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**Abstract** In this article, we have reviewed the work carried out by the UFRJ microbiology laboratory related to water quality and microorganisms associated with different aquatic ecosystems. We placed water at the center of the One Health concept, due to the integration that water makes between different living beings and the environment. We selected papers published between 2012 and 2022 by UFRJ microbiology laboratory related to bioinformatics genomic and metagenomics analysis. We described the main impacts caused in aquatic environments, about the microorganisms involved in biogeochemical cycles, microorganisms as bioindicators and their resistance genes. Finally, we identified the microorganisms that were most abundant in all the articles studied and pointed out some public policies that we consider important to maintain water quality and reduce anthropic impacts.

**Keywords:** Metagenomic of water, Quality of water, Biogeochemical cycles, Microorganisms

## 1 Introduction

It is well-known that the sustainability of our planet depends on the symbiotic relationship among humans, animals, and the environment. Consequently, human interference in the functioning of the ecosystem determines its importance as an agent in the preservation or extinction of animal and plant species on the planet [Burney and Flannery, 2005]. On the other hand, any environmental change has a direct or indirect effect on human health and its survival as a species [Lu *et al.*, 2008]. In particular, pollution (e.g., atmospheric, water, or soil pollution) is a direct consequence of the growing human potential in industrialization, globalization, and urbanization, which are linked by different ecological cycles [Ghaly and Ramakrishnan, 2015]. The water cycle is an example of this interaction, where pollution accumulated in the atmosphere and soil will eventually reach water bodies [Chaudhry and

Malik, 2017], directly affecting the ecological balance and the life dynamics of living beings in these ecosystems and possibly affecting human health indirectly in the long term [Haseena *et al.*, 2017].

In this context, the concept of One Health emerges in Figure 1, proposing an integrated approach among animal, human and ecosystem health sciences. This concept is recognized as highly relevant by researchers and international organizations such as the World Health Organization (WHO) and the G20 (an intergovernmental forum composed of 19 countries and the European Union) and is also embedded in the United Nations Sustainable Development goals. One Health's concept, which has been widely discussed since the mid-20th century, was improved in 2004 during a global symposium in New York [Panda *et al.*, 2021]. Therefore, the One Health concept is transdisciplinary, proposing a dialogue between different fields to improve and promote human and

animal health [Mazet *et al.*, 2009; Amuasi *et al.*, 2020].

Concerning the One Health concept, water is described in two of the United Nations Sustainable Development Goals: (i) “Clean Water and Sanitation”; and (ii) “Life Underwater”. Water is a vital resource for the survival of all species on the planet and the maintenance of ecosystems, could be used for watering, industry, and livestock. Hence, there is an evident interest in the quality of water resources due to the increase in consumption at a global level along with economic growth and the development of technologies in different areas linked to environmental pollution [Guimarães, 2012].

According to the National Water Agency of Brazil - NWA (Agência Nacional da Água ANA [2019]), only 3% of the water available in the world is freshwater, and just 0.5% is in liquid form and available for human consumption. Also, according to the agency, Brazil owns about 12% of surface fresh water and some of the largest underground water reservoirs. However, even with a spare percentage of water, the country is not free from the scarcity of this resource due to pollution. The other 97% belong to the oceans, which are the main reservoir of water on the planet and play an extremely important role in the hydrological cycle as they provide most (approximately 86%) of the water vapor in the atmosphere. In addition, 78% of the global precipitation occurs over the oceans; therefore, only a small fraction of this evaporated water is deposited on the continents and eventually returns to the sea [Campos, 2014].

The *Conselho Nacional do Meio Ambiente - CONAMA* (National Environment Council - NEC) ensures by law a maximum concentration for each class of pollutants. This is an effort to suppress the excessive release of pollutants and maintain human health and local biodiversity, both in freshwater and saltwater [Brasil, 2005]. Nonetheless, studies reported pollutants that are found bioaccumulating in animal tissues and biomagnifying in the trophic chain of aquatic organisms marketed for consumption [Di Benedetto *et al.*, 2019; Bauer *et al.*, 2021]. This fact has direct consequences for the rates of pollutants present in the human body and its implications for our health [Pamphlett *et al.*, 2021; Mello *et al.*, 2022]. In addition, according to the NWA, in each of the five Brazilian regions, an average of 50% of the population does not have access to basic sanitation. While the South region presents the best rate, where only 32% of the population does not have access to basic sanitation, the North region evidences the worst scenario, with a rate of 67%.

Pollution of aquatic environments has a direct impact on the diversity of microorganisms present in these places, leading to changes in biogeochemical cycles and the health of the aquatic biota. The study of these communities has been growing and has been demonstrated to be an important tool for the bioindication and bioremediation of impacted environments [Lopes *et al.*, 2016a; Campeão *et al.*, 2017; Appolinario *et al.*, 2020a]. Besides that, they are also highly relevant due to the potential to detect species of epidemiological importance. In this context, the use of microbial surveillance in the study of water bodies and wastewater from sewage discharges has gained attention, mainly because it has been proven that microorganisms that cause various diseases can be present in the feces and urine of contaminated individuals [Bacha *et al.*, 2022].

One of the most used approaches to tracing a taxonomic and functional profile of microorganisms is metagenomic analysis, which is a culture-independent technique that uses environmental DNA sequencing. Since only a small percentage of microorganism species in the environment can be identified by traditional and cultivation-dependent techniques, it is estimated that approximately 99% of microorganisms are not cultivable, due to the difficulty of identifying the necessary conditions for their cultivation and growth. Therefore, metagenomics consists of the extraction and direct sequencing of the environmental DNA. From that perspective, some further approaches can be used for analysis. A well-known approach is sequence-based screening, where a gene, a genomic fragment, or even a complete genome can be compared to sequences in a database, looking for sequence similarity or frequency of oligonucleotides (k-mers) [Vergin *et al.*, 1998; Markowitz *et al.*, 2012, 2014; Wood and Salzberg, 2014].

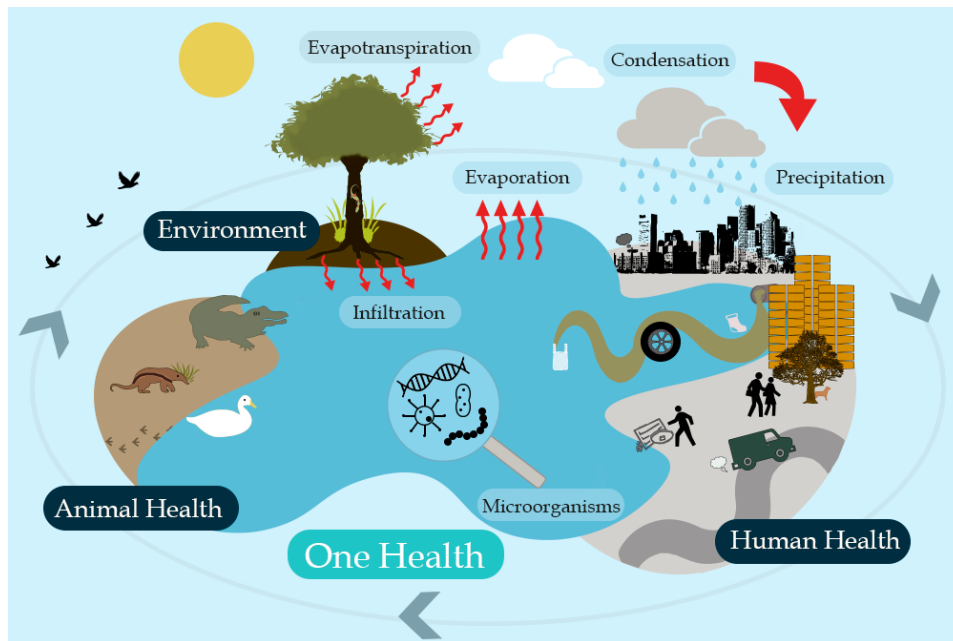
Thus, in this paper, we focused on the results of research on water-related metagenomics carried out by the Microbiology Laboratory at UFRJ and coordinated by Dr. Diogo Antonio Tschoeke and Dr. Fabiano Thompson in the last 10 years. Articles from 2012 to 2022 were selected in PubMed using the keywords “Diogo Tschoeke water”, “Fabiano Thompson water”, “Diogo Tschoeke water metagenomic” and “Fabiano Thompson water metagenomic” to have greater coverage of exclusive works of water-related metagenomics. Different methods of genomic sequencing were used in these articles, mostly using the Illumina MiSeq.

## 2 Impacts caused in aquatic environments

Freshwater is a fundamental resource for our survival as a species. The problem with its use is that the return of this resource to the natural environment is often done incorrectly and is harmful to the ecosystem, generating pollution. One of the most harmful forms of environmental imbalance is the excessive dumping of organic matter into the water. Even though organic nutrients are part of the natural cycle of water bodies [Lam *et al.*, 2020], the excessive availability of nutrients in these environments leads to the eutrophication process, causing an imbalance in the cycling of nutrients, the survival of the biota, and, in the long term, can generate the disappearance of water bodies [Khan and Ansari, 2005].

In eutrophic environments, a boom of cyanobacteria is expected due to the excess of available nutrients, so these bacteria can be used as bioindicators of polluted environments. Walter *et al.* [2021] studied the biogeochemical composition and microbial diversity of five different lakes in Campina Grande, Paraíba, which supplied the water consumption of the nearby population. They identified high nutrient loads, demonstrating that the lakes were under a severe eutrophication process, possibly conditioned by climatic factors and local pollution. This is promoted by the formation of potentially toxic cyanobacterial blooms that could cause disease for local consumers.

Another form of contamination of freshwater bodies by organic components comes from the use of pesticides in agri-



**Figure 1.** One Health concept and relationships between humans, animals, and environments. The wild and domestic animals, plants, humans, microorganisms, and industry use water as a resource, here represented by a lake, which is at the center of the One Health concept. The illustration shows untreated effluents (domestic and industrial) and solid waste being released into bodies of water, which is common in urban environments, especially in developing countries. The water cycle is an example of this interaction, where the pollution accumulated in the atmosphere and in the soil will eventually reach water bodies. The natural environment will make use of this potentially polluted water, as will the urban environment, perpetuating the water cycle with potentially toxic substances. In this context, the growing study of impacts caused in aquatic environments is extremely important for the preservation of human and animal health.

culture. These persistent organic pollutants reach aquatic ecosystems through processes such as leaching, soil surface runoff, and the water cycle. Some components can reach great distances from the place where they were applied due to their transport through the atmosphere and reach water bodies through rain [ChooChuay *et al.*, 2020; Potapowicz *et al.*, 2020]. Some pesticides have carcinogenic potential, high persistence in the environment, and are more complex to be remediated, thus affecting all aquatic life in the place through the absorption of these substances along the trophic chain; DDT (dichlorodiphenyltrichloroethane) is an example of this pesticide [Turusov *et al.*, 2002; Katagi, 2010].

Heavy metals from various sources of contamination have also reached and polluted water bodies over the years. These inorganic elements can bioaccumulate and biomagnify along the food chain, remaining in the environment for long periods [Ali and Khan, 2019]. One of the main sources of contamination by metals is mining. Mine tailings are the main polluters of lead and zinc [Häder *et al.*, 2020; Vendrell-Puigmitja *et al.*, 2020]. These elements are extremely harmful to the health of aquatic biota, causing genetic dysfunctions and decreased growth in invertebrate animals, as well as hyperglycemia, depletion of enzymatic activities, and dysfunction of the vertebrate immune system. Ingestion of contaminated animals can also harm human health [Ali and Khan, 2019; Paschoalini and Bazzoli, 2021].

Pollution by organic or inorganic compounds in freshwater can cause ecological imbalance, loss of diversity of aquatic organisms, and damage to human health [Haseena *et al.*, 2017]. Given the socio-environmental impacts caused, basic sanitation is needed. Unfortunately, this resource does

not reach every part of the globe, resulting in human contamination, and the perpetuation of several diseases, particularly in low-income areas [Silva *et al.*, 2021]. In addition, the quality of water and its treatment are also relevant issues. Studies such as the one carried out by Bacha *et al.* [2022] showed that some components used for water purification can further compromise its quality.

The pollution of water bodies caused by the flow of wastewater, in addition to compromising freshwater environments and the supply of drinking water, goes through the drain to reach marine ecosystems, generating negative impacts on their fauna [Beiras, 2018]. One of the biggest impacts that the oceans are suffering is plastic pollution; about 8 million tons of plastic enter the oceans every year. This pollutant reaches these environments in different ways, such as surface water runoff and fishing activities, but recent studies show that rivers are the dominant route for transporting plastics to the ocean [Helinski *et al.*, 2021; MacLeod *et al.*, 2021]. Among the different sizes of plastic that reach these ecosystems, the ones that are more difficult to remove and therefore cause great impacts on these environments are microplastics. Due to their small size, microplastics are easily found in the environment and can be ingested by aquatic organisms such as mollusks, echinoderms, corals, vertebrate larvae, green sea turtles, cetaceans, and seabirds, causing adverse health effects [Häder *et al.*, 2020].

Another factor that directly impacts the oceans are accidental spills, which can be consequences of offshore oil production [Gomes *et al.*, 2000]. The spillage of oily substances into saltwater has a major impact on marine systems, mangroves, and human health [Berenshtein *et al.*, 2020; Kup-

pusamy *et al.*, 2020; Ribeiro, 2012]. In Brazil, we have had several episodes involving oil spills. In 1997, a remarkable one was the rupture of a Petrobras pipeline, that connects the Duque de Caxias Refinery (Rio de Janeiro State), which caused the leakage of fuel oil into mangroves in Guanabara Bay. Besides that, several other accidents were cited by Kron *et al.* [2019], including the recent appearances of oil on several beaches along the Brazilian coast in 2019 and 2020, where 130 coastal municipalities were affected. Also, oil slicks were found in the São Francisco River, and a total of five thousand tons of oil were removed from the beach nearby mount river [de Oliveira Vicentini *et al.*, 2021].

The burning of fossil fuels over the years and deforestation have increased the availability of carbon dioxide (CO<sub>2</sub>) in the atmosphere. Marine environments are responsible for fixing about a third of the atmospheric carbon released by anthropogenic actions. CO<sub>2</sub> absorption results in an increase in the partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>), lower pH levels, promoting ocean acidification, and lower seawater carbonate saturation, all of which are responsible for controlling the calcification process [Doney *et al.*, 2009; Millero, 2007]. Within this context, red coralline algae (Corallinales, Rhodophyta) represent important targets for ocean acidification research due to their calcium carbonate skeleton. They are photosynthesizers and ecosystem engineers [Amado-Filho *et al.*, 2012]. According to Cavalcanti *et al.* [2018], high levels of CO<sub>2</sub> decrease the availability of carbonate in the water, affecting the calcification of algae.

Ecological interactions in coral reefs can be easily impacted, especially with the imbalance of food chains. Fishing is an important economic activity that has taken place for centuries on different continents [Jackson *et al.*, 2001]. It has already been shown that in unprotected reefs where fish biomass was lower, the cover of fleshy macroalgae was higher. In addition, they have an elevated amount of genomic sequences from archaea, viruses, and bacterial pathogens. In contrast, protected areas have lower *Vibrio* colony counts and a greater abundance of photosynthesis-related genes. Ecological conditions on unprotected reefs can promote the growth and rapid evolution of opportunistic microbial pathogens [Bruce *et al.*, 2012]. The indirect impact of overfishing results in alterations of habitat function and structure, causing an ecological imbalance in the communities [Valentine and Heck, 2005].

### 3 The role of microorganisms in biogeochemical cycles

Microorganisms are fundamental to aquatic ecosystems as they are part of the food chain and biogeochemical cycles linking abiotic and biotic processes [Grossart *et al.*, 2020]. They can be associated with plastic debris and, through metabolic products, contribute to the carbon (C) cycle [Rogers *et al.*, 2020]. Deep ocean gammaproteobacteria SAR324 depends on sulfur oxidation to fuel carbon fixation through the Calvin-Benson-Bassham cycle, changing the concept of carbon fixation in this environment [Coutinho *et al.*, 2018], as well as sulfur (S) producing dimethylsulfoxide (DMS), which is converted into dimethylsul-

fide (DMS) and then transformed into sulfate and other by-products responsible for cloud formation [Li *et al.*, 2021] and nitrogen (N) [Madsen, 2011].

Interestingly, Walter *et al.* [2016] found genes associated with the sulfur cycle (S oxidation and DMSP consumption) and nitrogen metabolism (N<sub>2</sub>, ammonia assimilation, masking nitrate, and nitrite ammonification) in the metagenome of turf algae on the Abrolhos reef. Furthermore, the study indicates that S and N metabolism are essential factors in distinguishing them from corals, rhodolites, and turfs. The latter organisms are more sulfide-tolerant and have a greater abundance of non-heterocystic and heterocystic cyanobacteria, which are important in the N cycle in other habitats of Abrolhos. In microbial mats in hypersaline lagoon systems of Araruama bacterial orders related to sulfate reduction, such as *Desulfobacterales*, *Desulfovibrionales*, and *Desulfuromonadales*, including a new *Thiohalocapsa* species with genes related to the sulfur metabolic pathway, and the genus *Nitrosococcus*, responsible for ammonia oxidation [Walter *et al.*, 2021], and important in the global N cycle [Wang *et al.*, 2020].

The sediment from Guanabara Bay also shows groups related to the S cycle, such as *Desulfurococcales*, *Thermoproteales*, and *Sulfolobales*, in addition to the *Sulfurimonas* genus, related to sulfur oxidation and nitrate reduction. Genes related to sulfur metabolic pathways were different according to sediment depth; deeper (4 m) the pathways of inorganic S assimilation were dominant, while at the surface was the organic S assimilation. Genes related to the methane cycle, such as Monomethylamine methyltransferase (mtmB), Trimethylamine methyltransferase (mttB), as well as bacteria that use acetate (the substrate for methanogenic and sulfate-reducing pathways) as carbon sources (*Candidatus Methanoregula boonei*, *Methanosarcina thermophila*, and *Methanospirillum hungatei*) were also found [Tschoeke *et al.*, 2020].

One of the most important ecosystems involved in biogeochemical cycles is the mangrove. Nóbrega *et al.* [2022] carried out metagenomics work on the mangrove located in the Amazon River estuary, finding that the genera *Allochromatium*, *Desulfovibrio*, *Thiobacillus* (S), *Nitrosococcus* and *Nitrospira* (N) increased at low salinity and low oxygen. In addition to metabolic genes involved with the S, N, and C cycles, the study showed a positive correlation with S metabolism, dissolved organic carbon (DOC), and respiration, possibly caused because the mangrove DOC increased anaerobic respiration through S metabolism and produced bicarbonate via sulfate reduction. Viruses also play an important role in nutrient cycling through lytic viral infection, releasing nutrients into the water column that become available to the microorganisms (viral shunting). Thus, it is estimated that virus-mediated death releases 10 billion tons of carbon, directly impacting the C cycle [Coutinho *et al.*, 2018]. In this process, they release particulate organic matter and dissolved organic matter through cell lysis, providing nutrients to other organisms rather than leaving the particle intact [Silva *et al.*, 2017].

## 4 Microorganisms as bioindicators

Human environmental action brings the need for technologies that point to and act on the impacts caused. Bioindicators are species, groups of species, or biological communities whose presence, quantity, and distribution indicate the magnitude of the impacts on an aquatic ecosystem and its drainage basin. Bioindicators are important for assessing the integrity of water bodies and are more efficient than traditional physical/chemical parameter analysis. The most useful bioindicators are those that can distinguish between natural phenomena – such as seasonal or rain/drought changes – and stresses of human origin related to point or diffuse sources of pollution [Callisto *et al.*, 2005]. The Paraguaçu River course has a section located inside the Caatinga environmental protection area. Metagenomic analysis of this location showed that microorganisms from the orders *Methylophilales*, *Xanthomonadales*, *Acidimicrobiales*, *Sphingomonadales*, *Pedosphaerales*, *Acidobacteriales*, and *Chthoniobacterales* were more abundant, while in the unprotected area, *Actinomycetales* and *Rhodocyclales* were significantly more abundant. Also, that last location has shown greater abundance in some genera responsible for the degradation of pesticides, such as *Arthrobacter*, pathogenic genus, such as *Flavobacterium*, and genera such as *Acidovorax* and *Polaromonas*, which are known for their arsenate oxidation abilities [Lopes *et al.*, 2016b].

Cordeiro *et al.* [2019] used microorganisms to check the water quality of the Doce River after the rupture of the ore dam, causing a disaster that contaminated about 670 km of the river. They observed changes in bacterial composition when compared to the microbiota of a control river (Paraguaçu River) [Lopes *et al.*, 2016b]. The changes observed at the family taxonomy level, showed that *Chitinophagaceae* and *Moraxellaceae* were more abundant in Doce River, while *Burkholderiaceae* was more abundant in Paraguaçu River. Furthermore, genomes recovered from the Doce River showed an increase in genes related to the Fe cycle, especially pertinent to Actinobacteria that are: commonly found in soil, responsible for the smell of wet soil [Pimentel *et al.*, 2016], known for their ability to produce antibiotics [Hussein *et al.*, 2018] and to grow in diverse habitats (surface waters; deep oceans; and extreme desert soils) [Amin *et al.*, 2020].

Studies carried out by Walter *et al.* [2018] in the five lakes of Campina Grande showed the presence of *Cyanobacteria* with a 10.6% average contribution. However, one of the samples showed a very high concentration, which suggests being in Class III of CONAMA Resolution 357, indicating that the sampled water needed advanced treatment for human consumption. Also, a metabolic annotation of the metagenomic sequences revealed gene sequences related to the production of toxins in all ponds, such as cyanopeptolin synthetase and microcystin synthetase.

Batista *et al.* [2018] performed a metagenomic analysis and investigated the effect of upwelling and anthropogenic pollution on sponge bioactivity to assess how environmental changes might affect them and their holobionts. Four sponge samples (*Dysidea etheria*, *Darwinella sp.*, *Hymeniacidon he-liophila*, and *Tedania ignis*) were collected in Arraial do

Cabo (Rio de Janeiro State) in areas without upwelling but with high pollution (ABC) and upwelling and low human influence (FI). *Acidovorax*, *Candidatus Puniceispirillum*, *Roseobacter*, *Rhodospirillum*, *Sphingopyxis*, *Ruegeria*, and *Burkholderia* were the genera most abundant in all samples, although, sponges of the same species had different abundances: *Candidatus puniceispirillum* was more abundant in *T. ignis* from the ABC than FI areas. *Roseobacter*, *Nitrosopumilus*, and *Burkholderia* were the most abundant in the FI areas. Also, *D. etheria* had a higher abundance of *Acidovorax* from the polluted site. *Darwinella sp.* and *D. etheria* from the ABC site had a higher abundance of *Myoviridae* than from the FI site. The study concluded that human activity in the environment and upwelling cause changes in temperature, nutrient supply, and the abundance of sponge-associated microorganisms.

Microbial communities in sediment samples of the Campos Basin (South Atlantic Ocean), collected from the seabed with different oil concentration gradients, report that metagenomics is a useful tool to monitor environmental changes. In studies from this location [Appolinario *et al.*, 2019, 2020b], an increase in microbial abundance and a decrease in oxygen and hydrocarbon concentrations were observed. Concomitantly, the metagenomic sequences of archaea, fungi, and oil-degrading bacteria (*Alcanivorax*, *Alteromonas*, *Collwellia*, *Marinobacter*, and *Pseudomonas*) were greater in high hydrocarbon concentrations. Furthermore, oil-sensitive organisms such as *Geobacter*, *Pelobacter*, and Sulfur and Sulfate Reducing Bacteria (SRB) can be used as warning tools for early detection of this compound in sediments.

According to Fistarol *et al.* [2015], microorganisms vary according to the Guanabara Bay pollution gradient, where *Alphaproteobacteria* and *Flavobacteria* classes were found in areas under greater ocean influence, that is, more oligotrophic environments, while sites under higher anthropic action have microbial communities enriched by *Betaproteobacteria*, *Gammaproteobacteria*, and *Actinobacteria*. Methanogenic Archaea, more common in anoxic environments, were also more abundant in areas of greater impact, possibly due to eutrophication by human action.

A change in the microbiota in the Abrolhos bank was verified when analyzing the aura-biomes of four different macroorganisms (coral, fleshy macroalgae, turf algae, and zoanthid). The coral aura-biome was associated with *Synechococcus* and functions suggesting oligotrophic growth, while the fleshy macroalgae aura-biome was associated with *Ruegeria*, *Pseudomonas*, and microbial functions suggesting low oxygen conditions. The turf algae aura-biome was associated with *Vibrio* and *Flavobacterium* and functions suggesting pathogenic activity, while zoanthids were associated with *Alteromonas* and functions suggesting a stressful environment Walsh *et al.* [2017].

Cavalcanti *et al.* [2018] performed a metagenomic analysis with live and dead rhodolites, extracted from Catalina Island, California, through a laboratory assay using seawater under ocean acidification conditions. They observed photosynthetic capacity, calcium carbonate production, and the associated microbiome using carbon absorption and decalcification assays. The microbiome of the living holobiont had no significant changes throughout the experiment, while the

biofilm present on the dead rhodolite showed changes such as the predominance of *Gammaproteobacteria* on the first day and was partially replaced by *Alphaproteobacteria* and *Flavobacteria* at the end of the experiment. They concluded that a microbial-host interaction that is strongly regulated, as shown by the stability of the rhodolite microbiome at similar conditions of ocean acidification, is important for host resistance to environmental stress.

Differences in microbiota could also be observed in the southeastern Atlantic Ocean through a study performed by Alves Junior *et al.* [2015], who carried out a wide analysis along the Brazilian coast (2000 km) and depth (up to 1200 m) covering euphotic and aphotic zones. They found that the microbial abundance was 3x lower in the south compared to the other locations, and there was a positive correlation between temperature and dissolved oxygen concentration. The abundance of *Alphaproteobacteria* and *Cyanobacteria* decreased with depth, while the abundance of *Gammaproteobacteria* increased. *Pelagibacter* was the most abundant genus in the mesopelagic zone, and 446 gene functions were exclusive to the Atlantic Ocean, showing a unique characterization of this environment. Overall, differences in microbial diversity and abundance seem to be influenced by temperature, dissolved organic carbon, and depth, showing a stratification between euphotic, SCM, and aphotic zones.

## 5 The resistance genes

Antimicrobial resistance is a natural event that gives bacteria the means to defend themselves against drugs and substances with antiseptic actions produced by other microorganisms. In this regard, antibiotics are essential for surgical procedures, organ transplantation, oncology, and treatment of other diseases [Huemer *et al.*, 2020]; for animal farming; and the environment [Zhuang *et al.*, 2021]. However, antibiotic-resistance genes (ARGs) and antibiotic-resistant bacteria (ARBs) are a worldwide public health problem that threatens farms, cities, hospitals, wastewater treatment stations, and various ecosystems since they can be found at these sites [Lamori *et al.*, 2019; Zhuang *et al.*, 2021].

Coutinho *et al.* [2019] performed the genome sequencing of a multidrug-resistant strain of *Vibrio parahaemolyticus* (JPA1) isolated from the Jacarepaguá lagoon system in Rio de Janeiro to establish the molecular basis of antibiotic resistance. This study revealed that the JPA1 genome has a wide range of antibiotic-resistance genes that confer a multidrug-resistant phenotype despite being a non-virulent strain. Efflux pump-related resistance genes associated with resistance to aminoglycosides, beta-lactams, fluoroquinolones, macrolides, streptogramin, and tetracycline were found. They found several resistance genes, such as those related to the AcrEF-TolC complex (multiple-drug efflux pump), MacAB-TolC complex (macrolide resistance), novA (ABC transporter type II - novobiocin resistance), vgaE (resistance to streptogramin), tet34 and tet35 (tetracycline resistance), and sav1866 (encodes a non-specific multidrug transporter). In addition, they also found antibiotic inactivation genes (APH(3'')-Ib, CARB-18, and catB8), as well as proteins related to antibiotic target protection (gene

dfrA3, otrA, tetW, tet32, qnrC, and qnrVC5).

In another work, Fistarol *et al.* [2015] described potentially pathogenic genera such as *Vibrio*, *Klebsiella*, *Pseudomonas*, *Clostridium*, and *Bacillus* in Guanabara Bay. *Vibrio cholerae*, *Klebsiella pneumoniae*, and *Shigella spp* strains were identified as resistant, super-resistant, and multi-resistant strains. The presence of ARGs was also detected between North Fundão Island and the mainland [Fistarol *et al.*, 2015]. The genus *Vibrio* is ubiquitous in marine aquatic environments, and it has been demonstrated that *Vibrio* abundance has a positive correlation with orthophosphate concentration and a negative correlation with salinity [Gregoracci *et al.*, 2012; Thompson *et al.*, 2013].

Hypersaline environments with concentrations of heavy metals provide perfect conditions for the newly classified genus *Salinivibrio sp.* Gorriti *et al.* [2014] analyzed three genomes isolated from a high-altitude hypersaline Andean lake and found several genes related to copper tolerance, the homeostasis of Co, Zn, Cd, and Pb (ATP-ases and transmembrane proteins), and other genes related to resistance to As, Hg, and Cr. In addition, other work also analyzed genomes isolated from the saline region of India and found the same genes cited above, providing strong corroboration for the existence of different cellular apparatus and genes for tolerance to high concentrations of salt and heavy metals (Cu, Zn, Co, Hg, Cr, and Pb) [Gorriti *et al.*, 2014; John *et al.*, 2019].

Costa *et al.* [2015] isolated and sequenced the genetic material of *Micrococcus sp.* MS-AsIII-49, was extracted from sediment in the Velhas River (MG), which is rich in metals, mainly arsenic. The genome indicates that there are specific genes for resistance to heavy metals such as arsenic (ArsR, ArsC, and ACR3), copper (CopZ, CopC, CopB, multicopper oxidase, and CtpA), mercury (MerR and MerA), cadmium, zinc, and cobalt (CzcD protein). The genus *Micrococcus* is present in different types of environments and ecosystems (such as human skin, water, and sediment). Because it is so diverse, it is constantly used as a consumer and transformer of organic matter in polluted environments [Santhini *et al.*, 2009].

The Actinobacteria phylum presents great diversity and is present in the most varied aquatic and terrestrial ecosystems. It is composed of gram-positive bacteria, whether aerobic, anaerobic, or facultatively anaerobic. The production of different secondary metabolites makes these organisms important tools in pharmaceutical, medical, and bioremediation areas [Alvarez *et al.*, 2017]. The *Frankia* genus has tolerance to heavy metals such as Cd(II), Co(II), Cu(II), Cr(VI), Ni(II), and Zn(II) and is an efficient symbiont in contaminated environments, promoting revegetation of disturbed ecosystems [Bélangier *et al.*, 2011]. The genus *Arthrobacter* is highly relevant in the biodegradation of toxic compounds, heavy metals, and/or pesticides, as it has catabolic pathways capable of detoxification and high resistance to environmental stress [Alvarez *et al.*, 2017].

## 6 Conclusion

The microorganisms change according to environmental gradients such as nutrients, geographical localization, temper-

ature, depth, and pollution, corroborating Thompson *et al.* [2013]. Our studies on different aquatic ecosystems showed that bacteria are the most common domain, whereas the most abundant phylum is *Proteobacteria*. *Vibrio* was the most observed genus in marine environments; they are well-known for their rapid responses to nutrient-rich environments and rapid replication under suitable conditions, and since some species are human and/or animal pathogens, it may be important to monitor *Vibrio* counts in coastal waters [Gregoracci *et al.*, 2012]. Besides that, they are also well-regarded pathogens associated with declines in coral health, coral bleaching, and disease [Walsh *et al.*, 2017]. In freshwater environments, the most common genus was *Limnohabitans*, an uncultivated bacterioplankton considered a “typical freshwater bacteria”, that is characterized by playing a significant role in these environments [Lindström *et al.*, 2005]. Beyond that, studies evidence the presence of antibiotic and heavy metal resistance genes in water also highlight metagenomics as an essential technique to identify uncultivated microorganisms associated with different aquatic ecosystems. In addition to being essential for understanding the gene functions and the resistance genes.

In the One Health concept, water performs an essential role in interaction by connecting environments and the living beings that inhabit them. Thus, it is important to emphasize that water quality is essential for the survival of ecosystems. In this context, the raw sewage released into water bodies is one of the biggest factors of water contamination and, consequently, directly influences water quality, resulting in microbiota modification. Therefore, public policies are fundamental to controlling pollution and restoring these environments. Hence, we suggest some crucial measures to mitigate these impacts:

1. The need to create a wide network of urban sewage and Sewage Treatment Stations (STs) in similar patterns to what Bacha *et al.* [2022] wrote about the Guandu River, which can be expanded to different freshwater bodies, increasing enforcement to prevent the release of clandestine sewage into all water environments;
2. Reforestation of the riparian forest, part of the Permanent Protection Area, is required according to the Brazilian Forest Code (Law n°. 12651/2012). The law guarantees a “protected area, covered or not by native vegetation, with the environmental function of preserving water resources, the landscape, geological stability, and biodiversity, facilitating the gene flow of fauna and flora, protecting the soil, and ensuring the well-being of human populations”. Thereby, the law ensures the preservation of water quality and the supply of nutrients downstream. In addition, most rivers flow into the sea; thus, the creation of STs and the reforestation of riparian forests will reduce the large contribution of substances coming from these environments, helping to clean up Guanabara Bay, for example, by achieving NEC 357 (CONAMA 357) standards;
3. Increase rigidity and efficiency in water treatment stations, since, according to Gil *et al.* [2021], many pathogenic microorganisms are not detectable by current techniques for monitoring water quality, thus being

ignored by conventional treatment. According to the World Health Organization [2004], treatment with chlorine alone is ineffective for inactivating oocysts of the waterborne protozoan *Cryptosporidium* that causes acute gastroenteritis, abdominal pain, and diarrhea [Gerace *et al.*, 2019]. The WHO [2021] suggests that *Enterococci* are used as an indicator of fecal pollution and can survive in the water longer than *E. coli*, even suggesting that Rotaviruses, Enteroviruses, and Noroviruses have been identified as potential reference pathogens because they survive conventional treatments and persist for a long time in water [WHO *et al.*, 2017];

4. Implementation of a progress tracking and evaluation system similar to what is already happening in Chesapeake Bay [Fistarol *et al.*, 2015];
5. Protect the mangroves that are also part of the Permanent Protection Area and are highly important for nutrient cycling;
6. Reduce overfishing, especially in Environmental Protection Areas, and increase inspection in these places;
7. Greater inspection at mining companies to prevent accidents, such as the Doce River, with more assiduous monitoring and greater rigor in forensic analysis, both dams and tailings tanks;
8. Raise public awareness of good personal hygiene practices, domestic cleaning, and the conscientious use and correct disposal of medicines and chemical substances to prevent the increase of ARGs and ARBs [Novak Babič *et al.*, 2020; Lamori *et al.*, 2019];
9. Greater inspection of ships and oil platforms to prevent oil spills, especially in reef or environmental protection areas;
10. Management of solid waste to prevent it from reaching aquatic environments;
11. Increasing research in areas not yet impacted aims to assess and comprehend the native microbial community, using this microbiota as biomarkers and bioremediators in case of accidents and/or disasters.

Ongoing and future works: the group is working on metagenome analysis, mainly environmental samples, applying bioinformatics and machine learning analysis to those data and we are planning to expand our work using the methodology to explore other types of samples, i.e. host-associated and clinical samples.

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## Authors' Contributions

DAT contributed to the conception of this study. All authors contributed equally to the writing of this manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare no conflicts of interests related to this work.

## Availability of data and materials

The manuscripts from 2012 to 2022 analysed in the current study are available in PubMed using the keywords “Diogo Tschoeke water”, “Fabiano Thompson water”, “Diogo Tschoeke water metagenomic” and “Fabiano Thompson water metagenomic”

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