Austral Journal of Veterinary Science

Austral J Vet Sci 56, 47-54 (2024)
https://doi.org/10.4206/ajvs.561.06

SHORT COMMUNICATION

Identification of potentially harmful bacterial genera of veterinary relevance in the Llanquihue urban wetlands

José Dellis Rocha[1,2], Catherine Opitz[2], Vicente Cárdenas[1], Cristal Mella[1], Daniel A. Medina*[1,2]

[1] Facultad de Ciencias de la Naturaleza, Escuela de Medicina Veterinaria, Universidad San Sebastián, Puerto Montt, Chile.

[2] Laboratorio Institucional Patagonia, Universidad San Sebastián, Puerto Montt, Chile.

Article History
Received: 09.08.2023
Accepted: 20.10.2023
Published: 25.01.2024

Corresponding author
*Daniel A. Medina
daniel.medina@uss.cl

ABSTRACT. Water bodies constitute natural, social, and ecological heritage under constant threat from the footprint of human action. They are the habitats of many species and play a key role in sustaining biodiversity in different ecosystems. However, anthropogenic activity can result in eutrophication of water bodies, favoring the excessive growth of microorganisms, which can be a risk factor for animal, human, and environmental health. The Agricultural and Livestock Service [Servicio Agrícola y Ganadero (SAG)] of Chile developed a list of Mandatory Reportable Diseases (MRD) to report suspected contagious diseases of veterinary relevance. In this sense, the detection of microorganisms in water bodies for the characterization of their sanitary status is important for the development of monitoring strategies and the prevention of possible diseases. This study aimed to determine the presence of potentially harmful bacteria in Llanquihue urban wetlands. Here, we used metagenomic sequencing of amplicons to characterize the composition of microorganisms in three urban wetlands in the city of Llanquihue, which could be a risk factor for animal health. Our main finding was the detection of genera that may be associated with MRD, in addition to other microbial genera that have pathogenic potential. On the other hand, we also report the presence of cyanobacteria genera that can be involved in toxin production in water bodies as another potential source of risk. This is the first study to use massive sequencing techniques to analyze the sanitary status of urban wetlands in the city of Llanquihue.

Keywords: Mandatory reportable diseases, Wetlands, DNA sequencing, bioinformatics.

INTRODUCTION

In the Los Lagos region of southern Chile, approximately 70% of the land corresponds to hydrographic basins. Rivers, lakes, wetlands, and groundwater have sustained the most important economic, social, and productive human activities, such as agriculture, livestock, forestry, and aquaculture, among others. However, poor management of water resources, excessive exploitation, and negligence in controlling anthropogenic organic and industrial waste harm ecosystems (Zedler & Kercher, 2005). These activities promote high levels of nutrients, such as nitrogen and phosphorous, in the aquatic network, a process called eutrophication (Akinnawo, 2023). Eutrophication is one of the most worrying consequences of anthropogenic activity in ecosystems and water bodies (Bhagowati & Ahamad, 2019; Chislock *et al.*, 2013; Khan & Mohammad, 2014; Pitois *et al.*, 2001), favoring the excessive growth (blooms) of microorganisms that decrease the quality and availability of resources (Amorim & Moura, 2021; Paerl *et al.*, 2014). In addition, microorganism blooms can be a risk factor for animal health because the abundance of certain pathogens in water bodies increases the risk of infectious or toxicological diseases in wildlife and domestic animals (Parlapani *et al.*, 2023; Lapointe *et al.*, 2015; Walters *et al.*, 2011; Zinia & Kroeze, 2015).

The Agricultural and Livestock Service of Chile [Servicio Agrícola Ganadero (SAG)] has drawn up a list of Mandatory Reportable Diseases (MRD) of veterinary relevance established for reporting suspected contagious diseases in animals (DECRETO 389 EXENTO, Ministerio de Agricultura, Chile. 2014). MRD can be caused by a wide variety of pathogens belonging to different bacterial genera. Some of these bacterial genera include *Mycobacterium*, *Brucella*, and *Mycoplasma*, which are involved in diseases such as bovine tuberculosis, paratuberculosis, brucellosis, and avian mycoplasmosis, among others (SAG, 2019). However, there are other diseases that, although not currently part of the MRD list, may constitute a risk to animal health, such as species members of *Flavobacterium*, *Microcystis*, and *Leptospira* genus. In the case of *Flavobacterium*, it has been reported that the species *Flavobacterium psychrophilum* can cause flavobacteriosis disease, being the second cause of mortality in fry and salmonids in culture center (Martínez, 2018). In addition, pathogenic species of the genus *Leptospira* are involved in a zoonosis with a worldwide distribution that occurs mainly in tropical, subtropical, and temperate zones (Céspedes, 2005). In this context, mammals such as horses, cows, dogs, cats, pigs, and rodents are highly relevant in the transmission of this pathogen because they are asymptomatic carriers (Faine, 1994). However, several reports suggest that animal health is not necessarily impaired only by pathogenic microorganisms, but also by microorganisms capable of producing toxic substances or products with noxious effects (Wood, 2016).

Cyanobacteria are widely distributed oxygenic, phototrophic microorganisms. Owing to eutrophication and the progressive increase in temperature associated with climate change, an increase in cyanobacterial blooms has been reported in marine and freshwater ecosystems worldwide (Markensten *et al*., 2010; Paerl & Huisman, 2008, 2009). One of the major concerns related to the presence of these microorganisms is the ability of some species to produce toxins (cyanotoxins) that can affect humans and animals as well as drastically decrease the health and quality of water bodies (Agrawal *et al*., 2006; Briand *et al*., 2003, 2005; Codd *et al*., 1999; Codd *et al*., 2005; Dadheech *et al*., 2001; Oberhaus *et al*., 2007). Cyanotoxins that damage the liver and/or nervous system are classified as hepatotoxins and neurotoxins (Dittmann *et al*., 2013). It has been reported that hepatotoxic and neurotoxic cyanotoxins can cause acute lethal poisoning (Roset *et al*., 2001). Microcystins are among the most common and toxic cyanotoxins in water bodies (Chorus & Welker, 2021), and toxins produced by members of the genus *Microcystis*. Microcystins can accumulate in environmentally exposed animal species, subsequently entering the food chain (Gkelis *et al*., 2006; Papadimitriou *et al*., 2010; Peng *et al*., 2010; Xie *et al*., 2005; Zhang *et al*., 2007, 2009) and affecting a wide variety of species. Therefore, the presence of microorganisms with noxious effects in water bodies is an important public health concern.

Urban wetlands are ecosystems located within an urban radius where a wide variety of animal species can interact (Jisha & Puthur, 2021). However, in very few of these ecosystems, initiatives aimed at the identification, monitoring, and risk assessment against the presence of bacterial genera that are pathogenic or pose any potential risk to other animal species have been conducted. Currently, massive sequencing tools are widely used in the study of microbial communities in different environments and allow, among other things, the detection of pathogens (Bass *et al*., 2023). In this study, we used amplicon sequencing to describe the composition of microorganisms in three urban wetlands in the city of Llanquihue, which may represent a risk factor for animal health.

MATERIAL AND METHODS

Samples

Water samples were collected from three wetlands in the city of Llanquihue (Figure 1), located in northern Patagonia, southern Chile. A volume of 2 liters of water samples was collected from the wetlands El Loto (41°15'16.4 "S 73°00'32.9 "W), Baquedano (41°15'01.2 "S 73°00'31.9 "W), and Las Ranas (41°15'43.4 "S 73°00'24.1 "W) in two different points of each wetland, using sterile glass bottles. The samples were recovered on May 5, 2021. The samples were transported using ice packs at 4°C for processing at the Institutional Laboratory of the Universidad San Sebastián, located in the city of Puerto Montt. The water was passed through MCE (Mixed Cellulose Ester) filters of 0.22 µm pore size, using a vacuum filtration system. The filters with biological material were stored in RNA Later (SIGMA, USA) at -20 °C until nucleic acid extraction.

Genomic DNA extraction

To recover gDNA, the filters were suspended in TRIS-EDTA-NaCl (STE) buffer at concentrations of 200 mM TRIS, 200 mM NaCl, and 20 mM EDTA prepared in DNAse-free water. Enzymatic digestion of the cell wall was performed using 20 µL lysozyme (20 mg/mL) and 20 µL proteinase K (20 mg/mL), incubated for 1 h at 37 °C, and then the temperature was raised to 55 °C for an additional hour. During incubation, the solution was vortexed to allow the biological material of microorganisms to be released from the filters. The obtained solution was used for gDNA extraction using affinity columns provided in the AccuPrep® Genomic DNA Extraction kit (BIONEER, Korea), following the manufacturer's instructions.

DNA Amplicon Sequencing

A minimum of 200 ng of total DNA was sent to Novogene Sequencing Service (USA) for DNA amplicon sequencing of the 16S rRNA taxonomic marker. For this, the V3-V4 region (F: CCTAYGGGRBGCASCAG, R: GGACTACNNGGGTATCTAAT) was amplified under previously established conditions (Behrendt *et al*., 2011). The amplicons obtained were sequenced using Illumina Novaseq 6000 equipment. Sequencing was performed using paired ends with a length of 150 bp (150 bp paired-end) and an output of 3 gigabases per sample. Sequences were delivered in FASTQ format.

Bioinformatic Analysis

The obtained sequences were imported into the R language version 4.1.0 (R Core Team, 2013) to be processed following the DADA2 v1.16 pipeline (Callahan *et al*., 2016) using the RStudio environment (Booth *et al*., 2018). The preprocessing performed included inspecting quality profiles, filtering, and trimming of low-quality data. The obtained files were used to learn the error rates and generate an error model based on the data structure. Subsequently, the information obtained was combined and a sequence table was constructed to remove chimeric artifacts resulting from PCR amplification.

Taxonomic assignment was performed using the SILVA SSU V138 database (Quast *et al*., 2013). The non-rarefied information was used to construct a phyloseq object (McMurdie & Holmes, 2013) to estimate taxonomic abundance and microbial diversity. The Microbiome package was used to estimate the core diversity (Lahti *et al*., 2017). Finally, all data were plotted using the ggplot2 library (Ginestet, 2011) following the instructions of the phyloseq package (McMurdie & Holmes, 2013). Relative abundance was plotted using the average of the replicates from each site.

RESULTS AND DISCUSSION

Microbial diversity of urban wetlands from Llanquihue City

Using 16S rRNA amplicon sequencing of water samples from the El Loto, Las Ranas, and Baquedano wetlands, we performed taxonomic assignment of microbes that inhabit these ecosystems. After describing the high diversity of microorganisms at the phylum level, sequencing data were separated into independent sets for each wetland studied (Figure 1A). The relative abundance at the phylum level was estimated for each wetland (Figure 1B). The results indicated a heterogeneous representation of the diversity of microorganisms present in the three water bodies considered in this study, with Las Ranas being the wetland with the highest diversity (Figure 1C). It is worth noting the high abundance of cyanobacteria in the El Loto wetland, which was considerably higher than that in the Baquedano and Las Ranas wetlands. Additionally, other phyla including potentially pathogenic species such as *Actinobacteriota*, *Spirochaetota*, and *Bacteroidota* were observed.

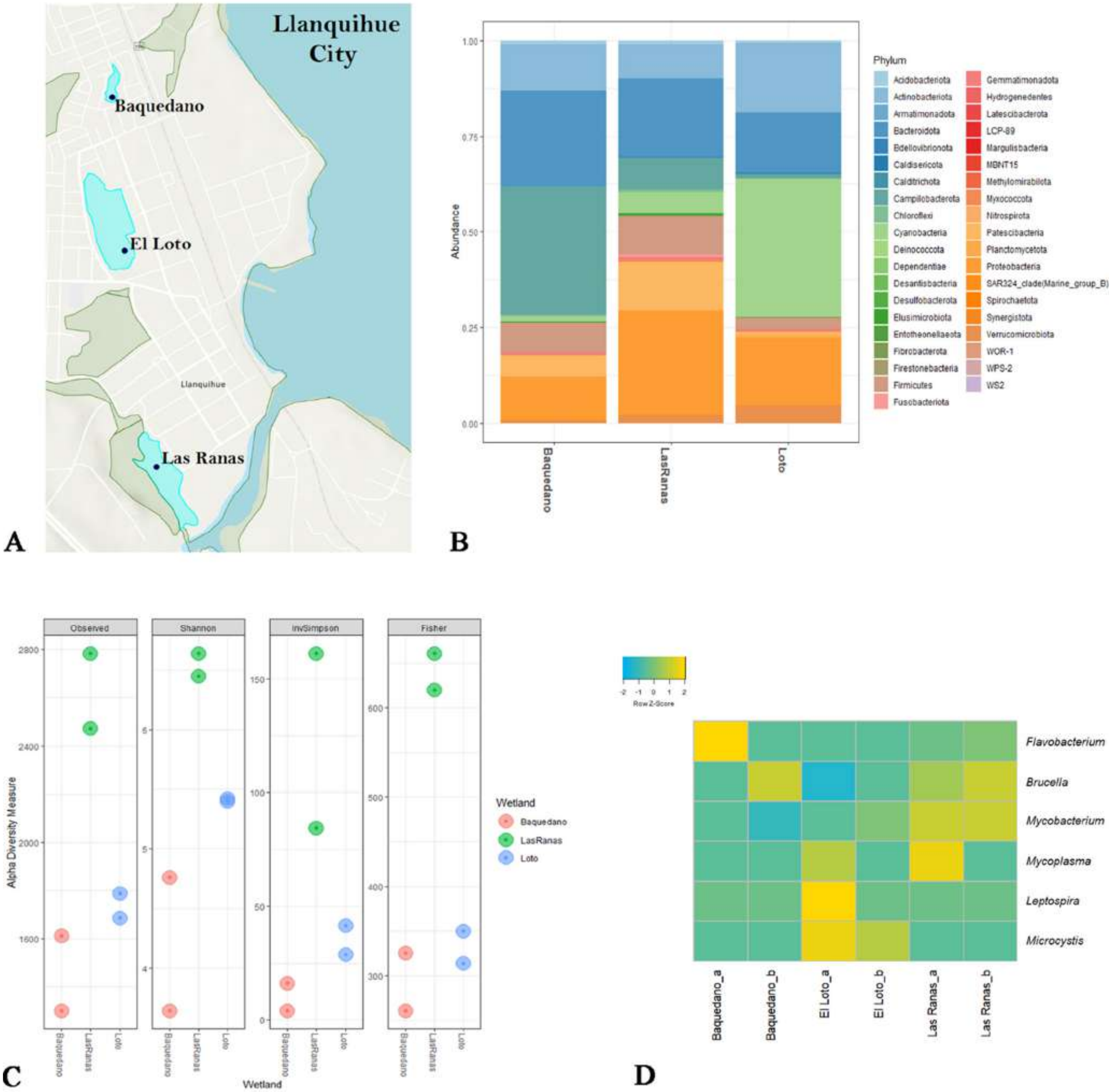

Figure 1.

A) Three Urban wetlands located in Llanquihue city were selected for microbiological description. Baquedano wetland (1, 41°15'01.2"S 73°00'31.9"W), El Loto wetland (2, 41°15'16.4"S 73°00'32.9"W), Las Ranas wetland (3, 41°15'43.4"S 73°00'24.1"W). B) Relative abundance at phylum level obtained from urban wetlands. C) Measure of alpha diversity. D) Z-score of relative abundance of genera that may be associated with MDR or with harmful species.

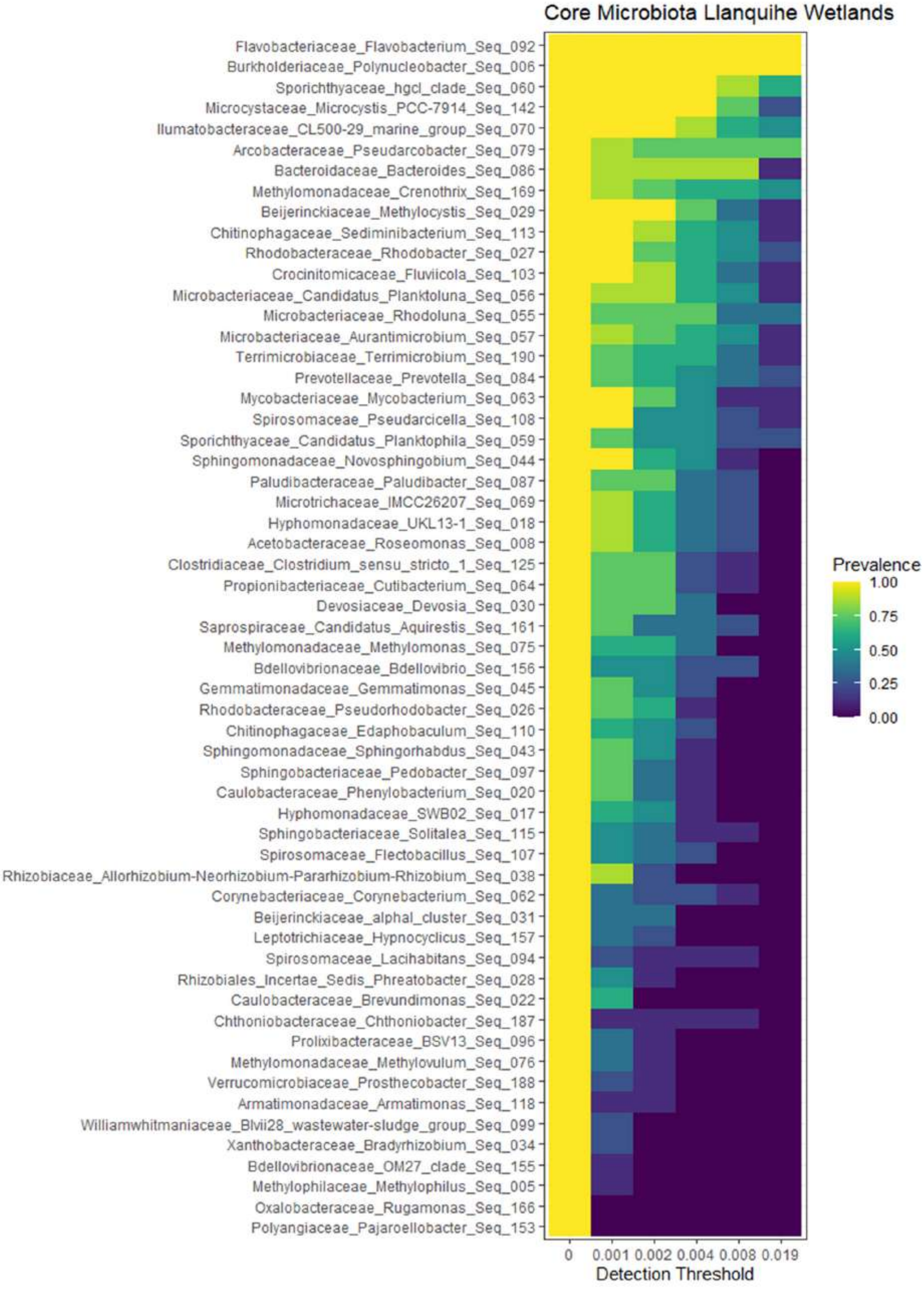

Figure 2.
Core diversity analysis of the three urban wetlands. Taxonomic abundance data obtained from the wetlands were used to estimate the core of the most prevalent microorganisms inhabiting wetlands. The detected genera are ordered along the Y-axis

When examining the taxonomic classification at lower levels, we found the presence of genera that possess species with pathogenic potential, which are even present in the list of MRD. Figure 1D shows the presence of these genera in the three wetlands: *Mycobacterium*, *Brucella*, *Mycoplasma*, *Leptospira*, *Flavobacterium*, and *Microcystis*. It is well known that a variety of species belonging to these genera have serious implications on health (for reviews, please see Bierque *et al*., 2020; Byndloss & Tsolis, 2016; Dow & Alvarez, 2022; Evangelista & Coburn, 2010; Garvey, 2020; Hilborn & Beasley, 2015; Irshath *et al*., 2023; Loch & Faisal, 2015; Olea-Popelka *et al*., 2017; Olsen & Palmer, 2014). Taken together, the presence of these bacterial genera corresponds to the first warning signal for the development of preventive measures.

Analysis of the Core diversity of the microbiota

Using the relative taxonomic abundance obtained, an analysis was carried out to identify the microorganisms that make up the microbial core, which consisted of the most prevalent shared microorganisms between niches (Shetty *et al*., 2017) in the three Llanquihue wetlands analyzed (Figure 2). The results indicate that cyanobacteria belonging to the taxa *Flavobacterium*, *Polynucleobacter*, *Sporichthyaceae*, and *Microcystis* are the four top bacterial groups, which include species of health concern, such as *Flavobacterium* (Nematollahi *et al*., 2003) and *Microcystis* (Carmichael, 1996). Interestingly, wetlands include genera that own species with pathogenic potential, such as *Roseomonas* and *Clostridium* (Mitchell *et al*., 2022; Loch & Faisal, 2015; Nematollahi *et al*., 2003). In addition, the genus *Microcystis* has toxicological potential (Carmichael, 1996).

The high abundance of genera with sanitary relevance, such as *Microcystis* and *Flavobacterium*, suggests that these water bodies can be reservoirs and dispersion media for MDRs and noxious species. In the case of *Microcystis* genera, there are few reports in Chile about the occurrence of a toxic bloom of *Microcystis aeruginosa* in lakes of the Biobío region, demonstrating that blooms occur periodically throughout the time, forming cumulative blooms during the summer; during the rest of the year, they can form a dispersive bloom (Almanza *et al*., 2016). Additionally, Nimptsch *et al.* (2016) detected toxins associated with cyanobacterial blooms in lakes in northern Chilean Patagonia. However, no reports are available on the urban wetlands in the Los Lagos region. In addition, there is no database in this region with reports of animal toxicosis or human illnesses associated with ingestion of toxin-producing cyanobacteria. Nevertheless, it is necessary to develop preventive monitoring strategies for potentially toxic cyanobacterial species, as this phenomenon is on the rise worldwide and its impact on health, the environment, and the economy can be harmful, mainly because the management of *Microcystis* blooms is complex (Wilhelm *et al*., 2020).

It is advisable to take preventive rather than reactionary action. In the microbial core, we only found the presence of *Bacteroides* and *Prevotella* (Figure 2). We detected the presence of genera whose species are described as belonging to intestinal microbiota, such as *Prevotella* (Tett *et al*., 2021), *Coprococcus* (Holdeman & Moore, 1974), *Bifidobacterium* (Bunesova *et al*., 2014), and *Ruminococcus* (La Reau & Suen, 2018), which are in concordance with the fecal presence in the studied urban wetlands (data not shown).

Currently, the One Health perspective for research that unifies animal, human, and environmental health, requires multidisciplinary approaches to deepen the understanding of microbial communities that inhabit a given environment (Oliveira *et al*., 2023; Banerjee & van der Heijden, 2023; Zinsstag *et al*., 2018; Farschtschi *et al*., 2022; Hilborn & Beasley, 2015). An increasing amount of evidence suggests that experimental strategies including global analyses of DNA, RNA, metabolites, and proteins (areas of research classified as “omics”) promising alternatives to conducting One Health studies (Gruszecka-Kosowska *et al*., 2022; Tigistu-Sahle *et al*., 2023) and the possibility to assess these studios in a variety of environments, ecosystems or conditions. Nevertheless, the presence of genera associated with MRD or noxious species raises the possibility that the same event may occur in other cities located around water bodies, which could affect the health of its inhabitants. Fortunately, the rise of molecular and DNA sequencing tools offers a new range of possibilities for the development of management strategies and monitoring of environmental microbial threats.

Further research is required to identify which species associated with MRD can be found in other water bodies in the region. It is necessary to implement techniques that allow characterization of the presence of microorganisms with sufficient resolution to determine the species inhabiting these ecosystems. Because water bodies are complex environments with a variety of microbial communities inhabiting them, Shotgun Metagenomics Sequencing may be an alternative to carry out deeper microbial and functional characterization of environmental communities. These methodologies are used for the massive detection of pathogen genomes in different environments and enable the study of interactions among human, animal, and environmental microbiomes (Trinh *et al.* 2018).

Authors’ contributions

JDR wrote the manuscript and discussed results. DAM directed the study, conceptualized the experiments, performed bioinformatics analyses, and edited the manuscript. CO performed the experiments and conceptualized the figures. VC and CM contributed to reviewing the manuscript and discussed results. All authors agree with the final version of the manuscript.

Acknowledgments

This research was funded through the FONDECYT Iniciación [#11230295] and FOVI220211 (DAM) and by the Universidad San Sebastián by grants VRID-PDOC22/06 (JDR) and VRID_FAPPE21-07 (DAM). Also, this work was supported by ‘Proyectos Colaborativos 2021 #2034’ from the ‘Vicerrectoría de Vinculación con el Medio’, and the ‘Vicerrectoría de Investigación y Doctorados’ – Fondo VRID_APC23/23, Universidad San Sebastián.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

REFERENCES

Agrawal, M. K., Ghosh, S. K., Divya, B., Juergen, W., Marcel, E., & Bagchi, S. N. (2006). Occurrence of Microcystin-Containing Toxic Water Blooms in Central India. *Journal of Microbiology and Biotechnology*, *16*(2), 212–218. https://www.koreascience.or.kr/article/JAKO200617033429134.page

Akinnawo, S. O. (2023). Eutrophication: Causes, consequences, physical, chemical and biological techniques for mitigation strategies. *Environmental Challenges*, *12*, 100733. https://doi.org/10.1016/j.envc.2023.100733

Almanza, V., Parra, O., Bicudo, C. E. De M., Baeza, C., Beltran, J., Figueroa, R., & Urrutia, R. (2016). Occurrence of toxic blooms of *Microcystis aeruginosa* in a central Chilean (36° Lat. S) urban lake. *Revista Chilena de Historia Natural* , *89*, 1–12. https://doi.org/10.1186/s40693-016-0057-7

Amorim, C. A., & Moura, A. do N. (2021). Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning. *Science of the Total Environment*, *758*, 143605. https://doi.org/10.1016/j.scitotenv.2020.143605

Banerjee, S., & van der Heijden, M. G. A. (2023). Soil microbiomes and one health. *Nature Reviews Microbiology*, *21*(1), 6–20. https://doi.org/10.1038/s41579-022-00779-w

Bass, D., Christison, K. W., Stentiford, G. D., Cook, L. S. J., & Hartikainen, H. (2023). Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. *Trends in Parasitology*, *39*(4), 285–304. https://doi.org/10.1016/j.pt.2022.12.010

Behrendt, L., Larkum, A. W. D., Trampe, E., Norman, A., Sørensen, S. J., & Kühl, M. (2011). Microbial diversity of biofilm communities in microniches associated with the didemnid ascidian *Lissoclinum patella*. *The ISME Journal*, *6*(6), 1222–1237. https://doi.org/10.1038/ismej.2011.181

Bhagowati, B., & Ahamad, K. U. (2019). A review on lake eutrophication dynamics and recent developments in lake modeling. *Ecohydrology & Hydrobiology*, *19*(1), 155–166. https://doi.org/10.1016/j.ecohyd.2018.03.002

Bierque, E., Thibeaux, R., Girault, D., Soupé-Gilbert, M.-E., & Goarant, C. (2020). A systematic review of *Leptospira* in water and soil environments. *PloS One*, *15*(1), e0227055. https://doi.org/10.1371/journal.pone.0227055

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* , *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Booth, Szmidt-Middleton, King, & Westbrook. (2018). RStudio: integrated development for R. Nature.

Briand, J.-F., Jacquet, S., Bernard, C., & Humbert, J.-F. (2003). Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water ecosystems. *Veterinary Research*, *34*(4), 361–377. https://doi.org/10.1051/vetres:2003019

Briand, J.-F., Jacquet, S., Flinois, C., Avois-Jacquet, C., Maisonnette, C., Leberre, B., & Humbert, J.-F. (2005). Variations in the microcystin production of *Planktothrix rubescens* (cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. *Microbial Ecology*, *50*(3), 418–428. https://doi.org/10.1007/s00248-005-0186-z

Bunesova, V., Vlkova, E., Rada, V., Killer, J., & Musilova, S. (2014). Bifidobacteria from the gastrointestinal tract of animals: differences and similarities. *Beneficial Microbes*, *5*(4), 377–388. https://doi.org/10.3920/BM2013.0081

Byndloss, M. X., & Tsolis, R. M. (2016). *Brucella* spp. Virulence Factors and Immunity. *Annual Review of Animal Biosciences*, *4*, 111–127. https://doi.org/10.1146/annurev-animal-021815-111326

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Carmichael, W. W. (1996). Toxic Microcystis and the environment. In: Watanabe, M. F. & Fujiki, H., (Eds.), T*oxic Microcystis*, CRC Press, Boca Raton, pp. 1–11.

Carvalho, L., Miller nee Ferguson, C. A., Scott, E. M., Codd, G. A., Davies, P. S., & Tyler, A. N. (2011). Cyanobacterial blooms: statistical models describing risk factors for national-scale lake assessment and lake management. *Science of the Total Environment*, *409*(24), 5353–5358. https://doi.org/10.1016/j.scitotenv.2011.09.030

Céspedes Z, M. (2005). Leptospirosis: Enfermedad Zoonótica Emergente. *Revista Peruana de Medicina Experimental y Salud Publica*, *22*(4), 290–307. http://www.scielo.org.pe/scielo.php?pid=S1726-46342005000400008&script=sci_arttext

Chislock, M. F., Doster, E., & Zitomer, R. A. (2013). Eutrophication: causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*. https://www.wilsonlab.com/wp-content/uploads/2021/05/2013_NE_Chislock_et_al.pdf

Chorus, I., & Welker, M. (2021). *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management* (2nd ed.). CRC Press, London. https://doi.org/10.1201/9781003081449

Codd, G. A., Lindsay, J., Young, F. M., Morrison, L. F., & Metcalf, J. S. (2005). Harmful cyanobacteria: from mass mortalities to management measures. In: Huisman, J., Matthijs, H. C. P., & Visser, P. M. (Eds.), *Harmful cyanobacteria*. Springer Dordrecht, pp. 1–23. https://doi.org/10.1007/1-4020-3022-3

Codd, G., Bell, S., Kaya, K., Ward, C., Beattie, K., & Metcalf, J. (1999). Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology*, *34*(4), 405–415. https://doi.org/10.1080/09670269910001736462

Decreto 389 Exento [Ministerio de Agricultura] Enfermedades De Declaración Obligatoria Para La Aplicación De Medidas Sanitarias Y Deroga Decretos Que Indica. 14-NOV-2014. https://bcn.cl/2rlwg

Dittmann, E., Fewer, D. P., & Neilan, B. A. (2013). Cyanobacterial toxins: biosynthetic routes and evolutionary roots. *FEMS Microbiology Reviews*, *37*(1), 23–43. https://doi.org/10.1111/j.1574-6976.2012.12000.x

Dreher, T. W., Collart, L. P., Mueller, R. S., Halsey, K. H., Bildfell, R. J., Schreder, P., Sobhakumari, A., & Ferry, R. (2019). *Anabaena/Dolichospermum* as the source of lethal microcystin levels responsible for a large cattle toxicosis event. *Toxicon: X*, 1, 100003. https://doi.org/10.1016/j.toxcx.2018.100003

Evangelista, K. V., & Coburn, J. (2010). *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology*, *5*(9), 1413–1425. https://doi.org/10.2217/fmb.10.102

Faine, S. (1994). *Leptospira and leptospirosis*. CRC Press Inc.

Farschtschi, S., Riedmaier-Sprenzel, I., Phomvisith, O., Gotoh, T., & Pfaffl, M. W. (2022). The successful use of -omic technologies to achieve the "One Health" concept in meat producing animals. *Meat Science*, *193*, 108949. https://doi.org/10.1016/j.meatsci.2022.108949

Garvey, M. (2020). *Mycobacterium Avium Paratuberculosis*: A Disease Burden on the Dairy Industry. *Animals*, *10*(10), 1773. https://doi.org/10.3390/ani10101773

Ginestet, C. (2011). ggplot2: Elegant Graphics for Data Analysis. *Journal of the Royal Statistical Society*. *Series A* , *174*(1), 245–246. https://ideas.repec.org/a/bla/jorssa/v174y2011i1p245-246.html

Gkelis, S., Lanaras, T., & Sivonen, K. (2006). The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. *Aquatic Toxicology* , *78*(1), 32–41. https://doi.org/10.1016/j.aquatox.2006.02.001

Gruszecka-Kosowska, A., Ampatzoglou, A., & Aguilera, M. (2022). Integration of Omics Approaches Enhances the Impact of Scientific Research in Environmental Applications. *International Journal of Environmental Research and Public Health*, *19*(14). https://doi.org/10.3390/ijerph19148758

Hilborn, E. D., & Beasley, V. R. (2015). One health and cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. *Toxins*, *7*(4), 1374–1395. https://doi.org/10.3390/toxins7041374

Holdeman, L. V., & Moore, W. E. C. (1974). New genus, *Coprococcus*, twelve new species, and emended descriptions of four previously described species of bacteria from human feces. *International Journal of Systematic Bacteriology*, *24*(2), 260–277. https://doi.org/10.1099/00207713-24-2-260

Huang, J., Ghaly, M., Hobson, P., & Chow, C. W. K. (2022). Innovative method of utilising hydrogen peroxide for source water management of cyanobacteria. *Environmental Science and Pollution Research International*, *29*(15), 22651–22660. https://doi.org/10.1007/s11356-021-17511-5

Ibelings, B. W., Fastner, J., Bormans, M., & Visser, P. M. (2016). Cyanobacterial blooms. Ecology, prevention, mitigation and control: Editorial to a CYANOCOST Special Issue. *Aquatic Ecology*, *50*(3), 327–331. https://doi.org/10.1007/s10452-016-9595-y

Irshath, A. A., Rajan, A. P., Vimal, S., Prabhakaran, V.-S., & Ganesan, R. (2023). Bacterial Pathogenesis in Various Fish Diseases: Recent Advances and Specific Challenges in Vaccine Development. *Vaccines*, *11*(2). https://doi.org/10.3390/vaccines11020470

Jančula, D., & Maršálek, B. (2011). Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. *Chemosphere*, *85*(9), 1415–1422. https://doi.org/10.1016/j.chemosphere.2011.08.036

Jisha, K. C., & Puthur, J. T. (2021). Ecological importance of wetland systems. In: Sharma, S., & Singh, P. (Eds.). *Wetlands Conservation*, pp. 40–54. Wiley. https://doi.org/10.1002/9781119692621.ch3

Khan, M. N., & Mohammad, F. (2014). Eutrophication: challenges and solutions. In: Ansari, A., Gill, S. (Eds.), *Eutrophication: Causes, Consequences and Control*, pp. 1-15. https://doi.org/10.1007/978-94-007-7814-6_1

La Reau, A. J., & Suen, G. (2018). The Ruminococci: key symbionts of the gut ecosystem. *Journal of Microbiology* , *56*(3), 199–208. https://doi.org/10.1007/s12275-018-8024-4

Lahti, L., Shetty, S., Blake, T., & Salojarvi, J. (2017). *Tools for microbiome analysis in R*. http://microbiome.github.com/microbiome

Lapointe, B. E., Herren, L. W., Debortoli, D. D., & Vogel, M. A. (2015). Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. *Harmful Algae*, *43*, 82–102. https://doi.org/10.1016/j.hal.2015.01.004

Loch, T. P., & Faisal, M. (2015). Emerging flavobacterial infections in fish: A review. *Journal of Advertising Research*, *6*(3), 283–300. https://doi.org/10.1016/j.jare.2014.10.009

Markensten, H., Moore, K., & Persson, I. (2010). Simulated lake phytoplankton composition shifts toward cyanobacteria dominance in a future warmer climate. *Ecological Applications*, *20*(3), 752–767. https://doi.org/10.1890/08-2109.1

Martinez, A. (2018). *Enfermedades infecciosas que afectan la producción de smolts en Chile Laboratorio Antares S.A. Salmonexpert*. Accessed on May 4 2023 de https://www.salmonexpert.cl/enfermedades-infecciosas/1236500

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mitchell, M., Nguyen, S. V., Macori, G., Bolton, D., McMullan, G., Drudy, D., & Fanning, S. (2022). *Clostridioides difficile* as a Potential Pathogen of Importance to One Health: A Review. *Foodborne Pathogens and Disease*, *19*(12), 806–816. https://doi.org/10.1089/fpd.2022.0037

Moore, C. E., Juan, J., Lin, Y., Gaskill, C. L., & Puschner, B. (2016). Comparison of Protein Phosphatase Inhibition Assay with LC-MS/MS for Diagnosis of Microcystin Toxicosis in Veterinary Cases. *Marine Drugs*, *14*(3). https://doi.org/10.3390/md14030054

Nematollahi, A., Decostere, A., Pasmans, F., & Haesebrouck, F. (2003). *Flavobacterium psychrophilum* infections in salmonid fish. *Journal of Fish Diseases*, *26*(10), 563–574. https://doi.org/10.1046/j.1365-2761.2003.00488.x

Nimptsch, J., Woelfl, S., Osorio, S., Valenzuela, J., Moreira, C., Ramos, V., Castelo-Branco, R., Leão, P. N., & Vasconcelos, V. (2016). First record of toxins associated with cyanobacterial blooms in oligotrophic North Patagonian lakes of Chile-a genomic approach. *International Review of Hydrobiology*, *101*(1-2), 57–68. https://doi.org/10.1002/iroh.201401780

Oberhaus, L., Gélinas, M., Pinel-Alloul, B., Ghadouani, A., & Humbert, J.-F. (2007). Grazing of two toxic *Planktothrix* species by *Daphnia pulicaria:* potential for bloom control and transfer of microcystins. *Journal of Plankton Research*, *29*(10), 827–838. https://doi.org/10.1093/plankt/fbm062

Olea-Popelka, F., Muwonge, A., Perera, A., Dean, A. S., Mumford, E., Erlacher-Vindel, E., Forcella, S., Silk, B. J., Ditiu, L., El Idrissi, A., Raviglione, M., Cosivi, O., LoBue, P., & Fujiwara, P. I. (2017). Zoonotic tuberculosis in human beings caused by *Mycobacterium bovis*—a call for action. *The Lancet Infectious Diseases*, *17*(1), e21–e25. https://doi.org/10.1016/S1473-3099(16)30139-6

Oliveira, M. M. E., Lopes, A. P., Pinto, T. N., da Costa, G. L., Goes-Neto, A., & Hauser-Davis, R. A. (2023). A Novel One Health Approach concerning Yeast Present in the Oral Microbiome of the Endangered Rio Skate (*Rioraja agassizii*) from Southeastern Brazil. *Microorganisms*, *11*(8), 1969. https://doi.org/10.3390/microorganisms11081969

Olsen, S. C., & Palmer, M. V. (2014). Advancement of knowledge of *Brucella* over the past 50 years. *Veterinary Pathology*, *51*(6), 1076–1089. https://doi.org/10.1177/0300985814540545

Paerl, H. W., Gardner, W. S., McCarthy, M. J., Peierls, B. L., & Wilhelm, S. W. (2014). Algal blooms: Noteworthy nitrogen. *Science*, *346*(6206), 175. https://doi.org/10.1126/science.346.6206.175-a

Paerl, H. W., & Huisman, J. (2008). Blooms like it hot. *Science*, *320*(5872), 57–58. https://doi.org/10.1126/science.1155398

Paerl, H. W., & Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, *1*(1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x

Papadimitriou, T., Kagalou, I., Bacopoulos, V., & Leonardos, I. D. (2010). Accumulation of microcystins in water and fish tissues: an estimation of risks associated with microcystins in most of the Greek Lakes. *Environmental Toxicology*, *25*(4), 418–427. https://doi.org/10.1002/tox.20513

Parlapani, F. F., Boziaris, I. S., & Mireles DeWitt, C. A. (2023). Pathogens and their sources in freshwater fish, sea finfish, shellfish, and algae. In: Knowles, M. E., Anelich, L. E., Boobis, A. R., & Popping, B. (Eds.), *Present Knowledge in Food Safety*, pp. 471–492. Academic Press. https://doi.org/10.1016/B978-0-12-819470-6.00056-1

Parulekar, N. N., Kolekar, P., Jenkins, A., Kleiven, S., Utkilen, H., Johansen, A., Sawant, S., Kulkarni-Kale, U., Kale, M., & Sæbø, M. (2017). Characterization of bacterial community associated with phytoplankton bloom in a eutrophic lake in South Norway using 16S rRNA gene amplicon sequence analysis. *PloS One*, *12*(3), e0173408. https://doi.org/10.1371/journal.pone.0173408

Peng, L., Liu, Y., Chen, W., Liu, L., Kent, M., & Song, L. (2010). Health risks associated with consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: significance for freshwater aquacultures. *Ecotoxicology and Environmental Safety*, *73*(7), 1804–1811. https://doi.org/10.1016/j.ecoenv.2010.07.043

Pitois, S., Jackson, M. H., & Wood, B. J. (2001). Sources of the eutrophication problems associated with toxic algae: an overview. *Journal of Environmental Health*, *64*(5), 25–32. https://www.ncbi.nlm.nih.gov/pubmed/11764678 PMID: 11764678

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(Database issue), D590–D596. https://doi.org/10.1093/nar/gks1219

Rankin, K. A., Alroy, K. A., Kudela, R. M., Oates, S. C., Murray, M. J., & Miller, M. A. (2013). Treatment of cyanobacterial (microcystin) toxicosis using oral cholestyramine: case report of a dog from Montana. *Toxins*, *5*(6), 1051–1063. https://doi.org/10.3390/toxins5061051

R Core Team. (2013). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. http://www.R-project.org/

Roset, J., Aguayo, S., & Muñoz, M. J. (2001). Cyanobacteria and toxins detection. A review. *Revista de Toxicologia 18*(2), 65-71 https://digital.csic.es/handle/10261/294473

Servicio Agrícola y Ganadero (2019). SAG: Lista de enfermedades de denuncia obligatoria https://www.sag.gob.cl/sites/default/files/enfermedades_denuncia_obligatoria_sag_9-10-2019.pdf

Shetty, S. A., Hugenholtz, F., Lahti, L., Smidt, H., & de Vos, W. M. (2017). Intestinal microbiome landscaping: insight in community assemblage and implications for microbial modulation strategies. *FEMS microbiology reviews*, *41*(2), 182-199. https://doi.org/10.1093/femsre/fuw045

Tett, A., Pasolli, E., Masetti, G., Ercolini, D., & Segata, N. (2021). *Prevotella* diversity, niches and interactions with the human host. *Nature Reviews Microbiology*, *19*(9), 585–599. https://doi.org/10.1038/s41579-021-00559-y

Tigistu-Sahle, F., Mekuria, Z. H., Satoskar, A. R., Sales, G. F. C., Gebreyes, W. A., & Oliveira, C. J. B. (2023). Challenges and opportunities of molecular epidemiology: using omics to address complex One Health issues in tropical settings. *Frontiers in Tropical Diseases*, *4*. https://doi.org/10.3389/fitd.2023.1151336

Trinh, P., Zaneveld, J. R., Safranek, S., & Rabinowitz, P. M. (2018). One Health Relationships Between Human, Animal, and Environmental Microbiomes: A Mini-Review. *Frontiers in Public Health*, *6*, 235. https://doi.org/10.3389/fpubh.2018.00235

van der Merwe, D., Sebbag, L., Nietfeld, J. C., Aubel, M. T., Foss, A., & Carney, E. (2012). Investigation of a *Microcystis aeruginosa* cyanobacterial freshwater harmful algal bloom associated with acute microcystin toxicosis in a dog. *Journal of Veterinary Diagnostic Investigation*, *24*(4), 679–687. https://doi.org/10.1177/1040638712445768

Walters, S. P., Thebo, A. L., & Boehm, A. B. (2011). Impact of urbanization and agriculture on the occurrence of bacterial pathogens and stx genes in coastal waterbodies of central California. *Water Research*, *45*(4), 1752–1762. https://doi.org/10.1016/j.watres.2010.11.032

Wang, Z., Akbar, S., Sun, Y., Gu, L., Zhang, L., Lyu, K., Huang, Y., & Yang, Z. (2021). Cyanobacterial dominance and succession: Factors, mechanisms, predictions, and managements. *Journal of Environmental Management*, *297*, 113281. https://doi.org/10.1016/j.jenvman.2021.113281

Wilhelm, S. W., Bullerjahn, G. S., & McKay, R. M. L. (2020). The Complicated and Confusing Ecology of Microcystis Blooms. *mBio*, *11*(3). https://doi.org/10.1128/mBio.00529-20

Wood, R. (2016). Acute animal and human poisonings from cyanotoxin exposure - A review of the literature. *Environment International*, *91*, 276–282. https://doi.org/10.1016/j.envint.2016.02.026

Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y., & Park, H.-D. (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Environmental Toxicology*, *20*(3), 293–300. https://doi.org/10.1002/tox.20120

Zamyadi, A., Choo, F., Newcombe, G., Stuetz, R., & Henderson, R. K. (2016). A review of monitoring technologies for real-time management of cyanobacteria: Recent advances and future direction. *Trends in Analytical Chemistry: TrAC*, *85*(A), 83–96. https://doi.org/10.1016/j.trac.2016.06.023

Zedler, J. B., & Kercher, S. (2005). WETLAND RESOURCES: Status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, *30*(1), 39–74. https://doi.org/10.1146/annurev.energy.30.050504.144248

Zhang, D., Xie, P., Liu, Y., Chen, J., & Liang, G. (2007). Bioaccumulation of the hepatotoxic microcystins in various organs of a freshwater snail from a subtropical Chinese lake, Taihu Lake, with dense toxic Microcystis blooms. *Environmental Toxicology and Chemistry*, *26*(1), 171–176. https://doi.org/10.1897/06-222r.1

Zhang, D., Xie, P., Liu, Y., & Qiu, T. (2009). Transfer, distribution and bioaccumulation of microcystins in the aquatic food web in Lake Taihu, China, with potential risks to human health. *Science of the Total Environment*, *407*(7), 2191–2199. https://doi.org/10.1016/j.scitotenv.2008.12.039

Zinia, N. J., & Kroeze, C. (2015). Future trends in urbanization and coastal water pollution in the Bay of Bengal: the lived experience. *Environment, Development and Sustainability*, *17*, 531–546. https://doi.org/10.1007/s10668-014-9558-1

Zinsstag, J., Crump, L., Schelling, E., Hattendorf, J., Maidane, Y. O., Ali, K. O., Muhummed, A., Umer, A. A., Aliyi, F., Nooh, F., Abdikadir, M. I., Ali, S. M., Hartinger, S., Mäusezahl, D., de White, M. B. G., Cordon-Rosales, C., Castillo, D. A., McCracken, J., Abakar, F., ... Cissé, G. (2018). Climate change and One Health. *FEMS Microbiology Letters*, *365*(11). https://doi.org/10.1093/femsle/fny085