Distribution of microbiota across different intestinal tract segments of a stranded dwarf minke whale, *Balaenoptera acutorostrata*

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Abstract
Marine mammals are an important part of ocean ecosystems, of which, whales play a vital role in the marine food chain. In this study, the mucosa and contents from different intestinal tract segments (ITSs) of a stranded dwarf minke whale (*Balaenoptera acutorostrata*) were analyzed. The gut microbiota were sequenced using high-throughput sequencing technology, based on a 16S rRNA approach. The microbial composition of the intestinal mucosa and its contents were similar in every single ITS. Large intestine microbiota richness and diversity were significantly higher when compared to the duodenum and jejunum. The dominant bacteria in the gut were *Firmicutes* and *Actinobacteria*; the former was enriched in the large intestine, whereas the latter was more abundant in the duodenum and jejunum. Our findings provide novel insights for microbiota in *B. acutorostrata*.

KEYWORDS
dwarf minke whale *Balaenoptera acutorostrata*, gut microbiota, high-throughput sequencing, intestinal tract segment

1 | INTRODUCTION

Animal digestive tracts are complex microecosystems composed of host cells and intestinal microbiota (Backhed, 2005). Their symbiotic relationship not only influences host metabolism, energy production, growth, and immunity but also underlies several pathological conditions (Flint, Scott, Louis, & Duncan, 2012; Malmuthuge, Li, Goonewardene, Oba, & Guan, 2013). Marine mammals are important microbial reservoirs thanks to their constant body temperature and large sizes (Higgins, 2000), and in recent years, the commensal microbiota of various marine mammals have been elucidated (Chiarello, Villéger, Bouvier, Auguet, & Bouvier, 2017; Numberger, Herlemann, Jürgens, Dehnhardt, & Schulz-Vogt, 2016). Monitoring the intestinal microbiota of marine mammals can help unravel adaptive mechanisms to different diets or habitats. For example, a metagenomic study reported high nutrient transport and cycling potential in the gut microbiota of the Australian sea lion, *Neophoca cinerea* (Lavery, Roudnew, Seymour, Mitchell, & Jeffries, 2012). Moreover, the gut microbiota of whales and terrestrial herbivores exhibit similar fermentative metabolism, while protein catabolic and amino acid synthesis pathways in the whale gut closely resemble terrestrial carnivores (Sanders et al., 2015).

The dwarf minke whale, *Balaenoptera acutorostrata*, is the most abundant species of baleen whale and is widely distributed in the North Atlantic and North Pacific regions (Walquist, Stormo, Jensen,
Bjarne, & Eilertsen, 2017). It forages on fish such as capelin, herring, mackerel, and codfish, as well as crustaceans, such as krill (Jonsgård, 1982; Windsland, Lindstrom, Nilssen, & Haug, 2007), suggesting the whale's gut microbiota are adaptive to diverse food sources. Due to its size, *B. acutorostrata* has a long digestive tract, suggesting a varied intestinal microbiota composition from different intestinal tract segments (ITSs). However, these compositions are currently unknown. Therefore, intestinal microbiota comparisons between different ITSs of *B. acutorostrata* could provide novel insights into food digestion and metabolism.

A freshly dead *B. acutorostrata* was found on a beach in Dalian, China, and its gut mucosa and contents were subsequently obtained. We analyzed microbiota from different ITSs using high-throughput sequencing technology based on 16S rRNA gene analysis. To the best of our knowledge, this is the first study to analyze microbiota of different ITSs from *B. acutorostrata*.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

The minke whale was found on a beach on ChangXing Island, Dalian, China (122°45′36.932″E, 39°1′24.872″N). As soon as local fishermen encountered the whale (which was still alive), they immediately contacted local authorities. By the time this personnel arrived, the whale had been dead for an hour. Thus, the stranded individual was considered a fresh stranding. Within two hours, it was transported to the laboratory (temperature = 10°C) in a sterilized plastic bag. The animal weighed 3350.0 kg and was dissected at the Anatomy Laboratory of Liaoning Ocean and Fisheries Research Institute. According to the clinical report of an attending veterinarian (Qualification Certificate No. C012015210021), there was no damage to the body surface and no pathological changes were observed, suggesting the animal was disease-free. Species identification and dissection were approved by the Ministry of Agriculture and Rural Affairs of the People’s Republic of China (No. 1376). The carcass was identified as a dwarf minke whale (*B. acutorostrata*). This identification was based on 256 pairs of yellow or milky white baleen in its mouth, a white transverse band on the lateral central part of the fin limb, and a distinct ridge on the back of the head. The body was dissected, and intestinal segments were harvested. Three samples of duodenum, jejunum, cecum, colon, and rectum contents and mucosa were collected and stored at −80°C (Figure 1).

### 2.2 | DNA extraction

Bacterial genomic DNA was extracted from 30 samples using the TIANamp Stool DNA Kit (Tiangen, China) according to the manufacturer's instructions. DNA was verified by electrophoresis on 1.5% agarose gels. DNA concentration and purity were measured on the Qubit™ 4 Fluorometer (Invitrogen, USA) and stored at −20°C until required.

### 2.3 | 16S rRNA gene amplification and sequencing

V3-V4 regions of 16S rRNA were amplified using the 341F (5′-CCTACGGGRBGCASCAGKVRVGAAT-3′) and 806R (5′-GGACTACNVGGGTWTCTAATCC-3′) primers. All PCR reactions were performed in a 25 µl master mix, consisting of 2.5 µl TransStart Buffer, 2 µl dNTPs (2.5 mM), 0.5 µl TransStartTaq DNA polymerase (TransGene, China), 1 µl each of forward and reverse primers (100 µM), and 20 ng DNA template. Cycling parameters included an initial denaturation at 94°C for 3 min, followed by 24 denaturation cycles at 94°C for 5 s, annealing at 57°C for 90 s, and elongation at 72°C for 10 s. PCR products were purified and quantified, and amplicon libraries were generated. Libraries were sequenced on an Illumina Miseq platform using a 250 base pair (bp) paired-end strategy.

### 2.4 | Processing sequencing data

All read sequences were analyzed by Bcl2fastq v 2.17.1.14. Lower quality reads were removed, based on average Phred scores <20, ambiguous bases, homopolymer runs >8, primer mismatches, and sequence lengths <200 bp. Paired-end reads with >20 bp overlaps, and without mismatches were assembled into tags using FLASH (Magoc & Salzberg, 2011). These tags were annotated to samples.
based on their unique barcodes, and then, the barcode and primer sequences were removed. Chimeric sequences were recognized and eliminated by the QIIME software package (Caporaso et al., 2010), and tags with ≥97% similarity were assigned to the same operational taxonomic unit (OTU) using VSEARCH v 1.9.6 (Edgar, 2010). Representative sequences of each OTU were chosen randomly and assigned taxonomy using the Ribosomal Database Project classifier (Wang, Garrity, Tiedje, & Cole, 2007) based on the SILVA database (Yilmaz et al., 2014). Finally, the relative abundances of different OTUs were calculated and normalized to the sample, with the least number of tags (Table S1).

### 2.5 Statistical analysis

Chao1 and Shannon indices representing alpha diversities were calculated, and rarefaction curves created using QIIME (Kemp & Aller, 2004). Indices from different ITSs were compared using Tukey’s HSD tests. The unweighted pair-group method, with arithmetic means (UPGMA) clustering, was used to analyze microbial composition similarities across the different ITSs. Species abundance at the phylum level was visualized using Circos software (Krzywinski et al., 2009).

### 3 RESULTS AND DISCUSSION

#### 3.1 Gut microbiota diversity

We sequenced 30 samples from different ITSs of *B. acutorostrata* and generated an average of 70,288 tags per sample. Rarefaction curves for each sample were close to a straight line (Figure 2a), indicating that sequencing data represented the entire microbial community. These tags were clustered into 172 OTUs at the 97% similarity level, and each OTU was annotated from phylum to species level.

We used alpha-diversity indices to analyze the gut microbiota of different ITSs. The Chao1 index was used to evaluate species richness, that is, the number of species in microbial communities (Colwell & Levin, 2009). As shown (Figure 2b), the microbial richness of intestinal mucosa and contents form the same ITSs were similar, except the duodenum. Gut microbiota richness ranged from 70 to 130, which was similar (Chao1 = 84) to microbial communities from beluga whale feces (Sanders et al., 2015). In contrast, gut microbiota richness from smaller cetaceans, such as bottlenose dolphins (Chao1 = 41) and Yangtze finless porpoises (Chao1 = 30), were lower (McLaughlin, Chen, Zheng, Zhao, & Wang, 2012). Furthermore, >500 OTUs were detected in the gut microbiomes from humpback and right whales (Sanders et al., 2015), which are larger than *B. acutorostrata*. Therefore, cetacean gut microbiota richness may be proportional to body size, although more evidence is required to validate this observation.

The Shannon index estimates microbial community diversity in terms of species richness and evenness (Moreno & Rodríguez, 2010). Unlike the Chao1 index, the microbial diversity of cecal and rectal mucosa and contents, were significantly different in each segment (Figure 2c, p < 0.05). Also, microbial richness and diversity in the large intestine (cecum, colon, and rectum) were significantly higher than the duodenum and jejunum (Figure 2b and c, p < 0.05). Due to the comparative whale studies are not available, we used other
animals as comparators. The result of this study was consistent with higher Shannon indices of the colon versus duodenum/jejunum microbiota reported in felines (Ritchie, Steiner, & Suchodolski, 2008). Furthermore, molecular fingerprinting technology revealed that bacterial diversity in the canine colon and rectum was significantly higher than the duodenum and jejunum (Suchodolski, Ruaux, Steiner, Fetz, & Williams, 2005). Moreover, bacterial communities in the midgut and hindgut of sea cucumbers had a significantly higher richness and diversity when compared with the foregut (Wang et al., 2018). Taken together, the posterior compartment of the intestinal tract could be more suitable for microbial colonization across diverse animal species.

3.2 Gut microbiota composition

Balaenoptera acutorostrata gut microbiota divergence was demonstrated by UPGMA clustering analysis, based on Bray–Curtis distances (Figure 3). The microbiota of mucosa and the contents of the same ITS were clustered together, indicating that attached and free bacteria across the B. acutorostrata gut were consistent. Furthermore, samples from the cecum, colon, and rectum formed a cluster, and the duodenum and jejunum microbiota were clustered, distinct to the large intestine. Thus, it appeared that gut microbiome composition in B. acutorostrata was significantly dependent on anatomical location.

The dominant phyla were Firmicutes and Actinobacteria, accounting for >90% of the total microbiome (Figure 4a). Firmicutes were the predominant group and comprised over 50% of the resident bacteria of the duodenum and jejunum and almost 90% of the large intestine microbiota. Also, Firmicutes primarily included the Clostridiales, Erysipelotrichales, and Selenomonadales orders (Figure 4b). The relative abundance of Clostridiales in cecum, colon, and rectum was significantly higher than the duodenum and jejunum. Clostridiales species ferment dietary fiber in the gut to generate short-chain fatty acids (SCFAs) (Chinda et al., 2004). Erysipelotrichales was significantly enriched in the jejunum, when compared to other ITSs, and is abundant in canine fecal microbiota when fed carbohydrate-rich diets (Hang et al., 2012). Erysipelotrichales is associated with chronic canine enteropathies due to its recognition by Toll-like receptors (Allenspach et al., 2010). Selenomonadales was only detected in the duodenum and jejunum. Thai children on high-fat diets exhibit a greater abundance of

![Figure 3](image-url) The unweighted pair-group method with arithmetic means (UPGMA) clustering analysis of gut microbiota composition in different ITSs from Balaenoptera acutorostrata. The names of each sample are duodenum (Du), jejunum (Je), cecum (Ce), colon (Co), and rectum (Re) contents (C) or mucosa (M) with the serial number of 1 to 3.

![Figure 4](image-url) Bacterial distribution at phylum (a) and order (b) levels among different samples. The names of each sample are duodenum (Du), jejunum (Je), cecum (Ce), colon (Co), and rectum (Re) contents (C) or mucosa (M) with the serial number of 1 to 3.
Selenomonadales in their gut when compared to children on largely vegetable-based diets (Kisuse et al., 2018). The Actinobacteria phylum was more abundant in the duodenum (46.61%) and jejunum (30.86%), when compared to other ITSs (6.19% –12.49%), and consisted almost entirely of the Coriobacterales order (Figure 4b). This latter order is saccharolytic and metabolizes a wide range of carbohydrates to lactate and other metabolites (Gupta, Anish, & Bijendra, 2017). Taken together, we observed distinct and varied bacterial compositions in different ITSs of B. acutorostrata, which could reflect different metabolic responses to different food types.

4 | CONCLUSIONS

In this study, the gut microbial composition of different ITSs from a minke whale was sequenced and compared. Significant differences were observed in gut microbiota composition across different ITSs; the microorganisms of posterior compartments were more diverse than the foregut. The duodenum and jejunum were enriched for Erysipelotrichales, Selenomonadales, and Coriobacterales, whereas Clostridiales were more abundant in the large intestine.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Jiashen Tian: Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Methodology (equal); Writing-original draft (equal). Jing Du: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal). Zhichuang Lu: Formal analysis (equal); Software (equal); Writing-review & editing (equal). Jiabo Han: Conceptualization (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). Zhen Wang: Investigation (equal); Methodology (equal); Writing-original draft (equal). Duohui Li: Formal analysis (equal); Project administration (equal). Xiaoyan Guan: Formal analysis (equal); Project administration (equal). Zhaohui Wang: Investigation (equal).

ETHICS STATEMENT

Species identification and dissection were approved by the Ministry of Agriculture and Rural Affairs of the People’s Republic of China (permit number: 1376). This study was conducted under a permit issued by the Liaoning Fisheries Administration Bureau, Liaoning Province, China (approval number: LSYXFZ20111105).

DATA AVAILABILITY STATEMENT

Raw sequence reads were uploaded to the National Center for Biotechnology Information’s Sequence Read Archive under the accession number PRJNA600130: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA600130

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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