Using rapid assessment of marine pollution (RAMP) techniques to assess the quality of marine sediments

ABESSA, D.M.S.1,*; BÍCEGO, M.C.2; SOUSA, E.C.P.M.2; ZARONI, L.P2,3; RACHID, B.R.F.2,4; ARNALOT, E.C.2,5; FILLMANN, G.6; READMAN, J.7 & DEPLEDGE, M.8

1. Universidade Estadual Paulista, Campus do Litoral Paulista, 11030-160, São Vicente, SP, Brazil
2. Universidade de São Paulo, Instituto Oceanográfico, 05508-900, São Paulo, SP, Brazil
3. Zaroni & Frazão. Oceanografia e Meio Ambiente, São Paulo, SP, Brazil.
4. BR Ambiental, São Paulo, SP, Brazil.
5. Instituto de Investigação das Pescas e do Mar, Lisboa, Portugal.
6. Universidade Federal do Rio Grande, Instituto de Oceanografia, 96203-900, Rio Grande, RS, Brazil
7. Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, UK
8. European Centre for Environment and Human Health, University of Exeter Medical School, Truro, Cornwall, TR1 3HD, UK.

Received December 15, 2017; Accept July 13, 2018

Abstract

Sediments represent an important environmental compartment, because they provide substrate for a range of species and may accumulate contaminants in high concentrations. However, the universe of methods to assess the quality of sediments is still small. This investigation aimed to assess the quality of sediments from some sites of Ubatuba (SE Brazil). To achieve that, sediments were analyzed for the presence of PAHs, by immunoassay ELISA kit for the carcinogenic PAH (c-PAH) RaPID Assay, and for toxicity to amphipods, sea-urchin embryos and direct exposure of mussel hemocytes and measurements of the neutral red retention time (NRTT). Results showed higher levels of PAHs in sediments from Itagua and Ribeira, those more intensely affected by contamination sources. The ecotoxicological analyses indicated these two sites as more degraded, together with the sediments from Lamberto Beach, which is also influenced by nautical activities. The NRRT correlated with the quantities of PAHs in sediments. We concluded that Ribeira Bay and Itaguá Beach need more detailed investigation on pollution and that the NRRT assay exposing directly hemocytes to sediment elutriates can be a useful tool to assessing sediment quality.

Keywords: coastal zone, contamination, toxicity, neutral red retention time, biomonitoring; early warning.

INTRODUCTION

Sediments are an important compartment of marine and estuarine ecosystems, providing substrate, food and shelter to a large number of benthic and epibenthic species (Abessa et al., 2006). However, sediments are also subjected to anthropic influences, because they consist of repositories for many of contaminants that are discharged in the aquatic systems (Swartz et al., 1985). Once in the sediments, contaminants may accumulate or be transformed by chemical and biological processes, may be assimilated by benthic organisms or released to the water column and may cause toxic effects to the aquatic biota (Nipper et al., 1989).

Despite the ecological importance, not many techniques are available for assessing the quality of estuarine and marine sediments. The available approaches comprise chemical analyses, toxicity tests, bioaccumulation assays, and the analysis of the benthic community structure (Chapman & Hollert,
2006); however, the number of biological models to evaluate the toxicity of sediments is quite small, especially in tropical environments. Although Brazil has a coastline of about 8,000 km sediment quality assessments have been conducted with less than 10 species, including embryos of the sea urchins *Lytechinus variegatus* and *Echinometra lucunter* (ABNT, 2012), amphipods *Tiburonella viscana* (ABNT, 2008; Melo & Abessa, 2002) and *Grandierella bonnieroides* (Moliani et al., 2013), tanaid *Kalliapseudes schubartii* (Zamboni & Costa, 2002), copepods *Nitocra* sp (Lotufo & Abessa, 2002) and *Tisbe biminiensis* (Araújo-Castro et al., 2009), tanaid *Kalliapseudes schubartii* (Zamboni & Costa, 2002), copepods *Nitocra* sp (Lotufo & Abessa, 2002) and *Tisbe biminiensis* (Araújo-Castro et al., 2009), tanaid *Kalliapseudes schubartii* (Zamboni & Costa, 2002), copepods *Nitocra* sp (Lotufo & Abessa, 2002) and *Tisbe biminiensis* (Araújo-Castro et al., 2009), shrimp juveniles (Nascimento & Evangelista, 2002) and the clam *Anomalocardia flexuosa* (Cruz, 2014). Thus, a much thorough ecotoxicological approach is urgently needed to better assess the sediment quality.

An additional aspect of tropical and subtropical regions is the scarcity of previous information about the studied area. Thus, contaminants levels and risks to the biota are often unknown. In these cases, screening techniques can used to identify hot spots and thus indicate priority sites where further studies should be conducted (Galloway et al., 2002). Such screening techniques should be rapid and cost effective allowing them to be used as the first step of tiered approaches, so that if the analysis of the initial set of data confirms the potential contamination, more thorough, reliable, and consequently expensive and time-consuming methods can be employed along this specific area.

Initial investigations of sediment quality have employed sediment toxicity tests, which are considered sensitive and provide reliable information on the sediment quality (Abessa et al., 2006). These techniques, however, may present some limitations, especially because they may not be sufficiently rapid in providing environmental information. Some studies
have used biomarkers (Bainy et al., 2000), which might be costly or require laboratorial efforts that are normally time consuming. The Neutral Red Retention Time (NRRT) assay has been used in studies concerning to evaluate the lysosomal membrane integrity, which can be used as a broad indicator of exposure to xenobiotics (Moore, 1990; Lowe et al., 1995; Cheung et al., 1997; Zaroni et al., 2001). Basically, it involves exposing blood cells to a colored dye, which is taken up by the lysosomes. Healthy cells retain the dye for more time than damaged cells, in which the dye rapidly leaks out into the cytoplasm. This technique has been used to study the health of field collected organisms (Abessa et al., 2005; Catharino et al., 2014; Maranho et al., 2017) or organisms exposed in laboratory, but to our knowledge no study has exposed directly the blood cells to the contaminants. Thus, the present study aimed to assess the quality of sediments from a tropical shallow region exposed to different pollution sources by using some simple and rapid techniques, including immunassays for analyzing the levels of PAHs, traditional toxicity tests (whole-sediment with amphipods and elutriates with embryos of sea urchins) an adaptation of the NRRT assay, which employed the direct exposure of mussels’ hemocytes to sediment elutriates.

**MATERIALS AND METHODS**

**Sediment Samples**

The sediment samples were collected at five different sites from Ubatuba, North Coast of the State of São Paulo (Fig. 1), in September 2000, by using a Petersen grab sampler. Some sites were located close to potential contamination sources such as sewage, urban drainage and marinas. Stations 1 (Ribeira) and 2 (Lamberto) were situated inside the Flamengo Bay, where several marinas are installed; these sites also receive some contribution of sewage. The Station 3 (Flamenguinho) was located in the same bay, but about 2 km southwards. The Station 4 (Itaguá) was situated in Ubatuba Bay, under the influence of domestic sewage inputs and a fishing terminal. Sediment from Station 5 was collected at Palmas Beach, in the Anchieta Island Marine State Park, and was used as reference; previous studies showed that sediments from this area are uncontaminated and do not present toxicity (Abessa et al., 2008). After collection, sediment samples were cooled and taken to the laboratory. Subsamples for chemical analyses were taken and frozen at -20 °C while those for ecotoxicological evaluation were stored at 4 °C.

Grain size distribution was analyzed by the wet and dry sieving method (McCave & Syvitski, 1991) and samples were classified following the textural classes of particles (Shepard, 1954; Folk & Ward, 1957), as sands or muds. The contents of organic matter (OM) in the sediment samples were estimated using the loss by ignition method (Luczak et al. 1997); 5g of dry sediment aliquots were separated from each sample and incinerated in a muffle (500°C) for 4 hours. Organic Matter contents were established by calculating the difference between initial and final weights.

**Enzyme-Linked Immunosorbent Assay (ELISA) for determining the concentrations of PAHs**

The concentration of PAHs was determined by the commercially available immunoassay ELISA kit for the carcinogenic PAH (c-PAH) RaPID Assay® (SDI Europe, Alton, UK). RaPID Assays® are tube-based immunoassays where the polyclonal antibodies are immobilized onto paramagnetic particles, being developed based on a competitive heterogeneous ELISA (Fillmann et al., 2007).

Initially, an extraction was performed prior to analysis using the SDI extraction kit for PAHs (SDI Europe, Alton, UK). Ten grams (10 g) of sediment and 20 mL of 100% methanol were added to an extraction jar (with 3 stainless steel ball bearings per jar) and capped. The extraction jar was shaken vigorously for 5 min and then allowed to settle for 15 min. About 1 mL of the supernatant was filtered using a filtration plunger fitted with a fiber glass filter. The filtered extracts were diluted 1:50-500 for c-PAH RaPID Assay® with 50% v/v methanol-buffered aqueous solution (SDI diluent). The c-PAH RaPID Assay® was used according to the manufacturer’s recommendations. The samples were analyzed in triplicate together with 4 calibration standards. Appropriate amounts of samples or standards, antibody-coated microbeads and enzyme conjugate were mixed and incubated. After washing twice with kit buffer using a magnetic rack to retain the antibodies, substrate (hydrogen peroxide) and chromogen (3,3’,5,5’-tetramethylbenzidine) were added and incubated. Stop solution (2 mol L⁻¹ sulphuric acid) was added and the color produced was measured at 450 nm using an microplate reader. Sample concentrations were calculated using a log-logit standard curve and multiplying results by the appropriate dilution factors. Details of this approach were described by Fillmann et al. (2007).

Analytical results were calculated from a standard curve of 0, 0.1, 1 and 5 ng benzo(a)pyrene mL⁻¹ (r² = 0.997; slope = -0.580; interception = -0.550) previously prepared for the c-PAH RaPID Assay®. Then, the concentrations in each sample were calculated by multiplying results by the appropriate dilution factor. As the c-PAH RaPID Assay® was developed using benzo(a)pyrene as the reference molecule, the compositions of the environmental extracts indicate a “quantitative” measure of benzo(a)pyrene “equivalents”.

Soxhlet extracted dry sediments (10 g) were fortified with the PAH standard mixture solution (SRM 1491) prepared in hexane, purchased from Promochem (Herts, UK). Fortified sediment samples were left to stabilize for 6 h before extraction.

**Sediment elutriate toxicity test**

The sediment elutriates were obtained by mixing a composite sediment sample and filtered clean seawater in the 1:4 ratio (USEPA, 1991), followed of an overnight resting period, allowing to the particles to settle and finally pipetting the elutriates. The elutriate samples were evaluated...
by the early life stage bioassay with embryos of the sea urchin *Lytechinus variegatus* (CETESB, 1992). In the beginning of the test, the salinity, pH, dissolved oxygen and temperature of the elutriates were checked. The total ammonia concentration was measured by a colorimetric method (Koroleff, 1970). Using these data, the unionized ammonia contents were estimated, using the method reported by Whitfield (1974).

Prior to the beginning of the test, adult individuals of *L. variegatus* were collected from a clean site at Ubatuba, and taken to the laboratory. The spawning was induced by the injection of 2-3 mL of KCl into the coelomic cavities of the animals, and gametes of 3 males and 3 females were collected. Ovules were collected by precipitation in beakers filled with filtered seawater, whereas the sperm was collected dry and transferred to keepers kept on ice. The sperm was activated by dilution in filtered seawater and the ovules were fertilized by adding 2 mL sperm solution to the eggs solution. The fertilization success was confirmed by examination under the microscope. The toxicity test was conducted in glass test tubes containing 10 mL test-solution. Four replicates were used for each sample. Only 100% elutriate samples were prepared and tested. The experiment was kept in a temperature controlled room, at 25 ± 2 ºC. After 24 h, the test was stopped by adding 0.75 mL of 10% buffered formaldehyde to each replicate. The embryos were analyzed microscopically for morphological anomalies and retarded development (100 per replicate). All the embryos which did not reach a well-developed pluteus larvae were considered affected.

**Whole sediment toxicity test**

Whole sediment acute toxicity tests were conducted using the amphipod *Tiburonella viscana*, as described by Melo & Abessa (2002). Five replicates per test sediment were prepared. One day before the beginning of the test, each sediment sample was thoroughly homogenized and aliquots were distributed into the test chambers (1-L polyethylene beakers). The test chambers were filled with 2 cm depth with the test sediments and filtered seawater up to 750 mL, and then maintained overnight at 25 ± 2 ºC with gentle aeration (maintained by air pumping). On the next day, 10 amphipods were added to each test chamber. The test chambers were conducted at 25 ± 2 ºC, under constant aeration and lighting. After ten days, the contents of the test-chambers were gently sieved through a 0.5-mm screen and the surviving amphipods were counted. Missing organisms were considered dead. Mortalities were compared with that of the reference sediment by Student t'- test. The dissolved oxygen concentration, salinity and pH of the overlying water in the test chambers were measured at the beginning and end of the tests. The water temperature was monitored daily.

**Neutral Red Retention Time Assay**

In the present investigation, the Neutral Red Retention Time (NRRT) assay consisted in exposing hemocytes of the mussel Perna perna directly to sediment elutriates. Adult mussels were collected from a clean site at Ponta Grossa, Ubatuba (Zaroni et al., 2001). They were taken to laboratory and immediately used. *Perna perna* is commonly used in experiments regarding biomarkers and bioaccumulation (Bainy et al., 2000; Zaroni et al., 2001; Ferreira et al., 2000) and there is a protocol for employing its hemocytes in studies using the NRRT assay (Abessa et al., 2005). For this study, 20 animals were used per treatment.

Prior to the beginning of the experiment, a physiological saline solution was prepared as described by Moore (1990), and the elutriates were prepared by adding 50 mL of each sediment sample into 150 mL physiological saline (1:4 proportion) and agitating for 30 min at speed or rpm (Burton, 1992). After mixing, each elutriate was left decanting overnight and the supernatants were exposed to the mussels’ hemocytes.

The assays were conducted at 25 ± 2 ºC. Initially, 0.2 mL hemolymph was collected from the posterior adductor muscle of each mussel, with syringes containing 0.2 mL elutriate, and transferred to an Eppendorf tube. Then, 40 µL of the hemocyte cells solution was pipetted and dropped onto a glass slide. The slides were placed into a dark and humid chamber and incubated for 15 min. During this interval, the NR working solution was prepared by the dilution of 10 µL previously prepared stock solution with 5 mL physiological saline solution. After incubation, 40 µL of NR working solution was dropped onto each slide. At the end of 15 min, the slides were quickly examined by microscopy. The cells were observed for structural abnormalities and for the retention time of the NR dye. The cell conditions were analyzed and recorded at 15 min intervals, until the majority of the cells were considered affected (i.e., if the dye was released to the cytosol; presence of vacuoles; modified cell shape; loss of fluids), as recommended by the protocols (Moore, 1990; Lowe et al., 1995).

**Statistical Analyses**

The results of the ecotoxicological assays were statistically analyzed by the student t-test (Zar, 1996), for comparison of the respective endpoints between the reference site (Anchieta) and the other tested sediments. The results were also compared by Spearman correlations, in order to find any relationship between the toxicity and the sediment properties.

**RESULTS AND DISCUSSION**

The texture of sediment samples ranged from sandy (Anchieta and Lamberto) to muddy sands (Itaguá and Ribeira) and sandy muds (Flamenguinho) (Table 1); besides, all the samples presented relatively low levels of organic matter (from 2.1 to 6.3 %). The concentrations of PAHs measured by the ELISA (expressed in BaP equivalents) showed relatively higher concentrations in the sediments from Ribeira and Itaguá (Table 1). No significant relationships were found between PAH levels and mud or OM contents (p > 0.05). Despite reasonable PAH contamination were found in sediments of Ribeira (104 (BaP equiv) ng g⁻¹) and Itaguá...
Using rapid assessment of marine pollution (RAMP)...

(88.2 (BaP equiv) ng g⁻¹), levels are lower in comparison to other more contaminated coastal areas of Brazil (i.e. Rio Grande Harbor, Patos Lagoon Estuary - Fillmann et al., 2007). That was expected since Ribeira is under direct influence of sewage, stormwater and especially marinas, whereas Itaguá is under influence of similar sources (mainly sewage and stormwater). Marinhas (King et al., 2004; Neira et al., 2017), sewage (Nichols & Espey, 1991; Włodarczyk-Makula, 2005; Gasperi et al., 2012) and stormwaters (Launay et al., 2016; Rogers, 2002) have been identified as local sources of PAHs. In addition, Itaguá waters are often considered by the State Environmental Agency as of poor quality for bathing (CETESB, 2008, 2013, 2016).

Results of the toxicity tests with elutriates and whole-sediments are shown in Figure 2. In general, physicochemical parameters were considered within the appropriate ranges for the species tested (see the Supplementary Material). Elutriates obtained from the Flamenguinho and Itaguá sediments caused significant effects on the embryolarval development of L. variegatus (Figure 2), although the toxicity related to Flamenguinho elutriate could have been influenced by the unionized ammonia, since the level found (0.14 mg L⁻¹) exceeded the toxic threshold to embryos of L. variegatus (0.05 mg L⁻¹); this sediment also presented the highest amount of mud (65.9%) and the lowest levels of PAHs. Regarding the whole sediment tests, the samples from Lamberto and Ribeira showed to be toxic to the amphipods (Figure 2). No correlation was seen between toxicity and either PAHs concentrations or sediment textures, although Ribeira presented the highest concentration of PAHs (p > 0.05).

The NRRT assay showed significant effects in hemocytes exposed to elutriates of Itaguá, Ribeira and Lamberto (Figure 3), despite the high variation between replicates. In this study, the concentrations of contaminants in the elutriates were not measured, but the results suggest that part of the substances from the sediment were transferred to the elutriates. The NRRT reduction correlated with the concentrations of PAHs ($r^2 = -0.86$; p < 0.05). In fact, the lower NRRT was observed in cells exposed to elutriates from Ribeira, where the highest concentration of PAH was found. Polycyclic aromatic hydrocarbons are known for causing several types of negative effects on aquatic organisms (Bihari et al., 2006; 2007) and considered highly toxic (Driscoll et al., 1998; Fishelson et al., 1999) and carcinogenic (Yu, 2002).

The results showed a worse condition in Itaguá and the internal portion of Ribeira Bay (Ribeira and Lamberto stations), where sediment toxicity and relatively higher levels PAHs were observed. As mentioned, Ribeira and Lamberto are located in a zone of marinas, where other contaminants, such as metals, antifouling biocides and other contaminants of emerging concern, are expected to occur. In fact, Godoi (2001) detected organotins, while metals (As, Cu, Pb, Hg, Zn) and sediment toxicity have been systematically observed in sediments of Ribeira Bay (CETESB, 2008; 2013, 2016). The

<table>
<thead>
<tr>
<th>Sediment sample</th>
<th>Sand (%)</th>
<th>Mud (%)</th>
<th>Organic Matter (%)</th>
<th>Classification</th>
<th>PAHs by ELISA (BaP equiv. ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchieta (ref.)</td>
<td>98.3</td>
<td>1.7</td>
<td>6.3</td>
<td>Sand</td>
<td>16.3</td>
</tr>
<tr>
<td>Lamberto</td>
<td>88.2</td>
<td>11.8</td>
<td>5.4</td>
<td>Sand</td>
<td>23.5</td>
</tr>
<tr>
<td>Flamenguinho</td>
<td>34.1</td>
<td>65.9</td>
<td>2.1</td>
<td>Sandy muds</td>
<td>1.0</td>
</tr>
<tr>
<td>Itaguá</td>
<td>73.7</td>
<td>26.3</td>
<td>6.3</td>
<td>Muddy sands</td>
<td>88.2</td>
</tr>
<tr>
<td>Ribeira</td>
<td>55.6</td>
<td>44.4</td>
<td>4.7</td>
<td>Muddy sands</td>
<td>104.2</td>
</tr>
</tbody>
</table>

Table 1. Texture, Organic Matter contents (%) and PAH levels (benzo(a)pyrene “equivalents”, ng g⁻¹) in the sediments from Ubatuba (southeastern Brazil).

Figure 2. Toxicity of sediments from some sites of Ubatuba, SP, Brazil. A) Whole-sediment toxicity to the amphipod Tiburonella viscana; B) Elutriate toxicity to embryos of the sea-urchin Lytechinus variegatus. Data are presented as means ± standard deviations. * indicates significant differences to the reference (Anchieta), at p <0.05.

Figure 3. Neutral Red Retention Time in hemocytes of Perna perna exposed to elutriates prepared from sediments of Ubatuba. Data are presented as means ± standard deviations. * indicates significant differences to the reference (Anchieta), at p <0.05.
results obtained to Flamenguinho indicate low contamination and a toxicity caused by natural levels of unionize ammonia. On the other hand, the results confirmed Ilha Anchieta as a less contaminated and non-toxic (under the tested conditions) environment, corroborating with official data (CETESB, 2007) and confirming that protected areas can provide better conditions of conservation.

The adaptation of NRRT assay for directly exposing sediment samples elutriate to mussel hemocyes showed to be viable and produced reliable results. Results showed good correspondence with classic toxicity test, and even better with the results of PAH contamination. Moreover, it has been a cost-effective, rapid and sensitive tool for evaluating toxicity of marine sediments. This technique can be used in environmental surveys, especially as a screening step whenever a lot of samples have to be scanned in order to identify hot spots. The results also showed that Ribeira Bay and Itaguá present signs of environmental degradation, requiring further efforts to diagnose the contamination levels and their effects and risks to the local biota.

ACKNOWLEDGEMENTS

This research was part of the Rapid Assessment of Marine Pollution (RAMP) programme funded by a DFID-UK (Department for International Development) grant to M.H. Depledge and J.W. Readman and by the ODA (Overseas Development Administration). Denis Abessa and Marcia Bícego were fellows of CNPq and Gilberto Fillmann was fellow of CAPES.

REFERENCES


### SUPPLEMENTARY MATERIAL

#### S1. Physicochemical variables of the overlying water during the whole-sediment toxicity test with the amphipod *Tiburonella viscana* and samples from Ubatuba (SP, Brazil).

<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>Dissolved Oxygen (mg.L(^{-1}))</th>
<th>pH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Anchieta (ref.)</td>
<td>33</td>
<td>34</td>
<td>5.45</td>
<td>5.67</td>
</tr>
<tr>
<td>Lamberto</td>
<td>34</td>
<td>34</td>
<td>4.88</td>
<td>4.92</td>
</tr>
<tr>
<td>Flamenguinho</td>
<td>34</td>
<td>35</td>
<td>4.97</td>
<td>5.17</td>
</tr>
<tr>
<td>Itaguá</td>
<td>33</td>
<td>34</td>
<td>4.33</td>
<td>5.25</td>
</tr>
<tr>
<td>Ribeira</td>
<td>34</td>
<td>34</td>
<td>4.08</td>
<td>4.76</td>
</tr>
</tbody>
</table>

#### S2. Physicochemical variables of sediment elutriates during the toxicity test with the embryos of the sea urchin *Lytechinus variegatus* and samples from Ubatuba (SP, Brazil). Dilutions were made in filtered sweater.

<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>Dissolved Oxygen (mg.L(^{-1}))</th>
<th>pH</th>
<th>NH(_3) (mg.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchieta (ref.)</td>
<td>34</td>
<td>5.65</td>
<td>8.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Lamberto</td>
<td>34</td>
<td>5.02</td>
<td>8.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Flamenguinho</td>
<td>34</td>
<td>5.39</td>
<td>8.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Itaguá</td>
<td>35</td>
<td>4.97</td>
<td>7.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Ribeira</td>
<td>34</td>
<td>4.83</td>
<td>7.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

#### S3. Physicochemical variables of sediment elutriates during the Neutral Red Retention Time assay using hemocytes of the brown mussel *Perna perna* and samples from Ubatuba (SP, Brazil). Dilutions were made in saline physiological solution.

<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>Dissolved Oxygen (mg.L(^{-1}))</th>
<th>pH</th>
<th>NH(_3) (mg.L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchieta (ref.)</td>
<td>33</td>
<td>5.2</td>
<td>7.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lamberto</td>
<td>33</td>
<td>5.2</td>
<td>7.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Flamenguinho</td>
<td>33</td>
<td>5.1</td>
<td>7.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Itaguá</td>
<td>33</td>
<td>5.0</td>
<td>7.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Ribeira</td>
<td>33</td>
<td>5.0</td>
<td>7.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>