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Development of environmental effects monitoring protocol in Brazil: a fish guide study of three river estuaries

Priscylla da Mata Pavione · Karla Gonçalves da Costa · Clayton Perônico · Mark E. McMaster · Joanne L. Parrott · L. Mark Hewitt · Kelly R. Munkittrick · Francisco Candido Cardoso Barreto · Thiago Hollanda Basilo · Marcelo Paes Gomes · Ricardo Wagner Reis Filho · Tatiana Heid Furley

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Abstract In Brazil, there are no unified and effective environmental monitoring models for bodies of water. Thus, several methodologies are used that result in information that is often difficult to compare, especially for stakeholders involved in regional water management. Studies in some countries such as Australia, Chile, the USA, and Sweden use the monitoring model implemented in Canada that was developed in the early 1990s. This model was designed to evaluate whether the current environmental regulations are sufficiently protective for pulp and paper effluents and for metal mining effluents. In this study, the Canadian Environmental Effects Monitoring methodologies were applied to three different Brazilian river basins, with the goal of constructing a framework for monitoring environmental effects. Pilot studies were carried out in the estuarine regions of the Benevente, Jucu, and Santa Maria da Vitória river basins, which are important rivers in the state of Espírito Santo. Evaluations included fish health, bioaccumulation studies, benthic invertebrate survey, and physical-chemical analyses of water and sediment. The quality of the environments was evaluated by

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P. da Mata Pavione
Instituto Nacional de Pesquisas da Amazônia, Rua Andre Araujo, 2936, Petrópolis Manaus, BR 69067-375, Brazil

K. G. da Costa
Centro Universitário Norte do Espírito Santo, São Mateus, Espirito Santo BR-101, Brazil

C. Perônico · T. H. Basilo
Instituto Federal do Espirito Santo, Rua Augusto Costa de Oliveira, 660, Praia Doce, Piúma, Espirito Santo 29285-000, Brazil

M. E. McMaster · J. L. Parrott · L. M. Hewitt
Environment and Climate Change Canada, 867 Lakeshore Road, Burlington, ON L7S 1A1, Canada

K. R. Munkittrick
Department of Biology, Wilfrid Laurier University, 75 University Ave W, Waterloo, ON N2L 3C5, Canada

F. C. C. Barreto
Universidade Federal do Espirito Santo, Av. Fernando Ferrari, 514 - Goiabeiras, Vitória, Espirito Santo 29075-910, Brazil

M. P. Gomes
Department of Earth, Ocean & Ecological Sciences, School of Environmental Sciences, University of Liverpool, Liverpool L69 3GP, UK

R. W. Reis Filho (✉)
APLYSIA Environmental Consulting, Rua Julia Lacourt Penna, 335, Jardim Camburi, Vitória, Espírito Santo 29090-210, Brazil
e-mail: ricardo@aplysia.com.br

T. H. Furley
Instituto Aplysia, Rua Julia Lacourt Penna, 335, Jardim Camburi, Vitória, Espírito Santo 29090-210, Brazil

means of seasonal samplings and comparisons between discharge, upstream, and downstream areas. This study made it possible to identify appropriate fish species to be used in environmental effects monitoring and the environmental quality of the rivers themselves as well as knowledge and policy gaps to implement such monitoring programs in Brazil. The study raises questions about the adequacy of Brazilian environmental legislation concerning tidal rivers.

Keywords Watershed · Estuary · Quality · Guideline · Tidal river

Introduction

In Brazil, federal environmental regulations are administered by the National Environment Council (CONAMA, Conselho Nacional do Meio Ambiente), which establishes water quality standards for different uses, such as swimming and potability. None of those ordinances considers effects in aquatic biota (Furley and Perônico 2015); however, a coherent biological approach must be established necessarily to link the conditions of the environment and the responses of organisms (Buss et al. 2008). Many regulatory agencies, for example, in Canada (Munkittrick et al. 2004), USA (Barbour et al. 1999), and the UK (CSM Guidance for Rivers 2016) have shown the importance and success of the integration of physical, chemical, and biological assessments for the evaluation of environmental health.

In Canada, this type of integrative monitoring was implemented in the mid-1990s, as part of the federal government's Environmental Effects Monitoring (EEM) program. This methodology is used to monitor pulp and paper mill (EEM 2010) and metal mining wastewater (EEM 2013) effects in the environment. Besides the physical and chemical data of water and sediment, fish health and benthic communities are also evaluated in the receiving environment. The EEM program is used as a scientific feedback tool to determine whether current regulations are sufficiently protective for rivers that receive different pollutant loads (Hewitt et al. 2008).

The Brazilian National Program of Water Quality Evaluation (PNQA, Programa Nacional de Avaliação da Qualidade das Águas) has not yet established a standardized methodology for monitoring or assessing environmental effects. This makes it difficult to compare results from different water bodies conducted by different groups.

The aim of this study was to adopt and apply the Canadian EEM protocol in a pilot study to evaluate the health of three tidal rivers in the state of Espírito Santo, Brazil: Benevente River, Jucu River, and Santa Maria da Vitória River. This pilot study was named the "Fish Guide Project," and it was designed to investigate and monitor aquatic environmental effects, with the goal of constructing a framework to monitor environmental effluents that in the future may be standardized in Brazil.

Materials and methods

Study area

The study was conducted in the estuary of three important rivers of Espírito Santo—Brazil: Benevente, Jucu, and Santa Maria da Vitória. These rivers receive urban and agricultural drainage, and the last two supply water to the largest city in the state, Vitória. As cited by Alvares et al. (2013), based on Koppen's climatic classification, the region comprising the Benevente and the Jucu basins is classified as a tropical zone with dry winter (Aw), while the basin of the Santa Maria da Vitória River is included in a tropical zone with monsoon (Am). According to the tide stations of the Brazilian Navy, the tide range for the three estuaries is from −0.2 to 1.8 m (DHN 2019). The rivers Benevente and Sta. Maria da Vitória are more influenced by the daily and seasonal cycles of the tides, while the Jucu River has less influence due to the dynamics of sediments that frequently form sand bars in its mouth, occasionally forcing its closure. The Q90 flow (flow of 90% in time) at the mouth region is very similar between the rivers: 9.57 m^3/s for the Benevente (AGERH 2015), 9.08 m^3/s for the Jucu, and 9.30 m^3/s for the Santa Maria da Vitória (AGERH 2016).

Briefly, the Benevente River has a drainage area of approximately 1260 km^2 and covers six municipalities of Espírito Santo state, with a length of 79 km. The main agricultural practices in its basin are vegetable, yam, banana, and coffee plantations (AGERH 2015). The Jucu River is 169.5 km long and drains an area of approximately 2183 km^2 spanning seven cities; the activities are predominantly rural (pastures and mainly coffee and fruit crops) with densely urbanized areas near the mouth (IEMA 2016). The Santa Maria da Vitória basin area is 1817 km^2, with a length of 126 km and an area that includes five cities. Its wider scope is rural (large coffee and vegetable crop areas, as well as various types of fruit). The regions bordering the estuarine area are also heavily urbanized (IEMA 2016).

Three sampling points were selected from upstream to downstream, along an expected (at least theoretically) contamination gradient in each river: the Benevente (B1, B2, and B3), the Jucu (J1, J2, and J3), and the Santa Maria da Vitória (S1, S2, and S3) (Fig. 1) to test EEM methodology. Among these sampling points, one had a higher degree of human influence with direct wastewater discharge and so was named the discharge point (Table 1).

Sampling

Previous meetings with scientific experts in collaboration with local fishermen (who knew the rivers in detail) were carried out in order to define the appropriate monitoring sites for sampling, to select the indicator species, and to define the most suitable fishing equipment.

Biological (fish and benthos) and chemical parameters were collected three times over a period of 2 years (2014 and 2015) in three campaigns. Samples of water and sediment were collected in parallel to the biota sampling. All sampling efforts were performed during low tides.

Fish sampling and analyses

According to EEM protocol, it is important to carefully select the indicator species. Biological and social factors such as small adult body size, preferentially omnivorous diet, economic relevance, ease of identification, abundance in the environment, and other criteria were considered (Furley and Perônico 2015; EEM 2013). Fish sampling was performed with 20 × 20, 30 × 35, and 40 × 40 mm mesh gillnets, with a 1.5 m approximate height and a 60 m average total length. The nets were set in parallel to the marginal vegetation of the mangrove in the three estuaries overnight for approximately 12 h. Every 6 h, the fish caught were taken from the nets and transported to the laboratory. Multiple days were required to catch sufficient sample numbers of fish (above 20 males and 20 females), and still that was not always successful.

Upon retrieval of fish from nets, the living specimens were immediately euthanized by severance of the spinal cord, packed in coolers, and transported to the biology laboratory of the Federal Institute of Education, Science and Technology (Instituto Federal de Educação, Ciência e Tecnologia, IFES) - Piúma campus and the University

Table 1 Overview and geographic coordinates of the sampling sites for studies in 2014 and 2015

River		Site	General description	Latitude and longitude
Benevente Jucu	B1	Upstream	Conservation area, artisanal crab fishing, tourism, and leisure.	328685.101435 X/7699482.91004 Y
	B2	Discharge	Sewage discharge area from agro-based industries plus urban sewage from small towns.	328302.378088 X/7699493.76298 Y
	B3	Downstream	Downstream of treated sewage Mouth of the river	327547.582016 X/7698450.21155 Y
	J1	Upstream	Suppression of vegetation along banks for livestock farming, diffuse sources of sewage discharge	358279.487628 X/7743483.91916 Y
	J2	Discharge	High domestic sewage discharge	361965.684524 X/7743657.89277 Y
	J3	Downstream	Low domestic sewage discharge Mouth of the river	361914.529548 X/7741155.77689 Y
Santa Maria da Vitória	S1	Upstream	Low human presence with diffuse agricultural and industrial (agro-based) activities	359502.121998 X/7760854.91655 Y
	S2	Discharge	Bubu River, near city of Cariacica—high domestic sewage discharge	356654.991547 X/7756970.13012 Y
	S3	Downstream	Low human activity	360163.996702 X/7760038.07506 Y

of Vila Velha for individual analysis of each specimen (according to this project environmental license SISBIO no. 42033-1).

In the laboratory, adult fresh fish were measured (fork length in millimeters and wet weight in grams), and the liver and gonads were removed and weighed (± 0.1 g). Indicators of growth and health, such as condition factor k: 100 × wet weight (g) / fork length^3 (mm), gonadosomatic index (GSI): 100 × gonads weight (g) / wet weight (g) (de Vazzoler 1996), and hepatosomatic index (HSI): 100 × liver weight (g) / wet weight (g), were calculated. The indices were evaluated as described in the Environment Canada 2010a, 2013) and in Kovacs et al. (2013). The evaluation of the health status of these fish species by means of the morphological parameters allows us to estimate (together with other measurements) the level of anthropogenic stress on these systems.

Fish trace metal bioaccumulation was analyzed in the fillets of 6 individuals from each site, during each sampling period. Each batch of samples was accompanied by a NIST calibration standard analysis of multiple elements (As, Cr, Pb, Cu, Al, Fe, Mn, and Zn). Samples were analyzed by ICP-OES following an operational protocol suggested by the AP 035 Merieus NutriSciences. The metal pollution index (MPI = $(CM_1 \times CM_2 \times CM_n)^{1/n}$)) was calculated according to Usero et al. 1997, Usero et al. 2005). The MPI was used to compare total metal accumulation among organisms inhabiting different areas. This index expresses the total contamination by metals and reflects the chronic exposure of an organism to these pollutants. Thus, the greater its value, the greater the possibility that the environments have metal concentrations that may affect the organisms studied.

Benthic community sampling and analyses

Samples were taken to evaluate benthic community structure in areas where the sediments were fine silt/clay, and the depth was less than 1.5 m. Three replicates were collected at each sampling point using a 0.08-m^2 Petersen grab sampler. Samples were sieved through 0.2-mm mesh bags in the field and then fixed in a 5% formalin solution. In the laboratory, samples were washed to remove the formalin preservative, and all organisms were counted and identified into taxonomic groups at the most refined level of detail possible.

Physico-chemical analysis of water and sediment

At each sampling site, bottom water samples were collected with Van Dorn Bottle Samplers (3 L capacity) and measurements of conductivity, pH, temperature, dissolved oxygen, turbidity, total suspended solids, salinity, and potential for oxide reduction were taken using a calibrated Horiba U-50 multiparameter (accredited by INMETRO - National Institute of Metrology, Quality and Technology). Samples were cooled and preservatives used, when necessary, for proper preservation. In the laboratory, samples were also analyzed for concentrations of metals (aluminum, arsenic, boron, cadmium, lead, zinc, chromium, iron, manganese), organic chemicals (NH_3, NO_2, NO_3, NH_3, chloride, fluoride, surfactants such as linear alkylbenzene sulfonates (LAS), and dissolved organic carbon), and *Escherichia coli*. The physico-chemical water results were compared to the Brazilian federal legislation water quality limits (CONAMA 2005). The sediment samples were collected with a Petersen grab (internal volume of 9890 mL) for analysis of metals (aluminum, arsenic, boron, cadmium, lead, zinc, chromium, iron, manganese), nutrients (total organic carbon, total Kjeldahl nitrogen, phosphorus), and particle size. The results were compared to the Brazilian federal legislation recommendations CONAMA (454/ 2012), and grain size classification followed ABNT (1995).

All tests were performed following the Standard Methods for the Examination of Water and Wastewater protocol (Rice and Bridgewater 2012) and the laboratories that carried out the analyses (Bioagri Ambiental and ArcelorMittal) are accredited by ISO 17025.

Statistical analysis

The statistical analysis of the biological data was performed by sampling sites and season.

For fish data sets, the evaluation was done by sex. Groups with sample sizes < 6 and groups with immature gonads were excluded from the analyses. For bioaccumulation analysis at each sampling site, data groups with < 3 samples were excluded. The responses were evaluated by analysis of variance (ANOVA, $p < 0.05$) after the normality among sites was checked and, when appropriate, post hoc Dunnett's tests were used to identify site differences. In the case of non-parametric data, the Kruskal-Wallis tests were used. All descriptive

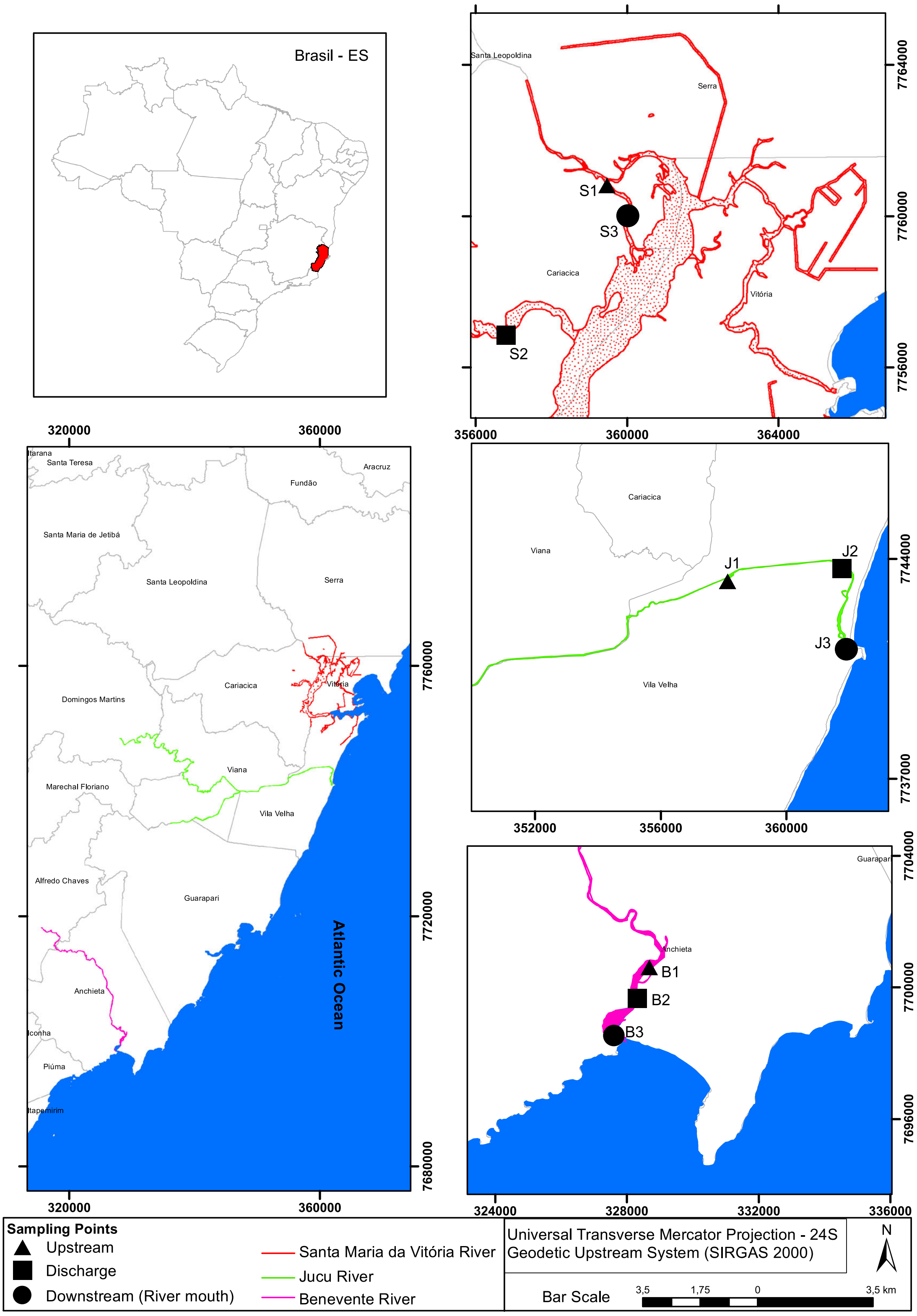

Fig. 1 Sampling points selected from upstream to downstream, along an expected (at least theoretically) contamination gradient in each river

Table 2 Sampling dates and selected bioindicator fish species

River	Fish species (common name)	Sampling campaign	Date
Benevente	*Ophioscion punctatissimus* (Cangoá; spotted croaker)	First	05/2014 (autumn)
		Second	11/2014 (spring)
		Third	07/2015 to 08/2015 (winter)
Jucu	*Genidens genidens* (Bagre; Guri sea catfish, urutu)	First	09/2014 (spring)
		Second	12/2014 (summer)
		Third	07/2015 (winter)
Santa Maria da Vitória	*Genidens genidens* (Bagre; Guri sea catfish, urutu) *Eleotris pisonis* (Moreia; spinycheek sleeper)	First	06/2014 to 09/2014 (winter)
		Second	12/2014 to 02/2015 (summer)
		Third	07/2015 to 08/2015 (winter)

statistics were performed using the free software R version 3.2.5.

The univariate descriptors of benthic fauna included numbers of taxa, density (individuals/m^2), the Shannon-Wiener diversity index (Log_2), and Pielou's evenness index (Begon et al. 2006). Non-parametric univariate analyses (Kruskal-Wallis) were performed to assess whether there were differences between sites or sampling periods. Multiple comparisons were applied when significant differences between the variables were observed.

Results and discussion

Fish health

Considering the sum of the biological and social factors used to select the indicator species by the EEM methodology, the fish species that best stood out and met almost 70% of the requirements were as follows: *Ophioscion punctatissimus* (Sciaenidae; Benevente River); *Genidens genidens* (Ariidade; Jucu River and Santa Maria da Vitória River); and *Eleotris pisonis* (Eleotridae; Santa Maria da Vitória River). Specifically for *G. genidens*, the most abundant fish, there were several other studies conducted in Brazilian coastal waters that employed this species as a bioindicator, including studies with biomarkers (Rodrigues et al. 2010; Sardi et al. 2016), and studies that highlight the species as focal organisms for environmental quality assessments in estuarine regions (Silva Junior et al. 2013; Blaber and Barletta 2016) since they have relatively low mobility and are widely geographically distributed (Azevedo et al. 2012).

These three fish species are not part of the current list of Brazilian fauna species threatened with extinction (ICMBio 2016); therefore, they can be collected for both environmental and commercial purposes. Briefly, spotted croakers (*O. punctatissimus*) inhabit muddy, sandy bottoms in shallow coastal regions (Menezes and de Figueiredo 1980) and feed mainly on bottom-dwelling organisms. Its geographic distribution, according to Chao et al. (2015), is more than in 50% of the country. The urutu catfish (*G. genidens*) lives in the sandy and muddy bottoms of estuaries, where it completes its entire life cycle. It is commonly found on the east coast of South America, from Bahia in Northern Brazil to the Rio de La Plata in Argentina (Fischer et al. 2011). The spinycheek sleeper (*E. pisonis*) is a bottom-dwelling species found in tropical estuarine systems, and adults seem to prefer shallow freshwater tributaries (Pezold et al. 2015). Its distribution is extensive and reported along the South American coast, from Venezuela to Southern Brazil (Pezold and Cage 2002).

It is still important to highlight that two of the species investigated, *G. genidens* and *E. pisonis*, are eurifagic and support and live in locations with broad variations of salinity according to the movement patterns and distribution data compiled by IUCN (Acero and Bentancur 2010; Pezold et al. 2015). In environmental assessment studies of estuarine zones, the use of bottom-feeding fish, such as catfish, is important since the sediment is the final site of contaminant deposition.

The biological descriptors measured in fish from the studied rivers (Online Resource 1) showed tenuous signs of both seasonal and spatial variations, mainly in females. Miller and Kendall (2009) mention that when males and females are compared, it is possible to observe that females invest much more energy in the

Table 3 Results of female gonadosomatic index (GSI♀) among the three evaluated rivers. Statistical significance (increase or decrease) evaluated by the Kruskal-Wallis tests

River	Biological descriptor	Sampling points	Statistical parameters	Statistical results
Benevente	GSI♀	B1 × B2	$H = 14.7$, $df = 2$, $p < 0.05$	Increased
(1st campaign)	*O. punctatissimus*	B1 × B3		
(2nd campaign)		B1 = B2 = B3	$H = 10.1$, $df = 2$, $p < 0.05$	Equal
(3rd campaign)		B1 × B2	$H = 9.4$, $df = 2$, $p < 0.05$	Equal
		B1 × B3		Increased
Jucu	GSI♀	J1 = J2 = J3	$H = 4.0$, $df = 2$, $p = 0.1$	Equal
(1st campaign)	*G. genidens*		$H = 2.7$, $df = 2$, $p = 0.3$	
(2nd campaign)			$H = 2.6$, $df = 2$, $p = 0.3$	
(3rd campaign)				
Sta. Maria da Vitória	GSI♀	S1 × S2	$H = 9.3$, $df = 1$, $p < 0.05$	Equal
(1st campaign)	*G. genidens*	S1 × S3	$H = 6.4$, $df = 1$, $p < 0.05$	Increased
(2nd campaign)		S1 = S2 = S3	$H = 0.7$, $df = 1$, $p = 0.4$	Equal
(3rd campaign)		S1 × S2		Equal
		S1 × S3		Decreased

The capital letter refers to the river name (B: Benevente; J: Jucu; S: Sta. Maria da Vitória). The number refers to the sampling points (1: reference; 2: discharge; 3: downstream)

gonads than males. Therefore, they are more likely to accurately indicate the reproductive condition of the population and were further evaluated in this work.

As a priori there was no scientific knowledge about the reproductive period of the species collected in the study area, the samplings were performed during both summer and winter periods (Table 2). For the Benevente River, in campaigns 1 (65% of a total of 122 (Nt) captured females) and 2 (80% Nt = 88), high proportions of mature females were found in relation to the third campaign (20% Nt = 73), which was in the winter period. In the Jucu River, during the first two campaigns, it was possible to capture sufficient mature females (42% Nt = 70 and 33% Nt = 77 respectively). This data corroborates the extended spawning period of the catfish described by Barbieri et al. (1992) and Gomes et al. (1999) from October to February in Jacarepaguá lagoon, a coastal system in Rio de Janeiro city. However, Dias et al. (2017) only found juveniles and immatures between September and December in Bertioga channel (the estuarine region of Santos-SP). Our results and these reports reinforce the need for local investigations to establish breeding population patterns before starting an EEM. For the Santa Maria da Vitória River, the number of mature specimens (both *G. genidens* and *E. pisonis*) was low (30% Nt = 66) in the first campaign, (25% Nt = 73) in the second, and lower (9% Nt = 63) in the third. In general, the campaigns carried out in the winter period were the ones that resulted in the highest percentages of immature fish. As a first evaluation, this pattern is quite consistent, since it is known that temperature is one of the main abiotic triggers responsible for reproduction in fish (da Ribeiro C and Moreira 2012; Wootton and Smith 2015), although this influence is more clearly manifested in temperate waters (Wang et al. 2010; Wootton and Smith 2015). One of the first recommendations from the study is the need to concentrate sampling efforts at

Table 4 Results of hepatosomatic index (HSI) of river sites that showed differences from the upstream site. Statistical significance (increase or decrease) evaluated by the Kruskal-Wallis test

River	Biological descriptor	Sampling points	Statistical parameters	Statistical results
Jucu	HSI♂	J1 × J2	$H = 7.4$, $df = 2$, $p < 0.05$	Increased
(1st campaign)	*G. genidens*	J1 × J2	$H = 7.9$, $df = 2$, $p < 0.05$	Increased
(2nd campaign)	HSI♀	J1 × J2	$H = 14.9$, $df = 2$, $p < 0.05$	Increased
(2nd campaign)				
Sta. Maria da Vitória	HSI♂	S1 × S2	$H = 8.9$, $df = 2$, $p < 0.05$	Decreased
(3rd campaign)	*G. genidens*			

all sites during periods of maximum gonadal development in the species of choice.

For the Benevente River, the female gonadosomatic index (GSI♀) of *O. punctatissimus* in the first campaign was significantly increased when comparing the upstream point B1 to the discharge point B2 and the downstream point B3 (Table 3). In the second sampling campaign, no point differences were detected, but in the third campaign, GSI♀ was again significantly higher at the downstream point B3. The GSI♀ calculated for *G. genidens* catfish in the Jucu River J2, J3 was not statistically different from the upstream point J1 in any sampling period. In the Santa Maria da Vitória River, *G. genidens* at downstream point S3 had a significant increase in GSI♀ in the first campaign but significantly decreased at the same point (S3) in the third campaign (Table 3). *E. pisonis* (captured in the river only in the first campaign) did not show differences in GSI♀ among sampling points. For *O. punctatissimus* and *E. pisonis*, it should be emphasized that there is a great lack of information for the species about their biology and reproductive cycles. In our study, both species had an increase in gonadal development in the months of October and November, indicating a pre-spawning period for upcoming reproductive events.

For males and females, the HSI (hepatosomatic index) results showed slight deviations from the upstream point values. The records showing significant differences in relation to the upstream point are summarized in Table 4. In the Jucu River, regardless of the campaign, the HSI from the discharge point was consistently elevated when compared to the upstream point. This tendency (liver hypertrophy) seen in the fish of J2 may be accompanied by histological damage caused by exposure to contaminants as seen by Filho et al. (2001) and Araújo et al. (2018). For the Santa Maria da Vitória River, the HSI decreased at the discharge point in the third campaign. Lower values may be associated with sublethal toxicity of effluents linked to zones of retention of pollution (Online Resource 6—Table 6.3—shows very low dissolved oxygen at this point) and consequently synergic effects causing liver intoxication (Araújo et al. 2018). Heath (2018) mentions that fish under chronic stressful situations often reduce or even cease feeding, therefore causing decline in liver mass. These two situations certainly occur and reflect the inferior quality of point B2.

The relative size of the liver is usually a good indicator for assessing fish health in impacted aquatic environments (Munkittrick et al. 1994; van der Oost et al. 2003). Several studies have shown that fish from contaminated sites have modified HSI values when compared to fish from less degraded sites (Pinkey et al. 2001; Martín-Díaz et al. 2005; Yang and Baumann 2006). The liver is important for metabolism of endogenous and exogenous chemicals (Hinton et al. 2008) and is the main detoxifying organ of the body (Schlenk et al. 2008). Fish often store energy in the liver, and in sites with increased food availability, fish often have larger livers as shown in J2. Therefore, HSI is an important index applied in the evaluation of aquatic environmental systems.

In the current studies, fish condition factor (*K*) for each species always differed slightly from the upstream sites along the contamination gradient; however, there was no statistically significant difference between the points for all rivers (Online Resource 1). The *K* in the Benevente River for female *O. punctatissimus* at the points B2 and B3 always showed a slight increase when compared to B1, regardless of when the campaign was carried out. However, the Jucu River did not present the same pattern, and the fish from J3 point had *K* lower than J1 in all campaigns, while the J2 point had higher *K* in all sampling campaigns. In the Sta. Maria da Vitória River, *K* values alternated between high and low during all of the sampling periods.

According to Sayer et al. (1996), the condition factor *K*, although varying among species, seasons, and geographic location, may help with the interpretation of environmental quality when analysed in conjunction with other parameters. There is substantial energy needed to regulate the effects of pollutants, so some of the energy that would be allocated to somatic growth may be diverted. Thus, fish with lower condition factors may be found in polluted waters (Lawrence and Hemingway 2003), while higher factors may be found in nutrient-enriched waters. In addition, variations in *K* are associated with reproductive activity, and maximum condition values often match with the period of higher GSI in mature females (Sato et al. 2003). This is why it is critical to sample fish from all sites at similar periods of the reproductive cycle and to remove immature fish from the comparisons as emphasized by the EEM protocol.

In general, domestic and industrial effluents, due to a complex mixture of chemical-biological contaminants, may be toxic to fish by interfering with their behavioral, feeding, and nutrient absorption processes and consequently reducing their growth and reproduction rates (Weis and Candelmo 2012; Al-Ghais 2013).

Despite the difficulty in establishing the ideal season for sampling fish species used as indicators in this study, signs of effects (GSI, HSI) could be distinguished at discharge and downstream points B2, B3, J2, S2, and S3 when compared with their respective upstream points.

Thus, the results found provide an indication that if efforts are directed to baseline studies of the population structure of the most abundant species in estuaries, they may provide more useful environmental quality responses. Therefore, it is essential to carefully establish the periods of pre-spawning, spawning, and post-spawning energy accumulation of the chosen sentinel fish species so that the applied indices better reflect the influence of environmental quality on the physiology of the organisms evaluated. Barrett and Munkittrick (2010) emphasize the need to determine the appropriate sampling period for each sentinel fish species so that the impacts in the studied environments are correctly measured. Results from the current Brazilian pilot study can be used to help in designing more appropriate monitoring studies for use in regional monitoring and in site-specific studies.

Bioaccumulation in fish muscle tissue

The Canadian Environmental Effects Monitoring program (EEM 2010) includes fish tissue contaminant analyses to monitor and confirm environmental effects on the fisheries resources. According to the Surface Water Ambient Monitoring Program (SWAMP 2008), bioaccumulation studies are also an effective method to evaluate the state of the investigated water bodies. Organisms integrate all aspects of their environment, as they can accumulate substances from ingested food and respired water through the gills.

The analysis of the fish musculature (fillets) of the captured species in this study led to the quantification of metals (aluminum, copper, lead, iron, manganese, zinc, and the metalloid arsenic) in organisms of all three river systems. Metal concentrations found (Online Resource 2), except for total arsenic, did not exceed Brazilian standards for human consumption. The decree No. 55.871/(D.O.U. 1965) and ANVISA (2013) established restriction limits for arsenic (1.0 mg/kg), lead (0.30 mg/kg), copper (30.0 mg/kg), chromium (0.1 mg/kg), and zinc (50.0 mg/kg).

In the Benevente River, *O. punctatissimus* bioaccumulation assessments showed that arsenic concentrations exceeded the legal limit at the downstream site, point B3, in the third campaign (arsenic medium concentration = As_{μ} = 1.15 mg/kg).

In the Jucu River, all results for the upstream point J1 in the three campaigns were below the arsenic limit in muscle tissue of *G. genidens.* For the J2 discharge point in the second and third sampling campaigns, the mean values for arsenic were higher than the legislation limit (As_{μ} = 3.07 mg/kg and As_{μ} = 1.17 mg/kg respectively). At the downstream point J3, the values of the first campaign (As_{μ} = 1.58 mg/kg), second campaign (As_{μ} = 2.33 mg/kg), and third campaign (As_{μ} = 1.37 mg/kg) exceeded the limits.

In the Santa Maria da Vitória River, the As concentration in fish tissue (*G. genidens*) was above the legal consumption limit only at the river mouth, point S3 in the second campaign (As_{μ} = 3.09 mg/kg). On the other hand, *E. pisonis* never presented measurable concentrations at any of the points sampled. This different response between these two sentinel fish species reinforces the recommendations of EEM (2013) to use at least two species of fish to avoid misinterpretation.

Angeli et al. (2013) studied trace elements in catfish of the Paranaguá estuary complex (South Brazil) and found that total As concentrations ranged from 0.91 to 4.78 mg/kg in muscle tissues. These values are comparable with those of our current studies. Furthermore, two points should be highlighted in our results: the presence of total arsenic in fish above Brazilian legal limits and the relevance of catfish as a sentinel organism. Azevedo et al. (2012) observed that for *G. genidens* in estuaries in the state of São Paulo (southwest Brazil), the liver and gills showed higher metal levels in relation to muscle tissues. Therefore, the high As concentrations found in the fillets in this study corroborate the hypothesis that the fish are under a high As exposure. It is known that inorganic arsenic can affect health and cause metabolic changes, immunological depression, body mass decrease, and reproductive problems in catfish (Ghosh et al. 2006; Yamaguchi et al. 2007; Kumar and Banerjee 2012; Balasubramanian and Kumar 2013). This can endanger fish populations chronically exposed to this contaminant and, consequently, the trophic food webs in which they are present. The FAO/WHO joint committee established that a tolerable daily intake of inorganic arsenic would be 0.002 mg/kg (WHO 2011), which

Table 5 Salinity measurements carried out in situ during sampling campaigns at the various study points

River	Upstream	Discharge	Downstream
Benevente	2.97 km	1.25 km	–
Course distance to the mouth of the river	1.90	1.15	2.54
(1st campaign)			
(2nd campaign)	19.54	24.12	25.68
(3rd campaign)	10.73	19.22	26.36
Jucu	6.39 km	2.57 km	–
Course distance to the mouth of the river	0	0	13
(1st campaign)	0	0	0
(2nd campaign)	0	0	14.2
(3rd campaign)			
Sta. Maria da Vitória	1.45 km	4.75 km	–
Course distance to the mouth of the river	0	2.37	0
(1st campaign)	0	22	0
(2nd campaign)	10.3	18.4	13.9
(3rd campaign)			

corresponds to the amount of 0.14 mg/day for a 70-kg adult (the average Brazilian adult weight).

Although Brazilian legislation stipulates total arsenic as a quality parameter for fish, the toxic inorganic form is generally less than 10% of the total fraction commonly bioaccumulated in fish tissues (WHO 2011). Recently, the Brazilian Federal Health Surveillance Agency (ANVISA) in a technical note (ANVISA 2019) recognized that the quantification of total arsenic may not characterize a health risk, since most of the arsenic detected probably refer to the non-toxic organic form of the element. Therefore, if the limit for total As is exceeded, ANVISA recommends speciation to verify the concentration of inorganic arsenic present in fish.

Therefore, the data for total As should be carefully evaluated, but they are nonetheless useful as a first characterization. These initial screening data provide a strong indication that more in-depth studies, including As speciation, are necessary.

In an attempt to standardize the evaluation of the total metal load bioaccumulated by fish, the metal pollution index (MPI) was calculated (Online Resource 3). Although some metals which do not have restrictive values in the legislation are often required for physiological activities, when in excess, they can also trigger harmful effects. Calculation of MPI allows comparisons among different areas because it generates an absolute value. The calculation of MPI for the different campaigns and among the sampling sites in most cases confirmed the absence of defined quality gradients in the water bodies investigated. In the Benevente River, there was a very slight difference between the index values for upstream points ($B_{1.2}$ = 0.14; $B_{1.3}$ = 0.10) and the downstream points ($B_{3.2}$ = 0.19; $B_{3.3}$ = 0.17) for the second and third campaigns for *O. punctatissimus*. As for the Jucu River, with *G. genidens*, in the first sampling campaign, the values were almost uniform. In the second campaign, the MPI for the upstream point ($J_{1.2}$ = 0.11) was low compared to that of the discharge point ($J_{2.2}$ = 0.25) and also the downstream river mouth point ($J_{3.2}$ = 0.17). In the third campaign, the values for the upstream, downstream, and discharge points were $J_{1.3}$ = 0.21, $J_{2.3}$ = 0.28, and $J_{3.3}$ = 0.18, respectively. The values calculated in the Santa Maria da Vitória River in the first campaign for *E. pisonis* and *G. genidens* were insignificant at the upstream and discharge sites; only at the downstream river mouth point, for the catfish, was there a higher value ($S_{1.3}$ = 0.12). The same pattern did not occur in subsequent campaigns, with higher MPIs found across all sampling points. In the third campaign, MPI calculations for *G. genidens* revealed the highest values ($S_{3.1}$ = 0.43; $S_{3.2}$ = 0.63; $S_{3.3}$ = 0.62). These data indicate a worsening of the habitat quality of the Santa Maria da Vitória River. It is interesting to note that during this third campaign, the highest salinity was recorded for the upstream point (S1) and also downstream (S3) (Table 5). So if this saline intrusion has occurred more systematically, the greater dynamics associated with resuspensions and various other physicochemical processes may favor higher metal exposure and bioaccumulation, and explain the highest values found.

Analysis of benthic communities

The average density of benthic invertebrates found in the samples of the Benevente River was 24,211 ind/m^2 (Online Resource 4). These organisms were distributed in eight taxonomic groups, with Tanaidacea being the most abundant group, representing 97.7% of the benthic fauna. The other groups found were Polychaeta, Amphipoda, Isopoda, Decapoda, Bivalvia, Gastropoda, and Insecta larvae. In the Jucu River, an average of 241.7 ind/m^2 belonging to 11 taxonomic groups was found. The Polychaeta were the most abundant in this river, accounting for 70.3% of the organisms found. In addition, Bivalvia, Gastropoda, Amphipoda, Isopoda, Tanaidacea, Ostracoda, Insecta larvae, Oligochaeta, Hirudinoida, and Nemertea were found. In the Santa Maria da Vitória River, 995.8 ind/m^2 were distributed in 10 taxonomic groups. The Polychaeta were the most abundant in this river, accounting for 52.6% of the organisms found. Bivalvia, Gastropoda, Oligochaeta, Isopoda, Amphipoda, Tanaidacea, Ostracoda, Nemertea, and Insecta larvae were the other groups found.

Assessment of invertebrate communities showed that the Benevente River was the least degraded among the three studied rivers. The general appearance of the samples collected suggested a cleaner environment, different than the Jucu and the Santa Maria da Vitória River samples, which distinctly contained finer sediment, higher amounts of organic matter, and untreated sewage residues (like feces and hair, especially at the discharge site).

For benthic fauna of the Benevente River, the high density of tanaids (*Monokalliapseudes schubarti* species) is characteristic of the region as shown in a previous study (Costa and Nalesso 2006). These organisms live buried or inside tubes in the sediment. They are important suspension feeders in the trophic structure of the community, being a common item in the diet of fish (Bemvenuti 1987; Figueiredo and Vieira 2005; Pennafirme and Soares-Gomes 2009). This species has been tested in toxicity assessments of estuarine environments and has demonstrated some resistance to Zn, Cd, SDS (sodium dodecyl sulfate), and NH_3 (Mottola et al. 2009; Resgalla Jr. and Laitano 2002).

For benthic fauna in the Benevente River, the Kruskal-Wallis test detected significant differences among sites and sampling periods (Online Resource 4). At the downstream point (B3), the lowest number of taxa and the lowest value for Pielou's evenness ($p <$ 0.05) were recorded. In the third sampling period, the lowest density of organisms was found, and there were higher values for Pielou's evenness and the Shannon-Wiener diversity relative to the first sampling period. Variations in the distribution of tanaids (which were more abundant at the downstream site during the first and second sampling periods, in addition to the lowest value recorded in the third sampling at all sites) probably explain the results found by these tests. Costa and Nalesso (2006) and Leite et al. (2003) also observed a reduction in the density of these tanaids in the colder months. Leite et al. (2003) suggest that sediment movement, algal growth, domestic sewage, and oil spills may cause local and short-term reductions in the abundance of these species.

In the Jucu River, significant spatial variation occurred for all univariate descriptors ($p < 0.05$) (Online Resource 4). At the discharge point (J2), no organisms were found in the first and third sampling periods. Samples from this river were the most difficult to work with due to the bad odor and the presence of untreated domestic sewage, especially in the discharge site sediment samples. On the other hand, the Kruskal-Wallis test with samples of the Santa Maria da Vitória River detected only significant variation in the density of organisms among samplings. In the first sampling, lower densities of organisms were found than in the second sampling period (Online Resource 4). However, this result occurred because there were no organisms at the discharge point (S2), which resulted in low density recorded for this sampling period. Like the samples collected at the discharge point (J2) in the Jucu River, samples of S2 were visually organically enriched. It is already widely known that organic enrichment causes changes in physical, chemical, and biological environment factors, which affect, directly or indirectly, the fauna present (Pearson and Rosemberg 1978). The organic matter discharge decreases the amount of dissolved oxygen in the bottom water, leading to eutrophication or even hypoxia. As a result, there are changes in the benthic fauna structure including organism mortality, reduced population densities, loss of biodiversity, and changes in trophic status (Pearson and Rosemberg 1978; Hall et al. 1997; Diaz and Rosenberg 2008).

The benthic infaunal communities in the present study clearly showed the gradients of environmental effects between rivers and sites. The benthic communities from the three rivers studied were characteristic of those generally seen in organically enriched sediments, with abundant opportunistic organisms, limited number of taxonomic groups, and even total absence of organisms. However, the results observed in the Benevente River do not seem to be as alarming as the ones seen in the Jucu and the Santa Maria da Vitória Rivers, where, in some samplings, no organisms were found at the discharge site, indicating a highly eutrophic environment.

Chemistry

Water

The driving forces of human development increasingly result in the input of substances into estuarine systems. For the three rivers evaluated, this finding becomes evident with high levels of metals, nutrients, surfactants, and microorganisms related to domestic sewage in the water (Online Resource 5). Among metals, the ones that most frequently exceeded regulatory limits (CONAMA Resolution 357/ 2005) were aluminum (0.1 mg/L), boron (0.5 mg/L), and iron (0.3 mg/L). The concentration of boron exceeded the limits at all sites of campaigns 2 and 3 in the Benevente River and at the discharge site of campaigns 1 and 2 of the Santa Maria da Vitória River. Among the uses of boron are agrochemicals, fertilizers, and stabilizers in cleaning products (Moss and Nagpal 2003). Therefore, boron can enter the rivers studied both via sewage effluents and through runoff. Schoderboeck et al. (2011) found predicted no-effect concentration (PNEC) values of 0.18 mg/L and 0.34 mg/L in an evaluation of the boron in aquatic environments using two different approaches. Boron exceeded these levels at all sites in our campaigns, which may indicate that the concentrations of this metal can potentially affect the evaluated ecosystems. Aluminum is widely used in several industrial and commercial sectors. Its use in water and effluent treatment processes stands out as one of the main sources of anthropogenic aluminum contamination (Wren and Stephenson 1991). In the Benevente River and in the Santa Maria da Vitória River, aluminum exceeded the limits during the second sampling campaign at all the sites investigated. The Jucu River was the one that most frequently had Al above the legal values. In six of the nine samples taken in three campaigns, aluminum exceeded the 0.1 mg/L threshold, including points upstream of campaigns 2 and 3. Metallic aluminum is innocuous under neutral or alkaline conditions (Sparling and Lowe 1996). However, under moderate acidity (pH between 5.5 and 7.0), fish and invertebrates may be affected due to the adsorption of aluminum to the gill surface and consequent asphyxiation (Sparling et al. 1997), and at pH of 4.5 to 5.5, there may be loss of ion regulation and resulting H^+ ion toxicity. Therefore, it is important to highlight that the pH measured in situ, especially for the Jucu River, was in the range of moderate acidity in many of the seasons and sample periods (Online Resource 6).

Besides the anthropogenic activities in the river basins, the pH of coastal waters is influenced by several factors including mixtures of substances, resuspension of bottom sediments, and anaerobic or aerobic dissolution of organic matter (Saraswat et al. 2015). The change in salinity can also induce pH changes (Greenwald and Hurlbert 1993; Sen Gupta 1999; Vadineanu 2004). Therefore, environments characterized as tidal rivers are especially influenced by these variations. Hackney et al. (1976) define tidal rivers as rivers that are influenced by tidal cycles and in which saltwater intrusions occur. As it can be observed in our measurements (Table 5, Online Resource 6), there are significant changes in the salinity of the three rivers studied, even at the most upstream points. These seasonal alternations between river discharge and tidal fluctuations strongly influence the physical and biological processes of monitored estuarine environments.

Elevated iron in water samples of these three rivers may have arisen from natural or anthropogenic sources. Iron also has many applications in mining and based on USEPA (1993) also in industry, including water and effluent treatment, insecticides, fertilizers, and pigments. In the monitored rivers, in the second campaign, iron exceeded the limits at all sites in the Benevente River and at the upstream and downstream river mouth sites of the Santa Maria da Vitória River. These results for the second campaign, in the summer, were even expected, as during these periods, there was more intense rainfall in the respective river basins (HIDROWEB 2019) that deposited on areas of deforested slopes. This resulted in iron-rich runoff. However, for the Jucu River, the values exceeded the legal limits in all three campaigns at all three sampling sites. Iron may trigger direct and indirect effects on river ecosystems (Vuori 1995) and may increase the toxicity of other substances (Sevcikova et al. 2011). Iron is a major constituent of soils, especially of clay soils (Phippen et al. 2008), and leaching processes can release iron particles that can cause irritation and damage to the gills of aquatic organisms, with consequent secondary bacterial or fungal infection.

Nutrients in the river waters (measured as various nitrogenous forms and total phosphorus) exceeded CONAMA limits at all sites (upstream, downstream, and discharge) in the third campaigns. Nitrate was the nitrogen component that most contributed to this scenario, and in waters of the Jucu River, concentrations were up to 100 times the maximum allowed (at the downstream site, second campaign, with a value of $J_{3.3}$ = 40 mg/L, versus the allowed limit of 0.40 mg/L). The mechanisms of nitrate toxic action and data related to its toxicity to aquatic organisms are quite variable (Poersch et al. 2007). However, the values observed at the mentioned river sites and campaigns are likely to trigger adverse effects when considering the levels of protection for freshwater (2 mg NO_3-N/L) and marine (20 mg NO_3-N/L) organisms, as evaluated in the review by Camargo et al. (2005).

Surfactants in the Benevente River were systematically above the limits at the discharge and downstream sites of all campaigns, and in the second and third campaigns, the upstream site also reached these high levels. One peculiarity on the Benevente River is that local boaters report frequent use of detergents by the artisanal fishing fleet to clean the boat decks. For the Jucu River, only the upstream point in the first campaign exceeded the limits of surfactants. However, in the third campaign, all points were over the limit. In the Santa Maria da Vitória River, the pattern in all campaigns was that the discharge site had high surfactant values (with the third campaign having the worst results as the upstream and downstream sites were also above the allowed thresholds). Surfactants damage cell membranes of organisms and result in denaturation of proteins (Pineda Flores et al. 2010). The Human and Environmental Risk Assessment (HERA) report (2013) indicates a PNEC value of 0.27 mg/L for linear alkylbenzene sulfonates (LAS). Thus, biota, especially those near the discharge sites of the rivers, are at risk due to high concentrations of surfactants at these sites.

Thermotolerant bacteria *Escherichia coli* are strongly associated with in natura sewer discharges because they inhabit the gastrointestinal tract of humans (Giatti et al. 2004; Rock and Rivera 2014). Studies indicate the possibility of fish retaining them for some time in their digestive and intestinal tracts (Del Rio-Rodriguez et al. 1997; Gordon and Cowling 2003; Hansen et al. 2008) and, therefore, becoming a contamination source for humans, especially if there is a lack of hygiene in fish handling and preparation (Novotny et al. 2004). By analyzing the levels of *E. coli* in the waters of the Benevente River and considering the standard of the CONAMA Resolution 274/ 2000 for waters with satisfactory quality of 800 *E. coli*/100 mL, the limits were exceeded in both the first and second campaigns at the upstream site and also at the discharge site of the second campaign. In the Jucu River, *E. coli* concentrations were usually above the limits, with only the downstream site of the first campaign and upstream site of the second campaign having *E. coli* levels below the criteria. For the sites exceeding the criteria, the values were extremely variable, from 1.2 times at the discharge site of the third campaign to more than 13,000 times at the downstream river mouth site of the second campaign. It should be noted that on this last occasion, the downstream river mouth was closed off due to low water levels. This special feature occurs in the Jucu River due to several factors that include low flow drought conditions and variable tidal forces, and these can result in upstream transport and sediment accumulation at the downstream river mouth site. In these situations, a large "lagoon" appears and the low water circulation in the system leads to deterioration of water quality. Still, it is interesting to note that even within a sample campaign, drastic variations occurred among sampling sites, a situation that implies multiple inputs and also possible illegal or non-regulated effluent discharge points. The Santa Maria da Vitória River also had several points with coliform levels above those expected, but the only site in which there was a significant discrepancy in relation to the others was at the discharge site during the second campaign, with 794,000 *E. coli*/100 mL, amounting to almost 1000 times the legal limits. Guillen and Wrast (2010) cite that *E. coli* levels in wild fish species tend to be similar to the environmental concentrations found in water and sediment. Thus, besides being very poor for human usage, the sites investigated can negatively impact both resident and visiting fish by possible alteration of their bacterial composition.

Although water parameters at the discharge areas of the three rivers often exceed regulatory standards for some parameters, it was not possible to establish a spatial pollution profile (as required by the EEM methodology for river health). This non-establishment was due to the values found at other points (upstream and downstream) which indiscriminately crossed the legal boundaries and therefore did not allow a clear definition of an upstream-downstream pollution gradation.

Sediment

Sediments are important compartments of any type of water system. Several contaminants adsorb or chelate into particles or debris that later settle on the bottom and so sediments may concentrate elements much higher than the water column where the processes are often more dynamic. The lower probability threshold for effects on the biota (Level 1 from CONAMA Resolution 454/ 2012) was used to compare the concentrations of contaminants determined in sediments in the three sampling campaigns. Few parameters exceeded the stipulated limits with only sporadic exceedance (considering all the sampling campaigns carried out). Paradoxically, relative to the other endpoints measured, the sediment of the Benevente River (the river that we assessed as in better condition) was the one that had highest As values in disagreement with CONAMA 454. The mean values found in the Benevente River at the discharge site (29.1 and 21.7 mg/kg) and the downstream river mouth site (32.4 and 20.7 mg/kg), respectively in campaigns 2 and 3, always exceeded the As limit which is 19 mg/kg (Level 1). These values, however, did not seem to affect the benthic community, as already discussed. The Benevente River had an abundant and a diverse benthic community. Espírito Santo soil is known to be rich in arsenic (Travassos et al. 2010; Mirlean et al. 2011), so unsustainable agricultural practice can accelerate As leaching to the aquatic environment. Arsenic is also used as a fertilizer and an agricultural pesticide as discussed by Henke (2009) in his book about arsenic issues around the globe. Although the concentrations of arsenic in sediments of the Jucu River and the Santa Maria da Vitória River were low, the fish fillet concentration at some sites (especially at the Jucu River) were high, showing the richness of this element in the aquatic environment. Cunha et al. (2013) found As up to eight times the allowed limit (CONAMA 2004) for stream sediments in other river basins in Espírito Santo state. Arsenic from weathering, rock dissolution, and erosive processes (Pimenta et al. 2015) can enter the watercourse and will settle, and over time will accumulate in the benthic environment. Therefore, this would reinforce the possibility of contamination arising from natural sources. In relation to biomagnification, Huff et al. (2010) showed that arsenic-laden organisms can be predated and arsenic biomagnified, resulting in high concentrations in tissues. These two lines of evidence must be considered in future studies in order to accurately determine the origin and pathways of elements in biological and physical matrices.

Salinity regime and water quality

An important point that is often not explored but cannot be neglected is the relationship between hydrological parameters and water quality of the analyzed environment. In tidal rivers, salinity varies according to the opposite forces of fluvial discharges and tidal phases, establishing in most cases the salt wedge. It is known that salt wedge intrusions influence water quality due to intensification of the precipitation and flocculation processes that occur at this interface (Carey 1990; Breault et al. 2000; Du Laing et al. 2008; Al-Maliki et al. 2015).

The highest salinities found in the upstream point were 19.54 (the Benevente River—second campaign) and 10.3 (the St. Maria da Vitória River—third campaign), classifying these zones, according to the international classification of Venice, as polyhaline and mesohaline respectively. These sampling sites are located approximately 3 and 1.5 km from the mouth of both rivers, and it is possible that the salt wedge extends beyond these zones.

As previously reported, boron, aluminum, and iron (for water) and arsenic (for sediment) were the elements most in disagreement with the quality standards. However, these did not vary in relation to the different salinities, but they appeared to be more related to both point and diffuse sources.

Although patterns between salinity and contaminant concentrations were not determined among sampling points, these important considerations should be included to assess the availability and possible effects on biota of both inorganic and organic compounds in tidal environments.

Conclusion

The EEM environmental effects assessment tools were applied to three tidal rivers in Brazil in order to discern differences in impacts between them. These rivers were already known to have different levels of water quality, so the applied methodology confirmed what was theoretically expected, being in increasing order of deterioration the Benevente River, the Sta. Maria da Vitória River, and the Jucu River. For assessments carried out within the same estuary watercourse, the results showed better spatial differences at the most deteriorated estuaries (the Jucu and the Santa Maria da Vitória). The Benevente River was the least impacted and presented clear saline intrusion in the estuary, so the spatial differences were not as clear.

The estuarine regions of the three rivers of this pilot study had concentrations of compounds typical of municipal effluents and runoff from agricultural areas. The Jucu River had the worst environmental conditions for fish health and subsequently for use as a fishing resource. The Benevente River had the best quality, regardless of the season or the sampling campaign. The catfish *Genidens genidens* and the spotted croaker *O. punctatissimus* proved to be suitable bioindicators for EEM assessments. Concerning physiological indices, although some significant signs of variation were observed (especially for GSI and HSI), it is believed that better results could have been achieved if sampling was performed just before the reproductive period, thus confirming the importance of deepening our basic understanding of the biology for both species.

The assessment of benthic invertebrate communities was an excellent tool for environmental evaluation and highlighted the Benevente River as the one that had the best environmental condition. This pilot study supports the importance of measures such as mapping illegal or unregulated points of effluent discharge, sewage treatment, river bank recovery, and the establishment of sustainable agricultural practices. This information and positive actions are needed urgently to improve water quality and sediment quality at these sites. Fish consumption should be carefully managed, due to the high concentrations of total arsenic in fish muscle tissue, and the levels of coliform bacteria found in the water at several points throughout the campaigns. Inorganic As bioaccumulation should be measured in future studies in Espírito Santo state.

It should be emphasized that the work constituted a pilot project where for the first time a standard framework for monitoring environmental effects (based on the Canadian model) was applied to tropical estuarine ecosystems. By adopting these consistent and published methods, it was possible to compare three different estuaries. Additional complementary studies (focused on specific questions and concerns such as As speciation) can be added to the outlined structure when needed or to identify the cause. Also, reliable databases, which allow tracking the environments investigated over time, can be used to permit modern and dynamic environmental management.

Finally, this study led to the understanding of the existence of a legal gap in relation to tidal rivers. The existing quality standards in the country do not take into account the influence of hydrological flow and tidal regimes in the stratification, dilution of pollutants, resuspension, and in the alterations of salinity that may alter bioavailability of contaminants (by causing precipitation and differentiated speciation of chemical elements). Therefore, the application of the environmental monitoring Fish Guide model, besides being essential for unifying investigations, has given rise to important questions that may contribute to the improvement of the water management and control policy in Brazil.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval The protocols of the study that involved the use of vertebrates were carried out according to the guidelines for the care and use of animals of CONCEA - National Council for Control of Animal Experimentation of the Brazilian Ministry of Science, Technology and Innovation.

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