Coral Reefs (2025) 44:145–162
https://doi.org/10.1007/s00338-024-02593-7

REPORT

# Microenvironments of black-tip reef sharks (*Carcharhinus melanopterus*) provide niche habitats for distinct bacterial communities

**Claudia Pogoreutz**[1,2] · **Mauvis Gore**[3,4] · **Gabriela Perna**[1] · **Rupert Ormond**[3,4] · **Christopher R. Clarke**[5] · **Christian R. Voolstra**[1]

Received: 20 June 2023 / Accepted: 2 November 2024 / Published online: 17 December 2024
© The Author(s) 2024

**Abstract** Animal holobionts constitute diverse yet interconnected landscapes of microenvironments that harbor specific bacterial communities with distinct functions. An increasing body of literature suggests a partitioning and distinct functional profiles of bacterial communities across shark microenvironments, which has led to the proposition that beneficial bacterial functions may contribute to shark health. Here, we provide a first assessment of bacterial communities in different microenvironments of black-tip reef sharks (*Carcharhinus melanopterus*), the most abundant reef shark species across the Indo-West Pacific. Collecting samples from 34 sharks from the Amirante Islands, Seychelles, we characterized the corresponding bacterial communities of two external skin locations, within the buccal cavity, and of the cloaca (representing the gut microbiome) using 16S rRNA gene amplicon sequencing. Overall, shark-associated bacterial communities were distinct from seawater, and skin, buccal, and cloaca samples were distinct from each other. Shark cloaca samples and seawater exhibited lower bacterial alpha diversity and richness compared to the other microenvironments. Predicted functional profiles and Linear Discriminant Effect Size analysis suggest potential differences in metabolic pathways present in the different shark-associated bacterial communities and in the seawater. Taxonomy-based functional inference suggests cloaca-associated bacterial communities specialize in the consumption and breakdown of various food items. Taken together, our data suggest distinct bacterial niche habitats within the 'microbial landscape' of black-tip reef sharks, as indicated by distinct bacterial communities and their predicted metabolic functions. Future (meta)genomic and functional work will help reveal potential roles of bacteria in the health of their shark hosts.

**Keywords** Marine bacteria · Fish microbiome · Skin microbiome · Gut microbiome · Western Indian Ocean

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00338-024-02593-7.

✉ Claudia Pogoreutz
c.pogoreutz@gmail.com

✉ Rupert Ormond
rupert.ormond.mci@gmail.com

✉ Christian R. Voolstra
christian.voolstra@uni-konstanz.de

[1] Department of Biology, University of Konstanz, 78457 Konstanz, Germany

[2] UAR 3278 CRIOBE, PSL Université Paris: EPHE-UPVD-CNRS, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France

[3] Marine Conservation International, Aros, Isle of Mull, Argyll, Scotland, UK

[4] Centre for Marine Biodiversity and Biotechnology, Heriot-Watt University, Riccarton, Edinburgh, Scotland, UK

[5] Marine Research Facility, Jeddah Beach Palace, North Obhur, Jeddah, Saudi Arabia

## Introduction

Animals and plants are complex holobionts that are typically comprised of a multicellular eukaryotic host and a diverse suite of eukaryotic and prokaryotic microbes, specifically bacteria, archaea, algae, fungi, and viruses (Rohwer et al. 2002; Rosenberg et al. 2007; Roik et al. 2022; Pogoreutz et al. 2022). In the terrestrial realm, examples of microbial contributions to overall holobiont function and health extending their host's physiological capabilities have been investigated for decades and include, among others, the gut microbiome (Koenig et al. 2011), nitrogen-fixing rhizobia



Konstanzer Online-Publikations-System (KOPS)
URL: http://nbn-resolving.de/urn:nbn:de:bsz:352-2-mcremo6oaxvz3

(Quides et al. 2021), and mycorrhizal fungi (Bennett and Groten 2022) associated with diverse plant hosts. In the marine realm, our understanding of beneficial host–microbe interactions is less developed (Roik et al. 2022; Pogoreutz et al. 2022; Voolstra et al. 2024). Some prominent examples of better understood systems include the squid-*Vibrio* symbiosis (McFall-Ngai 2014), the chemosynthetic deep sea *Riftia pachyptila* symbiosis (Hinzke et al. 2019), the cnidarian–dinoflagellate symbiosis (Davy et al. 2012; LaJeunesse et al. 2018), and the association of marine invertebrates with the bacterial genus *Endozoicomonas* (Neave et al. 2016a, b; Pogoreutz et al. 2022; Maire et al. 2023; Hochart et al. 2023; Pogoreutz and Ziegler 2024).

In recent years, work on the functional importance of marine microbes associated with bony fish (teleosts) or sharks and their relatives (elasmobranchs) has gained traction, covering diverse aspects, ranging from comparative work on bacterial community structures across native and captive environments to characterization of viral communities and functional potential of shark skin-associated microbial communities (Doane and Haggerty 2017; Perry et al. 2021; Clavere-Graciette et al. 2022; Correia Costa et al. 2022; Hesse et al. 2022; Kerr et al. 2023; Doane et al. 2023). Like other animal hosts, fish host diverse bacterial communities, and given their varied forms may constitute potentially complex, yet interconnected 'landscapes' of microenvironments. Overall, the notion is that such microenvironments may provide distinct habitats supporting bacterial communities with specialized functions. At the same time, elasmobranchs may exhibit traits that permit unique interactions with their microbial communities (Perry et al. 2021), as reflected in the presence of culturable bacteria present in the blood of visibly healthy sharks (Mylniczenko et al. 2007) and the presence of similar bacterial communities on healthy and injured shark skin (Pogoreutz et al. 2019). Human bacterial communities are structured by genotype, body cavity, environmental fluctuations, stressors, immunological state, usage (e.g., handedness and washing affecting bacterial diversity on hands), or diet (Costello et al. 2009).

Bacterial communities associated with the skin mucus of sharks and their relatives are host-specific, distinct from the surrounding seawater, and exhibit a degree of phylosymbiosis (Doane et al. 2020; Doane and Haggerty 2017; Kerr et al. 2023). They are further shaped by geographical location and may also be affected by environmental gradients or fluctuations in the water column (Pogoreutz et al. 2019). Previous studies have reported on the partitioning of bacterial communities across different locations of the shark body (Storo et al. 2021; Black et al. 2021), suggesting a degree of separation in bacterial community composition between the skin, oral, intestinal/cloaca, blood, and seminal microbiomes across several species of shark (Storo et al. 2021; Black et al. 2021; Muñoz-Baquero et al. 2023; Bregman et al. 2023) (partially summarized in Pratte et al. 2021; although see also Montemagno et al. 2024). Ultimately, such characterization of spatial partitioning of bacterial communities from specific shark body cavities may be important for our understanding of microbial ecology and ultimately of shark host–microbe relationships. Specifically, it may permit insight into putative bacterial functions and contributions to host health, for instance to skin health, wound healing, or digestion.

In the present study, we set out to provide a first characterization of how bacterial diversity and community composition are partitioned across different body cavities on black-tip reef sharks (*Carcharhinus melanopterus*). Following noninvasive swab sample collection from 34 sharks in the Amirante Islands, Seychelles, we conducted 16S rRNA gene amplicon sequencing from four different locations on the shark's body—two locations on the skin (on the back, i.e., the dorsal flank, and the skin covering the gills), as described previously in (Pogoreutz et al. 2019, 2020), the oral cavity (hereon referred to as buccal samples), and the cloaca (via 'rectal' probing, as a proxy for the gut microbiome), in addition to seawater collected at the shark sampling sites. We subsequently inferred genomic functions from taxonomic profiles in a functional inference approach using PICRUSt2 (Douglas et al. 2020) to gain putative insight into possible differences in bacterial metabolic pathways across bacterial microenvironments.

## Methods

### Sampling sites and swab sampling from black-tip reef sharks

Black-tip reef sharks and seawater were sampled over 15 days from November 16–December 8, 2018, at four different locations in the Amirante Islands, Seychelles (Fig. 1; Supp. Table S1). Throughout the manuscript, 'sampling sites' refer to the location in the Amirante Islands where sharks were caught, while 'microenvironments' refer to the location on the body of the shark where swab samples were collected from. The sampling sites were situated around St. Joseph Atoll and D'Arros Island. A total of 34 black-tip reef sharks were wild-caught alive using barbless circle hooks (to permit easy hook removal) and a line. During sampling, sharks were held, mostly submersed, at the side of the inflatable boat and subsequently released unharmed within 10–15 min. Skin areas from which mucus swab samples were taken were briefly exposed to air during the sampling. For each shark, the left side of the body was sampled for skin and buccal samples. Specifically, for each shark, one sample was collected from the back of the buccal cavity, one from the back area just below the first dorsal fin, and one from the skin covering the gills, by swabbing with individual

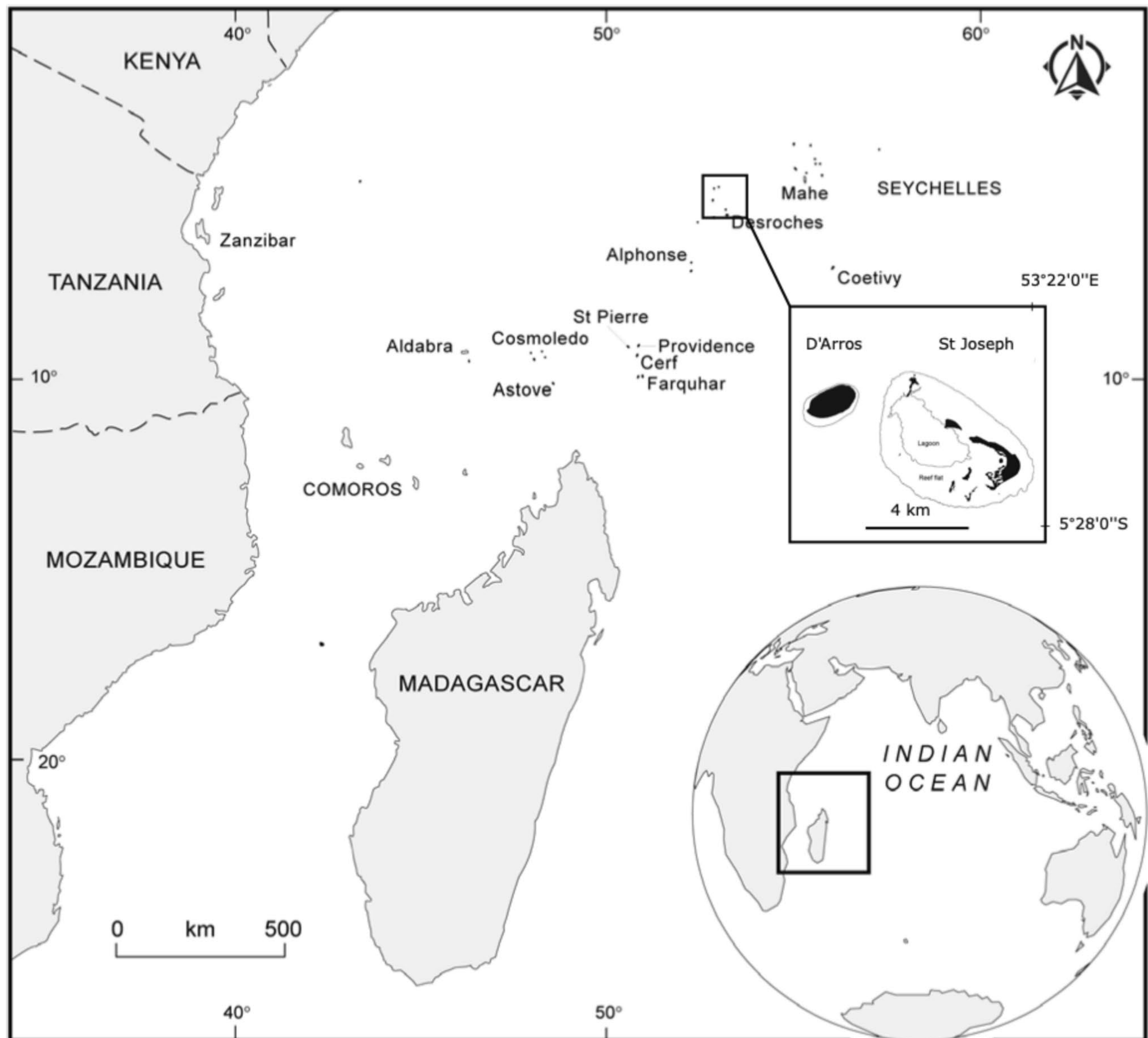


**Fig. 1** Sampling area of shark and seawater for the present study in the Southern Seychelles, Western Indian Ocean. Inset: All sampling took place off D'Arros Island and St. Joseph's Atoll in the Amirante Islands. Maps adapted from (Daly et al. 2018; Teleki et al. 1999)

forceps-held sterile cotton swabs (Nuova Aptaca, Italy) as a means of noninvasive sampling of live sharks (Pogoreutz et al. 2019). In addition, to obtain information on intestinal bacterial communities (i.e., the 'gut' microbiome), for each shark one cloacal sample was obtained by passing a sterile swab through the cloacal orifice. Cloacal (or rectal) probing with swabs has been demonstrated to be a legitimate and reliable method for determination of the intestinal bacterial community in mammals and arguably should work similarly well in sharks (Alfano et al. 2015; Artim et al. 2019; Choudhury et al. 2019). Cloacal (rectal) probing has been previously used on marine megafauna, including cetaceans and sea lions (Bik et al. 2016), for which fecal sample collection is impractical, as well as to obtain gut microbiota samples from fish in aquaculture (Piazzon et al. 2017). Swab samples were immediately transferred into RNAlater (Thermo Fisher Scientific, United Kingdom), stored at 5 °C during transport, and subsequently at − 20 °C until further processing. Sampling the same shark twice was avoided by taking pictures of each side of the first dorsal fin of each shark in order to document its individual markings, an approach which is commonly used for identification of individuals across a wide range of species (Castro and Rosa 2005; Domeier and Nasby-Lucas 2007; Gore et al. 2016). In addition, all sharks that were caught and sampled were marked by removing the extreme tip of the anal fin (Pogoreutz et al. 2019). Finally,

seawater bacterial communities were collected by filtering 2 L of seawater through a 0.22 µm GFF filter, with one sample being collected on each sampling day at each sampling location (total 15) (Supp. Table S1). The GFF filters holding the seawater bacteria were then transferred into glass vials containing RNAlater, stored at 5 °C during transport, and subsequently stored at − 20 °C until further processing. In summary, 34 sharks were caught and released, 4 swab samples taken from each, and 15 seawater samples (= one seawater sample for each collection day and location) were also collected.

### DNA extraction, PCR amplification, sequencing library preparation for 16S rRNA gene amplicon sequencing of bacterial communities

Swabs were thawed at room temperature, removed from RNAlater solution, and each placed in a sterile 1.5 ml Eppendorf tube and air-dried for 10 min. DNA extraction was conducted using the Qiagen Blood & Tissue 96 kit according to manufacturer's instructions (Qiagen, Germany). Briefly, 360 µl of ATL buffer and 40 µl of Proteinase K (at a final concentration of 20 mg/ml in a reaction volume of 400 µl) were added per swab to the 1.5 ml tubes. The individual tubes containing swab samples in ATL buffer and Proteinase K were vortexed vigorously for 15 s and incubated at 56 °C and 700 rpm for 90 min. Then, 1 µl of RNase A was added 15 min before the end of the incubation. After the incubation, samples were vortexed again, the swabs removed, and lysates transferred to individual wells of a Qiagen Blood & Tissue 96-well plate. Volumes for buffer AL and for 70% ethanol were adjusted to twice the volume (i.e., 400 µl per reaction each). Further steps were followed as per the manufacturer's instructions. All centrifugation steps were carried out at room temperature. Purified DNA bound to Qiagen spin column membranes was eluted once using 50 µl of AE buffer. DNA was quantified using a Qubit 2-Fluorometer using the HS dsDNA kit (Invitrogen, USA). In addition to DNA extractions from samples, two types of mock DNA extractions were conducted as follows: 1) no template, using kit reagents only; 2) using a clean swab with kit reagents.

For all samples, PCR amplifications were performed in triplicates using Qiagen Multiplex PCR Kit (Qiagen, Germany) with primers containing Illumina adapters (underlined below). For the 16S rRNA gene sequencing, we amplified the hypervariable regions V5 and V6 of the bacterial 16S rRNA gene. We used primers 16SMiSeqF-Andersson 5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA-3′ and 16SMiSeqR-Andersson 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCRRCACGAGCTGACGAC-3′, which had previously been shown to amplify well with different marine templates (Pogoreutz et al. 2018, 2019, 2022; Bayer et al. 2013). Individual PCRs were run using 5 µl Qiagen Mix, 0.3 µl of each 10 µM primer, 1 µl of DNA template (1–2 ng of DNA per 1 µl), and RNase-free water to adjust to a final reaction volume of 10 µl. In addition to the samples, PCRs were run for templates from the mock DNA extractions, which were also subjected to sequencing. Thermal cycling conditions for 16S rRNA gene PCRs were: 95 °C for 15 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 90 s, 72 °C for 30 s, and a final extension cycle of 72 °C at 10 min. Five microliters of each PCR product were run on an 1% agarose gel to visualize successful amplification. Sample triplicates were subsequently pooled and then purified with Illustra ExoProStar 1-Step (GE Healthcare Life Sciences, UK). Purified PCR products were subjected to an indexing PCR (8 cycles) to add Nextera XT indexing and sequencing adapters (Illumina, USA) according to the manufacturer's protocol. Successful addition of indexes was confirmed by comparing the length of the initial PCR product to the corresponding indexed sample on a 1% agarose gel. Indexed PCR products were purified and normalized with the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, USA), followed by quantification on the BioAnalyzer (Agilent Technologies, USA) and QuBit (Quant-IT dsDNA High Sensitivity Assay Kit; Invitrogen, USA), and pooled in equimolar ratios. The library was sequenced at the Bioscience Core Lab (BCL) at King Abdullah University of Science and Technology (KAUST) at 15 pM with 10% phiX on an Illumina MiSeq, 2 × 300 bp end, Rapid run, 500 cycles, according to the manufacturer's specifications. Overall, a total of 138 samples (15 seawater + 28 dorsal + 28 gill covering + 34 buccal + 33 cloacal samples) plus six blanks were prepared for sequencing.

### Sequencing data analysis

*Amplicon Sequence Variants (ASVs)*

Exact 16S rRNA ASVs were computed using the DADA2 package in R (Callahan et al. 2016). Raw reads were trimmed of residual primer and Illumina overhangs using cutadapt (Martin 2011). Of an initial total of 6,047,392 raw paired reads, 5,311,519 were retained after filtering. The error model was built and inspected using the *learnErrors* and *plotErrors* commands in DADA2 (Callahan et al. 2016). Denoised reads were then merged (4,834,555 merged read pairs retained) and chimeric contigs discarded using *mergePairs* and *removeBimeraDenovo*, respectively; after chimera removal, 4,592,489 merged read pairs were retained. Samples with fewer than 1,000 merged read pairs as well as ASVs with fewer than 10 read pairs cumulatively over all samples were omitted from further analyses. Finally, ASVs found in sequenced mock DNA extraction and mock PCR samples and present in more than 5% of samples were considered contamination and discarded, leaving a total of

3,827,454 merged read pairs distributed over 9,598 exact ASVs in 138 samples (15 seawater + 28 dorsal + 28 gill covering + 34 buccal + 33 cloacal samples). Taxonomic ranks were assigned to inferred ASVs using the SILVA database, using DADA2 function *assignTaxonomy*. All raw sequence data are accessible under NCBI's BioProject PRJNA966929.

### Community data analysis

To test for differences in bacterial community composition across habitats, specifically seawater and shark host-associated microenvironments, relative abundances of each ASV per sample were calculated. The resulting dataset was square-root transformed, converted into a Bray–Curtis similarity matrix, and employed to run global and pairwise *adonis* PERMANOVAs in the R package Vegan v2.6–4 (Oksanen et al. 2022), using site and microenvironment as fixed factors. Estimated richness (Chao 1 estimator) and alpha diversity (Shannon index) of bacterial communities associated with seawater and the shark-associated host habitats were calculated on rarefied data using the *Rarefy* function to subsample to the shallowest sequencing depth across all samples (5,140 sequences) in GUniFrac v1.7 (Chen et al. 2022). Statistical differences in alpha diversity between bacterial habitats were evaluated by a linear regression using the package lme4 v1.1–31 (Bates et al. 2017), and pairwise contrasts with Benjamini–Hochberg post hoc testing (i.e., fdr adjustments of *P*<s) were generated using the package emmeans v1.8.3 (Lenth et al. 2022). Beta diversity was calculated using Bray Curtis dissimilarities of square-root transformed relative abundance data and represented in an NMDS using Vegan (Oksanen et al. 2022). Multivariate dispersion as a measure of beta diversity of host microenvironments was calculated prior using the *betadisper* function as implemented in Vegan, followed by a Tukey's HSD post hoc comparison. Similarity percentage analysis (SIMPER) as implemented in Vegan was conducted to identify bacterial ASVs contributing most to similarity between investigated microenvironments on sharks. Indicator taxa, i.e., ASVs significantly associated with a particular host habitat or combination thereof, were identified using the *multipatt* function of the indicspecies v.1.7.12 package in R (De Caceres et al. 2016). Finally, to determine 'overrepresented' ASVs in the different habitats, Linear Discriminant Effect Size analysis (*LEfSe*; (Segata et al. 2011) was run on the Galaxy online server of the Huttenhower Lab (https://huttenhower.sph.harvard.edu/galaxy/).

#### *Taxonomy-based functional prediction with PICRUSt2*

PICRUSt2 predicts metagenomic gene content by inference from ribosomal sequencing data to identify the closest taxon with a sequenced genome (e.g., 16S rRNA gene amplicon sequences; Douglas et al. 2020). Thus, it does not identify the actual gene content or bacterial activity, i.e., predicted metagenomic content will have to be confirmed with follow-up experimental investigation.

The ASV relative abundance table was converted into a json formatted.biom file prior to running PICRUST2 (v2.5.1 beta; (Douglas et al. 2020)). First, ASVs were placed in the provided reference tree using *EPA-NG* and *GAPPA* (Czech et al. 2020). Next, hidden-state prediction of gene families was run for 16S rRNA gene copy and EC numbers (Enzyme Commission enzyme classification numbers) per genome using *castor* (Louca et al. 2018) based on ASV abundances and phylogenetic prediction. To minimize putative error in gene content prediction due to poor matches to available genomes, ASVs receiving a Nearest Sequenced Taxon Index (NSTI) > 2 were considered as noise and discarded. Relative abundances of biological pathways encoded by microbiomes were then predicted using *MinPath* (Ye and Doak 2009). To determine features (inferred EC accessions corresponding to putative metabolic processes) explaining differences between the different shark habitats, LEfSe (Segata et al. 2011) was run on the Galaxy online server of the Huttenhower Lab (https://huttenhower.sph.harvard.edu/galaxy/). Given the strong similarity in bacterial community composition on the shark skin (i.e., gills and back), samples were analyzed together as 'skin.'

Supplementary Data (Supp. Tables S2-S7) are publicly accessible via the zenodo depository: https://zenodo.org/record/8041781 (Pogoreutz et al. 2023).

## Results

### Distinct bacterial communities associated with microbial habitats on black-tip reef sharks and seawater

A total of 9,815 ASVs were determined, across the 138 samples—specifically 34 buccal, 28 dorsal skin, 28 skin covering the gills, and 33 cloaca samples from black-tip reef sharks (*Carcharhinus melanopterus*), in addition to 15 seawater samples, from the Amirante Islands, Seychelles (Fig. 1; Supp. Table S2; Pogoreutz et al. 2023). The highest numbers of ASVs were observed in the buccal cavity samples (5,493 ASVs), followed by the skin locations (3,395 and 3,509 for skin on the back and skin covering the gills, respectively). Cloaca samples exhibited the lowest numbers of ASVs among those from the shark-associated microenvironments (1,540 ASVs). The lowest total number of ASVs was observed in the seawater samples (589 ASVs, i.e., an order of magnitude lower than in the shark samples).

At the taxonomic class level, seawater and shark-related samples were dominated by Gammaproteobacteria (63.5%

relative abundance of 16S rRNA gene sequences), Alphaproteobacteria (14.7%), and Bacteroidia (11%). However, the mean proportions varied with sample origin: Gammaproteobacteria had 89.0% relative abundance in cloaca samples compared to between 58.6 and 66.3% in buccal, gill, and dorsal skin samples, Alphaproteobacteria had 3.1% relative abundance in cloacal samples, compared to between 11.8 and 14.6% in samples from other shark-related microbial habitats, and the Bacteroidia had 3.0% relative abundance in cloaca samples compared to between 9.4 and 14.9% in samples from other shark-related body cavities in varying proportions. While the same classes dominated the seawater samples, the proportions of each class differed markedly from the shark samples, with relative abundances of 39.0% for Alphaproteobacteria, 29.9% for Gammaproteobacteria, and 17.9% for Bacteroidia.

At the ASV level, seawater was highly distinct from shark-related samples (PERMANOVA, $F=9.86$, $p$ value $<< 0.001$). Given significant multivariate dispersion (betadisper, 99 permutations; $p$ value $=0.01$) between samples which was driven by the seawater samples (Tukey's HSD, multiple comparison of means; adjusted $p$ value for seawater paired with any of the shark-related samples, but not with each other: $p$ value $< < 0.001$), the latter were excluded from further statistical evaluation of the overall, shark-associated bacterial community (no significant multivariate dispersion: *betadisper*, 99 permutations; $F=0.9465$; $p=0.45$; Supp. Fig. S2, Supp. Table S3 A,B; Pogoreutz et al. 2023). After removal of seawater samples, bacterial communities between shark-associated habitats (using sampling site in the Amirante Islands as a factor) were statistically significantly different from each other (global *adonis* PERMANOVA; shark-associated microenvironments: $F=5.2744$, $R^2=0.11618$; $p<0.001$; sampling site: $F=2.0821$; $R^2=0.03057$, $p<0.003$; no significant interaction of shark-associated microenvironment with sampling site: $F=0.8689$; $R^2=0.03828$, $p=0.845$). A pairwise *adonis* PERMANOVA revealed statistically significant differences in bacterial community composition at the ASV level for all shark habitat pairings except when comparing the two skin locations, i.e., dorsal skin vs. gill-covering skin ($F=1.043981$; $R^2=0.0189663$; $p=0.366$). For full statistical details on the remaining habitat pairings, refer to Supp. Table S3; Pogoreutz et al. 2023).

### Bacterial community compositions and diversity indices of microbial shark habitats and seawater

Seawater samples were dominated by SAR11 Clade 1a (ASV17; 9.7% average relative abundance; Fig. 2**; Supp.** Fig. S1), *Acinetobacter* (ASV16: 5.9%), *Ca*. Actinomarina (ASV43: 4.6%), unclassified members of the families Lachnospiraceae (ASV58, 3.8%), Rhodobacteraceae (ASV52 and ASV60 at 3.6 and 2.4%, respectively), Flavobacteriaceae (ASV45: 2.8%), *Pseudomonas* (ASV36: 2.2%), and an unclassified clade of SAR86 (ASV64: 2.0%). Among shark-related samples, the cloaca samples were dominated by Vibrionaceae (60% comprised of *Vibrio* ASV1 and ASV4 at 27.6 and 10.1% relative abundance, respectively and *Photobacterium* ASV2 and ASV5 at 17.9 and 4.4%, respectively). Other notable taxa included *Providencia* and *Morganella*, both members of the Enterobacteriaceae (ASV19, ASV26 at 2.5 and 2.3% average relative abundance), and *Cetobacterium* (Fusobacteriaceae; ASV14 and ASV26 at 1.1 and 1.0% average relative abundance; Fig. 2). While Vibrionaceae were also prevalent in the external three shark-related bacterial habitats, their relative abundances were lower than in the cloaca samples (Fig. 2). Of the remaining three habitats, buccal samples cumulatively harbored the highest average number of sequences affiliated to Vibrionaceae (a total of 24% average relative abundance; 12.8, 8.3, and 2.4%, respectively, for *Vibrio* ASV1 and *Photobacterium* ASV2 and ASV5, respectively), followed by samples collected from the skin behind the dorsal fin and skin covering the gill. Further abundant bacteria present across skin and buccal samples were *Pseudoalteromonas* (ASV3: 8.2, 5.5, and 3.7% average relative abundance in dorsa, gill, and buccal samples), *Alteromonas* (ASV9, ASV10: average relative abundances ranging between 1.1 and 1.5%), and *Halomonas* (ASV13: around 1.3% relative abundance on skin habitats). The SIMPER analysis suggested that the differences in bacterial community compositions between sampled habitats were driven by several abundant ASVs, including *Vibrio* (ASV1, ASV4), *Photobacterium* (ASV2, ASV5), *Pseudoalteromonas* (ASV3), *Acinetobacter* (ASV16), SAR11 Clade Ia (ASV17), and *Actinomarina* (ASV43) (for a full overview of comparisons, refer to Supp. Table S3C; Pogoreutz et al. 2023). Overall, an nMDS analysis further highlights the contrasting relationships between bacterial communities of seawater and shark-associated habitats (Fig. 3A). Notably, bacterial communities of shark-associated external microenvironments show a high degree of overlap despite having many unique ASVs, which may in part be driven by the presence of few common, abundant, but also high numbers of rare, low-abundant ASVs (Fig. 3A).

Alpha diversity indices were significantly different between bacterial communities associated with seawater and the four shark-associated microenvironments (Chao1 index: F value $=14.46$; adjusted $R^2=0.2821$; $p$ value $< < 0.005$; Shannon index: F value $=45.87$, adjusted $R^2=0.5671$; $p$ value $< < 0.005$; Supp. Table S3D; Pogoreutz et al. 2023). Among all five bacterial habitats sampled, the highest bacterial alpha diversity was found for the external surfaces of sharks (Chao1 index of buccal samples: $312.15 \pm 32.79$

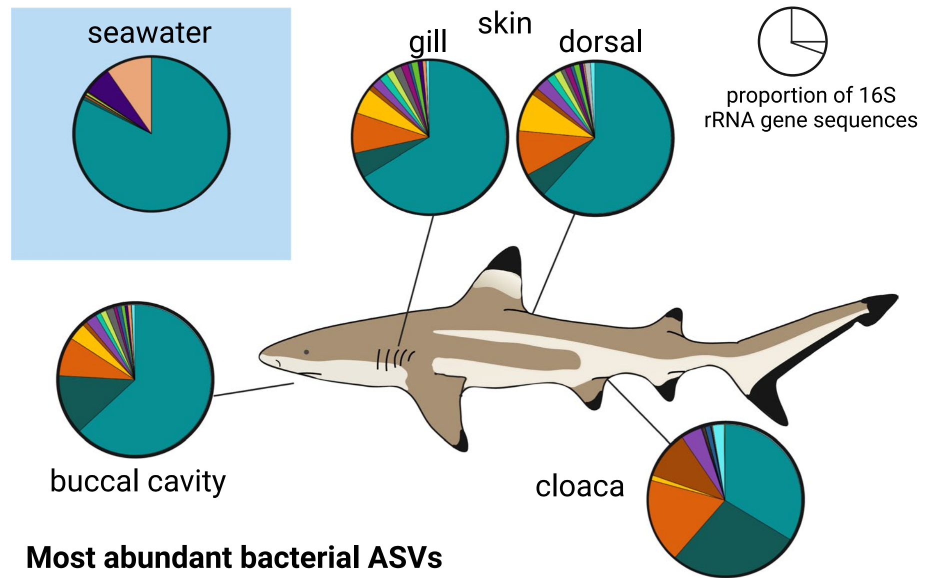


**Fig. 2** Pie charts showing relative presence of the 15 most abundant bacterial ASVs averaged over the five microbial habitats sampled: seawater, skin (dorsal and covering the gills), buccal cavity, and cloaca of black-tip reef sharks (*Carcharhinus melanopterus*) sampled in the Amirante Islands, Seychelles. The remaining ASVs are summarized in category 'others.' For full details on how these ASVs distribute over all samples and an ASV count table, refer to Supp. Fig. S1 and Supp. Table S2, respectively

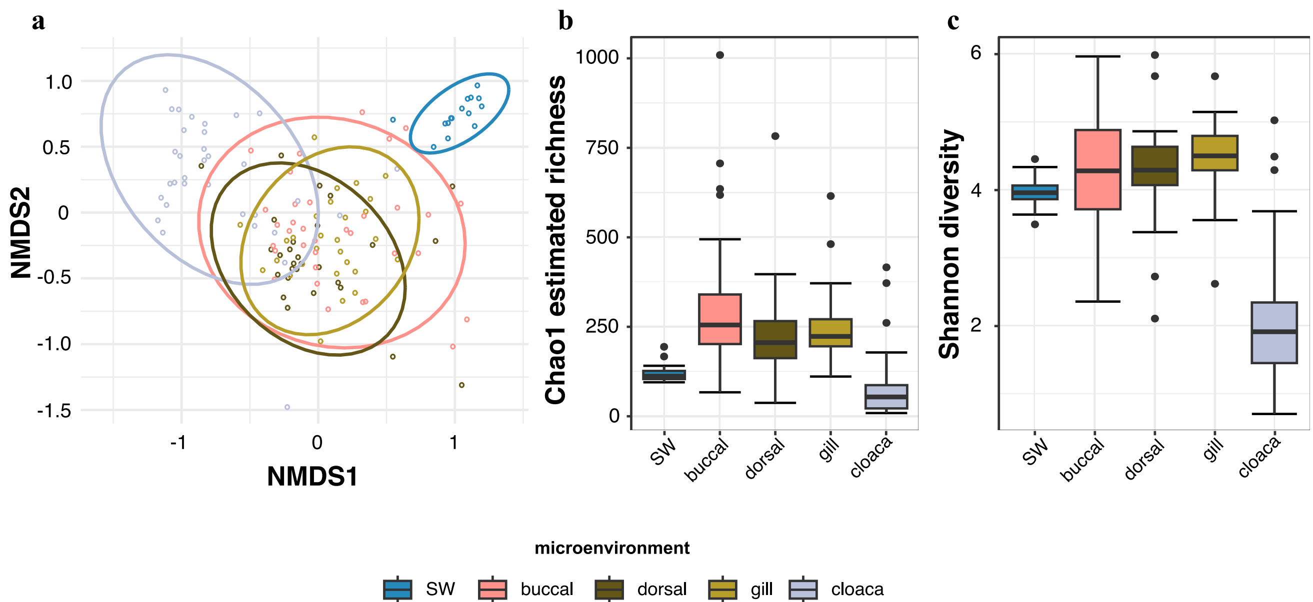


**Fig. 3** **A** Non-metric multidimensional scaling (nMDS) ordination and **B**, **C** Diversity indices (Chao1 estimator, Shannon Index) of bacterial communities sampled from seawater (SW) and different microenvironments on black-tip reef sharks (*Carcharhinus melanopterus*) from the Amirante Islands, Seychelles. Symbols of the same color represent individual samples of respective microenvironments. In **A,** ellipsoids represent standard deviations

ASVs; of the skin covering the gills and on the back: 253.0 ± 20.40 and 231.29 ± 26.03 ASVs, respectively; Shannon index of buccal samples: 4.31 ± 0.15; of skin covering the gills and on the back: 4.48 ± 0.11 and 4.29 ± 0.15, respectively). Lowest alpha diversity was observed in cloaca samples (Chao1 index: 87.91 ± 17.41 ASVs; Shannon index: 2.13 ± 0.18). Bacterial diversity in the seawater communities was comparable to shark external surfaces (Chao1 index: 121.93 ± 6.88 ASVs; Shannon index: 3.97 ± 0.06) (Fig. 3B, C; for details, refer to Supp. Table S3E; Pogoreutz et al. 2023). Excluding seawater, the buccal samples were associated with the highest number of distinct bacterial ASVs (3,270), followed by skin samples (1,671 and 1,560 ASVs for gill and dorsal samples, respectively), and the cloaca exhibiting the lowest number (484 ASVs) (Supp. Fig. S3A). Including seawater samples, buccal samples exhibited the greatest overlap in bacterial ASVs with samples from both skin locations combined (1,236 ASVs). The smallest overlap in bacterial ASVs was observed between seawater samples and the cloaca (9 ASVs), followed by cloaca with the external surfaces (overlap with skin samples combined: 10 ASVs; with buccal samples: 12 ASVs) (Supp. Fig. S3B).

### Bacterial ASVs indicative of black-tip reef shark microenvironments and seawater

Linear Discriminant Effect Size Analysis (LEfSe) revealed a total of 157 bacterial ASVs overrepresented in seawater samples, which included a range of marine pelagic groups including clades of SAR11, SAR86, HIMB11, as well as NS4 and NS2b (Fig. 4A; Supp. Fig. S4A). Shark buccal bacterial communities showed significantly increased abundances of 19 ASVs, including but not limited to ASV29 *Endozoicomonas* (LEfSe, log 10 LDA score = 4.1; $p < < 0.001$) along with ASV25 *Shewanella*, ASV39 *Vibrio*, and several *Tenacibaculum* ASVs (LEfSe, log 10 LDA score ≥ 3.0; $p > 0.001$; presented in decreasing order of LDA scores) in decreasing order of effect size (Fig. 4B; Supp. Fig. S4B). For the two shark skin locations, i.e., the back and covering the gills, 36 and 23 overrepresented bacterial ASVs were identified, respectively (Fig. 4C, D; Supp. Fig. S4C, S4D). This included several ASVs classified as *Pseudoalteromonas* (both skin locations), *Acinetobacter*, *Pseudomonas*, *Marinobacter*, *Psychrobacter* (dorsal), as well as *Alteromonas*, *Halomonas*, and *Idiomarina* (skin covering the gills), among others (Fig. 4C, D). Finally, bacterial communities in shark cloaca samples exhibited a total of 20 overrepresented ASVs compared to all other habitats sampled. This included ASV951 *Paraclostridium*, several *Providencia* and *Cetobacterium* ASVs, Vibrionaceae (*Vibrio* and *Photobacterium*, several ASVs), and ASV110 of the genus *Catenococcus* (Fig. 4E). For details on LEfSe results, refer to Supp. Fig. S4 and Supp. Table S5 (Pogoreutz et al. 2023).

Indicator species analysis identified 126 ASVs significantly associated with seawater; these included Rhodospirillales (4 ASVs belonging to the AEGEAN-169-marine group as well as 14 ASVs belonging to various Rhodobacteraceae taxa); Flavobacteriaceae (10 unclassified ASVs, 9 of which belong to the NS4, NS5, and NS7 marine groups within the family), Pelagibacteraceae-SAR11 (15 ASVs belonging to SAR11 Clades I, Ia, Ib, II, and IV), Acidimicrobiales (3 ASVs), and Rhodobacterales (2 ASVs) (for a full list including indicator values and *p* values, refer to Supp. Table S4; Pogoreutz et al. 2023). Further, a total of 41 and 49 ASVs significantly associated with the skin on the back and the skin covering the gills, respectively; the two skin locations together were characterized by 149 significantly associated ASVs, including several *Pseudoalteromonas* (11 ASVs), *Idiomarina* (7 ASVs), *Psychrobacter* (2 ASVs), *Halomonas* (8 ASVs), *Marinobacter* (8 ASVs), and *Erythrobacter* (2 ASVs) (for a detailed list including information on ASV numbers, indicator scores, and *p* values, please refer to Supp. Table S4; Pogoreutz et al. 2023). Fifty-eight ASVs were significantly associated with the buccal cavity, among them ASV29 *Endozoicomonas* (Endozoicomonadaceae) and members of Flavobacteriaceae (12 ASVs), Rhodobacteraceae (11 ASVs), Saprospiraceae (5 ASVs), Hyphomonadaceae (3 ASVs), and Vibrionaceae (3 ASVs). For the cloacal samples, 25 indicator ASVs where identified, including nine Vibrionaceae ASVs (*Catenococcus*; three *Vibrio*; *Photobacterium*; four unclassified Vibrionaceae ASVs) and six Enterobacterales (Morganellaceae, *Providencia,* and *Morganella*).

### Predicted metabolic pathways of bacterial communities associated with seawater and microenvironments on black-tip reef sharks

Overall, LEfSe predicted a total of 50 metabolic (MetaCyc) pathways (inferred from sequenced genomes of related 16S rRNA gene sequences) to be overrepresented across all shark-associated and seawater samples. Of these, 12 were related to seawater bacterial communities (PICRUSt2 output: Supp. Table S6; full LEfSe output: Supp. Table S7; Pogoreutz et al. 2023). These included pathways associated with ribonucleotide metabolism and salvage, lipid metabolism (fatty acid salvage), cell wall synthesis, and pathways associated with the biosynthesis of coenzymes, carriers, and vitamins (tetrapyrrole, heme b, and coenzyme A biosynthesis); further, amino acid metabolism was identified (L-arginine biosynthesis and L-tryptophan degradation; LEfSe*,* log10 LDA scores > 2.5, *p* value $< < 0.05$; Fig. 5A).

Only four of the overrepresented, predicted metabolic pathways were associated with bacterial communities from shark buccal samples, which included three pathways related to the biosynthesis of K vitamins (specifically menaquinone,

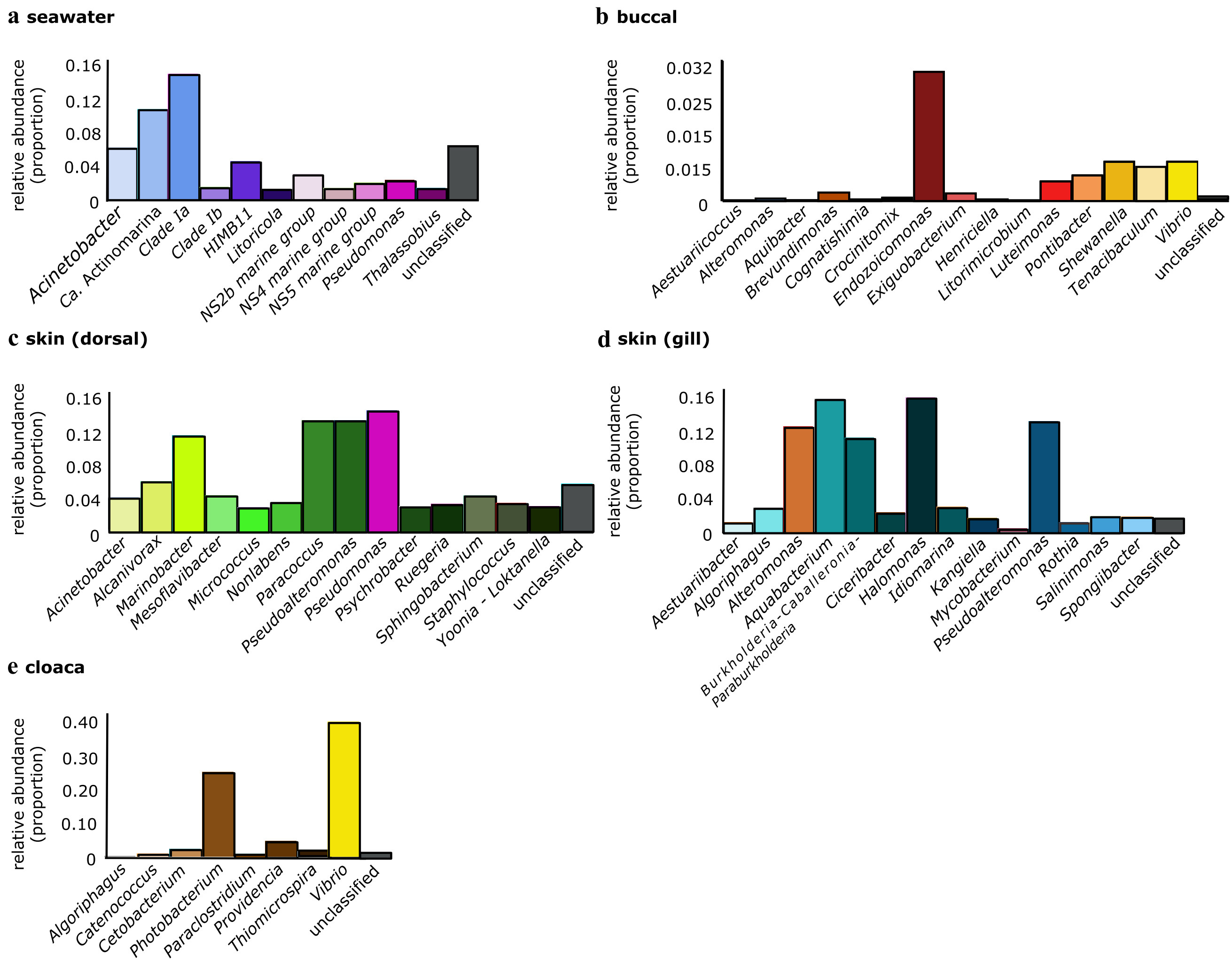


**Fig. 4** Overrepresented bacteria associated with seawater and shark-associated microenvironments. Relative abundances of overrepresented bacterial ASVs aggregated into their respective genera in **A** seawater and **B**–**E** different microenvironments on black-tip reef sharks (*C. melanopterus*) from the Amirante Islands, Seychelles. For **A**, **B**, **D**, only the top 20 statistically significant ASVs (based on decreasing order of LDA (log10) effect size scores of LEfSe analysis; all $p$ value $<0.05$) are considered. The same bacterial genera across sampled microenvironments have the same color

or vitamin K2) and one related to glycoprotein degradation (specifically, the breakdown of cartilage) (LEfSe*,* log10 LDA scores $>2.0$, $p$ values $<<0.05$; Fig. 5B; Supp. Fig. S5). A total of 16 metabolic features were overrepresented for microbial communities present on the two skin locations when combined and 18 for cloacal samples (for full table, please refer to Supp. Table S7; Pogoreutz et al. 2023). The 16 overrepresented predicted metabolic pathways on the skin included different pathways for fermentation (butanediol biosynthesis) and the degradation of alcohols ((methyl) catechol, ethanol, and methanol degradation) and one pathway each associated with the biosynthesis of amino acids (L-tryptophan) and of cofactors, carriers, and vitamins (biotin, i.e., vitamin B7; Fig. 5C). The 18 predicted metabolic functions overrepresented in bacterial communities of the cloaca included pathways associated with salvage pathways of pyrimidine (deoxy)ribonucleosides, amino acid metabolism (two pathways related to sulfur cycling and the biosynthesis of sulfur-containing amino acids, such as cysteine), carbohydrate metabolism (the degradation of D-galactose and sugar acids, and the biosynthesis of glycogen from ADP-D-glucose), the metabolism of cofactors, carriers, and vitamins (multiple pathways pertaining to the biosynthesis of vitamins B1, B6, and K vitamins) (LEfSe*,* log10 LDA scores $>3.0$, $p$ value $<<0.05$; Fig. 5D; for full details, see Supp. Table S7; Pogoreutz et al. 2023).

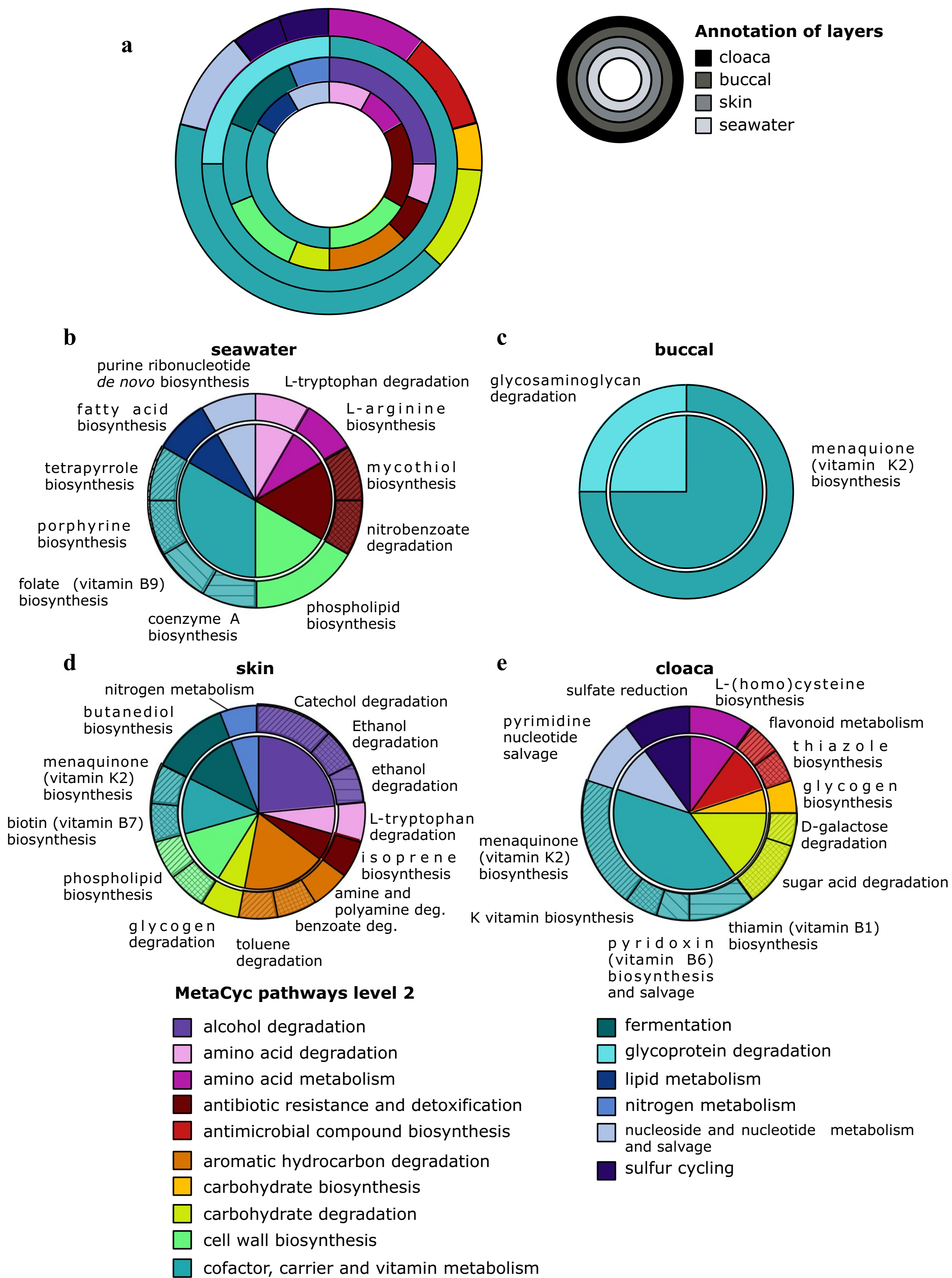


**Fig. 5** Overrepresented metabolic (MetaCyc) pathways predicted for seawater and shark-associated microenvironments. **A** Comparative overview of overrepresented metabolic pathways (MetaCyc level 2) across shark and seawater samples and detailed breakdowns for **B** seawater, **C** skin locations (skin from the back and covering the gills combined), **D** buccal, and **E** cloaca samples of black-tip reef sharks (*C. melanopterus*) from the Amirante Islands, Seychelles. Predicted metabolic pathways were inferred from 16S rRNA gene sequencing data using PICRUSt2 (Douglas et al. 2020). Detailed pathway information of differential metabolisms can be found in Supp. Fig. S4

## Discussion

The present study assessed the partitioning of bacterial communities across different locations on the body of black-tip reef sharks (*C. melanopterus*), generally the most abundant reef shark species across the Indo-West Pacific. We compared the bacterial communities from two locations on the skin (on the dorsal flank and on the covering of the gills), from the buccal cavity, and from the cloaca (as a proxy for the shark 'gut' microbiome) to that found in seawater from the shark sampling locations in the Amirante Islands, Seychelles. High-throughput sequencing of the 16S rRNA gene in combination with predictive metagenomic profiling not only demonstrated distinct bacterial communities present on shark skin compared to the surrounding seawater, but also suggests the presence of different microenvironments supporting distinct bacterial communities on black-tip reef sharks. The present study lends further support to the notion that the localization of associated microbes within marine holobionts is relevant (Hughes et al. 2022) and highlights the importance of expanding on genomic and functional approaches to assessing bacterial activity and metabolism, to better understand putative host–microbe interactions (Dörr et al 2023).

### Diversity of bacterial communities on microenvironments of black-tip reef sharks

Humans (and other multicellular terrestrial hosts) constitute a patchy landscape of microenvironments, where distinct skin microbiomes are associated with surface areas characterized by moist, dry, or sebaceous conditions (Grice et al. 2009; Byrd et al. 2018; Boxberger et al. 2021). In contrast, sharks are constantly surrounded by the ocean, which is likely a strong extrinsic (environmental) driver of bacterial community composition (Pogoreutz et al. 2019). In particular, it has been suggested that the thinness, texture (microscopic ridging, i.e., morphology and topography of epidermal denticles), resulting reduced hydrodynamic flow, as well as chemical properties (bioactivities) of shark skin and mucus may create a fairly challenging environment for microbial attachment and establishment and as such may be a significant factor structuring associated bacterial communities (Luer et al. 2014; Tsutsui et al. 2015; Doane et al. 2017; Moore et al. 1993; Kerr et al. 2023). Similar to what has been reported for other shark species, the bacterial communities associated with these external surfaces on black-tip reef sharks are more diverse, exhibit greater richness, and are distinct from those of both the surrounding seawater and of the cloaca (Storo et al. 2021; Doane and Haggerty 2017; Black et al. 2021; Pratte et al. 2022). This aligns with previous reports of greater bacterial community richness in the skin mucus of thresher sharks compared to the surrounding seawater (Doane et al. 2017) and the finding that skin-associated bacterial communities in animals, ranging from fish (Givens et al. 2015; Doane et al. 2020; Sylvain et al. 2020) and cetaceans (Li et al. 2022) to terrestrial vertebrates, including humans (Byrd et al. 2018), are distinct from and typically as diverse, or more diverse, than those of their gut (Eckburg et al. 2005; Fierer et al. 2008; Roggenbuck et al. 2014).

### Bacterial communities of shark surfaces: the skin and buccal cavity

Overall, we found distinct bacterial communities for two locations on the skin, the buccal cavity, and the cloaca in black-tip reef sharks. Such partitioning of bacterial communities across locations, tissues, and organs has been reported previously from the skin, oral, buccal cavity along with the intestine/cloaca, blood, and semen of other shark species (Storo et al. 2021; Black et al. 2021; Muñoz-Baquero et al. 2023; Bregman et al. 2023) (although see also (Montemagno et al. 2024)).

Bacterial communities associated with teleost fish and shark skin are influenced to a certain extent by the surrounding environment, such as fluctuations and seasonality in ocean geochemistry (Sylvain et al. 2020; Liston 1956; Larsen et al. 2013; Krotman et al. 2020) (but see also (Minniti et al. 2017). Indeed, the location of the sampling site was previously found to have an effect on the bacterial communities on the skin of black-tip reef sharks sampled from adjacent reef areas in the Amirante Islands, Seychelles, but also of bony fish from different environments (Pogoreutz et al. 2019; Sylvain et al. 2020; Liston 1956; Larsen et al. 2013; Krotman et al. 2020). At the same time, we found here that these shark skin-associated bacterial communities are distinct from those of the surrounding water. This finding aligns well with recent studies reporting distinct bacterial communities from the skin of thresher sharks and stingrays (Doane et al. 2017; Kerr et al. 2023), the scales of teleost fishes (e.g., Gomez and Primm 2021), and the skin of cetaceans (Apprill et al. 2014; Chiarello et al. 2017; Russo et al. 2018). It has hence been suggested that bacteria present on shark skin are likely not passive or transient associations of species acquired from the surrounding waters, but are characterized by different key taxa and generally show a higher overall bacterial diversity and functional potential than does the water column (Doane and Haggerty 2017; Doane et al. 2023; Kerr et al. 2023).

Several common and indicator taxa identified in the present study were previously reported from high-throughput sequencing studies of skin bacterial communities from black-tips and other sharks: e.g., *Pseudoalteromonas*, *Alteromonas*, *Marinobacter*, *Psychrobacter*, *Idiomarina*, *Vibrio*, *Erythrobacter* (Doane et al. 2017; Pogoreutz et al. 2019;

Bregman et al. 2023; Caballero et al. 2020), supporting their proposed status as stable members of the bacterial community on shark skin. While specific functions of these taxa have yet to be identified, it had been proposed that some of them may help mediate host skin health by reducing inflammatory responses (Doane et al. 2017; Kuepper et al. 2006) or by structuring the associated bacterial community through bioactivity against opportunistic microbes (Bowman 2007; Ballestriero et al. 2010). Several of these, most notably *Vibrio* are frequently isolated from different species of sharks (Grimes et al. 1985; Bertone et al. 1996; Black et al. 2021; Pogoreutz et al. *in prep*.), and *Vibrio* in particular have been implicated in bacterial wound infections in sharks (Grimes et al. 1984a,b; Unger et al. 2014), in humans following shark bite incidents (Pavia et al. 1989; Storo et al. 2021), and as part of the microbiota of various marine organisms, including diseased and bleached corals (Ben-Haim et al. 2003; Rosenberg and Falkovitz 2004; Banin et al. 2003). As such, while marine *Vibrio* are widely regarded as opportunistic pathogens (Austin and Zhang 2006; Sony et al. 2021; Ben-Haim et al. 2003; Ushijima et al. 2020, 2012), they are also commonly reported from a diversity of visibly healthy animal hosts. Further studies will be required to fully understand the nature of marine shark host–*Vibrio* interactions.

While in terms of abundant taxa the buccal cavity showed an overall similar bacterial community composition to the two skin locations, rare taxa and/or differential abundance of some common members may have driven statistical differences across the different microenvironments. Notably, the taxon with the highest indicator and LDA (log10) score identified for the buccal cavity was a bacterial taxon of the genus *Endozoicomonas*. This genus has previously been recovered in 16S rRNA gene sequencing data of coral reef fish gill filaments (Pratte et al. 2018), was previously linked to fish epitheliocystis in marine aquaculture (Mendoza et al. 2013; Katharios et al. 2015; Qi et al. 2018), and has also recently been isolated from the skin covering the gills of a black-tip reef shark from the Amirante Islands (Pogoreutz et al. *in prep*.). *Endozoicomonas* are also prevalent members of the bacterial communities of marine invertebrates (Neave et al. 2016a, b), and of reef-building corals in particular (Gignoux-Wolfsohn et al. 2017), in which they often occur at high relative abundances (Bayer et al. 2013; Neave et al. 2016a, b, 2017; Pogoreutz et al. 2018, 2022) and in dense aggregates deep in the tissues (Neave et al. 2016a, b; Wada et al. 2022; Maire et al. 2022). Members of this genus are commonly isolated from mucosal structures and surfaces of distantly related marine invertebrate hosts, including slugs (Kurahashi and Yokota 2007), bivalves (Hyun et al. 2014), and tunicates (Schreiber et al. 2016). While further study is required to reveal the potentially complex interactions of *Endozoicomonas* within their marine animal holobionts (Pogoreutz et al. 2022; Maire et al. 2022; Wada et al. 2022; Hochart et al. 2023; Pogoreutz and Ziegler 2024), our findings suggest that the mucus associated with the external environments of the shark, such as the buccal cavity (and/or potentially the gills, which are spatially connected to the buccal cavity), may select for a specific bacterial community highly adapted to likely challenging prevailing conditions.

### Bacterial communities associated with the cloaca of black-tip reef sharks

The bacterial communities in shark cloaca samples exhibited an overall lower (albeit fairly variable) bacterial diversity compared to those of the external surfaces. The observed spread in the Shannon index may stem from various factors, such as age, diet, immunological state, retention time of digesta in the intestine, the time since last intestinal evacuation, and any underlying (chronic) health conditions (Reese and Dunn 2018; Carmody et al. 2019). Overall, it is presumed that the taxonomic composition of the bacterial community in cloaca samples may reflect the environment of the shark distal intestine, i.e., of an environment shaped by the distinct physicochemical environment associated with digestive processes, but also the presence of digesta, which may represent substrates and nutrients available to microbes. Notably, the observation of distinct cloacal bacterial communities in black-tip reef sharks contrasts with that of another study (Pratte et al. 2022) that found similar bacterial communities associated with the cloaca and skin of three large roving shark species with distinct feeding ecologies, which was attributed to strong environmental effects from the surrounding seawater. These contrasting observations may further be explained by differences in sampling design and conditions between the two studies. Potentially, swabs may penetrate deeper into the cloaca into the distal intestine of the physically smaller black-tip sharks and be more reflective of a 'gut' bacterial community compared to in large roving shark species.

The cloaca samples were associated with several abundant and/or indicator taxa of the families Vibrionaceae (*Vibrio*, *Photobacterium, Catenococcus*) and Fusobacteriaceae (*Cetobacterium*), which are frequently reported as prevalent and abundant members of bacterial communities in the gut (intestinal or cloacal) of different elasmobranchs, including *Carcharhinus brevipinna*, *C. plumbeus*, *Rhizoprionodon terraenovae* (Givens et al. 2015), the omnivorous bonnethead shark *Sphyrna tiburo* (Leigh et al. 2021), and the white spotted eagle ray *Aetobatus narinari* (Clavere-Graciette et al. 2022). Other common and/or indicator taxa, such as Enterobacterales, a diverse order of gram-negative bacteria, contain numerous members associated with the gut microbiomes of humans and animals (Wüst et al. 2011; Martinson et al. 2019; Skrodenytė-Arbačiauskienė et al.

2008). Among these, *Providencia* have previously been isolated from the shark oral cavity (Interaminense et al. 2010). Finally, the bacterial taxon with the highest LDA (log10) score in the gut (as identified by LEfSe analysis) was *Paraclostridium*. This genus belongs to a group of fermenting, obligate anaerobes, representatives of the genus, and the family Clostridiaceae in general having been isolated from a range of terrestrial and marine environments, including marine sediment and sponges (Rai et al. 2019, Jyothsna et al. 2016; de Oliveira et al. 2019), as well as the gut of the omnivorous bonnethead shark (Leigh, Papastamatiou, and German 2021).

### Distinct inferred metabolic profiles of bacterial communities associated with different shark microenvironments

The current study found distinct differences in the predicted metabolic profiles between microenvironments of black-tip reef sharks and the water column, which aligns with previous reports on the gene content of the metagenomes for other elasmobranchs, such as the thresher shark (Doane and Haggerty 2017) and stingrays (Kerr et al. 2023). Of the 18 predicted metabolic pathways overrepresented on the skin of black-tip reef sharks, several are related to the degradation of a variety of aromatic compounds, specifically of the environmental pollutant toluene (specifically through the (methyl) catechol degradation pathways and via p-cresol cleavage; (Parales et al. 2008)), of harmful nitrogenous biogenic amines (via the decarboxylation of several amino acids (Mah et al. 2019)), as well as of the amino acid L-tryptophan. The former may suggest potential responses of bacterial communities to environmental pollutants, while the latter two suggest bacterial cycling of nitrogen, including amino acids and cofactors, respectively. This finding aligns with recent work, which identified genes associated with the metabolism of several amino acids and their derivatives and, at lower abundances, genes for major nitrogen cycling pathways in the skin metagenomes of leopard sharks (Doane et al. 2023). Finally, one predicted pathway associated with shark skin was related to the production of isoprenoids through the mevalonate pathway. Isoprenoids and their derivatives constitute the largest class of organic compounds in nature and cover diverse bioactivities found across the Tree of Life (Hoshino and Gaucher 2018). While the predicted function or subclass of isoprenoid is not more closely specified, this result supports previous findings of epidermal microbiomes of the leopard shark *Triakis semifasciata* being enriched in functional genes for isoprenoid metabolism (Doane et al. 2023). In this context, recent metagenomic studies reported an abundance of genes associated with 'defense functions,' including bioactivities against microorganisms overrepresented in skin mucus and fecal microbiomes of different sharks as well as the regulation of virulence (Doane et al. 2017; Pratte et al. 2022; Goodman et al. 2024), and antibiotic activity has been reported from bacteria isolated from the skin of other elasmobranchs, specifically rays and skates (Ritchie et al. 2017). In this light, it will be of interest to further elucidate the role of shark skin-associated microbiota in shark health by assessing their genomic potential for various secondary metabolite biosynthesis pathways as well as their associated chemodiversity.

The bacterial taxa found in the cloacal samples are predictive of several overrepresented predicted metabolic pathways. While specific bacterial capabilities and activities remain to be confirmed directly and *in hospite*, the composition of cloacal 16S rRNA gene sequencing data suggests a metabolic diversity reflective of a bacterial community adapted to the intestinal environment of sharks. Indeed, the availability and diversity of molecules and nutrients present in the digesta may support bacterial metabolisms and lifestyles different from the essentially oligotrophic environment of the mucus on shark skin (Sylvain et al. 2020) or in the water column. In bony fishes (teleosts), skin-associated bacteria are considered generalists, while those contributing to the gut bacterial communities include many specialized taxa (Sylvain et al. 2020). In the present study, overrepresented predicted metabolic pathways included for instance ribonucleotide salvage pathways, which may reflect bacterial use of 'exogenous' (dietary) nucleotides derived from digestive processes (Grimble 1994). Further pathways include the biosynthesis of sulfur-containing amino acids (e.g., cysteine), the degradation and biosynthesis of different carbohydrates, and their derivatives, along with the salvage and (de novo) biosynthesis of different vitamins, specifically vitamin B1 (thiamin), B6 (pyridoxin), and K vitamins (menaquinones). While it remains to be determined whether these biosynthetic and/or catabolic pathways are indeed present and active in the gut bacterial communities of black-tip reef sharks, isolates of *Vibrio* from fish intestinal tracts have been described to produce a diversity of hydrolytic enzymes under laboratory conditions, such as amylases, lipases, cellulases, chitinases, or others (Hamid et al. 1979; Henderson & Millar 1998; Itoi et al. 2006; Egerton et al. 2018; Gatesoupe et al. 1997; (summarized in (Leigh et al. German 2021). There is further first evidence of microbial fermentation in the omnivorous bonnethead shark (*Sphyrna tiburo*), as reflected by moderate concentrations of short-chain-fatty-acids along with greater epithelial surface area in the spiral intestine (Leigh et al. 2021). Finally, it is currently unclear whether sharks are physiologically dependent on or benefit from microbial digestion (Jhaveri et al. 2015; Leigh et al. 2021). However, it is established that animals are auxotrophic for B vitamins and have to acquire them from their diet or their bacterial symbionts (Douglas 2017; Salem et al. 2014). Further experimental study will hopefully help

elucidate the roles of different functional groups of cloacal ('gut') bacteria communities in shark host nutrition and physiology.

## Conclusion

Combining high-throughput marker gene sequencing with functional prediction, our study suggests the presence of several different bacterial microenvironments on black-tip reef sharks (*Carcharhinus melanopterus*). Importantly, while shark 'microbial landscapes' may not be as diverse as those of other, especially terrestrial animals, shark-associated microenvironments nevertheless appear to constitute distinct habitats on and in sharks, supporting distinct bacterial communities. The structure of these bacterial communities is likely driven in part by extrinsic factors, i.e., the physicochemical environment (surrounding seawater for external surfaces, digestive processes and digesta for the intestinal microbiome) and in part by intrinsic factors (i.e., host factors and microbe-microbe interactions). For future studies, it will be of interest to not only disentangle such drivers of bacterial community assembly and dynamics, but also to assess the diversity, activity, and roles for shark health. Combined culture-dependent and culture-independent applications will permit functional experimental interrogations leading to an integrated understanding of host–microbe interactions in elasmobranchs.

**Acknowledgements** MAG, GP, RO, and CC are grateful for the assistance of Stephen Morgan, Michael (Darnsley) Simara, and other members of the Danah Divers dive team in Seychelles, and to the scientific and other staff of the Save Our Seas Foundation D'Arros Research Centre (SOSF-DRC), for their considerable support during fieldwork and sample collection. This research was supported by an Independent Research Grant 2020 of the Center for Advanced Studies (Zukunftskolleg) of the University of Konstanz and Junior Professorship Grant *'A connected underwater world'* ANR-22-CPJ2-0113-01 awarded by the French National Research Agency (ANR) to CP. Funding covering fieldwork expenses was provided to MAG, RO, and GP by the Marine Research Facility, Jeddah. CRV acknowledges baseline funding by the King Abdullah University of Science and Technology and AFF funding by the University of Konstanz to CP and CRV (Project MetaFit; grant number 15902919 FP 029/19). Figure 2 was designed by CP in BioRender. The authors thank the two anonymous reviewers, whose constructive feedback greatly improved the manuscript.

**Author contributions** CP, MAG, RO, CC, and CRV conceived study; CP, MAG, RO, CC, and CRV provided funding; MAG, GP, CM, and RO conducted field work; CP performed DNA extractions and PCRs; GP performed library preparation and sequencing; CP analyzed and interpreted data; data curation was performed by CP and CRV; and CP wrote the first manuscript draft. All authors edited the manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Data availability** Raw sequence data are accessible under NCBI's BioProject PRJNA966929 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA966929). Supplementary Tables S2-S7 are available via the zenodo depository: https://zenodo.org/record/8041781 (Pogoreutz et al. 2023).

**Code availability** All code used for data analysis as part of this project is available at: https://github.com/Pogozoicomonas/Microbial-habitats-on-black-tip-reef-sharks-Carcharhinus-melanopterus-

**Declarations**

**Conflict of interest statement** None declared.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

## References

Alfano N, Courtiol A, Vielgrader H, Timms P, Roca AL, Greenwood AD (2015) Variation in koala microbiomes within and between individuals: effect of body region and captivity status. Sci Rep 5:10189

Apprill A, Robbins J, Eren AM, Pack AA, Reveillaud J, Mattila D, Moore M, Niemeyer M, Moore KMT, Mincer TJ (2014) Humpback whale populations share a core skin bacterial community: towards a health index for marine mammals? PLoS ONE 9:e90785

Artim SC, Sheh A, Burns MA, Fox JG (2019) Evaluating rectal swab collection method for gut microbiome analysis in the common marmoset (*Callithrix Jacchus*). PLoS ONE 14(11):e0224950

Austin B, Zhang XH (2006) *Vibrio harveyi*: A significant pathogen of marine vertebrates and invertebrates. Lett Appl Microbiol 43:119–124

Ballestriero F, Thomas T, Burke C, Egan S, Kjelleberg S (2010) Identification of compounds with bioactivity against the nematode caenorhabditis elegans by a screen based on the functional genomics of the marine bacterium *Pseudoalteromonas tunicata* D2. Appl Environ Microbiol 76(17):5710–5717

Banin E, Vassilakos D, Orr E, Martinez RJ, Rosenberg E (2003) Superoxide dismutase is a virulence factor produced by the coral bleaching pathogen *Vibrio shiloi*. Curr Microbiol 46(6):418–422

Bates, Douglas, Martin Maechler, Ben Bolker, Steven Walker, R. H. B. Christensen, H. Singmann, and Others. 2017. "lme4: Linear Mixed-Effects Models Using Eigen and S4, 2014." *R Package Version* 1 (4).

Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T, Hughen K, Apprill A, Voolstra CR (2013) The microbiome of the red sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. Appl Environ Microbiol 79(15):4759–4762

Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, Rosenberg E (2003) *Vibrio coralliilyticus* Sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. Int J Syst Evol Microbiol 53(Pt 1):309–315

Bennett AE, Groten K (2022) The costs and benefits of plant-arbuscular mycorrhizal fungal interactions. Annu Rev Plant Biol 73(1):649–672

Bertone S, Gili C, Moizo A, Calegari L (1996) *Vibrio carchariae* associated with a chronic skin ulcer on a shark, *Carcharhinus plumbeus* (Nardo). J Fish Dis 19(6):429–434

Bik EM, Costello EK, Switzer AD, Callahan BJ, Holmes SP, Wells RS, Carlin KP, Jensen ED, Venn-Watson S, Relman DA (2016) Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. Nat Commun 7(February):10516

Black C, Merly L, Hammerschlag N (2021) Bacterial communities in multiple tissues across the body surface of three coastal shark species. Zool Stud 60(November):e69

Bowman JP (2007) Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas*. Mar Drugs 5(4):220–241

Boxberger M, Cenizo V, Cassir N, La Scola B (2021) Challenges in exploring and manipulating the human skin microbiome. Microbiome 9(1):125

Bregman G, Lalzar M, Livne L, Bigal E, Zemah-Shamir Z, Morick D, Tchernov D, Scheinin A, Meron D (2023) Preliminary study of shark microbiota at a unique mix-species shark aggregation site, in the eastern mediterranean sea. Front Microbiol 14(February):1027804

Byrd AL, Belkaid Y, Segre JA (2018) The human skin microbiome. Nat Rev Microbiol 16(3):143–155

Caballero S, Galeano AM, Lozano JD, Vives M (2020) Description of the microbiota in epidermal mucus and skin of sharks (*Ginglymostoma cirratum* and *Negaprion brevirostris*) and one stingray (*Hypanus americanus*). PeerJ 8(December):e10240

De Caceres, Jansen, and Dell. (2016). “Indicspecies: Studying the Statistical Relationship between Species and Groups of Sites.” *R Package Version*.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Amy Jo A, Johnson-Holmes SP (2016) DADA2: high-resolution sample inference from illumina amplicon data. Nature Methods 13(7):581–583. https://doi.org/10.1038/nmeth.3869

Carmody RN, Bisanz JE, Bowen BP, Maurice CF, Lyalina S, Louie KB, Treen D et al (2019) Cooking shapes the structure and function of the gut microbiome. Nat Microbiol 4(12):2052–2063

Castro ALF, Rosa RS (2005) Use of natural marks on population estimates of the nurse shark, *Ginglymostoma cirratu*m, at atol das rocas biological reserve, Brazil. Environ Biol Fishes 72(2):213–221

Chen, J., X. Zhang, and H. Zhou. (2022). “GUniFrac: Generalized UniFrac Distances, Distance-Based Multivariate Methods and Feature-Based Univariate Methods for Microbiome Data Analysis.” *R Package Version*.

Chiarello M, Villéger S, Bouvier C, Auguet JC, Bouvier T (2017) Captive bottlenose dolphins and killer whales harbor a species-specific skin microbiota that varies among individuals. Sci Rep 7(1):15269

Choudhury R, Middelkoop A, Bolhuis JE, Kleerebezem M (2019) Legitimate and reliable determination of the age-related intestinal microbiome in young piglets; rectal swabs and fecal samples provide comparable insights. Front Microbiol 10(August):1886

Clavere-Graciette AG, McWhirt ME, Hoopes LA, Bassos-Hull K, Wilkinson KA, Stewart FJ, Pratte ZA (2022) Microbiome differences between wild and aquarium whitespotted eagle rays (*Aetobatus narinari*). Animal Microbiome 4(1):34

Costa C, Ivana MA, de Oliveira N, Wosnick RA, Hauser-Davis SS, Nunes JLS (2022) Elasmobranch-associated microbiota: a scientometric literature review. PeerJ 10(November):e14255

Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R (2009) Bacterial community variation in human body habitats across space and time. Science 326(5960):1694–1697

Czech L, Barbera P, Stamatakis A (2020) Genesis and gappa: processing, analyzing and visualizing phylogenetic (placement) data. Bioinformatics 36(10):3263–3265

Daly R, Stevens G, Daly CK (2018) Rapid marine biodiversity assessment records 16 new marine fish species for seychelles, West Indian Ocean. Marine Biodiv Rec 11(1):6

Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol Mol Biol Rev 76(2):229–261

Doane MP, Haggerty JM (2017) The skin microbiome of the common thresher shark (*Alopias vulpinus*) has low taxonomic and gene function b -diversity. Environ Microbiol 9:357–373

Doane MP, Haggerty JM, Kacev D, Papudeshi B, Dinsdale EA (2017) The skin microbiome of the common thresher shark (*Alopias vulpinus*) has low taxonomic and gene function β-Diversity. Environ Microbiol Rep 9(4):357–373

Doane MP, Morris MM, Papudeshi B, Allen L, Pande D, Haggerty JM, Johri S et al (2020) The skin microbiome of elasmobranchs follows phylosymbiosis, but in teleost fishes, the microbiomes converge. Microbiome 8(1):1–15

Doane MP, Johnson CJ, Johri S, Kerr EN, Morris MM, Desantiago R, Turnlund AC et al (2023) The epidermal microbiome within an aggregation of leopard sharks (*Triakis semifasciata*) has taxonomic flexibility with gene functional stability across three time-points. Microb Ecol 85(2):747–764

Domeier ML, Nasby-Lucas N (2007) Annual re-sightings of photographically identified white sharks (*Carcharodon carcharias*) at an Eastern Pacific aggregation site (Guadalupe Island, Mexico). Mar Biol 150:977–984

Dörr M, Denger J, Maier CS, Kirsch JV, Manns H, Voolstra CR (2023) Short-term heat stress assays resolve effects of host strain, repeat stress, and bacterial inoculation on Aiptasia thermal tolerance phenotypes. Coral Reefs 42:1271–1281

Douglas AE (2017) The B vitamin nutrition of insects: the contributions of diet, microbiome and horizontally acquired genes. Curr Opin Insect Sci 23(October):65–69

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MGI (2020) PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. https://doi.org/10.1038/s41587-020-0548-6

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA (2005) Diversity of the human intestinal microbial flora. Science 308(5728):1635–1638

Egerton S, Culloty S, Whooley J, Stanton C, Paul Ross R (2018) The gut microbiota of marine fish. Front Microbiol 9(May):873

Fierer N, Hamady M, Lauber CL, Knight R (2008) The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proceed Natl Acad Sci 105(46):17994–17999

Gatesoupe F-J, Infante J-L, Cahu C, Quazuguel P (1997) Early weaning of seabass larvae, *Dicentrarchus labrax*: the effect on microbiota, with particular attention to iron supply and exoenzymes. Aquaculture 158(1):117–127

Gignoux-Wolfsohn SA, Aronson FM, Vollmer SV (2017) Complex interactions between potentially pathogenic, opportunistic, and resident bacteria emerge during infection on a reef-building coral. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fix080

Givens CE, Ransom B, Bano N, Hollibaugh JT (2015) Comparison of the gut microbiomes of 12 bony fish and 3 shark species. Mar Ecol Prog Ser 518:209–223

Gomez JA, Primm TP (2021) A slimy business: the future of fish skin microbiome studies. Microb Ecol 82(2):275–287

Goodman, Asha Z., Bhavya Papudeshi, Maria Mora, Emma N. Kerr, Melissa Torres, Jennifer Nero Moffatt, Laís F. O. Lima, et al. 2024. "Elasmobranchs Exhibit Species-Specific Epidermal Microbiomes Guided by Denticle Topography." *bioRxiv*. https://doi.org/10.1101/2024.04.05.588334.

Gore MA, Frey PH, Ormond RF, Allan H, Gilkes G (2016) Use of photo-identification and mark-recapture methodology to assess basking shark (*Cetorhinus maximus)* populations. PLoS ONE 11(3):e0150160

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Comparative Sequencing Program NISC et al (2009) Topographical and temporal diversity of the human skin microbiome. Science 324(5931):1190–1192

Grimble GK (1994) Dietary nucleotides and gut mucosal defence. Gut 35(1 Suppl):S46-51

Grimes DJ, Colwell RR, Stemmler J, Hada H, Maneval D, Hetrick FM, May EB, Jones RT, Stoskopf M (1984a) *Vibrio* Species as agents of elasmobranch disease. Helgolaender Meeresuntersuchungen 315(37):309–315

Grimes DJ, Stemmler J, Hada H, May EB, Maneval D, Hetrick FM, Jones RT, Stoskopf M, Colwell RR (1984b) *Vibrio* Species associated with mortality of sharks held in captivity. Microb Ecol 10(3):271–282

Grimes DJ, Brayton P, Colwell RR, Gruber SH (1985) Vibrios as autochthonous flora of neritic sharks. Syst Appl Microbiol 6(2):221–226

Hamid A, Sakata T, Kakimoto D (1979) Microflora in the alimentary tract of gray mullet. IV. Estimation of enzymic activities of the intestinal bacteria. Nippon Suisan Gakkaishi 45(1):99–106

Hamid A, Sakata T, Kakimoto D (1979) Microflora in the alimentary tract of gray mullet. IV. Estimation of enzymic activities of the intestinal bacteria. Bull Jap Soc Sci Fish 45:99–106

Henderson RJ, Millar RM (1998) Characterization of lipolytic activity associated with a *Vibrio* Species of bacterium isolated from fish intestines. J Mar Biotechnol 6(3):168–173

Hesse RD, Roach M, Kerr EN, Papudeshi B, Lima LFO, Goodman AZ, Hoopes L et al (2022) Phage diving: an exploration of the carcharhinid shark epidermal virome. Viruses 14(9):1969. https://doi.org/10.3390/v14091969

Hinzke T, Kleiner M, Breusing C, Felbeck H, Häsler R, Sievert SM, Schlüter R et al (2019) Host-microbe interactions in the chemosynthetic *Riftia pachyptila* symbiosis. mBio. https://doi.org/10.1128/mBio.02243-19

Hochart C, Paoli L, Ruscheweyh HJ et al (2023) Ecology of Endozoicomonadaceae in three coral species across the Pacific Ocean. Nat Comm. https://doi.org/10.1038/s41467-023-38502-9

Hoshino Y, Gaucher EA (2018) On the origin of isoprenoid biosynthesis. Mol Biol Evol 35(9):2185–2197

Hughes DJ, Raina JB, Nielsen DA, Suggett DJ (2022) Disentangling compartment functions in sessile marine invertebrates. Trends Eco Evo 37:740–748

Hyun DW, Shin NR, Kim MS, Sei Joon O, Kim PS, Whon TW, Bae JW (2014) *Endozoicomonas atrinae* Sp. Nov., isolated from the intestine of a comb pen shell *Atrina pectinata*. Int J Syst Evol Microbiol 64:2312–18

Interaminense JA, Nascimento DCO, Ventura RF, Batista JEC, Souza MMC, Hazin FHV, Pontes-Filho NT, Lima-Filho JV (2010) Recovery and screening for antibiotic susceptibility of potential bacterial pathogens from the oral cavity of shark species involved in attacks on humans in recife, Brazil. J Med Microbiol 59(Pt 8):941–947

Itoi S, Okamura T, Koyama Y, Sugita H (2006) Chitinolytic bacteria in the intestinal tract of japanese coastal fishes. Can J Microbiol 52(12):1158–1163

Jhaveri P, Papastamatiou YP, German DP (2015) Digestive enzyme activities in the guts of bonnethead sharks (*Sphyrna tiburo*) provide insight into their digestive strategy and evidence for microbial digestion in their hindguts. Comp Biochem Physiol A Mol Integr Physiol 189:76–83

Katharios P, Seth-Smith HMB, Fehr A, Mateos JM, Qi W, Richter D, Nufer L et al (2015) Environmental marine pathogen isolation using mesocosm culture of sharpsnout seabream: striking genomic and morphological features of novel *Endozoicomonas* Sp. Sci Rep 5(December):17609

Kerr EN, Papudeshi B, Haggerty M, Wild N, Goodman AZ, Lima LFO, Hesse RD et al (2023) Stingray epidermal microbiomes are species-specific with local adaptations. Front Microbiol 14(March):1031711

Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE (2011) Succession of microbial consortia in the developing infant gut microbiome. Proceed Natl Acad Sci U S A 108:4578–4585

Krotman Y, Yergaliyev TM, Shani RA, Avrahami Y, Szitenberg A (2020) Dissecting the factors shaping fish skin microbiomes in a heterogeneous inland water system. Microbiome 8(1):9

Kuepper FC, Gaquerel E, Boneberg EM, Morath S, Salauen JP, Potin P (2006) Early events in the perception of lipopolysaccharides in the brown alga *Laminaria digitata* include an oxidative burst and activation of fatty acid oxidation cascades. J Exp Bot 57:1991–1999

Kurahashi M, Yokota A (2007) *Endozoicomonas elysicola* Gen. nov., Sp. nov., a gamma-proteobacterium isolated from the sea slug *Elysia ornata*. Syst Appl Microbiol 30(3):202–206

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol: CB 28:2570–2580

Larsen A, Tao Z, Bullard SA, Arias CR (2013) Diversity of the skin microbiota of fishes: evidence for host species specificity. FEMS Microbiol Ecol 85(3):483–494

Leigh SC, Papastamatiou YP, German DP (2021) Gut microbial diversity and digestive function of an omnivorous shark. Mar Biol. https://doi.org/10.1007/s00227-021-03866-3

Lenth, R. V., P. Buerkner, M. Herve, J. Love, and F. Miguez. n.d. "Emmeans: Estimated Marginal Means, Aka Least-Squares Means. 2022." *R Package Version*.

Li C, Xie H, Sun Y, Zeng Y, Tian Z, Chen X, Sanganyado Edmond et al (2022) Insights on gut and skin wound microbiome in stranded indo-pacific finless porpoise (*Neophocaena phocaenoides*). Microorganisms. https://doi.org/10.3390/microorganisms10071295

Liston J (1956) Quantitative variations in the bacterial flora of flatfish. J Gen Microbiol 15(2):305–314

Louca S, Doebeli M, Parfrey LW (2018) Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem. Microbiome 6(1):41

Luer, Carl A., Catherine Walsh, Kimberly Ritchie, Laura Edsberg, Jennifer Wyffels, Vicki Luna, and Ashby Bodine. 2014. "Novel Compounds from Shark and Stingray Epidermal Mucus with Antimicrobial Activity against Wound Infection Pathogens." MOTE MARINE LAB SARASOTA FL. https://apps.dtic.mil/sti/citations/ADA600463.

Mah J-H, Park YK, Jin YH, Lee J-H, Hwang H-J (2019) Bacterial production and control of biogenic amines in Asian fermented soybean foods. Foods (Basel, Switzerland) 8(2):85. https://doi.org/10.3390/foods8020085

Maire J, Tandon K, Collingro A, van de Meene A, Damjanovic K, Gotze CR, Stephenson S et al (2023) Colocalization and potential interactions of *Endozoicomonas* and chlamydiae in

microbial aggregates of the coral *Pocillopora acuta*. Sci Adv 9(20):eadg0773
Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMB.net J 17:10–12
Martinson JNV, Pinkham NV, Peters GW, Cho H, Heng J, Rauch M, Broadaway SC, Walk ST (2019) Rethinking gut microbiome residency and the *Enterobacteriaceae* in healthy human adults. ISME J 13(9):2306–2318
McFall-Ngai MJ (2014) The importance of microbes in animal development: lessons from the squid-*vibrio* symbiosis. Annu Rev Microbiol 68(June):177–194
Mendoza M, Güiza L, Martinez X, Caraballo X, Rojas J, Aranguren LF, Salazar M (2013) A novel agent (*Endozoicomonas elysicola*) responsible for epitheliocystis in cobia *rachycentrum canadum* larvae. Dis Aquat Org 106(1):31–37
Minniti G, Hagen LH, Porcellato D, Jørgensen SM, Pope PB, Vaaje-Kolstad G (2017) The skin-mucus microbial community of farmed atlantic salmon (*Salmo salar*). Front Microbiol 8(October):2043
Montemagno F, Romano C, Bastoni D, Cordone A, De Castro, O, Stefanni S, Giovannelli D (2024) Shark Microbiome Analysis Demonstrates Unique Microbial Communities in Two Distinct Mediterranean Sea Shark Species. Microorganisms, 12(3):557. https://doi.org/10.3390/microorganisms12030557
Moore KS, Wehrlit S, Roder H, Rogers M, Forrest JN, McCrimmon D, Zasloff M (1993) Squalamine : an aminosterol antibiotic from the shark. Proc Natl Acad Sci 90(February):1354–1358
Mylniczenko ND, Harris B, Wilborn RE, Young FA (2007) Blood culture results from healthy captive and free-ranging elasmobranchs. J Aquat Anim Health 19(3):159–167
Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR (2016a) Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-016-7777-0
Neave MJ, Rachmawati R, Xun L, Michell CT, Bourne DG, Apprill A, Voolstra CR (2016) Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. ISME J 11(1):186–200
Oksanen, Jari, F. Guillaume Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, et al. 2022. "Vegan: Community Ecology Package. R Package Version 2.5--7. 2020."
Parales RE, Parales JV, Pelletier DA, Ditty JL (2008) Diversity of microbial toluene degradation pathways. Adv Appl Microbiol 64:1–73
Pavia AT, Bryan JA, Maher KL, Hester TR Jr, Farmer JJ 3rd (1989) *Vibrio carchariae* infection after a shark bite. Ann Intern Med 111(1):85–86
Perry CT, Pratte ZA, Clavere-Graciette A, Ritchie KB, Hueter RE, Newton AL, Christopher Fischer G et al (2021) Elasmobranch microbiomes: emerging patterns and implications for host health and ecology. Animal Microb 3(1):61
Piazzon MC, Calduch-Giner JA, Fouz B, Estensoro I, Simó-Mirabet P, Puyalto M, Karalazos V, Palenzuela O, Sitjà-Bobadilla A, Pérez-Sánchez J (2017) Under control: how a dietary additive can restore the gut microbiome and proteomic profile, and improve disease resilience in a marine teleostean fish fed vegetable diets. Microbiome. https://doi.org/10.1186/s40168-017-0390-3
Pogoreutz C, Ziegler M (2024) Frenemies on the reef? resolving the coral–*Endozoicomonas* association. Trends Microbiol 32(5):422–434. https://doi.org/10.1016/j.tim.2023.11.006
Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2018) Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome. Ecol Evol 8(4):2240–2252
Pogoreutz C, Gore MA, Perna G, Millar C, Nestler R, Ormond RF, Clarke CR, Voolstra CR (2019) Similar bacterial communities on healthy and injured skin of black tip reef sharks. Animal Microb 1(1):1–16
Pogoreutz C, Oakley CA, Rädecker N, Cárdenas A, Perna G, Xiang N, Peng L, Davy SK, Ngugi DK, Voolstra CR (2022) Coral holobiont cues prime Endozoicomonas for a symbiotic lifestyle. ISME J 16(8):1883–1895. https://doi.org/10.1038/s41396-022-01226-7
Pogoreutz, Claudia, Gabriela Perna, Mauvis A. Gore, Rupert F. Ormond, Christopher R. Clarke, and Christian R. Voolstra. 2020. "Isolation of Bacteria Associated with Mucus on Shark Skin". *protocols.io* 2–5. https://www.protocols.io/view/isolation-of-bacteria-associated-with-mucus-on-sha-bmdik24e/metadata
Pogoreutz, Claudia, Mauvis A. Gore, Gabriela Perna, Rupert F. Ormond, Christopher R. Clarke, and Christian R. Voolstra. 2023. Data for NCBI BioProject PRJNA966929 (Version 2). *Zenodo*: https://zenodo.org/record/8041781.(2023)
Pratte ZA, Besson M, Hollman RD, Stewarta FJ (2018) The gills of reef fish support a distinct microbiome influenced by host-specific factors. Appl Environ Microbiol 84(9):1–15
Pratte ZA, Perry C, Dove ADM, Hoopes LA, Ritchie KB, Hueter RE, Fischer C, Newton AL, Stewart FJ (2022) Microbiome structure in large pelagic sharks with distinct feeding ecologies. Animal Microb 4(1):17
Qi W, Cascarano MC, Schlapbach R, Katharios P, Vaughan L, Seth-Smith HMB (2018) *Ca.* Endozoicomonas Cretensis: a novel fish pathogen characterized by genome plasticity. Genome Biol Evol 10(6):1363–1374
Quides KW, Weisberg AJ, Trinh J, Salaheldine F, Cardenas P, Lee H-H, Jariwala R, Chang JH, Sachs JL (2021) Experimental evolution can enhance benefits of rhizobia to novel legume hosts. Proceed Royal Soc B Biol Sci 288(1951):20210812. https://doi.org/10.1098/rspb.2021.0812
Reese AT, Dunn RR (2018) Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. mBio 9(4):10–1128. https://doi.org/10.1128/mBio.01294-18
Ritchie KB, Schwarz M, Mueller J, Lapacek VA, Merselis D, Walsh CJ, Luer CA (2017) Survey of antibiotic-producing bacteria associated with the epidermal mucus layers of rays and skates. Front Microbiol. https://doi.org/10.3389/fmicb.2017.01050
Roggenbuck M, Schnell IB, Blom N, Bælum J, Bertelsen MF, Sicheritz-Pontén T, Sørensen SJ, Thomas M, Gilbert P, Graves GR, Hansen LH (2014) The microbiome of new world vultures. Nat Commun 5:5498
Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10
Roik A, Reverter M, Pogoreutz C (2022) A roadmap to understanding diversity and function of coral reef-associated fungi. FEMS Microbiol Rev June. https://doi.org/10.1093/femsre/fuac028
Rosenberg E, Falkovitz L (2004) The *Vibrio Shiloi / Oculina Patagonica* model system of coral bleaching. Annu Rev Microbiol 58(1):143–159
Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5(5):355–362
Russo CD, Weller DW, Nelson KE, Chivers SJ, Torralba M, Jay Grimes D (2018) Bacterial species identified on the skin of bottlenose dolphins off Southern California via next generation sequencing techniques. Microb Ecol 75(2):303–309
Salem H, Bauer E, Strauss AS, Vogel H, Marz M, Kaltenpoth M (2014) Vitamin supplementation by gut symbionts ensures metabolic homeostasis in an insect host. Proceed Royal Soc B Biol Sci 281(1796):20141838. https://doi.org/10.1098/rspb.2014.1838

Schreiber L, Kjeldsen KU, Funch P, Jensen J, Obst M, López-Legentil S, Schramm A (2016) *Endozoicomonas* are specific, facultative symbionts of sea squirts. Front Microbiol 7:1042

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12(6):R60

Skrodenytė-Arbačiauskienė V, Sruoga A, Butkauskas D, Skrupskelis K (2008) Phylogenetic analysis of intestinal bacteria of freshwater salmon *Salmo salar* and sea trout *Salmo trutta* and diet. Fish Sci: FS 74(6):1307–1314

Sony M, Sumithra TG, Anusree VN, Amala PV, Reshma KJ, Alex S, Sanil NK (2021) Antimicrobial resistance and virulence characteristics of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio harveyi* from natural disease outbreaks of marine/estuarine fishes. Aquaculture 539(June):736608

Storo R, Easson C, Shivji M, Lopez JV (2021) Microbiome analyses demonstrate specific communities within five shark species. Front Microbiol 12(February):605285

Sylvain FÉ, Holland A, Bouslama S, Audet-Gilbert É, Lavoie C, Val AL, Derome N (2020) Fish skin and gut microbiomes show contrasting signatures of host species and habitat. Appl Environ Microbiol 86(16):1–15

Teleki, K. A., Nigel Downing, B. Stobart, and Raymond Buckley. 1999. "Aldabra Marine Programme." *Cambridge Coastal Research Unit, Department of Geography, University of Cambridge* 31. https://www.researchgate.net/profile/Ben-Stobart/publication/280134888_Aldabra_Marine_Programme/links/55ac69ae08aea9946727a805/Aldabra-Marine-Programme.pdf.

Tsutsui S, Dotsuta Y, Ono A, Suzuki M, Tateno H, Hirabayashi J, Nakamura O (2015) A C-type lectin isolated from the skin of japanese bullhead shark (*Heterodontus japonicus*) binds a remarkably broad range of sugars and induces blood coagulation. J Biochem 157(5):345–356

Unger NR, Ritter E, Borrego R, Goodman J, Osiyemi OO (2014) Antibiotic susceptibilities of bacteria isolated within the oral flora of florida blacktip sharks: guidance for empiric antibiotic therapy. PLoS ONE 9(8):e104577

Ushijima B, Smith A, Aeby GS, Callahan SM (2012) *Vibrio owensii* induces the tissue loss disease *Montipora* white syndrome in the hawaiian reef coral *Montipora capitata*. PLoS ONE 7(10):e46717

Ushijima B, Meyer JL, Thompson S, Pitts K, Marusich MF, Tittl J, Weatherup E et al (2020) Disease diagnostics and potential coinfections by *Vibrio Coralliilyticus* during an ongoing coral disease outbreak in Florida. Front Microbiol. https://doi.org/10.3389/fmicb.2020.569354

Voolstra CR, Raina J-B, Dörr M, Cárdenas A, Pogoreutz C, Silveira CB et al (2024) The coral microbiome in sickness, in health and in a changing world. Nat Rev Microbiol 22:460–475

Wada N, Hsu M-T, Tandon K, Hsiao SS-Y, Chen H-J, Chen Y-H, Chiang P-W et al (2022) High-resolution spatial and genomic characterization of coral-associated microbial aggregates in the coral *Stylophora pistillata*. Sci Adv 8(27):2431

Wüst PK, Horn MA, Drake HL (2011) *Clostridiaceae* and *Enterobacteriaceae* as active fermenters in earthworm gut content. ISME J 5(1):92–106

Ye Y, Doak TG (2009) A parsimony approach to biological pathway reconstruction/inference for genomes and metagenomes. PLoS Comput Biol 5(8):e1000465

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.