APPENDIX

Primer details

Primers used to amplify DNA from fecal, soil and prey samples.

Primer	**Sequence 5'->3'**	**Reference**
Aves-16S-1AF	CATAAGACGAGAAGACCCTGTGGA	Dalén *et al.* 2017
Aves-16S-1AR	TCCAAGGTCGCCCCAACCGAA	Dalén *et al.* 2017
Aves-16S-1A-Block	AGACCCTATGGAGCTTTAATTTATTAATGCAAAC/3SpC3/	Dalén *et al.* 2017
TuftedPuffin-16S-Rblock	CCCTGGGGTAGCTTGGTCCATTAATC/3SpC3/	Based on Bowser *et al.* 2013
16S1F-Ext	[1]-ATAAGACGAGAAGACCCTAT	Based on Deagle *et al.* 2007
16S2R	[2]-CGCTGTTATCCCTADRGTAACT	Deagle *et al.* 2007
16S-inv-R	[2]-CCAACATCGAGGTGSYAA	Fountain *et al.* 2023
Mala16S-R	[2]-TCAACATCGAGGTCGYAA	Fountain *et al.* 2023
Actinopterygii-12S-F	ACAAACTRGGATTAGATACC	This study
Actinopterygii16SLRpcr_R	ATCCAACATCGAGGTCGTAAAC	Deiner *et al.* 2017

[1] Illumina forward overhang: tcgtcggcagcgtcagatgtgtataagagacag,

[2] Illumina reverse overhang: gtctcgtgggctcggagatgtgtataagagacag

PCR amplification details

DNA Extraction

Fecal samples from the captive zoo population (n = 9), and fecal (n = 9) and soil samples (n = 8) from the field were extracted with QIAamp Fast DNA Stool Mini Kit (Qiagen 51604). Modifications to the published protocol included an extended lysis period for fecal samples of 0.5-1.5 hours, and elution in 100 µl of ATE for all samples (Ford *et al.* 2016). The DNA was then quantified using the dsDNA High Sensitivity Kit on a Qubit 3.0 Fluorometer (Invitrogen, ThermoFisher). Tissue samples from the four prey items the zoo provided were extracted with Qiagen DNeasy Blood & Tissue Kit (Qiagen 69504).

Predator/Host Amplification

Both the island and the zoo exhibit host several marine bird species, so all samples were tested for species origin by amplifying and Sanger sequencing a 125-bp fragment using primers Aves16S-1AF and Aves16S-1AR (Dalén *et al.* 2017). To avoid human cross-amplification, a human blocking primer containing a C3-spacer was added as per Dalén *et al.* 2017 (see **Primer details** section above). Amplifications were carried out in a 25 µl reaction using 0.05 U GoTaq® Flexi DNA Polymerase with 1× buffer and 2mM $MgCl_2$ (Promega M8295), with final concentrations of 0.1 mg ml^{-1} BSA (NEB B9000S), 0.2 µM dNTPs (Promega U1240), 0.2 µM primers, and 2µM human blocker (Aves-16S-1A-Block) and 4 µl of DNA at ≤ 1 ng $µl^{-1}$. PCR conditions were 95°C for 2 min, 45 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 30 s, and a 72°C extension for 5 min. Resulting amplicons were sequenced on an ABI3500 Genetic Analyzer in only the forward direction. Samples identified with Tufted Puffin DNA were taken to next steps.

Prey Amplification

To identify the prey component of samples, we used fish primers (16S1F-Ext and 16S2R, Deagle *et al.* 2007) and crustacean primers (Mala16S-R, and 16S-inv-R, Fountain *et al.* 2023) with Illumina overhangs to amplify fragments expected to be between 190-325 bp, depending on primer set and species (see **Primer details** section above). Primer 16S1F was redesigned after having studied its specificity against both an alignment of potential prey *in silico* using Geneious Prime and zoo prey tissue samples in vitro. The two primer sets were used to increase capture of diversity across taxa and were tested on prey tissue samples provided by the zoo prior to use on fecal and soil samples (data not shown). To prevent origin (host) species amplification a puffin blocker, TuftedPuffin-16S-Rblock, based on Bowser *et al.* (2013) was used. PCR reaction and amplification was as above, except the blocker was used at 1.25 µM, and the annealing temperature was 55°C. In addition to negative controls, the same primer pairs were used on DNA extractions of Pink Abalone *Haliotis corrugata*, shrimps from the genus *Palaemon* and *Penaeus,* and Tilapia *Oreochromis niloticus* to act as positive controls.

Successful PCR products of the prey component were purified using 1×AMPure XP magnetic beads to remove primer dimer and individually indexed using the IDT for Nextera UD Indices (Illumina, sets A and B, 20027213, 20027214). The reaction for the indexing PCR contained 12.5 µl of Phusion High Fidelity Master Mix (Thermo Fisher, F531L), 1.5µl of indices, and 5µl of cleaned PCR product and 6 µl nuclease free water. PCR conditions were 95°C for 3 min, 8 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a 72°C extension for 5 min. The resulting amplicons were purified again with 0.8×AMPure XP magnetic beads and quantified

using Qubit dsDNA High Sensitivity Kit (Invitrogen, Q33230) before pooling them equimolarly. The pooled library was metabarcoded using an Illumina MiSeq Reagent Kit v3 (600 cycle; Illumina MS-102-3003) on a MiSeq System (Illumina).

Nanopore sequencing

The remaining DNA samples (n=25) were used in a pilot study to determine the length of the prey DNA and the capability of long fragment metabarcoding using an Oxford Nanopore Technologies sequencing platform with adaptive sampling. Primers Actinopterygii-12S-F and Actinopterygii16SLRpcr_R were used to amplify a ca. 2 kbp fragment in 25 µl using 5 µl of DNA, 1x LongAmpHotStart Master mix (NEB), 16 µg of bovine serum albumin and 0.4 µM of each primer. PCR conditions were 94ºC for 1 min, followed by 45 cycles of 94ºC for 30 s, 54ºC for 1 min and 65ºC for 1 min 40 s and a final extension of 5 min at 65ºC.

Eleven subsamples (originally from two fecal and five soil samples from the wild and one fecal sample from the zoo) produced visible bands. These were then subjected to a free-end preparation for barcode ligation by adding 1.75 µl of EndPrep buffer and 0.75 µl of EndPrep Enzyme mix (NEB) and incubating at 20ºC for 5 min and at 65ºC for 5 min. Subsequently, 2 µl of barcodes from EXP-NBD196 kit were added with 13 µl of Blunt TA ligase (NEB) and incubated for 20min at 20ºC and for 5 min at 65ºC. Each sample was cleaned individually with 30 µl of AMPure XP magnetic beads, washed twice with 70% ethanol, and eluted in 12 µl nuclease free water. Samples were pooled equimolarly, and a library containing a total mass of 720 ng was loaded on a R10.3 flow cell, following the Oxford Nanopore loading instructions. The run was set up to enrich the prey and to exclude Tufted Puffin signal using a partial

sequence of 12S and 16S rRNA (ca. 2000 bp, GenBank Accession OP603960) for the adaptive sampling setting on the MinKnow sequencing system (Oxford Nanopore).

Nanopore sequencing devices produce a raw output in the form of a voltage *squiggle* that can be translated to a DNA sequence using a variety of algorithms; we used the *Guppy 4.3.4 sup* (Oxford Nanopore). Individual samples were demultiplexed using the native barcodes, clusters of similar sequences were delimited using Decona v 1.3.1 (https://github.com/Saskia-Oosterbroek/decona, Oosterbroek *et al.* 2021), and the number of sequences associated with each sample and cluster was obtained with a custom script (see **16S rRNA haplotypes** section below). The taxonomic identification of the clusters consensus sequences was obtained in the same way as with the Illumina-generated sequences.

16S rRNA haplotypes

Tufted Puffin haplotypes as partial sequencing of 12SrRNA and 16SrRNA genes.

>*Fratercula cirrhata* H1 12S-16S rRNA

CCTTGTACCTTTTGCATCATGATTTAGCAAGAATAACCAAGCAAAACGAATTTAAGCTTGCCACCCCGAAACCTAA
GCGAGCTACTTGCAAGCAGCTACTTATGAGCGAACCCGTCTCTGTTGCAAAAGAGTGGGATGACTTGCTAGTAGAG
GTGAAAAGCCAACCGAGCTGGGTGATAGCTGGTTGCCTGTGAAATGAATCTCAGTTCCTCCTTGACCTCCCTCCCC
GGACACAACTTTAACCCTCATGTGGAAGGTCAAGAGTAATTTAAAGGAGGTACAGCTCCTTTAAAAAAGGATACA
ACCTTCACTAGCGGCTATACCACCTAACTTCTTCCCATTACTGTAGGCCTTCAAGCAGCCATCAATAAAGAGTGCG
TCAAAGCTCTACGCATAAAATCCAACAATAACATGATTCCCTTCCCACTAACAGGCCAACCTATGCCAATAGGAGT
ATTAATGCTAAAATGAGTAACTAGGGGTTCACCCTCCACAAGCGCAAGCTTACATCCTCGTATTATTAACAGATAA
CAAACCAATACCTCAATTATAACAAGACTAGCATATTAAACCCACCCTGTTACACCGACCAAGGAGCGCCCACTA
GAAAGATTAAAATCTGTAAAAGGAACTAGGCAAACCTAAGGCCCGACTGTTTACCAAAAACATAGCCTTCAGCCC
ACCAAGTATTGAAGGTGATGCCTGCCCAGTGACATAACGTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAG
CGCAATCAATTGTCCCATAAATCGAGACTTGTATGAATGGCTAAACGAGGTCTTAACTGTCTCTTACAGATAATCA
GTGAAATTGATCTTCCTGTGCAAAAGCAGGAATACTAACATAAGACGAGAAGACCCTGTGGAACTTAAAAATCAG
CGGCCACTACACACAAACTCAAACCTACTAGGCCTCCTTCCTCCCCAAATACTGGCCCGCATTTTTCGGTTGGGGC
GACCTTGGAGAAAAACAGATCCTCCAAAAATAAGACCATACCTCTTAACCAAGAACAACCCCTCAACGTACTAAC
AGTAACCAGACCCAATACAATTGATTAATGGACCAAGCTACCCCAGGGATAACAGCGCAATCTCCTCTAAGAGCC
CGCATCGACGAGGAG

>*Fratercula cirrhata* H2 12S-16S rRNA

TTGGCGCGATAGAGACTTCGTACCGTAAGGGAAAGATGAAATAGCAATGAAAAACCAAGCAATAAATAGCAAAG
ATAAACCCTTGTACCTTTTGCATCATGATTTAGCAAGAATAACCAAGCAAAACGAATTTAAGCTTGCCACCCCGAA
ACCTAAGCGAGCTACTTGCAAGCAGCTACTTATGAGCGAACCCGTCTCTGTTGCAAAAGAGTGGGATGACTTGCTA
GTAGAGGTGAAAAGCCAACCGAGCTGGGTGATAGCTGGTTGCCTGTGAAATGAATCTCAGTTCCTCCTTGACCTCC
CTCCCCGGACACAACTTTAACCCTCATGTGGAAGGTCAAGAGTAATTTAAAGGAGGTACAGCTCCTTTAAAAAAG
GATACAACCTTCACTAGCGGCTATACCACCTAACTTCTTCCCATTACTGTAGGCCTTCAAGCAGCCATCAATAAAG
AGTGCGTCAAAGCTCTACGCATAAAATCCAACAATAACATGATTCCCTTCCCACTAACAGGCCAACCTATGCCAAT
AGGAGTATTAATGCTAAAATGAGTAACTAGGGGTTCACCCTCCACAAGCGCAAGCTTACATCCTCGTATTATTAAC
AGATAACAAACCAATACCTCAATTATAACAAGACTAACATATTAAACCCACCCTGTTACACCGACCAAGGAGCGC
CCACTAGAAAGATTAAAATCTGTAAAAGGAACTAGGCAAACCTAAGGCCCGACTGTTTACCAAAAACATAGCCTT
CAGCCCACCAAGTATTGAAGGTGATGCCTGCCCAGTGACATAACGTTCAACGGCCGCGGTATCCTAACCGTGCGA
AGGTAGCGCAATCAATTGTCCCATAAATCGAGACTTGTATGAATGGCTAAACGAGGTCTTAACTGTCTCTTACAGA
TAATCAGTGAAATTGATCTTCCTGTGCAAAAGCAGGAATACTAACATAAGACGAGAAGACCCTGTGGAACTTAAA
AATCAGCGGCCACTACACACAAACTCAAACCTACTAGGCCTCCTTCCTCCCCAAATACTGGCCCGCATTTTTCGGT
TGGGGCGACCTTGGAGAAAAACAGATCCTCCAAAAATAAGACCATACCTCTTAACCAAGAACAACCCCTCAACGT
ACTAACAGTAACCAGACCCAATACAATTGATTAATGGACCAAGCTACCCCAGGGATAACAGCGCAATCTCCTCTA
AGAGCCCGCATCGACGAGGAG

>*Fratercula cirrhata* H3 12S-16SrRNA

AAAGATAAACCCTTGTACCTTTTGCATCATGATTTAGCAAGAATAACCAAGCAAAACGAATTTAAGCTTGCCACCC
CGAAACCTAAGCGAGCTACTTGCAAGCAGCTACTTATGAGCGAACCCGTCTCTGTTGCAAAAGAGTGGGATGACTT
GCTAGTAGAGGTGAAAAGCCAACCGAGCTGGGTGATAGCTGGTTGCCTGTGAAATGAATCTCAGTTCCTCCTTGAC
CTCCCTCCCCGGACACAACTTTAACCCTCATGTGGAAGGTCAAGAGTAATTTAAAGGAGGTACAGCTCCTTTAAAA
AAGGATACAACCTTCACTAGCGGCTATACCACCTAACTTCTTCCCATTACTGTAGGCCTTCAAGCAGCCATCAATA
AAGAGTGCGTCAAAGCTCTACGCATAAAATCCAACAATAACATGATTCCCTTCCCACTAACAGGCCAACCTATGCC
AATAGGAGTATTAATGCTAAAATGAGTAACTAGGGGTTCACCCTCCACAAGCGCAAGCTTACATCCTCGTATTATT
AACAGATAACAAACCAATACCTCAATTATAACAAGACTAGCATATTAAACCCACCCTGTTACACCGACCAAGGAG
CGCCCACTAGAAAGATTAAAATCTGTAAAAGGAACTAGGCAAACCTAAGGCCCGACTGTTTACCAAAAACATAGC
CTTCAGCCCACCAAGTATTGAAGGTGATGCCTGCCCAGTGACATAACGTTCAACGGCCGCGGTATCCTAACCGTGC
GAAGGTAGCGCAATCAATTGTCCCATAAATCGAGACTTGTATGAATGGCTAAACGAGGTCTTAACTGTCTCTTACA
GATAATCAGTGAAATTGATCTTCCTGTGCAAAAGCAGGAATACTAACATAAGACGAGAAGACCCTGTGGAACTTA
AAAATCAGCGGCCACTACACACAAACTCAAACCTACTAGGCCTCCTTCCTCCCCAAATACTGGCCCGCATTTTTCG
GTTGGGGCGACCTTGGAGAAAAACAGATCCTCCAAAAATAAGACCATACCTCTTAACCAAGAACAACTCCTCAAC
GTACTAACAGTAACCAGACCCAATACAATTGATTAATGGACCAAGCTACCCCAGGGATAACAGCGCAATCTCCTC
TAAGAGCCCGCATCGACGAGGAG

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