Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.69338

Characterization of the digestive microbiome of invasive *Limnoperna fortunei* (Dunker, 1857) collected in different seasons in the upper Uruguay River, Santa Catarina, Brazil

Tamiris Henrique Ferreira[1*], Lúvia Souza de Sá[1], Paula Brando de Mederios[1], Domickson Silva Costa[1], Grasiela Fagundes Minatto Cardoso[4], Bruno da Silva Pierri[2], Alex Pires de Oliveira Nuñer[3], Maurício Laterça Martins[1] and José Luiz Pedreira Mouriño[1]

[1]Sanidade de Organismos Aquáticos, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga 1346, 88040-900, Florianópolis, Santa Catarina, Brazil. [2]Departamento de Geologia e Geofísica, Instituto de Geociências, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil. [3]Laboratório de Biologia e Cultivo de Peixes de Água Doce, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. [4]Engie Brasil Energia S/A, Florianópolis, Santa Catarina, Brazil. *Author for correspondence. E-mail: tamirishenrique@hotmail.com

ABSTRACT. We aimed to identify the digestive microbiota of the golden mussel *Limnoperna fortunei*, and characterize its structure in different seasons. *Limnoperna fortunei* specimens were sampled in winter (September 2019) and summer (March 2020) in five reservoirs of hydroelectric power plants in southern Brazil. In each reservoir, we sampled 15 individuals at three different points. We collected the digestive diverticulum and portions of the intestine for bacterial DNA extraction, and subsequently conducted metagenomic analysis. The intestinal microbial communities of *L. fortunei* occurring in the upper Uruguay River region showed significant differences during winter and summer. Although the phyla Proteobacteria, Bacteroidetes, and Cyanobacteria were dominant regardless of the season, the microbial communities showed greater richness and bacterial diversity in the summer. Additionally, we found 143 species of bacteria in the digestive samples collected during both winter and summer, which may indicate a central microbial community for the species. The microbial communities in the digestive tract of *L. fortunei* showed greater bacterial richness and diversity in summer samples, which existed at a significantly higher temperature than those in winter samples. We observed seasonal variations in the microbiota of *L. fortunei* in the upper Uruguay River region, with increased bacterial richness and diversity in the digestive tract during summer, attributed to higher temperatures. However, Proteobacteria, Bacteroidetes, and Cyanobacteria consistently dominated the digestive tract of the golden mussel, regardless of seasonal fluctuations.

Keywords: Metagenomic; Exotic species; Bacteria; Gut; Temperature.

Received on September 28, 2023
Accepted on February 06, 2024

Introduction

Bivalves are organisms that filter large amounts of water for nutrition and respiration. They are found in the initial levels of many aquatic food webs and play an important ecological role, regulating the turbidity of the water column, recycling nutrients and organic matter, and controlling phytoplankton biomass (Binelli & Provini, 2003).

Limnoperna fortunei, popularly known as the golden mussel, is a freshwater bivalve native to rivers and streams in China and Southeast Asia. This species has a high reproductive and adaptive potential, and does not contain natural pathogens in invaded aquatic systems. Furthermore, the golden mussel can colonize wide habitats with water temperatures, current velocities, water depths, dissolved oxygen levels, and pH levels ranging from 0 to 35 °C, 0.1 to 2 m s^{-1}, 0.1 to 40 m, 0.2 to 11.33 mg L^{-1}, and 6.0 to 7.8, respectively (Darrigran, Damborenea, Drago, Drago, & Paira, 2011). It is considered as an ecosystem engineer, implying that it can change the abiotic environment by physically altering its structure. Consequently, they often—but not invariably—have effects on other members of the biota and their interactions, and on overall ecosystem processes. The concept of ecosystem engineering connects a several important ecological and evolutionary concepts, and is particularly relevant to environmental management (Darrigran & Damborenea, 2011).

The golden mussel is considered to be an engineering species owing to the potential structural changes it can cause due to its high filtering capacity and macrofouling (Morton, 1973). Macrofouling has been defined

as the attachment and subsequent growth of a community of visible plants and animals on structures (abiotic and biotic) exposed to water (Gizer, Önal, Ram, & Sahiner, 2022). The characteristics that make this invasive species robust include their resistance to adverse environmental conditions and different chemical pollutants (Mackie & Claudi 2010; João et al. 2014).

The presence of the golden mussel in Brazilian reservoirs has resulted in significant environmental and economic impacts, necessitating frequent investment for maintenance and control. Damage to hydroelectric plants, pipelines, pumps, turbines, boat hulls (Mansur et al. 2003), and net cages in fish farms are some of the main impacts caused by the spreading of this species. Considering the numerous negative impacts induced by this species and its prevalence in invaded ecosystems, identifying effective methods for managing its populations is imperative. Management approaches should adequately address the issues of incrustation without compromising the well-being of local populations or triggering adverse environmental impacts (Furlan-Murari et al. 2019).

However, certain gaps exist in the literature regarding the microbial ecology of mussels, which is complex and influenced by the environment (Sui et al. 2017). As microbial communities are strictly linked to the characteristics of the environments, their profiles are directly influenced by the physicochemical parameters, mainly temperature. The physicochemical parameters of the environment directly affect the metabolic activity of these microorganisms, especially in environments with well-defined seasons, such as cold winters and hot summers (Coulon, McKew, Osborn, McGenity, & Timmis, 2007).

Environmental temperatures can directly induce changes in a host's microbiome structure, but whether these changes subsequently feed-back into mediating the thermal responses of the host remain unclear. Considering that host and resident microbes are likely constantly interacting, differences in the microbiome structure at different temperatures may be driven by both host biological and environmental factors (Kers et al. 2018; Woodhams et al. 2020). The strength of these respective factors may vary. Hosts with adaptive immune systems are known to have greater microbiome diversity than those with only innate immunity (Woodhams et al. 2020), suggesting that different defense mechanisms have different levels of host control over the microbiome.

Host biology and environment not only have separate impacts but may also interact to shape the microbiome. The ambient temperature and thermal variation can affect the adaptive thermal tolerance of organisms. Hosts from tropical areas may be more stressed under climate change (Deutsch et al. 2008; Sunday et al. 2014), considering their relatively narrow thermal safety margins (the difference between a species' maximum tolerance to heat and the regularly experienced temperature of the species, as defined in Sunday et al. 2014). Consequently, species will have more disrupted microbiomes at thermal extremes. Similarly, aquatic ectotherms have smaller thermal safety margins than terrestrial ectotherms (Pinsky, Eikeset, McCauley, Payne, & Sunday, 2019), potentially making them more susceptible to disturbed microbiomes under thermal changes.

However, a better understanding of the gut microbiome of aquatic animals may reveal potential methods for controlling these invasive species as dysbiosis of the gut microbiome has been found to contribute to disease manifestation in humans and aquatic animals. Thus, characterizing the gut microbiome of invasive aquatic animals is an important step toward understanding the community that comprises this "hidden organ," which may eventually be exploited for species control purposes (Eichmiller, Hamilton, Staley, Sadowsky, & Sorensen, 2016). We aimed to evaluate the effect of different seasons on the microbial community in the digestive tract of exotic mussels, namely, *L. fortunei*, in five reservoirs in southern Brazil. This is the first study regarding the characterization of the digestive microbiota of *L. fortunei* and its structure during different seasons of the year in the upper Uruguay River.

Material and methods

Study area and sampling design

Limnoperna fortunei were sampled in winter (September 2019) and summer (March 2020) in five reservoirs of hydroelectric plants (HEs): Campos Novos (CN), Barra Grande (BG), Machadinho (MA), Itá (IT), and Foz de Chapecó (FC), located in the upper Uruguay River region, Santa Catarina, Brazil. Three points were sampled in each of the reservoirs (Figure 1).

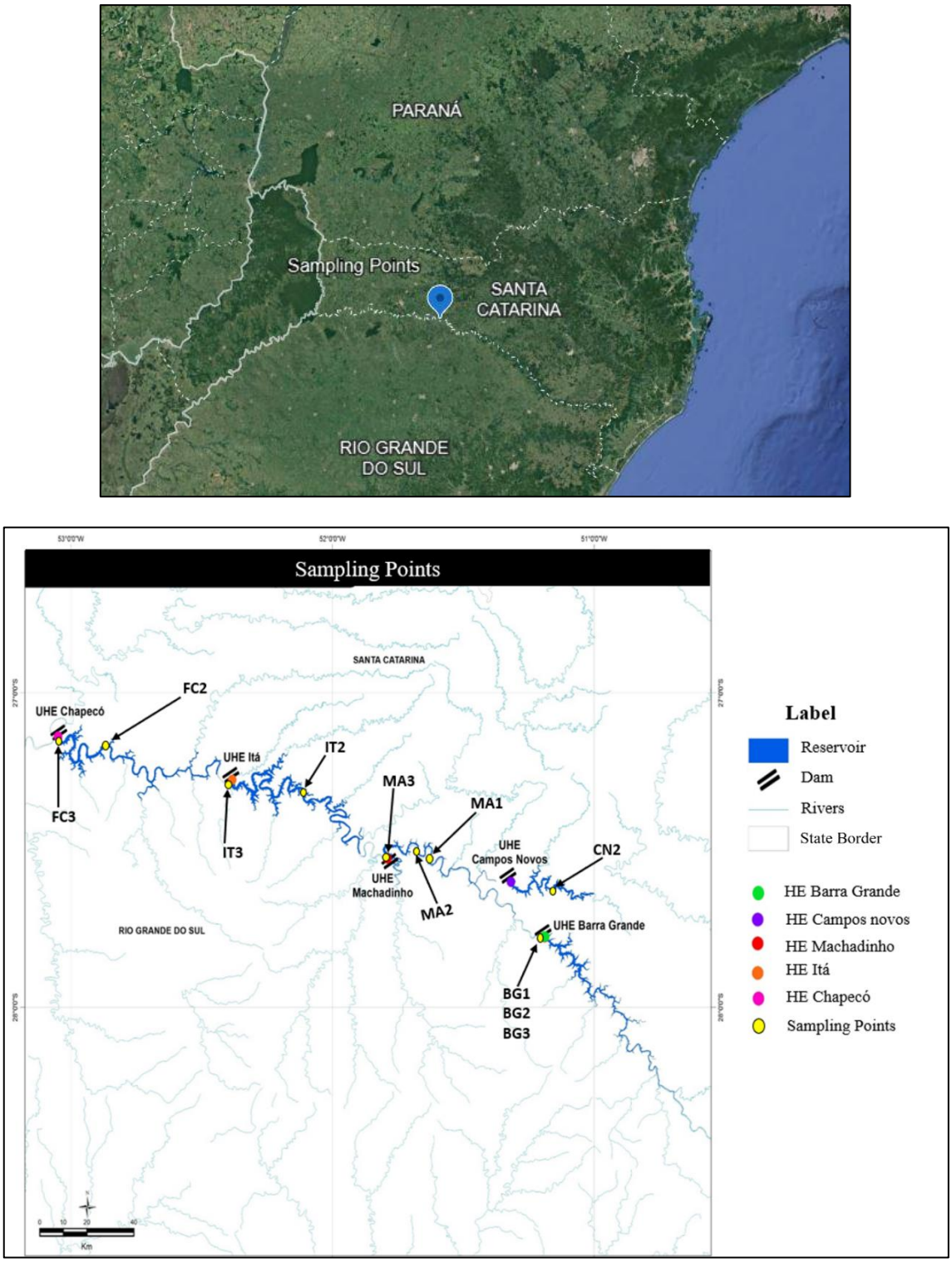

Figure 1. Location of the sampling points of the reservoirs of hydroelectric power plants on the upper Uruguay River. HE is related to the reservoirs and UHE is the specific point where the energy plant is located. Upper image font: Google Earth.

Sample storage and preparation

We collected fifteen adult mussels (average length of 2.92 ± 0.21 cm in winter and 2.99 ± 0.35 cm in summer; values showed as mean ± SD) at each point at depths of up to 1.0 m from hard surfaces, including stones, rocks, and clusters of mussels. They were stored in refrigerated thermal boxes (4.0 °C) until dissection. Each animal was aseptically opened with a sterilized scalpel to remove the digestive diverticula and portions of the intestine. This material was identified and stored individually in microtubes free of ribonucleic acids (RNAse) and deoxyribonucleic acids (DNAse), and preserved at -20 °C and transported to the Aquatic Organisms Sanity Laboratory (AQUOS) at the *Universidade Federal de Santa Catarina* (UFSC). The water quality

parameters were measured during samplings, including the temperature, pH, and dissolved oxygen concentration at each collection point.

DNA extraction

For the extraction of DNA from the bacteria present in the collected material, 200 mg of the portion comprising the digestive diverticula and gut of the mussels was used at each of the 15 collection points (three pools of five mussels). The extraction was conducted using the QIAamp® Fast DNA Stool Mini kit (QIOGEN, Hilden, Germany, DE), following the supplier's specifications. Subsequently, the extracted DNA was quantified in a NanoDrop™ 1000 spectrophotometer (Thermo Scientifc DE, US) and maintained at a concentration of above 100 μg μL^{-1}.

PCR amplification

After DNA extraction, the samples were sent to Macrogen® for metagenomic analysis. Amplification of the 16S ribosomal RNA gene (16S rRNA) was conducted through polymerase chain reaction (PCR), targeting the sequence between the preserved V3 and V4 regions.

High Throughput Sequencing (HTS)

Illumina SBS technology was used in the sequencing of data, wherein the nucleotides are marked by fluorescence when they bind to the complementary strand in each cycle. Noisy sequences were removed, and the remaining representative reads from the clusters were grouped using complex algorithms into operational taxonomic units (OTUs) through fast length adjustment of short reads (FLASH). Reads were grouped with 100% identity (ID) using Cluster Database at High Identity with Tolerance (CD-HIT-DUP) into a single file. OTUs were recorded using a quality filter to ensure 97% ID at the species level. For sequencing, we employed a minimum alignment of 300 pb and 100 k of readings per sample.

Statistical Analyses

We obtained quantitative insights into microbial ecology based on the methodology of Caporaso et al. (2010) through the Quantitative Insights Into Microbial Ecology (QIIME) program, wherein the created OTUs were related to their corresponding taxa using the "assign_taxonomy.py" tool through comparison with the Greengenes database (http://qiime.org).

The following indices were calculated: Shannon diversity index to determine the number and uniformity of species distribution; Chao1 richness index, wherein the Chao1 richness estimate is used to define an OTU; and the Margalef index. We conducted a Student's t test to compare winter and summer data. A Venn diagram was designed to determine unique bacterial OTUs, and those shared between different seasons through the InteractiVenn program (Heberle, Meirelles, Silva, Telles, & Minghim, 2015). To estimate bacterial richness and diversity in the sampled periods, an α rarefaction analysis was conducted; the sequencing coverage index, Chao1 index, and Shannon index were determined using the "alpha_diversity.py" tool. The sequencing coverage index was calculated as follows: C = 1- (S/n), where S is the number of unique OTUs and n is the number of individuals in the sample; this index is used to express how much the sample represents the environment in relative measure.

Initially, three collection points were scheduled for each HE. However, owing to the weather conditions on the days of winter collection, samples could not be collected in two points of BG, one point of IT, and one point of FC. Thus, we could not separately conduct comparative analyses between the digestive microbiota of mussels collected in winter and summer by HE as data for statistical analysis were insufficient. Thus, the data presented for the seasons in this study represent a pool of all points where collection was possible during each season.

Results

The sequencing coverage of all RNA of collected samples, considering all samples, was greater than 99%. This indicated that the sequencing of the 16S rRNA gene provided a solid basis for the alignment of the reads and enabled the identification of most bacteria present in the samples.

The digestive microbial community of the golden mussel was directly affected by the seasons. The microbial species richness of specimens collected in summer was almost three-fold greater than that of specimens collected in winter. However, both the total and average of richness and abundance showed an

inverse relation, namely, the highest richness in summer was accompanied by a community that was 1.3-fold less abundant than that collected in winter.

This inverse relation between the richness and abundance was directly reflected in the other ecological indices, wherein a greater bacteria biodiversity was observed in the microbial community in summer (reinforced by the Shannon and Margalef diversity indices and by lower dominance); this community was less abundant and more diverse compared with that collected in winter. All diversity indices were significantly higher ($p < 0.05$) in summer. Additionally, the determined dominance index was four-fold higher in winter than in summer, corroborating the results of lower diversity during this season, wherein some species were significantly more abundant in proportion in the winter microbial community.

Enterobacter tabaci, *Chryseobacterium vietnamense*, *Cyanobium gracile*, and *Arenimonas maotaiensis* were the most abundant species in winter and substantially contributed to the formation of the highest dominance index in winter. Overall, these species corresponded to 72% of the total abundance in winter, corroborating the lowest diversity indices and highest dominance index during this season (Table 1).

Table 1. Summary of data analysis obtained from high-throughput sequencing (HTS) in samples from the digestive tract of the golden mussel *Limnoperna fortunei* present in five reservoirs from the upper Uruguay River. Different letters mean statistical difference between the columns (season).

Taxonomy	Winter	Summer
Phylum	16[b]	20[a]
Class	39[b]	55[a]
Order	74[b]	101[a]
Family	129[b]	195[a]
Genus	218[b]	572[a]
Species	281[b]	836[a]
Ecological Indices		
Total Abundance	573,111[a]	454,888[b]
Average Abundance*	52,101[a]	41,353[b]
Richness	281[b]	836[a]
Chao1	74[b]	267[a]
Shannon	2.87[b]	6.35[a]
Dominance	0.154[a]	0.0380[b]
Margalef	21.12[b]	64.02[a]
Coverage	99.99%	99.99%

* Average Abundance $= \left(\frac{\text{Total Abundance}}{11}\right)$, where 11 is the collection points amount.

The rarefaction curves reached their saturation level, indicating that the bacterial richness of the sample could be determined, and that most of the microbial diversity was reached. With high α indices, especially in the summer, a significant difference ($p < 0.05$) was observed in all indices, confirming that greater richness and diversity were observed in summer than in winter. This can be explained by the difference in the water quality parameters found during winter and summer. The water quality parameters revealed significant differences in the sustained temperature and oxygen levels between summer and winter (Table 2).

Table 2. Water quality parameters (mean ± SD) sampled at different reservoirs on the upper Uruguay River. Different letters mean statistical difference between the columns.

Parameters	Winter	Summer
Temperature, ºC	19.30 ± 1.58[b]	26.15 ± 1.37[a]
pH	6.79 ± 0.19	6.61 ± 0.28
Dissolved Oxygen, mg L^{-1}	8.75 ± 0.50[b]	7.20 ± 0.34[a]

Among a total of 16 phyla found in winter, seven represented 74% of the entire bacterial community in the coldest season, wherein the phylum Proteobacteria was the most abundant, with 37% of the microorganisms being identified. In summer, the number of phyla was higher than that in winter, with a total of 20 identified phyla. Among these phyla, the most abundant was Proteobacteria, with a similar proportion to those in winter, representing approximately 39% of the identified microorganisms (Figure 2). As observed with Proteobacteria, the relative abundances of the phyla Bacteroidetes and Cyanobacteria were stable between the two seasons. Additionally, the phyla whose relative abundances were not greater than 0.8% in the different seasons of the analyzed year (9 phyla in winter and 13 in summer) were classified as "Other."

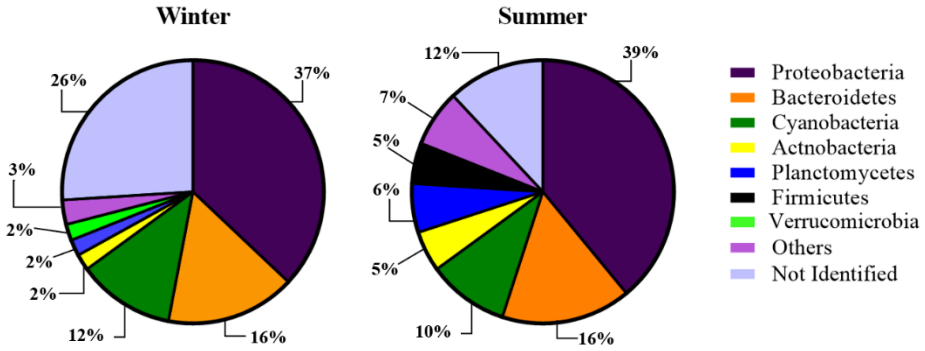

Figure 2. Relative abundance (%) of phyla present in the digestive microbial community of the golden mussel *Limnoperna fortunei* present in the upper Uruguay River.

Based on SAR data, we identified the ten most abundant genera of microorganisms present in the samples from the digestive tract of the golden mussel. The genus *Enterobacter* (23%) had the highest abundance in the winter samples, followed by *Cyanobium* (13%) and *Chyseobacterium* (10%) (Figure 3). Conversely, in the summer samples, the genus *Synechococcus* (9%) had the highest relative abundance, followed by the *Enterobacter* (7%) and *Elizabethkingia* (5%) (Figure 3). Three of these genera were common between the two seasons of the year, namely, *Enterobacter*, *Elizabethkingia*, and *Gimesia*.

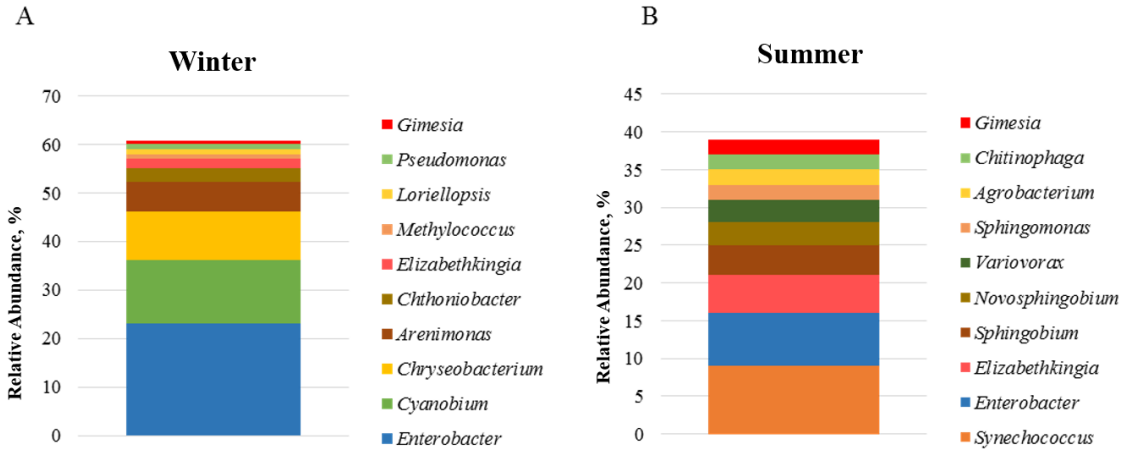

Figure 3. Relative abundance of digestive microbiota composition of the golden mussel *Limnoperna fortunei* present in the upper Uruguay River. Describes the frequency (%) of bacteria distribution, in terms of genera. A) Dominant genera in winter samples. B) Dominant genera in summer samples. The y-axes have different scales, adapting to the results found in each season.

Considering the elementary microbiota, we created a Venn diagram to search for unique or shared OTUs at the species level in quantitative terms (Figure 4). As indicated by the Venn diagram, 143 species were shared between the winter and summer samples, namely, approximately 13% of the species were not influenced by the collection period. Considering season-specific species, 138 and 693 species were observed exclusively in winter and summer, respectively.

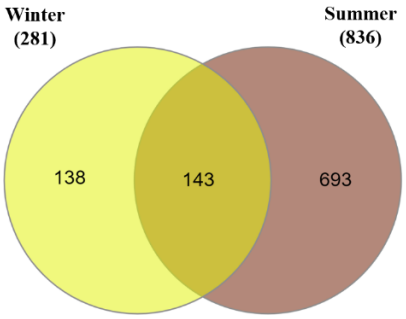

Figure 4. Venn diagram indicating singular and shared OTUs (Operational Taxonimic Units), at the species level, of the composition of the digestive microbiota of the golden mussel *Limnoperna fortunei* present in the upper Uruguay River. Each ellipse represents a set. The intersection of the two ellipses denotes the number of OTUs shared between pools. Numbers outside the intersection area correspond to the number of unique OTUs in a given pool.

Although 143 species were observed in both winter and summer, these species showed substantial changes in relation to their absolute and relative abundances in both seasons. The species common to the two most abundant stations in winter were *Novosphingobium ginsenosidimutans*, *Conexibacter stalactite*, and *Ereboglobus luteus*, with 72,269, 62,390, and 10,952 observations, respectively.

Although these three species (*Novosphingobium ginsenosidimutans*, *Conexibacter stalactite*, and *Ereboglobus luteus*) were also observed in the summer, their abundances in the hottest season decreased to 90, 220, and 26 observations, respectively. The same trend was observed with the species *Synechococcus rubescens*, with 47,265 observations in the hottest season and only 111 observations in winter. Conversely, the abundances of five species showed variations of less than 15% between the two seasons. These species were *Limnohabitans curvus*, *Aciditerrimonas ferrireducens*, *Pseudolabrys taiwanensis*, *Lactococcus lactis*, and *Mycoplasma mobile*, wherein the changes in abundance between the two seasons were 15, 14, 14, 9, and 3%, respectively.

Furthermore, the analysis and characterization of the profile of the studied microbiota also showed the presence of five species of bacteria relatively more abundant for each sampled period. In winter, *Enterobacter tabaci* (23%), belonging to the phylum Proteobacteria, had the highest relative abundance among all identified species, followed by *Cyanobium gracile* (13%), *Chryseobacterium vietnamense* (10%), *Arenimonas maotaiensis* (6%), and *Chthoniobacter flavus* (3%), which accounted for 55% of the total OTUs. Conversely, in the samples collected in summer, the microorganism with the highest relative abundance was *Synechococcus rubescens* (9%), belonging to the phylum Cyanobacteria, followed by *Enterobacter mori* (7%), *Elizabethkingia anophelis* (4%), *Sphingobium czechense* (4%), and *Variovorax boronicumulans* (2%), represented in the cladogram in Figure 5.

In this study, we characterized, for the first time, the digestive microbiota and its structure in different seasons of the year for *Limnoperna fortunei* present in the upper Uruguay River. The microbial community of an animal in a natural environment can reflect the conditions imposed by the external environment. The results of our study indicate that the different seasons of the year, influenced by variations in the abiotic factors, such as temperature, induced a change in the digestive microbiota of *L. fortunei* in the upper Uruguay River.

Aquatic animals are present in an environment with constant exposure to microbes as their diet, water, and sediment harbor high levels of microorganisms that can colonize their external and internal body surfaces (Hector, Hoang, Li, & King, 2022). The intestinal microbiota of mollusks is considered to be dependent on the microbial content of water owing to the large volume of water flowing through this tissue (Martínez, Ibáñez, Monroy, & Ramírez, 2012). Although the exact mechanisms of interaction between the host and bacteria are elusive, changes in bacterial communities in the gut may be closely related to host physiology (Tan, Xu, & Long, 2020). Moreover, high temperatures can lead to heterogeneity in the composition of the bacterial community (Erwin, Pita, López-Legentil, & Turon, 2012). This is indicated by studies conducted on mussels of the species *Mytilus galloprovincialis*, wherein a significant increase in microbial diversity was observed in the intestinal microbiota (Li et al. 2019). Moreover, a previous study indicated that the greatest influence on the structure of the microbial community present in *Dreissena polymorpha* was caused by the temperature of the water in which they resided (Mathai, Magnone, Dunn, & Sadowsky, 2020).

Studies conducted on *L. fortunei* in South America have indicated that the increase in temperature influenced several factors caused by thermal changes, including filtration rate, reproductive activity, mortality, and distribution (Boltovskoy, 2015). Our analyses based on high-throughput sequencing demonstrated that the microbial communities in the digestive tract of *L. fortunei* showed greater bacterial richness and diversity in samples collected in summer, wherein the temperature was significantly higher. It remains to be seen whether changes in the bacterial community were ascribed to a change in mussel physiology caused by the increased water temperature, or a direct response of the bacteria to temperature.

The microbiome composition is correlated with the thermal tolerance of the host. The transplantation of microbiomes from heat-tolerant *Drosophila melanogaster* improved the ability of recipient flies to cope with higher temperatures (Moghadam et al. 2018). In contrast, experimentally depleting gut microbiome diversity was found to reduce the thermal tolerance of tadpoles to both heat and cold, with impacts on survival under acute heat stress (Fontaine, Mineo, & Kohl, 2022). In coral-related systems, the abundance of particular bacterial taxa is related to the response of the host to short-term heat-stress (Hartman, Van Oppen, & Blackall, 2019). These findings suggest that microbial communities are associated with improved thermal tolerance of hosts, but no common indicator has been determined across diverse host species. Thus, in some systems, species interactions in microbial communities or the functions of the entire microbial community may play a more important role than individual symbionts under heat stress conditions.

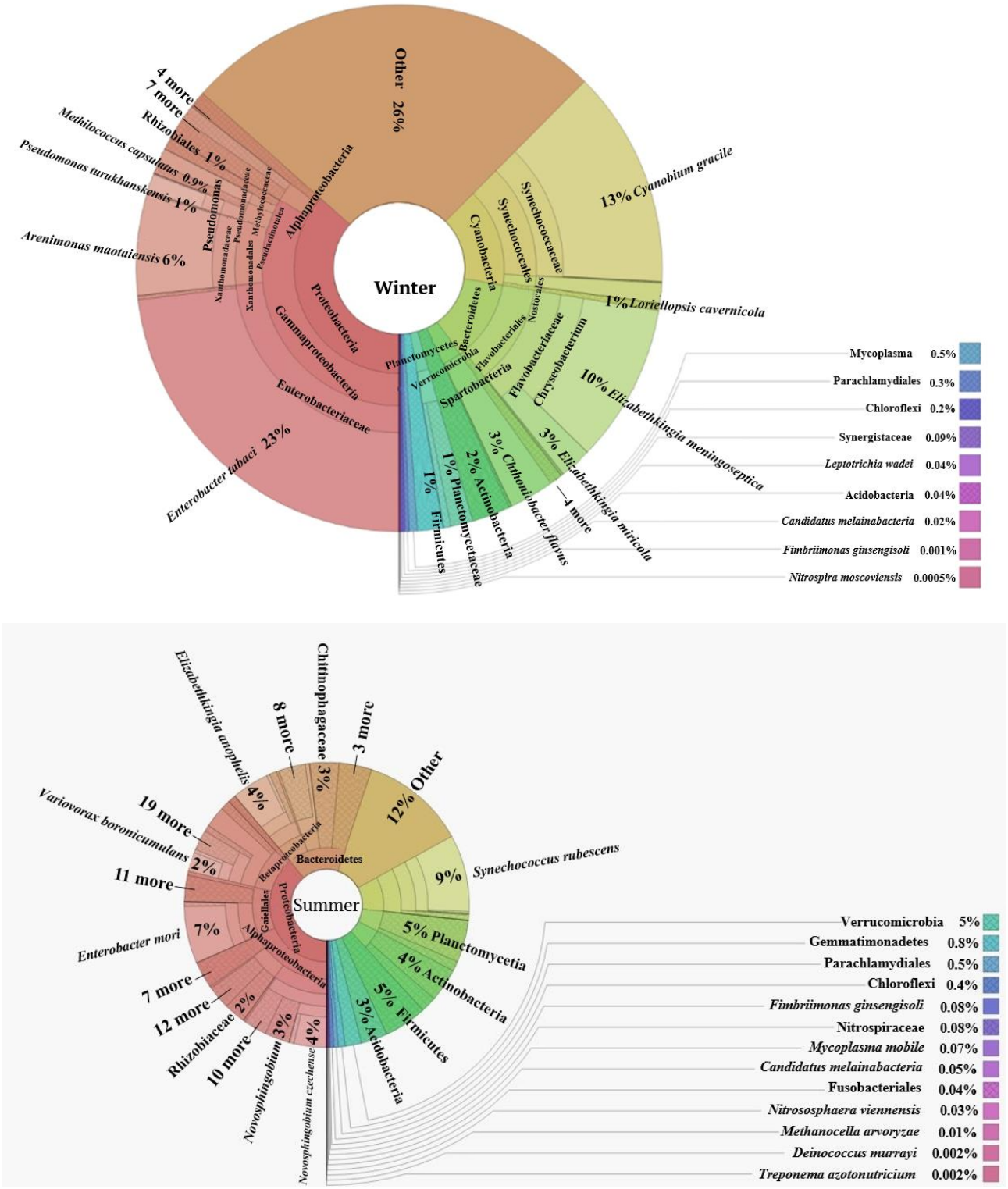

Figure 5. Bacterial taxonomic composition in golden mussel digestive samples from upper Uruguay River shown as relative abundance. A) Samples shown at species level in the winter period. B) Samples shown at species level in the summer period. The Krona chart was generated using the Excel template. Percentages of assigned readings are shown with taxa.

Heat stress can lead to a breakdown of symbiosis between a host and its mutualistic symbiont, wherein the host no longer benefits from association. For example, bacteria that increase survival of pea aphids against parasitoid wasps no longer provide the same level of protection under heat stress (Doremus et al. 2018). In cnidarian-algae symbioses, heat can affect symbiont uptake and prevent the symbiont from synthesizing proteins necessary for photosynthesis (Takahashi, Whitney, Itoh, Maruyama, & Badger, 2008). Many heritable symbionts are heat-sensitive, which can hinder their transmission to the next host generation, making symbioses unstable under warming conditions (Hooper, Midtvedt, & Gordon, 2002). In our study, the results

indicate a direct relationship between the effects of temperature on the digestive microbial community of *L. fortunei*. Changes in temperature can influence competitive relationships between different microbial species, and this can lead to changes in the microbial community composition. Considering that the golden mussel is a species native to environments with mild to cold temperatures, it has evolved and created a relationship with the characteristics of this type of environment. This may be a factor that can explain the greater abundance of microorganisms in winter, wherein microorganisms favored in these conditions may find suitable conditions for the colonization of the digestive tract of *L. fortunei*.

Host microbiomes are regulated by both host-related and environmental factors. Host factors, such as immunity, can be affected by environmental stress, which in turn shapes the interactions between the host and microbiome (Posadas, Baquiran, Nada, Kelly, & Conaco, 2022). Although studies on an array of host species (from plants to animals, ectotherms to endotherms, terrestrial to aquatic) have shown that microbiomes can be altered by temperature (Vargas, Leiva, & Wörheide, 2021), it is unclear whether microbial alteration is the direct result of temperature, the physiological response of the host, or both.

The implications for species persistence owing to the impact of symbiosis on the thermal performance of the host will be substantial. For example, species and populations in tropical regions are often more resistant to thermal stress than those in temperature regions, suggesting adaptation to local conditions (Hector, Sgrò, & Hall, 2021). However, as tropical species and populations already live close to their upper thermal limits, and therefore have small thermal safety margins, they could be disproportionately harmed by infection (Hector et al. 2021). In the absence of genetic and phenotypic variation in the upper thermal limits, tropical populations may be under stronger selection to facilitate the colonization of beneficial symbionts. Nevertheless, the harm caused by infection may be compounded by the likely greater parasite abundance in the tropics (Møller, 1998).

Alternatively, temperate species may be most vulnerable from infection and thermal stress. Models incorporating the thermal performance of parasites have predicted that warming will increase parasite prevalence in temperate regions but decrease it in the tropics (Cohen, Sauer, Santiago, Spencer, & Rohr, 2020). Any geographic shift in parasite prevalence owing to global warming may alleviate the impacts of parasites on the thermal performance of tropical species. Temperate species may not be this fortunate. In conjunction with an increase in parasite prevalence, hosts in temperate regions will frequently experience thermal stress owing to an increase in thermal variability and rising average temperatures (Kingsolver, Diamond, & Buckley, 2013). Warmer temperatures may lengthen the season suitable for the growth and transmission of parasites in temperate regions (Gehman, Hall, & Byers, 2018). Warmer average temperatures may therefore increase the abundance of parasites, as well as the prevalence and severity of disease outbreaks. Subsequent infections will dramatically impair the responses of these hosts to the increasing frequency of thermal stress.

In this study, we revealed that the gut of the golden mussel was dominated by three bacterial phyla: Proteobacteria, Bacteroidetes, and Cyanobacteria. Although the samples collected in summer showed a significantly higher relative abundance than those in winter, the most abundant phyla in both seasons remained the same. The presence of Proteobacteria in freshwater reservoirs is a common feature, and this phylum is known to actively participate in the biogeochemical processes of lake ecosystems (Yuan, Nogi, Tan, Zhang, & Lv, 2014). This taxon was also found in the total visceral mass of *D. polymorpha* collected in lake environments (Mathai et al. 2020). Microorganisms belonging to the phylum Bacteroidetes were abundant in the intestines of the small abalone *Haliotis diversicolor* (Zhao, Ling, Zhang, Ke, & Hong, 2018), crab *Callinectes sapidus* (Givens, Burnett, Burnett, & Hollibaugh, 2013), and sea urchin *Lytechinus variegatus* (Hakim et al. 2015).

Transient microorganisms acquired from the environment can be abundant in the intestinal microbial groups of animals (King, Judd, Kuske, & Smith, 2012; Pierce, Ward, Holohan, Zhao, & Hicks, 2016). The third most abundant taxon in our dataset belonged to the phylum Cyanobacteria, which are absorbed as food by bivalves (Avila-Poveda, Torres-Ariño, Girón-Cruz, & Cuevas-Aguirre, 2014). This indicated that the abundance of this phylum in the digestive tract of *L. fortunei* may be related to its temporary passage during digestive processes.

Furthermore, the taxonomic classification of the microbiota, based on the 16S rRNA gene, revealed that the microbiota of *L. fortunei* contained members of the genera *Enterobacter*, *Elizabethkingia, Gimesia*, and *Chyseobacterium* among the OTUs. Some of these bacterial genera have been detected in marine mussels, such as *Mytilus sp.* (William, Deborah, & Cynthia, 2017; Vezzulli et al. 2018; Li et al. 2019) and *Brachidontes sp.* (Cleary, Becking, Polónia, Freitas, & Gomes, 2015; Cleary & Polónia 2018). Furthermore, the analysis and

characterization of the profile of the studied microbiota indicated the species of bacteria that were relatively more abundant for each sampled period. In winter, *Enterobacter tabaci* had the highest relative abundance among all the identified species. In summer, *Synechococcus rubescens* showed the highest relative abundance was. The high abundances of these taxa present in the digestive tract of *L. fortunei* indicate a functional association with the host, such as microbial involvement in the metabolism of nutrients and its source of nutrition.

Considering the elemental microbiota, the Venn diagram revealed that 143 OTUs (13%) were shared between the samples collected in winter and summer, namely, these microorganisms were not influenced by the collection period, indicating a core microbiota (i.e., the group of microorganisms commonly found within the microbiome of a host across the boundaries of space and time based on its persistence; Hernandez-Agreda, Gates, & Ainsworth, 2017). Among the species common to the two seasons of the year, *Chryseobacterium vietnamense*, *Cyanobium gracile*, *Synechococcus rubescens*, *Arenimonas maotaiensis*, and *Chthoniobacter flavus* accounted for 59% of the total OTUs shared between the sets. These species are present in natural environments, such as *C. vietnamense* isolated from forest soil in Vietnam (Li & Zhu, 2012), *C. gracile* isolated from freshwater from Adongji Lagoon, Korea (Kwon, Jo, Jang, Lee, & Nam, 2021), *S. rubescens* isolated from soil in the Netherlands (Reimer et al. 2022), *A. maotaiensis* isolated from freshwater Chishui River, China (Yuan et al. 2014), and *C. flavus* isolated from pasture soil in Australia (Sangwan, Chen, Hugenholtz, & Janssen, 2004). Reports on the presence of these microorganisms are scarce. However, as these microorganisms are mainly isolated from soil and water, their high relative abundance in the digestive tract of *L. fortunei* collected during different seasons of the year is likely related to the nutrition of the mollusk.

Conclusion

The microbiota in *Limnoperna fortunei* varied seasonally in the upper Uruguay River. Summer samples showed greater bacterial richness and diversity due to warmer temperatures compared to winter. The dominant phyla in the digestive tract of *L. fortunei* were Proteobacteria, Bacteroidetes and Cyanobacteria, regardless of the season. 143 OTUs shared between seasons suggest a core microbiota is not affected by season. Temperature emerges as the main factor that influences the modulation of the intestinal microbiome. Understanding seasonal variations in the golden mussel's immune system is crucial for managing this exotic species and mitigating its environmental impacts.

Acknowledgments

The authors would like to thank the PhD J. Ribolli for reviewing the manuscript and to PhD. V. A. Pontinha (in memoriam) for the intellectual contribution and assistance in the development of this research and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq 303822/2022-8) for grant to M. L. Martins. This study was partially financed by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES financial code 001.

References

Avila-Poveda, O. H., Torres-Ariño, A., Girón-Cruz, D. A., & Cuevas-Aguirre, A. (2014). Evidence for accumulation of *Synechococcus elongatus* (Cyanobacteria: Cyanophyceae) in the tissues of the oyster *Crassostrea gigas* (Mollusca: Bivalvia). *Tissue Cell, 46*(5), 379-387. DOI: https://doi.org/10.1016/j.tice.2014.07.001

Binelli, A., & Provini, A. (2003). The PCB pollution of Lake Iseo (N. Italy) and the role of biomagnification in the pelagic food web. *Chemosphere, 53*(2), 143–151. DOI: https://doi.org/10.1016/S0045-6535(03)00441-7

Boltovskoy, D. (Ed.) (2015). *Limnoperna Fortunei*: *The ecology, distribution and control of a swiftly spreading invasive fouling mussel*. Springer International Publishing, Cham

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*, 335-336. DOI: https://doi.org/10.1038/nmeth.f.303

Cleary, D. F. R., Becking, L. E., Polónia, A. R. M., Freitas, R. M., & Gomes, N. C. M. (2015). Composition and predicted functional ecology of mussel-associated bacteria in Indonesian marine lakes. *Antonie van Leeuwenhoek, 107*, 821-834. DOI: https://doi.org/10.1007/s10482-014-0375-1

Cleary, D. F. R., & Polónia, A. R. M. (2018). Bacterial and archaeal communities inhabiting mussels, sediment and water in Indonesian anchialine lakes. *Antonie van Leeuwenhoek, 111*, 237-257. DOI: https://doi.org/10.1007/s10482-017-0944-1

Cohen, J. M., Sauer, E. L., Santiago, O., Spencer, S., & Rohr, R. J. (2020). Divergent impacts of warming weather on wildlife disease risk across climates. *Science, 370*(6519). DOI: https://doi.org/10.1126/science.abb1702

Coulon, F., McKew, B. A., Osborn, A. M., McGenity, T. J., & Timmis, K. N. (2007). Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. *Environmental Microbiology, 9*(1), 177-186. DOI: https://doi.org/10.1111/j.1462-2920.2006.01126.x

Darrigran, G, & Damborenea, C. (2011). Ecosystem engineering impact of *Limnoperna fortunei* in South America. *Zoological Science, 28*(1), 1-7. DOI: https://doi.org/10.2108/zsj.28.1

Darrigran, G., Damborenea, C., Drago, E. C., Drago, I. E., & Paira, A. (2011). Environmental factors restrict the invasion process of *Limnoperna fortunei* (Mytilidae) in the Neotropical region: A case study from the Andean tributaries. *Annales de Limnologie-International Journal of Limnology 47*(3), 221-229. DOI: https://doi.org/10.1051/limn/2011025

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences, 105*(18), 6668-6672. DOI: https://doi.org/10.1073/pnas.0709472105

Doremus, M. R., Smith, A. H., Kim, K. L., Holder, A. J., Russell, J. A., & Oliver, K. M. (2018). Breakdown of a defensive symbiosis, but not endogenous defences, at elevated temperatures. *Molecular Ecology, 27*(8), 2138-2151. DOI: https://doi.org/10.1111/mec.14399

Eichmiller, J. J., Hamilton, M. J., Staley, C., Sadowsky, M. J., & Sorensen, P. W. (2016). Environment shapes the fecal microbiome of invasive carp species. *Microbiome, 4*(44). DOI: https://doi.org/10.1186/s40168-016-0190-1

Erwin, P. M., Pita, L., López-Legentil, S., & Turon, X. (2012). Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Applied and Environmental Microbiology, 78*(20), 7358–7368. DOI: https://doi.org/10.1128/AEM.02035-12

Fontaine, S. S., Mineo, P. M., & Kohl, K. D. (2022). Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. *Nature Ecology & Evolution, 6*, 405–417. DOI: https://doi.org/10.1038/s41559-022-01686-2

Furlan-Murari, P. J., Ruas, C. F., Ruas, E. A., Benício, L. M., Urrea-Rojas, A. M., Poveda-Parra, A. R., & Lopera-Barrero, N. M. (2019). Structure and genetic variability of golden mussel (*Limnoperna fortunei*) populations from Brazilian reservoirs. *Ecology and Evolution, 9*(5), 2706–2714. DOI: https://doi.org/10.1002/ece3.4941

Gehman, A. M., Hall, R. J., & Byers, J. E. (2018). Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proceedings of the National Academy of Sciences, 115*(4), 744–749. DOI: https://doi.org/10.1073/pnas.1705067115

Givens, C. E., Burnett, K. G., Burnett, L. E., & Hollibaugh, J. T. (2013). Microbial communities of the carapace, gut, and hemolymph of the Atlantic blue crab, *Callinectes sapidus*. *Marine Biology, 160*, 2841–2851. DOI: https://doi.org/10.1007/s00227-013-2275-8

Gizer, G., Önal, U., Ram, M., & Sahiner, N. (2022). Biofouling and mitigation methods: A Review. *Biointerface Research in Applied Chemistry, 13*. DOI: https://doi.org/10.33263/BRIAC132.185

João, G., Pereira, J. L., Rosa, I. C., Saraiva, P. M., Gonçalves, F., & Costa, R. (2014). Evaluation of candidate biocides to control the biofouling Asian clam in the drinking water treatment industry: An environmentally friendly approach. *Journal of Great Lakes Research, 40*(2), 421–428. DOI: https://doi.org/10.1016/j.jglr.2014.03.013

Hakim, J. A., Koo, H., Dennis, L. N., Kumar, R., Ptacek, T., Morrow, C. D., ... Watts, S. A. (2015). An abundance of Epsilonproteobacteria revealed in the gut microbiome of the laboratory cultured sea urchin, *Lytechinus variegatus*. *Frontiers in Microbiology, 6*, 1–12. DOI: https://doi.org/10.3389/fmicb.2015.01047

Hartman, L. M., van Oppen, M. J. H., & Blackall, L. L. (2019). The effect of thermal stress on the bacterial microbiome of *Exaiptasia diaphana*. *Microorganisms, 8*(1), 20. DOI: https://doi.org/10.3390/microorganisms8010020

Heberle, H., Meirelles, G. V., Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. *BMC Bioinformatics, 16*(1), 1–7. DOI: https://doi.org/10.1186/s12859-015-0611-3

Hector, T. E., Hoang, K. L., Li, J., & King, K. C. (2022). Symbiosis and host responses to heating. *Trends in Ecology & Evolution, 37*(7), 611–624. DOI: https://doi.org/10.1016/j.tree.2022.03.011

Hector, T. E., Sgrò, C. M., & Hall, M. D. (2021). Thermal limits in the face of infectious disease: How important are pathogens? *Global Change Biology, 27*(19), 4469–4480. DOI: https://doi.org/10.1111/gcb.15761

Hernandez-Agreda, A., Gates, R. D., & Ainsworth, T. D. (2017). Defining the core microbiome in corals' microbial soup. *Trends in Microbiology, 25*(2), 125–140. DOI: https://doi.org/10.1016/j.tim.2016.11.003

Hooper, L. V., Midtvedt, T., & Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual Review of Nutrition, 22*, 283–307. DOI: https://doi.org/10.1146/annurev.nutr.22.011602.092259

Kers, J. G., Velkers, F. C., Fischer, E. A. J., Hermes, G. D. A., Stegeman, J. A., & Smidt, H. (2018). Host and environmental factors affecting the intestinal microbiota in chickens. *Frontiers in Microbiology, 9*,235. DOI: https://doi.org/10.3389/fmicb.2018.00235

King, G. M., Judd, C., Kuske, C. R., & Smith, C. (2012). Analysis of stomach and gut microbiomes of the eastern oyster (*Crassostrea virginica*) from Coastal Louisiana, USA. *PLoS One, 7*(12), 1–11. DOI: https://doi.org/10.1371/journal.pone.0051475

Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology, 27*(6), 1415–1423. DOI: https://doi.org/10.1111/1365-2435.12145

Kwon, D. R., Jo, B. Y., Jang, S. W., Lee, C. S., & Nam, S. W. (2021) New records of the genus *Cyanobium* and *Cyanobium gracile* (Synechococcales, Cyanophyceae) in Korean freshwater. *Environmental Biology Research, 39*(1), 32-38. DOI: https://doi.org/10.11626/kjeb.2021.39.1.032

Li, Y., Xu, J., Chen, Y., Ding, W., Shao, A., Liang, X., ... Yang, J. (2019). Characterization of gut microbiome in the mussel *Mytilus galloprovincialis* in response to thermal stress. *Frontiers in Physiology, 10,* 1086. DOI: https://doi.org/10.3389/fphys.2019.01086

Li, Z., & Zhu, H. (2012). *Chryseobacterium vietnamense* sp. nov., isolated from forest soil. *International Journal of Systematic and Evolutionary Microbiology, 62*(4), 827–831. DOI: https://doi.org/10.1099/ijs.0.027201-0

Mackie, G. L., & Claudi, R. (2010). Monitoring and control of macrofouling mollusks in freshwater systems. (2nd edn.). CRC Press, Boca Raton. DOI: https://doi.org/10.1201/9781439804414

Mansur, M. C. D., Santos, C. P., Darrigran, G., Heydrich, I., Callil, C. T., & Cardoso, F. R. (2003). Primeiros dados quali-quantitativos do mexilhão-dourado, *Limnoperna fortunei* (Dunker), no Delta do Jacuí, no Lago Guaíba e na Laguna dos Patos, Rio Grande do Sul, Brasil e alguns aspectos de sua invasão no novo ambiente. *Revista Brasileira de Zoologia, 20*(1), 75–84. DOI: https://doi.org/10.1590/S0101-81752003000100009

Martínez, C. P., Ibáñez, A. L., Monroy, H. O. A., & Ramírez, S. H. C. (2012). Use of probiotics in aquaculture. *ISRN Microbiol, 2012*, 1–13. DOI: https://doi.org/10.5402/2012/916845

Mathai, P. P., Magnone, P., Dunn, H. M., & Sadowsky, M. J. (2020). Water and sediment act as reservoirs for microbial taxa associated with invasive dreissenid mussels. *Science of the Total Environment, 703*, 1–7. DOI: https://doi.org/10.1016/j.scitotenv.2019.134915

Moghadam, N. N., Thorshauge, P. M., Kristensen, T. N., Jonge, N., Bahrndorff, S., Kjeldal, H., & Nielsen, J. L. (2018). Strong responses of *Drosophila melanogaster* microbiota to developmental temperature. *Fly, 12*(1), 1–12. DOI: https://doi.org/10.1080/19336934.2017.1394558

Møller, A.P. (1998). Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos, 82*(2), 265-270. DOI: https://doi.org/10.2307/3546966

Morton, B. (1973). Some aspects of the biology and functional morphology of the organs of feeding and digestion of *Limnoperna fortunei* (Dunker) (Bivalvia: Mytilacea). *Malacologia, 12*(2), 265–281

Pierce, M. L., Ward, J. E., Holohan, B. A., Zhao, X., & Hicks, R. E. (2016). The influence of site and season on the gut and pallial fluid microbial communities of the eastern oyster, *Crassostrea virginica* (Bivalvia, Ostreidae): community-level physiological profiling and genetic structure. *Hydrobiologia, 765*(1), 97–113. DOI: https://doi.org/10.1007/s10750-015-2405-z

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature, 569*(7754), 108–111. DOI: https://doi.org/10.1038/s41586-019-1132-4

Posadas, N., Baquiran, J. I. P., Nada, M. A. L., Kelly, M., & Conaco, C. (2022). Microbiome diversity and host immune functions influence survivorship of sponge holobionts under future ocean conditions. *The ISME Journal, 16*(1), 58–67. DOI: https://doi.org/10.1038/s41396-021-01050-5

Reimer, L. C., Sardà, C. J., Koblitz, J., Ebeling, C., Podstawka, A., & Overmann, J. (2022). BacDive in 2022: The knowledge base for standardized bacterial and archaeal data. *Nucleic Acids Research, 50*(1), D741–D746. DOI: https://doi.org/10.1093/nar/gkab961

Sangwan, P., Chen, X., Hugenholtz, P., & Janssen, P. H. (2004). *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, *Spartobacteria* classis nov., of the phylum *Verrucomicrobia*. *Applied and Environmental Microbiology, 70*(10), 5875–5881. DOI: https://doi.org/10.1128/AEM.70.10.5875-5881.2004

Sui, Y., Hu, M., Shang, Y., Wu, F., Huang, X., Dupont, S., & Wang, Y. (2017). Antioxidant response of the hard shelled mussel *Mytilus coruscus* exposed to reduced pH and oxygen concentration. *Ecotoxicology and Environmental Safety, 137*, 94–102. DOI: https://doi.org/10.1016/j.ecoenv.2016.11.023

Sunday, J. M., Bates, A. E., Kearney, M. R. Colwell, R. K. Dulvy, N. K. Longino, J. T. & Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences, 111*(15), 5610–5615. DOI: https://doi.org/10.1073/pnas.1316145111

Takahashi, S., Whitney, S., Itoh, S., Maruyama, T., & Badger, M. (2008). Heat stress causes inhibition of the *de novo* synthesis of antenna proteins and photobleaching in cultured *Symbiodinium*. *Proceedings of the National Academy of Sciences, 105*(11), 4203–4208. DOI: https://doi.org/10.1073/pnas.0708554105

Tan, K., Xu, C., & Long, C. (2020). Association of microbiota in the stomach of *Sinanodonta woodiana* and its cultured soil. *3 Biotech, 10*(7), 1–9. DOI: https://doi.org/10.1007/s13205-020-02313-2

Vargas, S., Leiva, L., & Wörheide, G. (2021). Short-term exposure to high-temperature water causes a shift in the microbiome of the common aquarium sponge *Lendenfeldia chondrodes*. *Microbial Ecology, 81*(1), 213–222. DOI: https://doi.org/10.1007/s00248-020-01556-z

Vezzulli, L., Stagnaro, L., Grande, C., Tassistro, G., Canesi, L., & Pruzzo, C. (2018). Comparative 16SrDNA gene-based microbiota profiles of the Pacific oyster (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) from a Shellfish Farm (Ligurian Sea, Italy). *Microbial Ecology, 75*(2), 495–504. DOI: https://doi.org/10.1007/s00248-017-1051-6

William, B. S., Deborah, I., & Cynthia, A. (2017). Endozoicomonas dominates the gill and intestinal content microbiomes of *Mytilus edulis* from Barnegat Bay, New Jersey. *Journal of Shellfish Research, 36*(2), 391–401. DOI: https://doi.org/10.2983/035.036.0212

Woodhams, D. C., Bletz, M. C., Becker, C. G., Bender, H. A., Buitrago-Rosas, D., Diebboll, H., ... Whetstone, R. (2020). Host-associated microbiomes are predicted by immune system complexity and climate. *Genome Biology, 21*(1), 23. DOI: https://doi.org/10.1186/s13059-019-1908-8

Yuan, X., Nogi, Y., Tan, X., Zhang, R., & Lv, J. (2014). *Arenimonas maotaiensis* sp. nov., isolated from fresh water. *International Journal of Systematic and Evolutionary Microbiology, 64*(12), 3994–4000. DOI: https://doi.org/10.1099/ijs.0.067769-0

Zhao, J., Ling, Y., Zhang, R., Ke, C., & Hong, G. (2018). Effects of dietary supplementation of probiotics on growth, immune responses, and gut microbiome of the abalone *Haliotis diversicolor*. *Aquaculture, 493*(1), 289–295. DOI: https://doi.org/10.1016/j.aquaculture.2018.05.011