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Ecotoxicological diagnosis of Aratu Bay, Bahia, Brazil: a new approach to validate a reactive short-term toxicity end-point by comparison with intertidal benthic activity

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Abstract

The Aratu Bay ecosystem (12°48′S; 38°28′W), located in an industrialized area, was compared with a similar but undisturbed ecosystem, Iguape Bay, in order to determine the impact of industry on water quality during the last forty years. Comparison was based on simple and low cost methodologies using bioassays on oyster embryos, validated by a survey of a benthic activity index.

A bioassay method based on oyster embryo development was used to compare water quality from the study areas. A high percentage (93.2 and 97.3) of abnormal oyster embryo development was observed in water samples from one area (south and southeast) of the bay where, besides receiving heavy discharges of industrial effluents, water circulation is restricted. At the north end of the bay where the area is shallow, and some industrial discharges are present, water quality data indicated that the area was stressed. Little effect of industrial impact has been noted around the Cotegipe channel, a deep area where the water circulation pattern is heavy and the water well mixed.

Validation of this survey has been achieved by the simple method of investigating benthic activity in the area. Quadrat counts of active benthic galleries were used as an index of community activity for comparative analysis between sampling stations and for discriminating between polluted and unpolluted sites. This research has shown that the quadrat counts of active benthic galleries, at least in areas of comparable homogeneous substrate, can be used as a viable index of environmental impact, when interpreted together with other source of data, such as early-life-stage tests. This new approach proved to be effective and led to the recommendation of remedial policies in the area.

Keywords: Early-life-stages test; Ecotoxicological diagnosis

1. Introduction

Aratu Bay (12°48′S; 38°28′W) is a shallow estuarine bay of 1215 ha with a heterogeneous shoreline, varying from mixed substrates of rock, gravel, sand and mud bordered by upland forest, to uniform tidal mud-flats bordered by a mixed mangrove community. It is linked to the more extensive Todos os Santos Bay (Fig. 1) by a deep, narrow channel approximately 3 km long. Water exchange is severely limited in the shallow northern and southern arms of the bay (Nascimento et al., 1982a,b).

An industrial complex was established in the area surrounding Aratu Bay in the late 1960s, and it has received both domestic discharges and industrial effluents since that time. A large variety of manufacturing enterprises, primarily metallurgic, have been delivering their wastes directly to the surrounding areas, with only

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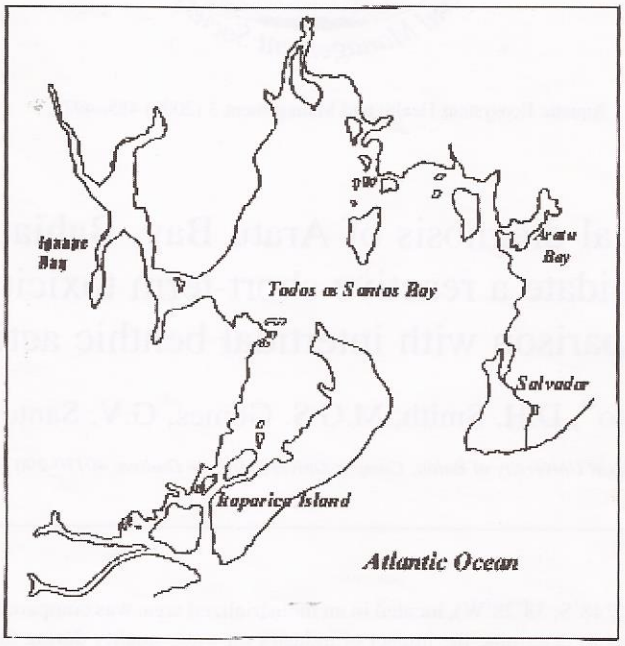

Fig. 1. Location of Aratu and Iguape Bays in relation to Todos os Santos Bay.

primary treatment which is not effective. The nature of these effluents is complex, containing a variety of both organic and inorganic wastes, including heavy metals. There is no quantitative data available for the majority of these wastes. The water volume of the bay is 47×10^6 m^3 and drainage to the sea is limited. These two characteristics significantly increase the influence of waste sediment material on water quality. Except for those studies referred to above, no assessment to evaluate the potential adverse environmental effects of contaminants has been done in Aratu Bay.

Trying to make recommendations concerning the establishment of new industries Nascimento (1989) and Smith et al. (1989) carried out the first environmental studies in this area. Unfortunately, there was no baseline data for the ecological conditions of this bay before the development of industry. For this reason, it was necessary to use a reference area to allow the effects of industrialization to be assessed by comparison.

Iguape Bay (12°47′S, 38°55′W), Maragojipe, Bahia, (Fig. 1) was selected as a control area for comparisons. This bay is situated approximately 45 km due west of Aratu Bay, on the opposite side of Todos os Santos Bay. It is a shallow bay of comparable size, characterized primarily by homogeneous tidal mud-flats and an extensive mangrove community, with a few widely separated outcrops of rocky shoreline. The area is not industrialized and, except for agricultural runoff and small boatyards, receives only domestic discharges from the small city of Maragojipe.

It has been demonstrated that bivalve embryos and larvae respond to many chemical and physical variables by failing to develop, or by developing in an abnormal manner (Woelke, 1960, 1961, 1965, 1968, 1972; Okubo and Okubo, 1967; Loosanoff and Davis, 1963; Davis and Calabrese, 1964; Dimick and Breese, 1965; Hidu, 1965; Stewart et al., 1967; Calabrese and Davis, 1970; Woelke et al., 1970; Calabrese et al., 1973; McInnes and Calabrese, 1978, 1979; McInnes, 1981; Martin et al., 1981). Based on most of these findings Woelke (1972) made the general assumption that the response of bivalve embryos is a reflection or indicator of the response of juveniles and adults to the same variable. He succeeded in establishing the relationships between embryonic development of *Crassostrea gigas* and water quality. He stated that the same level of stress or toxicant which causes an increase in the number of abnormalities of *C. gigas* embryos during development will also have an adverse effect on other species of clam and oysters and, in many cases, on fish, sea urchins, crustacea, algae and other forms of marine life.

Based on the above statement and on the fact that the responses of *Crassostrea rhizophorae* and *C. gigas* to toxicants are similar (Nascimento, unpublished data), we have used *C. rhizophorae* embryonic development to test the quality of water from Aratu Bay.

The use of a short term bioassay based on the oyster embryonic development to predict water quality has been advised by The Marine Pollution Monitoring Management Group (UK). Although there are clear limitations in extrapolating laboratory experiments to field effects (Kimball and Levin, 1985) it is important that the end point measured in the laboratory be linked to meaningful effects at the organism or population level (Underwood and Petersen, 1988). Studies of the effects of chemical contaminants on embryos and larvae can be so linked, since increase in natural mortality may decrease population and be highly detrimental to the ecosystem (Weis and Weis, 1989). However, ecological effects are best understood if examined at several levels, from individual to population or community levels within any ecosystem (USEPA, 1992; Rand, 1995). In view of this, comparisons were made between intertidal benthic activity in Aratu bay as an industrialized area, and the relatively unimpacted Iguape Bay, as a possible additional and complementary support for the toxicity data obtained through the bioassays on oyster embryos.

The study of benthic communities, especially of those organisms in the intertidal zone, is of great interest and importance in the evaluation of marine habitats subjected to natural and man-made perturbations. The animals of this zone are subjected to great variations in environmental conditions, primarily salinity, temperature, concentrations of dissolved oxygen and solar radiation as they are exposed at low tide. In addition, many species of this community have little or no mobility, and thus are unable to migrate in response to abnormal conditions, and may consequently suffer to a great extent from environmental degradation. According to Hale (1975), the principal function attributed to the benthos in shallow marine ecosystems is the regeneration of inorganic nutrients. Thus, benthic species play a vital role in the lower food web and their decline may be reflected throughout the marine shallow water ecosystem.

The aim of the present work was to try to validate the data obtained through bioassays of water quality based on the embryonic development of *C. rhizophorae* by comparison with an index of intertidal activity.

2. Materials and methods

For the purpose of the bioassay study, eight collection stations were chosen inside the bay (Fig. 2) according to the following criteria: (1) areas chosen for future industrial development; (2) areas near the drainage points of already developed industries; (3) areas having different water circulation patterns.

In the northeast section of the bay, two stations (Ponta da Conceição, station 3 and Santa Maria, station 2) were established. These areas, which are supposed to drain the effluents of future industrial installations, are characterized by shallow and very warm, calm waters. The station Ponta do Criminoso (station 1) is located at the entrance of the bay and is probably influenced by the impact of atmospheric pollution from the Aratu cement plant given the direction of the prevailing northeast winds during the summer and southeast during the winter time. Another three stations were established in one area of intensive water circulation: Matanga (station 6), marking the end of Cotegipe Channel which connects the Bays, Todos os Santos and Aratu, Tapua da Fonte (station 8), marking the beginning of Cotegipe channel in Todos os Santos Bay and Mangueira (station 7), where the Dow Chemical plant is located. Located at the east and southeast of Aratu Bay are Cimento Aratu North (station 4) and Cimento Aratu South (station 5) where most of the effluents coming from the industries in the area drain into the river Macaco. At Cimento Aratu North there are relatively strong water currents coming from the Cotegipe channel. At Cimento Aratu South the water circulation is weaker, as there are a number of small islands which affect circulation.

Iguape Bay, used as a control area, is a typical estuarine region. Only one station was placed here (station Maragojipe, located near Ponta do Chiqueiro). The same stations were selected for the benthic study at Aratu Bay. However, station Cimento Aratu North (station 4) was divided into four substations separated from one another by a distance of 1000 m. These

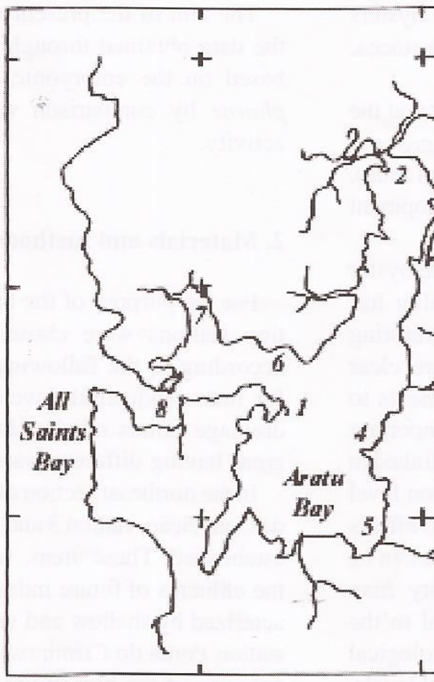

Fig. 2. Map of Aratu Bay (12°47′S, 38°, 29′W) showing the locations of the sampling stations. The grid system indicated by tic marks has an interval of 4 km. 1 = Ponta do Criminoso, 2 = Santa Maria, 3 = Ponta da Conceição, 4 = Cimento Aratu North, 5 = Cimento Aratu South, 6 = Matanga, 7 = Mangueira and 8 = Tapua da Fonte.

eleven stations represented both heterogeneous rock and homogeneous muddy substrates and were located at varying distances from sources of potential pollution and from the tidal influxes of fresh seawater from Todos os Santos Bay. Four stations were selected for the benthic study in Iguape Bay (Fig. 3): Manjuba, Estaleiro, Caiote, Ponta do Chiqueiro. All were of homogeneous mud substrate with mangrove margins. Stations Estaleiro and Caiote were adjacent to the harbor of Maragojipe city and the others were in relatively undisturbed areas of the bay.

Counts of active galleries in one square meter quadrats were used as an index of benthic activity. Three transects of ten quadrats each were arrayed parallel to the edge of the water; one at the seaward margin of the mangrove, one at low tide level and one intermediate transect equidistant from the first two. The distance of each line from the margin of the vegetation was recorded. The elevation of each transect was measured, relative to mean high-tide level, using a taut-line with suspended bubble level and a 2 m stadia rod. The status of the tide (rising or falling) was also noted for each count, along with the substrate type and a general description of the sampling station.

Statistical analyses were performed using the Statistical Package for the Social Sciences (Nie et al., 1975). The 'Index of Dispersion' ($I = S_x^2/\bar{X}$) and the associated Chi-square test ($\bar{X}^2 = (n-1)I$) were employed in a preliminary analysis of spatial dispersion of galleries among quadrats (Elliott, 1971). The galleries were significantly aggregated, but did not conform to a negative binomial distribution. Consequently, the original gallery counts were normalized by log-transformation, before applying parametric comparisons among stations (Elliott, 1971). A factorial design analysis of variance and covariance (SPSS; Nie et al., 1975) was used to determine and to control the effects of factors (such as type of substrate and tidal conditions) and covariates (such as relative elevation of the substrate) prior to comparing differences among stations or between the two bays. The resultant, adjusted mean counts were compared using Duncan's multiple-range procedure (SPSS; Nie et al., 1975).

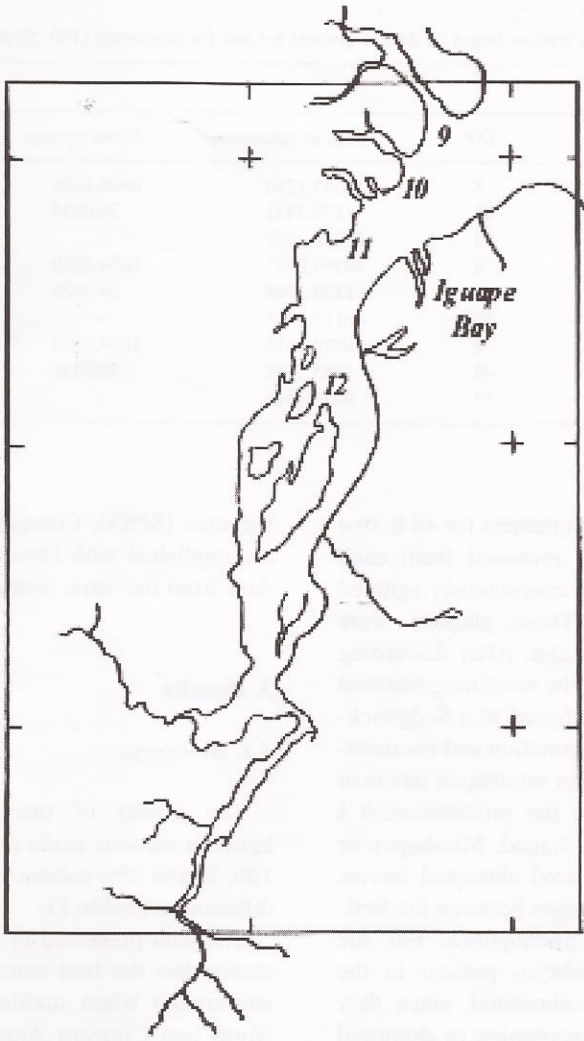

Fig. 3. Map of Iguape Bay (12°47′S, 38°55′W), showing the locations of the four sampling stations. 9 = Manjuba, 10 = Estaleiro, 11 = Caiote, 12 = Ponta do Chiqueiro. The grid system indicated by tic marks has an interval of 4 km.

For the laboratory toxicity bioassays the methodology used to determine the effect of water quality on *C. rhizophorae* embryos followed, in general, that indicated by the American Society for Testing and Materials (ASTM, 1995). Mature *C. rhizophorae* were obtained from Salinas das Margaridas (12°52′S; 32°44′W), a previously studied area, where bioassays with oyster embryos gave results not statistically different from those obtained for Ondina seawater, used as a control.

Gametes were collected from adult oysters by stripping the gonads from at least three individuals of each sex. The eggs were then fertilized and allowed to develop to the first cleavage stage before being counted. Embryos were distributed in the experimental jars, at a density of 10 ml^{-1}. Four treatments were tested in triplicate for each station at a dilution factor of 100, 50, 25 and 0% test seawater with control Ondina seawater. The salinity was determined by refractometry and adjusted to 30‰, using distilled water. Considering all experiments, the pH varied from 7.8 to 8.2 and the dissolved oxygen from 7.5 to 8.3 ppm. The tests were set up in filtered (glass fiber GF-C filter) seawater and maintained under an environmental temperature which ranged from 27 to 28°C.

Table 1
One-way analysis of variance between stations based on data of percent net risk for treatments (100, 50 and 25% local water)

Treatments	Results					
	Source	D.F.	Sum of squares	Mean squares	*F*-ratio	*F*. prob
100%	Between groups	8	51585.1250	6448.1406	247.954	0.000
	Within groups	45	1170.2432	26.0054	–	–
	Total	53	52755.3682	–	–	–
50%	Between groups	8	44997.2637	5624.6580	225.955	0.000
	Within groups	45	1120.1748	24.8928	–	–
	Total	53	46117.4385	–	–	–
25%	Between groups	8	8032.2416	1004.0302	31.041	0.000
	Within groups	45	1455.5532	32.3456	–	–
	Total	53	9487.7948	–	–	–

After incubation in the test containers for 48 h, two aliquots of 15 ml each, were removed from each beaker while the contents were continuously agitated with a perforated plunger. Those aliquots were preserved in 5% buffered formalin. After discarding about 14 ml of the supernatant, the remaining material containing the larvae was transferred to a Sedgwick-Rafter cell for microscopic examination and enumeration of either normal, abnormal or misshapen larvae or embryos. Normal larvae were the prodissoconch I (first larval shell) perfectly D-shaped. Misshapen or incomplete shells were considered abnormal larvae. Embryos were defined as the stages between the fertilized egg and the ciliated trochophore. For the purposes of analysis, any embryos present in the sample were also considered abnormal, since they had incomplete development. Incomplete or abnormal shell development was based upon the proportion of larvae failing to develop normally after 48 h (abnormal) relative to the total number of normal plus abnormal larvae. Final comparative results between stations were given in terms of percent net risk according to Abbott's formula (Woelke, 1972):

$$\%\text{Net risk} = \frac{(\%\ \text{Abnormals in Treatment} - \%\ \text{Abnormals in Control})}{100 - \%\ \text{Abnormals in control}} \times 100$$

Differences in net risk between test and control stations were tested statistically using analysis of variance (SPSS). Comparisons between means were accomplished with Duncan's Multiple Range Procedure from the same source (Nie et al., 1975).

3. Results

3.1. Bioassays

The results of one-way analysis of variance between stations made on data of percent net risk in 100, 50 and 25% habitat water show highly significant differences (Table 1).

The data presented in Table 2 show that Matanga station has the best water quality (8.8% net risk of abnormals) when undiluted, while Cimento Aratu North and Cimento Aratu South have the water of the worst quality (respectively, 93.17 and 97.33% net risk of abnormals when undiluted). All the other stations are intermediate, presenting values in the range of 21.63–29.60% net risk of abnormals, when undiluted. Within this intermediate group, Ponta do Criminoso, Mangueira and Maragojipe comprise a low extreme, while Tapua da Fonte, Ponta da Conceição and Santa Maria occupy the other extreme, having higher values for percent net risk of abnormals. The seawater from the stations at Cimento Aratu, even when diluted with 75% of control water, present higher values of abnormals than the undiluted water from Santa Maria and Ponta da Conceição. The 50% dilution of water from other stations considerably lowers the values of percent net risk.

Table 2
Results of multiple range tests between stations based on data of percent net risk for treatments (100, 50 and 25% local water). Mean values united by lines are not significantly different ($P < 0.05$)

TREAT-MENTS		MEAN PERCENT NET RISK AT EACH STATION								
		Matanga	Ponta do Criminoso	Mangueira	Maragojipe	Tapua da Fonte	Ponta da Conceição	Santa Maria	Cimento Aratu Norte	Cimento Aratu Sul
100%	Mean % net risk	8.80	21.63	22.43	22.82	24.12	24.67	29.60	93.17	97.33
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		Matanga	Mangueira	Maragogipe	Ponta do Criminoso	Tapua da Fonte	Santa Maria	Ponta da Conceição	Cimento Aratu Norte	Cimento Aratu Sul
50%	Mean % net risk	1.17	10.03	11.97	14.47	14.68	16.20	21.43	80.27	82.08
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		Matanga	Mangueira	Maragojipe	Tapua da Fonte	Ponta do Crimonoso	Santa Maria	Ponta da Conceição	Cimento Aratu Norte	Cimento Aratu Sul
25%	Mean % net risk	1.33	3. 55	7.48	7.93	8.67	10.55	16.37	35.52	35.93
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Table 3
Preliminary analysis of variance of the number of active galleries in the intertidal zone of Aratu Bay, Bahia, as a function of type of substrate, relative elevation of substrate and of rising or falling tide. The effects of sampling stations were not analyzed at this stage and are included in the unexplained sum-of-squares. Counts were log-transformed prior to analysis

	Anova				
Source of variation	df	SS	MS	*F*	Signif.
Covariable					
Relative elevation	1	1.254	1.254	13.652	0.001
Main effects	3	3.843	1.281	13.951	0.001
Substrate	2	1.515	0.758	8.250	0.001
Tide	1	2.328	2.328	25.351	0.001
Explained	4	5.097	1.274	13.876	0.001
Unexplained	245	22.497	0.092	–	–
Total	249	27.594	–	–	–

Table 4
Preliminary analysis of variance of the number of active galleries in the intertidal zone of Iguape Bay, Bahia, as a function of relative elevation of the substrate and of rising or falling tide. Homogeneous mud was the only substrate sampled quantitatively in Iguape Bay. The effects of sampling stations were not analyzed at this stage and are included in the unexplained sum-of-squares. Counts were log-transformed prior to analysis

	Anova				
Source of variation	df	SS	MS	*F*	Signif.
Covariable					
Relative elevation	1	0.501	0.501	3.540	0.059
Main effects					
Tide	1	0.972	0.972	6.859	0.010
Explained	2	1.473	0.737	5.199	0.007
Unexplained	107	15.160	0.142	–	–
Total	109	16.633	–	–	–

3.2. Benthic activity quantitative analysis

The spatial dispersion of active galleries in meter-square quadrats was significantly aggregated ($P < 0.05$), as indicated by the Index of Dispersion and the associated chi-square test. An initial analysis of variance and covariance among the stations of Aratu Bay revealed that the number of galleries varied significantly as a function of the type of substrate, relative elevation of the substrate and of rising or falling tide (Table 3). In Iguape Bay only sites with homogeneous mud substrates were sampled. The number of galleries there also varied significantly with changes in relative elevation of the substrate and with changing tides (Table 4). Quantitative comparisons between the two bays were necessarily limited to homogeneous mud substrate. The separate initial analysis showed that the effects of relative elevation and of tide were nearly identical between the two areas.

Comparisons between the two areas among stations with homogeneous mud substrate (Santa Maria, station 2; Ponta da Conceição, station 3; Cimento Aratu North, station 4; Cimento Aratu South, station 5 and Mangueira, station 7 at Aratu Bay and all the stations at Iguape Bay) revealed that a significant difference in benthic activity existed between Aratu Bay and Iguape Bay, as well as between individual sampling stations (Table 5). Comparisons of adjusted mean counts among stations and between bays showed that in general, the number of active galleries was greater in Iguape Bay than in Aratu Bay (Table 6, A–C). The station Caiote in Iguape Bay had lower benthic activity than the stations Santa Maria (station 2), Ponta da Conceição (station 3) and Cimento Aratu South (station 5), even though the differences were not statistically significant. The lowest counts were observed at Cimento Aratu North, an area of heavy calcium

Table 5
Analysis of variance of the number of active galleries in the intertidal zones of Aratu Bay and Iguape Bay, Bahia, as a function of relative elevation of substrate, rising or falling tide, the difference between bays and the differences among sampling stations. Mud was the only substrate sampled quantitatively in Iguape Bay. Consequently, other substrate were excluded from this analysis. Counts were log-transformed prior to analysis

	Anova				
Source of variation	df	SS	MS	*F*	Signif.
Covariable					
Relative elevation	1	1.115	1.115	13.698	0.001
Main effects	10	15.291	1.529	18.781	0.001
Tide	1	3.490	3.490	42.864	0.001
Bay	1	0.676	0.676	8.304	0.004
Station	8	11.125	1.391	17.080	0.001
Explained	11	16.406	1.491	18.319	0.001
Unexplained	288	23.449	0.081	–	–
Total	299	39.854	–	–	–

Table 6
Comparisons among adjusted geometric means, and their respective 95% confidence intervals, of the number of active galleries/m^2 in the intertidal zone of stations with homogeneous mud substrate. The stations are arranged in order of increasing geometric means. Stations connected by the same line do not differ significantly in number of active galleries (Duncan's multiple-range test)

A. Aratu Bay

Cimento Aratu Norte	Santa Maria	Ponta da Conceição	Cimento Aratu Sul	Mangueira
GM = 29.03	GM = 44.66	GM = 45.57	GM = 45.77	GM = 60.13
18.28-46.10	32.79-60.83	33.39-62.19	40.01-52.37	46.22-78.22
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B. Iguape Bay

Caiote	Manjuba	Ponta do Chiqueiro	Estaleiro
GM = 31.36	GM = 53.83	GM = 78.51	GM = 131.60
25.50-38.57	40.00-72.44	59.99-102.73	98.90-175.11
!---!			
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C. Aratu and Iguape Bay

Cimento Aratu Norte	Caiote	Santa Maria	Ponta da Conceição	Cimento Aratu Sul	Manjuba	Mangueira	Ponta do Chiqueiro	Estaleiro
GM = 29.03	GM = 31.36	GM = 44.66	GM = 45.57	GM = 45.77	GM = 58.83	GM = 60.13	GM = 78.51	GM = 131.60
18.28-46.10	25.50-38.57	32.79-60.83	33.39-62.19	40.01-52.37	40.00-72.44	46.22-78.22	59.99-102.73	98.90-175.11
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carbonate siltation adjacent to a deactivated cement factory ($\bar{X} = 29.03$ galleries m^{-2}), and Caiote, a station immediately adjacent to the city of Maragojipe ($\bar{X} = 31.36$ galleries m^{-2}). The highest count was observed at Estaleiro, a station adjacent to a small boat-building yard near Maragojipe ($\bar{X} = 131.60$ galleries m^{-2}). This station was considered atypical because the majority of the galleries were fine tubes of polychaete annelids with few crustacean burrows.

At stations of heterogeneous substrate (Ponta do Criminoso, Matanga and Tapua da Fonte) galleries were more difficult to count in areas of rock and gravel, and the number of galleries varied greatly as a function of the texture of the substrate. Two adjacent stations of similar rock and mud substrates and of equal water quality, Ponta do Criminoso and Matanga, were also very similar in their mean numbers of galleries m^{-2} ($\bar{X} = 88.7$ and $\bar{X} = 95.9$, respectively). However, because the type of substrate varied greatly, both within and between stations of heterogeneous substrate, statistical comparisons of mean counts were not considered meaningful.

4. Discussion and conclusions

Estuarine and coastal marine waters are ultimately the repositories of wastes released from anthropogenic activities. In Brazil, where industrialization is recent, the effort to develop techniques and approaches for an evaluation of the impact of various pollutants on estuarine ecosystems is just beginning. In these environments, responses to stressing factors is seldom determined by a single driving function. Generally, they act synergistically affecting the estuarine communities and should be evaluated through biological procedures which represent a more realistic response of the damaged environment.

Bivalve molluscs possess several characteristics making them good models for pollution studies (Cunningham, 1979). The use of embryos of the oyster *C. gigas* as a bioindicator for assessing water quality was first proposed by Woelke (1972). As the native oyster *C. rhizophorae* is commonly found in mangrove communities and its biology and ecology are known (Nascimento, 1991a,b), we used *C. rhizophorae* embryos to assess water quality in Aratu Bay, as a means of determining the impact of industrial wastes in this area and in order to make recommendations relative to the establishing of new factories in the area.

The criterion of water quality based on Pacific oyster embryo development (Woelke, 1972) states that the percentage of abnormals shall, under no circumstances, exceed 20% in a single sample. He considers the misshapen or malformed larva as normal. However, according to Martin et al. (1981), the criterion of abnormal shell development in bivalve larvae represents a sublethal response that leads to certain non-recruitment into the population due to increased predatory pressure from longer pelagic existence. In addition, morphological deformities generally are associated with abnormal physiological behavior which decreases the chance of survival. Considering misshapen larvae and embryos as abnormals, we believe that the 20% level established by Woelke (1972) should be increased to 25%. There is not yet an established criterion of water quality based on *C. rhizophorae* embryo development, even though many similarities in the behavioral development have been found between this oyster and *C. gigas* (Nascimento, unpublished data). We could, however, evaluate the impact of industrialization by comparing the water quality in Aratu Bay with the quality of water in a similar bay having the same gross physical and biological characteristics, but not industrialized (Iguape Bay). Based on these comparisons the only area inside Aratu Bay where industrial operation did not show any detrimental effect was at the western end of the Bay, at Matanga station (station 6). In the area of the Cotegipe channel, the stations Ponta do Criminoso (station 1) Mangueira (station 7) and Tapua da Fonte (station 8) had values of abnormals not significantly different from the control area (Maragojipe). This suggests that the possible contribution to the pollution of Aratu Bay by Todos os Santos Bay is negligible, even though the percentage of abnormals from Tapua da Fonte (station 8), located at the connection area within Todos os Santos Bay, was not significantly different from Santa Maria (station 2) and Ponta da Conceição (station 3).

In north-eastern Aratu bay the effects of pollution are shown by the decrease in water quality from Ponta da Conceição and Santa Maria stations, as shown by the increase in the percentage of abnormal embryonic development. The meaning of these results are not restricted to *C. rhizophorae*, if we accept the assumption of Okubo and Okubo (1962) that whatever causes an increase in percent abnormal of oyster embryos will have an adverse effect on other estuarine species. The benthic activity and the species diversity of the benthos in this area showed evidence of an increased perturbation when related to the control area (Nascimento et al., 1982a)

The north-eastern section of Aratu Bay drains the basins of Cotegipe and Petecada Rivers which receive a variety of waste effluents, including heavy metals, delivered from the same industries located in the northern section of the bay. Although this area has been planned for the future installation of factories, the present study serves to discourage the increase of any industrial discharge, since the area is shallow and has poor water circulation. This condition means that conditions of dilution and transport of the effluents are difficult, and wastes have a long retention time, a factor of great importance in the evaluation of pollution effects. Considering that many forms of edible benthic organisms accumulate heavy metals and other toxicants, the consumption of these contaminated sea food products might cause public health problems and therefore the introduction of toxicants to the bay should be prevented.

The area where the stations Cimento Aratu North and Cimento Aratu South were located receives a great variety of effluents (hydrocarbons, heavy metals and carbohydrate addition from petrochemical industries) delivered by the Macaco River. In addition, the Cimento Aratu plant has contributed a great quantity of suspended material, which is further dispersed by shipping traffic. Although nutrient levels and phytoplankton diversity at Cimento Aratu North indicate good water circulation in the area, the quantitative activity of benthos is very low compared to the control area and other stations in Aratu (Nascimento et al., 1982a). Cimento Aratu South is partially isolated from the rest of the bay by Aratu Island, which limits the water circulation. This area receives sewage effluents from Mapele and Paripe in addition to industrial wastes brought by the Macaco River. The bioassays at the Cimento Aratu stations showed the highest levels of abnormalities, indicating that the area is seriously polluted and will not support an increase of effluent discharges.

The comparisons among sampling stations and between Aratu and Iguape Bays were additionally based on quantitative counts of active galleries in the various substrates. However, interpretation of the results must be made with caution. Quantitative counts of galleries may indicate high benthic activity, but polluted environments are often characterized by dominance of a few very active species (Smith et al., 1989). Clearly, our conclusions in this study had to include consideration of other data on environmental quality such as the bioassays on oyster embryos referred to above.

Quadrat counts of active benthic galleries were made in all substrates encountered in Aratu and Iguape Bays. The fact that variations due to relative elevation of the substrate and to tidal flux were analyzed and adjusted prior to analyzing between station effects, resulted in conservative estimates of the differences between stations. In general, the counts from heterogeneous substrates were considerably higher than those from homogeneous mud substrates. In Aratu Bay the mean count of galleries in heterogeneous rock/gravel/sand/mud substrates was 72.9 ± 9.6 galleries m^{-2}, as compared with a mean of 57.1 ± 10.0 galleries m^{-2} in homogeneous mud substrates. Statistical comparisons were not made between stations of heterogeneous substrates, since the texture and combination of substrate materials varied greatly both within and between stations.

In contrast, statistical comparisons among stations of homogeneous substrate were possible, not only because of the larger number of stations sampled in the two bays, but also because of the relative uniformity of the substrate. In Aratu Bay the minimum benthic activity was observed in a homogeneous mud substation of Cimento Aratu North ($\bar{X} = 29.0$ galleries m^{-2}). It was an area highly polluted by calcareous sediment from a deactivated cement factory. The highest benthic activity was observed at station Mangueira ($\bar{X} = 60.1$ galleries m^{-2}). This station was located in a small lateral bay, off the main channel to Aratu Bay, received a flux of fresh seawater from Todos os Santos Bay with each tidal cycle, and was relatively isolated from the principal sources of pollution by the prevailing water currents. It was judged to have the best water quality among all stations sampled according to the bioassays results.

In Iguape Bay the station with the lowest count of active galleries was Caiote ($\bar{X} = 31.4$ galleries m^{-2}). This station was adjacent to and received a large quantity of domestic discharges from the city of Maragojipe. The station with the highest count was Estaleiro ($\bar{X} = 11.6$ galleries m^{-2}). It was adjacent to a boat-building yard, and probably received chemical effluents in addition to the domestic discharges from Maragojipe. The high activity at this site was interpreted to be a result of dominance by a few surviving species. This conclusion was supported by the fact that the single species of polychaete there was a nereid, a group which is highly tolerant of pollution and which often occurs in high numbers in polluted areas (Smith et al., 1989).

Among the stations with intermediate gallery counts, the three relatively polluted stations from Aratu Bay (Santa Maria, Ponta da Conceição and Cimento Aratu South) had fewer galleries than the relatively unperturbed stations of Iguape Bay, but the differences were not statistically significant ($P > 0.05$). In overview, the rank-order of stations, in relation to their mean gallery counts, corresponded quite well with our final site evaluations of the impact of pollution based on bioassays with the single exception of Estaleiro. It appears that quadrat counts of active benthic galleries, at least in areas of comparable homogeneous substrate, can be a viable index of environmental impact when interpreted together with other sources of data. Based on the conclusions discussed in this paper it is recommended that further industrial discharge in Aratu Bay should be avoided and a better treatment of the effluents should be installed as a remedial approach.

Chapman (1995) states that laboratory toxicity bioassays, such as the early-life-stages-tests, do work, and are proving themselves as effective tools for environmental research. However, tests and end-points should reflect primary adverse effects of concern within the ecosystem. Tests that use indigenous species and end-points that reflect constraints to the population level such as recruitment are important choices (Cairns and Pratt, 1993), even though these tools do not provide the whole answer. The early-life-stage test using oysters fills these requirements.

It is advisable for diagnosis and for pollution prevention to use different end-points, different species and different levels of biological organization, considering that simple methods and answers do not exist for a complex system like environment (Chapman, 1995). Validation is not required (Chapman 1995; Cairns and Pratt, 1993); however, it is safer to take decisions based on more than one type of evidence. The simple and low cost methodology discussed in the present work was shown to be able to infer the deleterious effects of industrial and urban activities in Aratu Bay and to show the immediate necessity of remedial policies in the area.

References

ASTM (American Society for Testing and Materials), 1995. Guide for conducting static acute toxicity testing with embryos of four species of saltwater bivalve molluscs. E724-89. In: Annual Book of ASTM Standards. Water and Environmental Technology. Vol. 11.05, Philadelphia, PA, USA, pp. 221–238.

Cairns Jr, J., Pratt, J.R., 1993. Trends in ecotoxicology. Sci. Tot. Environ. Suppl. 1, 7–22.

Calabrese, A., Davis, H.C., 1970. Tolerance and requirements of embryos and larvae of bivalve molluscs. Helgolander eiss. Meeresunters 20, 553–554.

Calabrese, A., Collier, R.S., Nelson, D.A., McInnes, J.R., 1973. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar. Biol. 18, 162–166.

Chapman, P.M., 1995. Extrapolating laboratory toxicity tests to the field. Environ. Toxicol. Chem. 14, 927–930.

Cunningham, P.A., 1979. The use of bivalve molluscs in heavy metal pollution research: functional responses. In: Vernberg, W.C., Thurberg, F.P., Calabrese, A., Vernberg, F.J. (Eds.). Marine Pollution. Academic Press, New York, pp. 183–221.

Davis, H.C., Calabrese, A., 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *Mytilus mercenaria* and *C. virginica*. US Bur. Com. Fish. Bull. 63 (3), 643–655.

Dimick, R.E., Breese, W.P., 1965. Bay mussel embryo biossay. Proc. 12th Pacific Northwest Industrial Waste Conf. University of Washington, Seattle, WA, pp. 165–175.

Elliott, J.M., 1971. Some Methods for the Statistical Analysis of Benthic Invertebrates. Sci. Publ. No. 25. Freshwater Biol. Assoc., UK.

Hale, S.S., 1975. The role of benthic communities in the nitrogen and phosphorus cycles of an estuary. In: Gentry, J.B., Smith, M.H. (Eds.), Mineral Cycling in Southeastern Ecosystems. CONF-740513, National Technical Information Service, US Department of Commerce, Springfield, VA, pp. 291–313.

Hidu, H., 1965. Effects of synthetic surfactants on the larvae of clams (*Mytilus mercenaria*) and oysters (*Crassostrea virginica*). J. Water Pollut. Contr. Fed. 37 (2), 262–270.

Kimball, K.D., Levin, S.A., 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing. Bioscience 35, 165–171.

Loosanoff, V.L., Davis, H.C., 1963. Rearing bivalves molluscs. Adv. Mar. Biol. 1, 1–136.

Martin, M., Osborn, K.E., Billig, P., Glickstein, N., 1981. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. Mar. Pollut. Bull. 12, 305–308.

McInnes, J.R., 1981. Response of embryos of the American oyster *Crassostrea virginica*, to the heavy metal mixtures. Mar. Environ. Res. 4, 217–227.

McInnes, J.R., Calabrese, A., 1978. Response of embryos of the American oyster *Crassostrea virginica* to the heavy metals at different temperatures. In: McLusky, D.S., Berry, A.J. (Eds.). Physiology and Behaviour of Marine Organisms. Pergamon Press, Oxford, pp. 195–202.

McInnes, J.R., Calabrese, A., 1979. Combined effects of salinity, temperature, and copper on embryos and early larvae of the American oyster, *Crassostrea virginica*. Arch. Environ. Contam. Toxicol. 8, 553–562.

Nascimento, I.A., 1989. Bioassays of water quality in Aratu Bay, Bahia, Brazil using embryonic development of the mangrove oyster *Crassostrea rhizophorae* (Guilding, 1828). Proc. SIUEC Rio Grande/RS: Universidade do Rio Grande. Vol. 2, pp. 195–212.

Nascimento, I.A., 1991a. *Crassostrea rhizophorae* (Guilding) and *C. brasiliana* (Lemarck) in South and Central Americas. In: Menzes, W. (Ed.). Estuarine and Marine Bivalve Mollusk Culture. CIRC Press, Florida, pp. 125–134 (chap. 10).

Nascimento, I.A., 1991b. Brazilian characteristics of the mangrove oyster in Brazil as a basis for their cultivation. In: Newkirk, G., Field, B. (Eds.). Oyster Culture in the Caribbean. Dalhousie University Press, Nova Scotia, Canada, pp. 17–33.

Nascimento, I.A., Santos, A.E., Aragão Faria, L.A.M., 1982a. Determinação da qualidade da água na Baía de Aratu usando-se bioensaios com embriões de ostras. In: Diagnóstico ecológico da Baía de Aratu. Vol. III, Sistema de coleta, transporte, tratamento e disposição final dos fluentes líquidos e restos sólidos do Centro Industrial de Aratu, Convênio SIC/CIA Governo do estado da Bahia.

Nascimento, I.A., Smith, D.H., Brito, R.C., Leite, M.B.N.L., 1982b. Diagnose Ecológica da Bahia de Aratu. Geotécnica adendo AO-RTI-PR-15-0041/81. Secretaria da Industria e Comercio, Governo do estado da Bahia.

Nie, N.H., Hull, C.H., Jenkins, J.G., Stainbrenner, S., Bent, D.H., 1975. Statistical Package for the Social Sciences. McGraw-Hill, Inc., St. Louis, MO.

Okubo, K., Okubo, T., 1967. Study on the biossay method for the evaluation of water pollution. II. Use of fertilized eggs of sea urchins and bivalves. Bull. Tokai Regional Fish. Res. Lab. 32, 131–140.

Rand, G.M., 1995. Fundaments of Aquatic Toxicology: Effects, Environmental fate, and Risk Assessment. 2nd ed. Taylor and Francis, Washington, DC.

Smith, D.H., Gomes, M.C.S., Santos, G.V., 1989. An ecological diagnosis of Aratu Bay: preliminary survey and analysis of the intertidal benthos of Aratu Bay and Iguape Bay, Bahia, Brazil. Proc. SIUEC Rio Grande/RS: Universidade do Rio Grande. Vol. 2, pp. 33–58.

Stewart, N.E., Milleman, R., Breese, W.P., 1967. Acute toxicity of the insecticide sevin and its hydrolyct product 1-naphthol to some marine organisms. Trans. Am. Fish. Soc. 96 (1), 25–30.

Underwood, A.J., Peterson, C.H., 1988. Towards an ecological framework for investigating pollution. Mar. Ecol. Prog. Ser. 46, 227–234.

USEPA (United States Environmental Protection Agency), 1992. Report on ecological risk assessment guidelines strategic planning workshop. EPA/ 630/R-92-002. Washington, DC.

Weis, J.S., Weis, P., 1989. Effects of environment pollutants on early fish development. Rev. Aquat. Sci. 1, 45–73.

Woelke, C.E., 1960. Reports of sulfite waste liquor in a marine environment and its effect on oyster larvae. Was. Dept. Fish. Res. Bull. 6, 81–161.

Woelke, C.E., 1961. Bioassay. The bivalve larvae tool. Proc. 10th Pacific Northwest Symposium on Waste Pollution Research. US Public Health Service, Portland, Oregon, pp. 113–123.

Woelke, C.E., 1965. Bioassay of pulp mill wastes with oysters. In: Biological Problems in Waste Pollution. Third Seminar. Tech. Report 999-WP-US Public Health Service, Ohio, pp. 67–77.

Woelke, C.E., 1968. Development and validation of a field bioassay method with the pacific oyster *Crassostrea gigas* embryo. PhD Thesis. Univ. of Was. Coll. Fish.

Woelke, C.E., 1972. Development of receiving waste quality bioassay criterion based on the 48-hour Pacific oyster (*Crassostrea gigas*) embryo. Wash. Dept. Fish. Tech. Report 9, Seattle, WA.

Woelke, C.E., Schink, T.D., Sasnborn, E.W., 1970. Development of in situ marine bioassay with clams. Annual Report (Sept. 1970) Wash. Dept. Fish. Washington, Seattle, WA.