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Research article

Environmental contamination alters the intestinal microbial community of the livebearer killifish *Phalloceros caudimaculatus*

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ABSTRACT

Intestinal microbiota perform important functions for the health of fishes. Knowing the microbial composition and evaluating the possible effects caused by anthropogenic pollution in the intestinal microbiota of fish populations might represent an important step in defining microbial biomarkers for water pollution. This study evaluated the impact of environmental contamination on the gut microbiota of the livebearer killifish *Phalloceros caudimaculatus*. The 16S survey using the V4 region of the 16S rRNA gene was used to characterize and compare the microbiota of two *P. caudimaculatus* populations from streams with different levels of environmental contamination in Rio Grande, RS, Brazil. Twelve bacterial operational taxonomic units (OTUs) (around one-third of the total) were shared between both fish populations. They represent the core microbiota of the gut in this species. The dominant phyla were *Protebacteria* and *Firmicutes,* with more than 80% of relative abundance. The dominant genus was *Burkholderia* with more than 35% of the relative abundance irrespective of the environmental condition. We detected a lower microbial diversity (Shannon index and observed OTUs) in fish from the polluted stream compared to the reference stream. The PERMANOVA analysis showed that the intestinal microbial communities from fish living in the polluted stream were distinct from those found in the reference stream ($p < 0.05$). Finally, we identified *Luteolibacter*, *Methylocaldum* and *Rhodobacter* genera, which correlated strongly with the polluted stream. These taxa might represent potential microbial biomarkers of exposure to environmental contaminants in the guts of fish. Confirmation of these findings in other polluted environments might allow the development of a microbiota-based screening approach for environmental evaluation in ecotoxicological studies in aquatic ecosystems.

1. Introduction

Currently, there is a great and global concern about the pollution of aquatic ecosystems. Development of anthropic activities led many countries to a critical level of pollution due to the huge amount and diversity of residues that are produced and discharged into water bodies (Hu and Cheng, 2013). Many aquatic ecosystems near to urban areas, such as streams or lagoons, are often the final receptors of urban waste and industrial effluents (Garcia et al., 2010). Anthropogenic waste contains a mixture of contaminants that can cause physiological and functional alterations, and even reduced survival, in fishes (McCallum et al., 2016; Ndiaye et al., 2012).

The livebearer killifish *Phalloceros caudimaculatus* (Cyprinodontiformes; Poecillidae; Hensel, 1868) is widespread in freshwater and estuarine environments of South American countries, such as Brazil, Argentina, Paraguay and Uruguay, while also being introduced in countries on other continents, such as Malawi and New Zealand (Lucinda, 2008). Similar to other cyprinodontiform fishes, e.g., *Poecilia reticulata* and *Fundulus heteroclitus*, it presents characteristics that favor its use as a model in environmental toxicology studies (Zanette, 2013). The *P. caudimaculatus* fish has been suggested as a model to evaluate the effects of pollution in the aquatic environment (Araújo et al., 2009; Chivittz et al., 2016; Ferreira et al., 2016).

It has been suggested recently that the exposure to anthropogenic waste in the aquatic environment could cause alterations on the intestinal

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microbiome composition of fish (Giang et al., 2018). The gut microbial community in fishes are involved in important biological functions such as nutrition (Clements, 1997), physiology, and immunology (Nayak, 2010) that help to maintain a healthy state in fishes (Pérez et al., 2010). Nonetheless, there are environmental and ecological factors that could shape the gut microbiota in fish (Dehler et al., 2017) including the exposure to multiple environmental contaminants such as microplastics (Jin et al., 2018), organochlorine biocide (Kan et al., 2015), and metal (Zhai et al., 2017). Ecotoxicology studies were carried out to evaluate the effects of isolated environmental contaminants in the gut microbiota under laboratory conditions (Evariste et al., 2019). In contrast, little is known about how anthropogenic activities influence the gut microbiota of fish populations that inhabit the aquatic environment.

From an ecotoxicological point of view, little attention has been given to analyzing the gut microbiota of fish as a parameter for the evaluation of the environmental quality. The understanding of the composition of the gut microbiota of fish could enable the assessment of the host's health as well as the quality of the surrounding environment (Giang et al., 2018). The aims of this study were to characterize the gut microbiota of *P. caudimaculatus* livebearer killifish from two streams with different anthropogenic impact levels (reference and polluted) in order to identify the microbial groups that are shared (i.e., core microbiota) and the microbial groups that are unique for each stream. A metagenomics approach was used to evaluate the effect of pollution on the microbial diversity and composition of communities as well as to identify the existence of microbial groups that could be used as potential biomarkers for environmental contamination.

2. Materials and methods

2.1. Sampling, selection and site characterization

Male *P. caudimaculatus* fish (lengths 2.4 ± 0.1 cm) were collected in October of 2017 from two streams with different anthropogenic impacts in the city of Rio Grande, RS, Brazil (n = 20 fish in each site). The sampling site in the polluted stream (32° 02′ 56.48 ″S, 52° 05′ 06.98″ W) is located in the margin of a petroleum refinery, between an urban area with high population density and an industrial area. This area receives urban sewage and industrial effluents, and it has been historically contaminated with polycyclic aromatic hydrocarbons (PAHs) (Medeiros et al., 2005). The sampling site in the reference stream (32° 33′ 32.48″ S, 52° 23′ 54.59″ W) is a watercourse located 14 km away from the closest inhabited urban area at Cassino Beach, so it is relatively less impacted by anthropogenic contamination (Figure 1). Chivittz et al. (2016) analyzed the sum of 16 EPA priority PAHs in sediment in those sites, confirming very different levels of PAHs between the polluted and reference streams (4414.0 and 1.7 ng g^{-1} dry weight, respectively).

All fish collected were transported to the Laboratory of the Institute of Biological Sciences in the Federal University of Rio Grande (FURG). The use of fish and euthanasia procedures were approved by the Committee on Ethics and Use of Animals (CEUA-P003/2018, FURG) and by the System of Authorization and Information on Biodiversity in Brazil (SISBIO/60693-1). Fish were euthanized with an overdose of tricaine methanesulfonate (MS222) (Sigma-Aldrich, St Louis, MO, USA) and exterior surfaces were swabbed with the same water with MS222 solution before dissection of the whole intestine using sterile instruments. All intestines were extracted and stored individually at -20 °C.

2.2. Microbial DNA extraction, 16S rRNA gene amplification and sequencing

Entire fish intestines were mashed using PowerLyzer homogenizer in a bead tube with glass beads for 45 s. The microbial DNA was isolated using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. DNA quality was defined by spectrophotometry using a NanoVueTM spectrophotometer (GE Healthcare, Chicago, IL, USA). Due to a low initial DNA concentration, all samples were centrifuged for 1 h at 60 °C in a vacuum centrifuge to obtain higher concentrations of microbial DNA. All the DNA samples were stored at -20 °C until their use in PCR reactions. The determination of the intestinal microbial community was based on partial 16S rRNA gene (V4 region) sequences, directly amplified using bacterial/archaeal primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2010). PCR was performed in a 25 μL total volume of reaction containing 2U of Platinum® Taq DNA High Fidelity Polymerase (Invitrogen, Carlsbad, CA, USA), 4 μL 10X High Fidelity PCR Buffer, 2 mM MgSO4, 0.2 mM dNTPs, 0.1 μM of both the 806R barcoded primer and the 515F primer, 25μg of Ultrapure BSA (Invitrogen, Carlsbad, CA, USA), and approximately 50 ng of DNA template. After an initial denaturation step of 5 min at 95 °C, 30 cycles of 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 1 min were performed, followed by a final extension step of 10 min at 72 °C. The PCR products were purified with Agencourt® AMPure® O reagent XP (Beckman Coulter, Brea, CA, USA), quantified using the Qubit fluorometer kit (Invitrogen, Carlsbad, CA, USA) and combined in equimolar concentrations to create a mixture composed by amplified fragments of the 16S of gene of each sample. The Ion OneTouch™ 2 system with the Ion PGM™ Hi-Q™ View OT2 Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used for the library preparation. Sequencing was performed using the Ion PGM™ Sequencing with Ion PGM™ Hi-Q™ View Sequencing Kit and the Ion 318™ Chip v2.

2.3. 16S sequence processing for downstream analyses

The 16S rRNA reads from the Ion PGM™ system were analyzed using the BMP Operational System (BMPOS) (Pylro et al., 2016) following the recommendations of the Brazilian Microbiome Project (Pylro et al., 2014) for removal the errors and chimeric sequences. Raw reads were trimmed at 200 bp and quality filtered using a maximum expected error of 0.5. Quality filtered reads were de-replicated and singletons were removed. The sequences were clustered into operational taxonomic units (OTUs) at 97% similarity cutoff and chimeras were identified and removed. Thus, we obtained representative sequences for each microbial phylotype (Edgar, 2013). Finally, the sequences were clustered, aligned and taxonomically classified in the software QIIME (Caporaso et al., 2010) based on the UCLUST method against the Greengenes 13.5 database (McDonald et al., 2012) with a confidence interval of 80%. Sampling effort was estimated using Good's coverage (Good, 1953). Samples with coverage smaller than 80% were excluded from the analysis. The profile of OTUs was used to visualize the relative abundances of phyla in fish from the two streams (reference and polluted) and the relative abundances of phyla and genera in individual samples of the two streams. The core microbiota in the gut of *P. caudimaculatus* was identified by detecting the taxa with prevalence equal or higher than 90 % in all gut samples (reference plus polluted). The taxa with detection thresholds (relative abundance, %) lower than 0.001 were not considered for the core microbiota count.

2.4. Statistical analysis of data

All statistical analyses were carried out using R (R Development Core Team, 2008). Downstream analyses were carried out after the normalization of the number of sequences in all samples as recommended by Lemos et al. (2011). Alpha diversity was calculated and plotted using the "phyloseq" package (McMurdie and Holmes, 2013) and was measured by observed species and the Shannon diversity index. The observed species measures the number of different species or richness per each sample and Shannon index measures diversity using the OTUs richness and the relative abundance of the different species. Significant differences in the diversity of the gut microbiota, comparing fish from polluted and reference streams, were evaluated using Mann-Whitney non-parametric tests ($P < 0.05$) after testing the normality of the data by Shapiro–Wilk W test.

Beta-diversity was applied to compare the microbial community between different samples through principal coordinates analysis (PCoA) using the "phyloseq" package (McMurdie and Holmes, 2013). This method was based on multivariate statistical analysis where the dissimilarity among microbial communities was calculated with Bray-Curtis and binary distance metrics. We analyzed the significance of the differences between the groups observed by PCoA using a non-parametric permutational multivariate analysis of variance (PERMANOVA) with the "Adonis" function available in the "vegan" package (Oksanen et al., 2015) with 999 permutations.

Identification and classification of bacterial OTUs correlated with exposure to environmental contaminants was performed by ANOVA-like differential expression analysis using ALDEx2 (Fernandes et al., 2013). The method is based on the principal that microbiome datasets generated by high-throughput sequencing are compositional (Gloor et al., 2017). The software models the contingency table as proportions of the data available rather than as counts. This analysis was based on the microbiota profile at the OTU-level.

3. Results

3.1. Microbiota composition in the gut and the OTUs shared between the P. caudimaculatus populations

We characterized the intestinal contents of wild *P. caldimaculatus* collected in two streams with distinct levels of contaminants. After excluding samples with low sequence coverage, 267,003 high-quality sequences were obtained from the 32 samples used in this study (17 fish from the reference stream and 15 from the polluted stream). There were 604 microbial genera distributed in 42 phyla that were identified. The most dominant phyla were Proteobacteria and Firmicutes. Proteobacteria presented a relative abundance of 68.9 ± 8.5% and 69.9 ± 9.5% in the gut of fish from reference and polluted streams, respectively (average ±standard deviation). The Firmicutes presented a relative abundance of 13.0 ± 6.7% and 14.7 ± 6.8% in the gut of fish from reference and polluted streams, respectively (Figure 2A). At the genus

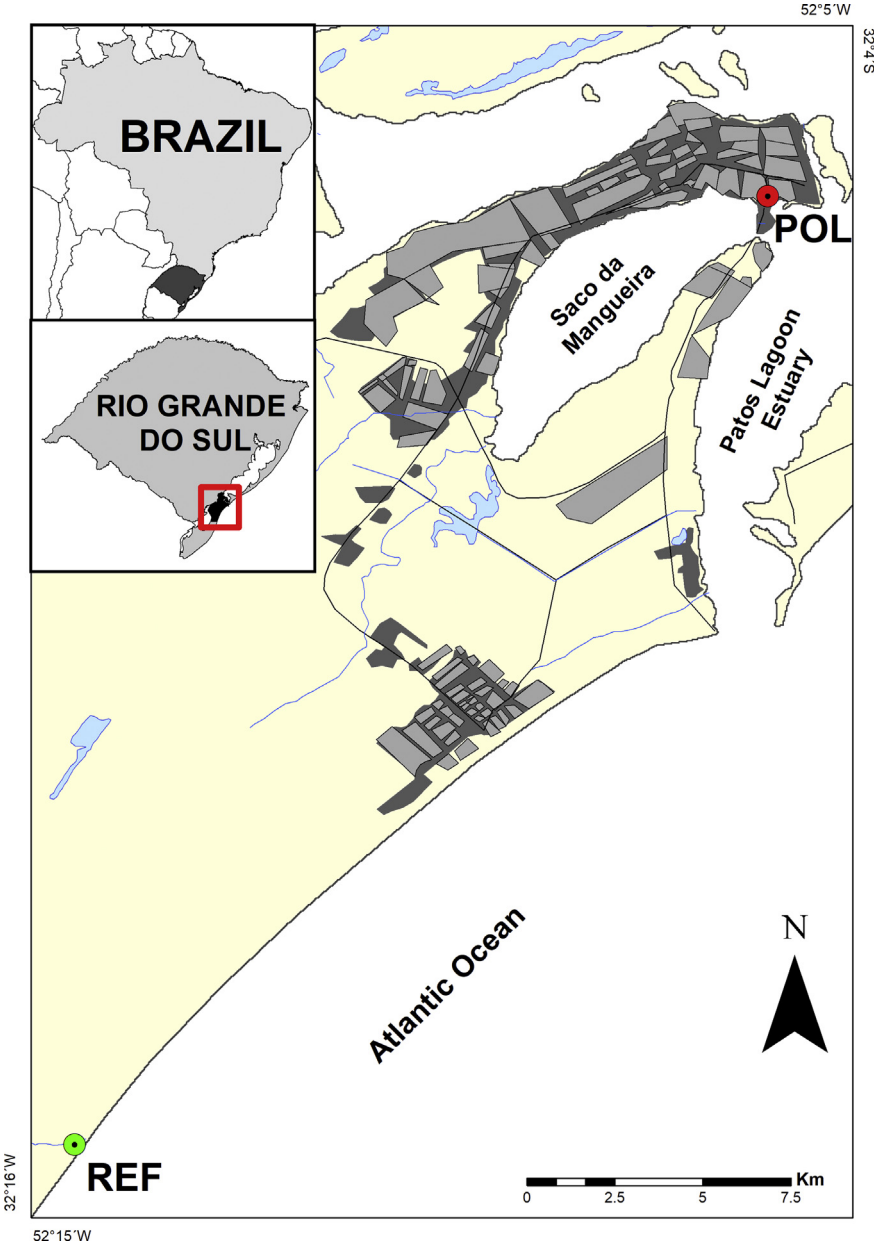

Figure 1. Study area. Sampling sites of *P. caudimaculatus* in the polluted stream (POL) and the reference stream (REF) in the city of Rio Grande, RS, Brazil. The POL site is located between a highly urbanized area and an industrial area (gray).

level, the most dominant bacterial OTU was *Burkholderia* with a relative abundance of 38.1 ± 16.9% in the gut of fish from reference stream and 31.0 ± 19.1% in the gut of fish from polluted stream (Figure 2B).

The core microbiota analysis indicated that *Burkholderia* was the most prevalent and abundant genus associated with *P. caudimaculatus,* irrespective of the environmental conditions. This genus was present in 90% of the samples with a relative abundance higher than 10% (Figure 3). Another 11 genera with lower abundances than *Burkholderia* were also found at the 90% prevalence cutoff. They were *Streptococcus, Sphingonomas, Staphylococcus, Lactobacillus, Veillonella, Acinetobacter,*

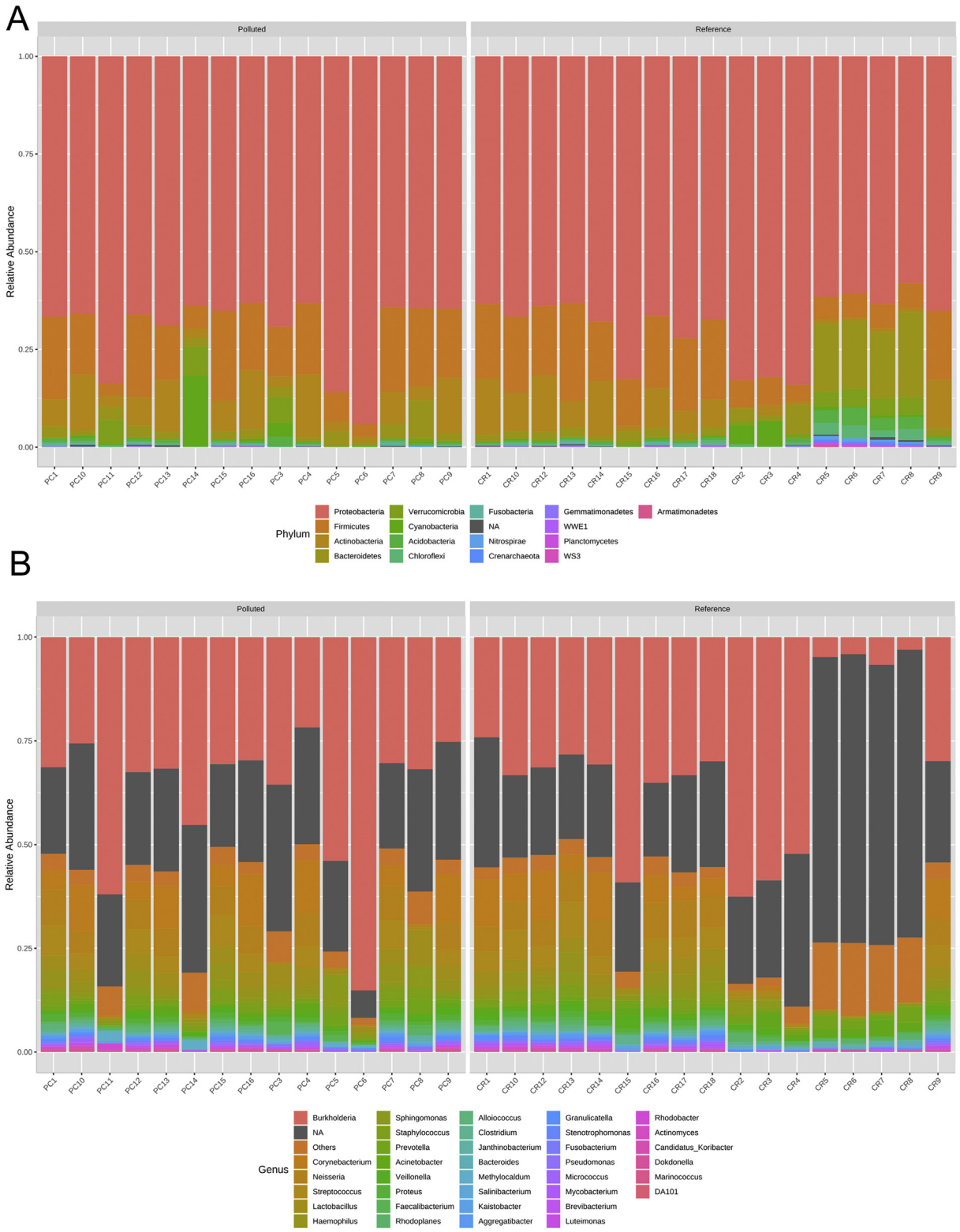

Figure 2. Microbiota composition of *P. caudimaculatus* populations. Bar graphs showing: A. Relative abundance of phyla in the intestinal microbiota of each fish. B. Relative abundances of genera in the intestinal microbiota of each fish. The samples PC are the guts of fish from polluted site and CR are the guts of fish from reference site. Low abundant taxa (total counts <10) were grouped into the NA class.

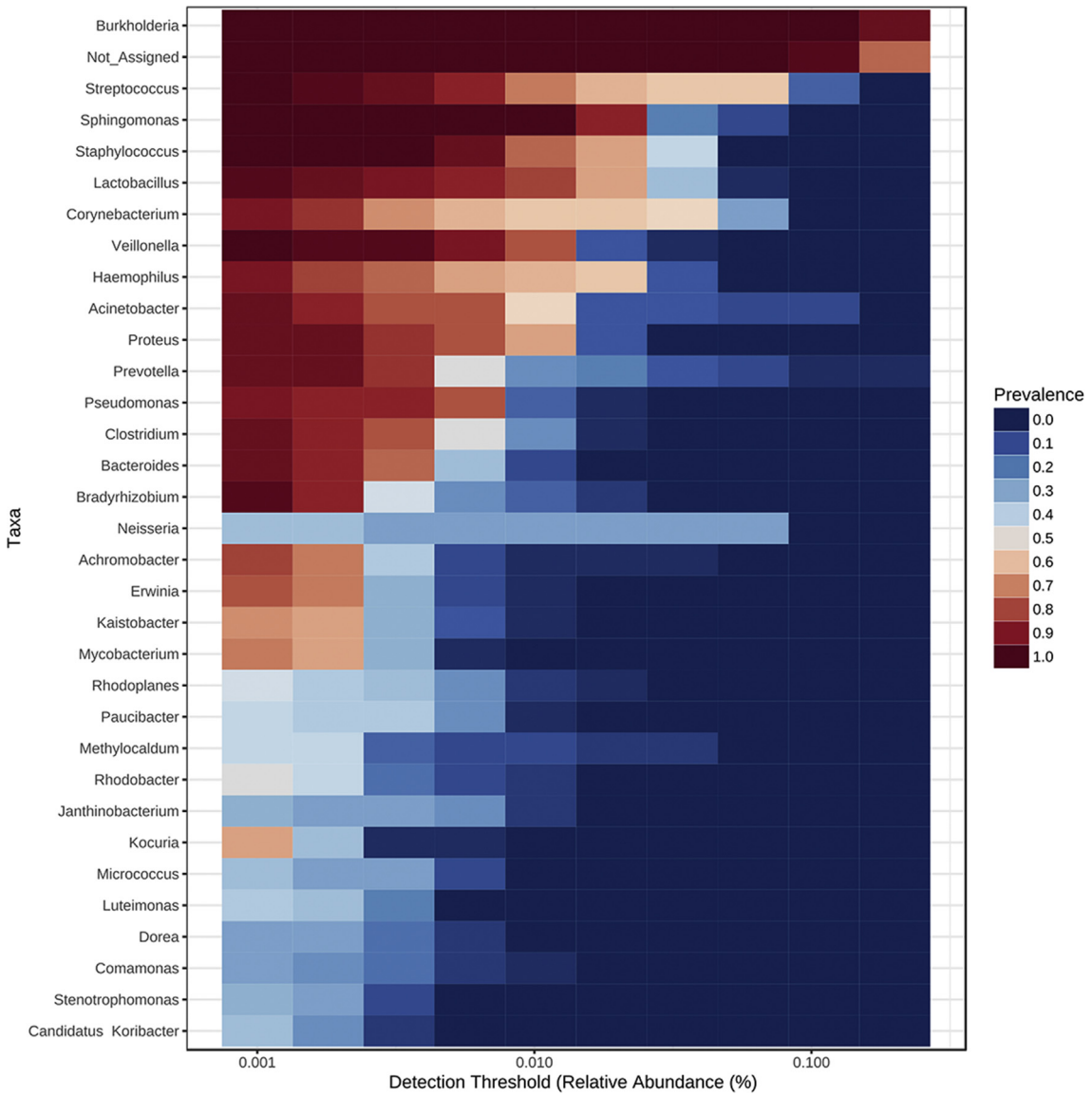

Figure 3. Heatmap showing the prevalence of the microbiota taxa in the gut of *P. caudimaculatus* considering detection thresholds (relative abundance, %) equal or higher than 0.001. The prevalence varies from 0 % (blue, $value_{mín} = 0.0$) to 100 % (red, $value_{máx} = 1.0$) in the gut samples of fish from polluted and reference sites (total of n = 32 fish).

Proteus, Prevotella, Clostridium, Bacteroides, and *Bradyrhizobium*. These 12 genera represented approximately one-third of all genera detected within this study. Altogether they were considered here as the core microbiota in the gut of *P. caudimaculatus*.

3.2. Decreased microbial diversity and the possible influence of the environmental contaminants in microbial communities

Microbial diversity analysis was performed to evaluate differences in the gut microbiota of *P. caudimaculatus* from the reference and polluted streams. The gut microbiota of fish from the polluted stream showed lower microbial diversity compared to the reference stream according to the number of observed species (p-value = 0.0028) and the Shannon diversity index (p-value = 0.008) (Figure 4).

Using dissimilarity matrices to evaluate the beta diversity, we found that the microbial communities were grouped differently depending on the origin of the *P. caudimaculatus* population (reference or polluted stream). The ordination analysis using a presence-absence of species (i.e., binary distance) showed that the gut microbial communities in fish from thepolluted stream were widely different from those found in the gut of fish from the reference stream (Figure 5A). When the relative abundance was taken into consideration (i.e., Bray-Curtis distance), the groups of the two streams were less different to each other (Figure 5B). PERMANOVA analysis using the "Adonis" function (number of permutations: 999) was carried out using either binary or Bray-Curtis metrics and confirmed the difference between microbial groups of the reference and polluted streams. The value of R^2 (effect size) was 0.468 (p = 0.001) for binary distance and 0.095 (p = 0.01) for the Bray-Curtis distance.

3.3. Microbial OTUs correlated with exposure to environmental contaminants

To associate bacterial OTUs with exposure to environmental contaminants, we used a compositional data analysis approach. Our interest was to detect microbial biomarkers for water pollution. A total of 11 bacterial OTUs were found to be differentially abundant between the gut of fish from polluted stream and reference stream (Table 1). Ten OTUs were more abundant in the gut of fish from polluted stream, while only one OTU was more abundant in the gut of fish from reference stream. Three OTUs associated with the gut of fish from polluted stream were

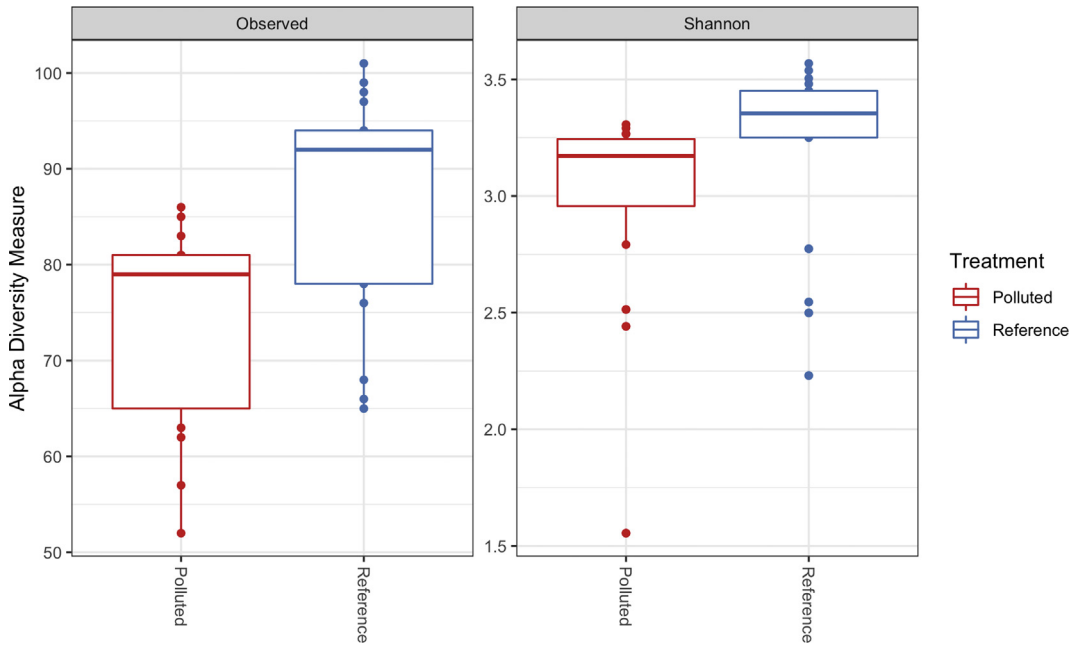

Figure 4. Alpha diversity of the two *P. caudimaculatus* populations. Observed diversity = total number of OTUs observed (p = 0.0028), and Shannon diversity (p = 0.0082). The boxes cover the first to the third quartile; the horizontal line inside the boxes represents the median. Whiskers extending vertically from the boxes indicate variability outside the upper and lower quartiles, and the single red and blue circles indicate outliers.

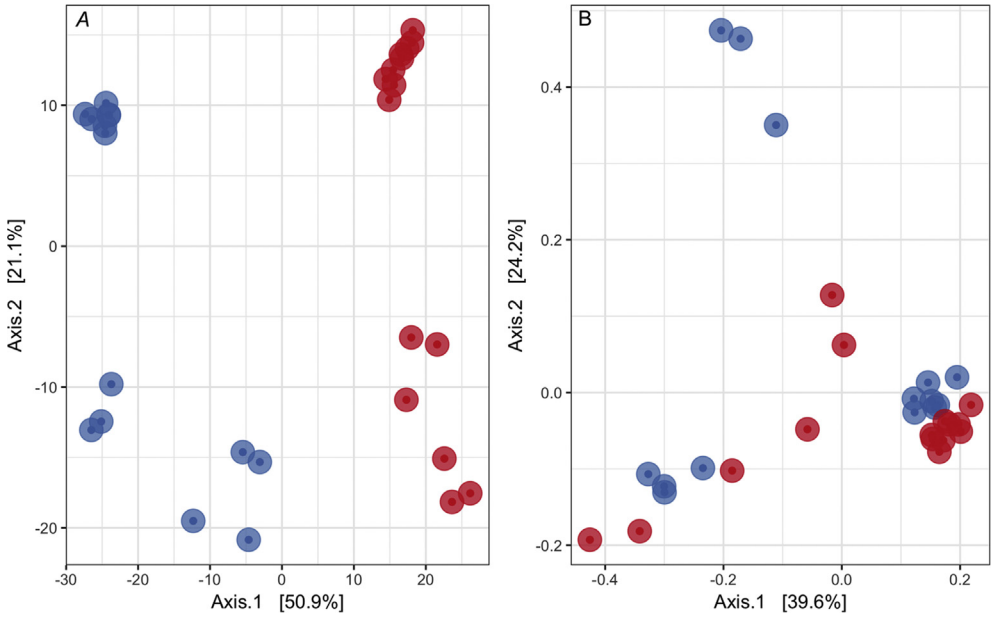

Figure 5. Comparisons of microbial communities based on principal coordinates analysis (PCoA) by binary (A) and Bray-Curtis (B) distance metrics. Each point represents a microbial community, and the blue and red points represent the reference and polluted streams, respectively. Closer points represent similar microbial communities, while the more distant points represent more different microbial communities. The statistical significance of the groupings of samples was tested by PERMANOVA. The R^2 values were 0.468 (p = 0.001) for binary distance metrics and 0.095 (p = 0.01) for Bray-Curtis distance metrics.

identified to genus. They were *Luteolibacter, Methylocaldum,* and *Rhodobacter*. An OTU from the genus *Acinetobacter* was found in higher abundance in the gut of fish from reference stream. Altogether, those results indicate the different characteristics present in the gut of fish from each stream seem to favor the appearance of possible microbial biomarkers of environmental quality.

4. Discussion

Previous studies have shown that the gut microbiota of fishes is dominated mostly by the phyla *Proteobacteria* and *Firmicutes* (Larsen et al., 2014; Li et al., 2014; Liu et al., 2016). The same two phyla dominated the gut microbiota of *P. caudimaculatus* in the present study. One possible explanation is that the presence of those bacterial groups could be determined by inherent factors from the host, such as genetics, anatomy, or evolutionary relationships (Goodrich et al., 2014; Roeselers et al., 2011). Another important finding from this research was to identify *Burkholderia* as the most dominant bacterial genus in the fish intestines. *Burkholderia* has been identified before as part of the normal gut microbiota of fish (Nayak, 2010; Sun et al., 2009). However, the understanding of the biological functions of *Burkholderia* in fish guts is rather rudimentary. The *Burkholderia* genus contains clades that could be considered beneficial for the host (Mahenthiralingam et al., 2005). The present study is the first to register *Burkholderia* as the most dominant genus of microbiota in the gut of a fish species. Therefore, we suggest focusing on *Burkholderia* for future studies, since its exploration could

Table 1. Differential abundance analysis of perspective microbial biomarkers associated with polluted or reference streams.

median clr Pol.	median clr Ref.	p-value	Closest microbial relative				
			Phylum	Class	Order	Family	Genus
Increased in the polluted steam							
6.31	5.31	0.017	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*	*Sphingomonadaceae*	-
6.13	4.57	0.036	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	-
5.72	4.55	0.044	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	*Rhodobacter*
5.17	3.01	0.006	*Proteobacteria*	*Alphaproteobacteria*	*Rhodobacterales*	*Rhodobacteraceae*	-
5.11	2.82	0.042	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	-	-
4.90	2.20	0.024	*Proteobacteria*	*Gammaproteobacteria*	*Methylococcales*	*Methylococcaceae*	*Methylocaldum*
4.64	2.39	0.046	*Proteobacteria*	*Gammaproteobacteria*	*Methylococcales*	*Methylococcaceae*	*Methylocaldum*
4.00	0.88	0.046	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Luteolibacter*
3.92	1.34	0.032	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	-
3.51	0.24	0.019	*Verrucomicrobia*	*Verrucomicrobiae*	*Verrucomicrobiales*	*Verrucomicrobiaceae*	-
Increased in the reference stream							
6.15	6.83	0.035	*Proteobacteria*	*Gammaproteobacteria*	*Pseudomonadales*	*Moraxellaceae*	*Acinetobacter*

reveal significant biological functions in the intestinal microbiota of *P. caudimaculatus* living in the wild, and possibly in other fishes living in distinct environmental or laboratory conditions.

Discovering shared bacterial OTUs in the gut of fish from different populations and geographic locations could indicate that these bacterial groups may be performing important biological functions in the host (Roeselers et al., 2011). In the present study, we found that one-third of the total OTUs (12 genus) were shared in all gut samples analyzed. The results of this study were partially supported by Salonen et al. (2012) who indicated that one-third of the phylotypes shared among all samples could be considered a conserved community that does not change with the genetic or dietary variation within individuals. Knowing the microbial core is very important because it allows us to define a stable and healthy bacterial community of the host (Shade and Handelsman, 2012). These 12 OTUs identified in the present study might be playing a key role in the health of *P. caudimaculatus*.

Once the core microbial community has been determined, the functional characterization of the microbiota can be focused on those salient members that possess the potential to benefit fish health. Some of these bacteria identified, such as *Lactobacillus*, correlate to normal gut microbiota in fishes and act in biological processes such as digestion, stress response, and reproduction (Butt and Volkoff, 2019). The genera *Clostridium* is associated with cellulose-decomposing capacity (Liu et al., 2016), and *Bacteroides* produces vitamin B_{12} (Tsuchiya et al., 2007). The *Burkholderia* genus is a clade that presents characteristics that could be helping the fishes to resist to some environmental contaminants, such as chemical substances (Rhodes and Schweizer, 2016), insecticides (Itoh et al., 2018), and hydrocarbons (Yang et al., 2016). As this genus was less abundant in the polluted stream population, its presence in the core microbiota may indicate a co evolution with *P. caudimaculatus* as a general mechanism of defense for these animals. In actuality, there are many bacterial groups that are used commonly as probiotics in aquaculture (Carnevali et al., 2017). It is important to characterize these genera and their functions in future studies, because those bacterial groups could be participating in important biological process in the fishes, e.g., adaptation and evolution (Zilber-Rosenberg and Rosenberg, 2008).

In the present study, we found a loss of bacterial diversity and alterations in the intestinal microbial community in fish inhabiting the polluted stream when it was compared to the reference stream that is geographically distant from possible sources of human activity, and possesses very low levels of contaminants, such as PAHs (Chivittz et al., 2016). This result is in agreement with previous studies demonstrating that environmental contaminants would lead to changes in the gut flora of aquatic organisms (Evariste et al., 2019), and fish exposed to anthropic waste in the environment present changes in the intestinal microbiome composition (Giang et al., 2018). In general, the anthropic waste could contain a diversity of compounds, for example, pesticides, PCBs, PBDEs, heavy metals, nanoparticles, PPCPs, microplastics, and endocrine disruptors that could lead to negative effects in the gut microbiota (Evariste et al., 2019). Previous studies have documented a significant environmental contamination in the downtown of Rio Grande city (RS, Brazil) in the locality of the polluted stream used in the present study. This environmental contamination was denoted by high levels of chemicals, such as metals (Mirlean et al., 2003), PAHs (Chivittz et al., 2016; Garcia et al., 2010; Medeiros et al., 2005), and high levels of nutrients and microbiological markers for domestic sewage discharges in the water, i.e., total and fecal coliforms (Niencheski and Baumgarten, 2010; Niencheski et al., 2006). Therefore, we suggest that the loss of intestinal microbial diversity and changes in the microbial community observed in *P. caudimaculatus* could represent a direct effect of environmental contamination in this fish population at the polluted site downtown of Rio Grande city.

The beta-diversity metrics indicated that the bacterial community of the fish from the two sites differed by both absence/presence (i.e., binary distances metric) and species abundance (i.e., Bray-Curtis metric). The differences in the community composition between microbial groups are most likely a reflection of the environment around the host, as previously suggested (Nayak, 2010). We suppose that the environmental quality influences the development of bacteria groups that could colonize the fish intestines. This would explain the difference between the intestinal microbial communities of the *P. caudimaculatus* populations. It is clear that when the host is exposed to different external factors, such as environmental contaminants, it could lead to microbial dysbiosis (Evariste et al., 2019; Jin et al., 2017; Teyssier et al., 2018). So far, little importance has been given to the alterations caused by anthropic waste in the gut microbiota of wild fish (Giang et al., 2018). Most studies performed with fish in-situ relate anthropic waste with genetic, behavioral, and biomarker alterations (Ballesteros et al., 2017; Kim and Jung, 2016; McCallum et al., 2016). We suggest considering the loss of diversity and alteration of the gut microbiota of fish as additional effects related to urban waste. Our results provide insights about the negative consequences of the environmental contamination for the intestinal microbiota of fish that live in industrial areas.

In the present study, we found bacterial groups in the guts of fish that could represent potential biomarkers of environmental contamination by anthropogenic activities. The bacterial groups *Methylocaldum* and *Rhodobacter* were strongly correlated with the *P. caudimaculatus* populations from the polluted stream, and could be further validated as environmental biomarkers. The *Methylocaldum* are methanotrophic bacteria that use methane as a sole source of carbon and energy (Bodrossy et al., 1997). Moreover, *Methylocaldum* occasionally develops in environments

with high temperatures, between 40 and 50 °C (Bodrossy et al., 1997; Cvejic et al., 2000; Saidi-Mehrabad et al., 2013). *Rhodobacter* are bacteria that are widely distributed from marine environments to freshwater. In addition, these bacteria have been related to processes of anoxygenic photosynthesis, carbon fixation, and nitrogen fixation (Mackenzie et al., 2007; Masepohl and Hallenbeck, 2010). It is very important to state that this bacterium can be harmful to fish, and that *Rhodobacter* is related to microbial dysbiosis in zebrafish induced by polystyrene microplastics (Jin et al., 2018). The presence of these two genera would indicate a contamination of the aquatic environment by anthropogenic residues and petrogenic compounds, e.g., PAHs. This coincides with the historical contamination present in the polluted stream from urban sewage and industrial effluents (Medeiros et al., 2005). Our findings may represent a new functional attribution for those groups as biomarkers of environmental contamination.

5. Conclusion

The metagenomic analysis in the gut of *Phalloceros caudimaculatus* in populations from two streams with different anthropogenic impacts allowed us to: 1. Provide insight into the existence and dimensions of the common core microbiota in this model fish used in ecotoxicology, 2. Associate environmental contamination with microbial diversity, modifications in taxonomic composition, and changes in structure community of intestinal bacteria of fish, and 3. identify microbial groups that could be potential environmental biomarkers of contamination. Our research provides the first evidence of the core microbiota of *P. caudimaculatus*, which may be constituted by 12 bacterial genera. We also found loss of bacterial diversity and alterations in the intestinal microbial community in fish inhabiting the polluted stream. The bacterial groups *Luteolibacter*, *Methylocaldum,* and *Rhodobacter* were identified in the gut of *P. caudimaculatus* as biomarkers for the polluted stream, and further studies could validate its use for environmental monitoring.

Declarations

Author contribution statement

Christian Deyvis Nolorbe Payahua: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anderson Santos de Freitas: Performed the experiments; Analyzed and interpreted the data.

Luiz Fernando Wurdig Roesch, Juliano Zanette: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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