Mapping the Green-Lipped Mussel (*Perna canaliculus*) Microbiome: A Multi-Tissue Analysis of Bacterial and Fungal Diversity

Siming Li1 · Tim Young1,2 · Stephen Archer3 · Kevin Lee3 · Shaneel Sharma1 · Andrea C. Alfaro1

Received: 10 August 2021 / Accepted: 28 December 2021
© The Author(s) 2022

Abstract
Poor health and mortality events of the commercially important and endemic New Zealand green-lipped mussel (*Perna canaliculus*) pose a threat to its industry. Despite the known importance of microbiomes to animal health and environmental resilience, the host-associated microbiome is unexplored in this species. We conducted the first baseline characterization of bacteria and fungi within key host tissues (gills, haemolymph, digestive gland, and stomach) using high-throughput amplicon sequencing of 16S rRNA gene and ITS1 region for bacteria and fungi, respectively. Tissue types displayed distinctive bacterial profiles, consistent among individuals, that were dominated by phyla which reflect (1) a fluid exchange between the circulatory system (gills and haemolymph) and surrounding aqueous environment and (2) a highly diverse digestive system (digestive gland and stomach) microbiota. **Gammaproteobacteria** and **Campylobacterota** were mostly identified in the gill tissue and haemolymph, and were also found in high abundance in seawater. Digestive gland and stomach tissues were dominated by common gut bacterial phyla, such as **Firmicutes**, **Cyanobacteria**, **Proteobacteria**, and **Bacteroidota**, which reflects the selectivity of the digestive system and food-based influences. Other major notable taxa included the family **Spirochaetaceae**, and genera **Endozoicomonas**, **Psychrilyobacter**, **Moritella** and **Poseidonibacter**, which were highly variable among tissue types and samples. More than 50% of fungal amplicon sequence variants (ASVs) were unclassified beyond the phylum level, which reflects the lack of studies with marine fungi. However, the majority of those identified were assigned to the phylum **Ascomycota**. The findings from this work provide the first insight into healthy tissue microbiomes of *P. canaliculus* and is of central importance to understanding the effect of environmental changes on farmed mussels at the microbial level.

Introduction
The New Zealand green-lipped mussel (*Perna canaliculus*) is an endemic bivalve commonly found within intertidal and subtidal coastal habitats. Mussel beds provide important ecological functions, such as removing suspended sediment and particulate organic material, resulting in improved water quality. *P. canaliculus* is also a highly valued species for the New Zealand’s growing aquaculture industry, which supports a mussel sector worth over NZ$300 million in export revenues [1].

Given their ecological and economic importance, monitoring health of wild mussels and maintaining the health of domesticated stocks is of utmost importance. While infections from pathogenic microbes may lead to deleterious outcomes, host-microbe interactions are also thought to play a key role in maintaining mussel health and organ-level functioning. Filter-feeding marine mussels are in continuous and direct contact with a dynamically shifting microbial environment [2, 3]. Indeed, the high filter feeding capability of marine mussels allows them to filter large volumes of seawater, while capturing different types of particulate water-borne pollutants as well as microorganisms [4, 5]. Growing evidence suggests that microbes can offer their host organisms probiotic functions, such as enhanced pathogen defence, immunological regulation, and improved digestion efficiency and nutrient uptake, among other factors.
Thus, characterizing the microbial structures of host compartments can inform the underlying functionalities of host-microbiota interactions and associations.

Although a previous report of microbiota characterizations of marine mussels (*Mytilus galloprovincialis*) revealed that different tissues harbour unique microbial communities which serve specific functional purposes [9], the functions of microbial communities in the gut, stomach, and digestive gland tissues are less explored among the diverse range of marine molluscs. Additionally, there have been no microbiota characterization studies for the endemic New Zealand green-lipped mussel *P. canaliculus*. The aim of this study was to profile the microbiota associated with different tissues of farmed green-lipped mussels using high-throughput sequencing. The main objectives were to: (1) profile marine bacteria and fungi in different mussel tissues and the surrounding seawater, (2) describe microbiome variability among individual samples, (3) determine bacterial and fungal community similarity/dissimilarity among the different tissue types, and 4) Identify key dominant host-associated taxa across the tissue types.

### Material and Methods

#### Sample Collection

Five healthy adult mussels (length = 95.8 mm ± 6.6; weight = 66.3 g ± 9.9) and a sample of seawater (1 L) were collected in September 2020 (autumn) from a mussel farm located in Kaiaua, Firth of Thames, New Zealand (GPS coordinate: −37.0610, 175.3002). Mussels were cleaned and washed externally with fresh filtered seawater to remove biofouling. Haemolymph was extracted from the abductor muscle using sterile disposable 1 mL syringes and transferred to sterile 2 mL cryovials (BioStor™) containing 20 µL RNA stabiliser (Qiagen, Germany). The digestive gland, stomach and gill tissues were dissected and the samples were placed in 2 mL cryovials with RNA stabiliser (200 µL), then immediately snap-frozen in liquid nitrogen and stored at −80 °C until further analyses. Sub- aliquots of seawater were filtered through single use 25 mm diameter Whatman filters with 0.2 µm pore size (Cytiva, USA) using 20 mL syringes flooded with RNA stabiliser. Filters were sealed in parafilm and stored at 4 °C for 2 weeks before DNA extractions.

#### Microbial DNA Extraction

Total microbial DNA was extracted from tissue samples (each 20–30 mg) and haemolymph (200 µL) using the DNeasy PowerSoil kit (Qiagen, Germany) according to the manufacturer's instructions and the adapted protocol of Musella et al. (2020). Tissues were lysed using a FastPrep system (MP Biomedicals; Irvine, California) at six movements per second for one minute prior to extraction. The elution step from the DNeasy PowerSoil kit was repeated twice with 50 µL Tris elution buffer, incubating the columns for five minutes at room temperature before centrifugation. DNA samples were stored at −20 °C before subsequent processing. To extract microbial DNA from seawater filters, samples were flooded with 1 mL of extraction buffer 1, incubated at 60 °C for 30 min. Then, the fluid was pushed into a clean 2 mL bead tube for processing with the DNeasy PowerSoil kit according to the manufacturer’s instructions. Multiple tubes of seawater were pooled at the column stage.

### PCR Amplicon and Sequencing

Purified DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen; USA). MiSeq (Illumina, USA) libraries were prepared as per manufacturer’s protocol (16S Metagenomic Sequencing Library Preparation; Part # 15044223; Rev. B [Illumina; San Diego, CA, USA]) and as previously described (Archer et al. 2020). PCR was conducted with primer sets targeting the V3-V4 regions of the bacterial 16S rRNA gene: PCR1 forward (5’ CTCAG GGNGGCWGCAG 3’) and PCR1 reverse (5’ GACTAC HVGGGTATCTAATCC 3’) and the internal transcribed spacer region (ITS) between the fungal 18S and 5.8S rRNA genes: ITS1 forward (5’-CTTGGTCATTTAGAGGA GTAA-3’) and ITS2 reverse (5’ GCTGCGTTCCTTCATC GATGC 3’).

### Bioinformatics and Statistical Analysis

Data were pre-processed using our established workflow [10]. Briefly, 16S rRNA gene and fungal ITS1 amplicons were processed using the R package DADA2 v1.8 [11] and cutadapt v3.4 [12] to remove forward (CCTACGCGCGCC WGCAG) and reverse (GACTACHVGGGTATCTAATCC) primer sequences for 16S rRNA gene, and forward (CTT GGTATTAGAGGAA GTAAGC) and reverse (CTTGTG CATTATTAGAGGA GTGAA) primer sequences for fungal ITS1 region. High quality bacterial reads (forward base reads < 230 and reverse base reads < 220 were trimmed and removed) were then clustered into amplicon sequence variants (ASVs) which were assigned taxonomic ranks using SILVA nr v132 database [13]. Fungal reads were taxonomically classified using the UNITE v7.2 database [14]. R v3.5.2 [15] and the R packages MicrobiomeAnalyst [16], phyloseq [17], and ggplot2 [18] were used for downstream statistical analysis and data visualisation (i.e., relative bacterial/fungal abundances, ordination [principal coordinates analysis; Bray–Curtis], hierarchical cluster analysis
Mapping the Green-Lipped Mussel (Perna canaliculus) Microbiome: A Multi-Tissue Analysis…

Results & Discussion

Bacterial communities were distinct by tissue type (PERMANOVA; $F$ value = 6.1784; $R^2 = 0.59246$; $P$ value < 0.001) (Fig. 1a, b) with the exception of stomach and digestive gland tissues that were highly similar to one another, but distinct from the gill tissues and haemolymph. The seawater samples were also clustered closely with the gill tissue and haemolymph, indicating similar bacterial communities. The gills of bivalves perform respiratory, excretory, and feeding functions, which require them to interact directly with seawater. Therefore, our results suggest that close contact between gills and haemolymph allows waterborne microbiota from the external environment to be transferred to the haemolymph via the gills as has been shown previously [19]. Higher species richness were observed in digestive gland and stomach tissues compared to seawater, haemolymph and gill tissues ($P$ value: 0.017667; [ANOVA] $F$ value: 4.034) (Fig. 2b). This is most likely reflective of the difference in functions and selection of a host-associated microbiota of symbionts with nutrition-related roles [20, 21]. These findings indicate a highly selective host recruitment of the mussel microbiome which aligns with previous studies in fish; gill microbial communities tend to be more associated with interactions and communication processes involving the circulatory system, signal transduction, and cell motility, whereas gut microbiota are associated with metabolism and genetic information processing [22].

Profiling the microbiome of P. canaliculus at multiple taxonomic levels revealed distinct bacterial community structures (Fig. 2a) (for family, class, order, genus levels refer to Supplementary Fig. S1–S5; for significance values of the phyla refer to Supplementary Table 1). The dominance of Proteobacteria ($P$ value = $1 \times 10^{-3}$; $q$ value = $2 \times 10^{-3}$), specifically gamma, and Campylobacterota ($P$ value = $1.12 \times 10^{-5}$; $q$ value = $7.06 \times 10^{-5}$) (a new phylum that contains Epsilonproteobacteria based on Genome Taxonomy Database) in the gill tissues and haemolymph are consistent with prior findings in other mussel species [23, 24], oysters [25], and abalone [26]. These findings suggest that host-associated bacterial community in distantly related marine molluscs may be more tightly linked with general tissue types, as a potential consequence of organ-level function and/or environmental interaction. Proteobacteria have been found to dominate fish gill tissues where they are thought to play crucial roles in supporting the mucosa’s microbial barrier, and, with many being opportunistic pathogens, they may even contribute to the development and maintenance of the host immune system through stimulatory mechanisms [27, 28]. However, their functional roles, if any, are yet to be established in mussel gill mucosa.

In regard to the bacterial profile of digestive gland and stomach tissues, the high abundances of anaerobic phyla Firmicutes ($P$ value = $1 \times 10^{-3}$; $q$ value = $6 \times 10^{-4}$) and Bacteroidota ($P$ value = 0.02; $q$ value = 0.03), as well as Cyanobacteria ($P$ value = $3 \times 10^{-4}$; $q$ value = $1 \times 10^{-3}$) were to be expected. Microbes within the phylum Firmicutes can produce short-chain fatty acids from complex polysaccharides, which provide nutrition for the intestinal mucosal cells [29, 30]. High levels of Firmicutes may also contribute to the maintenance of the normal function of the intestinal mucosa.
and the regulation of the intestinal microbial environment \cite{29, 31}. Bacteroidetes participate in carbohydrate transport and protein metabolism, which are involved in digestive processes \cite{32}. Interestingly, Bacteroidota/Firmicutes ratios have been extensively researched as markers for gut health and dysbiosis in humans and mice \cite{33–36}, however, their significance in mussel gut functioning is yet to be explored. The high abundances of Cyanobacteria in digestive gland and stomach tissues in the present study are most likely derived from the environment (ingested food and seawater). However, the absence of this group of bacteria in the gills and haemolymph could be due to said tissues’ undesirable conditions for proliferation of this type of bacteria.

In contrast to bacterial communities, the fungal profiles in this study were more ambiguous due to the large amounts of unmatched/unidentified ASVs. Furthermore, the relative

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Microbiome profiling of \textit{P. canaliculus}: a Bacterial relative abundances at the phylum level in different tissue types and seawater; b Bacterial alpha diversity; c Fungal alpha diversity; d Fungal relative abundances at the phylum level in different tissue types and seawater.}
\end{figure}
abundances of identified fungi did not reveal any specific patterns or tissue-specific associations (Supplementary information S6–S10). Identified fungal phyla were almost entirely dominated by Ascomycota, except for seawater, which contained a large proportion of unidentified phyla. The alpha diversity for fungal species revealed a slightly higher diversity in the digestive gland, and lower diversity in the gills compared to stomach, seawater and haemolymph (Fig. 2c). The lack of clear trends in the distribution of fungi found in this study is not surprising given the stochastic nature of fungal dispersion [37, 38] and the lack of studies on marine fungi [39].

Finally, to describe microbiome variability among individuals, and to identify key dominant host-associated taxa across tissue types, the top 20 bacterial genera were ranked from highest to lowest in terms of abundances across all samples (Fig. 3). The results visualized via a heatmap revealed that bacterial genera, such as an unclassified genera of families Spirochaetaceae, Moritella and Poseidonibacter were more abundant in gill tissue, haemolymph and/or seawater. Bacteria, such as Mycoplasma, Synechococcus and Psychrilyobacter were elevated in digestive gland and stomach tissues. Interestingly, high relative abundance of Vibrio spp. was observed across seawater and all tissue types. The presence of Vibrio spp. is to be expected as they are ubiquitous in marine and estuarine environments, and on surfaces and intestinal contents of marine animals [40]. Although many Vibrio species are harmless, several can be highly pathogenic for humans and/or marine animals [41–44]. Warm temperature favours the proliferation of Vibrio spp. and has contributed to mass mortalities in shellfish farms [45, 46]. Higher abundances of Moritella and Poseidonibacter in the gill tissues were expected because these bacteria are of marine origin [47–49].

Higher abundances of Mycoplasma in the stomach were not surprising as they are common members of the intestinal bacterial flora of many marine species (e.g., fish, abalone) where they may provide nutrients to their hosts [50–52]. Cyanobacterium Synechococcus are one of the most important components of photosynthetic picoplankton [53–55], and their presence in digestive samples of P. canaliculus represents their dietary origin. Psychrilyobacter is a marine member of the phylum Fusobacteria. This genus is an obligate anaerobic halophile that is able to grow well in low temperatures, and it has been recently isolated and described from marine sediments and marine animals.
Interestingly, the most abundant genus identified (Endozoicomonas) across samples was elevated in gill and stomach tissues. A study using comparative analysis revealed that Endozoicomonas species are likely to participate in nutritional symbiosis and their genomes may be enriched for transport and secretion processes, such as transfer of carbohydrates, amino acids, and proteins between the symbiont and host [59]. In addition, Endozoicomonas species seem to have symbiotic relationships with the host by producing antimicrobial substances to deter potential invading microbes [60]. Previous reports have also shown that Endozoicomonas dominates the gut of M. galloprovincialis in response to thermal stress (27 °C), suggesting that the microbes from this genus play a crucial role in maintaining health [61]. Contrary to these reports, the presence of Endozoicomonas has been associated with mortalities of shellfishes, such as green-lipped mussels, clams, and scallops in New Zealand [62], and infecting the gill tissues of king scallop [63]. The identification of major microbial genera in P. canaliculus microbiomes demonstrates key associations and similarities with other marine organisms. These taxa also represent targets for future microbial-host interaction research in P. canaliculus for the potential development of host health biomarkers.

Conclusions

Marine bacteria and fungi were profiled in different tissues of P. canaliculus and surrounding seawater. Distinct compositional patterns of microbes were identified at various taxonomic levels. Seawater, gills, and haemolymph contained Proteobacterial groups, while digestive gland and stomach tissues were dominated by common anaerobic gut microbes involved in fatty acid synthesis, carbohydrate digestion and gut maintenance. Fungal profiles in all samples were dominated by taxa within the phylum Ascomycota, but could not be identified beyond this taxonomic level. This study also highlights the open association between the circulatory physiology (gills and haemolymph) of mussels and surrounding seawater, and the high selectivity of microbiomes in the digestive system (digestive gland and gut). Furthermore, by comparing individual sample variability, we identified key genera of interest, such as Endozoicomonas, which could potentially be used as markers for mussel health in the future. Our study represents the first detailed characterization of microbiome profiles of P. canaliculus within different tissues, hence providing a baseline for future physiological and health studies of this important aquaculture species.

Acknowledgements

We would like to thank Kaiaua Mussel Farms for providing the mussels for this research and for their ongoing support in the field. We also thank Leonie Venter and Awanis Azizan from the Aquaculture Biotechnology Research Group, Auckland University of Technology (AUT) for their assistance with sampling.

Author Contributions

SL: formal analysis, conceptualization, writing—original draft preparation, data curation. TY, SA: conceptualization, methodology, writing—review & editing. KL: software, data curation, writing—review & editing. SS: resources. ACA: supervision, project administration, funding acquisition, conceptualization, writing—review & editing, resources.

Funding

Open Access funding enabled and organized by CAUL and its Member Institutions. This project was funded by the New Zealand Ministry of Business, Innovation and Employment (MBIE; CAWX1707) under a collaboration between AUT and Cawthron Institute (Nelson, New Zealand).

Data Availability

All sequence data are published in NCBI BioProject (Accession: PRJNA788989). Raw data and outputs may also be available upon request.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Informed Consent

Not applicable.

Consent for Publication

Not applicable.

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

1. Aquaculture New Zealand (2020) New Zealand aquaculture a sector overview with key facts and statistics. https://drive.google.com/file/d/1yAInzMbUPyuvfXxtD80aoLXydl5Vic/view
2. Pita L, Rix L, Slaby BM, Franke A, Hentschel U (2018) The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome. https://doi.org/10.1186/s40168-018-0428-1
3. Glasl B, Herndl GJ, Frade PR (2016) The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J. https://doi.org/10.1038/ismej.2016.9
4. Pagano M et al (2016) Evaluation of functionality and biological responses of mytilus galloprovincialis after exposure to quaternium-15 (methenamine 3-chloroallylochloride). Molecules. https://doi.org/10.3390/molecules21020144
5. Neori A et al (2004) Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture. https://doi.org/10.1016/j.aquaculture.2003.11.015
6. O’Brien PA, Webster NS, Miller DJ, Bourne DG (2019) Host-microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts. mBio. https://doi.org/10.1128/MBio.02241-18
7. Rausch P et al (2019) Comparative analysis of amplicon and metatagene sequencing methods reveals key features in the evolution of animal metagenomics. Microbiome. https://doi.org/10.1186/s40168-019-0743-1
8. Simon JC, Marchesi JR, Mougell C, Selosse MA (2019) Host-microbiota interactions: from holobiont theory to analysis. Microbiome. https://doi.org/10.1186/s40168-019-0619-4
9. Musella M et al (2020) Tissue-scale microbiota of the Mediterranean mussel (*Mytilus galloprovincialis*) and its relationship with the environment. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137209
10. Archer SDJ et al (2020) Air mass source determines airborne microbial diversity at the ocean–atmosphere interface of the Great Barrier Reef marine ecosystem. ISME J. https://doi.org/10.1038/s41396-019-0555-0
11. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. https://doi.org/10.1038/nmeth.3869
12. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10. https://doi.org/10.14806/ej.17.1.200
13. Quast C et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. https://doi.org/10.1093/nar/gks1219
14. Nilsson RH et al (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. https://doi.org/10.1093/nar/gky1022
15. R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
16. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J (2017) MicrobiomeAnalyzer: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Res. https://doi.org/10.1093/nar/gkx295
17. McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. https://doi.org/10.1371/journal.pone.0061217
18. Wickham H (2011) ggplot2. Wiley Interdiscip Rev Comput Stat. https://doi.org/10.1002/wics.147
19. Brito TL et al (2018) The gill-associated microbiome is the main source of wood plant polysaccharide hydrolases and secondary metabolite gene clusters in the mangrove shipworm *Neoteredo reynesi*. PLoS ONE. https://doi.org/10.1371/journal.pone.0200437
20. Ikuta T et al (2019) Identification of cells expressing two peptidoglycan recognition proteins in the gill of the vent mussel, *Bathy Tellinidae, September.* Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2019.08.022
21. Burgos-Aceves MA, Faggio C (2017) An approach to the study of the immunity functions of bivalve haemocytes: physiology and molecular aspects. Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2017.06.042
22. Kuang T, He A, Lin Y, Huang X, Liu L, Zhou L (2020) Comparative analysis of microbial communities associated with the gill, gut, and habitat of two filter-feeding fish. Aquac Rep. https://doi.org/10.1016/j.aqrep.2020.100501
23. Li YF et al (2018) Elevated seawater temperatures decrease microbial diversity in the gut of *Mytilus coruscus*. Front Physiol. https://doi.org/10.3389/fphys.2018.00839
24. Li YF et al (2019) Temperature elevation and Vibrio cyclitrophicus infection reduce the diversity of haemolymph microbiome of the mussel *Mytilus coruscus*. Sci Rep. https://doi.org/10.1038/s41598-019-52752-y
25. Lokmer A, Wegner KM (2015) Hemolymph microbiome of Pacific oysters in response to temperature, temperature stress and infection. ISME J. https://doi.org/10.1038/ismej.2014.160
26. Mizutani Y, Mori T, Miyazaki T, Fukuizaki S, Tanaka R (2020) Microbial community analysis in the gills of abalone suggested possible dominance of epibiontproteobacterium in *Haliotis gigantea*. PeerJ. https://doi.org/10.7717/peerj.9326
27. Wu Z et al (2021) Taxonomic and functional characteristics of the gill and gastrointestinal microbiota and its correlation with intestinal metabolites in new gift strain of farmed adult nile tilapia (*Oreochromis niloticus*). Microorganisms. https://doi.org/10.3390/microorganisms9030617
28. Gomez D, Sunyer JO, Salinas I (2013) The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens. Fish Shellfish Immunol. https://doi.org/10.1016/j.fsi.2013.09.032
29. Koh A, De Valder F, Kovatcheva-Datchary P, Bäckhed F (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. https://doi.org/10.1016/j.cell.2016.05.041
30. Muegge BD et al (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332:970–974. https://doi.org/10.1126/science.1198719
31. Hao YT, Wu SG, Jakovlić I, Zou H, Li WX, Wang GT (2017) Impacts of diet on hindgut microbiota and short-chain fatty acids in grass carp (*Ctenopharyngodon idellus*). Aquac Res. https://doi.org/10.1111/are.13381
32. Karlsson FH, Ussery DW, Nielsen J, Noorhaw I (2011) A closer look at bacterioides: phylogenetic relationship and genomic implications of a life in the human gut. Microb Ecol. https://doi.org/10.1007/s00248-010-9796-1
33. Mariat D et al (2009) The firmicutes/bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. https://doi.org/10.1186/1471-2180-9-123
34. Magne F et al (2020) The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients. https://doi.org/10.3390/nu12051474
35. Jamé E, White BA, Mizrahi I (2014) Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. PLoS ONE. https://doi.org/10.1371/journal.pone.0085423
36. Nguyen TLA, Vieira-Silva S, Liston A, Raes J (2015) How look at bacteroides: phylogenetic relationship and genomic implications of a life in the human gut. Microb Ecol. https://doi.org/10.1111/are.13381
41. Froelich BA, Noble RT (2016) Vibrio bacteria in raw oysters: managing risks to human health. Philos Trans R Soc B: Biol Sci. https://doi.org/10.1098/rstb.2015.0209

42. Petton B, Bruto M, James A, Labreuche Y, Alunno-Bruscia M, Le Roux F (2015) Crassostrea gigas mortality in France: the usual suspect, a herpes virus, may not be the killer in this polymicrobial opportunistic disease. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00686

43. EFSA Panel on Animal Health and welfare (2010) Scientific opinion on the increased mortality events in Pacific oysters, Crassostrea gigas. EFSA J 8:1894. https://doi.org/10.2903/j.efsa.2010.1894

44. Castinel A, Webb S, Jones J, Peeler E, Forrest B (2019) Disease threats to farmed green-lipped mussels Perna canaliculus in New Zealand: review of challenges in risk assessment and pathway analysis. Aquac Environ Interact. https://doi.org/10.3354/aei00314

45. Eiston RA, Hasegawa H, Humphrey KL, Polyak IK, Hase CC (2008) Re-emergence of Vibrio tubiashii in bivalve shellfish aquaculture: severity, environmental drivers, geographic extent and management. Dis Aquat Organ. https://doi.org/10.3354/dao01982

46. Le Roux F, Wegner KM, Polz MF (2016) Oysters and vibrios as a model for disease dynamics in wild animals. Trends Microbiol. https://doi.org/10.1016/j.tmb.2016.03.006

47. Kautharapu KB, Jarboe LR (2012) Genome sequence of the Psychrophilic deep-sea bacterium moritella marina MP-1 (ATCC 15381). J Bacteriol. https://doi.org/10.1128/JB.01382-12

48. Kim MJ, Baek MG, Shin SK, Yi H (2021) Characterization of gut microbiome in the mussel Mytilus galloprovincialis in response to thermal stress. Front Physiol. https://doi.org/10.3389/fphys.2021.609979

49. Guo XH et al (2019) Poseidonibacter parvus sp. nov., isolated from a squid. Int J Syst Evol Microbiol. https://doi.org/10.1099/ijsem.0.004590

50. Bano N, DeRae Smith A, Bennett W, Vasquez L, Hollibaugh JT (2007) Dominance of mycoplasma in the guts of the long-jawed mudsucker, Gillichthys mirabilis, from five California salt marshes. Environ Microbiol 9:2636–2641. https://doi.org/10.1111/j.1462-2920.2007.01381.x

51. Bin Huang Z, Guo F, Zhao J, Li WD, Ke CH (2010) Molecular analysis of the intestinal bacterial flora in cage-cultured adult small abalone, Haliotis diversicolor. Aquac Res 41:260–260. https://doi.org/10.1111/j.1467-7155.2009.02577.x

52. Tanaka R, Ootsubo M, Sawabe T, Ezura Y, Tajima K (2004) Biodiversity and in situ abundance of gut microflora of abalone (Haliotis discus hannai) determined by culture-independent techniques. Aquaculture. https://doi.org/10.1016/j.aquaculture.2004.08.032

53. Sohn JA et al (2016) Co-occurring Synechococcus ecotypes occupy four major oceanic regimes defined by temperature, macronutrients and iron. ISME J. https://doi.org/10.1038/isemj.2015.115

54. Partensky F, Blanchot J, Vaulot D (1999) Differential distribution and ecology of Prochlorococcus and Synechococcus in oceanic waters: a review. Marine Cyanobacteria. Institut Oceanographique, Monaco, pp 457–475

55. Flombaum P et al (2013) Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.1307701110

56. Schuett C, Doepke H (2010) Endobiotic bacteria and their pathogenic potential in cnidian tentacles. Helgol Mar Res. https://doi.org/10.1007/s10152-009-0179-2

57. Zhao JS, Manno D, Hawari J (2009) Psychrilyobacter atlanticus gen. nov., sp. Nov., a marine member of the phylum Fusobacteria that produces H2 and degrades nitramine explosives under low temperature conditions. Int J Syst Evol Microbiol 59:491–497. https://doi.org/10.1099/ijs.0.65263-0

58. Navarrete P, Espejo RT, Romero J (2009) Molecular analysis of microbiota along the digestive tract of juvenile Atlantic salmon (Salmo salar L.). Microb Ecol 57:550–561. https://doi.org/10.1007/s00248-008-9448-x

59. Neave MJ, Michell CT, Apprill A, Voolstra CR (2017) Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. Sci Rep. https://doi.org/10.1038/srep40579

60. Bourne D, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. ISME J. https://doi.org/10.1038/isemj.2007.112

61. Li YF et al (2019) Characterization of gut microbiome in the mussel Mytilus galloprovincialis in response to thermal stress. Front Physiol. https://doi.org/10.3389/fphys.2019.01086

62. Howells J, Jaramillo D, Brosnahan CL, Pande A, Lane HS (2021) Intracellular bacteria in New Zealand shellfish are identified as Endozoicomonas species. Dis Aquat Organ. https://doi.org/10.3354/dao03547

63. Hooper PM, Ross SH, Feist SW, Cano I (2019) Shedding and survival of an intracellular pathogenic Endozoicomonas-like organism infecting king scallop Pecten maximus. Dis Aquat Organ. https://doi.org/10.3354/dao03375

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