Effect of Geography and Captivity on Scat Bacterial Communities in the Imperiled Channel Island Fox

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**Effect of geography and captivity on scat bacterial communities in the imperiled Channel
Island Fox**

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17 Abstract**18 Background**

19 With developing understanding that host-associated microbiota play significant roles in
20 individual health and fitness, taking an interdisciplinary approach combining microbiome
21 research with conservation science is increasingly favored. Here we establish the scat
22 microbiome of the imperiled Channel Island fox (*Urocyon littoralis*) and look at the effects of
23 geography and captivity on the variation in bacterial communities.

24 Results

25 Using high throughput 16S rRNA gene amplicon sequencing, we discovered distinct bacterial
26 communities in each island fox subspecies. Weight, timing of the sample collection, and sex
27 contributed to the geographic patterns. We uncovered significant taxonomic differences and an
28 overall decrease in bacterial diversity in captive versus wild foxes.

29 Conclusions

30 Understanding the drivers of microbial variation in this system provides a valuable lens through
31 which to evaluate the health and conservation of these genetically depauperate foxes. The
32 island-specific bacterial community baselines established in this study can make monitoring
33 island fox health easier and understanding the implications of inter-island translocation clearer.
34 The decrease in bacterial diversity within captive foxes could lead to losses in the functional
35 services normally provided by commensal microbes and suggests that zoos and captive breeding
36 programs would benefit from maintaining microbial diversity.

37 Keywords

38 16S rRNA gene, captivity, Channel Island fox, conservation, microbiota

Background

The gut microbiome plays an essential role in the health and fitness of its host. As such, it is becoming increasingly clear that to fully understand a host one must examine their associated microbiome and the forces driving variation among microbial communities. This approach is burgeoning in the field of conservation biology, where anthropogenic effects such as habitat fragmentation [1] and increasing temperatures [2] have been shown to alter host-associated microbial communities. Integrating microbiome research into conservation science is beneficial at multiple levels from health monitoring [3, 4] to microbiome transplants in captive individuals [5, 6]. Therefore, characterizing the current bacterial communities in endangered host species is crucial to identify future changes in microbial diversity.

Here we investigated the gut microbiome of the Channel Island fox, *Urocyon littoralis*, a charismatic species that was recently rescued from the brink of local extinction and that still faces continuing threats. *Urocyon littoralis* is a dwarfed insular canid with a distinct subspecies on six of the Channel Islands off the coast of southern California, USA. Formerly endangered, four of the six inhabited islands went through extensive population crashes losing upwards of 90-96% of foxes, but have since recovered in population size [7]. Only one subspecies is still federally listed as threatened but all island fox populations face continued threats of human activity and climate change, including introduced animals, novel pathogens, and worsening prolonged droughts. With low genetic diversity within populations, the microbiome may be particularly important in disease identification and protection. In fact, changes in the microbiome have been linked to mange and ear tumors in *U. littoralis* [8, 9]. Potential differences in microbiomes between populations (signifying underlying adaptive differences)

could have profound conservation implications considering proposed inter-island translocation of foxes [10].

In addition to being of conservation concern, this system presents a natural experiment to test the effect of geography on the gut microbiome because the foxes originated from one mainland source, and migrated to the other islands in a known pattern with little further movement between islands [11]. Variation in the size and topography of the islands cause differences in temperature, precipitation, and wind [12] and likely has contributed to the divergence of the gut microbiome. These geographically differing variables, as well as differences in anthropogenic impacts, across the islands influence the diet [13, 14] and pathogen prevalence [15–18] across fox populations. Knowing that diet and the immune system affect the gut microbiome, this system is ideal to test if geography correlates with gut bacterial communities.

Broadly, geographic isolation of populations can lead to divergence in microbiota over time due to drift and/or local adaptation. While geography has been shown to play a role in shaping the gut microbiome in a number of taxa [19–22], few studies have looked at these effects in intraspecific island populations. Relative to interspecific comparisons, intraspecific comparisons can be expected to minimize differences in host factors such as physiology, behavior and ecology, and therefore allow better resolution of factors such as host sex and condition, as well as the effects of geography. Here we investigate a species with substantial geographic subdivision, allowing a strong test of the correspondence between geography and microbial variation.

Captivity, often a tool in conservation management, has also been shown to alter gut microbial diversity [23–28]. Conditions in captivity are markedly different than those in the wild

including changes in diet, interspecific interactions, and health care, any of which can impact microbial community makeup. Across multiple taxa, studies have shown a decrease in microbial diversity in captive mammals compared to wild individuals [23, 25–28], which could impact individual health and/or release success [29]. However, a recent study showed that the microbiome of Tasmanian devils was restored upon release in the wild [30]. The effects of captivity on the microbial community appear to affect diet specialists more than generalists [23] and carnivores more than herbivores [27]. Currently, there are island foxes in multiple mainland zoos, and previously four wild populations went through captive breeding programs, yet it remains unknown how captivity affects the microbiome of *U. littoralis*.

The objective of this study was to characterize the bacterial communities in island fox scat and to determine if geography shapes the composition of those taxa. We also compare natural populations to captive populations. We predict that geography and captivity will drive shifts in bacterial community composition. For the effects of captivity our predictions are two-fold: 1) we predict that bacterial diversity will decrease in captive foxes compared to their wild counterparts, as shown in other canids [27] and 2) we predict that bacterial communities from captive foxes will be more similar to other captive foxes than they are to those from the host source population. Understanding the drivers of the microbiome will provide additional tools for the conservation of these threatened animals and potentially provide a minimally invasive way to gauge health.

Materials and Methods

Sampling and DNA extraction

Scat samples were collected during annual trap monitoring of *U. littoralis* during the fall and winter of 2014-2015 by managers or contractors on the Channel Islands off the coast of

southern California, USA (San Miguel Island, SMI; Santa Rosa Island, SRI; Santa Cruz Island, SCZ; Santa Catalina Island, CAT; San Clemente Island, SCL; San Nicolas Island, SNI; Fig. 1, Table S1). Scat samples from captive *U. littoralis* were collected opportunistically in 2014 (Orange County Zoo, OCZ; Santa Barbara Zoo, SBZ). Scat samples were collected in ethanol and then stored at -80°C until DNA extraction could be performed. Total genomic DNA was extracted from the scat samples using the E.Z.N.A Genomic DNA Isolation Kit for stool samples (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions with minor changes. Modifications to the protocol included incubating with Proteinase K for 20 minutes (an increase of 10 minutes) and eluting the DNA with 100µL of elution buffer. Each batch was extracted alongside an experimental blank containing molecular grade water instead of sample to monitor potential contamination. Genomic DNA was quantified using an Invitrogen Qubit 2.0 Fluorometer (Carlsbad, CA, USA).

Library preparation and sequencing

The V4 hypervariable region of the 16S rRNA gene was amplified from DNA extracted from *U. littoralis* scat using bacterial “universal” primers 515F and 806R, to which 4 ambiguous bases and variable barcodes on the forward primer and variable indexes on the reverse primers were added (Table S2; Caporaso et al. 2010; Bartram et al. 2011). We followed a modified Earth Microbiome Project protocol [33]. Briefly, the 16S V4 region was amplified in triplicate reactions using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA) and then the reactions were cleaned with AMPure XP beads (Beckman Coulter, Brea, CA, USA) at a 1:1 ratio of beads to PCR product volume. The amplicon quantity was again evaluated with the Qubit and the quality was further evaluated with a Bioanalyzer analysis (Agilent Technologies, Santa Clara, CA, USA). Finally, samples were pooled equimolarly for

sequencing. 95 samples (Table S1) were sequenced as 300bp paired-end reads on an Illumina MiSeq at UCLA's Genotyping Core (Los Angeles, CA, USA). Blank samples (N = 5), technical replicates (repeat sequencing library preparations on the same DNA extraction; N = 10), and a positive control (an even mock community) were sequenced as part of the 95 samples. For the Orange County Zoo samples (sample 1 = OCZ.1a and OCZ.1b) and one Santa Barbara Zoo sample (sample 102003 = SBZ.2a and SBZ.2b) biological replicates were sequenced (meaning two different scat samples from the same organism). These two zoo individuals (SBZ.2a/b and OCZ.1a/b) were born in captivity but are descended from San Clemente Island foxes. One zoo sample (SBZ.1) was born on San Clemente Island but was brought into captivity.

Sequence processing

Leading ambiguous bases were trimmed from forward reads with Trimmomatic v. 0.33 [34]. Barcodes were extracted with `extract_barcodes.py`, and samples were demultiplexed in QIIME1 v. 1.9.1 [35]. Demultiplexed reads were merged into one artifact with QIIME1 and then imported back into QIIME2. Sequences were processed using `denoise-paired` in the DADA2 v. 2018.6.0 plugin [36] in QIIME2. Processing included the following steps with default settings unless otherwise noted: denoising, trimming primers (`--p-trim-left-f 19, --p-trim-left-r 20`), removing chimeras and dereplicate reads, trimming forward reads to a minimum of 270bp and reverse reads to a minimum of 210bp based on quality scores, filtering out targets below those length cutoffs, and inferring amplicon sequence variants (ASVs) for both forward and reverse reads. Non-clustering methods that generate sequence variants are convincingly argued to be preferred over traditional 97% sequence clustering approaches due to their higher resolution and reproducibility [37]. Taxonomy was assigned using `feature-classifier classify-sklearn` [38] in QIIME2 using the Greengenes database v. 13-8 [39], then non-target ASVs (mitochondria,

archaea, and chloroplasts) were removed. The mock community sample was removed from the dataset for analysis.

Decontamination, filtering, and transformation

The data were imported into RStudio v. 1.1.456 (RStudio Team, 2016) with R v. 3.5.1 (R Core Team, 2018) using the Qiime2r v. 0.99.1 package [40] creating an object to use with the Bioconductor R package Phyloseq v. 1.24.2 [41]. Extraction blanks were addressed first by identifying potential contaminants using Decontam v. 1.0.0 [42]. We used the “combined” method, which is based on frequency (variation based on DNA concentration) and prevalence (increased prevalence in negative controls) probabilities in tandem with a Fisher’s combined probabilities test. This method identified 12 ASVs as potential contaminants which were then removed from the dataset (Table S3). After decontamination, extraction blanks were also removed from the dataset. Then technical replicates were merged using mean counts in Phyloseq (see Fig. S1 for principal coordinates analysis (PCoA) of technical replicates). The ASV count table was transformed using a variance stabilizing transformation [43, 44] in DESeq2 v. 1.20.0 (type = “poscounts” to estimate size factors; Love et al. 2014) for beta diversity assessment.

General taxonomy and beta diversity visualization

The dominant taxa present in the scat bacterial microbiome were identified based on ASV proportions (not transformed), and differences among sources were evaluated with analysis of variance (ANOVA) and Tukey HSD post hoc tests. The Proteobacteria phylum was split into its major classes for analysis due to its complexity [46], and phyla representing less than 5% of the ASVs were grouped together as “other”. To address beta diversity, a Euclidean distance matrix was calculated on variance-stabilizing transformed data and used with hclust (method =

ward.D2) to generate a hierarchical clustering dendrogram. The transformed data were also used to conduct a principal coordinates analysis (PCoA) based on Euclidean distances with Phyloseq.

Testing for group differences and differential abundance

Testing for statistical differences in scat bacterial communities was done on the transformed ASV counts with Vegan v. 2.5-3 (Oksanen, 2018). Permutational ANOVAs were calculated using adonis (permutations = 99999) based on Euclidean distances [47, 48] only on samples with no missing data (Table S4A). The data were re-transformed for each dataset after samples with missing data were removed. We did this across all samples and then again for each island. The assumption of homogeneity in dispersion within groups was tested with betadisper. We evaluated the best models by including all variables regardless of dispersion and then we sequentially removed variables with low variance explained (R^2). The models were evaluated with Akaike information criterion (AIC), taking the lowest value as the best model that was used in the permutational ANOVA. The source code for the AIC method is available at [49]. The effects of age, body condition, captivity (wild vs captive), month and year collected, sex, source (each island or zoo), and weight were analyzed. Body condition is a common measurement scale used to gauge animal health [50] that goes from emaciated (body condition = 1) to obese (body condition = 5) and was recorded by wildlife managers in the field. We then examined differential abundances in ASVs for comparisons with significant permutational ANOVA results using the R package DESeq2 and a significance level of 0.01 (with the input being the untransformed, but filtered, count table). We looked at alpha diversity in comparisons with significant permutational ANOVA results by calculating Chao1 richness index and Shannon diversity index using untransformed count tables and plot_richness in Phyloseq to understand relative differences.

Results

Taxonomy

At the phylum level, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria (classes Gammaproteobacteria and Betaproteobacteria) dominated the scat samples; the first two accounting for over 67% of the recovered sequences and the top 6 major taxa collectively accounting for about 94% of the total sequences in each group (Fig. 2). Seven major taxa were significantly different between sources. Firmicutes had the highest number of significant pairwise comparisons and was predominantly elevated in zoo foxes compared to island foxes (Table S5, Fig. S2). Foxes from SMI and SNI were significantly different between 3 out of 7 major taxa, the most of any pairwise comparison (Table S5). Predominantly foxes from SMI had significantly elevated proportions of Fusobacteria (Table S5, Fig. S2). Bacteroidia made up the largest average proportion ($37.8 \pm 12.2\%$; mean $\pm$ 1SD) of identified classes followed by Clostridia ($27.4 \pm 10.0\%$). The same pattern held for order; Bacteroidales and Clostridiales made up the largest represented orders at comparable percentages to their respective classes.

Between-group diversity visualization

The bacterial communities largely clustered into two main groups based on geography (Fig. 3, Fig. 4A). We identified two main clusters based on the dendrogram. One cluster is entirely composed of samples from the southern islands (SNI, SCL and CAT), while the other contains all of the northern and zoo samples, as well as a subset of southern samples including about half of the SCL samples, 4 CAT and 2 SNI. In the PCoA, axis 1 largely described the distance between the northern/zoo samples group and the southern islands group (10.6%), including the aforementioned exceptions, and axis 2 predominantly described the variation within those groups (8.0%, Fig. 4A).

Drivers of the bacterial communities

The strongest driver of differences among bacterial communities was source (Table 1, Fig. 4B). Across all samples, weight and month of sample collection also significantly influenced the bacterial communities (Table 1). When broken out by island, month was the driving factor on SRI, sex the largest factor on CAT, and on SCL weight and year drove the difference in bacterial communities (Table 1, Fig. S3). To look further into the influence of collection timing on SRI and SCL, we evaluated precipitation over the period looked at for each island (Fig. S4). Sex was also found to be the driving factor on SNI; however, the pattern was driven by one male, and when removed there were no significant factors (Table 1, Fig. S3). We investigated the difference between the southern samples that grouped with the northern samples and those that did not and found the best model included weight, month collected, and the group in which the sample was extracted as significant drivers of bacterial community difference. For results from our model testing see Table S4B-H. It should be noted that not all variables were homogeneously distributed, but the heterogeneity in the source term is likely biologically relevant (Table S6, Fig. 4B). Further, SCZ did not have enough samples ($N = 3$) to conduct the permutational ANOVA, and there were no significant factors identified for SMI likely due to small sample size ($N = 6$) after removing samples with missing data.

We found 173 differentially abundant ASVs significant at the $\alpha = 0.01$ level across our significant factors. On SRI we found 72% of the significantly differentially abundant ASVs were elevated in samples collected in November, while 28% of the ASVs were elevated in December (Table 2). On CAT we found 38% of the significantly differentially abundant ASVs were elevated in female samples, opposed to 62% of the ASVs being elevated in males (Table 2).

Additionally, on SCL we found the majority of the significantly differentially abundant ASVs were elevated in samples collected from 2014 (87%) and heavier foxes (88%).

To explicitly test for the effect of captivity on the scat bacterial microbiome, we probed differences between the captive and SCL samples because all zoo individuals were either descendants of SCL foxes or born on the island and then taken into captivity (Fig. 5). We found that the best model for these data included only the captivity factor ($AIC = 275.58$), which was significant (Table 1). Body condition and weight could not be considered due to missing data. We found that the majority of the significantly differentially abundant ASVs were elevated in wild samples (81%) compared to captive (Table 2).

Patterns of alpha diversity

Statistical analyses on alpha diversity can be misleading [51], therefore we explore qualitative alpha diversity patterns to gain insight into the statistically significant factors we identified through the PERMANOVAs. When comparing across sources, scat bacterial communities from the three southern islands, especially CAT, generally had higher ASV richness and evenness compared to the northern Channel Islands (Fig. S5A). Across all samples analyzed, ASV richness and evenness peaked in October, while richness, but not evenness, trended toward increasing with weight (Fig. S5B, S5C). In bacterial communities from SRI, scat collected in November had higher richness and evenness compared to those collected in December (Fig. S5D). Males had higher evenness, but not richness, compared to females on CAT (Fig. S5E). Meanwhile, the evenness of bacterial communities on SCL declined with increasing weight while richness was constant (Fig. S5F). Both richness and evenness were higher in samples collected in 2014 compared to those collected in 2015 on SCL (Fig. S5G).

Samples collected from captive foxes had lower richness and evenness than those collected from wild foxes (Fig. 5C).

Discussion

Recent advances and cost efficiency in high-throughput sequencing has made culture-independent methods of surveying microbial communities tractable. Combined with the insight that host-associated microbiota may aid in rapid adaptation to environmental change [52], both have led to conservation biologists adopting this approach as a valuable tool to maintain biodiversity at yet another level—the microbiome. We sought to characterize a baseline and identify determining factors in each isolated subspecies within the threatened *U. littoralis* by using high-throughput amplicon sequencing. Few studies address microbiome differences among geographically isolated intraspecific populations, yet such studies provide insight into the mechanisms driving the composition of commensal microbes. We report distinct bacterial communities on each island as well as between wild and captive foxes.

Geography drives the island fox microbiome

Geographic source is a large contributor to bacterial community composition in *U. littoralis* scat (Fig. 3; Fig. 4). This result is expected, given that many biotic and abiotic factors change with biogeography which have been previously shown to affect the bacterial microbiome [20]. Overall, the scat bacterial communities of *U. littoralis* were made up of the usual mammalian gut taxa; Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria are consistent, abundant players in the canid scat bacterial communities [53, 54]. In other studies, bacterial community differences among populations have been shown to be driven by differences in diet [1], which could also contribute to the differences we see across islands in our dataset. For example, the lower relative abundance of Firmicutes classes Bacilli and Clostridia on SMI and

SRI, and the higher abundance of Fusobacteriales, compared to the southern islands could reflect a more protein-rich diet [55–57]. This hypothesis is consistent with data showing that foxes on SRI consume the lowest amount of fruit among the islands and that on SMI the only fruit consumed by foxes is that of a non-native plant and instead they rely heavily on insects [14]. The month the sample was collected likely reflects differences in diet as well, as described below. Interestingly, we found a unidirectional pattern in which a subset of samples from southern islands grouped with northern samples, but none of our predictor variables were able to explain this pattern (data not shown). More work is therefore needed to understand this pattern.

While the largest signal was from geography itself, weight, timing of the collection, and sex also contributed to the geographic patterns. Weight was a significant factor across all samples, although it is important to note that there is a known significant difference in body size among the islands [58], which was also found in our weight data ($\text{ANOVA}_{\text{source}}, P = 5.0 \times 10^{-9}$). The highest mean weight was found on CAT, and SNI had the lowest mean weight. Weight was also a significant factor within an island, specifically on SCL, where the majority of the significantly differentially abundant ASVs in high weight samples were associated with Clostridia. Similarly, Clostridiales was found to be more abundant in obese pet dogs [59]. Weight is a well-studied factor, especially in humans and mice, that affects the microbiome [60–63], and thus also an expected result of our study. Obesity has been associated with increased levels of Firmicutes [61–64], and while foxes are rarely overweight especially in the wild, we found similar patterns in heavier animals.

The timing of sample collection also significantly influenced the scat bacterial communities in *U. littoralis* both among and within islands, specifically on SCL and SRI. The top NCBI Blast hit for the ASV with the largest effect in foxes in 2014 on SCL was

Parasutterella secunda, which is proposed to be a core taxon that has a role in bile acid maintenance and cholesterol metabolism [65]. The top NCBI Blast hit for the ASV with the largest effect in foxes in 2015 on SCL was *Bacteroides coprocola*, which is also associated with bile and the breakdown of plant polysaccharides [66]. Temporal changes in the gut microbiome can be associated with seasonal changes in diet [67]. The winter is typically the wet season in southern California, which drastically changes the flora and fauna for the subsequent months. Exemplary of this process, the total rainfall on SRI and SCL in December of 2014 was 9-25 times greater than the surrounding months (Fig. S4; [68]) and likely changed food source availability for the foxes. The top NCBI Blast hit for the ASV with the largest effect in foxes in November on SRI was *Diplorickettsia massiliensis*, which is an obligate bacterium of arthropods [69]. The top NCBI Blast hit for the ASV with the largest effect in foxes in December on SRI was *Rosenbergiella australiborealis*, which was isolated from plant nectar [70]. The taxa that correlate with collection timing, especially on SRI, appear to be more associated with environmental drivers of diet than with host metabolism.

Sex contributed to the differences in bacterial communities, which is consistent with several mammalian gut studies [71–74]. However, this result was only true for one island (CAT). The top NCBI Blast hit for the ASV with the largest effect in females was *Fusobacterium mortiferum*, which metabolizes sucrose [75]. The top NCBI Blast hit for the ASV with the largest effect in males was *Faecalibacterium prausnitzii*, which is a well-studied gut commensal that produces butyrate and is indicated as a sentinel in gut health [76, 77]. As the function of these bacteria indicate, sex-biased differences in diet can explain differences in gut microbiota [78]. However, the previous study of the island fox’s diet did not look at differences

between sexes [14] so more work is needed here to tease apart that relationship. Alternatively, sex hormones have been proposed as a mechanism for sex-specific microbiome differences [79].

Captivity alters the fox microbiome

Captivity significantly altered the scat microbiome of *U. littoralis* as evidenced by shifts in specific taxa (i.e., Firmicutes) and lower overall bacterial diversity. Despite the small sample sizes including biological replicates, this strong pattern emerged and is consistent with previous studies [23, 27, 80–82]. The top NCBI Blast hit for the ASV with the largest effect in wild foxes was *Ruficoccus amylovorans*, which is a diazotroph that reduces nitrate and degrades starch [83]. In contrast, the top NCBI Blast hit for the ASV with the largest effect in captive foxes was *Streptococcus alactolyticus*, a potentially pathogenic species associated with diseases (ruminal acidosis, infective endocarditis, colorectal cancer) in humans and animals [84, 85]. Furthermore, several subspecies in the *Streptococcus bovis*/*Streptococcus equinus* Complex, to which *S. alactolyticus* belongs, have antimicrobial resistance [85, 86]. While the mechanism of the bacterial shifts seen in this study remains unknown, previous studies have suggested changes in diet between captive individuals and their wild counterparts drive community differences in microbiota [26, 27, 87–89]. In the wild, island foxes are opportunistically omnivorous feeding on small mammals, insects, and native fruits [14], while individuals in captivity are fed food items not found on the Channel Islands (e.g. canned wet and dry food, bananas, carrots, pasta, yams). Our results point to the influence that captivity can have on the fox immune system and subsequently the scat bacterial community. Reduced gut microbiome diversity can affect nutrient absorption and immune system function, ultimately affecting the overall health of the organism [4, 29, 90]. While there is some evidence that captive animals can gain back lost microbes upon release into the wild [30], carnivores have been shown to be more susceptible to

losing microbial diversity in captivity [87] and there is evidence that it is more difficult to gain back wild microbes with an increasing number of generations in captivity [91, 92]. Going forward it will be imperative to manage host-associated microbiomes for captive foxes, especially if they will be released into the wild. To encourage a more diverse gut microbiome in captivity, managers could increase microbial reservoirs in enclosures (i.e., [93]), shift diets to be more diverse and mimic natural food items, and decrease the use of antibiotics, as presented in [30]. More intensive approaches, including using prebiotics and probiotics that reflect the wild microbiome and/or fecal transplants could also be included to maintain diverse gut microbes.

For the captive samples, the PCoA (Fig. 5A) and multivariate dispersion (Fig. 5B) both show that SBZ2 and OCZ1 (both born in captivity) are similar to each other, while SCZ1 (born on SCL) is more similar to SCL. This pattern, while not confirmed statistically, suggests that inoculation from the natal environment may be more important than current environmental effects, which warrants further study. This legacy effect has been well-characterized in humans [94] and identified in other taxa [95–97]. Some organisms have been able to maintain 60-70% of their wild microbes in captivity [98, 99], which could suggest that animals born in the wild but housed in captivity may have better health outcomes than those born in captivity.

Conservation implications

The evidence provided here suggests that the scat microbiome of *U. littoralis* is largely driven by geography, and that a strong contributing factor seems to be food availability. These results add to the mounting evidence of distinctions among the six subspecies. Discussions of a genetic rescue to augment island populations of low genetic diversity by introducing animals from islands of higher genetic diversity will have to consider the microbiome differences as they represent possible divergences in natural history (diet) and immune systems that could be

incompatible with other island-specific pathogens [100]. Secondly, even though there is extremely low genome-wide diversity in *U. littoralis*, a recent study found no signs of inbreeding depression in skeletal morphological traits [101]. The prevailing thought is that, on some of the islands, foxes have been able to persist despite the negative consequences of small population sizes and low genetic diversity. Perhaps other factors, such as the microbiome, contribute to their longevity and health, and the plasticity of the microbiome contributes to acclimating and eventually adapting to demographic and environmental changes [52]. The insights provided in this study attest to the potential that microbiome research has for informing conservation science.

Conclusions

We conducted the first scat microbiome analysis across all islands of the Channel Island fox. Our results revealed that scat bacterial communities are primarily comprised of Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. The scat microbiome was driven by the geography of the isolated subspecies, while weight, timing of the sample collection, and sex also contributed to the geographic patterns. Captive foxes had significantly different scat bacterial communities, specifically an enrichment of Firmicutes and lower diversity compared to their wild counterparts. Our results suggest that the microbiome is a fruitful tool to use in *U. littoralis* conservation management.

Abbreviations

ANOVA: Analysis of variance

ASV: amplicon sequencing variant

CAT: Santa Catalina Island

PERMANOVA: Permutational multivariate analysis of variance

403 SCL: San Clemente Island

404 SCZ: Santa Cruz Island

405 SMI: San Miguel Island

406 SNI: San Nicolas Island

407 SRI: Santa Rosa Island

408

409 **Declarations**

410 **Ethics approval and consent to participate**

411 Not applicable

412 **Consent for publication**

413 Not applicable

414 **Availability of data and material**

415 The dataset generated for this study (BioProject PRJNA560412) can be found in the NCBI SRA

416 <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA560412/>.

417 **Competing interests**

418 The authors declare that they have no competing interests.

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422 **Authors' contributions**

423 SE and NEA conceived and designed the study. NEA and MAB performed the laboratory work

424 and analyzed the data. NEA and SE wrote the manuscript.

425 **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Additional files

Additional file 1: Supplementary Tables and Figures (Supplemental Information_3-23-2021.pdf)

Additional file 2: Supplementary Table of sample sizes and model testing (modelTesting_1-18-2021.xlsx)

Additional file 3: Amplicon sequence variants count table (feature-table.biom)

Additional file 4: Taxonomic assignment for amplicon sequence variants (taxonomy.tsv)

Additional file 5: Metadata associated with samples (sample_metadata_UPDATED_4-13-2020.txt)

Additional file 6: Data analysis in R (Adams_code4ms.html)

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Adams et al. Tables and Figures

Table 1. Results from the permutational ANOVAs (Euclidean distance, permutations = 99999) for the best models. P-values for significant factors are in bold.

	Df	sumSq	meanSq	F	R ²	P	AIC
Islands (N = 62)							890.79
Source	5	448613	89722.57	3.8	0.25	1.00E-05	
Month collected	4	118065	29516.19	1.2	0.065	0.0232	
Weight	1	45732	45731.68	1.9	0.025	0.00173	
Residuals	51	1218009	23882.52	NA	0.67	-	
Total	61	1830418	-	-	1	-	
SMI (N = 6)							63.59
Age	1	8038	8037.51	1.1	0.19	0.368	
Year collected	1	8135	8134.7	1.1	0.2	0.361	
Weight	1	8340	8339.91	1.1	0.2	0.293	
Extracted group	1	9354	9354.43	1.2	0.23	0.0944	
Residuals	1	7572	7571.83	-	0.18	-	
Total	5	41438	-	-	1	-	
SRI (N = 13)							158.29
Month collected	1	25848	25848.18	2	0.15	0.00218	
Residuals	11	142736	12976.02	-	0.85	-	
Total	12	168584	-	-	1	-	
CAT (N = 15)							193.45
Sex	1	35034	35034.06	1.5	0.1	0.0155	
Residuals	13	305670	23513.04	-	0.9	-	
Total	14	340704	-	-	1	-	
SCL (N = 15)							190.01
Year collected	1	28305	28304.96	1.6	0.1	0.0414	
weight	1	39466	39466.12	2.2	0.14	0.00416	
Residuals	12	212639	17719.95	-	0.76	-	
Total	14	280410	-	-	1	-	
SNI (N = 10)							117.49
Sex	1	21653	21653.19	2.1	0.2	0.0137	
Condition	1	11634	11633.55	1.1	0.11	0.254	
Age	1	10918	10917.97	1	0.1	0.326	
Month collected	1	12581	12581.41	1.2	0.11	0.137	
Weight	1	11056	11055.98	1.1	0.1	0.339	
Extracted group	1	10387	10386.84	1	0.095	0.481	
Residuals	3	31223	10407.57	-	0.29	-	
Total	9	109452	-	-	1	-	
SNI (females, N = 9)							102.91
Month collected	1	10093	10092.74	1.2	0.15	0.11	
Residuals	7	59271	8467.26	NA	0.85	-	

Total	8	69364	-	-	1	-
Captivity (N = 21)						275.58
Wild vs captive	1	67512	67511.98	3.1	0.14	4E-5
Residuals	19	413486	21762.42	-	0.86	-
Total	20	480998	-	-	1.00	-

Df = degrees of freedom, sumSq = sum of squares, meanSq = mean squares, F = F-statistic, R^2 = variance explained, P = p-value, AIC = Akaike information criterion.

Table 2. Top differentially abundant ASVs for significant comparisons. The values in parentheses are the total number of differentially abundant ASVs at a significance level of $\alpha=0.01$ for that comparison. Adjusted p-values are reported from a Wald test. Top Blast hits were found using the 16S database in NCBI and reporting the top hit with the highest score. LfcSE indicates the standard error of the logarithmic fold change, and the lowest classification is from the taxonomic assignment based on the Greengenes database.

Comparison	ASV	log2Fold Change	lfcSE	padj	Lowest classification	BlastHit	Accession	Identity (%)	Score
SRI									
Nov (33)	79b4767bedcb8bbb569be837c0e91fc9	27.7	3.1	6.17E-17	<i>Rickettsiella</i>	<i>Diplorickettsia massiliensis</i> 20B	NR_117407.1	98.01	431
Dec (13)	e42382ea3fa1149094d507e3167e462b	-28.7	3.01	7.72E-19	Enterobacteriaceae	<i>Rosenbergiella australiborealis</i> strain CdVSA 20.1	NR_126305.1	100	453
CAT									
Female (19)	290aac7b5cfcb9e70ca98c874f099965	26	2.99	3.83E-15	<i>Fusobacterium</i>	<i>Fusobacterium mortiferum</i> strain DSM 19809	NR_117734.1	98.8	448
Male (31)	b15f5845058247119ae560a6b19e2e4d	-25.5	2.98	6.80E-15	<i>Faecalibacterium prausnitzii</i>	<i>Faecalibacterium prausnitzii</i> strain ATCC 27768	NR_028961.1	98.41	442
SCL									
2014 (46)	13173fa1a3d63abc7f566a5a4cbf27bc	25.1	3.42	1.43E-11	<i>Sutterella</i>	<i>Parasutterella secunda</i> strain JCM 16078	NR_113328.1	95.63	403
2015 (7)	3c994ad0917947698cf5235a951c4df0	-28.9	3.32	1.79E-15	<i>Bacteroides</i>	<i>Bacteroides coprocola</i> DSM 17136 strain M16	NR_041278.1	98.41	442
Low weight (3)	06c214f56026f8ffd260f153bd0cf856	19.8	5.11	0.00637	<i>Prevotella</i>	<i>Prevotellamassilia timonensis</i> strain Marseille-P2831	NR_144750.1	93.63	375
High weight (21)	f62358a1dcad7653dddf044a1b7277c3	-23.7	5.22	0.00212	<i>Anaerofilum</i>	<i>Anaerofilum pentosovorans</i> strain Fae	NR_029313.1	94.8	390
Captivity									
Wild (147)	845be19afef094e4686bb348b963a973	28.0	2.83	3.36E-21	Cerasicoccaceae	<i>Ruficoccus amylovorans</i> strain CC-MHH0563	NR_156844.1	88.49	303
Captive (34)	386622a3b30e3e131e44a1db5038508d	-27.9	3.43	9.25E-15	<i>Streptococcus luteciae</i>	<i>Streptococcus alactolyticus</i> strain ATCC 43077	NR_041781.1	100	464

Figure 1. Map of scat samples across the Channel Islands and the mainland. The sample sizes are listed followed by the number of replicates, which are listed in parentheses.

Figure 2. Percent relative abundance of the major taxa recovered from *U. littoralis* scat per sample. Sample names are colored by source (island or zoo).

Figure 3. Dendrogram of transformed data based on Euclidean distance. Sample names are colored by island or zoo (see Fig. 1) and shapes correspond to region: circle = northern islands, triangle = southern islands, and square = captive samples.

Figure 4. **A)** Principal coordinates analysis (PCoA) based on Euclidean distance. The points are samples colored by source (island or zoo) and the shapes represent northern or southern Channel Islands and captive samples. **B)** Boxplot of multivariate dispersion as measured by the distance to the centroid per sample and displayed by source.

A **B** **C**

Figure 5. **A)** Principal coordinates analysis (PCoA) based on Euclidean distance of samples from SCL and captivity (SBZ, OCZ). **B)** Boxplot of multivariate dispersion as measured by the distance to the centroid per sample and displayed by source. **C)** Chao 1 and Shannon diversity metrics between wild and captive samples.

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Table S1. Details for scat samples. Scat samples were collected from all six inhabited islands which included: San Miguel (SMI), Santa Rosa (SRI), Santa Cruz (SCZ), Santa Catalina (CAT), San Clemente (SCL), and San Nicolas (SNI) as well as the Orange County Zoo (OCZ) and the Santa Barbara Zoo (SBZ). Samples were extracted along with extraction blanks (BLANK) and sequenced along with a mock community (MOCK).

ID	Specimen number	Source	Region	Captivity status	Sex	Age	Body condition	Weight (kg)	Year collected	Month collected
SMI.4	2249	SMI	north	wild	M	3	2	2.29	2015	Jan
SMI.5	8875	SMI	north	wild	F				2015	Jan
SMI.6	15754	SMI	north	wild	F				2015	Jan
SMI.7	15761	SMI	north	wild	M				2015	Jan
SMI.8	24411	SMI	north	wild	F				2015	Jan
SMI.9*	26235	SMI	north	wild	M				2014	Nov
SMI.10	77488	SMI	north	wild	F	3	2	1.75	2014	Nov
SMI.11	87117	SMI	north	wild	F				2015	Jan
SMI.12	88361	SMI	north	wild	F	2	2	2.3	2015	Jan
SMI.13	1124C	SMI	north	wild	M	4	3	2.68	2015	Jan
SMI.14	24902-F388	SMI	north	wild	F	1	2	1.9	2014	Dec
SMI.15*	C4F60-M281	SMI	north	wild	M	2	2	2.45	2014	Nov
SMI.16	C7602-F348	SMI	north	wild					2015	Jan
SRI.17	15792	SRI	north	wild	F	2	3	1.95	2014	Nov
SRI.18	15793	SRI	north	wild	M	3	4	2.53	2014	Nov
SRI.19*	16090	SRI	north	wild	F	0	2	1.9	2014	Nov
SRI.20*	16101	SRI	north	wild	F	2	4	2	2014	Nov
SRI.21	16122	SRI	north	wild	F	2	2	2.01	2014	Nov
SRI.22	16123	SRI	north	wild	F	1	4	2.6	2014	Nov
SRI.23	16444	SRI	north	wild	M	0	3	2.17	2014	Dec
SRI.24	16500	SRI	north	wild	F	2	4	2.37	2014	Dec
SRI.25	37599	SRI	north	wild	F	2	4	2.21	2014	Nov
SRI.26	38956	SRI	north	wild	F	3	2	2.07	2014	Nov
SRI.27	62035	SRI	north	wild	M	3	3	2.3	2014	Dec
SRI.28	86634	SRI	north	wild	F	3	3	2.31	2014	Dec

SRI.29	3604E	SRI	north	wild	F	3	3	2.28	2014	Dec
SCZ.1	12846	SCZ	north	wild	M	1	3	1.89	2015	Jan
SCZ.2	36422	SCZ	north	wild	M	2	2	1.46	2015	Jan
SCZ.3	51026	SCZ	north	wild	F	1	3	1.71	2015	Jan
CAT.50	21133	CAT	south	wild	F	2	3	2.61	2014	Oct
CAT.51	20852	CAT	south	wild	F	2	2.5	2.06	2014	Oct
CAT.53	63425	CAT	south	wild	F	1	3	2.59	2014	Oct
CAT.54	95519	CAT	south	wild	M	1	3	2.5	2014	Oct
CAT.55	45F62	CAT	south	wild	M	3	5	3.36	2014	Oct
CAT.57	3792D	CAT	south	wild	F	1	4	2.07	2014	Nov
CAT.58	B0F74	CAT	south	wild	M	1	3	2.7	2014	Oct
CAT.59	1477A	CAT	south	wild	M	3	2	2.43	2014	Nov
CAT.60	35E15	CAT	south	wild	F	3	3	2.4	2014	Dec
CAT.63	97338	CAT	south	wild	F	3	2	1.87	2014	Oct
CAT.16C09	16C09	CAT	south	wild	M	4	4.5	2.86	2014	Oct
CAT.20377	20377	CAT	south	wild	F	3	2	1.86	2014	Oct
CAT.8383D	8383D	CAT	south	wild	M	1	3	2.5	2014	Oct
CAT.E2211*	E2211	CAT	south	wild	F	3	3	2.36	2014	Oct
CAT.F0534*	F0534	CAT	south	wild	M	1	4.5	3.08	2015	Oct
SCL.1	982000190551073	SCL	south	wild	M	1	3	2.07	2014	Oct
SCL.2	982000190686094	SCL	south	wild	F	2	2	1.56	2014	Nov
SCL.3	982000167831348	SCL	south	wild	F	2	3	1.91	2014	Oct
SCL.4	982000363935561	SCL	south	wild	F	1	2	1.76	2015	Jan
SCL.5	4A0A021174	SCL	south	wild	M	2	2	1.77	2015	Jan
SCL.7	982000190642413	SCL	south	wild	F	1	3	1.64	2014	Nov
SCL.8	985121017331033	SCL	south	wild	F	2	2	1.81	2014	Nov
SCL.9	900236000199222	SCL	south	wild	F	1	2	1.55	2014	Nov
SCL.10*	985121017379629	SCL	south	wild	M	1	3	2.28	2015	Jan
SCL.11	985120032372226	SCL	south	wild	M	3	3	2.08	2014	Oct
SCL.13	900236000199213	SCL	south	wild	M	1	2	1.98	2015	Jan
SCL.14	982000363932112	SCL	south	wild	F	1	3	2.05	2014	Dec
SCL.15	900236000199202	SCL	south	wild	F	1	2	1.2	2014	Oct
SCL.17*	900236000202580	SCL	south	wild	F	1	3	1.71	2014	Nov
SCL.19*	900118001238833	SCL	south	wild	F	1	3	2.33	2014	Oct

SCL.20.2	985120032249793	SCL	south	wild	F	2	2	1.56	2014	Nov
SNI.2B	1804	SNI	south	wild	M	1			2014	Aug
SNI.4B	1797	SNI	south	wild	F	2	2	1.55	2014	Aug
SNI.6A.mix	76372	SNI	south	wild	F	4	1	1.29	2014	Sep
SNI.7A	17828	SNI	south	wild	F	4	1	1.39	2014	Aug
SNI.9A	38106	SNI	south	wild	F	2	2	1.69	2014	Sep
SNI.10A	1807	SNI	south	wild	M	1			2014	Sep
SNI.11	32952	SNI	south	wild	F	1	2	1.56	2014	Sep
SNI.12A	82F61	SNI	south	wild	F	4	1	1.5	2014	Sep
SNI.13A	40749	SNI	south	wild	M	2	1	1.21	2014	Sep
SNI.14A	2609	SNI	south	wild	F	1	3	1.53	2014	Sep
SNI.15	5141C	SNI	south	wild	F	1	2	1.8	2014	Sep
SNI.17	40530	SNI	south	wild	M	3			2014	Sep
SNI.19	76917	SNI	south	wild	F	3	3	2.02	2014	Sep
SNI.20*	38057	SNI	south	wild						
OCZ.1a+§	1	OCZ	mainland	captive	M	11			2014	Oct
SBZ.1§	700607	SBZ	mainland	captive	M	2			2015	Jan
SBZ.2a+§	102003	SBZ	mainland	captive	M	12			2015	Jan
BLANK.2	BLANK.2	BLANK	NA	NA						
BLANK.7	BLANK.7	BLANK	NA	NA						
BLANK.9	BLANK.9	BLANK	NA	NA						
BLANK.10	BLANK.10	BLANK	NA	NA						
BLANK.11	BLANK.11	BLANK	NA	NA						
MockCom.E	MockCom.E	MOCKCOM	NA	NA						

* Technical replicate IDs: SMI.9.2, SMI.15.2, SRI.19.2, SRI.20.2, CAT.E2211.2, CAT.F0534.2, SCL.10.2, SCL.17.2, SCL.19.2, SNI.20.2

+ Biological replicate IDs: OCZ.1b, SBZ.2b

§ Original lab IDs: OCZ1a = OCZOO.1, OCZ.1b = OCZ.2, SBZ.1 = SBZOO.1, SBZ.2a = SBZ.3, SBZ.2b = SBZ.4

Table S2. 16S rRNA V4 primer constructs for **A)** forward and **B)** reverse primers.**A**

Number	Illumina adapter	Sequencing primer	Ns	Barcode	16S 515F
515F_1	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	TCAGC	GTGCCAGCMGCCGCGGTAA
515F_2	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	GTATC	GTGCCAGCMGCCGCGGTAA
515F_3	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	GCTAC	GTGCCAGCMGCCGCGGTAA
515F_4	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	ACGCA	GTGCCAGCMGCCGCGGTAA
515F_5	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	GAGAC	GTGCCAGCMGCCGCGGTAA
515F_6	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	GACTC	GTGCCAGCMGCCGCGGTAA
515F_7	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	CTAGC	GTGCCAGCMGCCGCGGTAA
515F_8	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	CGCTC	GTGCCAGCMGCCGCGGTAA
515F_9	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	AACAT	GTGCCAGCMGCCGCGGTAA
515F_10	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	AGTGG	GTGCCAGCMGCCGCGGTAA
515F_11	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	TGACC	GTGCCAGCMGCCGCGGTAA
515F_12	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	TGGAT	GTGCCAGCMGCCGCGGTAA
515F_13	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	TACTG	GTGCCAGCMGCCGCGGTAA
515F_14	AATGATACGGCGACCACCGAGATCTACAC	TCTTTCCCTACACGACGCTCTTCCGATCT	NNNN	CCCCA	GTGCCAGCMGCCGCGGTAA

B

Number	Illumina adapter	Index	Sequencing primer	16S 806R primer
806R_1	CAAGCAGAAGACGGCATACGAGAT	ATCACG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_2	CAAGCAGAAGACGGCATACGAGAT	CGATGT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_3	CAAGCAGAAGACGGCATACGAGAT	TTAGGC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_4	CAAGCAGAAGACGGCATACGAGAT	TGACCA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_5	CAAGCAGAAGACGGCATACGAGAT	ACAGTG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_6	CAAGCAGAAGACGGCATACGAGAT	GCCAAAT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_7	CAAGCAGAAGACGGCATACGAGAT	CAGATC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_8	CAAGCAGAAGACGGCATACGAGAT	ACTTGA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_9	CAAGCAGAAGACGGCATACGAGAT	ATGTCA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_10	CAAGCAGAAGACGGCATACGAGAT	CCGTCC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_11	CAAGCAGAAGACGGCATACGAGAT	GTCCGC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_12	CAAGCAGAAGACGGCATACGAGAT	GTGAAA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT
806R_13	CAAGCAGAAGACGGCATACGAGAT	CACCGG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	GGACTACHVGGGTWTCTAAT

806R_14 CAAGCAGAAGACGGCATAACGAGAT CACGAT GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT GGACTACHVGGGTWTCTAAT

Table S3. Number of samples (excluding the mock community), ASVs, and total target amplicons at each analysis step.

Step	N	ASVs	Target amplicons
Start	94	8054	9421247
Decontamination	94	8042	9283245
Filtering	89	8042	9281918
Technical replicates merged	79	8042	8231785

Table S5. Results of the ANOVA and Tukey HSD post hoc tests for differences in relative abundance of recovered ASVs from 7 major taxa between sources. Significant p-values (adjusted for multiple comparisons) are in bold.

	Bacteroidetes	Firmicutes	Fusobacteria	Gammaproteobacteria	Tenericutes	Spirochaetes	Actinobacteria
Mean % proportion (sd)	38(12.2)	30.1(11.6)	13.2(12)	8.03(7.53)	0.653(1.3)	0.613(1.01)	0.361(0.67)
ANOVA	0.00895	7.05E-09	4.07E-06	0.0219	0.0385	0.0352	7.30E-15
Tukey HSD comparisons							
SRI-SMI	0.404	0.996	0.054	0.905	0.999	0.98	0.98
SCZ-SMI	0.903	0.807	0.233	0.999	1	1	1
CAT-SMI	1	0.002	0	0.274	0.882	0.995	0.995
SCL-SMI	1	0.113	0	0.998	0.126	0.892	0.892
SNI-SMI	1	0.997	0.019	0.406	1	0.028	0.028
SBZ-SMI	0.769	0.006	0.043	0.056	1	1	1
OCZ-SMI	0.225	0	0.476	1	1	1	1
SCZ-SRI	1	0.53	0.999	0.886	1	0.984	0.984
CAT-SRI	0.373	0	0.032	0.965	0.546	1	1
SCL-SRI	0.288	0.015	0.694	0.997	0.028	1	1
SNI-SRI	0.648	0.842	1	0.991	0.961	0.273	0.273
SBZ-SRI	0.094	0.001	0.901	0.31	1	0.986	0.986
OCZ-SRI	0.014	0	1	0.98	1	0.997	0.997
CAT-SCZ	0.903	0.97	0.852	0.51	0.969	0.993	0.993
SCL-SCZ	0.872	1	1	0.98	0.585	0.951	0.951
SNI-SCZ	0.967	0.957	1	0.597	0.999	0.28	0.28
SBZ-SCZ	0.346	0.601	0.999	0.101	1	1	1
OCZ-SCZ	0.077	0.007	1	1	1	1	1
SCL-CAT	1	0.772	0.68	0.62	0.833	0.999	0.999
SNI-CAT	1	0.013	0.065	1	0.99	0.144	0.144
SBZ-CAT	0.748	0.879	0.998	0.688	0.942	0.994	0.994
OCZ-CAT	0.21	0.009	0.921	0.816	0.973	0.999	0.999
SNI-SCL	0.999	0.41	0.859	0.77	0.323	0.38	0.38
SBZ-SCL	0.785	0.34	1	0.128	0.499	0.956	0.956
OCZ-SCL	0.233	0.001	1	0.998	0.689	0.988	0.988
SBZ-SNI	0.605	0.021	0.954	0.617	0.998	0.29	0.29
OCZ-SNI	0.142	0	1	0.866	0.999	0.547	0.547

OCZ-SBZ	0.979	0.396	0.999	0.259	1	1	1
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Table S6. Dispersion tests based on Euclidean distances. Factors with homogenous dispersions are in bold.

	Df	sumSq	meanSq	F	P
Islands (N = 62)					
Island	5	19798	3959.62	16	7.55E-10
Sex	1	36	36.41	0.11	0.744
Condition	6	61935	10322.53	21	9.14E-13
Age	4	11705	2926.21	7.3	7.62E-05
Year collected	1	3169	3168.78	9.1	0.00379
Month collected	5	23410	4681.96	11	1.50E-07
Weight	52	200872	3862.92	2400	1.44E-14
Extracted group	13	74688	5745.23	15	1.54E-12
SMI (N = 6)					
Sex	1	1	1.47	0.053	0.829
Condition	1	5701	5701.48	400	3.62E-05
Age	3	5223	1741.07	6.30E+31	1.59E-32
Year collected	1	4	3.79	0.028	0.876
Month collected	2	4071	2035.38	11	0.0401
Weight	5	0	0	-	-
Extracted group	2	7740	3869.89	57	0.00413
SRI (N = 13)					
Sex	1	463	463.13	5	0.0474
Condition	2	278	139.21	0.55	0.593
Age	3	10807	3602.3	22	0.00018
Month collected	1	96	96.21	0.85	0.377
Weight	12	0	0	-	-
Extracted group	2	10459	5229.36	37	2.36E-05
CAT (N = 15)					
Sex	1	0	0.13	0.00054	0.982
Condition	5	44714	8942.9	29	2.84E-05
Age	3	19756	6585.4	24	3.79E-05
Year collected	1	20713	20712.82	130	4.50E-08
Month collected	2	22287	11143.71	94	4.55E-08
Weight	13	24547	1888.26	1.90E+32	5.64E-17
Extracted group	2	18914	9457.14	50	1.59E-06
SCL (N = 15)					
Sex	1	354	353.62	2.3	0.155
Condition	1	93	92.84	0.7	0.418
Age	2	16413	8206.62	76	1.53E-07
Year collected	1	2029	2028.89	5.1	0.0413
Month collected	3	14185	4728.37	10	0.00174
Weight	14	0	0	-	-
Extracted group	2	240	120.21	0.64	0.543

SNI (N = 10)					
Sex	1	8735	8735.19	140	2.54E-06
Condition	2	1089	544.31	2.4	0.157
Age	3	7381	2460.44	7.7	0.0174
Month collected	1	1578	1577.69	5.6	0.0457
Weight	9	0	0	-	-
Extracted group	3	14662	4887.19	13	0.005
Captivity (N = 21)					
Source	2	18649	9324.31	24.00	9.00e-06
Sex	1	87	87.30	0.31	5.87e-01
Age	4	39035	9758.66	63.00	0.00e+00
Year Collected	1	1390	1389.89	5.20	3.40e-02
Month Collected	3	18058	6019.48	21.00	5.40e-06
Wild vs Captive	1	3752	3751.56	33.00	1.57e-05
Wild or Captive born	1	5250	5250.23	50.00	1.00e-06

Df = degrees of freedom, sumSq = sum of squares, meanSq = mean squares, F = F-statistic, P = p-value.

Figure S1. Principal coordinates analysis (PCoA) based on Euclidean distance showing technical replicates that were merged for later analyses.

Figure S2. Boxplots of major taxa compared by source. Note the y-axis is free to vary according to the proportions of each taxon.

Figure S3. Principal coordinate analyses for significant factors including **A)** month that the scat was collected on SRI, **B)** sex on CAT, **C)** year the scat was collected and fox weight on SCL, and **D)** sex on SNI.

Figure S4. The total precipitation from October 2014 to March 2015 with the dates of sample collection indicated with vertical dashed lines for **A)** SRI and **B)** SCL.

Figure S5. A-C) Alpha diversity (Chao1 richness and Shannon diversity indexes) between sources, the month the samples were collected, and weight. Samples with missing data were kept for A. **D-G)** Chao 1 and Shannon diversity metrics for significant factors identified by PERMANOVA tests within an island.

Figures

Figure 1

Map of scat samples across the Channel Islands and the mainland. The sample sizes are listed followed by the number of replicates, which are listed in parentheses. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Percent relative abundance of the major taxa recovered from *U. littoralis* scat per sample. Sample names are colored by source (island or zoo).

Figure 3

Dendrogram of transformed data based on Euclidean distance. Sample names are colored by island or zoo (see Fig. 1) and shapes correspond to region: circle = northern islands, triangle = southern islands, and square = captive samples.

Figure 4

A) Principal coordinates analysis (PCoA) based on Euclidean distance. The points are samples colored by source (island or zoo) and the shapes represent northern or southern Channel Islands and captive samples. B) Boxplot of multivariate dispersion as measured by the distance to the centroid per sample and displayed by source.

A **B** **C** **Figure 5**

A) Principal coordinates analysis (PCoA) based on Euclidean distance of samples from SCL and captivity (SBZ, OCZ). B) Boxplot of multivariate dispersion as measured by the distance to the centroid per sample and displayed by source. C) Chao 1 and Shannon diversity metrics between wild and captive samples.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [modelTesting1182021.xlsx](#)
- [featuretable.biom](#)
- [taxonomy.tsv](#)
- [samplemetadataUPdated4132020.txt](#)
- [Adamscode4ms.html](#)
- [SupplementaryMaterials.pdf](#)