__Firmicutes__g__Clostridium_248626	0.12	0.19
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_65627	0.12	-0.12
p__Actinobacteria__g__Mobiluncus__s__Mobiluncuscurtisii_267940	0.11	0
p__Proteobacteria__g__Achromobacter_324477	0.11	-0.04
p__Proteobacteria__g__Klebsiella_558424	0.1	0.11
p__Actinobacteria__g__Dietzia_70231	0.09	0.13
p__Proteobacteria__g__Aeromonas_217733	0.09	0.14
p__SR1__c___526194	0.09	0.14
p__Firmicutes__g__Aerococcus__s__Aerococcusurinae_72529	0.08	0
p__Firmicutes__g__Anaerofilum_460336	0.08	0
p__Actinobacteria__g__Mycobacterium_393834	0.07	0.14
p__Proteobacteria__g__Pseudomonas_704131	0.07	0.14
p__Actinobacteria__g__Arthrobacter__s__Arthrobacterureafaciens_610242	0.06	0.06
p__Bacteroidetes__g__Alistipes_17519	0.06	-0.24
p__Bacteroidetes__o__Bacteroidales_63588	0.06	-0.2
p__Firmicutes__o__Clostridiales_403067	0.06	0.15
p__Proteobacteria__c__Gammaproteobacteria_692699	0.06	0.13
p__Proteobacteria__g__Arcobacter_130909	0.06	0.09
p__Proteobacteria__g__Pseudomonas_639712	0.06	0
p__Bacteroidetes__g__Tenacibaculum_241145	0.05	0.06
p__Proteobacteria__f__Alcaligenaceae_541229	0.04	0.04
p__Proteobacteria__g__Serratia__s__Serratiamarcescens_432257	0.04	0.04
p__Acidobacteria__o__Acidobacteriales_743933	0.02	0.23
p__Actinobacteria__g__Actinomyces__s__Actinomycesmarimammalium_667873	0.02	0.23
p__Firmicutes__o__Clostridiales_66573	0.02	0.23
p__Fusobacteria__g__J229_224256	0.02	-0.21
p__Proteobacteria__f__Enterobacteriaceae_452665	0.02	-0.04
p__Proteobacteria__g__Providencia__s__Providenciaalcalifaciens_414599	0.02	0.23
p__Chloroflexi__c__SOGA31_42333	0.01	-0.02
p__Proteobacteria__f__Rhodobacteraceae_649099	0.01	-0.09
p__Proteobacteria__g__Methylobacterium_99391	0.01	-0.26
p__Actinobacteria__g__Arcanobacterium_246345	0	0.21
p__Proteobacteria__g__Bacteriovorax_64787	0	0.3
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_301898	-0.01	-0.31
p__Firmicutes__f__Lachnospiraceae_351119	-0.02	-0.19
p__Firmicutes__g__Acidaminococcus_127229	-0.02	-0.25
p__Firmicutes__g__Coprococcus_628747	-0.02	0.1
p__Firmicutes__g__Eubacterium_622811	-0.02	-0.24
p__Proteobacteria__f__Alcaligenaceae_503155	-0.02	0.08
p__Proteobacteria__g__Brucella_305522	-0.02	0
p__Firmicutes__g__Oscillospira_381776	-0.03	-0.23
p__Firmicutes__o__Clostridiales_425694	-0.03	-0.24
p__Firmicutes__o__Clostridiales_676690	-0.03	-0.19
p__Bacteroidetes__f__Flavobacteriaceae_374120	-0.04	-0.23
p__Bacteroidetes__g__Bacteroides_120561	-0.04	-0.18
p__Proteobacteria__f__Enterobacteriaceae_641353	-0.04	0.14
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_466829	-0.04	-0.23
p__Proteobacteria__g__Paracoccus_110077	-0.04	-0.23
p__Proteobacteria__g__Vibrio__s__Vibrioorientalis_705701	-0.04	-0.23
p__Actinobacteria__g__Rhodococcus__s__Rhodococcusequi_412056	-0.06	0.3
p__Proteobacteria__f__Enterobacteriaceae_231744	-0.06	0.26
p__Proteobacteria__g__Enterobacter__s__Enterobacterhormaechei_313908	-0.08	-0.02
p__Firmicutes__g__Bacillus_388773	-0.09	0.08
p__Firmicutes__g__Bacillus_719832	-0.09	0.16

p__Proteobacteria__f__Enterobacteriaceae_84333	-0.09	0.19
p__Proteobacteria__g__Nitratireductor_5045	-0.09	-0.06
p__Proteobacteria__g__Pseudomonas_132221	-0.09	-0.03
p__Firmicutes__o__Clostridiales_748404	-0.1	-0.17
p__Bacteroidetes__f__Porphyromonadaceae_665191	-0.11	-0.14
p__Bacteroidetes__g__Sphingobacterium_176945	-0.11	0.15
p__Proteobacteria__f__SUP05_224259	-0.11	0.15
p__Firmicutes__g__Bacillus_207834	-0.12	0.17
p__Proteobacteria__g__Thermomonas_132820	-0.12	0.1
p__Firmicutes__f__Planococcaceae_152858	-0.13	0.22
p__Firmicutes__g__Bacillus__s__Bacilluscoahuilensis_23748	-0.13	0.22
p__Firmicutes__g__Brevibacillus_405240	-0.13	0.38
p__Firmicutes__g__Paenibacillus_212495	-0.13	0.31
p__Proteobacteria__f__Brucellaceae_698093	-0.14	0.25
p__Actinobacteria__g__Brevibacterium__s__Brevibacteriumaureum_674805	-0.15	-0.15
p__Firmicutes__f__Bacillaceae_724791	-0.15	0.08
p__Firmicutes__g__Eubacterium_647632	-0.15	-0.32
p__Firmicutes__g__Bacillus__s__Bacillusfirmus_749341	-0.17	0.23
p__Proteobacteria__g__Acinetobacter_168646	-0.17	-0.15
p__Bacteroidetes__g__Sphingobacterium__s__Sphingobacteriummizutaii_632930	-0.18	-0.03
p__Firmicutes__g__Lysinibacillus_81925	-0.18	0.25
p__Proteobacteria__g__Klebsiella_347396	-0.18	-0.03
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_694969	-0.19	-0.19
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasmendocina_126983	-0.19	-0.19
p__Firmicutes__f__Bacillaceae_185216	-0.2	0.27
p__Firmicutes__f__Lachnospiraceae_285759	-0.23	-0.24
p__Firmicutes__g__Oceanobacillus_357784	-0.23	-0.24
p__Firmicutes__o__Clostridiales_430603	-0.23	-0.24
p__Firmicutes__f__Bacillaceae_664374	-0.24	-0.21
p__Firmicutes__g__Bacillus_444784	-0.24	0.24
p__Fusobacteria__g__Cetobacterium__s__Cetobacteriumsomerae_541959	-0.24	-0.21
p__Proteobacteria__g__Lysobacter_433400	-0.24	-0.21
p__Proteobacteria__g__Magnetococcus_659945	-0.24	-0.21
p__Firmicutes__f__Planococcaceae_570623	-0.25	0.18
p__Bacteroidetes__g__Myroides_398565	-0.26	-0.26
p__Firmicutes__g__Bacillus__s__Bacillusamyloliquefaciens_585304	-0.26	-0.1
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_209517	-0.26	-0.26
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_428427	-0.26	-0.26
p__Firmicutes__g__Sediminibacillus_653187	-0.27	0.12
p__Firmicutes__f__Bacillaceae_341269	-0.28	-0.12
p__Firmicutes__f__Bacillaceae_117340	-0.32	-0.03
p__Firmicutes__g__Bacillus_535651	-0.32	0.25
p__Firmicutes__g__Bacillus__s__Bacillusendophyticus_410037	-0.34	0.18
p__Firmicutes__g__Virgibacillus__s__Virgibacillusproomii_522633	-0.35	-0.08
p__Proteobacteria__g__Acinetobacter__s__Acinetobacterrhizosphaerae_297583	-0.35	0.06
p__Firmicutes__g__Bacillus_245239	-0.37	0.27
p__Firmicutes__g__Paenibacillus__s__Paenibacilluschondroitinus_746934	-0.4	-0.22
p__Firmicutes__g__Bacillus_220083	-0.44	-0.35
p__Firmicutes__g__Bacillus_161559	-0.6	-0.29
p__Firmicutes__f__Planococcaceae_668479	-0.61	-0.21
p__Actinobacteria__f__Coriobacteriaceae_718543	NA	NA
p__Actinobacteria__f__Kineosporiaceae_653080	NA	NA
p__Actinobacteria__f__Microbacteriaceae_56401	NA	NA
p__Actinobacteria__g__Actinotalea_764221	NA	NA
p__Actinobacteria__g__Agromyces__s__Agromycesulmi_291388	NA	NA
p__Actinobacteria__g__Demequina_705827	NA	NA
p__Actinobacteria__g__Dermacoccus_337626	NA	NA
p__Actinobacteria__g__Jonesia_14545	NA	NA
p__Actinobacteria__g__Leucobacter_145505	NA	NA
p__Actinobacteria__g__Leucobacter_153685	NA	NA

p__Actinobacteria__g__Leucobacter_418594	NA	NA
p__Actinobacteria__g__Microbacterium_545806	NA	NA
p__Actinobacteria__g__Pimelobacter_394756	NA	NA
p__Actinobacteria__g__Propionibacterium_705851	NA	NA
p__Actinobacteria__g__Pseudoclavibacter_17723	NA	NA
p__Actinobacteria__g__Pseudoclavibacter_340276	NA	NA
p__Actinobacteria__g__Pseudoclavibacter_588006	NA	NA
p__Actinobacteria__g__Pseudoclavibacter_74986	NA	NA
p__Actinobacteria__g__Rhodococcus_413286	NA	NA
p__Actinobacteria__g__Tessaracoccus_287453	NA	NA
p__Actinobacteria__g__Tropheryma_445164	NA	NA
p__Actinobacteria__o__koll13_727662	NA	NA
p__Bacteroidetes__c__Flavobacteria_105335	NA	NA
p__Bacteroidetes__f__Cryomorphaceae_690566	NA	NA
p__Bacteroidetes__f__Cryomorphaceae_96414	NA	NA
p__Bacteroidetes__f__Flavobacteriaceae_555335	NA	NA
p__Bacteroidetes__f__Flavobacteriaceae_555372	NA	NA
p__Bacteroidetes__f__Flexibacteraceae_122867	NA	NA
p__Bacteroidetes__f__Porphyromonadaceae_384223	NA	NA
p__Bacteroidetes__f__Porphyromonadaceae_438273	NA	NA
p__Bacteroidetes__f__Sphingobacteriaceae_540060	NA	NA
p__Bacteroidetes__g__Bacteroides_9387	NA	NA
p__Bacteroidetes__g__Chitinophaga_80088	NA	NA
p__Bacteroidetes__g__Chryseobacterium_171211	NA	NA
p__Bacteroidetes__g__Dyadobacter_763044	NA	NA
p__Bacteroidetes__g__Myroides__s__Myroidesodoratimimus_295565	NA	NA
p__Bacteroidetes__g__Myroides__s__Myroidesodoratimimus_489867	NA	NA
p__Bacteroidetes__g__Odoribacter_244028	NA	NA
p__Bacteroidetes__g__Parabacteroides__s__Parabacteroidesgordonii_340135	NA	NA
p__Bacteroidetes__g__Prevotella_15418	NA	NA
p__Bacteroidetes__g__Sphingobacterium_246851	NA	NA
p__Bacteroidetes__g__Sphingobacterium_250053	NA	NA
p__Bacteroidetes__g__Sphingobacterium_298499	NA	NA
p__Bacteroidetes__g__Sphingobacterium_512955	NA	NA
p__Bacteroidetes__g__Wautersiella_232450	NA	NA
p__Bacteroidetes__o__Sphingobacteriales_152865	NA	NA
p__Bacteroidetes__o__Sphingobacteriales_571041	NA	NA
p__Chlamydiae__g__Chlamydophila__s__Chlamydophilapecorum_581037	NA	NA
p__Firmicutes__f__Ruminococcaceae_49610	NA	NA
p__Firmicutes__f__Ruminococcaceae_499927	NA	NA
p__Firmicutes__g__Clostridium_110727	NA	NA
p__Firmicutes__g__Clostridium_356361	NA	NA
p__Firmicutes__g__Clostridium__s__Clostridiumsubterminale_449559	NA	NA
p__Firmicutes__g__Coprococcus_219830	NA	NA
p__Firmicutes__g__Coprococcus_517519	NA	NA
p__Firmicutes__g__Epulopiscium_234442	NA	NA
p__Firmicutes__g__Faecalibacterium_341196	NA	NA
p__Firmicutes__g__Fusibacter_19731	NA	NA
p__Firmicutes__g__Lactobacillus__s__Lactobacillusreuteri_192675	NA	NA
p__Firmicutes__g__Megamonas__s__Megamonashypermegale_267977	NA	NA
p__Firmicutes__g__Paenibacillus_494536	NA	NA
p__Firmicutes__g__Ruminococcus__s__Ruminococcustorques_740297	NA	NA
p__Firmicutes__g__Tissierella_366131	NA	NA
p__Firmicutes__o__Clostridiales_139048	NA	NA
p__Firmicutes__o__Clostridiales_340076	NA	NA
p__Firmicutes__o__Clostridiales_688183	NA	NA
p__Fusobacteria__g__Streptobacillus_131821	NA	NA
p__Fusobacteria__g__Streptobacillus_398372	NA	NA
p__Fusobacteria__g__Streptobacillus_640775	NA	NA
p__Fusobacteria__g__Streptobacillus__s__Streptobacillusmoniliformis_287396	NA	NA

p__Proteobacteria__c__Alphaproteobacteria_462900	NA	NA
p__Proteobacteria__c__Deltaproteobacteria_239572	NA	NA
p__Proteobacteria__c__Gammaproteobacteria_417606	NA	NA
p__Proteobacteria__c__Gammaproteobacteria_747652	NA	NA
p__Proteobacteria__f__Acetobacteraceae_614109	NA	NA
p__Proteobacteria__f__Acetobacteraceae_741892	NA	NA
p__Proteobacteria__f__Alcaligenaceae_126597	NA	NA
p__Proteobacteria__f__Alcaligenaceae_301955	NA	NA
p__Proteobacteria__f__Alcaligenaceae_310612	NA	NA
p__Proteobacteria__f__Alcaligenaceae_662472	NA	NA
p__Proteobacteria__f__Alcaligenaceae_663917	NA	NA
p__Proteobacteria__f__Comamonadaceae_2152	NA	NA
p__Proteobacteria__f__Enterobacteriaceae_172645	NA	NA
p__Proteobacteria__f__Enterobacteriaceae_245606	NA	NA
p__Proteobacteria__f__Enterobacteriaceae_297365	NA	NA
p__Proteobacteria__f__Enterobacteriaceae_410502	NA	NA
p__Proteobacteria__f__Hyphomicrobiaceae_600020	NA	NA
p__Proteobacteria__f__Methylophilaceae_93397	NA	NA
p__Proteobacteria__f__Pasteurellaceae_161712	NA	NA
p__Proteobacteria__f__Pasteurellaceae_565362	NA	NA
p__Proteobacteria__f__Pasteurellaceae_642119	NA	NA
p__Proteobacteria__f__Pseudomonadaceae_99078	NA	NA
p__Proteobacteria__f__SUP05_502024	NA	NA
p__Proteobacteria__f__Sinobacteraceae_668199	NA	NA
p__Proteobacteria__f__Vibrionaceae_561226	NA	NA
p__Proteobacteria__f__Xanthomonadaceae_258602	NA	NA
p__Proteobacteria__f__Xanthomonadaceae_702358	NA	NA
p__Proteobacteria__g__Achromobacter_155903	NA	NA
p__Proteobacteria__g__Achromobacter_375749	NA	NA
p__Proteobacteria__g__Achromobacter_376840	NA	NA
p__Proteobacteria__g__Achromobacter_468982	NA	NA
p__Proteobacteria__g__Achromobacter_678564	NA	NA
p__Proteobacteria__g__Achromobacter__s__Bordetellahinzii_629547	NA	NA
p__Proteobacteria__g__Alcaligenes_538514	NA	NA
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_38406	NA	NA
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_386688	NA	NA
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_462593	NA	NA
p__Proteobacteria__g__Alcaligenes__s__Alcaligenesfaecalis_478078	NA	NA
p__Proteobacteria__g__Averyella_710470	NA	NA
p__Proteobacteria__g__Bosea_424613	NA	NA
p__Proteobacteria__g__Campylobacter__s__Campylobacterfetus_297665	NA	NA
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumhominis_407530	NA	NA
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumvalvarum_155355	NA	NA
p__Proteobacteria__g__Cardiobacterium__s__Cardiobacteriumvalvarum_368504	NA	NA
p__Proteobacteria__g__Castellaniella__s__Castellanielladefragrans_166109	NA	NA
p__Proteobacteria__g__Cohaesibacter_664815	NA	NA
p__Proteobacteria__g__Comamonas_41770	NA	NA
p__Proteobacteria__g__Defluvibacter_651784	NA	NA
p__Proteobacteria__g__Desulfovibrio__s__Desulfovibriodesulfuricans_176573	NA	NA
p__Proteobacteria__g__Enterobacter_572676	NA	NA
p__Proteobacteria__g__Enterobacter__s__Enterobacterpulveris_433956	NA	NA
p__Proteobacteria__g__Erwinia_25313	NA	NA
p__Proteobacteria__g__Haliangium_138177	NA	NA
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_567033	NA	NA
p__Proteobacteria__g__Helicobacter__s__Helicobactermustelae_709097	NA	NA
p__Proteobacteria__g__Kaistia_437081	NA	NA
p__Proteobacteria__g__Klebsiella_117934	NA	NA
p__Proteobacteria__g__Klebsiella_430511	NA	NA
p__Proteobacteria__g__Klebsiella_664333	NA	NA
p__Proteobacteria__g__Lampropedia_284463	NA	NA

p__Proteobacteria__g__Marinobacter_334925	NA	NA
p__Proteobacteria__g__Mesorhizobium_4131	NA	NA
p__Proteobacteria__g__Methylophaga__s__Methylophagamarina_521544	NA	NA
p__Proteobacteria__g__Nitratireductor__s__Nitratireductoraquibiodomus_479777	NA	NA
p__Proteobacteria__g__Paracoccus__s__Paracoccussolventivorans_641772	NA	NA
p__Proteobacteria__g__Pelistega__s__Pelistegaeuropaea_751309	NA	NA
p__Proteobacteria__g__Photobacterium__s__Photobacteriumdamselae_224255	NA	NA
p__Proteobacteria__g__Pseudomonas_10820	NA	NA
p__Proteobacteria__g__Pseudomonas_110207	NA	NA
p__Proteobacteria__g__Pseudomonas_142124	NA	NA
p__Proteobacteria__g__Pseudomonas_159349	NA	NA
p__Proteobacteria__g__Pseudomonas_161575	NA	NA
p__Proteobacteria__g__Pseudomonas_165065	NA	NA
p__Proteobacteria__g__Pseudomonas_174392	NA	NA
p__Proteobacteria__g__Pseudomonas_235588	NA	NA
p__Proteobacteria__g__Pseudomonas_281622	NA	NA
p__Proteobacteria__g__Pseudomonas_308359	NA	NA
p__Proteobacteria__g__Pseudomonas_34101	NA	NA
p__Proteobacteria__g__Pseudomonas_391544	NA	NA
p__Proteobacteria__g__Pseudomonas_403884	NA	NA
p__Proteobacteria__g__Pseudomonas_466613	NA	NA
p__Proteobacteria__g__Pseudomonas_511660	NA	NA
p__Proteobacteria__g__Pseudomonas_572655	NA	NA
p__Proteobacteria__g__Pseudomonas_711667	NA	NA
p__Proteobacteria__g__Pseudomonas_731148	NA	NA
p__Proteobacteria__g__Pseudomonas_89597	NA	NA
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_256671	NA	NA
p__Proteobacteria__g__Pseudomonas__s__Pseudomonasstutzeri_665176	NA	NA
p__Proteobacteria__g__Pseudoxanthomonas__s__Pseudoxanthomonasmexicana_117378	NA	NA
p__Proteobacteria__g__Rheinheimera__s__Rheinheimeraaquimaris_486023	NA	NA
p__Proteobacteria__g__Shewanella__s__Shewanellaalgae_485511	NA	NA
p__Proteobacteria__g__Shinella_386729	NA	NA
p__Proteobacteria__g__Sodalis__s__Sodalisglossinidius_202548	NA	NA
p__Proteobacteria__g__Stenotrophomonas_168758	NA	NA
p__Proteobacteria__g__Stenotrophomonas_247119	NA	NA
p__Proteobacteria__g__Stenotrophomonas_502872	NA	NA
p__Proteobacteria__g__Stenotrophomonas_505642	NA	NA
p__Proteobacteria__g__Stenotrophomonas_531558	NA	NA
p__Proteobacteria__g__Stenotrophomonas_657476	NA	NA
p__Proteobacteria__g__Stenotrophomonas_695630	NA	NA
p__Proteobacteria__g__Stenotrophomonas_729526	NA	NA
p__Proteobacteria__g__Stenoxybacter_367464	NA	NA
p__Proteobacteria__g__Stenoxybacter_396392	NA	NA
p__Proteobacteria__g__Stenoxybacter_51163	NA	NA
p__Proteobacteria__g__Stenoxybacter_589139	NA	NA
p__Proteobacteria__g__Stenoxybacter_651794	NA	NA
p__Proteobacteria__g__Stenoxybacter_687490	NA	NA
p__Proteobacteria__g__Trabulsiella__s__Kluyveraascorbata_152515	NA	NA
p__Proteobacteria__g__Vibrio_303061	NA	NA
p__Proteobacteria__g__Vibrio__s__Vibrioshilonii_386760	NA	NA
p__Proteobacteria__g__Vibrio__s__Vibriosp.AND4_634776	NA	NA
p__Proteobacteria__o__Chromatiales_298301	NA	NA
p__Proteobacteria__o__Chromatiales_658840	NA	NA
p__Proteobacteria__o__Chromatiales_726756	NA	NA
p__Proteobacteria__o__Myxococcales_441863	NA	NA
p__Proteobacteria__o__Rhizobiales_602739	NA	NA
p__Proteobacteria__o__Rhizobiales_675457	NA	NA
p__Spirochaetes__g__Brachyspira_27753	NA	NA
p__TM7__o__EW055_125519	NA	NA
p__Tenericutes__g__Bulleidia_166205	NA	NA

p__Tenericutes__g__Mycoplasma__s__Mycoplasmazalophi_300459	NA	NA
p__Tenericutes__g___Unclassified.Erysipelotrichaceae__s__Clostridiuminnocuum_474350	NA	NA

Table 6.4 Results of statistical comparisons of microbiota versus blood results in flatback turtles from Crab Island, Queensland, and Port Hedland, Western Australia. Significant results are indicated by bold text.

Analyte		**Estimate**	**Std. Error**	**t**	**p**
PCV	(Intercept)	30.46	1.20	25.49	<0.01
	V1	-1.94	3.60	-0.54	0.59
	LocationPortHedland	8.04	2.19	3.67	**<0.01**
	V1:LocationPortHedland	-6.38	7.36	-0.87	0.39
	(Intercept)	30.85	0.94	32.68	<0.01
	V2	0.31	4.76	0.07	0.95
	LocationPortHedland	5.62	1.39	4.04	**<0.01**
	V2:LocationPortHedland	-6.77	6.30	-1.07	0.29
TWCC	(Intercept)	8.17	0.84	9.69	<0.01
	V1	1.53	2.54	0.60	0.55
	LocationPort Hedland	-2.66	1.55	-1.72	0.09
	V1:LocationPort Hedland	2.59	5.19	0.50	0.62
	(Intercept)	7.85	0.67	11.76	<0.01
	V2	1.76	3.37	0.52	0.60
	LocationPort Hedland	-1.33	0.98	-1.36	0.19
	V2:LocationPort Hedland	1.48	4.46	0.33	0.74
Lymphocytes	(Intercept)	2.91	0.45	6.40	<0.01
	V1	1.50	1.37	1.09	0.28
	LocationPort Hedland	-0.85	0.83	-1.02	0.32
	V1:LocationPort Hedland	1.09	2.80	0.39	0.70
	(Intercept)	2.61	0.37	7.13	<0.01
	V2	-0.06	1.85	-0.03	0.98
	LocationPort Hedland	0.08	0.54	0.16	0.88
	V2:LocationPort Hedland	2.01	2.44	0.82	0.42
Heterophils	(Intercept)	4.46	0.43	10.50	<0.01
	V1	0.37	1.28	0.29	0.77
	LocationPort Hedland	-1.84	0.78	-2.36	**0.02**
	V1:LocationPort Hedland	1.01	2.62	0.39	0.70
	(Intercept)	4.38	0.34	13.04	<0.01
	V2	0.90	1.69	0.53	0.60
	LocationPort Hedland	-1.42	0.50	-2.87	**0.01**
	V2:LocationPort Hedland	0.24	2.24	0.11	0.92
H:L	(Intercept)	2.00	0.28	7.25	<0.01
	V1	-0.87	0.83	-1.05	0.30

	LocationPort Hedland	-0.41	0.51	-0.81	0.42
	V1:LocationPort Hedland	-0.12	1.70	-0.07	0.94
	(Intercept)	2.17	0.22	9.78	<0.01
	V2	0.60	1.12	0.54	0.60
	LocationPort Hedland	-0.82	0.33	-2.52	**0.02**
	V2:LocationPort Hedland	-1.25	1.48	-0.84	0.41
Monocytes	(Intercept)	0.23	0.05	4.54	<0.01
	V1	0.02	0.16	0.13	0.90
	LocationPort Hedland	0.12	0.09	1.32	0.20
	V1:LocationPort Hedland	-0.49	0.32	-1.55	0.13
	(Intercept)	0.23	0.04	5.59	<0.01
	V2	0.14	0.21	0.69	0.49
	LocationPort Hedland	0.02	0.06	0.26	0.80
	V2:LocationPort Hedland	-0.43	0.27	-1.56	0.13
Eosinophils	(Intercept)	0.48	0.11	4.24	<0.01
	V1	-0.45	0.34	-1.31	0.20
	LocationPort Hedland	-0.14	0.21	-0.66	0.51
	V1:LocationPort Hedland	1.13	0.70	1.62	0.11
	(Intercept)	0.57	0.09	6.32	<0.01
	V2	0.63	0.45	1.39	0.17
	LocationPort Hedland	-0.05	0.13	-0.42	0.68
	V2:LocationPort Hedland	-0.13	0.60	-0.22	0.83
Basophils	(Intercept)	0.09	0.03	3.14	<0.01
	V1	0.17	0.09	2.01	0.05
	LocationPort Hedland	0.08	0.05	1.55	0.13
	V1:LocationPort Hedland	-0.30	0.18	-1.70	0.10
	(Intercept)	0.05	0.02	2.29	0.03
	V2	0.14	0.12	1.17	0.25
	LocationPort Hedland	0.09	0.03	2.48	**0.02**
	V2:LocationPort Hedland	-0.23	0.16	-1.46	0.15
AST	(Intercept)	181.44	25.84	7.02	<0.01
	V1	-13.69	77.79	-0.18	0.86
	LocationPort Hedland	30.26	55.46	0.55	0.59
	V1:LocationPort Hedland	-97.59	178.77	-0.55	0.59
	(Intercept)	184.72	20.46	9.03	<0.01
	V2	-67.85	103.18	-0.66	0.52
	LocationPort Hedland	-0.69	31.43	-0.02	0.98
	V2:LocationPort Hedland	6.84	145.22	0.05	0.96
CK	(Intercept)	517.07	99.58	5.19	<0.01
	V1	-52.71	299.71	-0.18	0.86
	LocationPort Hedland	131.44	213.68	0.62	0.54
	V1:LocationPort Hedland	-682.57	688.80	-0.99	0.33

	(Intercept)	528.11	79.17	6.67	<0.01
	V2	-53.40	399.26	-0.13	0.89
	LocationPort Hedland	-60.12	121.60	-0.49	0.63
	V2:LocationPort Hedland	-419.44	561.92	-0.75	0.46
UA	(Intercept)	58.67	5.91	9.93	0.00
	V1	-1.41	17.79	-0.08	0.94
	LocationPort Hedland	-36.15	12.68	-2.85	**0.01**
	V1:LocationPort Hedland	-7.78	40.88	-0.19	0.85
	(Intercept)	58.83	4.67	12.60	<0.01
	V2	15.36	23.55	0.65	0.52
	LocationPort Hedland	-38.62	7.17	-5.38	**0.00**
	V2:LocationPort Hedland	-19.89	33.15	-0.60	0.55
Glu	(Intercept)	4.89	0.19	26.38	<0.01
	V1	0.94	0.56	1.68	0.10
	LocationPort Hedland	-0.37	0.40	-0.94	0.36
	V1:LocationPort Hedland	-1.24	1.28	-0.97	0.34
	(Intercept)	4.71	0.15	31.35	<0.01
	V2	-0.92	0.76	-1.22	0.23
	LocationPort Hedland	-0.26	0.23	-1.14	0.27
	V2:LocationPort Hedland	0.63	1.07	0.59	0.56
Ca	(Intercept)	2.90	0.28	10.49	<0.01
	V1	0.21	0.83	0.25	0.80
	LocationPort Hedland	-1.66	0.59	-2.80	**0.01**
	V1:LocationPort Hedland	5.51	1.91	2.88	0.01
	(Intercept)	2.86	0.22	13.30	0.00
	V2	-0.24	1.09	-0.22	0.83
	LocationPort Hedland	-0.22	0.33	-0.68	0.50
	V2:LocationPort Hedland	4.10	1.53	2.69	**0.01**
Phos	(Intercept)	3.10	0.26	12.14	<0.01
	V1	0.19	0.77	0.24	0.81
	LocationPort Hedland	0.89	0.55	1.62	0.12
	V1:LocationPort Hedland	-2.05	1.76	-1.16	0.25
	(Intercept)	3.06	0.21	14.85	<0.01
	V2	-0.39	1.04	-0.37	0.71
	LocationPort Hedland	0.44	0.32	1.39	0.18
	V2:LocationPort Hedland	-0.11	1.46	-0.08	0.94
TP	(Intercept)	36.74	2.14	17.16	<0.01
	V1	10.88	6.45	1.69	0.10
	LocationPort Hedland	16.76	4.60	3.65	**<0.01**
	V1:LocationPort Hedland	-34.99	14.81	-2.36	**0.02**
	(Intercept)	34.65	1.77	19.59	<0.01
	V2	-13.00	8.92	-1.46	0.16

	LocationPort Hedland	12.75	2.72	4.70	**<0.01**
	V2:LocationPort Hedland	2.93	12.55	0.23	0.82
Alb	(Intercept)	16.92	1.19	14.26	<0.01
	V1	7.55	3.57	2.11	0.04
	LocationPort Hedland	9.49	2.55	3.73	**<0.01**
	V1:LocationPort Hedland	-22.35	8.21	-2.72	**0.01**
	(Intercept)	15.46	0.99	15.56	<0.01
	V2	-7.85	5.01	-1.57	0.13
	LocationPort Hedland	7.25	1.53	4.75	**<0.01**
	V2:LocationPort Hedland	0.55	7.05	0.08	0.94
Glob	(Intercept)	20.03	1.24	16.11	<0.01
	V1	3.63	3.74	0.97	0.34
	LocationPort Hedland	6.96	2.67	2.61	**0.01**
	V1:LocationPort Hedland	-12.09	8.60	-1.41	0.17
	(Intercept)	19.35	1.00	19.41	<0.01
	V2	-5.91	5.03	-1.18	0.25
	LocationPort Hedland	5.47	1.53	3.57	**<0.01**
	V2:LocationPort Hedland	3.47	7.07	0.49	0.63
Na	(Intercept)	151.72	1.25	121.86	<0.01
	V1	10.27	3.75	2.74	0.01
	LocationPort Hedland	-0.03	2.67	-0.01	0.99
	V1:LocationPort Hedland	-18.88	8.61	-2.19	**0.04**
	(Intercept)	149.69	1.09	137.03	<0.01
	V2	-5.19	5.51	-0.94	0.35
	LocationPort Hedland	-0.13	1.68	-0.08	0.94
	V2:LocationPort Hedland	0.42	7.75	0.05	0.96
K	(Intercept)	6.54	0.33	20.11	<0.01
	V1	0.63	0.98	0.64	0.53
	LocationPort Hedland	3.75	0.70	5.37	**<0.01**
	V1:LocationPort Hedland	-9.04	2.25	-4.02	**<0.01**
	(Intercept)	6.41	0.28	22.97	<0.01
	V2	0.09	1.41	0.06	0.95
	LocationPort Hedland	1.78	0.43	4.16	**<0.01**
	V2:LocationPort Hedland	-4.66	1.98	-2.35	**0.03**

6.5 Discussion

A major finding in this investigation was the correlation between intestinal microbiota composition and PCV, heterophils, uric acid, calcium, total protein, albumin, globulin, and potassium. Further, we detected significant differences in a number of blood analytes between the two populations of flatback turtles (Table 6.1). We discovered that an increase in the bacterial OTUs 668479 (p_Firmicutes, f_*Planococcaceae*), 161559 (p_Firmicutes, g_*Bacillus*), and 746934 (p_Firmicutes, *Paenibacillus paenibacilluschondroitinus*) was correlated with a decrease in PCV, uric acid, total protein, albumin, globulin, and potassium in animals from Port Hedland (Figure 6.4 and Table 6.3). In contrast, an increase in 104 different OTUs was correlated with an increase in calcium and heterophils in Port Hedland animals (Table 6.3). The observed correlation between microbiota and PCV may be attributable to bile acid production, secondary to the periods of complete or reduced fasting that sea turtles undergo during the breeding and nesting phases of their life (Hays *et al.* 2002). In mammals, periods of inappetence, such as during hibernation, results in a decrease of expression of the rate limiting enzyme of bile acid production CYP7A1 in the liver of hibernating animals (Fedorov *et al.* 2011; Otis *et al.* 2011; Sommer *et al.* 2016). Given that the microbiota contributes to bile acid production, this decreased bile acid regulation may result in a relative increase in circulating bile acids (Sayin *et al.* 2013). Certain bile acids, such as deoxycholic acid, and lithocholic acid, both of which are dependent on the microbiota, are known to have haemolytic activity, which may result in destruction of red blood cells, thus lowering PCV (Sommer *et al.* 2016). Differences in microbiota observed between the two populations may have influenced bile acid production, and as a corollary, PCV in this study. We were not able to measure bile acids, nor were we able to assess if there were differential foraging efforts between animals of the two populations during nesting. Furthermore, no data exists for PCV levels of foraging flatback turtles, so we were unable to determine if the PCV in our results were different than would be expected in non-breeding turtles.

A second explanation for differences in PCV may be attributed to hydration. In comparison to Crab Island animals, Port Hedland flatbacks had a relative increase in PCV and total protein, which may be an indication of mild dehydration in these animals (Martinez-Jimenez and Hernandez-Divers 2007). In humans, the microbiota has been shown to influence the cellular transport of solutes through the gut mucosa and contribute to the hydration of individuals, due to changes in plasma osmolality (Mach and Fuster-Botella 2017).

During this study we noted a significant correlation between microbiota composition and total protein, albumin, and globulin in individual turtles. In humans suffering from primary biliary cirrhosis, alterations in the microbiota are implicated in changes to total protein and globulin, as well as a range of liver-specific enzymes (Lv *et al.* 2016). Superficially, there was no evidence that any of the turtles in either of our study sites were obviously unwell, but we did not perform any liver-specific functional testing. The role that the microbiota plays in hepatic protein synthesis and metabolism in sea turtles remains unknown. Related to the observed alterations in serum proteins in this study, was the correlation with microbiota composition and uric acid concentration. The role that both the small and large intestinal microbes play in dietary protein metabolism are well known (Zhao *et al.* 2019). Secondary metabolites from bacterial degradation of peptides is dependent on the type of proteins ingested, as well as the composition of the intestinal microbial community (Zhao *et al.* 2019). In chelonians, the end product of purine metabolism may be a combination of ammonia, urea and uric acid, with the proportion of each metabolite -dependent on species and life history (Moyle 1949; Scheelings 2019). Hyperuricaemia is associated with alteration of the gut microbiota in mice (Yu *et al.* 2018), and it is possible that differences in microbial communities between flatback turtle populations may account for differences in uric acid levels. We did not assess microbial function and protein metabolism as a component of this study, but the role that the microbiota plays in protein degradation in sea turtles warrants further investigation, and may also explain the correlations that we observed with total protein, albumin, and globulin.

Hyperkalaemia is commonly associated with chronic renal disease in humans, and may be exacerbated by alterations in the microbiome (Vaziri 2016). Although we saw a correlation between microbiome and potassium levels in flatback turtles, assessing renal function in chelonians is difficult without the use of endoscopic biopsies (Hernandez-Divers 2004) or other techniques beyond the scope of this study. The blood parameters reported in this investigation are the first reported for the flatback turtle and determining what is normal for blood potassium levels is difficult, so it may be that animals from Crab Island are hypokalaemic.

We found that microbiota composition was correlated with absolute heterophil and basophil numbers. In mammals, the function of the immune system is intrinsically linked to the microbiota due to the immunomodulatory effects of a number of bacterial metabolites, such as, short-chain fatty acids (Trompette *et al.* 2014), bacterial polysaccharides (Mazmanian *et al.* 2005), and aryl hydrocarbon receptor ligands (Rooks and Garrett 2016). These metabolites are important regulators of haematopoiesis (Khosravi *et al.* 2014), they promote immune cell emigration (Shi *et al.* 2011), guide lymphoid organogenesis (Mazmanian *et al.* 2005), control inflammatory responses (Trompette *et al.* 2014), and directly inhibit pathogens (Rooks and Garrett 2016). There have been no investigations into the role that microbial metabolites play in reptilian immune development, but it is likely that similar relationships exist in all vertebrates, based on the highly conserved nature of immune function in animals (Zimmerman *et al.* 2010). Alterations in the relative abundance of bacterial phyla within the microbiota of mice have been associated with increased risk of inflammatory disease (Trompette *et al.* 2014), and deficits in innate immune cell populations (Khosravi *et al.* 2014). Therefore, it is plausible that the relative increase in *Firmicutes*, associated with the decrease in circulating heterophils and basophils in animals from Port Hedland, may be due to inhibition of cell maturation, or egression from the bone marrow due to alterations in bacterial metabolite concentrations. The role that the microbiota plays in reptile immunity warrants further investigation and is an important step in understanding the vertebrate-microbiota relationship and its evolution.

We detected a difference in stress indicators between the two populations of turtles, with animals from Crab Island having a higher H:L ratio than animals from Port Hedland (p=0.015, Table 6.1). Some researchers have shown that specific bacterial populations may exacerbate or dampen the stress responses in mice, and that it may be controlled with supplementation of probiotics (Dinan and Cryan 2012). However, in our data we did not find any correlation between bacterial OTUs and stress, and interpreting indicators of stress in wild animals is complex (Johnstone *et al.* 2012). The observed differences in our study may be due to the presence of saltwater crocodiles (*Crocodylus porosus*) in the water and on the beaches of Crab Island, which are predators of both adult turtles and hatchlings and have been reported to occur over the nesting season on Crab Island (Sutherland and Sutherland 2003). While there are no specific investigations exploring the effects of crocodile predation on sea turtle physiology, a number of studies have shown that sea turtles will compromise foraging strategies in an effort to avoid shark predation (Heithaus *et al.* 2008). In a situation such as nesting on a remote island, behavioural modifications to avoid aggregations of predators are not possible, and so sea turtles are forced into a situation where they are in constant close proximity to these predators. This type of interaction has been shown to be a key stressor in a number of vertebrate species (Boonstra and Fox 2013), and is likely to be having a similar effect on the turtles in this study..

Calcium levels did not differ significantly between the populations of turtles (p=0.87, Table 1), but we observed a significant correlation between calcium and microbiota between the different turtle populations. In mice and humans, bacterial genera such as *Lactobacillius*, *Bifidobacterium*, *Prevotella*, *Bacteroides*, and *Clostridium* have all been shown to impact calcium metabolism (Skrypnik and Suliburska 2018). We identified a range of OTUs belonging to *Clostridium, Prevotella* and *Bacteroides* (Supplementary Table S5), but none of these were associated with calcium levels in the turtles. Given that *Lactobacillus* and *Bifidobacterium* are common inhabitants of the human microbiota (Turroni *et al.* 2013), and the vast physiological differences that exist between endotherms and ectotherms, it may be that reptiles have specific bacteria specially adapted to mineral metabolism that better suits their unique physiological requirements.

We provide preliminary correlative data to show that microbiota may be having an influence on blood parameters in a reptilian species. A manipulative study involving adult sea turtles eating a natural diet would not be possible because of the life-history traits and environmental needs of these species, so much of our interpretation is by necessity drawn from human and laboratory animal research. Inter-species interpretation of microbiota studies is frequently used in the human field, when it may be impractical or unethical to use human subjects. The breadth of study species utilised in these experiments is wide, and may include fish (Lescak and Milligan-Myhre 2017), mice (Nguyen *et al.* 2015), rats (Alpert *et al.* 2008; Wos-Oxley *et al.* 2012; Liou *et al.* 2013), guinea pigs (Hildebrand *et al.* 2012), dogs (Middelbos *et al.* 2010), pigs (Heinritz *et al.* 2013), chimpanzees (Moeller *et al.* 2012), and macaques (McKenna *et al.* 2008). Such a diversity of model species spans a significant proportion of the evolutionary tree, yet they are considered valid experiments as there appears to be some conservation of basic microbe-host interactions among vertebrates (Ley *et al.* 2008). Therefore, in the absence of more specific, chelonian-derived data, we feel that it is appropriate to discuss the results of our investigation using similar experiments. What we provide is associative rather than categorical evidence, but we hope that it will lead to future investigations in this field. Furthermore, it is critical to understand that we did not undertake a thorough health assessment of the turtles used in this experiment. While haematology and biochemistry can be useful tools in assessing the health of individual chelonians (Heatley and Russell 2019), their value is dependent on the establishment of robust reference ranges that take into consideration factors such as sex, time of year, reproduction, and nutrition (Campbell and Ellis 2007; Heatley and Russell 2019). To our knowledge, the blood parameters presented in this paper are the first to have been reported for the flatback turtle, and given that they were collected at a very specific, single point in time, they cannot be used as an accurate measure of health. This is especially pertinent as it has been shown that in some chelonian species maternal health may be sacrificed in preference to reproductive output (Rafferty *et al.* 2014). Finally, we could not categorise all of the possible environmental and other external factors that may be exerting their effects on the physiology and microbiotas of the turtles in this investigation, as this would not be possible for any wild animal study.

In this investigation we show results that indicate that microbiota composition may play a part in determining some of the blood parameters of flatback turtles. The role that specific OTUs play in modulating inflammation, immune maturation, protein and mineral metabolism, and overall health of reptiles is an area that requires further study. This research highlights the importance of interpreting both blood parameters and microbiota in the context of locality. Not only should future investigations focus on the role that the microbiota plays in the health of sea turtles, but also consider if these results may act as sentinels for ecosystem health.

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Chapter 7. General Discussion

7.1 Summary of major findings

The aims of the studies described in this thesis were to gain a better understanding of the role that the microbiota has played in vertebrate evolution, how it affects sea turtle physiology, and explore factors that may influence the microbiota in sea turtles. Through this work I made new discoveries on factors that influence the microbiota composition of vertebrates, how the microbiota of sea turtles differs between species and how sea turtles acquire their microbiotas. I also showed that the microbiota of sea turtles differs between geographically distinct populations of the same species, and that the microbiota may have an influence on blood parameters of sea turtles. Collectively, my research showed that the microbiota of sea turtles plays a pivotal role in physiology and understanding its importance may play a key role in sea turtle conservation. How each experimental chapter (Chapters 2-6) addressed my general aim and the specific aims of each chapter is displayed in Figure 7.1. A brief summary of the major findings of each chapter is presented below.

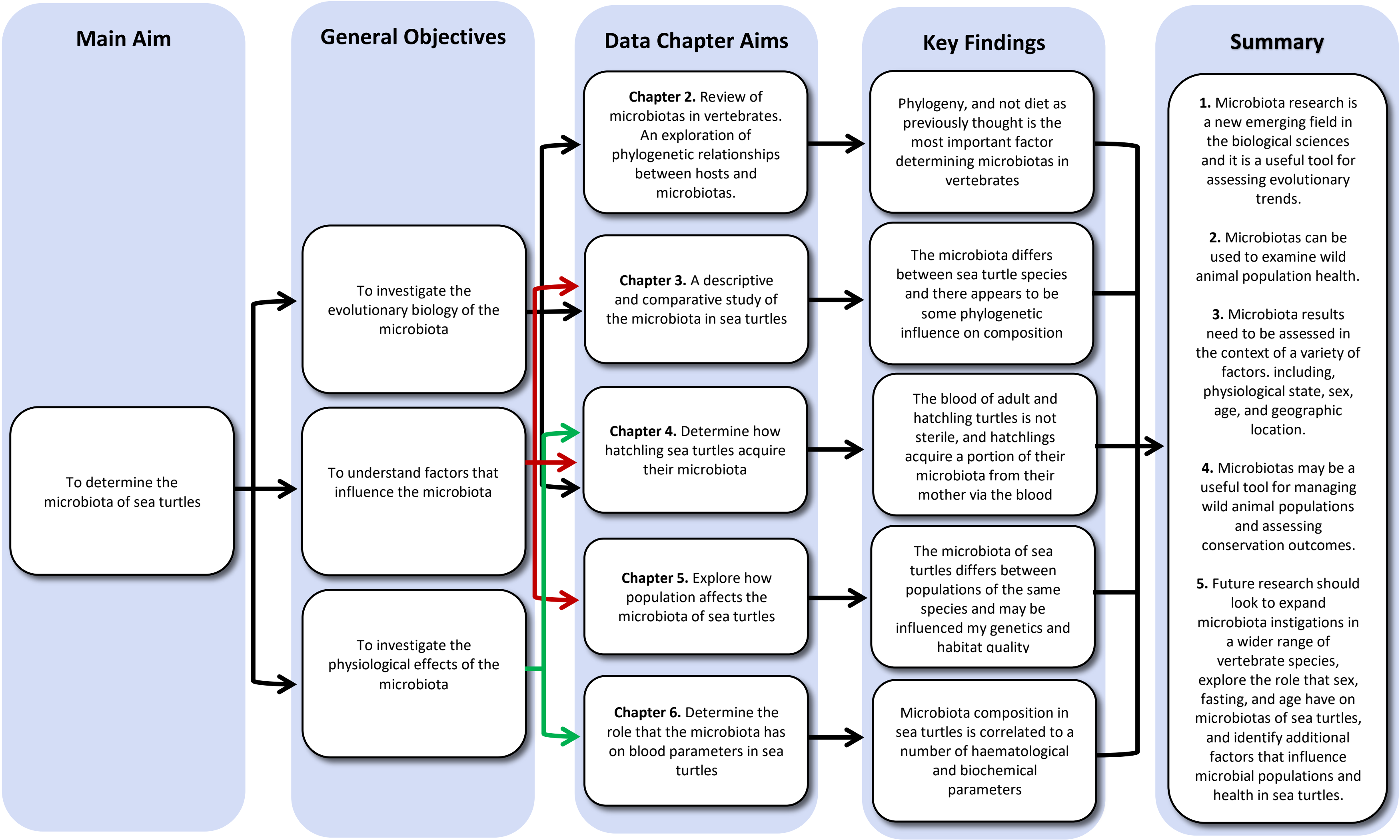

Figure 7.1 Research aims, general structure and key findings for thesis

7.1.1 Vertebrate host microbiota is determined by phylogenetic history (Chapter 2)

Historically, studies into the microbial communities of vertebrates have focused on the role that diet plays in convergence of the microbiota across various vertebrate taxa (Ley *et al.* 2008a; Ley *et al.* 2008b; Muegge *et al.* 2011; Delsuc *et al.* 2014). However, these investigations typically do not take into consideration host evolutionary history in their analysis. This is an important oversight because microbes are considered to be a key driver of vertebrate evolution (Lee and Mazmanian 2010; McFall-Ngai *et al.* 2013), and so they should not be considered separate from the host genome, but rather as an important component of whole organism phylogeny. Although some investigators have suggested that phylogeny may play an important role in determining vertebrate microbiotas (e.g. Amato *et al.* 2018), these studies are limited to a small number of species, with relatively close taxonomic relationships. The results of my analysis indicate that when microbiota composition is combined with host phylogeny over a broad taxonomic range, as well as incorporating basic natural history data, an animal's evolutionary history is the most important factor in determining gastrointestinal microbial communities. These results indicate that vertebrates have co-evolved with their microbial inhabitants, and that microbiome community composition within a species is largely evolutionarily determined, regardless of diet or other natural history factors. Further, my results suggest that the influence of diet on the microbiome is likely to be at the individual organism level, rather than at a broader taxonomic level. Interestingly, I also found that of the more than 50 bacterial phyla described to date (Yarza *et al.* 2014), community composition is dominated by relatively few groups, that are common to all vertebrate taxa.

7.1.2 The microbiota differs among sea turtle species (Chapter 3)

This chapter forms the largest investigation into the microbiota of sea turtles to date, and is one of the most complete microbiota studies of any taxon, as I was able to obtain samples from all extant species and thus have sampled an entire clade of the evolutionary tree. I showed that microbiota composition differs among sea turtle species, but in all species, it is dominated by the bacterial phyla Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. I speculated that composition of the microbiota in sea turtles may be related to phylogenetic history, although this requires further investigation. These preliminary results provide a sound platform from which to launch a number of further investigations into the microbiota of sea turtles, as described in section 7.3 below.

7.1.3 Sea turtles acquire their proportion of their microbiota from their mothers (Chapter 4)

Acquisition of the microbiota is the critical first step in developing the host-microbiota relationship. For those species dependent on parent-offspring transfer of microbiota, the failure to do so may have lasting effects on the intestinal bacterial composition into later life, with important ramifications for immune system development and other fitness consequences (Torrazza and Neu 2011; Taschuk and Griebel 2012). In many species, due to the peculiarities of their natural history, there are multiple opportunities for the transmission of the microbiota to their offspring during birth or through subsequent parental care. However, in sea turtles, which are oviparous, and provide no parental care (Rafferty *et al.* 2013), there are fewer options. I showed that hatchling turtles emerge from the egg with a microbiota that partially resembles that of the mother, that this microbiota is consistent among an entire nest but that the environment in which the eggs are incubated may have some effect on its composition. This is an indication that the microbiota of hatchling sea turtles is predetermined at some point prior to oviposition on the nesting beach. Furthermore, I showed that the blood of mother turtles is not sterile, and is actually populated by a range of microbes. It is possible that mother turtles use these circulating bacteria to seed developing embryos while in utero, prior to oviposition. This ensures transgenerational transmission of the microbiota and means that hatchling turtles emerge from the egg with a microbiota that is maternally derived, rather than by less predictable events. This is the first time that this mechanism of vertical transmission of the microbiota has been shown to occur in any vertebrate species and improves our knowledge of the role that microbes have played in vertebrate evolution.

7.1.4 Population and genetics influence the microbiota composition of sea turtles (Chapter 5)

While it is widely accepted that the gut microbiota of humans varies due to geography (Yatsunenko *et al.* 2012), such analyses are less common in wild animal populations. My analysis of the microbiota of wild nesting sea turtles, is the first of its kind in reptiles, and indicates that microbial populations differ significantly in both composition and diversity among animals of the same species that originate from different populations. This chapter highlights the importance of interpreting microbiota data in concert with environmental factors and potential genetic differences among populations. Factors that may be influencing microbiota composition in sea turtle populations include genetics, environment and physiology but they may also co-vary, making identification of the most important factors difficult.

7.1.5 The composition of the microbiota is correlated with a number of haematology and biochemistry values in nesting sea turtles (Chapter 6)

Investigations into how the microbiota affects the health of humans have shown that microbes are important for priming and directing maturation of the immune system (Mazmanian *et al.* 2005), and contributing to the function of both the innate and adaptive immune systems (Lee and Mazmanian 2010; Thaiss *et al.* 2016). However, outside humans, studies exploring these relationships are rare. In this chapter I showed that differences in microbiota composition are correlated with differences in blood parameters in two populations of nesting flatback turtles. The data outlined in this study is preliminary, and an important first step in closing the knowledge gap on the role that microbiota plays in wild animal health. I found that the microbiota composition had a correlation with a number of analytes in animals from Port Hedland including packed cell volume, heterophils, uric acid, calcium, total protein, albumin, globulin, and potassium. A number of operational taxonomic units were identified to be contributing to this correlation. Additionally, a correlation was seen in heterophil/lymphocyte ratio, and basophils, associated with microbiota composition in Port Hedland animals, but no specific OTUs could be identified to be significantly contributing to these observations. This study is the first of its kind in reptiles and highlights the importance of the link between microbiota and physiology in this taxon. Further investigation is required to determine the exact mechanisms by which the microbiota exerts these effects in reptiles.

7.2 Significance of results

A growing understanding of the microbiota is changing our definition of metazoan life. No longer can we consider animals separate from the microbial world, but more as an aggregation of trillions of organisms, working in concert to ensure mutual survival and propagation. The first multicellular collaboration, over 2 billion years ago (Hedges *et al.* 2004), was a defining moment in the history of Earth's biodiversity, and is directly responsible for the assortment of form and function we see today. Unravelling the pivotal role that microbes have played in shaping the course of our planet's extraordinary natural history, is an exciting and novel frontier in the biological sciences. It is only by recognising how all life is bonded, regardless of how seemingly insignificant it is, that we can truly hope to preserve what persists today. This is especially important in the face of modern mass climactic upheaval, in which all living creatures face unprecedented challenges.

The results outlined in the various chapters of this thesis contribute significantly to our knowledge of how the microbiota has shaped vertebrate evolution and ecology. While I elected to focus on sea turtles as a model taxon, the findings outlined in each of my experiments may be applicable to an array of vertebrate species. In Chapter 2, I showed that vertebrate microbiota composition is largely shaped by phylogenetic past. This was further reinforced in Chapter 3, by the finding that although there are differences in microbial composition between sea turtle species, ultimately it appears that there is phylogenetic signal driving these microbial community structures. Importantly, in Chapter 4, I demonstrated that a portion of the microbiota in sea turtles is heritable, further confirming the role that genetic history has in regulating the microbial communities of vertebrates. In Chapter 5, I showed that geographic location plays a role in sea turtle microbiotas, which may be mediated by genetic differences. Finally, in Chapter 6, I correlated microbial composition with health parameters in sea turtles, further strengthening the theory that microbes have played a fundamental part in determining vertebrate physiological function.

Investigations such as those presented in this thesis are essential to future conservation efforts (West *et al.* 2019), but in comparison to human studies, research into wild animal microbiotas are scant. Given the importance of microbes in both an individual sense, as well as at a population level, a thorough understanding of the normal microbiota of wild animal hosts is fundamental in assessing population health (Redford *et al.* 2012; Bahrndorff *et al.* 2016; West *et al.* 2019). In addition to describing inter-individual and inter-species variation in microbial species, consideration also needs to be given to functional variation, as this is likely to be a more meaningful measure of host health (West *et al.* 2019). By creating a catalogue of host microbiomes, ecologists and conservation biologists can then identify instances of community collapse and dysbiosis, which may be used as a detection system for potentially deleterious physiological derangements within animal populations (Apprill 2017). Furthermore, such information may also prevent the future mis-identification of isolates as being potentially abnormal, and only present as pathogens, while in reality, they may be part of the natural microbiota of particular animals. Incorrectly labelling such isolates as harmful, and attempting to treat or eliminate them from the microbiota, could unnecessarily halt conservation efforts (Shaw *et al.* 2014).

7.3 Limitations and directions for future work

During the course of my study there have been many ideas and questions regarding the microbiota not only in sea turtles, but in vertebrates in general, that I could not investigate. The following section outlines some of the limitations of my observations and experiments as well as some directions for future investigations.

7.3.1 Deciphering evolutionary trends in vertebrate microbiotas is hampered by a lack of data (Chapter 2)

I showed that the most important factor in determining vertebrate microbiotas was phylogeny, and not diet as previously thought. However, the analysis of this investigation was completed with relatively few representatives of the diversity of vertebrate life. In this analysis I examined the microbiotas of 203 vertebrate species, yet it is estimated that there are more than 66,000 extant vertebrate species in the world (IUCN 2019). Many of the species included in this analysis were chosen by investigators for microbiota studies because they are either charismatic species, or animals that are easily captured, thus potentially skewing the representativeness of the sample considerably. This means that the available data is deficient in a number of important taxa including Chondrichthyes (rays and sharks), Amphibia (amphibians), Reptilia (reptiles), Palaeognathae (ratites), Psittaciformes (parrots), Strisores (nightjars), Columbaves (pigeons and relatives), Mirandornithes (flamingos and grebes), Accipitriformes (hawks and relatives), Afroaves (owls, kingfishers, woodpeckers, etc.), Monotremata (echidna and platypus), Eulipotyphla (moles, shrews, hedgehogs, etc.), Lagomorphs (rabbits), and Pholidota (pangolins). It is plausible that by adding species from this list that the results of my analysis might change. Therefore, it should be a priority of future microbiota investigations to attempt to obtain samples from each of these major clades.

A second major limitation of this review was the level of detail of microbiota reporting available in the literature. Most investigations only reported the major bacterial phyla identified, with relatively few describing bacterial genera or OTUs. This meant that only a limited analysis could be conducted and it is possible that the effects of diet etc., may be more profound if investigations focused at lower taxonomic levels with respect to both microbiota and host. However, despite these limitations, seeing such a strong phylogenetic signal, across a broad taxonomic scale, is a convincing indication of the validity of these results.

7.3.2 Impact of life-stage, and sex on microbiotas of sea turtles (Chapter 3)

I identified that microbiotas differ among the seven extant sea turtle species. However, a limitation of this investigation was that I was only able to sample nesting female turtles. I chose to focus on this cohort because of the predictability of nesting in sea turtles, in order to maximise my chances of obtaining sufficient samples for meaningful results. Most importantly, future investigations should focus on obtaining samples from male turtles to compare with females, and improve our understanding of sea turtle microbiotas and the evolutionary forces which shape them. Additionally, sampling animals from a broader range of life-stages, i.e. from hatchling, to juvenile and then to adult, would give greater insight into how the microbiota changes with the ecological and physiological demands of sea turtles over the course of their lives. This would be especially useful in the green turtle, which undergoes an ontogenetic dietary shift from hatchlings to adults (Arthur *et al.* 2008), and initial results have shown that their microbiota may change accordingly (Price *et al.* 2017).

7.3.3 Determining mechanisms by which sea turtles become bacteraemic (Chapter 4)

I showed that the blood of both mother sea turtles and their hatchlings is not sterile, and that bacteraemia may be a possible route for transference of a proportion of the maternal microbiota to offspring. How turtles become bacteraemic, and are able to maintain a population of circulating bacteria without becoming septicaemic, remains a mystery at this stage. Future investigations should focus on the methods by which selective bacteria enter the blood stream, and then are able to exist harmoniously within the host's circulatory system. It is also important to determine the step at which developing embryos become seeded with these blood-borne microbes. This is likely to only be possible by accessing fresh turtle carcasses, in order to sample the entire reproductive tract. A potential source of these may be animals killed by native hunters still living a subsistence lifestyle. It would also be interesting to investigate if mother turtles are persistently bacteraemic throughout life, or only during reproductive phases. Additionally, it should also be investigated whether or not adult male turtles harbour haematogenous bacteria in a similar manner to females and therefore whether bacteria in the blood stream serve a solely reproductive function or whether there may be additional reasons for this phenomenon

7.3.4 Identify additional factors involved in determining microbiotas of sea turtles (Chapter 5)

I showed that geographic locality had a major effect on microbiota composition in sea turtle populations. A major factor that should be the focus of future studies is the effect of fasting on sea turtle microbiotas. Sea turtles may undergo long periods of fasting, particularly during breeding and migration (Hays *et al.* 2002; Jessop *et al.* 2004), and prolonged periods of inappetence have been shown to have a significant effect on the microbial communities of a variety of species including the Burmese python (*Python molurus*) (Costello *et al.* 2010), humans (Remely *et al.* 2015), fish (Xia *et al.* 2014), brown bears (*Ursus arctos*) (Sommer *et al.* 2016), alligators (*Alligator mississippiensis*) (Keenan *et al.* 2013), mice (Beli *et al.* 2018), and penguins (*Eudyptula minor* and *Aptenodytes patagonicus*) (Dewar *et al.* 2014). These differences have been shown to alter physiological function in animals, and thus similar observations may be made in sea turtles in response to feast or famine. Additional factors that should be investigated include sex, captivity, hospitalisation and rehabilitation of sick or injured turtles, disease, and human disturbance as all have been shown to influence microbiotas and physiology in a range of species (Sekirov *et al.* 2010; Amato *et al.* 2013; Nelson *et al.* 2013).

7.3.5 Examining the biochemical pathways by which microbes influence sea turtle health (Chapter 6)

I demonstrated that sea turtle microbiotas are correlated with a variety of basic health indices in sea turtles. However, determining the exact biochemical pathways by which microbes exhort these effects was beyond the scope of this investigation. Future studies should focus on exploring these relationships to gain a better understanding of the OTUs responsible for critical physiological functions and the implications for the host in their absence. In particular, future investigations should focus the role that the microbiota of sea turtles has on metabolites such as short-chain fatty acids, bile acids, choline metabolites, phenol derivatives, indole derivatives, vitamins, polyamines, and lipids (Nicholson *et al.* 2012).

7.4 Conclusions

Fundamental to the existence of metazoan life on planet Earth has been the collaboration between microbe and host. Extricating the intricacies of these relationships, and the key role that they have played in driving evolution, are essential to understanding the origins of all multicellular organisms. In this age of mass extinction, a reflection on our beginnings, and the forces that helped to shape such extraordinary biodiversity, may provide insight into how species will adapt to widespread, anthropomorphic driven change. Recognising the importance of the symbiosis between the micro and macro world, and preserving biodiversity on all scales, has never been more important in conservation efforts. The outcomes of this thesis have further highlighted the vital role that microbes have played in the evolution and physiology of endangered species, and it is hoped that these results will be able to assist in preserving sea turtles for future generations to appreciate.

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