Microbiomes of native Aotearoa New Zealand animals

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Abstract: Microbiome research is revealing the profound effects that microbial inhabitants can have on their animal hosts. Recent and rapid advances in sequencing technologies have allowed biologists to characterise the microbial constituents of a variety of host organisms, giving greater insights into these intimate relationships than ever before. For many animal species, microbiomes serve as an interface between host and environment, with associated microorganisms playing functional roles in nutrition, immunity, reproduction, and even behaviour. In this Review, we offer a brief overview of microbiome research methodology before summarising previous and ongoing research into the microbiomes of native New Zealand animals. Our unique endemic fauna, evolved during tens of millions of years of geographic isolation, offers exciting opportunities for microbiome research across a range of diverse taxa and we highlight key findings of relevant studies. Moreover, while recognising the crucial role that 16S rRNA gene sequencing plays in microbiome research, we conclude the Review with a look beyond 16S and consider what other technologies can bring to this field. We encourage further investigation into the functional roles of microbial species across a broader range of host-animal taxa across New Zealand, both in wild and captive states.

Keywords: 16S rRNA, animal host, bacteria, fungi, microbiota, microbiology

Introduction

Animals and microorganisms share a long evolutionary history. A vast diversity of microorganisms existed long before complex multicellular animals appeared on earth; the emergence of these new host species provided microbial life with novel ecological niches to occupy. As we continue to expand our awareness of interactions between animals and microorganisms it has become necessary to rethink certain ideas surrounding animal biology and what it means to be an animal (McFall-Ngai et al. 2013; Rees et al. 2018). With a few notable exceptions (Hammer et al. 2019), today’s animals do not exist as single entities; rather, they may be considered as “metaorganisms” comprising their own animal cells plus those of their associated microbial communities (Bosch & McFall-Ngai 2011). The communities of microbes that inhabit specific regions of an animal host are commonly referred to as microbiota. Potentially comprised of bacteria, archaea and viruses, as well as microbial eukaryotes such as fungi and protozoa, these microorganisms colonise a variety of tissue types both within and on the surfaces of host animals. Within humans, undoubtedly the best-studied of all animal hosts (Human Microbiome Project Consortium 2012; Gilbert et al. 2018), by far the largest abundances of microorganisms are in the large intestine, where they contribute to the breakdown of otherwise indigestible dietary components. Other human-associated niches, including the oral cavity, sinuses, skin, small intestine, and even the healthy lung, also appear to harbour resident microorganisms (Hoggard et al. 2017; Moffatt & Cookson 2017; Byrd et al. 2018; Verma et al. 2018; Kastl et al. 2020). Another term that is often—incorrectly—used interchangeably with microbiota is the microbiome. The microbiome encompasses the microbiota itself plus its “theatre of activity”, i.e. structural elements (such as nucleic acids, proteins and lipids), microbial metabolites, as well as the surrounding environmental conditions (Berg et al. 2020). Collectively, the members of an animal’s microbiome confer upon the host an enormous genomic and metabolic repertoire that may greatly exceed the capabilities encoded within its own genome (Bordenstein & Theis 2015). Animal-associated microbiota are thus not mere idle bystanders, passively collecting within and upon animal hosts, but may contribute substantially to nutrition and metabolism, defence against pathogens, priming of the immune system, and even influencing animal behaviour (Zhu et al. 2011; Ezenwa et al. 2012; Desbonnet et al. 2014; Colombo et al. 2015; McLaren & Callahan 2020).

While the microbiome field has understandably focused extensively on humans (Gilbert et al. 2018), dramatic reductions in the costs of molecular techniques used in these studies have facilitated the inevitable expansion into non-human microbiome research. Aotearoa New Zealand is no exception, with this expanded focus encompassing numerous studies of our native animals and their microbiomes. With some 80 000
endemic species of animals, plants and fungi, New Zealand has been described as a biodiversity hotspot (Myers et al. 2000). This high level of endemism reflects c. 80 million years of geographic isolation and is characterised by lineages of animal taxa in New Zealand that often hold particular evolutionary importance (Gibbs 2006). For example, the predominance of terrestrial mammals found across Earth is not mirrored in New Zealand. With only two species of bat as terrestrial mammalian representatives, many ecological niches typically occupied by mammals elsewhere are instead taken by birds, reptiles or even insects. In assuming these roles, many of New Zealand’s native animals have acquired adaptations and appearances that are bizarre in comparison to other animals across the globe. Indeed, our unique fauna was once described by Jared Diamond as “the nearest approach to life on another planet” (Gibbs 2006, p. 7). Evolved in New Zealand are flightless birds such as the iconic kiwi (genus *Apteryx*) and enigmatic kākāpō (*Strigops habroptilus*), the living reptilian relic tuatara (*Sphenodon punctatus*), and the entirely terrestrial Archey’s frog (*Leiopelma archeyi*) all of which garner national and international attention from biologists (Fig. 1). Studying the microbiomes of native New Zealand animals may, therefore, present a unique opportunity to describe communities of microorganisms that conceivably differ from those described elsewhere. With continuing loss of animal biodiversity an ever-present threat in New Zealand as elsewhere (Hare et al. 2019; WWF 2020), there additionally comes a certain pressure to describe these microbiomes before the opportunity to do so no longer exists.

The purpose of this review is to describe previous and ongoing research into the microbiomes of native New Zealand animals. We acknowledge, but do not cover here, the outstanding research undertaken in this country on animals of agricultural importance, such as that focusing on methane mitigation in cattle and sheep (e.g. Jeyanathan et al. 2011; Seshadri et al. 2018). Where applicable, we do touch upon selected studies of introduced wild animals, albeit only briefly and mainly to alert the reader to the existence of this work. At the end of the article, we highlight which New Zealand animal taxa have received the most research attention to date from microbiome scientists, and suggest potential foci for future research endeavours.

**How to study the microbiome**

**Sampling**

One of the first stages in any microbiome study is to obtain a suitable sample(s) from the host animal. However, a crucial step that must often precede sampling is engagement with conservation authorities and iwi (Māori) interests, with many native species holding a taonga (prized treasure) status among Māori. Sampling of native vertebrate species, or any sampling on conservation land, requires prior permission from the Department of Conservation (DOC). In accordance with Aotearoa New Zealand’s Te Tiriti o Waitangi (Treaty of Waitangi) obligations, the DOC process also involves

![Figure 1. A selection of native New Zealand animals for which the microbiome has received research attention. Clockwise from top left: the marine sponge *Raspailia topsenti*, North Island brown kiwi (*Apteryx mantelli*), silver drummer (*Kyphosus sydneyanus*), Archey’s frog (*Leiopelma archeyi*), honeydew-producing native scale insect (species uncertain), adult kākāpō with egg (*Strigops habroptilus*). Photo credits: sponge: M. Taylor; kiwi: Maungatautari Ecological Island Trust; silver drummer: K. Clements; frog: *Leiopelma archeyi* (Green, 2010) CC BY-SA 2.5; scale insect: M. Taylor; kākāpō: A. Digby.](image)
consultation with relevant iwi for informed decision making. The building of genuine, enduring relationships between researchers and iwi is increasingly recognised as a crucial step in the conduct of microbiome research in this country, as well as a growing requirement of national research funding agencies. Addressing issues surrounding the sovereignty of genomic (including microbiome) data of relevance to Indigenous populations has become increasingly complex due to the relative ease of generating such data and the requirements of many journals and funders to make all data available (Hudson et al. 2020).

Whilst sampling specifics will inevitably depend upon the aims of the intended work as well as the identity of the target animal, there are some general considerations for any such study. Avoiding contamination from environmental microbes by sampling as aseptically as possible is a key step, albeit a challenging one when sampling in remote environments or underwater. Preservation and storage are also important considerations, with numerous studies highlighting variation in results arising from different preservation methods (Song et al. 2016; Marotz et al. 2021). For field studies the analysis of fresh samples is typically not feasible, thus alternatives such as freezing in liquid nitrogen or immediately immersing the sample in a fixative (e.g. 95% ethanol) or bacteriostatic agent (e.g. RNA later) should be considered. Irrespective of the method of choice, it is imperative that conditions be kept consistent across all samples. Sample replication in space and time is another key consideration, albeit an area in which microbial ecologists have not always excelled (Prosser 2010). Fortunately, the ready availability of affordable analysis techniques means that today there is little excuse for inadequate replication and sampling design – notwithstanding access to the respective host animal(s).

The majority of microbiome studies on vertebrate hosts focus on the gastrointestinal tract (gut). This is due to both the recognised importance of gut microbes to animal health, as well as the relative ease of sample collection. Faecal samples are widely used as a proxy for the gut microbiota: sampling is non-invasive (of particular importance for research on threatened species) and repeated sampling of a given individual is possible in longitudinal studies. On the other hand, while faecal material may provide a reasonable representation of microbes from the large intestine (Yasuda et al. 2015) it does not necessarily reflect the small intestine microbiota, nor those microbes which are specifically associated with the gut mucosa (Ringel et al. 2015; Donaldson et al. 2016; Ingala et al. 2018). A somewhat more invasive means of gaining insights into the gut microbiota, at least for some animal taxa, is via swabbing of the cloaca, a shared reproductive and excretory chamber in birds, reptiles and amphibians. The extent to which faecal or cloacal swab samples best represent the intestinal microbiota is not clear and may differ depending on host taxonomy (Videvall et al. 2018; Zhou et al. 2020). Swabbing also provides a suitable mechanism for sampling other body sites of vertebrates, such as the skin, fur, feathers, oral cavity or the gills of fish (Avena et al. 2016; van Veelen et al. 2017; Emami-Khoyi et al. 2019; Ross et al. 2019; Clinton et al. 2021). When studying the gut, fish are often sampled destructively, with dissection following death upon catch (Moran et al. 2005; Clements et al. 2007; Kim et al. 2021).

Whether body sites other than the gut necessarily even harbour their own microbiome also warrants consideration (Hammer et al. 2019), with the study of low-microbial-biomass samples particularly fraught with methodological challenges (Marsh et al. 2018; Eisenhofer et al. 2019). DNA contamination of reagents or other laboratory sources may have a negligible impact on how we perceive the rich microbial communities of the gut, but can dramatically influence the microbiota profiles of samples with low starting concentrations of DNA (Salter et al. 2014). For example, laboratory contamination appears to account for earlier reports proclaiming the existence of a human placental microbiome (Lauder et al. 2016).

Invertebrate taxa may be sampled in various ways. Marine sponges, for example, do not possess true tissues or organs, enabling a subsample of an individual sponge to be taken without causing death of the organism. By contrast, studies of the insect microbiome typically involve the death and subsequent dissection of the animal in order to sample the gut (Colman et al. 2012; Dhami et al. 2012; Martinson et al. 2012; Reid et al. 2014; Waite et al. 2015). Sometimes, researchers must be creative in their efforts to obtain samples from elusive animals. For example, the collection of cetacean blow (exhaled breath condensate) has been achieved through the use of unmanned aerial vehicles (drones) (Apprill et al. 2017; Centelleghie et al. 2020).

Sample analysis – from lab bench to computer
The study of complex microbial communities underwent a revolution in the 1980s and 1990s with the advent of ground-breaking molecular biology techniques including ribosomal RNA (rRNA) gene sequencing (Lane et al. 1985), polymerase chain reaction (PCR) (Zhu et al. 2020), and fluorescence in situ hybridisation (FISH) (Wagner et al. 2003). Prior to these advances, microbial ecology research depended heavily on the isolation of environmental microorganisms in pure culture. However, given that many or even most bacteria have thus far resisted cultivation on artificial laboratory media (Amann et al. 1995), the use of cultivation-independent (molecular) techniques has provided a more complete picture of microbial diversity; particularly with the dramatically reduced costs associated with the rise of so-called next-generation sequencing since the mid-2000s (Sogin et al. 2006; Hu et al. 2021). As with sampling strategy, the method of choice depends on the research question being asked, with a plethora of options available to today’s microbiome researcher.

When describing the composition of microbial communities within any sample type, the initial step often involves the extraction and purification of DNA via mechanical, chemical or enzymatic lysis of microbial cells (Fig. 2). If bacteria and/or archaea are the intended target, the 16S rRNA gene will typically be amplified by PCR from the extracted DNA. The generated PCR products (amplicons) can then be sequenced and interrogated against freely available online databases to establish the identities of the organisms present. A parallel protocol, targeting the homologous 18S rRNA gene, or in some cases the internal transcribed spacer (ITS) portion of the genome, can be applied for eukaryotic microorganisms such as fungi or protozoa (Bharti & Grimm 2021). rRNA genes are present in all living organisms and contain both variable and conserved regions, making them attractive targets for comprehensive surveys of microbial diversity.

If one wishes to move beyond description of the microbiome (i.e. “who’s there?”) and gain insight into microbial function (i.e. “what are they doing?”), there are again many options available. While some inferences about microbial function can be made from rRNA amplicon sequencing (Langille et al. 2013), a more direct approach is to sequence the extracted DNA itself (i.e. the metagenome) (Fig. 2).
Figure 2. A simplified overview of experimental procedures used in microbiome studies. Cultivation studies use specific media to grow microorganisms in vitro for later phenotypic analysis. Cultivation-independent techniques allow identification of microbiome members by PCR amplification of target genes such as 16S rRNA genes. Functional profiling may be obtained from other -omics approaches, such as shotgun metagenomics, whereby DNA from the entire sample is randomly broken into small fragments for individual sequencing.

Each of the aforementioned approaches brings its own biases, including (but not limited to) differences in DNA extraction efficiency between different microbial cell types, PCR bias whereby the rRNA genes of some organisms are preferentially amplified over others (sometimes due to selection of PCR primers), and choice of database for assigning taxonomic identities to obtained sequence data (Pollock et al. 2018). Differences in methodological approaches among different research labs can make it difficult to compare between studies, and there are increasing calls for standardisation of methods within the microbiome field (Costea et al. 2017; Broderick et al. 2021; Bodawatta et al. 2022). While global initiatives such as the Earth Microbiome Project (Thompson et al. 2017) speak to the power of methods standardisation, one must also acknowledge the challenges with attempting to develop a one-size-fits-all approach; indeed, in the authors’ laboratory alone, several different DNA extraction protocols are used when working with different host animals. Though beyond the scope of this review to provide anything more than a cursory look at the methods available, we here draw...
Sponges and other marine invertebrates

With > 15 000 km of coastline, it is hardly surprising that marine animals have come to the attention of New Zealand’s microbiome scientists. Among marine invertebrates, sponges in particular have been the subject of much research interest. Sponges (Phylum Porifera) are an evolutionarily ancient taxon of filter-feeding benthic invertebrates with > 8 500 extant species (Van Soest et al. 2012). Though morphologically simple, sponges carry out a variety of key ecological functions including nutrient cycling, bio-erosion and the facilitation of primary production (Bell 2008). Many marine sponges harbour dense and diverse microbial communities, with microbial cells comprising up to 35–40% of total sponge volume (Fig. 3; Taylor et al. 2007; Hentschel et al. 2012). At least 60 bacterial and archaeal phyla, as well as eukaryotic microbes such as dinoflagellates and diatoms, have been described from sponges.
Research on New Zealand’s sponge microbiomes has tended to focus on the host specificity of microbial associates and/or the potential involvement of symbiotic microbes in the production of biologically active natural products. Regarding the former, the microbiomes of several local sponge species have been described, either in isolation (Kamke et al. 2010; Schmitt et al. 2011; Simister et al. 2013; Cardenas et al. 2014) or as part of more extensive global studies (Schmitt et al. 2012). New Zealand’s sponges contain similar bacterial taxa to sponges from around the world, with the likes of Chloroflexi, Actinobacteria, and Proteobacteria being among the dominant phyla. Analysis of microbes from 13 phylogenetically distinct deep-sea Demospongiae and Hexactinellida sponges from around the New Zealand coast revealed species-level host specificity among bacterial communities, whereas members of archaeanal communities were specific to individual sponges even within the same host species (Steinert et al. 2020).

Sponges are famed producers of bioactive metabolites, many of which are of suspected or proven microbial origin (Carroll et al. 2021). Obtaining sufficient quantities of many marine bioactives has been problematic, though establishing a microbial source has the potential (potentially inexhaustible) to thrive despite harsh conditions have often interested researchers. Hydrothermal vents are one such environment, in which well-adapted bacterial and animal communities are able to survive (Van Dover 2000). Around 450 km from the New Zealand mainland is the submarine and hydrothermally active Brother’s volcano. This volcano hosts a suite of unique marine life including bacteria, tubeworms, and barnacles. The endemic stalked barnacle (Vulcanoopaleas osheai) lives on deep-sea hydrothermal vents of the caldera and possesses cirral setae populated by filamentous bacteria (Suzuki et al. 2009). These are considered epibionts, with 16S rRNA gene amplicon sequencing and FISH indicating relatedness to bacteria associated with other hydrothermal vent fauna. Perhaps not unusually for an animal living on hydrothermal vents, isotopic analysis indicated that the barnacle relies on bacteria, including its epibionts, for nutrition (Suzuki et al. 2009).

Insights into the host microbiome can also come about via research aimed at identifying microbial agents of aquatic disease. One such study investigated the potential bacterial contribution to tail fan necrosis (TFN) in wild Jasus edwardsii (crayfish) (Zha et al. 2019). As an economically important seafood species in New Zealand and Australia, crayfish are subject to TFN mortality during transport. 16S rRNA gene amplicon sequencing revealed that bacterial communities on affected tail fan cuticles differ significantly from those on unaffected cuticle samples. While Flavobacteriales was the dominant bacterial order among both affected and unaffected crayfish, affected tail cuticles exhibited lower bacterial species richness and diversity, with greater variation among bacterial communities, compared with unaffected cuticles (Zha et al. 2019). Further research will be required to unequivocally determine the role (if any) of resident bacteria in the disease process.

Finally, the toheroa (Paphies ventricosa) is a threatened endemic surf clam that, despite decades of protection due to previous over-exploitation, has failed to recover. Recent work suggests that anthropogenic impacts on toheroa beaches may cause shifts in the microbiome composition of these animals, perhaps inhibiting their proliferation (Bennion et al. 2022).

Terrestrial invertebrates

Insects

The global diversity of insects is astounding, with the million named species likely a significant underestimate (Stork 2018). Of the c. 20 000 insect species within New Zealand, approximately 18 000 are endemic (Cranston 2010). For only a handful of these do we have information about their microbiome, with much discussion centred around the role of the microbiome in aiding host nutrition.

Among the best-studied insect microbiomes globally are those of termites, which rely on gut microbes for diverse functions including the breakdown of lignocellulosic biomass and provision of nitrogen via fixation of atmospheric dinitrogen (Brune 2014). The New Zealand damp wood termite (Stolotermes ruficeps), one of three endemic termite species, contributes to the breakdown of decaying wood, potentially making a not insignificant contribution to forest turnover (Reid & Lloyd-Jones 2009). The first of two published studies on the S. ruficeps microbiome described the diversity of nitrogen-fixing bacteria in the termite’s gut (Reid & Lloyd-Jones 2009). Nitrogen-fixing bacteria supply nitrogen to the host which survives on an exclusive diet of carbon-rich, but nitrogen-poor, wood. Amplification of the nifH gene, a biomarker for nitrogen-fixing bacteria that encodes a part of the nitrogenase
A type of scarab beetle material is the New Zealand grass grub (Costelytra zealandica) (Viswam et al. 2019). A subsequent study by the same authors utilised 16S rRNA gene amplicon sequencing to compare S. ruficeps bacterial communities in multiple colonies to those of two other endemic termite species, S. inopinus and Kalotermes brouni (Reid et al. 2014). The microbiotas of the two congenic hosts were more similar to each other compared to that of K. brouni. Both of these studies hinted at phyllosymbiosis, whereby similarities in the microbiotas of different host species correspond to a shared evolutionary history (Lim & Bordenstein 2020). Members of the bacterial families Spirochaetaeaceae, Elusimicrobiaceae and Porphyromonadaceae were dominant across all S. ruficeps locations (Reid et al. 2014).

Another animal that feeds on decaying wood is the larvae (grub) of the huhu beetle (Prionoplus reticularis). Nitrogen fixation within the gut of the huhu grub was indicated via the acetylene reduction assay, with parallel 16S rRNA gene amplicon sequencing identifying some 1800 different bacterial phylotypes (Reid et al. 2011). By sequencing both extracted DNA (i.e. the 16S rRNA gene) and RNA (i.e. the 16S rRNA itself), the authors concluded that 71% of identified phylotypes were metabolically active. The underlying premise here is that only active microorganisms will be producing RNA. A subsequent study indicated that diet may affect the diversity of fungi in the huhu grub gut microbiome, though the dietary influence on the bacterial component remains somewhat obscure (Viswam et al. 2019).

Yet another insect capable of digesting recalcitrant organic material is the New Zealand grass grub (Costelytra zealandica), a type of scarab beetle. Costelytra zealandica is a significant pasture pest during its larval stage, causing damage through root-feeding of grass and clover (Townsend et al. 2004). 16S rRNA gene-based bacterial community fingerprinting showed that diet and location influence the composition of the midgut microbiota, whereas the hindgut maintains a more diverse but consistent community profile irrespective of these factors (Zhang & Jackson 2008). The presence of Clostridium spp. across larvae from five geographically separate populations indicates this genus as containing potentially autochthonous, functionally important symbionts, with a suggested role for these bacteria in the fermentation of sugar as well as in cellulose metabolism (Zhang & Jackson 2008). The relationship between C. zealandica and the bacterium Serratia entomophila is also of note. Colonisation of the digestive tract during the larval stage by S. entomophila results in the eventual death of the organism through amber disease (Hurst et al. 2004). As this disease is highly host-specific, S. entomophila has been developed for commercial use as a biopesticide (Townsend et al. 2004).

Alongside the huhu beetle, wētā are arguably the most iconic of New Zealand’s insect fauna. A variety of cultivation-independent techniques were employed to establish the microbial density and community composition in the gut of the Auckland tree wētā (Hamideina thoracica) (Waite et al. 2015). Fluorescence in situ hybridisation revealed that bacterial density was highest in the hindgut, and lowest in the foregut, while 16S rRNA gene amplicon sequencing revealed a diverse bacterial community with Firmicutes and Bacteroidetes as dominant phyla. In a comparison with publicly available sequences from the more commonly studied termites and cockroaches, tree wētā microbiota was most similar to that of cockroaches, perhaps unsurprisingly given their overlapping dietary tendencies. However, unlike the cockroach gut, which harbours methane-producing (methanogenic) archaee, no archaeeal 16S rRNA gene sequences were recovered from the wētā gut (Waite et al. 2015).

Sap-sucking insects such as aphids, psyllids and scale insects represent a very different type of host-microbe association, typically one of much lower bacterial diversity compared with the aforementioned host insects. Whilst arguably lacking the charismatic appeal of a wētā or huhu beetle, scale insects of the Coelostomidiidae family are keystone species in parts of New Zealand, with far-reaching importance for entire forest ecosystems (Beggs & Wardle 2006). Feeding on plant phloem sap and excreting surplus carbon-rich “honeydew” droplets from their long anal tube, scale insects provide an essential food source for a variety of other native species including lizards, fungi (Dhami et al. 2013a; Evans et al. 2015), and birds such as tūi (Prosthemadera novaeseelandiae), kākā (Nestor meridionalis), and bellbird (Anthornis melanura) which can spend up to 80% of their daily foraging time seeking out honeydew (Beggs & Wardle 2006). This constant supply of food is particularly important for these avian species during the food-scarce winter months. Though rich in carbon, honeydew lacks essential amino acids, implying a dependence on bacterial symbionts to provide essential nitrogen to the host insect. Indeed, seminal studies by Dhami and colleagues used a combination of DNA- and microscopy-based techniques to document symbiotic bacteria in the kākā giant scale insect (Coelostomidia wairoensis) and sooty beech scale insect (Ultracoelostoma brittini) (Dhami et al. 2012), with all nine endemic members of the Coelostomidiidae further analysed to demonstrate co-speciation of the host and its main Bacterioideetes symbiont (Dhami et al. 2013b).

The commercial relevance of many of New Zealand’s introduced insect species has also seen them attract interest from microbiome researchers. These include the honey bee (Apis mellifera), which is widely used for pollination of commercial crops (Taylor et al. 2019), and the blowfly (Lucilia sericata) which can be of veterinary concern due to its role in myiasis (flystrike) of New Zealand livestock (Palevich et al. 2021). The common wasp (Vespula vulgaris) is an aggressive and highly effective competitor with native species for honeydew (Gruber et al. 2019), while certain mealybug species are vectors of viruses connected to grapevine leafroll disease, with their endosymbiotic bacteria potentially facilitating this transmission (Gatehouse et al. 2012).

Other terrestrial invertebrates

Microbiome research on other New Zealand terrestrial invertebrates is limited. The New Zealand mud snail Potamopyrgus antipodarum is an unusual organism from Aotearoa in that it has been a successful intruder in foreign ecosystems and is now considered an invasive pest in Europe. In their invasive range, these snails form novel microbial relationships, with a core microbiome comprising some 30 microbial taxa (Bankers et al. 2021). The singular core taxon found in New Zealand resident snails (Areninomnas) is retained by the invasive snails (Bankers et al. 2021). Earlier work suggested a link between the bacterial microbiome of this freshwater snail and its reproductive state (Takecs-Vesbach et al. 2016). Significant differences in bacterial community composition occur between sexually and asexually reproducing snails, the former of which hosts Rickettsialis bacteria, and the latter Rhodobacter. This paper did discuss the need for additional work, due to some uncertainty around
phylogeography, but it is interesting to consider that invasive lines of snails in Europe and North America reproduce asexually, and have distinct differences in microbiomes (Takacs-Vesbach et al. 2016; Bankers et al. 2021).

Fish

Fish represent one of the oldest classes of vertebrates and certainly the most species rich, with over 30 000 extant species and accounting for around half of all vertebrates (Nelson 2016). As with terrestrial animals, much fish microbiome research has focused on the role of ecological, evolutionary and environmental factors in shaping the composition and function of host-associated microbial communities (Sullam et al. 2012). In New Zealand, research has largely focused on the gut symbionts of marine herbivorous fish.

It has been established for some time that marine herbivorous fish species harbour specialised gut microbiota (Fishelson et al. 1985), with early studies suggesting that these symbionts facilitate the fermentation of algal products (Rimmer & Wiebe 1987). The hindgut microbiota of some of these symbionts facilitate the fermentation of algal products and short-chain fatty acids (a product of Firmicutes) (Waite et al. 2014; Perry et al. 2017), though the artificial diet provided to hand-reared chicks does elicit a substantial, albeit transient, alteration in bacterial community composition (West et al. 2022a). Shotgun metagenomic analyses suggested that Escherichia/Shigella may maintain its prominent position via its capacity to metabolise a varied range of nutrients within the gut of the kākāpō; indeed, it appears to be the only constituent of the kākāpō microbiota that is able to utilise all forms of carbohydrate ingested by the host bird (Waite et al. 2018).

Captive birds are a number of anthropogenic factors known to influence the microbiomes of various vertebrate taxa (McKenzie et al. 2017). Among New Zealand birds, human-related alterations in microbial diversity have been documented for takahē (Porphyrio hochstetteri) and brown kiwi (Apteryx mantelli; Fig. 3) (San Juan et al. 2021; West et al. 2022b). In each case, 16S rRNA gene sequencing revealed that Lactobacillus bacteria were more prevalent in individual birds which were subjected to more intensive management (takahē) or captivity (brown kiwi). Takahē are subjected to different levels of anthropogenic management depending on their location, including differences in feeding regimes. Lactobacillus aviarius was ubiquitously detected in 57 takahē from across New Zealand, with its relative abundance reflecting the location from which the sample was obtained (West et al. 2022b). Notably, the highest Lactobacillus abundances were at Burwood Takahē Centre, which provides the most intensive management. For captive kiwi, the shift in bacterial composition towards over-representation of Lactobacillus was suggested to be the result of probiotic feeding supplements administered after antibiotic treatment of the birds (San Juan et al. 2021).

Until recently, little was known about the virus communities (viromes) of New Zealand birds. Characterisation of the viromes of 12 passerine species was recently achieved through metatranscriptomic sequencing of apparently healthy birds (French et al. 2022). This study included six endemic and six introduced species. Of the 470 virus species identified, 436 are likely to be associated with diet or microbiome members due to their specificity in infecting plants, fungi or microorganisms (French et al. 2022). Three novel viruses, potentially infectious in nature, were identified in three of the introduced species, with current unknown risk of transmission to endemic species. Further investigation into the viromes of endemic species is thus warranted.

Other native New Zealand birds were among > 900 species included in a large 16S rRNA gene sequence-based
comparative study of vertebrate gut microbiomes (Song et al. 2020). The New Zealand birds were sampled in captivity at Auckland Zoo. A key finding was that birds capable of flight exhibited similar microbiomes to those of bats, with neither strongly influenced by either host phylogeny or diet, suggesting a potential association between the gut microbiome and the physiological adaptation to flight (Song et al. 2020). Such a link is considered a key research question within the avian microbiome field (Bodawatta et al. 2022).

Amphibians and reptiles

Amphibians are a class of ectothermic vertebrates divided into frogs (Anura), salamanders (Urodela) and caecilians (Gymnophiona). Of the c. 8400 species globally, 41% are considered threatened (IUCN 2021; AmphibiaWeb 2022). There are only four extant native pepeketua (frog) species in New Zealand. Belonging to the genus Leiohela, and regarded as the most archaic frogs in the world, these animals represent the only endemic amphibians in the country. Sadly, the smallest of the genus, Archey’s frog (L. archeyi), is critically endangered and one of the world’s most threatened amphibians, while the Maud Island (L. pakeka) and Hamilton’s frogs (L. hamiltoni) are considered vulnerable (IUCN 2021). Data surrounding the microbial inhabitants of New Zealand’s native frogs are still somewhat scarce.

The fungal skin disease chytridiomycosis, caused by Batrachochytrium dendrobatidis (Bd), is a leading cause of global amphibian declines (Fisher & Garner 2020). Studies of amphibian microbiomes around the world have thus largely focused on the role of skin bacteria as a potential barrier against Bd infection (Rebollar et al. 2020). Following a dramatic population crash of L. archeyi between 1996–2001, conjectural links were made to Bd as a driver of mortality. Experimental evidence has since suggested that L. archeyi, L. pakeka, and L. hochstetteri are somewhat resistant to Bd infection (Shaw et al. 2010; Ohmer et al. 2013). In understanding this apparent resistance, baseline cutaneous bacteria from free-living L. archeyi and L. hochstetteri were cultivated consisting of 92 distinct bacterial isolates (Shaw et al. 2014). A Flavobacterium sp. strain was able to inhibit Bd growth in vitro (Fig. 3), suggesting a potential functional role for this bacterium in the innate defence of Leiopelma against chytridiomycosis (Shaw et al. 2014). Should this bacterium exemplify the characteristics of an effective probiotic against chytridiomycosis (Bletz et al. 2013), it may prove useful in future bioaugmentation strategies for other at-risk amphibians. Bioaugmentation is regarded as a feasible intervention method against Bd, essentially involving the inoculation of an organism with a suitable probiotic - perhaps isolated from an amphibian living as normal despite the presence of Bd (Woodhams et al. 2016).

As previous cultivation of dorsal skin bacteria revealed differences between captive and free-living L. archeyi (Potter & Norman 2006), the role of captivity in the microbiome composition of frogs should also be considered. Should the reduced interaction of captive frogs with the wild environment be the cause of these changes, it may also be beneficial to consider another role for bioaugmentation via wild frogs as a restorative measure for captive individuals.

Native reptilian life in New Zealand is almost exclusively comprised of lizards, with > 60 representative species. The only other reptile group present is the distinct lineage of Rhynchocephalia (tuatara), as well as the occasional visiting turtle. Reptiles in New Zealand are entirely underrepresented in microbiome studies, however there are some related studies that have been performed on tuatara. Having diverged from other reptiles some 250 million years ago, the tuatara (Sphenodon punctatus) is a well-known relic of New Zealand and an important link to the extinct stem reptiles from which modern amniotes evolved. Indeed, the recently sequenced genome of the tuatara has revealed features seen in both reptiles and mammals (Gemmill et al. 2020). While the tuatara microbiome is yet to be comprehensively documented, this reptile is notable for its apparent absence of Salmonella bacteria (Gartrell et al. 2007; Middleton et al. 2014). While Salmonella can be pathogenic to reptiles, many healthy individuals within this class routinely harbour Salmonella (Whiteley et al. 2017), including some New Zealand native lizard species (Middleton et al. 2010; Middleton et al. 2014). It has therefore been suggested that some Salmonella serovars are commensals of reptiles, maintaining their pathogenicity in an opportunistic capacity. Unusually, tuatara appear entirely resistant to Salmonella, despite the bacterium being prevalent in their living environment (Middleton et al. 2015). Ongoing studies (D Middleton, Manaaki Whenua - Landcare Research, unpubl. data) should help to elucidate any potential roles of the microbiome in this resistance, as well as more generally the composition and function of the tuatara microbiome.

Mammals

The list of native New Zealand mammals is a short one, with terrestrial mammals limited to the lesser short-tailed bat Mystacina tuberculata and the New Zealand long-tailed bat Chalinolobus tuberculatus. Although bats are the only mammals capable of true flight, the bats of New Zealand prefer to spend much of their time on the ground. M. tuberculata and C. tuberculatus are considered vulnerable and critically endangered, respectively (IUCN Redlist, 2021). There are relatively few global studies on bat microbiomes, and fewer still in New Zealand. Globally, one large study has indicated that bat microbiomes likely do not follow the same patterns as seen in other mammals, with phylogeny having little influence over bacterial community dissimilarity (Lutz et al. 2019). Rather, host species and geographic location were better predictors of microbiome community structure in bats (Lutz et al. 2019). Furthermore, and as mentioned earlier, bat microbiomes may be associated with the physiological adaptation to flight, as they host more bird-like gut microbiomes that do not appear to be strongly influenced by phylogeny (Song et al. 2020). The contributing faecal samples from M. tuberculata in the study by Song and colleagues were obtained from captive representatives at Auckland Zoo, with much thus remaining unknown about the microbial inhabitants of New Zealand’s native bats.

Although not native to New Zealand, we note here some of the work conducted on two invasive predators that have become naturalised: the brushtail possum (Trichosurus vulpecula) and the carnivorous stoat (Mustela erminea). The introduction of these two species, among many others, in the 1800s has had devastating consequences for native biodiversity. While native animals did not evolve defensive traits appropriate for mammalian predators (Dowding & Murphy 2001; O’Donnell et al. 2015), plant life has also suffered. Possums, in particular, are damaging to native flora, destroying tall canopy species such as southern rātā (Metrosideros umbellata) and pōhutukawa (M. excelsa) through intensive browsing (Leutert 1988; Hosking & Hutcheson 1993). Functional aspects of the possum and stoat microbiomes are of particular research interest due to the potential microbial contribution towards
the evolutionary success of these host species, distinct from host-specific genomic adaptations. With an eye to the potential future development of novel, microbiome-targeted biocultures for these destructive mammal species, recent work has identified that the dominant bacterial phyla Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria are characteristic core members of the brushtail possum and stoat oral cavities (Emami-Khoyi et al. 2020), though continued work into microbial function should be pursued.

Marine mammals in New Zealand are more speciose than their terrestrial counterparts. Of the 90 known cetacean species, 47 have been reported in New Zealand waters, giving Aotearoa the status of a cetacean diversity hotspot (Stephenson et al. 2021). Endemic cetaceans include Hector’s dolphin (Cephalorhynchus hectori) and the closely related Maui dolphin (C. hectori maui) subspecies. New Zealand pinnipeds include the endemic New Zealand sea lion (Phocarctos hookeri) and the widely distributed but native New Zealand fur seal/kekeno (Arctocephalus forsteri). Marine animals are, by their nature, more difficult to sample than terrestrial organisms. Offshore habitat use and rarer contact with humans have left them comparatively less well studied across the globe, although some recent attempts have been made to study marine mammal microbiomes (Nelson et al. 2015). The only native marine mammal microbiome so far studied in New Zealand is that of the oral cavity of A. forsteri pups (Emami-Khoyi et al. 2019). Sequencing of PCR-amplified 16S rRNA genes from five seal pups revealed that bacterial assemblages found in the kekeno oral cavity are similar to previously reported oral bacteria from some dolphins and sea lions (Bik et al. 2016). The oral cavity of kākāpō pups also shows a high incidence of Firmicutes, as seen in the gut microbiomes of Arctic and Subarctic seals, but particularly common were Lachnospiraceae and Streptococcaceae spp., often seen in the oral cavities of canines (Dewhirst et al. 2012).

Perspectives/Future directions

The studies outlined in this review exemplify the current state of microbiome research in Aotearoa New Zealand’s native animals. By far the most common are studies which taxonomically profile the bacterial members of animal microbiomes, through 16S rRNA gene amplicon sequencing. Comparatively little attention has been given to other members of the microbiome, such as archaea, viruses, fungi, or protozoa. Additionally, while the use of 16S rRNA gene amplicon sequencing has proved a valuable tool in deriving taxonomic information for a select few animal microbiomes, in most cases the functional roles of these bacteria and their interactions with the host organism are yet to be thoroughly explored. The recent metatranscriptomic study into passeriine viromes provides a good example of how microbiome studies need not be limited to bacteria or 16S rRNA gene amplicon sequencing (French et al. 2022).

Unsurprisingly given the relative ease of faecal sampling, for native vertebrates the faecal (gut) microbiome has received the most research attention. For these taxa some insights into the role of bacteria in host digestion have been reported. Conversely, the role of the microbiome in the digestive processes of marine invertebrates remains largely unknown, with research in these organisms branching across several other areas of interest, including the role of the microbiome in disease and in the production of bioactives.

The last 1–2 decades of research into native animal microbiomes in New Zealand have provided data for animals from a broad phylogenetic range. There remains, however, an overwhelmingly large proportion of New Zealand animal species for which we are entirely data deficient when it comes to the microbiome. To place this in perspective, while in this review c. 10 insect species are represented in the microbiome literature (a seemingly large number compared to the single marine mammal studied to date), this is a minute fraction of the 20 000 extant insect species present in Aotearoa. Moreover, for the individual species for which we do have data, only a select few have been subjected to repeated study allowing for a richer understanding of their microbiomes, for example the kākāpō and silver drummer.

Outside the gut: the need for studying other microbiomes

While the gut microbiome is rightly a major research focus, more extensive investigations of non-gut microbiomes are also required. Currently, there is no animal in New Zealand for which we have attempted to investigate all microbial niches. While microbiome sites of interest will depend on host physiology, common features may be an appropriate place to start. For example, the vertebrate skin can be colonised by a variety of different microorganisms (Ross et al. 2019). Among New Zealand animals, the skin microbiome has only been briefly explored for native frogs. As host species appears to be an important predictor of skin microbial communities (Chiarello et al. 2018; Ross et al. 2018) and studies of domestic animals in healthy or diseased states have made clear the relevance of the skin microbiome (Weese 2013), we should at the very least attempt to establish a baseline understanding of this microbiome for key host species. A caveat here is that, as mentioned earlier, some body sites of a given animal may simply not have a microbiome (Hammer et al. 2019). It is thus imperative that caution be exercised when describing such sites for the first time, particularly when samples have low microbial biomass and are therefore more prone to the effects of DNA contamination.

Beyond the 16S rRNA gene

While the use of 16S rRNA gene amplicon sequencing has been imperative in increasing our understanding of bacterial taxonomic diversity, many of the above-mentioned papers have indicated a need for further investigation to fully confirm the functional roles of microbiome members. In addition, while bacteria tend to be the most dominant constituents of microbiomes, remaining ignorant of non-bacterial community members severely constrains our overall understanding. Indeed, for humans, 454 pyrosequencing of the human oral microbiome revealed a diversity of fungal species that were hitherto unknown (Ghannoum et al. 2010), with later investigation into fungal colonisation of the human gut indicating that all fungal species present may only be transient, derived from the diet or the mouth (Auchtung et al. 2018).

Increasing the use of shotgun metagenomics for our native animal microbiomes should provide a more in-depth view of microbiome taxonomy and function in future studies. With metagenomics able to identify the presence of novel genes, functional genes, and antibiotic resistance genes as well as indicating interactions between microbiota and host (Wang et al. 2015), the rationale to increase our use of metagenomics is evident. The application of shotgun metagenomics to kākāpō, for example, yielded novel insights into the likely roles of key
gut bacteria (Waite et al. 2018), with near-complete genomes able to be assembled even for organisms that are yet to be obtained in pure culture. While still a more expensive option than 16S rRNA gene sequencing, the ever-decreasing costs of DNA sequencing make the shotgun approach increasingly attractive for the study of animal microbiomes.

Despite some drawbacks, however, the relatively low expense, ease of data processing, and ability to circumvent contamination from host DNA (via the use of microbe-specific PCR primers) should ensure a place for 16S rRNA gene amplicon analyses for some time to come. The global popularity of 16S rRNA gene sequencing has led to the existence of a wide range of publicly accessible taxonomic datasets e.g. Greengenes and SILVA (DeSantis et al. 2006; Pruesse et al. 2007). As the frequency of shotgun metagenomic sequencing does not yet correspond to that of 16S amplicon sequencing, less reference data exist, thus sometimes fewer taxa may actually be identified in a sample (Tessler et al. 2017). This may be particularly true of samples that are taken from an animal group whose microbiomes are poorly characterised globally, e.g. marine mammals. Over time, with continued collaborative effort, and ongoing cost reductions, further microbial sequence data should become available from shotgun metagenomics, resolving this issue.

The microbiome in conservation

The role of the microbiome in conservation is an area that is receiving increasing attention (Trevelline et al. 2019; West et al. 2019; Dallas & Warne 2022). It is a topic of particular relevance to New Zealand given the large number of native animals under active conservation management. A key part of many conservation strategies is captive maintenance of the species in question. However, captive animals are exposed to a variety of environmental shifts that do not necessarily reflect conditions in their wild habitat. Animals that are either born into captivity or translocated from the wild are subject to dietary changes, veterinary manipulation (such as the use of antibiotics), increased human interaction and reduced habitat range. Changes in bacterial diversity for captive animals have been described for numerous species (Clayton et al. 2016; Kueneman et al. 2016; McKenzie et al. 2017; Tang et al. 2020), including some native New Zealand animals (Potter & Norman 2006; San Juan et al. 2021; West et al. 2022b). The consequences of such changes remain unclear. While alterations to the microbiome composition of captive animals may not have inherently negative impacts on the host animal, it is difficult to fully comprehend the implications of these changes until further comparative analyses are undertaken. Once more, we must move past the point of establishing microbiome composition and begin to elucidate the functions of the microbiome, particularly if we wish to integrate this knowledge into practical applications. With captive rearing of threatened species an important conservation tool in New Zealand, any further perspective gained about the influence of captive environments on the microbiome may have far-reaching implications in conservation and management strategies, as well as the design of future animal-microbiome studies.

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Data and code availability

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