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Research Article

Gut bacterial communities in Atlantic bottlenose dolphins (*Tursiops truncatus*) throughout a disease-driven (*Morbillivirus*) unusual mortality event

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Abstract

Gut microbiomes are important determinants of animal health. In sentinel marine mammals where animal and ocean health are connected, microbiome impacts can scale to ecosystem-level importance. Mass mortality events affect cetacean populations worldwide, yet little is known about the contributory role of their gut bacterial communities to disease susceptibility and progression. Here, we characterized bacterial communities from fecal samples of common bottlenose dolphins, *Tursiops truncatus*, across an unusual mortality event (UME) caused by dolphin *Morbillivirus* (DMV). 16S rRNA gene sequence analysis revealed similar diversity and structure of bacterial communities in individuals stranding before, during, and after the 2013–2015 Mid-Atlantic Bottlenose Dolphin UME and these trends held in a subset of dolphins tested by PCR for DMV infection. Fine-scale shifts related to the UME were not common (10 of 968 bacterial taxa) though potential biomarkers for health monitoring were identified within the complex bacterial communities. Accordingly, acute DMV infection was not associated with a distinct gut bacterial community signature in *T. truncatus*. However, temporal stratification of DMV-positive dolphins did reveal changes in bacterial community composition between early and late outbreak periods, suggesting that gut community disruptions may be amplified by the indirect effects of accumulating health burdens associated with chronic morbidity.

Keywords: 16S rRNA, bacterial community, cetacean microbiome, dolphin *Morbillivirus*, marine mammal stranding, UME

Introduction

Multicellular organisms host diverse consortia of microorganisms, including viruses, archaea, and bacteria (Blaser and Kirschner 2007, Bello et al. 2018, Salazar et al. 2020). These microorganisms create resident communities ("microbiomes") in body sites exposed to the external environment, including the gastrointestinal tract, skin surface, and oral cavity (Backhed et al. 2005, Bello et al. 2018, Salazar et al. 2020). The gastrointestinal tract ("gut") hosts the highest microbial diversity and abundance ($>10^{14}$ cells), is dominated by bacteria, and has the largest documented impacts on host health and homeostasis (Backhed et al. 2005, Salazar et al. 2020). Gut bacterial communities expand the functional genetic diversity of their hosts, and have, thus, been conceptualized to function as a "microbial organ" within the host (Backhed et al. 2005). In humans and many other mammals, gut bacterial communities develop at birth, quickly diversifies in the early stages of host life, then stabilize during adulthood (Yatsunenko et al. 2012, Derrien et al. 2019, Salazar et al. 2020). Disruptions to gut bacterial communities ("dysbiosis"), including community shifts (destabilization) and diversity loss, generally correlate with declines in host health from disease and aging (Salazar et al. 2020). The composition and diversity of gut bacterial communities play a vital role in host health by influencing metabolism, immune function, hormonal activity, and digestion (Shreiner et al. 2015, Finlayson-Trick et al. 2017, Metcalf et al. 2017, Bello et al. 2018, Zheng et al. 2020). Accordingly, disruptions to gut communities may negatively impact host health, particularly through decreased immunity and increased susceptibility to disease (Wang et al. 2017).

Recent investigations have focused on characterizing the microbiomes of marine mammals to gain new insights into their roles in host biology, ecology, and evolution (e.g. Apprill et al. 2014, Nelson et al. 2015, Apprill et al. 2017, Erwin et al. 2017, Godoy-Vitorino et al. 2017, Nishida and Ochman 2018, Suzuki et al. 2019, Apprill et al. 2020, Centelleghe et al. 2020, Denison et al. 2020, Robles-Malagamba et al. 2020). Microbiome characterization has also been identified as an important tool for monitoring the health of marine mammals in a rapidly changing ocean environment (reviewed by Nelson et al. 2015, Apprill 2017). Some marine mammal species have been identified as "ecosystem sentinels" because of their sensitivity to environmental stressors, including infectious disease, and their pivotal roles within trophic systems (Bossart 2011, Nelson et al. 2015). One such species is the common bottlenose dolphin (*Tursiops truncatus*), a long-lived, apex predator in coastal and estuarine environments, and one that has experienced multiple epizootic events in the United States due to dolphin *Morbillivirus* (DMV), a strain of *Cetacean Morbillivirus* (CeMV) (Lipscomb et al. 1994, McLellan et al. 2002, Wells et al. 2004, Rosel

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et al. 2009, Bossart 2011, Rowles et al. 2011). *Cetacean Morbillivirus* causes metabolic impacts, immunosuppression, pneumonia, and skin lesions, and is often fatal (Barrett et al. 1991, Barrett 1999, van de Bildt et al. 2005, Bossart et al. 2011, Di Guardo et al. 2018, Pfeffermann et al. 2018). Along the US Atlantic coast, *T. truncatus* experienced *Morbillivirus* epizootics in 1987–1988 and again in 2013–2015 (reviewed by Morris et al. 2015). The recent epizootic was classified as an Unusual Mortality Event (UME) by the National Oceanographic and Atmospheric Administration (NOAA), and its cause was identified as DMV (NOAA Active and Closed Unusual Mortality Events 2022).

This study investigated the association between DMV infection and gut bacterial community structure in *T. truncatus* and the diagnostic potential of gut bacteria in predicting susceptibility to infection and health outcomes. Previous studies have characterized the gut bacterial communities of free-ranging and captive bottlenose dolphins (Bik et al. 2016, Soverini et al. 2016, Suzuki et al. 2019, Robles-Malagamba et al. 2020) and differentiated these host-associated communities from environmental microbiomes (Bik et al. 2016, Robles-Malagamba et al. 2020). Adult *T. truncatus* gut bacterial communities are dominated by the phyla Firmicutes, Proteobacteria, and Fusobacteria (Bik et al. 2016, Soverini et al. 2016, Suzuki et al. 2019, Robles-Malagamba et al. 2020), with some variability among captive animals correlating with environmental conditions (Suzuki et al. 2019). The gut microbial composition of *Cetaceans* associated with a disease outbreak has not been explored and may help define bacterial components associated with overall host health (Apprill et al. 2014, Nelson et al. 2015, Li et al. 2019). Such "biomarkers" of cetacean health can include specific bacterial taxa or community-level patterns of diversity and similarity that correspond to host health status and disease outcomes (Soares-Castro et al. 2019). For example, infection with *Morbillivirus* has been shown to impact gut bacterial community structure in giant pandas (*Ailuropoda melanoleuca*) which led to increased disease progression and severity (Zhao et al. 2017).

Here, we characterized the gut bacterial communities from fecal samples of 63 *T. truncatus* individuals that stranded in the mid-Atlantic between 2003–2019. Taxonomic composition, diversity and community structure of gut bacteria were compared across individuals sampled before, during and after the 2013–2015 Mid-Atlantic Bottlenose Dolphin UME. Further, a subset of individuals was tested by PCR for DMV infection, allowing for direct comparisons of gut community differentiation between confirmed DMV-positive and DMV-negative dolphins. Additional host factors were investigated to account for bacterial community variation independent of the mortality event, including sex, carcass condition, life history stage, and evidence of fisheries interaction. This study represents the first investigation of cetacean gut bacterial communities across a disease-driven UME, an important step in integrating microbiology into the health assessment of sentinel ocean species.

Methods

Sample collection and ethics statement

Stranding response activities were carried out under a NOAA Stranding Agreement to the University of North Carolina Wilmington (UNCW) and protocols were approved by UNCW's Institutional Animal Care and Use Committee (protocols 00–11, 2003–13, 2006–15, A0809-019, A1112-013, A1415-015, and A1718-011). Five coastal stocks of bottlenose dolphins along the US Atlantic coast are listed as depleted as defined by the Marine Mammal Protection Act (MMPA) 1972. This study included postmortem sampling of stranded *T. truncatus* that were either found freshly dead, died during initial response, or underwent humane euthanasia, following consultation with the NOAA's National Marine Fisheries Service and under the supervision of a licensed veterinarian in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2013 Edition).

In total, fecal samples were collected from 63 *T. truncatus* individuals that stranded along Virginia ($n = 2$), North Carolina ($n = 60$), and South Carolina ($n = 1$) coasts between 2003 and 2019 (Table S1), encompassing the 2013–2015 Mid-Atlantic Bottlenose Dolphin UME (*post hoc* identified as from 1 July 2013 to 15 March 2015) associated with a *Morbillivirus* outbreak. Samples were stored in a -80°C freezer after collection until processing for DNA extraction. For each individual, metadata were collected for the factors UME status, sex, life history stage, and carcass condition. For a subset of samples, metadata were collected for the factors DMV infection ($n = 33$), DMV period ($n = 20$), and evidence of fisheries interaction ($n = 54$, Table S1).

Three host factors defined by the mortality event (UME status, DMV infection, and DMV period) were the primary focus of bacterial community analyses. "UME status" factor levels were defined by individual stranding dates occurring before (pre, $n = 23$), during (UME, $n = 26$), and after (post, $n = 14$) the UME. "DMV infection" factor levels were positive ($n = 21$) or negative ($n = 12$) based upon polymerase chain reaction (PCR) testing for DMV RNA performed using the previously described consensus universal *Morbillivirus* primers (sense: 5′-ATGTTTATGATCACAGCGGT-3′; anti-sense: 5′-ATTGGGTTGCACCACTTGTC-3′) targeting a 429 bp fragment of the phosphoprotein gene (Barrett et al. 1993), followed by nested primers for DMV (sense: 5′-ATGTTTATGATCACAGCGGT-3′, anti-sense: 5′-ATCTCTCTCCTGTGCCCTTT-3′) that amplify a 384 bp fragment of the phosphoprotein gene. PCR reactions consisted of 1 µl of each primer, 18.15 µl molecular grade water, 2.5 µl 10X PCR Buffer, 0.75 µl $MgCl_2$ (50 mM), 0.5 µl dNTP (10 mM), 0.1 µl Invitrogen Platinum Taq DNA polymerase, and 1 µl cDNA template. Thermocycler conditions consist of an initial denaturing step of 94°C for 2 min, followed by 35 cycles of denaturing at 94°C for 20 sec, annealing at 56°C for 20 sec, and extension at 72°C for 60 sec; and a final extension step of 72°C for 15 min. PCR products were visualized via gel electrophoresis. Samples were tested with mammalian ß-actin primers to demonstrate amplifiable RNA in extracts (i.e. positive control). Individuals that tested positive for DMV were further divided into three approximately equal bins for the factor "DMV period": bin 1 (July and August 2013; $n = 6$) and bin 2 (September and October 2013; $n = 7$) corresponded to early outbreak periods and peak stranding incidence, and bin 3 (November 2013 and after; $n = 7$) to later outbreak periods. Separate analyses of all samples divided into these bins (i.e. assessment of the factor "Season") revealed no significant impacts on bacterial community diversity and structure, indicating that gut community shifts across DMV periods were not a result of seasonal trends (see Supplemental Text for details).

Four host factors defined independently of the mortality event (sex, carcass condition, life history stage, and fisheries interaction status) were also investigated to account for other sources of bacterial community variation. "Sex" factor levels were male ($n = 38$) and female ($n = 25$), as determined during necropsy. "Carcass condition" was defined upon initial observation of each stranding event and factor levels were alive ($n = 14$), fresh dead ($n = 35$), and moderately decomposed ($n = 14$) following the Smithsonian Institution Condition Code (Geraci and Lounsbury 1993). "Life history stage" factor levels were calf ($n = 12$), subadult ($n = 23$),

and adult ($n = 28$), as determined by total body length, presence of neonate characteristics, and necropsy examination (following Mallette et al. 2016 definitions). "Fisheries interaction" levels were positive ($n = 13$) and negative ($n = 41$), as determined by standardized HI examination and completion of the Marine Mammal Human Interaction Report (OMB No. 0648-0178, National Oceanic and Atmospheric Administration National Marine Fisheries Marine Mammal Health and Stranding Response Program). Fisheries interactions are categorized as a subset of human interaction, and included the presence of entanglement, lesions, and/or scarring associated with anthropogenic fisheries interaction (Moore and Barco 2013).

DNA extraction, sequencing, and sequence processing

Whole genomic DNA was extracted from 200 to 250 mg of fecal material using the DNeasy PowerSoil Kit (Qiagen) and used as templates for bacterial community characterization via partial 16S rRNA gene sequencing at the V4 region corresponding to the primer pair 515f and 806r (Caporaso et al. 2011). While this primary pair can amplify DNA of bacterial and archaeal origin (e.g. Denison et al. 2020), no sequences affiliated with the domain Archaea were recovered herein. Gel electrophoresis and DNA quantification (NanoDrop® One Spectrophotometer) were conducted to verify the quality and quantity of DNA extractions. PCR viability of DNA extractions was determined using PCR reactions consisting of 0.5 μL of each primer, 11 μL of PCR water, 12.5 μL of MyTaq HS Red Mix, and 0.5 μL of DNA extract. Thermocycler conditions consisting of an initial denaturing step of 95°C for 2 min, 35 cycles of denaturing at 95°C for 15 sec, annealing at 50°C for 15 sec, and extension at 72°C for 20 sec; and a final extension step of 72°C for 2 min. PCR products were visualized via gel electrophoresis, resulting in a single band for each sample.

DNA extractions were subsequently sent to Zymo Research (Irvine, CA) for library construction, standardization, and sequencing on an Illumina MiSeq Platform. Briefly, DNA extracts were amplified by real-time PCR and quantified using qPCR fluorescence readings. PCR products were then pooled in equimolar solutions and purified with Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA) before being quantified with TapeStation® (Agilent Technologies, Santa Clara, CA) and Qubit® (Thermo Fisher Scientific, Waltham, WA). The final library was sequenced on an Illumina® MiSeq™ platform. Negative (blank library preparations) and positive controls (ZymoBIOMICS® Microbial Community DNA Standard) were processed simultaneously with experimental samples. Controls confirmed no bioburden during the processing (i.e. no amplification of blanks) and high output accuracy (i.e. no significant differences in actual and theoretical standard composition, $\chi^2 = 5.49$, $P = 0.60$)

Raw sequences were processed in the mothur software package version 1.43.0 (Schloss et al. 2009) as described previously (Denison et al. 2020) and detailed in Table S2. Briefly, raw sequences were quality-filtered, aligned, and taxonomically identified (SILVA database, version 132, Quast et al. 2013), and then clustered into operational taxonomic units (OTUs) at 97% identity. Singleton OTUs were removed and sequences were subsampled to the lowest read count to standardize sequencing depth ($n = 50\,460$).

Composition of gut bacterial communities

The taxonomic composition of gut bacterial communities in *T. truncatus* was compared at the phylum and OTU levels across host factors. At the phylum level, the relative abundance of the top four phyla (accounting for >99.6% of all sequences) was compared across all host factors (UME status, DMV infection, DMV period, sex, carcass condition, life history stage, and fisheries interaction) using analyses of variance (ANOVA) accompanied by *post hoc* Tukey's Honest Significant Difference (HSD) tests in Sigmaplot (version 12). Datasets that violated ANOVA assumptions (normality and equal variance) were ranked prior to statistical assessment (Kruskal–Wallis) and post hoc comparisons (Dunn's method). At the OTU level, all 968 OTUs were analyzed for differences in relative abundances across all host factors using the DESeq2 algorithm as implemented in MicrobiomeAnalyst (Chong et al. 2020), with significance defined at $P < 0.05$ following false detection rate (FDR) corrections. While there is no perfect method of differential analysis, DESeq2 has shown consistent performance across datasets (Calgaro et al. 2020) and produces conservative estimates of P values, even in datasets with high OTU sparsity (Thorsen et al. 2016).

Diversity and structure of gut bacterial communities

Alpha diversity statistics were performed in mothur for each sample, calculating observed richness (S), Simpson evenness ($E_{1/D}$), Inverse Simpson diversity ($1/D$), and Berger–Parker dominance (d) of bacterial communities in *T. truncatus*. Significant differences in each alpha diversity statistic were calculated in Sigmaplot (version 12) for all host factors with an analysis of variance (ANOVA) accompanied by *post hoc* Tukey's HSD tests.

Beta-diversity statistics were performed based on OTU-dependent metrics (Bray–Curtis similarity) calculated in Plymouth Routines in Multivariate Ecological Research (PRIMER, version 6.1.11, PRIMER-e Ltd.) and OTU-independent metrics (UniFrac distance) calculated in mothur. Both metrics were analyzed based on relative abundance (OTU relative abundance Bray–Curtis, weighted UniFrac) and membership (OTU presence-absence Bray–Curtis, unweighted UniFrac). Significant differences in bacterial community similarity across all host factors were determined by main and pairwise permutational multivariate analyses of variance (PERMANOVA). Permutation multivariate analyses of dispersion (PERMDISP) were conducted to test for homogeneity of multivariate dispersions among factor levels. In addition, PERMANOVAs and PERMDISPs were conducted following centered log-ratio data transformation in MicrobiomeAnalyst and based on Euclidean distance calculations in PRIMER to confirm congruence between "standard" and "compositional" approaches (sensu Gloor et al. 2017; see Table S3).

Results

Composition of gut bacterial communities

A total of 968 OTUs were obtained from all 63 *T. truncatus* individuals and represented 14 bacterial phyla (Fig. 1, Table 1). Firmicutes and Fusobacteria were particularly abundant phyla, accounting for over 80% of gut communities and over 66% of total OTUs. Combined with Proteobacteria and Bacteroidetes, OTUs affiliated with these four phyla accounted for nearly all (>99%) of the gut bacterial community in *T. truncatus*. The remaining 10 phyla (Actinobacteria, Chlamydiae, Chloroflexi, Cyanobacteria, Dependentiae, Epsilonbacteraeota, Planctomycetes, Spirochaetes, Tenericutes, and Verrucomicrobia) combined accounted for <1% of the total gut composition (Fig. 1, Table 1). A total of 14 bacterial OTUs were dominant, together accounting for 90.7% of gut communities

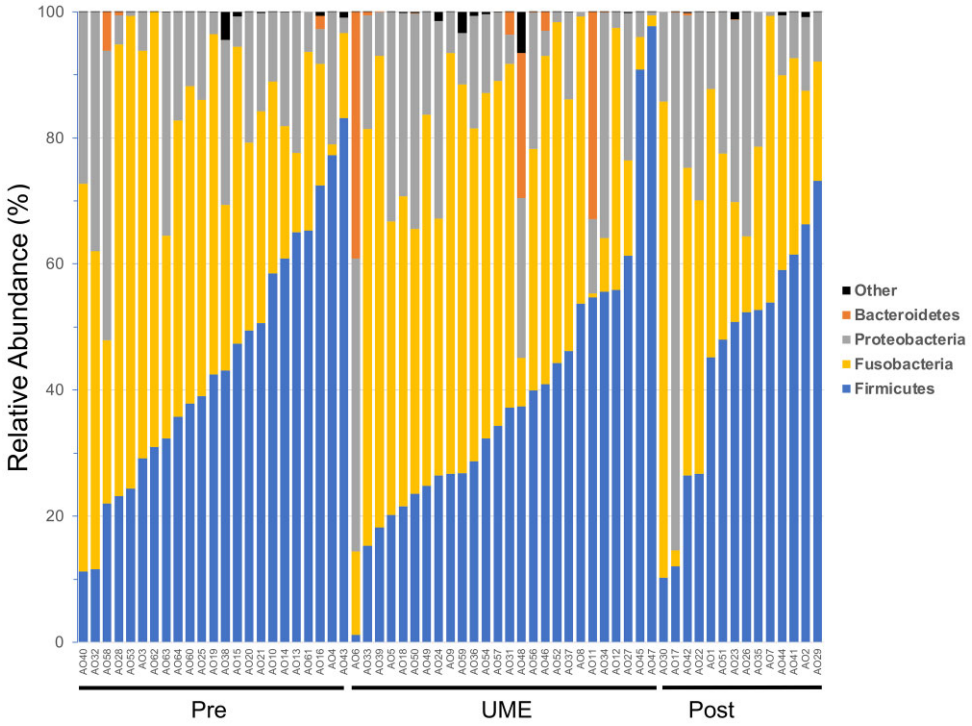

Figure 1. Phylum-level composition of gut bacterial communities in stranded *T. truncatus* individuals sorted by pre-, during, and post-UME time periods. Data are shown as relative abundances. The category "Other Phyla" consists of the rare phyla (<1% relative abundance) Actinobacteria, unclassified bacteria, Chlamydiae, Chloroflexi, Cyanobacteria, Dependentiae, Epsilonbacteraeota, Planctomycetes, Spirochaetes, Tenericutes, and Verrucomicrobia.

Table 1. Relative abundance of phyla present in gut bacterial communities of 63 stranded *T. truncatus* samples.

Phylum	Relative abundance
Firmicutes	42.34 ± 2.60
Fusobacteria	38.18 ± 2.61
Proteobacteria	17.32 ± 1.87
Bacteroidetes	1.78 ± 0.88
Tenericutes	0.17 ± 0.08
Verrucomicrobia	0.09 ± 0.09
Actinobacteria	0.07 ± 0.03
Epsilonbacteraeota	0.04 ± 0.02
Chlamydiae	0.01 ± 0.01
Unclassified Bacteria	≤0.001
Cyanobacteria	≤0.001
Planctomycetes	≤0.001
Dependentiae	≤0.001
Spirochaetes	≤0.001
Chloroflexi	≤0.001

Relative abundance is expressed as a percentage ±1 standard error.

Table 2. The 14 most abundant OTUs in gut bacterial communities of stranded *T. truncatus* by percentage of total sequences.

OTU	% Abundance	Phylum	Genus
Otu0001[(a,s,c)]	30.82	Fusobacteria	*Cetobacterium*
Otu0002[(a,s,c)]	17.00	Firmicutes	*Clostridium_sensu_stricto_1*
Otu0003[(a,s,c)]	10.26	Firmicutes	*Paeniclostridium*
Otu0004	6.33	Proteobacteria	*Photobacterium*
Otu0005[(a,s,c)]	5.93	Firmicutes	*Clostridium_sensu_stricto_1*
Otu0006[(c)]	4.73	Fusobacteria	*Fusobacterium*
Otu0007[(s)]	3.28	Proteobacteria	*Edwardsiella*
Otu0008	2.65	Proteobacteria	*Escherichia-Shigella*
Otu0009	2.03	Firmicutes	*Peptostreptococcus*
Otu0010	1.69	Firmicutes	*Clostridiaceae_1_unclassified*
Otu0011	1.91	Proteobacteria	*Actinobacillus*
Otu0012	1.51	Bacteroidetes	*Bacteroides*
Otu0013[(c)]	1.42	Proteobacteria	*Actinobacillus*
Otu0014	1.18	Firmicutes	*Clostridium_sensu_stricto_1*

Phylum and genus-level taxonomy are listed for each OTU. Core OTUs (those detected in all samples, $n = 4$ or in all samples of a given life history stage, $n = 3$) are indicated for adult (a), subadult (s), and calf (c) OTUs.

(Table 2), 4 of which represented core OTUs (detected in all 63 *T. truncatus* samples). Three core OTUs were in the phylum Firmicutes and one core OTU belonged to the phylum Fusobacteria. The analysis of core OTUs by life history stage revealed two additional core OTUs in all calves, *Fusobacterium* sp. (Fusobacteria) and *Actinobacillus* sp. (Proteobacteria), and one additional core OTU in all subadults, *Edwardsiella* sp. (Proteobacteria, Table 2).

Phylum-level composition of gut bacterial communities was similar across samples of *T. truncatus*. No significant differences in phylum-level composition were detected across UME status (Fig. 1, Table 3), DMV infection (Fig. 2, Table 4), DMV period

Table 3. Relative abundance of phyla in gut bacterial communities of *T. truncatus* that stranded before (pre-UME), during (UME), and after (post-UME) the mortality event.

Phylum	Pre-UME	UME	Post-UME	P-value
Bacteroidetes	0.39 ± 0.28	3.94 ± 2.06	0.04 ± 0.03	0.392
Firmicutes	44.05 ± 4.22	39.07 ± 4.28	45.59 ± 5.20	0.567
Fusobacteria	39.65 ± 4.27	40.26 ± 4.36	31.92 ± 4.90	0.445
Proteobacteria	15.58 ± 2.63	16.21 ± 2.51	22.23 ± 5.55	0.529

Relative abundances (± standard error) are expressed as a mean percentage. No significant differences in relative abundances ($P < 0.05$) were found between phyla in dolphins across UME status periods.

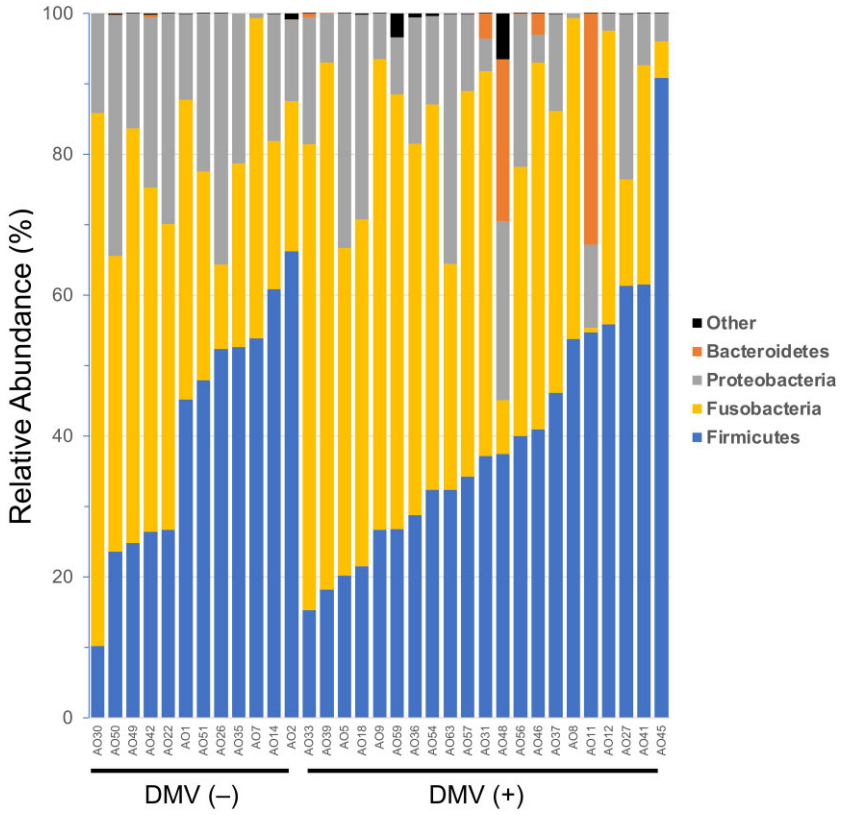

Figure 2. Phylum-level composition of gut bacterial communities in stranded *T. truncatus* individuals sorted by *Morbillivirus* negative [DMV(-)] and *Morbillivirus* positive [DMV(+)] status. Data are shown as relative abundances. The category "Other Phyla" consists of the rare phyla (<1% relative abundance) Actinobacteria, unclassified bacteria, Chlamydiae, Chloroflexi, Cyanobacteria, Dependentiae, Epsilonbacteraeota, Planctomycetes, Spirochaetes, Tenericutes, and Verrucomicrobia.

Table 4. Relative abundance of the top four phyla in gut bacterial communities of dolphin *Morbillivirus*-positive [DMV (+)] and negative [DMV (-)] stranded *T. truncatus* samples. Relative abundances (± standard error) are expressed as a mean percentage.

Phylum	DMV(+)	DMV(–)	P-value
Bacteroidetes	3.01 ± 1.85	0.037 ± 0.03	0.195
Firmicutes	39.81 ± 3.98	40.90 ± 5.12	0.869
Fusobacteria	42.42 ± 4.53	38.87 ± 5.18	0.623
Proteobacteria	14.18 ± 2.27	20.06 ± 2.92	0.125

No significant differences in relative abundances ($P < 0.05$) were found between phyla in dolphins that were positive or negative for *Morbillivirus* infection.

Table 5. Relative abundance of the top four phyla in gut bacterial communities of DMV (+) individuals by DMV period in stranded *T. truncatus* samples.

Phylum	Bin 1	Bin 2	Bin 3	P-value
Bacteroidetes	0.12 ± 0.10	5.64 ± 4.58	3.29 ± 3.28	0.701
Firmicutes	36.24 ± 7.91	43.76 ± 8.77	39.99 ± 5.56	0.243
Fusobacteria	49.59 ± 9.00	38.43 ± 9.21	41.76 ± 7.08	0.711
Proteobacteria	13.46 ± 3.54	11.97 ± 3.48	13.97 ± 4.32	0.927

Relative abundances (± standard error) are expressed as a mean percentage. No significant differences in relative abundances ($P < 0.05$) were found between phyla in dolphins across the DMV period.

(Table 5), carcass condition (Table S4), or fisheries interaction (Table S5). Significant differences in phylum-level composition were detected across sexes (Table S6) and life history stages (Table S7). Female dolphins exhibited lower Firmicutes relative abundances ($P = 0.035$) than males, whereas calves hosted a significantly lower relative abundance of Fusobacteria ($P = 0.045$) compared to subadults and adults.

Differential OTU-level analyses were conducted to assess finer-scale taxonomic shifts in bacterial community composition by UME-related and other host factors. Of the 968 OTUs investigated, 20 (2.1%) exhibited significant differences in relative abundance across any factor, with low overlap among factors (Fig. 3, Table S8). Four OTUs differed by UME status, including OTU0012 (*Bacteroides*) that peaked in abundance during the UME (Fig. 3). Three additional OTUs were rare or absent before and during the UME and increased significantly in relative abundance post-UME (Fig. 3). One of these OTUs (OTU0011 *Actinobacillus delphinicola*) also exhibited high relative abundance (3.4%) in dolphins that tested negative for DMV and very low relative abundance (0.1%) for dolphins testing positive (Fig. 3). OTU0023 represented an unclassified and rare OTU in the family Barnesiellaceae that was significantly more abundant in DMV-positive vs. DMV-negative dolphins (0.7% vs. <0.01%). When comparing by DMV period, five OTUs were differentially abundant in animals during early and late outbreak periods. Two of these OTUs (OTU0048 *Ureaplasma* and OTU0088 *Morganella*) matched to genera that contain opportunistic pathogens (O'Hara et al. 2000, von Chamier et al. 2012) and were most common in the earliest outbreak period (bin 1) and rare or absent in later periods (bins 2 and 3). The remaining three OTUs (OTU0008 *Escherichia*, OTU0010 Clostridiaceae_1, and OTU0060 *Aeriscardovia*) were most common in the latest outbreak period (bin 3) and rare or absent in earlier periods (bins 1 and 2). Additional OTUs were

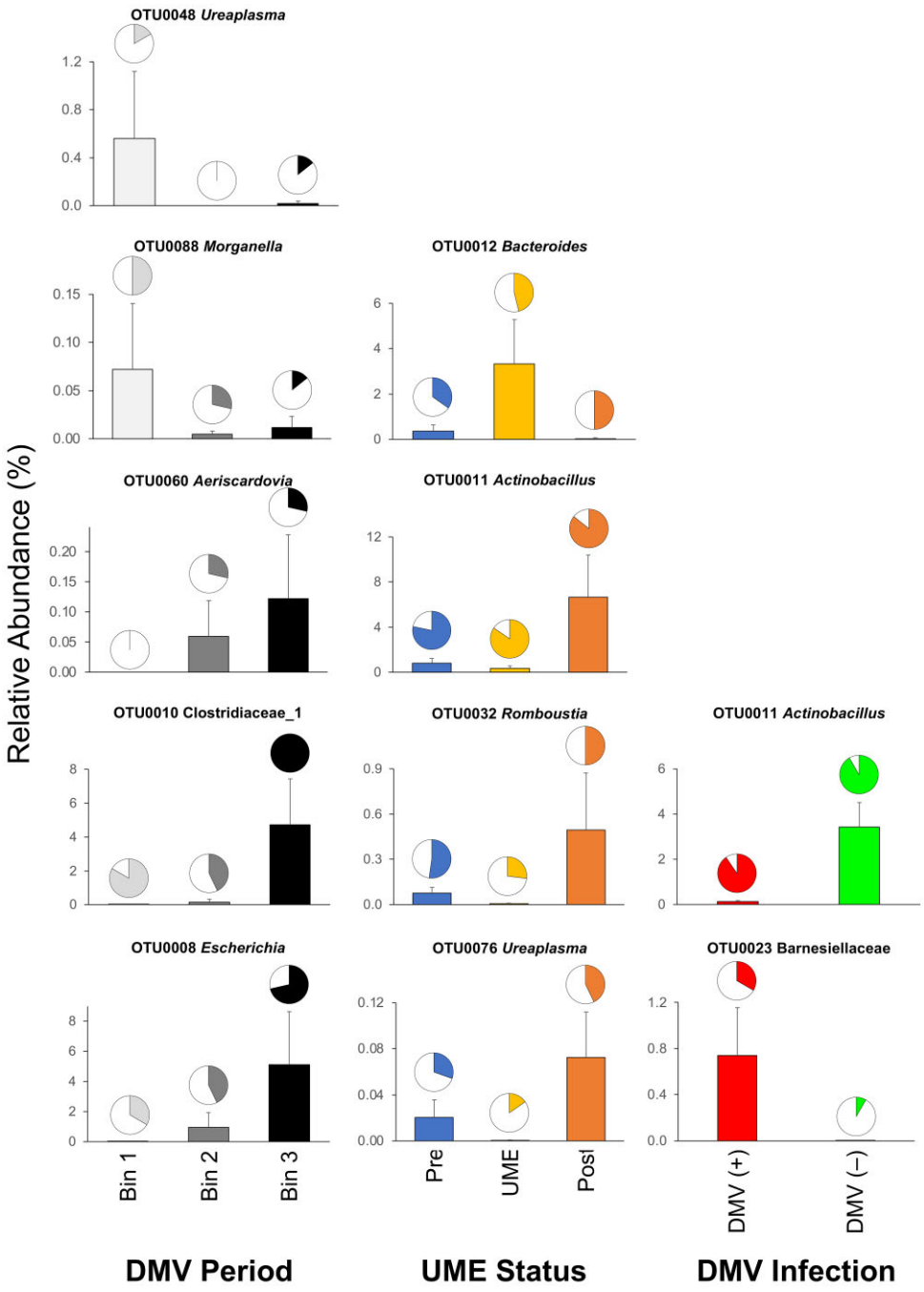

Figure 3. Relative abundance (bar charts) and incidence (pie charts) of OTUs that differed significantly across UME factors. The lowest taxonomic classification of each OTU is shown. Error bars represent ± 1 standard error.

detected as differentially abundant across the host factors carcass condition ($n = 7$), life history stage ($n = 4$), and fisheries interaction ($n = 2$, Table S8).

Diversity and structure of gut bacterial communities

Overall, gut bacterial communities of *T. truncatus* displayed minimal differences in diversity across UME-related and other host factors (Table 6). Indeed, no significant differences in diversity ($1/D$) and dominance (d) metrics were detected across any host factor. All alpha-diversity metrics were similar across UME status, while evenness (E) varied across DMV infection status and richness (S) varied across the DMV period (Table 6, Fig. 4). The evenness of the gut microbial communities was significantly lower in DMV-positive as compared to DMV-negative dolphins ($P = 0.026$, Fig. 4), indicating greater dominance of select bacterial taxa in dolphins infected with DMV. In addition, gut bacteria richness was significantly higher in animals testing positive for DMV later in the outbreak ($P = 0.045$, Fig. 4), increasing 50%–77% compared to earlier outbreak periods. Significant differences in the evenness of gut communities were also detected across sex ($P = 0.042$), where male dolphins exhibited lower evenness compared to females (Fig. S1).

Beta-diversity metrics of community structure in gut bacterial communities of *T. truncatus* exhibited high overall similarity

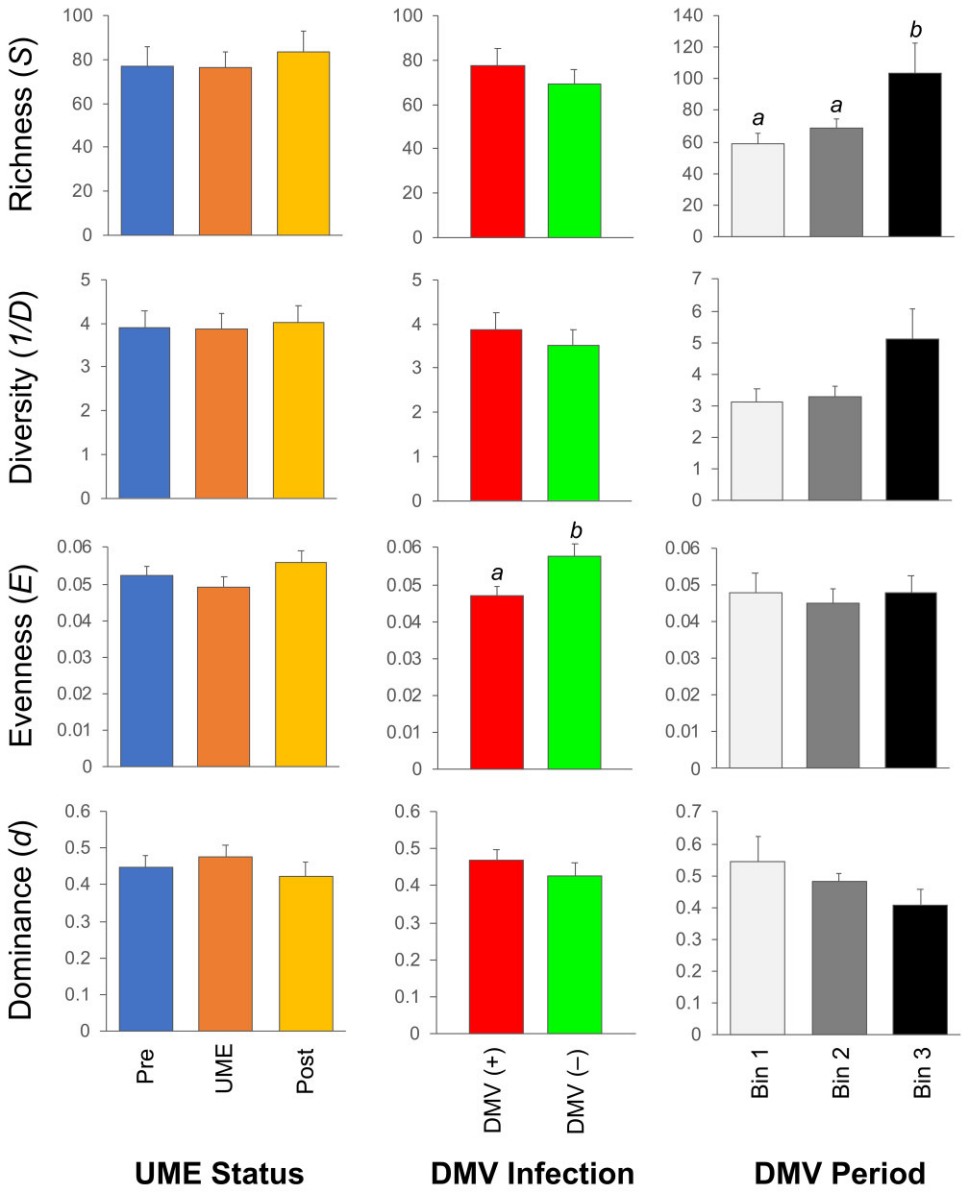

Figure 4. Alpha-diversity metrics of gut bacterial communities in stranded *T. truncatus* individuals grouped by UME status, DMV infection, and DMV period. Error bars represent ± 1 standard error. Significant differences ($P < 0.05$) are labeled with different letters (*a* and *b*).

Table 6. Statistical analysis of variance (ANOVA) of gut bacterial communities in stranded *T. truncatus* across host factors based on the alpha-diversity metrics richness (*S*), diversity (*1/D*), evenness (*E*), and dominance (*d*).

Factor	*n*	Richness (S)	Diversity (1/D)	Evenness (E)	Dominance (d)
UME status	63	0.556	0.967	0.290	0.595
DMV infection	33	0.452	0.547	0.026*	0.417
DMV period	20	0.045*	0.302	0.874	0.239
Sex	63	0.584	0.440	0.042*	0.544
Carcass condition	63	0.358	0.659	0.392	0.904
Life history stage	63	0.574	0.242	0.197	0.421
Fisheries interaction	61	0.620	0.288	0.671	0.422

Significant differences ($P < 0.05$) are denoted by an asterisk (*).

across factors related to the mortality event, with a trend toward higher variability in animals that tested positive for DMV late in the outbreak. Non-metric multidimensional scaling (NMDS) plots revealed no distinct clustering of bacterial communities in pre-UME, UME and post-UME animals (Fig. 5), and significant differences in gut community similarity across UME status were detected for only one of four metrics (Table 7) and only between pre-UME and UME animals in pairwise tests for this metric (Table S9). No significant differences in gut bacterial community similarity and dispersion were detected between DMV-positive and DMV-negative *T. truncatus* individuals (Table 7, Fig. 6). Temporal stratification of DMV-positive dolphins revealed significant differences in community similarity and dispersion for two of four metrics (both based on membership, Table 7, Fig. 7). For both metrics, differences in bacterial community structure were driven by late outbreak animals, as pairwise tests revealed significant differences between early and late periods (bin 1 vs. bin 3 and bin 2 vs. bin 3), but not among early periods (bins 1 and 2, Table S10).

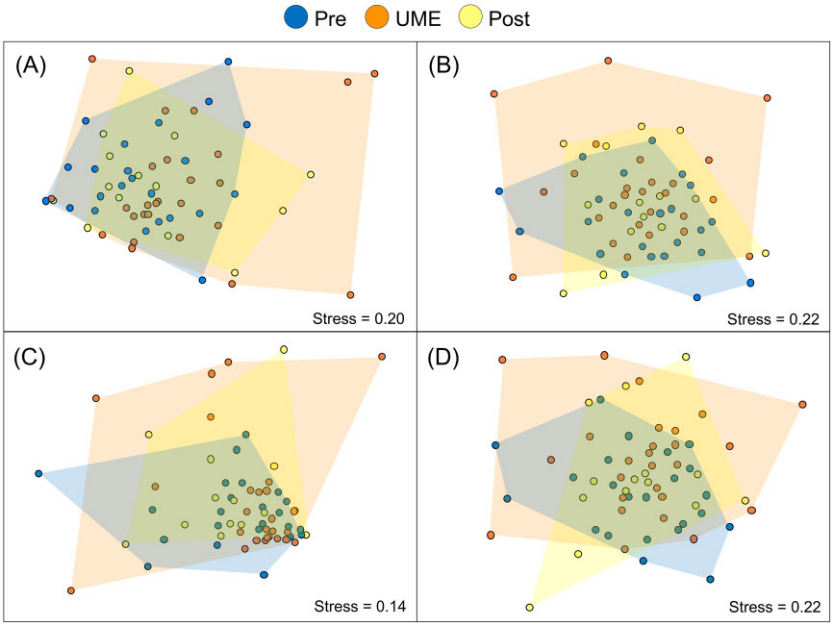

Figure 5. NMDS of gut bacterial communities in stranded *T. truncatus* colored by individuals collected before (pre-UME, blue), during (UME, orange), and after (post-UME, yellow) the mortality event. Ordination is based on (A) Bray–Curtis similarity of relative abundance (RA); (B) Bray–Curtis similarity of presence/absence (PA); (C) Weighted UniFrac **(W)** distances; and (D) Unweighted UniFrac (UW) distances. Significant differences in bacterial community structure (PERMANOVA, $P < 0.05$) were detected between pre-UME and UME strandings for one metric **(D)**. No significant differences in bacterial community dispersion (PERMDISP) were detected among groups.

Table 7. Statistical analyses of variance (PERMANOVA, P-ANOVA) and dispersion (PERMDISP, P-DISP) of gut bacterial communities in stranded *T. truncatus* across factors based on OTU-dependent (Bray–Curtis) and OTU-independent (UniFrac) metrics.

		Bray–Curtis (RA)		Bray–Curtis (PA)		UNIFRAC-W		UNIFRAC-UW	
Factor	*n*	P-ANOVA	P-DISP	P-ANOVA	P-DISP	P-ANOVA	P-DISP	P-ANOVA	P-DISP
UME status	63	0.134	0.777	0.089	0.974	0.413	0.957	0.037*	0.667
DMV infection	33	0.606	0.713	0.562	0.737	0.454	0.803	0.525	0.149
DMV period	20	0.115	0.088	0.013*	0.001*	0.862	0.662	0.015*	0.003*
Sex	63	0.138	0.224	0.237	0.074	0.091	0.599	0.310	0.323
Carcass condition	63	0.030*	0.481	0.028*	0.212	0.010*	0.348	0.020*	0.445
Life history stage	63	0.015*	0.048*	0.012*	0.299	0.026	0.046*	0.006*	0.869
Fisheries interaction	62	0.289	0.028*	0.221	0.001*	0.827	0.924	0.127	0.001*

These metrics include relative abundance (Bray–Curtis RA and UniFrac-W) and presence-absence (Bray–Curtis PA and UniFrac-UW) measures. Significant differences are denoted by an asterisk (*).

Other host factors also impacted bacterial community structure in *T. truncatus* individuals. Most prominent were life history stage and carcass condition, where significant differences in community structure or dispersion were detected for all four beta-diversity metrics (Table 7). NMDS plots showed considerable overlap across life history stages but also a general trend of gut community convergence (i.e. tighter sample clustering) with maturity (Fig. S2). Indeed, all significant pairwise comparisons occurred between the earliest life history stage (calves) and later life history stages (subadults and adults, Table S11). Carcass condition significantly impacted bacterial community structure despite the visual overlap in gut communities across all carcass conditions (Fig. S3). These differences were driven by strandings that experienced moderate decomposition (condition 3), which differed consistently from condition 1 strandings (four of four tests) and occasionally from condition 2 (one of four tests, Table S12). Weaker signals were detected when comparing bacterial communities across fisheries interaction status, where differences were restricted to dispersion (Table 7, Fig. S4). No differences in structure or dispersion of gut communities were found between the sexes for any metric (Table 7).

Discussion

This study provided the first comprehensive analysis of the composition, diversity, and structure of the gut bacterial communities in common bottlenose dolphins, *T. truncatus*, associated with a disease-driven mortality event. Gut bacteria were similar in dolphins that were stranded before, during, and after the 2013–2015 Mid-Atlantic Bottlenose Dolphin UME in terms of broad-scale taxonomic composition, diversity, and community similarity. These trends held between a subset of animals that tested PCR-positive or PCR-negative for DMV infection, where bacterial shifts in response to infection status were minimal on the community level. OTU-level analyses identified potential biomarkers for health monitoring within the complex gut bacterial communities (e.g. OTU0011 *Actinobacillus delphinicola*) though at a low incidence (10 of 968 OTUs). The similarity of *T. truncatus* gut

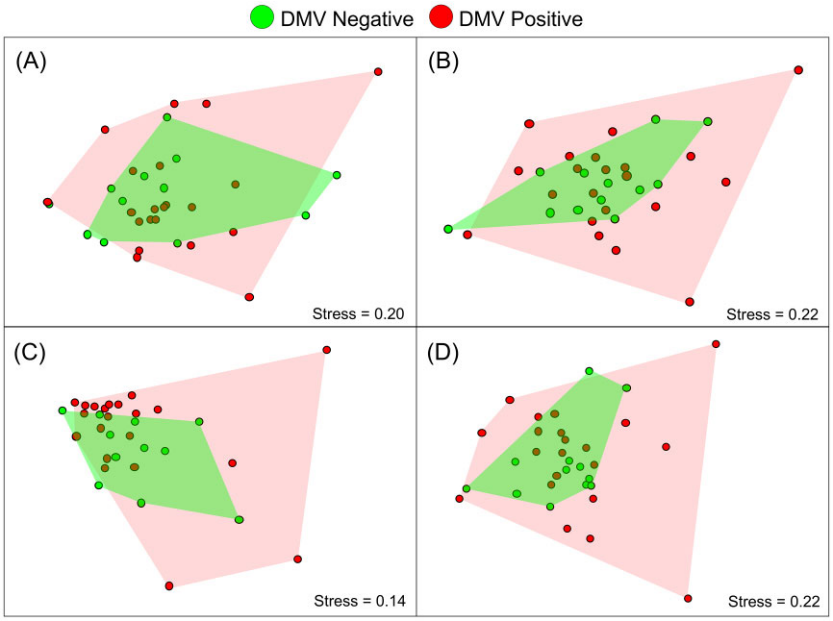

Figure 6. NMDS of gut bacterial communities in stranded *T. truncatus* in dolphin *Morbillivirus* negative (green) and positive (red) individuals. Ordination is based on (A) Bray–Curtis similarity of relative abundance (RA); (B) Bray–Curtis similarity of presence/absence (PA); (C) Weighted UniFrac **(W)** distances; and (D) Unweighted UniFrac (UW) distances. No significant differences in bacterial community structure (PERMANOVA) or dispersion (PERMDISP) were detected among groups.

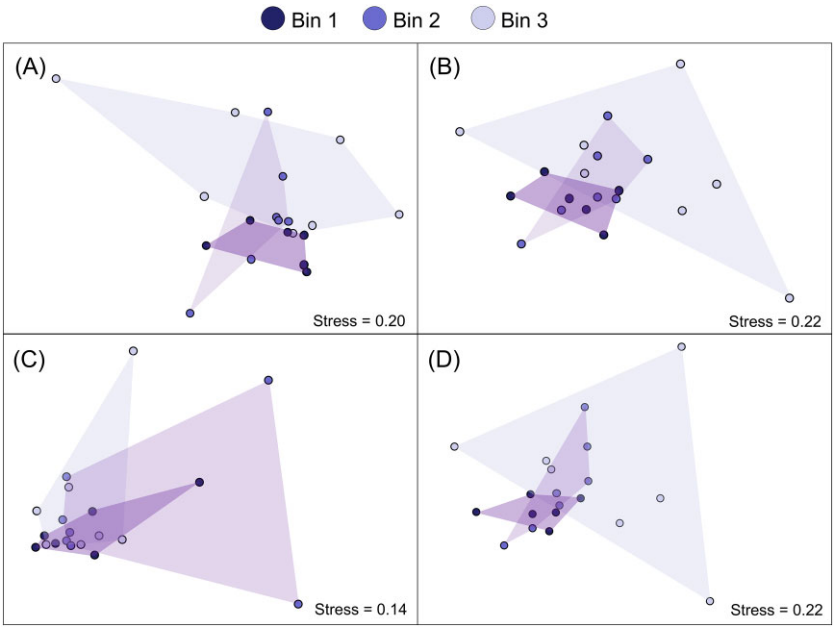

Figure 7. NMDS of gut bacterial communities in stranded *T. truncatus* colored by early (dark purple, bin 1; purple, bin 2) and late (light purple, bin 3) outbreak bins. Ordination is based on (A) Bray–Curtis similarity of relative abundance (RA); (B) Bray–Curtis similarity of presence/absence (PA); (C) Weighted UniFrac **(W)** distances; and (D) Unweighted UniFrac (UW) distances. Significant differences in bacterial community structure (PERMANOVA, $p<0.05$) were detected between early and late periods (bin 1 vs. bin 3 and bin 2 vs. bin 3) for two metrics **(B, D)**. No significant differences were detected between early periods (bins 1 and 2) for any metric.

bacterial communities before, during, and after the disease-driven mortality event suggests that acute DMV infection is not associated with a distinct and consistent gut community signature, thereby limiting the diagnostic potential of structural characterization of gut bacteria for forecasting infection susceptibility of their hosts. Further, temporal delineation of DMV-positive dolphins revealed larger shifts in bacterial diversity in late vs. early outbreak periods, suggesting that long-term morbidity may disrupt gut communities as a secondary impact of the accumulating health burden in chronic infections.

Consistent with previous studies, gut bacterial communities in *T. truncatus* displayed low diversity and were dominated by OTUs affiliated with the phyla Firmicutes, Fusobacteria, and Proteobacteria (Bik et al. 2016, Soverini et al. 2016, Suzuki et al. 2019, Abdelrhman et al. 2020, Robles-Malagamba et al. 2020). Gut bacteria richness in *T. truncatus* was low (<100 OTUs), typical of

odontocetes (with the notable exception of kogiids, Erwin et al. 2017, Denison et al. 2020) and lower than mysticetes (>400 OTUs, Sanders et al. 2015, Erwin et al. 2017). Indeed, a small core community (four OTUs) dominated gut bacterial communities in *T. truncatus* (64.0% relative abundance), consisting of the genera *Cetobacterium*, a bacterium endemic to *Cetaceans* (Staley and Whitman 2010); *Paeniclostridium*, a newly reclassified *Clostridium* genus also found in marine sediment (Sasi Jyothsna et al. 2016); and *Clostridium sensu stricto 1*, a genus frequently associated with gut communities (Schleifer 2009). Notably reduced or absent from gut communities in *T. truncatus* were members of Bacteroidetes, a dominant phylum in the gastrointestinal tract of other *Cetaceans* and terrestrial mammals including humans (Nishida and Ochman 2018).

Our data from stranded *T. truncatus* revealed the same bacterial community trends previously reported from bottlenose dolphins in the wild and under human care (Bik et al. 2016, Robles-Malagamba et al. 2020). Within this broad structural similarity, finer-scale patterns of variability among individuals were noted herein and in previous studies. For example, the most dominant bacterial phylum in the gut community of *T. truncatus* varied among individuals: some were dominated by Firmicutes ($n = 31$), others by Fusobacteria ($n = 28$), and a few animals by Proteobacteria ($n = 4$). Indeed, the relative abundance of each of these dominant phyla ranged from less than 2% to over 85% of gut communities. Previous studies have documented similar intra-specific variation, in some cases ascribing variation to environmental factors like aquaria conditions for animals in human care (Suzuki et al. 2019). The consistent general features and noted plasticity of bacterial communities in *T. truncatus* suggest that gut profiles from stranded animals are representative of healthy individuals and that these communities can change in response to environmental variables.

The present study assessed whether gut bacterial communities changed before, during, and after a virus-induced mortality event and if patterns of variability were associated with infection status and UME period. Remarkably, gut bacterial communities in *T. truncatus* were similar regardless of stranding period or *Morbillivirus* infection status. Broad patterns in gut community composition (phylum level), diversity (richness), and similarity were consistent across the UME and between DMV-positive and DMV-negative dolphins. Finer scale shifts in diversity were detected, namely significantly lower evenness of gut bacterial communities in DMV-positive vs. DMV-negative dolphins. Recent work on Indo-Pacific bottlenose dolphins (*T. aduncus*) also reported changes in gut community evenness (but not richness) when comparing captive and wild animals (Suzuki et al. 2021), suggesting this alpha-diversity metric may be most sensitive to environmental impacts on dolphin bacterial communities. Overall, these results indicate that DMV infection is not associated with large changes in gut bacterial community richness and composition in *T. truncatus*, and future work is required to investigate whether subtle changes in gut communities signal deviation from a healthy host state.

OTU-level analyses revealed fine-scale changes in specific gut bacteria related to the UME and promising biomarker candidates for future microbiome diagnostics. Only 2% of the 968 different OTUs detected in gut bacterial communities in *T. truncatus* differed across host factors, supporting the overall similarity highlighted above. However, several potential biomarkers were identified within the complex gut bacterial communities. Most notably, a bacterium in the genus *Actinobacillus* (OTU0011) was common in dolphins post-UME and those testing negative for DMV, yet rare during the UME and in DMV-positive dolphins. This OTU matched identically to *Actinobacillus delphincola* (strain NCTC12871, NCBI Acc. No. LR134510), a bacterium first isolated and described from three *Cetacean* species (*Phocoena phocoena*, *Mesoploden bidens*, and *Stenella coeruleoalba*; Foster et al. 1996) and later detected as an abundant gut bacterium in free-living *T. truncatus* using DNA-based methods (Bik et al. 2016). The loss of this common gut community member may signal incipient dysbiosis or play a contributory role in morbidity following viral infection, thereby representing a promising target for developing bacterial biomarkers for health monitoring.

Alternatively, shifts in gut bacterial communities may signal dysbiosis *following* infection, an indirect impact of disease-related health declines associated with infection course. Dolphin *Morbillivirus* usually manifests as an acute infection, with sudden symptomatic onset and death of the animal, and as a chronic infection, where the host survives the initial viral infection but ultimately succumbs to secondary causes of death from prolonged host immunosuppression and opportunistic infections (van Bressem et al. 2014, Pfeffermann et al. 2018). In this study, shifts in gut community diversity were found when DMV-positive *T. truncatus* individuals were stratified temporally across the outbreak period. Early outbreak bins (1 and 2) more likely contained animals that died soon after DMV exposure (i.e. acute infections), whereas the later bin (3) included animals with suspected chronic infections. The longer infection periods and slower declines in host health associated with chronic infections may result in a dysbiotic state not seen in acute infections, characterized by higher bacterial diversity and compositional shifts. While this hypothesis requires additional testing and incorporation of histological and gross anatomy data to confirm acute vs. chronic infection stages, future studies accounting for infection course with clinical diagnoses may be key to documenting bacterial community impacts (dysbiosis) in acute and chronic infections.

Notably, acute *Morbillivirus* infection has been associated with gut community dysbiosis in other mammalian species, namely giant pandas (*Ailuropoda melanoleuca*), where animals positive for canine distemper virus (CDV) exhibited an increased inflammatory response and altered bacterial communities compared to healthy controls (Zhao et al. 2017). Specifically, the gut communities of CDV-positive pandas had proportionally more Firmicutes and fewer Proteobacteria, with an increase in overall diversity and community variability, compared to uninfected animals. Two animals were sampled on the date of infection and longitudinally over 18 and 40 days until death, documenting changes to bacterial communities over the course of infection and the corresponding reduction in dominant resident taxa (e.g. *Escherichia* and *Clostridium*, Zhao et al. 2017). Although this type of longitudinal sampling was not possible with the dolphins investigated in this study, similar increases in gut bacteria diversity and community variability were detected in late outbreak DMV-positive *T. truncatus* individuals. However, no corresponding changes at the phylum level were observed, even when separating acute from chronic infections, indicating that the link between *Morbillivirus* infection and gut bacterial community structure differs by host species and virus type.

Other host factors were correlated with differences in gut community structure in *T. truncatus*, including carcass condition and life history stage. Moderately decomposed carcasses (code 3) showed signs of minor bacterial community shifts: community similarity differed between carcass condition extremes, though no corresponding changes in alpha-diversity (richness, evenness) or phylum-level taxonomic composition occurred. These results are

consistent with past studies of bacterial communities in stranded *Cetaceans* (Erwin et al. 2017, Denison et al. 2020) and experimental studies of postmortem shifts in mammals (murine models, Metcalf et al. 2013), where gut bacterial communities remain largely stable until more advanced stages of decomposition. Animals that were stranded in advanced stages of decomposition (codes 4 and 5) were not included in this study. Shifts in gut community similarity and membership were also detected between the earliest life history stage investigated (calves) and later life history stages (subadults and adults) of *T. truncatus*. Gut bacterial communities displayed convergence across host ontogeny, with more similar communities and fewer core OTUs in later life history stages, a trend also observed in kogiid whales (Denison et al. 2020). These changes may reflect dietary differences and transitions across life history stages, from a dependency on a mother's milk to a piscivorous diet (Soverini et al. 2016, Suzuki et al. 2019).

In summary, we show that gut bacterial communities in *T. truncatus* were similar before, during and after a disease-driven mortality event and accordingly have a limited ability to predict infection susceptibility at the community level. However, finer-scale investigations (OTU level) identified individual taxa that correlated with the disease outbreak and host infection status (notably, *Actinobacillus delphincola*), providing promising candidates for future studies targeting the development of bacterial biomarkers for health monitoring. Evidence of indirect impacts from chronic infections warrant further investigation to better understand the interactions between gut bacterial community changes and chronic disease that may precipitate secondary infections and accelerate health declines. Further, these results highlight the host-specific link between *Morbillivirus* infection and gut community structure (Zhao et al. 2017) and encourage the investigation of additional body sites for microbial diagnostics (sensu Apprill et al. 2017). For example, respiratory microbiomes may readily shift as the primary site of DMV infection, and skin bacteria changes may precede characteristic skin lesions associated with DMV infection. Additional investigations of marine mammal microbiomes in the context of disease susceptibility and progression may ultimately help elucidate outcomes of *Morbillivirus* exposure and infection that contribute to recurrent mass mortality events threatening cetacean populations worldwide.

Author contributions

Alyssa R.B. Olmstead (Data curation [supporting], Formal analysis [lead], Investigation [lead], Writing—original draft [lead], Writing—review and editing [supporting]), Olivia L. Mathieson (Data curation [supporting], Formal analysis [supporting], Investigation [supporting], Writing—original draft [supporting], Writing—review and editing [supporting]), William A. McLellan (Conceptualization [lead], Data curation [supporting], Funding acquisition [lead], Investigation [lead], Writing—review and editing [supporting]), D. Ann Pabst (Conceptualization [lead], Data curation [supporting], Funding acquisition [lead], Investigation [lead], Writing—review and editing [supporting]), Tiffany F Keenan (Data curation [lead], Funding acquisition [supporting], Investigation [lead], Writing—review and editing [supporting]), Tracey Goldstein (Formal analysis [lead], Investigation [supporting], Writing—review and editing [supporting]), and Patrick M. Erwin (Conceptualization [lead], Data curation [supporting], Formal analysis [supporting], Funding acquisition [lead], Investigation [supporting], Writing—original draft [supporting], Writing—review and editing [lead])

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Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

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References

Abdelrhman KFA, Ciofini A, Bacci G *et al.* Exploring the resident gut microbiota of stranded odontocetes: high similarities between two dolphin species *Tursiops truncatus* and *Stenella coeruleoalba*. *J Mar Biol Ass* 2020;**100**:1181–8.

Apprill A, Miller CA, Moore MJ *et al.* Extensive core microbiome in drone captured whale blow supports a framework for health monitoring. *Msystems* 2017;**2**:e00119–17.

Apprill A, Miller CA, Van Cise AM *et al.* Marine mammal skin microbiotas are influence by host phylogeny. *R Soc Open Sci* 2020;**7**:192046.

Apprill A, Robbins J, Murat Eren A *et al.* Humpback whale populations share a core skin bacterial community: towards a health index for marine mammals? *PLoS One* 2014;**9**:e90785.

Apprill A. Marine animal microbiomes: toward understanding host-microbiome interactions in a changing ocean. *Front Mar Sci* 2017;**4**:9.

Backhed F, Ley RE, Sonnenburg JL *et al.* Host-bacterial mutualism in the human intestine. *Science* 2005;**307**:1915–20.

Barrett T, Subbarao SM, Belsham GJ *et al.* The molecular biology of the *Morbilliviruses*. In: Kingsbury DW (ed.), *The Paramyxoviruses*. Boston, MA: Springer, 1991, 83–102.

Barrett T, Visser IKG, Mamaev L *et al.* Dolphin and porpoise *Morbilliviruses* are genetically distinct from phocine distemper virus. *Virology* 1993;**193**:1010–2.

Barrett T. *Morbillivirus* infections, with special emphasis on *Morbilliviruses* of carnivores. *Vet Microbiol* 1999;**69**:3–13.

Bello MGD, Knight R, Gilbert JA *et al.* Preserving microbial diversity. *Science* 2018;**362**:33–4.

Bik EM, Costello EK, Switzer AD *et al.* Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nat Commun* 2016;**7**:10516.

Blaser MJ, Kirschner D. The equilibria that allow bacterial persistence in human hosts. *Nature* 2007;**449**:843–9.

Bossart G, Romano T, Peden Adams M *et al.* Clinicoimmunopathologic findings in Atlantic bottlenose dolphins *Tursiops truncatus* with positive *Cetacean Morbillivirus* antibody titers. *Dis Aquat Org* 2011;**97**:103–12.

Bossart G. Marine mammals as sentinel species for oceans and human health. *Vet Pathol* 2011;**48**:676–90.

Calgaro M, Romualdi C, Waldron L *et al.* Assessment of statistical methods from singe cell, bulk RNA-seq, and metagenomics applied to microbiome data. *Genome Biol* 2020;**21**:191.

Caporaso JG, Lauber CL, Walters WA *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *P Natl Acad Sci USA* 2011;**108**:4516–22.

Centelleghe C, Carraro L, Gonzalvo J *et al.* The use of Unmanned Aerial Vehicles (UAVs) to sample the blow microbiome of small *Cetaceans*. *PLoS One* 2020;**15**:14.

Chong J, Liu P, Zhou G *et al.* Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nat Protoc* 2020;**15**:799–821.

Denison ER, Rhodes RG, McLellan WA *et al.* Host phylogeny and life history stage shape the gut microbiome in dwarf (*Kogia sima*) and pygmy (*Kogia breviceps*) sperm whales. *Sci Rep* 2020;**10**:15162.

Derrien M, Alvarez AS, de Vos WM. The gut microbiota in the first decade of life. *Trends Microbiol* 2019;**27**:997–1010.

Di Guardo G, Centelleghe C, Mazzariol S. Cetacean host-pathogen interaction(s): critical knowledge gaps. *Front Immunol* 2018;**9**:3.

Erwin PM, Rhodes RG, Kiser KB *et al.* High diversity and unique composition of gut microbiomes in pygmy (*Kogia breviceps*) and dwarf (*K. sima*) sperm whales. *Sci Rep* 2017;**7**:7205.

Finlayson-Trick ECL, Getz LJ, Slaine PD *et al.* Taxonomic differences of gut microbiomes drive cellulolytic enzymatic potential within hind-gut fermenting mammals. *PLoS One* 2017; **12**:e0189404.

Foster G, Ross HM, Malnick H *et al. Actinobacillus delphinicola* sp. nov., a new member of the family Pasteurellaceae Pohl (1979) 1981 isolated from sea mammals. *Int J Syst Bacteriol* 1996;**46**:648.

Geraci JR, Lounsbury V. *Marine Mammals Ashore: A Field Guide for Strandings*. Galveston: Texas A&M Sea Grant Publications, 1993.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V *et al.* Microbiome datasets are compositional: and this is not optional. *Front Microbiol* 2017;**8**:2224.

Godoy-Vitorino F, Rodriguez-Hilario A, Alves AL *et al.* The microbiome of a striped dolphin (*Stenella coeruleoalba*) stranded in Portugal. *Res Microbiol* 2017;**168**:85–93.

Li C, Tan XX, Bai J *et al.* A survey of the sperm whale (*Physeter catodon*) commensal microbiome. *PeerJ* 2019;**7**:16.

Lipscomb TP, Schulman FY, Moffett D *et al.* Morbilliviral disease in Atlantic bottlenose dolphins (*Tursiops truncatus*) from the 1987–1988 epizootic. *J Wildl Dis* 1994;**30**:567–71.

Mallette SD, McLellan WA, Scharf FS *et al.* Ontogenetic allometry and body composition of the common bottlenose dolphin (*Tursiops truncatus*) from the US mid-Atlantic. *Mar Mam Sci* 2016;**32**:86–121.

Marine Mammal Protection Act. 16 U.S.C. 1361 §§101-509. 1972; https://uscode.house.gov/view.xhtml?path=/prelim@title16/chapter31&edition=prelim (15 September 2022, date last accessed).

McLellan WA, Friedlaender AS, Mead JG *et al.* Analyzing 25 years of bottlenose dolphin (*Tursiops truncatus* Montagu 1821) strandings along the U.S. Atlantic coast: do historic records support the coastal migratory stock hypothesis? *Jcrm* 2002;**4**:297–304.

Metcalf JL, Parfrey LW, Gonzalez A *et al.* A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. *eLife* 2013;**2**:e01104.

Metcalf JL, Song SJ, Morton JT *et al.* Evaluating the impact of domestication and captivity on the horse gut microbiome. *Sci Rep* 2017;**7**:15497.

Moore KT, Barco SG. *Handbook for Recognizing, Evaluating, and Documenting Human Interaction in Stranded Cetaceans and Pinnipeds*. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-510, 2013.

Morris SE, Zelner JL, Fauquier DA *et al.* Partially observed epidemics in wildlife hosts: modelling an outbreak of dolphin *Morbillivirus* in the northwestern Atlantic, June 2013–2014. *J R Soc Interface* 2015;**12**:20150676.

Nelson TM, Apprill A, Mann J *et al.* The marine mammal microbiome: current knowledge and future directions. *Microbiol Aust* 2015;**36**:8–13.

Nishida AH, Ochman H. Rates of gut microbiome divergence in mammals. *Mol Ecol* 2018;**27**:1884–97.

NOAA Active and Closed Unusual Mortality Events. 2022. https://www.fisheries.noaa.gov/national/marine-life-distress/2013-2015-bottlenose-dolphin-unusual-mortality-event-mid-atlantic (15 September 2022, date last accessed).

O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia* and *Morganella*. *Clin Microbiol Rev* 2000;**13**:534.

Pfeffermann K, Dorr M, Zirkel F *et al. Morbillivirus* pathogenesis and virus-host interactions. In: Kielian M, Mettenleiter TC, Roossinck MJ (eds.), *Advances in Virus Research, Vol 100*. San Diego, CA: Elsevier Academic Press, 2018, 75–98.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:D590.

Robles-Malagamba MJ, Walsh MT, Ahasan MS *et al.* Characterization of the bacterial microbiome among free-ranging bottlenose dolphins (*Tursiops truncatus*). *Heliyon* 2020;**6**:10.

Rosel PE, Hansen L, Hohn AA. Restricted dispersal in a continuously distributed marine species: common bottlenose dolphins *Tursiops truncatus* in coastal waters of the western North Atlantic. *Mol Ecol* 2009;**18**:5030–45.

Rowels TK, Schwacke LS, Wells RS *et al.* Evidence of susceptibility to *Morbillivirus* infection in *Cetaceans* from the United States. *Mar Mamm Sci* 2011;**27**:1

Salazar N, Gonzalez S, Nogacka AM *et al.* Microbiome: effects of ageing and diet. *Curr Iss Mol Biol* 2020;**36**:33–61.

Sanders JG, Beichman AC, Roman J *et al.* Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivours. *Nat Commun* 2015;**6**:8285.

Sasi Jyothsna TS, Tushar L, Sasikala C *et al. Paraclostridium benzoelyticum* gen. nov., sp. nov., isolated from marine sediment and reclassification of *Clostridium bifermentans* as *Paraclostridium bifermentans* comb. nov. Proposal of a new genus *paeniclostridium* gen. nov. to accommodate *Clostridium sordelli* and *Clostridium ghonii*. *Int J Syst Evol Microbiol* 2016;**66**:1268–74.

Schleifer KH. Phylum XIII. Firmicutes Gibbons and Murray 1978, 5 (Firmacutes [sic] Gibbons and Murray 1978, 5). In: De Vos P, Gar-

rity GM, Jones D *et al.* (eds.), *Bergey's Manual® of Systematic Bacteriology: volume Three the Firmicutes*. New York: Springer, 2009, 19–1317.

Schloss PD, Wescott SL, Ryabin T *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microb* 2009;**75**:7537–41.

Shreiner AB, Kao JY, Young VB. Gut microbiome in health and disease. *Curr Opin Gastroenterol* 2015;**31**:67–75.

Soares-Castro P, Araújo-Rodrigues H, Godoy-Vitorino F *et al.* Microbiota fingerprints withinthe oral cavity of *Cetaceans* as indicators for population biomonitoring. *Sci Rep* 2019;**9**:13679.

Soverini M, Quercia S, Biancani B *et al.* The bottlenose dolphin (*Tursiops truncatus*) faecal microbiota. *FEMS Microbiol Ecol* 2016;**92**: 8.

Staley JT, Whitman WB. Phylum XIX. Fusobacteria Garrity and Holt 2001, 140. In: Krieg NR, Staley JT, Brown DR *et al.* (eds.), *Bergey's Manual® of Systematic Bacteriology: volume Four the Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes*. New York: Springer, 2010, 747–74.

Suzuki A, Akuzawa K, Kogi K *et al.* Captive environment influences the composition and diversity of fecal microbiota in Indo-Pacific bottlenose dolphins, *Tursiops aduncus*. *Mar Mam Sci* 2021;**37**: 207–19.

Suzuki A, Segawa T, Sawa S *et al.* Comparison of the gut microbiota of captive common bottlenose dolphins *Tursiops truncatus* in three aquaria. *J Appl Microbiol* 2019;**126**:31–9.

Thorsen J, Brejnrod A, Mortensen M *et al.* Large-scale benchmarking reveals false discoveries and count transformation sensitivity in 16S rRNA gene amplicon data analysis methods used in microbiome studies. *Microbiome* 2016;**4**:62.

van Bressem MF, Duignan PJ, Banyard A *et al.* *Cetacean morbillivirus*: current knowledge and future directions. *Viruses* 2014;**6**: 5145–81.

van de Bildt MWG, Kuiken T, Osterhaus A. *Cetacean morbilliviruses* are phylogenetically divergent. *Arch Virol* 2005;**150**:577–83.

von Chamier M, Allam A, Brown MB *et al.* Host genetic background impacts disease outcome during intrauterine infection with *Ureaplasma parvum*. *PLoS One* 2012;**7**:e44047.

Wang JZ, Du WT, Xu YL *et al.* Gut microbiome-based medical methodologies for early-stage disease prevention. *Microb Pathog* 2017;**105**:122–30.

Wells RS, Rhinehart HL, Hansen LJ *et al.* Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth* 2004;**1**:246.

Yatsunenko T, Rey FE, Manary MJ *et al.* Human gut microbiome viewed across age and geography. *Nature* 2012;**486**: 222–7.

Zhao N, Li M, Luo J *et al.* Impacts of canine distemper virus infection on the giant panda population from the perspective of gut microbiota. *Sci Rep* 2017;**7**:39954.

Zheng DP, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020;**30**: 492–506.

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