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Colony Location and Captivity Influence the Gut Microbial Community Composition of the Australian Sea Lion (*Neophoca cinerea*)

Tiffany C. Delport,[a] Michelle L. Power,[a] Robert G. Harcourt,[a] Koa N. Webster,[a] Sasha G. Tetu[b]

Department of Biological Sciences[a] and Department of Chemistry and Biomolecular Sciences,[b] Macquarie University, Sydney, New South Wales, Australia

ABSTRACT

Gut microbiota play an important role in maintenance of mammalian metabolism and immune system regulation, and disturbances to this community can have adverse impacts on animal health. To better understand the composition of gut microbiota in marine mammals, fecal bacterial communities of the Australian sea lion (*Neophoca cinerea*), an endangered pinniped with localized distribution, were examined. A comparison of samples from individuals across 11 wild colonies in South and Western Australia and three Australian captive populations showed five dominant bacterial phyla: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*. The phylum *Firmicutes* was dominant in both wild (76.4% ± 4.73%) and captive animals (61.4% ± 10.8%), while *Proteobacteria* contributed more to captive (29.3% ± 11.5%) than to wild (10.6% ± 3.43%) fecal communities. Qualitative differences were observed between fecal communities from wild and captive animals based on principal-coordinate analysis. SIMPER (similarity percentage procedure) analyses indicated that operational taxonomic units (OTU) from the bacterial families *Clostridiaceae* and *Ruminococcaceae* were more abundant in wild than in captive animals and contributed most to the average dissimilarity between groups (SIMPER contributions of 19.1% and 10.9%, respectively). Differences in the biological environment, the foraging site fidelity, and anthropogenic impacts may provide various opportunities for unique microbial establishment in Australian sea lions. As anthropogenic disturbances to marine mammals are likely to increase, understanding the potential for such disturbances to impact microbial community compositions and subsequently affect animal health will be beneficial for management of these vulnerable species.

IMPORTANCE

The Australian sea lion is an endangered species for which there is currently little information regarding disease and microbial ecology. In this work, we present an in-depth study of the fecal microbiota of a large number of Australian sea lions from geographically diverse wild and captive populations. Colony location and captivity were found to influence the gut microbial community compositions of these animals. Our findings significantly extend the baseline knowledge of marine mammal gut microbiome composition and variability.

It is predicted that global change will have many major, but as yet unknown, impacts on aquatic ecosystems. Marine mammal species, including bottlenose dolphins, sea otters, manatees, and gray whales, have been proposed as sentinels for diverse threats to aquatic health, including disease transmission, changes in food webs, and contaminant levels (1–6). The key roles and contributions of microbes to mammalian host health are becoming increasingly recognized, yet remain largely unexplored for marine mammals (7). An understanding of the impacts of anthropogenic pressures on marine mammal microbial communities and of the potential cascade of impacts on marine health is necessary for full exploration of the roles and suitability of different marine mammal species as sentinels for aquatic ecosystem health.

The mammalian gastrointestinal tract is home to a diverse array of microbial species that are essential for daily regulatory functions of the host and that contribute to maintenance of metabolic processes, immune defense, and intestinal tissue maturation and health (8–10). Gut microbiota also contribute to gut function through the digestion of food and absorption of nutrients and minerals (11). Gut microbial communities have coevolved with their hosts and within distinct evolutionary lineages (12–14), yet many evolutionarily unrelated hosts share a similarity in gut microbiota, as diet is a primary driver of the microbial composition (13, 15). Extrinsic factors occurring throughout the host life span may also alter gut composition (16).

Comparisons of the compositions of gut microbiota of terrestrial and marine mammals have shown that the predominant phyla differ significantly between the two groups and that marine carnivores display richer microbial diversity than do terrestrial carnivores (17). However, marine mammal samples used for studies and comparisons of microbiota have almost entirely come from seals of various species (5, 17–21). Such studies have found that the microbiota are largely dominated by four phyla, *Firmic-*

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Address correspondence to Sasha G. Tetu, sasha.tetu@mq.edu.au.

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utes, *Bacteroidetes*, *Fusobacteria*, and *Proteobacteria*, and, to a lesser extent, by *Actinobacteria* (5, 18–21).

The Australian sea lion (*Neophoca cinerea*), a member of the suborder Pinnipedia, is one of the rarest seal species in the world and Australia's only endemic otariid seal. The current species census estimate is ~14,700 individuals, and it is listed as endangered on the International Union for Conservation of Nature (IUCN) Red List (22). In the late 18th and early 19th centuries, the Australian sea lion was subjected to unregulated harvests, resulting in a reduction in numbers and extirpation of colonies in the Bass Strait and within the current range (23, 24). Unlike the two sympatric fur seal species, the Australian fur seal (*Arctocephalus pusillus doriferus*) and the New Zealand fur seal (*Arctocephalus forsteri*), the Australian sea lion is not recovering (22).

The Australian sea lion population is dispersed over approximately 76 small island colonies and protected mainland coves (22). This geographic range extends over 2,700 km of Australian coastline, with the result that some colonies are situated in close proximity (<25 km) to high-density metropolitan areas, while more isolated colonies are located further than 100 km from the nearest coastal settlement. Despite their broad population distribution, both male and female sea lions from individual colonies exhibit strong natal-site philopatry and a tendency for localized foraging (25, 26). In the wild, these animals are opportunistic foragers; their diet includes a broad range of shallow-water benthic prey, such as teleost fish, cuttlefish, octopus, squid, rock lobster, rays, small sharks, penguins, and small crustaceans (27, 28).

The distribution of sea lions along the coastline and Australian mainland brings some animals into contact with humans and environments influenced by terrestrial processes. As a tourist icon, a few South Australia (SA) and Western Australia (WA) sea lion colonies also experience high levels of human visitation at close proximity. For example, at the most popular observing area, Seal Bay, Kangaroo Island, SA, the number of visitors exceeds 150,000 annually (29). Sea lions are also extremely popular in zoological and marine park collections. These animals originate from captive breeding programs and/or rehabilitation of rescued injured animals. Previous work looking at the gut community compositions of captive pinnipeds focused on a small number of samples (5) or selected bacterial strains (30). These studies indicated that the carefully controlled diet and habitat, as well as potential interactions with a different set of foreign animals, are likely to influence the gut microbiome.

The ecology, conservation status, localized distribution, and site fidelity of Australian sea lions make them a potential sentinel species for ocean health in the Southern Hemisphere. Sentinel species can provide early information on the impacts on individuals and at the population level (31). Given the likely contribution of gut bacteria to mammalian host health, the characterization of fecal microbial communities may provide a useful, relatively noninvasive, tool to assess the health of Australian sea lion populations and perhaps the health of the wider marine community. To assess this, baseline information on the variations in Australian sea lion fecal microbial communities at both the individual level and the population level is required.

The aim of this study was to address the question of how colony location and captivity influence the gut microbial community composition of the Australian sea lion (*Neophoca cinerea*). Fecal samples were collected from geographically disparate colonies encompassing 75% of the animal's range and from captive animals housed at three different locations. The compositions of the fecal microbial communities from captive and wild samples were analyzed with next-generation sequencing of V1 to V3 region 16S rRNA gene amplicons.

MATERIALS AND METHODS

Host description and sample collection. Australian sea lions are large animals. Adult males weigh 250 to 300 kg and are 185 to 225 cm in length, while females are smaller, weighing 61 to 104 kg with an average length of 130 to 180 cm (32). The endangered status of these animals, together with their large size, has meant that trapping and sampling of adult individuals are rarely undertaken.

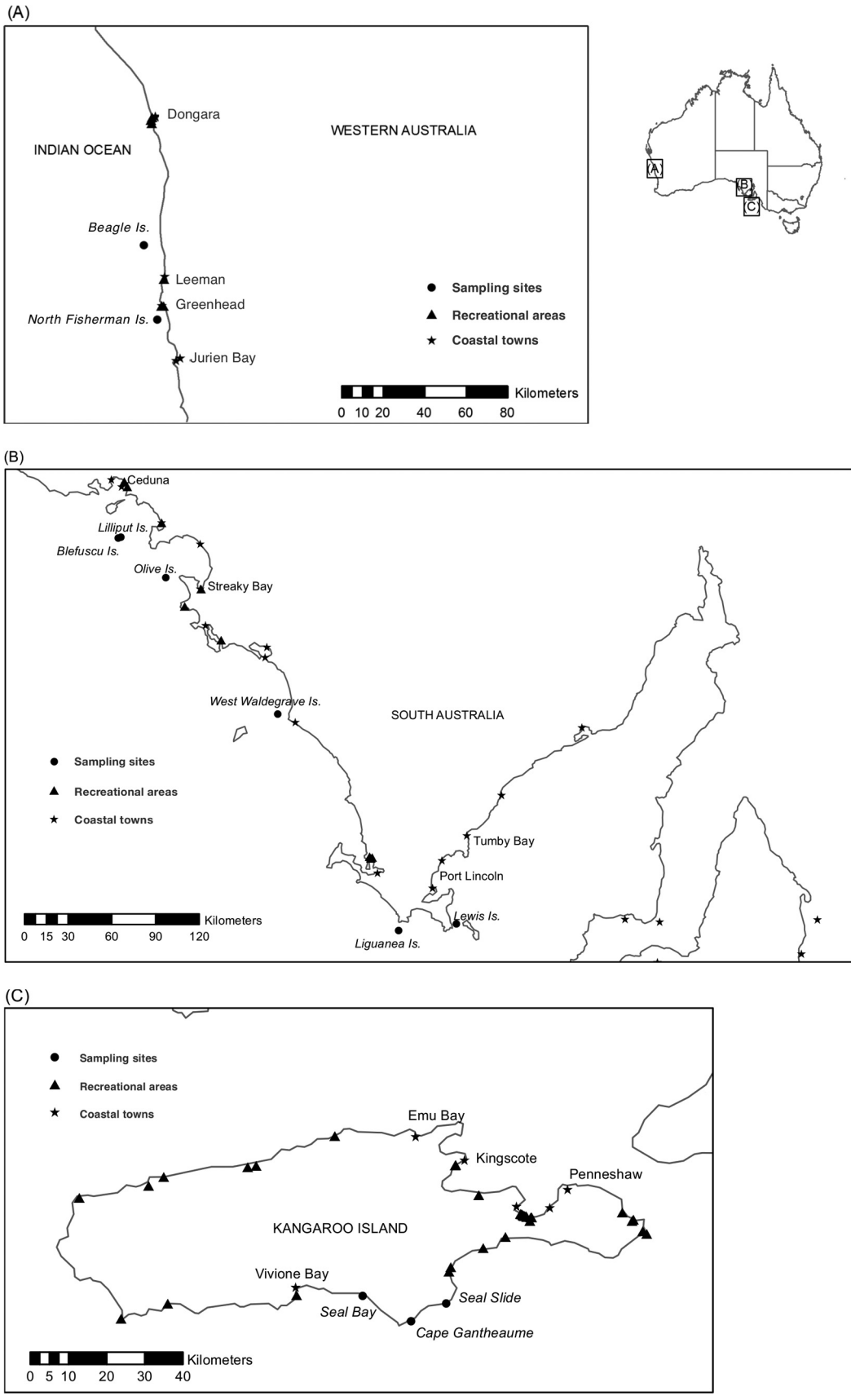
Fecal samples from wild Australian sea lions were collected opportunistically over a period of 18 months (March 2009 to September 2010) from 11 coastal and island colonies in Western Australia (Fig. 1; see also Table S1 in the supplemental material). Once adult sea lions left the colony to feed each morning, fecal samples deemed to be fresh on the basis of visibly high moisture content and dark color were collected from haul-out sites. For captive sea lions, fecal samples were collected from haul-out areas in enclosures over a period of more than 2 years (March 2011 to May 2013) from the resident animals held at Dolphin Marine Magic, Coffs Harbor, New South Wales, Australia; Taronga Zoo, Sydney, New South Wales, Australia; and Sea World, Gold Coast, Queensland, Australia. Care was taken with all fecal samples collected to ensure that material in direct contact with the environment was excluded. Fecal samples were transported to the laboratory and stored at 4°C until processing for genomic DNA extraction.

DNA isolation and subsampling. Total genomic DNA was extracted from wild and captive sea lion fecal samples (approximately ~150 mg from each homogenized sample) using the ISOLATE fecal DNA kit (Bioline, Sydney, Australia), and extraction was performed according to the manufacturer's protocol. Extracted sea lion DNA was quantified using a Qubit 2.0 fluorometer (Invitrogen; Life Technologies) and stored at −20°C until further analysis.

PCR and sequencing of 16S rRNA gene amplicons. PCR amplification and sequencing of a region of the 16S rRNA gene were conducted by the Ramaciotti Centre for Genomics at the University of New South Wales (Sydney, Australia). PCR was performed using the bacterial universal forward primer 27F and reverse primer 519R, producing an ~530-bp fragment spanning the hypervariable regions V1 to V3 (33, 34). Reverse primers contained an Illumina MiSeq adaptor sequence, a 12-base barcode, and the universal primer sequence, as described previously (33). PCR mixtures (25 μl) were prepared using 200 nM deoxynucleoside triphosphates (dNTPs), 2.5 mM $MgCl_2$, 500 nM each primer (27F/519R), 1× Immolase Immobuffer (Bioline), 1 U of Immolase DNA polymerase (Bioline), and 1 μl of template DNA (ranging from 2.5 to 87 ng/μl). Thermocycling was performed as follows: activation for 10 min at 95°C; 35 cycles at 94°C for 30 s, 55°C for 10 s, and 72°C for 45 s; and a final extension step at 72°C for 10 min.

PCR products were purified using the AMpure XP purification kit (Beckman Coulter, Australia), following the manufacturer's protocol. To assess the integrity of total RNA, each sample was quantified on an Agilent bioanalyzer RNA nano 6000 chip. After integrity assessment, sequencing was carried out on a MiSeq sequencer (Illumina), yielding 250-bp paired-end reads.

Computational analyses. The MiSeq forward and reverse reads were merged into single contiguous sequences with the mergepairs tool in USEARCH version 7.0 (35). Quantitative Insights Into Microbial Ecology (QIIME) version 1.8.0 was used for all subsequent sequence analysis unless otherwise noted (36). Sequences were filtered for quality using the default settings with a total of 9.8 million reads obtained for a total of 43 samples, 33 wild and 10 captive. These were clustered into operational taxonomic units (OTU) with a closed-reference OTU picking protocol at a 97% sequencing identity level using UCLUST (35) against the August 2013 release of Greengenes, core data set 18_3 (37). OTU at very low


(A)
Dongara
WESTERN AUSTRALIA
INDIAN OCEAN
Beagle Is.
Leeman
Greenhead
North Fisherman Is.
Jurien Bay
Sampling sites
Recreational areas
Coastal towns
Kilometers
0 10 20 40 60 80
(B)
Ceduna
Lilliput Is.
Blefuscu Is.
Olive Is.
Streaky Bay
West Waldegrave Is.
SOUTH AUSTRALIA
Sampling sites
Recreational areas
Coastal towns
Tumby Bay
Port Lincoln
Lewis Is.
Liguanea Is.
Kilometers
0 15 30 60 90 120
(C)
Sampling sites
Recreational areas
Coastal towns
Emu Bay
Kingscote
Penneshaw
KANGAROO ISLAND
Vivione Bay
Seal Slide
Seal Bay
Cape Gantheaume
Kilometers
0 5 10 20 30 40

abundance, <0.00005% of the total number of sequences, were filtered out following the QIIME default settings. Each library was subsampled to an even sequencing depth of 10,000 reads per sample to mitigate biases arising from different depths of sequence across all samples. All subsequent analyses, including beta diversity analyses (described below), were conducted on rarefied data.

Mapping and statistical analyses. Maps illustrating wild Australian sea lion colonies sampled were developed using ArcGIS version 10.0 (38).

The QIIME software package (36) was used for preliminary statistical analyses and visualizations, including phylum- and family-level analysis of relative taxon abundance and exploration of beta diversity patterns, using principal-coordinate analyses (PCoA) with phylogeny-based (UniFrac) unweighted distances. To determine the taxa driving dissimilarities of the fecal microbial communities, Bray-Curtis SIMPER (similarity percentage procedure) analysis was performed in PAST 3.0.1 (39) at the family level. Differences in the community structures (relative microbial abundances) of phyla between wild and captive habitats were determined using the Mann-Whitney U test in IBM SPSS Statistics version 20.0 for Mac. Analyses to look at differences in the OTU frequencies between wild and captive sample groups were also conducted in QIIME using the Kruskal-Wallis nonparametric test.

Metagenome sequence accession numbers. The sequences generated in this study were submitted to MG-RAST as the project titled "Australian Sea Lion Fecal Collection" under reference identification numbers 4629998.3 to 4630040.3.

RESULTS

Taxonomic composition of Australian sea lion fecal microbial communities. Following all quality filtering steps in QIIME, a data set of 4,993,234 sequences spanning the hypervariable V1 to V3 region of the 16S rRNA gene from wild ($n = 33$) and captive ($n = 10$) sea lion fecal samples (mean, 116,122; standard deviation, 99,605; $n = 43$) was compiled. Analyses performed on rarefied data subsampled to 10,000 reads per sample clustered sequences into 309 OTU from 7 bacterial phyla: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Fusobacteria*, *Chloroflexi*, and *Cyanobacteria*.

The community compositions of fecal microbiota were examined at different taxonomic levels. At the phylum level, OTU classified as *Firmicutes* were dominant in the majority of sampled individuals (range, 7.1% to 99.5%) (Fig. 2A). In individuals with lower proportions of *Firmicutes*, either *Proteobacteria* or *Bacteroidetes* was the dominant phylum. *Bacteroidetes* contributed to more than 50% of the total abundance in two wild individuals, and *Proteobacteria* contributed to more than 50% of three captive and two wild individuals (Fig. 2A). The only other phyla that contributed more than 1% in multiple individuals were *Fusobacteria* and *Actinobacteria*.

At the level of bacterial family, community relative abundance profiles were highly variable for individual samples (Fig. 2B). There were 15 bacterial families that were dominant in the majority of samples, and these represented >1% of overall abundance. These dominant groups include a number of bacterial order *Clostridiales* representatives (*Clostridiaceae*, *Ruminococcaceae*, *Peptostreptococcaceae*, *Lachnospiraceae*, *Peptococcaceae*, and *Clostridiales* unclassified at the family level). Three samples from locations on South Australian islands (A187, A188, and A243) had higher proportions of bacterial families that were at overall low abundance. The taxonomic composition of sample A243 showed the most notable deviation, with families contributing <1% to overall abundance in this study, comprising 86% of this particular sample (*Porphyromonadaceae*, *Aerococcaceae*, and *Oxalobacteraceae* all contributed >10%).

Comparison of wild fecal microbiota. Samples from wild sea lion colonies showed some variations in the relative abundances of their dominant fecal bacterial phyla (Fig. 3). Three South Australian islands (Lilliput, Olive, and West Waldegrave Islands) and one Western Australian island (North Fisherman Island) had notable differences in the distributions of certain phyla (Fig. 3). On Lilliput Island, sea lion microbial communities contained a higher relative abundance of OTU from the phylum *Proteobacteria* than any other colony. OTU from two *Proteobacteria* families, *Xanthomonadaceae* (20% ± 16.1%) and *Moraxellaceae* (18.1% ± 14.8%), contributed to the observed increase in *Proteobacteria*.

In sea lions from Olive Island, gut microbial community profiles showed high proportions of taxa from *Bacteroidetes* (19.8% ± 14.9%) and *Proteobacteria* (18.5% ± 8.51%), while *Firmicutes* (54.8% ± 23.2%) represented a smaller contribution to the microbial community composition.

On West Waldegrave Island, the *Bacteroidetes* phylum (44.0% ± 9.43%) represented a greater contribution to the sea lion gut microbial composition than for other wild colonies. The phylum *Proteobacteria* (22.4% ± 17.0%) also represented a greater composition of gut microbial communities in this location.

In sea lions from North Fisherman Island, OTU characteristic of the phylum *Firmicutes* (98.1% ± 0.445%) contributed more to gut microbiota. The *Proteobacteria* (0.323% ± 0.102%) and *Bacteroidetes* (0.26% ± 0.0737%) phyla represented a smaller contribution to gut microbiota than for other colonies. The *Firmicutes* families *Clostridiaceae* (58.6% ± 28.0%) and *Carnobacteriaceae* (29.2% ± 29.2%) were more abundant in gut microbiota of North Fisherman sea lions.

Collectively, the microbiota of sea lions from Lilliput Island and Olive Island were composed of a greater number of genera ($n = 72$) than those of most other wild colonies (mean, 55 ± 4.01). Fewer genera were observed in the microbiota of West Waldegrave Island and North Fisherman Island seals ($n = 40$ and $n = 42$, respectively).

Comparison of captive and wild fecal microbiota. The fecal microbiota of wild and captive sea lions showed differences in overall community memberships. The average relative abundances of the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were higher for wild animals, while *Proteobacteria* contributed more to the fecal communities of captive animals (Fig. 3). However, neither of these differences was statistically significant (Mann-Whitney U test, $P > 0.05$). Comparison of the community memberships of fecal microbial communities from wild and captive animals, using PCoA ordination of unweighted UniFrac distances, showed a degree of grouping of samples based on habitat

FIG 1 Sea lion sampling locations in Western Australia (A), South Australia (mainland) (B), and Kangaroo Island, South Australia (C). In Western Australia, the sea lion fecal samples were collected from colonies on Beagle and North Fisherman Islands. Colonies sampled in South Australia included those from Blefuscu, Lewis, Liguanea, Lilliput, Olive, and West Waldegrave Islands and three colonies from Kangaroo Island (Cape Gantheaume, Seal Bay, and Seal Slide). Coastal settlements and recreational locations within close proximity to sea lion colonies are indicated. (Adapted from reference 55 [published under a CC BY-NC-ND license {http://creativecommons.org/licenses/by-nc-nd/4.0}].)

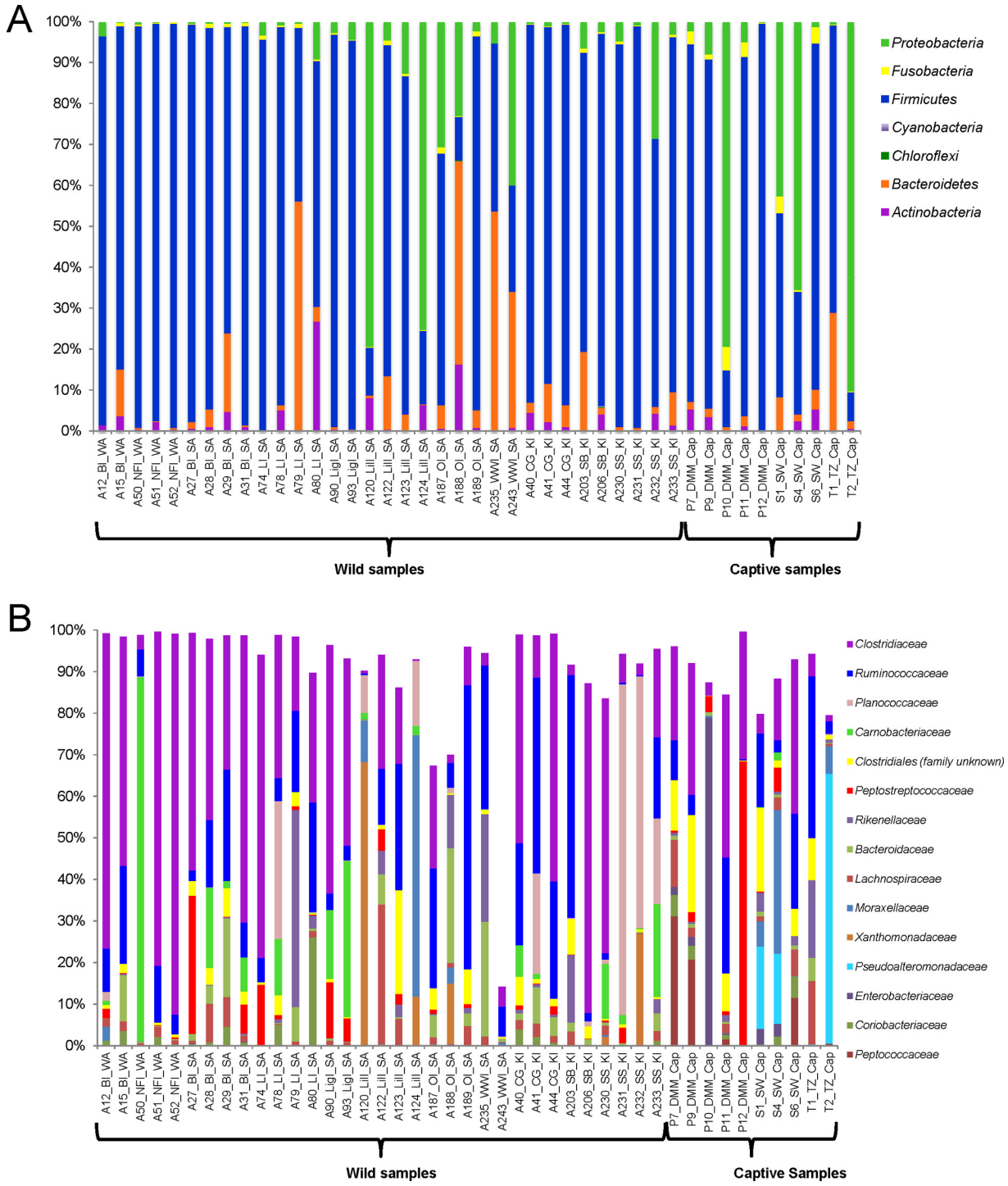


FIG 2 Relative taxon abundances for sampled Australian sea lion fecal microbial communities at both phylum (A) and family (B) levels. Each bar represents an individual fecal sample from a sea lion. In panel B, only the taxonomic groups with mean relative abundances of >1% are shown. Samples are grouped according to colony and colony location (for colony abbreviations, see Table S1 in the supplemental material).

(Fig. 4), indicating that samples from these different environments do show some qualitative differences. Differences in the abundances of specific OTU between wild and captive sample groups were found for only a small number of OTU (Kruskal-Wallis test, $P < 0.01$) (Table 1). In all cases, the significantly different OTU were more abundant in captive samples.

To determine the likely drivers of differences in fecal microbial communities between wild and captive animals, SIMPER analysis was conducted at the family level. Of the bacterial families observed ($n = 67$), 13 contributed ≥2% to the dissimilarity of wild and captive groups (SIMPER overall average dissimilarity of 73.4). Characteristic OTU from the bacterial families *Clostridiaceae* and

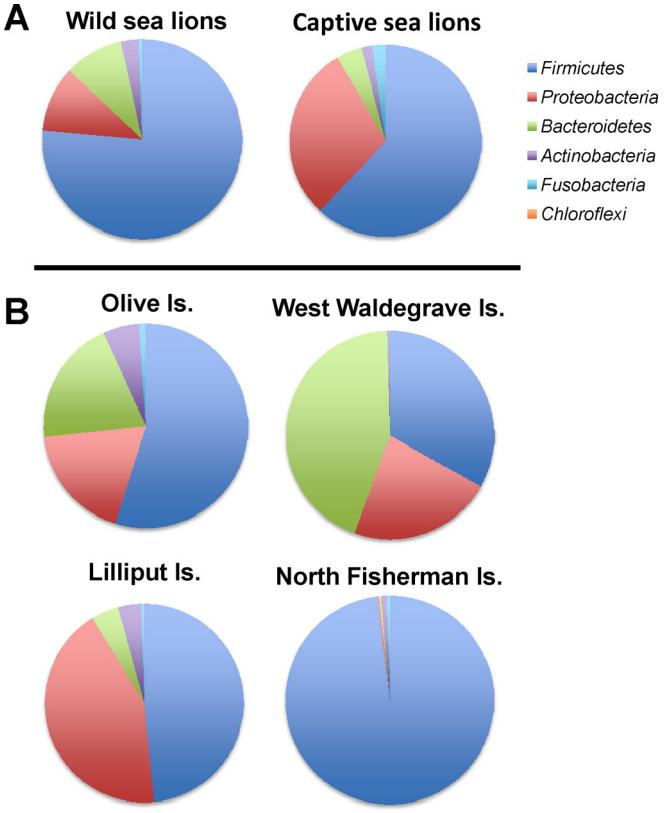


FIG 3 Relative abundances of bacterial phyla from geographically disparate sea lion populations. The collective fecal microbial communities of most wild colonies showed patterns of abundance that were similar at the phylum level. Notable differences in phylum distribution were observed in three colonies from South Australia (Lilliput, Olive, and West Waldegrave Islands) and in one Western Australian colony (North Fisherman Island). The pie graphs represent the collective data from all wild samples and all captive samples and for the four notably different colonies.

Ruminococcaceae were more abundant in wild (34.8% ± 4.98% and 16.7% ± 2.98%, respectively) than in captive (19.2% ± 4.77% and 12.9% ± 4.24%, respectively) animals and contributed most to the average dissimilarity between the groups (SIMPER contributions of 19.1% and 10.9%, respectively) (Table 2). In captive animals, higher average abundances of OTU characteristic of the *Pseudoalteromonadaceae* (10.2% ± 6.55%), *Peptostreptococcaceae* (8.27% ± 6.66%), and *Enterobacteriaceae* (8.91% ± 7.71%) families contributed the most to fecal microbiota dissimilarity (SIMPER cumulative contribution of 19.6%) (Table 2).

DISCUSSION

In this study, the fecal microbiota of a number of Australian sea lions representing both wild and captive individuals were compared. Studies of marine mammals (and other wildlife) often involve only a small number of samples due to limitations in sourcing samples from wild animals. For large-sized species, such as marine mammals, only a few animals are kept in captive facilities, presenting further sampling limitations in studies contrasting individuals from wild and captive sources. Here, our sample size of 33 wild and 10 captive animal samples from a single species was far greater than that of previous studies investigating the microbiota of marine mammals (5, 17, 19).

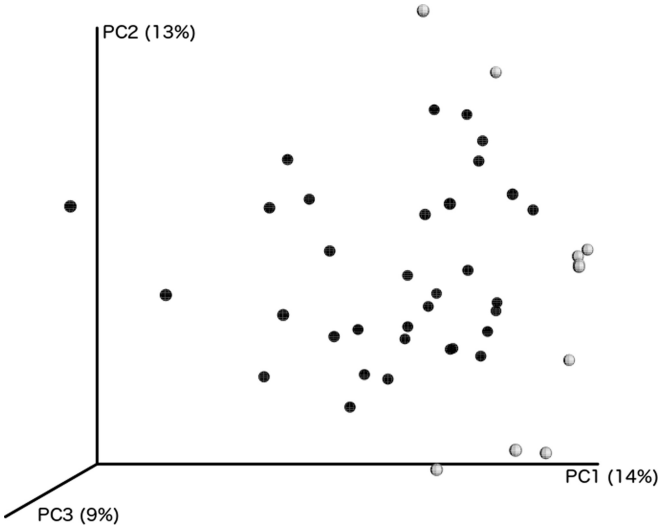


FIG 4 PCoA ordination of unweighted UniFrac distances for fecal microbial communities of wild and captive Australian sea lions. Dark shaded circles, fecal microbiota from wild samples; circles with light shading, fecal microbiota from captive communities.

Our analysis showed that the fecal microbiota of Australian sea lions was dominated by five bacterial phyla: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*. Our findings greatly expand upon observations describing the fecal microbiota of a single sea lion determined from feces collected from Seal Bay, Kangaroo Island, Australia, in which *Firmicutes* contributed ~80% to the microbial community composition (5, 18, 20). Similar fecal microbiota composition and *Firmicutes* dominance have been observed in other pinniped species, including Australian fur, leopard, southern elephant, and Weddell seals, suggesting that the *Firmicutes* are a core group within the pinniped microbiota. In endothermic mammals, the *Firmicutes* have been shown to be associated with layering of body fat to assist thermoregulation in the cool ocean environment (40, 41).

Although pinnipeds appear to have a group of core fecal microbiota, variations in the community compositions between wild sampling sites were observed. Colony dynamics, sea lion behavior, and foraging site fidelity are likely to play important roles in the compositions of gut microbial communities in sea lions from geographically disparate colonies. Three samples in particular had markedly different family-level community compositions, with the bacterial families at low abundances in other samples making up higher proportions of the overall populations in these compared to those in all other samples. Two of these samples were from Olive Island, and the other was from West Waldegrave Island, both in South Australia. Olive and Lilliput Islands, South Australia, also showed the greatest OTU diversity at the genus level.

Olive Island hosts a relatively high-density sea lion colony that is sympatric with a small colony of New Zealand fur seals (*Arctocephalus forsteri*) that also breed on the island (24). Socialization and other interactions between these species are known to occur and could increase the potential for microbial transmission, thereby contributing to the richness of South Australian sea lion gut microbiota (42). Similar findings have been reported in studies observing southern elephant and leopard seal gut microbiota.

TABLE 1 Differences in the OTU frequencies between wild and captive sample groups[a]

OTU identification no.	Taxonomy		Mean OTU frequency (%)		Test statistic[b]	Bonferroni *P*
	Family	Genus	Wild	Captive		
328628	*Lachnospiraceae*	*Blautia*	0.000303	0.54	27.8	0.00004
4380886	*Enterobacteriaceae*		0	5.84	22.3	0.0007
304757	*Ruminococcaceae*		0	0.09	22.3	0.0007
320553	*Peptococcaceae*	*Peptococcus*	0.000909	6.59	20.7	0.0017
178885	*Alcaligenaceae*	*Sutterella*	0.001212	0.14	18.4	0.0055
3070686	*Moraxellaceae*		0	0.88	18.2	0.0063
165489	*Enterobacteriaceae*		0	1.11	18.1	0.0063
185668	*Lachnospiraceae*	*Dorea*	0.03	0.22	17.7	0.0079

[a] The OTU frequency is reported as the percent mean frequency of each individual OTU within each group, using the rarefied subsample of 10,000 reads. The analysis was performed in QIIME using the Kruskal-Wallis nonparametric test. Only OTU for which the Bonferroni-corrected *P* value was <0.01 are shown.
[b] Kruskal-Wallis test statistic.

There, increased microbial richness in elephant seals was attributed to the social nature of elephant seals, as they aggregate ashore in great numbers during the breeding and molting periods, in stark contrast with the solitary nature of leopard seals (5).

Lilliput Island is a very small island that can be completely awash during severe storms. It is close to the mainland and is frequently visited by terrestrial bird species, including the rock parrot (*Neophema petrophila*), shorebirds such as the ruddy turnstone (*Arenaria interpres*), and wide-ranging, beach-roosting seabirds such as the crested and Caspian terns (*Thalasseus bergii* and *Hydroprogne caspia*), increasing the potential for dissemination of terrestrial microbes to sea lions (43, 44). Seabirds visiting terrestrial sources are exposed to a variety of microbes atypical to the natural habitat of marine mammals (45). In addition to having visitation from terrestrial species, sea lions from Lilliput Island forage inshore in very shallow coastal waters close to the mainland (26), increasing their likelihood of exposure to microbes from wastewater runoff and terrestrial sources, which may further explain the elevated microbial diversity observed in fecal samples from this colony.

TABLE 2 SIMPER analysis results comparing wild and captive fecal samples[a]

Taxon (family level)	Avg dissimilarity[b]	Contribution (%)[c]	Mean abundance (%)	
			Wild	Captive
Clostridiaceae	14	19.1	34.8	19.2
Ruminococcaceae	8	10.9	16.7	12.9
Pseudoalteromonadaceae	5.11	6.96	0.09E–06	10.2
Peptostreptococcaceae	4.81	6.56	2.91	8.26
Enterobacteriaceae	4.45	6.07	0.0127	8.91
Clostridiales family, unclassified	3.91	5.33	3.03	8.4
Planococcaceae	3.77	5.15	7.55	0.034
Carnobacteriaceae	3.59	4.89	7.15	0.187
Peptococcaceae	3.32	4.53	1.82E–05	6.65
Moraxellaceae	3.26	4.45	2.47	4.69
Rikenellaceae	2.70	3.69	3.77	2.91
Lachnospiraceae	2.34	3.19	2.92	4.21
Bacteroidaceae	2.13	2.91	4.35	1.24

[a] The Bray-Curtis average dissimilarity between wild and captive was >2% for these 13 microbial families. The overall average dissimilarity was 73.4.
[b] Bray-Curtis average dissimilarity between wild and captive groups, expressed as a percentile.
[c] Contribution to dissimilarity between wild and captive groups.

Only two samples were collected from West Waldegrave Island. One of these, A243, was observed to have a family-level composition which diverged from those of all other samples quite markedly, including A235 from the same colony. A number of bacterial families that were absent or at low abundance (>1%) in other samples contributed significantly (>10%) to the community sampled in A243. West Waldegrave Island is rarely visited either by the general public or by researchers, reducing the likelihood of habitat disturbance. It is unclear whether absence of disturbance contributes in some way to the variable composition or whether it is linked to other intrinsic factors of the colony.

Sea lions from North Fisherman Island, WA, showed substantially lower OTU richness at the phylum level than the majority of South Australian colonies. At this site, sea lions demonstrate a strong tendency for limited dispersal from breeding colonies and a high level of foraging site fidelity (25, 26, 46). Significant differences in trophic diversity between South and Western Australia colonies were identified, and western foraging sites showed lower richness (25). Colony-centric foraging and limited dispersal suggest that habitat and the availability of prey may contribute to decreased richness of microbes colonizing the guts of Western Australian sea lions. Future investigations into dietary variations between South and Western Australia colonies may help further explain the observed differences in gut microbiota compositions and diversity.

We also observed variations in the compositions of the fecal microbiota of captive and wild sea lions. While differences in phylum abundances between the two groups were not statistically significant, qualitative differences were observed at the OTU level. Dietary resources have been shown to exert a strong influence on microbial community compositions of both terrestrial and marine mammals (5, 47, 48). Recent studies observing the influence of diet on gut microbiota of fish and mammalian livestock species have shown that animals that forage manifest greater microbial diversity than those fed from artificial or concentrate sources (47–49). The varied diet of wild Australian sea lions includes a number of species with chitinous body parts, including small crustaceans, rock lobster, and cephalopods such as cuttlefish, octopus, and squid (27, 28). This contrasts with the diet of captive animals, which are fed fresh or frozen fish almost entirely (28). Studies looking at the chitinolytic bacteria in the feces of wild herbivores

(50), sheep (51), and humans (52) found that the majority of identified bacteria belonged to the genus *Clostridium*. It is plausible that the higher levels of *Clostridiaceae* observed in the gut communities of animals from wild populations than in captive animals may be linked to this dietary difference. In addition, environmental microbes from ocean and coastal sources may contribute to gut bacterial richness in wild animals, as has been seen in leopard and southern elephant seals (5).

In captivity, animals are exposed to organisms atypical of their natural environment. This may lead to establishment of microbes from nonendemic sources (30, 53), potentially influencing the composition of microbiota. Captive marine mammals are exposed to a variety of nonendemic microbes via interactions with zookeepers, through animal interaction programs involving the general public, and through social interactions in holding pens with mammalian species not usually within their natural environment. In the present study, the OTU characteristic of the *Proteobacteria* families *Enterobacteriaceae* and *Pseudoalteromonadaceae* were more abundant in captive sea lions, driving the dissimilarity from wild animals. While many members of *Enterobacteriaceae* are harmless intestinal symbionts, this family also includes many well-known pathogenic species (54).

Long-term monitoring studies of microbial communities would be useful for determining what factors are primary drivers of microbial diversity in Australian sea lions. Information on the specificities of diet, medicinal treatment history, cohabitation procedures, and enclosure sampling would enable better understanding of microbial flow through the captive environment. Such information would be beneficial for maintaining the health of sea lions in captivity and also informative for conservation programs that involve breeding and release of other endangered species.

The utility of marine mammals as sentinels for aquatic health requires information on the baseline parameters that drive host health. In this study, we describe the fecal microbial community taxonomic compositions and variability across a large number of wild and captive Australian sea lions. We report a high level of variability across the sampled fecal microbiomes, including variability within many of the studied colony populations. This finding suggests that, for Australian sea lions, fecal microbial community analyses may have limited utility in providing an indication of overall colony health. Larger-scale studies involving greater numbers of samples from each colony may increase the potential for identification of specific taxa that provide indicators of the health of the colony at large or perhaps marine health more broadly, but this remains to be determined.

Our data provide a starting point for hypothesis-based investigations into the complex microbial interactions and microbial movement between populations of a marine species experiencing a range of exposure to anthropogenic sources. Future observations regarding dissemination routes of potentially pathogenic microbes and their establishment will assist in better assessing the suitability of these marine mammals as sentinel species and aid in the long-term conservation of this endangered, diminishing species.

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