Coliform accumulation in *Amphibalanus amphitrite* (Darwin, 1854) (Cirripedia) and its use as an organic pollution bioindicator in the estuarine area of Recife, Pernambuco, Brazil

Farrapeira, CMR.[a]*, *Mendes, ES.*[b], *Dourado, J.*[b] *and Guimarães,J.*[b]

[a]Departamento de Biologia/Zoologia, Universidade Federal Rural de Pernambuco – UFRPE, Rua Dom Manoel de Medeiros, s/nº, Dois Irmãos, Recife, PE, Brazil

[b]Laboratório de Sanidade de Animais Aquáticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco – UFRPE, Rua Dom Manoel de Medeiros, s/nº, Dois Irmãos, Recife, PE, Brazil

*e-mail: c.farrapeira@db.ufrpe.

Received: January 28, 2009 – Accepted: March 6, 2009 – Distributed: May 31, 2010

(With 1 figure)

Abstract

Samples of water and barnacles *Amphibalanus amphitrite* were collected from Recife, Brazil, to assess if it accumulates total (TC) and thermotolerant coliforms (TTC) related with sewage pollution. The Most Probable Number (MPN) values and the standard procedures for examination of shellfish were used. Comparatively with the water samples, the highest coliform values came from the barnacles, with TC values ranging from $< 3.0 \times 10^3$ to $\geq 2.4 \times 10^6$ MPN.g^{-1}, and TTC ranging from $\geq 2.4 \times 10^3$ to 2.9×10^5 MPN.g^{-1}. Barnacles accumulate the TC *Ewingella americana*, and the TTC *Escherichia coli, Enterobacter gergoviae, Enterobacter aerogenes,* and *Enterobacter sakazakii*. The results provided an indication of the level of organic contamination at the sampling locations and that this species could be a good organic pollution bioindicator.

Keywords: barnacle, bioaccumulation, bioindicator, estuary, organic pollution.

Acumulação de coliformes em *Amphibalanus amphitrite* (Darwin, 1854) (Cirripedia) e seu uso como bioindicador de poluição orgânica na área estuarina do Recife, Pernambuco, Brazil

Resumo

Amostras de água e cirrípedes *Amphibalanus amphitrite* foram coletados em Recife, Brasil, para avaliar se estes acumulam coliformes totais (CT) e termo-tolerantes (TTC) relacionados à poluição por esgoto doméstico. Foram utilizados os valores de Números Mais Prováveis (NMP) e os procedimentos padrões para exame de bivalves. Comparativamente às amostras de água, os valores mais altos de coliformes foram dos cirrípedes, com valores de TC variando de $< 3,0 \times 10^3$ a $\geq 2,4 \times 10^6$ NMP.g^{-1}, e TTC variando de $\geq 2,4 \times 10^3$ a $2,9 \times 10^5$ NMP.g^{-1}. Os cirrípedes acumularam TC *Ewingella americana* e TTC *Escherichia coli, Enterobacter gergoviae, Enterobacter aerogenes* e *Enterobacter sakazakii*. Os resultados proveram uma indicação de que há contaminação orgânica nas localidades de amostras e que esta espécie pode ser um bioindicador bom de poluição orgânica.

Palavras-chave: cirrípedes, bioacumulação, bioindicador, estuário, poluição orgânica.

1. Introduction

The biological effects of pollution are closely related to the increasing man-made changes to nature. A number of investigations have focused their attention on the ecological, physiological, or biochemical consequences of pollution, as marine organisms could potentially be used as pollution indicators (Patarnello et al., 1991). Benthic animals are useful for the monitoring of environmental quality due to their habitat and lifestyle. Attributes of benthic community structure (species composition, quantitative parameters, trophic groups, and species-indicators) may therefore reflect the quality of marine environments (Pearson and Rosenberg 1978). Assessing patterns in benthic community structures has several advantages over other experimental methods to detect anthropogenic disturbances as the benthos can integrate conditions over time rather than just reflect the conditions of the time the sampling occurred; thus, benthic animals are more useful in assessing local effects of monitoring programmes (Rivero et al., 2005).

According to Zauke et al. (1988), the main goal of biomonitoring is to evaluate the potential of chemical bioaccumulation in ecological systems, to identify corresponding accumulation strategies, and to analyse biological effects using different scales of observation – ranging from intracellular sequestration of substances to effects on the population, community, or ecosystem level. Biological effects at the individual or population levels (e.g., reduced reproduction) may lead to effects at the ecosystem level (e.g., due to changes in resource use and biological interactions such as predation or competition), eventually changing the structure and dynamics of marine systems.

Gestel and Brummelen (1996) considered as bioindicators organisms or groups of organisms that provide information on the environmental conditions of their habitat through their presence or absence or through ecosystem parameters. Rinderhagen et al. (2000) complemented this definition affirming that these organisms should be benthic species, distributed over the whole region of interest in the littoral zone, to enable easy sampling. For Blackmore et al. (1998), these organisms should accumulate trace contaminants in their tissues, responding essentially to the fraction in the environment which is of direct ecotoxicological relevance, i.e. the bioavailability of chemical forms. The required biological properties for using an animal as a bioindicator can be summarised as: sessility or very low mobility; widespread distribution and abundance in the area that will be monitored; sampling ease; and capacity of filtration and accumulation of pollutants (Barbaro et al., 1978, Westman, 1985).

Among the marine and estuarine benthic organisms present in the littoral zone, barnacles constitute an important, characteristic, and successful group in the intertidal region of hard substrata throughout the world's oceans in terms of abundance and diversity (Newman and Ross, 1976). As they are sessile and relatively long-living, they reflect prolonged conditions of the place where they occur and, as bioindicators, they can be used to differentiate between general background levels and responses to increased bioavailable environmental supplies (Al-Thaqafi and White, 1991).

Since barnacles do not tightly regulate the metal burdens in their bodies, they have been suggested as good indicators of metal pollution (Walker et al., 1975). They tolerate and selectively uptake a wide range of toxic metal pollution and accumulate high levels of metals in their body tissues, egg masses and shells in parallel concentrations to the metals in the seawater, which justifies their use as metal pollution bioindicators (Al-Thaqafi and White, 1991; Watson et al., 1995). Among the barnacles used as suitable biomonitors of trace metal pollutant elements, *Amphibalanus amphitrite* (Darwin, 1854) has been cited as a bioaccumulator of 5 trace metals: cadmium, chromium, copper, lead, zinc (Blackmore et al., 1998), and fluoride (Barbaro et al., 1978).

Nevertheless, the focus of the newly developed monitoring programmes are the evaluation of coastal seawater quality and the assessment of the impacts of human sewage on the benthic invertebrate population, using the bioaccumulation of total and thermotolerant coliforms (Alonso et al., 1999; Chiroles-Rubalcaba et al., 2007). Coliforms include a heterogeneous group of lactose-fermentative bacteria belonging to the Enterobacteriaceae Family (APHA, 2000). Among them, *Escherichia coli* (Escherich, 1885) is generally considered a more reliable sanitary indicator of the quality of shellfish, and is also used to classify water (Hood et al., 1983; Silva et al., 2003).

Estuaries are usually highly contaminated by organic and inorganic residues, which increase in concentration in the brackish waters due to the sedimentation of most residues, where there is severe oxygen depletion and increased inputs of untreated organic matter due to urbanization (Cross and Rebordinos, 2003). Furthermore, the composition and structural variation of benthic communities has been used to infer the prevalent environmental factors when creating models for these communities (Gorostiaga et al., 2004). In domestic sewage, the presence of thermotolerant coliforms indicates sewage pollution and contamination of benthic animals as their presence could mean that pathogenic bacteria and viruses might be concentrated in them (Waldichuk, 1989; Chigbu et al. 2005).

Therefore, among the barnacles to date, only *Amphibalanus amphitrite* has been proposed as a potential biomonitor for sewage-derived nutrients in coastal marine ecosystems. The high percentage cover of the barnacle *A. amphitrite* in estuarine areas with waste input and little or no efficient circulation showed that this species is also tolerant of polluted environments; thus, it could be considered a good organic pollution indicator when occurring alone without other typical euryhaline species that are common in these estuarine areas (Lacombe and Monteiro, 1986; Calcagno et al., 1998; Junqueira et al., 2000; Breves-Ramos et al., 2005; Farrapeira, 2006; Farrapeira et al., 2009).

Amphibalanus amphitrite is a warm temperate to tropical barnacle distributed throughout the world (Newman and Ross, 1976). It is distinctly euryhaline and eurythermic and is commonly found in the intertidal zone, settled on several substrates such as mangrove roots, rocky shores, and artificial substrates (Newman, 1967; Baker et al., 2004; Desai et al., 2006; Farrapeira, 2008). This species has an average lifespan of 22 months and a maximum of 5-6 years (Calcagno et al., 1998). In the estuarine area of Recife, Pernambuco, it was considered the most euryhaline marine component, even in the most polluted area when no other species were found, and always occupied a wide zone in the intertidal area (Farrapeira, 2006; Farrapeira et al., 2009). This study aimed to assess whether *A. amphitrite* accumulates total and thermotolerant coliforms in this estuarine area, and thus to demonstrate that it is a good bioindicator of sewage coliform pollution.

2. Methodology

2.1. Study area

Recife (08° 04' 03" - 08° 05' 06" S and 34° 52' 16"- 34° 53' 58" W), located in the northeastern region of

Brazil, in the state of Pernambuco, has a population of approximately 1.40 million people, and suffers with problems such as lack of basic urban services (water, sanitation, solid waste collection, etc.) and physical and social infrastructure (The World Bank, 2003). It was built on a coastal plain constituted of fluvial-marine sediments, where the mangroves were one of the most important deposition systems that provided substrata for urban development (Silva, 2004). The climate is tropical warm and wet, with an annual average air temperature of around 25 °C and water temperatures that range from 23.5 to 32.0 °C. Relative air humidity is of approximately 80% and the area has an average precipitation of 1763 mm, of which 80% falls during the period of April to July – the winter months – with rare rain events occurring during the months of September and February (summer) (Somerfield et al., 2003).

The estuarine area comprises the rivers Capibaribe, Jiquiá, Tejipió, Jordão, Pina, and Beberibe, which flow towards the Atlantic Ocean through a single opening located in the Port of Recife. There are two important basins in this area. The Pina Basin is formed by the confluence of the southern branch of the Capibaribe River and the Jiquiá, Tejipió, Jordão, and Pina rivers. The Harbor Basin is formed by the confluence of the Pina Basin, and by the northern branch of the Capibaribe and Beberibe rivers, enclosed by the Recife and Olinda moles (Figure 1) (Farrapeira, 2006).

Due to its proximity to the city, the estuarine region suffers from extreme environmental degradation, especially mangrove degradation and water pollution from domestic organic sewage. Moreover, only 39% of the urban population has sewerage, and less than 50% is effectively treated; the rest of the sewage is discharged in natura into the rivers (Recife, 1995).

2.2. Sampling and laboratorial analysis

Sampling was designed to test the pollution gradient in two areas, in April and May 2007. Station 1, Pina Bridge (08° 05' 142" S and 34° 53' 419" W), potentially the most polluted site, is an artificial rocky substrate located in the Pina Basin. Station 2, at Marco Zero- Port of Recife, located in the Harbor Basin (08° 03' 795" S and 34° 52' 248" W), represented the place of little or null impact due to the constant renovation of seawater, and is an artificial rocky wall in the Harbour Basin (Figure 1).

Environmental variables from the water (pH, salinity and temperature) were taken by portable pHmeter and densimeter during low tide. The water samples from the estuarine areas were collected in sterile containers and processed within 12 hours of collection. Furthermore, the unpublished data of salinities carried out by the Oceanographic Department of Federal University of Pernambuco sampled during the year was used.

Living barnacles were collected from both stations, with similar heights and diameters to avoid possible distinct effects caused by differences in age. They were taken from the middle range of the intertidal zone during low tides. The samples were placed into clean plas-

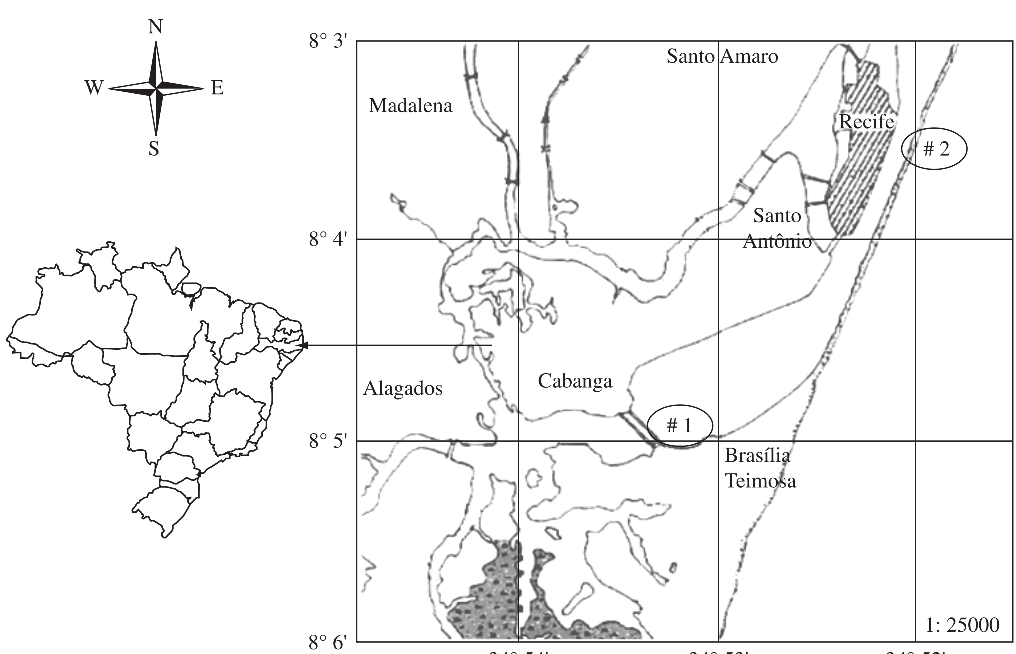

Figure 1. Map of the estuarine area of Recife, Pernambuco, Brazil, showing station 1 at Pina Basin and station 2 at Harbour Basin.

tic bags and stored at room temperature until further processing.

In the Laboratório de Sanidade de Animais Aquáticos, Universidade Federal Rural de Pernambuco – UFRPE all specimens were washed and lightly scrubbed under running tap water to clean any possible contamination and to remove sediments and any microorganisms from the shell plates. They were then measured (basal diameter and height) using calipers (mm), and the whole sample was weighed with an electronic balance (two decimals). The soft tissues of the barnacles (including bodies, muscles, mantle tissue and egg masses) were then aseptically separated from their shells using sterile stainless steel instruments, transferred to appropriate sterile glass jars, and weighed again.

The bacterial analyses were performed according to the standard methods for seawater and shellfish examination (Silva et al., 1997; Feng et al., 1998), using the Most Probable Number (MPN) technique for the total (TC) and thermotolerant coliforms (TTC) test. Thus, the barnacle soft tissues were homogenised with 0.1% peptone water to obtain a dilution of 10^{-1}; the mixture was then disintegrated using sterilised blender equipment. Further dilutions (10^{-4}, 10^{-5}, 10^{-6}) were prepared from this one, inoculating 1 ml of each dilution into a series of three tubes containing 9 mL Brilliant Green Lactose Bile broth (BGLB) with a Durham tube and incubated at 35 °C for 48 hours. The water samples were analysed using the same method, and dilution was 10^{-1}, 10^{-2}, 10^{-3}. All BGLB tubes that showed turbidity and gas production were quantified for the Most Probable Number (MPN) of TC per gram using the MPN table for three tubes.

To analyse the TTC from each gassing BGLB tube, a loopful of suspension was transferred to two tubes of tryptone broth and *E. coli* broth (EC), which remained in a double boiler at 44.5 °C during 48 hours. The presence of TTC was considered positive when gas production was observed in the EC broth tubes. To confirm it, 0.2-0.3 mL of Kovacs' reagent was inoculated in the tube of tryptone broth to test for indole. The distinct red colour in the upper layer (in a ring) was considered a positive result. The MPN of TTC per gramme was determined using the MPN table for three tubes (APHA, 2000). The biochemical identification of bacteria in barnacles and water was carried out according to specific procedures in each station sample.

3. Results

The environmental parameters of the Pina Station (08° 05' 142" S and 34° 53' 419" W) and Port Station (08° 03' 795" S and 34° 52' 248" W) are indicated in Table 1. The remarkably low salinity recorded in the first collection was a result of torrential rain which had occurred in the previous week. The pluviometric pattern and the presence of several rivers in the studied area produce great variation of salinity. According to the Oceanographic Department of the Federal University of Pernambuco, the Pina Basin has a minimal salinity of 0.35 at low tide and 6.14 at high tide during the rainy months, while in the summer period, the maximal values of salinities varies from 26.63 to 33.70 at low and high tides, respectively. At the Harbor Basin, the salinity varies from 0.77 at low tide and 18.98 at high tide during the rainy months, and from 33.72 to 36.09 at low and high tides in the summer period, respectively.

The barnacles collected from Station 1 (Pina) measured 7.9 ± 1.8 mm in height and 11.5 ± 1.9 mm in diameter; their total weight in April was 141.23 g (including shells and opercula) and 6 g (soft tissue weight), and 190.23 and 7 g in May, respectively. The barnacles from Station 2 (Port) measured 8.7 ± 1.7 mm in height and 12.9 ± 2.6 mm in diameter; weight was of 135.79 g (complete animals) and 6 g (soft tissues) in April, and 201.26 and 10 g in May, respectively.

TC and TTC densities in water had the same values in both months, but were clearly different at each site; values decreased from the most polluted sample area (Pina Basin: $\geq 0.24 \times 10$ to $\geq 2.4 \times 10^3$ MPN.100 mL^{-1}) to the lesser contaminated ones, near the sea bar (Harbor Basin: 0.46×10 to 0.9×10 MPN.100 mL^{-1}) (Table 1). Bacteria composition also varied. Three TC species of *Salmonella* were identified in the water at Pina Station, as well as three TTC: *Citrobacter amalonaticus* (Werkman and Gillen,

Table 1. Abiotic parameters and total (TC) and thermotolerant coliform (TTC) values in water (MPN.100 mL^{-1}) and barnacle soft tissues (MPN.g^{-1}) from the estuarine area of Recife, Pernambuco, Brazil.

Parameters	Pina Station		Port Station	
	23/04/2007	17/05/2007	23/04/2007	17/05/2007
Salinity	10.0	24.0	16.0	22.0
pH	6.8	7.1	7.3	7.3
T °C water	30.5 °C	29 °C	29.6 °C	29 °C
T °C air	26.8 °C	29 °C	27.8 °C	29 °C
TC water	$\geq 0.24 \times 10$	$\geq 2.4 \times 10^3$	0.46×10	0.9×10
TTC water	$\geq 0.24 \times 10$	$\geq 2.4 \times 10^3$	0.46×10	0.9×10
TC barnacles	4.6×10^5	$< 3.0 \times 10^5$	$\geq 2.4 \times 10^6$	$\geq 2.4 \times 10^8$
TTC barnacles	$\geq 2.4 \times 10^5$	$< 3.0 \times 10^5$	$\geq 2.4 \times 10^6$	2.9×10^7

1932), *Escherichia coli, and Klebsiella pneumonia* (Edwin Klebs, 1887). In Port Station, three TC (*Kluyvera ascorbata* (Farmer, 1981), *Providencia alcalifaciens* (Ewing, 1962), and *Providencia rettgeri* (Brenner et al., 1978)) and three TTC were found (*Escherichia coli*, *Klebsiella pneumoniae* and *Obesumbacterium* spp).

Bacterial concentrations in barnacle soft tissues showed an inverse distribution in the two sampling sites. The station considered the most polluted (Pina Station), showed a lower TC concentration ($< 3.0 \times 10^5$ to 4.6×10^5 MPN.g^{-1}), with only one species present, *Ewingella americana* Grimont et al., 1983. There were four TTC ($\geq 2.4 \times 10^5$ to $< 3.0 \times 10^5$ MPN.g^{-1}): *Escherichia coli, Enterobacter gergoviae* Brenner et al., 1980, *Enterobacter aerogenes* (Edwards e Ewing, 1962), and *Enterobacter sakazakii* (Farmer et al., 1980). At the Port station, the concentration of TC (*Ewingella americana*) varied from $\geq 2.4 \times 10^6$ to $\geq 2.4 \times 10^8$ MPN.g^{-1}, while the TTC varied from $\geq 2.4 \times 10^6$ to 2.9×10^7 MPN.g^{-1}, with two species: *Escherichia coli* and *Enterobacter gergoviae*. It was remarkable that in both stations the concentration of both types of coliforms were higher than in the water samples (Table 1).

4. Discussion

The coliforms present in the water varied in value for the commonest bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) that were found in both stations, but the highest values of TC and TTC were only recorded at the most polluted station. It is important to highlight that in addition to these species, the TTC *Citrobacter amalonaticus* also occurred in this locality, a species considered of fecal origin (Leclerc et al., 1981). When studying the coliforms present in Valencia, Spain, Alonso et al. (1999) found *Escherichia coli*, *Klebsiella pneumoniae*, and 12 other species, while Chiroles-Rubalcaba et al. (2007) found the same TTC coliforms and another four species when researching bacterial indications of fecal contamination in the Almendares River, Cuba. The specific variation, especially in TC species composition between the two stations sampled, may be explained based on their ecological specificity. The survival of coliforms in marine waters depends on salinity, predation, competition with autochthonous microorganisms, heavy metals and nutrients (Hood and Ness, 1982).

Although several other disease-causing organisms might be present, the results found here are useful to recognise the high density of bacterial pathogens at the Pina Basin. The presence of the pneumonia-causing TTC *Klebsiella pneumoniae*, for instance, is cause for concern (Grisi et al., 1983), as well the TC *Salmonella* spp. (which causes typhoid fever and salmonellosis) and the TTC *Citrobacter amalonaticus*, which is frequently present in humans as normal intestinal inhabitants and can cause a range of diseases that include bacteremia/sepsis and several other infectious diseases (Suwansrinon et al., 2005).

The coliform count of this study showed that Pina Station was the most contaminated site, and this fact is cited in the literature. According to Recife (1995), high levels of TTC ($> 3 \times 10^4$ MPN.100 ml^{-1}) have been recorded in the Pina Basin, which shows the strong influence of domestic sewage at that locality. Similarly, Castro et al. (2006) described the Pina Basin as hypereutrophic and organically polluted by the constant influx of nutrients from the five rivers that enter the basin through urban areas with poor sanitation or none at all. Moreover, these rivers pick up a large range of pollutants, particularly high levels of nutrients from the domestic sewage derived from the Boa Viagem and Pina neighbourhoods and also the downtown area. The situation of sewerage is even more dramatic. The World Bank (2003) reported that overall coverage is around 36% and as low as 7% in the poorer areas. Sewage is discharged into open canals which flow into the river systems without any treatment whatsoever. Only an estimated 20% of total sewage is treated; as a result, the heavily contaminated river system affects water quality along the coast.

According to Tommasi (1987), the domestic sewage composition of a large Brazilian city has a pH of approximately 6.8 and a total bacteria count that varies from 0.1×10 to 3×10^6 MPN.100 mL^{-1}. This author also estimated that sewage pollution per inhabitant is of 1×10^5 MPN.100 mL^{-1} (without treatment) and 5×10^3 MPN.100 mL^{-1} (with primary treatment). Kolm and Absher (2008), when studying the bacterial density in waters of the Paranaguá estuarine complex (Paraná, Brazil), found a correlation between the area that receives waters from city sewers and the highest values of TC, *E. coli*, and seston in the water; the highest TC values were obtained from the polluted estuarine area (6.7×10^3 MPN.100 mL^{-1}). At the mouth of the Nervión River (Biscay Bay, Iberian Peninsula), which receives an enormous load of untreated sanitary sewage, Pagola-Carte and Saiz-Salinas (2001) found TC values that ranged from 1.0×10 to 2.2×10^4 MPN.100 mL^{-1} and TTC values between 0.1×10 and 2.7×10^3 MPN.100 mL^{-1}. Jeng et al. (2005) found the highest TTC concentrations (1.6×10^2 MPN.100 mL^{-1}) at the mouth of the Lake Pontchartrain canal, an estuary in Louisiana, Mississippi (USA).

The maximum values observed by these authors were somewhat similar to those obtained from Pina Station in this study and are higher than the maximum allowed by Brazilian law regarding TTC counts in water from where the bivalve mollusks will be extracted and/or cultivated to be consumed by humans (1.4×10 MPN.100 mL^{-1}) (CONAMA, 2005). The lower coliform values at Port Station, nevertheless, can be explained because of the proximity to the Atlantic Ocean and the tide movements, where seawater is continuously renovated. According to Tommasi (1987), seawater has a high ability to purify itself and to inactivate thermotolerant organisms. Additionally, bacteria could travel some distances (nearly 2.4 km from the discharge point) as a result of pumping, depending on wind direction/intensity and current (Jeng et al., 2005). Chigbu et al. (2005) noted that after a peak in TTC values the numbers decline rapidly in 0.3 to 13 days (an average of 6 days); their dynamics in coastal waters is a function of the bacterial load from inflowing streams and rivers, mass transport, and losses due to death and sedimentation.

An interesting fact was finding the highest concentrations of coliforms inside the barnacles rather than in the water surface. Kolm and Absher (2008), when comparing the bacterial density in waters and oysters of the Paranaguá estuarine complex, Paraná- Brazil, always found lower TC values in the water than in the oysters during the period sampled. The same was observed by Burkhardt III and Calci (2000), who found TTC concentrations inside the oysters *Crassostrea virginica* (Gmelin, 1791) from an estuary of the Mexican Gulf as much as 4.4 times than that of the surrounding water. Similarly, Lucena et al. (1994) noted that bacterial concentrations of the black mussel *Mytilus edulis* d`Orbigny, 1846 from the Mediterranean Sea were far greater than bacterial levels in the water, which indicates bioaccumulation.

It might be expected that coliforms also penetrate the soft tissues of the barnacles. Zauke et al. (1988) defines bioaccumulation as the net uptake of a substance by aquatic organisms through the interactive effect of bioconcentration (via non-dietary uptake routes). Paralleling an observation by Cerutti and Barbosa (1991) about mollusks, bacterial selection in barnacles may be related to factors such as the bacteria's adaptation to the marine environment, resistance to enzymatic degradation, and use of the host's gut content as a source of nutrients. Although there is a lot of literature on many aspects of barnacle biology, there is very little quantitative information on suspension feeding rates. Barnacles are sessile facultative active-passive suspension-feeders and the volume of seston filtered from the water is a function of current speed and the surface area of the extended basket of modified legs or cirri (Foster, 1978). *Amphibalanus amphitrite* is more microphagous in nature and is capable of feeding on phytoplankton and debris (Desai and Anil, 2004). Regarding the nutrition of this species' larvae, Gosselin and Qian (1997) observed that when bacteria concentrations were added, barnacle larvae did not grow and did not accumulate particulate material in their gut, which suggests that *A. amphitrite* larvae are unable to capture bacteria.

Comparing coliform concentrations in other marine invertebrate organisms – mainly shellfish (oysters and mussels) – the coliform values inside the barnacles were astonishingly higher than for any other marine or estuarine invertebrates. Although there are no established TTC standards for shellfish, according to Hood et al. (1983) the National Shellfish Sanitation Program (USA) has suggested a standard of $\geq 2.3 \times 10$ MPN.g^{-1} of tissue. These authors found TTC levels of 8.6×10 MPN.g^{-1} in *Crassostrea virginica* oysters from the Apalachicola and Tampa bays, USA. In Brazil, the estimated TC and TTC values in *Crassostrea rhizophorae* (Guilding, 1828) oysters ranged from $< 0.2 \times 10$ to $> 1.6 \times 10^3$ MPN.g^{-1} and from $< 0.2 \times 10$ to $> 9.2 \times 10^2$ MPN.g^{-1}, respectively, in the Cocó River estuary, State of Ceará (Silva et al., 2003). Values ranged from $< 0.2 \times 10$ to 9.2×10^3 MPN.g^{-1} for TC and from $< 0.2 \times 10$ to 4.3×10^2 MPN.g^{-1} for TTC in samples taken from the Jaguaribe River estuary, in the same state (Vieira et al., 2007). Kolm and Absher (2008) found that in the oysters from the Paranaguá estuarine complex the TC values varied from 0.6×10 MPN.g^{-1} to 4.8×10^2 MPN.g^{-1} in polluted areas. In addition, coliform values in mussels were not similar to those in barnacles. Jorge et al. (2002), when studying *Perna perna* (Linnaeus, 1758) mussels from Niterói, Rio de Janeiro, found 0.3×10 MPN.g^{-1} TC and 0.1×10 MPN.g^{-1} TTC.

Another interesting fact observed in this study was the bacteria composition within the barnacles. With the exception of *Escherichia coli* – which was also found in water samples – all other coliform species were only sampled from barnacles, regardless of their occurrence in other rivers, as noted by Alonso et al. (1999) in Valencia, Spain. *Escherichia coli* and *Enterobacter aerogenes* are usually isolated from human feces, while the other thermotolerant coliforms (*Enterobacter gergoviae* and *Enterobacter sakazakii*) are probably of non-fecal origin (Leclerc et al., 1981). *E. gergoviae* has been isolated from a variety of clinical and environmental sources (Brenner et al., 1980), as well as *E. sakazakii*; the latter has been linked to outbreaks of meningitis or enteritis, especially in infants (Kandhai et al., 2004). The TC *Ewingella americana* – also found solely inside the barnacles – is the only species of the genus in Family Enterobacteriaceae, first described from clinical specimens (Grimont et al., 1983). This organism has low pathogenic potential and rarely causes infections in immunocompromised humans; peritonitis and bacteremy has been identified from various clinical specimens, although the reservoir's niches have not been clarified (Pound et al., 2007).

When observing this lack of pattern of coliform numbers in water and animal samples, Escobar Nieves (1988) suggested that TC and TTC detected in the water do not represent a reliable measurement of oyster quality. To justify the occurrence of a specific coliform in an organism and not in the water, Pommepuy et al. (1996) proposed that enterobacteria are best preserved when the microorganisms are ingested by organisms, which may account for the great differences observed in bacterial counts. Chigbu et al. (2005) noted that TTC in surface waters decrease or disappear from the water column with time through death and sedimentation processes, and can concentrate in sediments at high densities. Likewise, Troussellier (1998) stated that water microbes directly discharged into receiving waters could remain in the water column and be transported for some distance prior to their die-off, precipitate to the bottom sediment, or remain suspended in the surface water overlying the bottom sediment. Therefore, the presence of the TC and

TTC identified in the barnacles sampled means that at any moment in the past these bacteria were in the water and were carried out to the estuarine area.

The high concentration of coliforms in body tissues of the barnacle *Amphibalanus amphitrite* at an estuarine area of Recife showed not only that this species is tolerant to polluted environments (Calcagno et al., 1998), but especially that it is a good coliform bioaccumulator; thus, it proved to be a good choice as a sentinel species for biomonitoring. According to Westman (1985), this occurs because they are unusually tolerant of degraded conditions and – considering the literature on their history in estuarine areas contaminated with sewage – they can survive where others organisms cannot.

References

ALONSO, JA., SORIANO, A., CARBAJO, O., AMOROS, I. and GARELICK, H., 1999. Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 °C. *Applied and Environmental Microbiology*, vol. 65, no. 8, p. 3746-3749.

AL-THAQAFI, K. and WHITE, KN., 1991. Effect of shore position and environmental levels on body metal burdens in the barnacle, *Elminius modestus*. *Environmental Pollution*, vol. 69, no. 2-3, p. 89-104.

American Public Health Association - APHA, 2000. *Standard methods for the examination of water and wastewater*. 21th ed. Washington: APHA. 937 p.

BAKER, P., BAKER, SM. and FAJANS, J., 2004. *Nonindigenous marine species in the Greater Tampa Bay ecosystem*. Gainesville: Tampa Bay Estuary Program Technical Publication. no. 02-04, 118 p.

BARBARO, A., FRANCESCON, A., POLO, B. and BILIO, M., 1978. *Balanus amphitrite* (Cirripedia Thoracica); a potential indicator of fluoride, copper, lead, chromium and mercury in North Adriatic lagoons. *Marine Biology*, vol. 46, no. 3, p. 247-258.

BLACKMORE, G., MORTON, B. and HUANG, ZG., 1998. Heavy metals in *Balanus amphitrite* and *Tetraclita squamosa* (Crustacea: Cirripedia) collected from the coastal waters of Xiamen, China. *Marine Pollution Bulletin*, vol. 36, no. 1, p. 32-40.

BRENNER, DJ., RICHARD, C., STEIGERWALT, AG., ASBURY, MA. and MANDEL, M., 1980. *Enterobacter gergoviae* sp. nov.: a new species of Enterobacteriaceae found in clinical specimens and the environment. *International Journal of Systematics Bacteriology*, vol. 30, no. 1, p. 1-6.

BREVES-RAMOS, A., LAVRADO, HP., JUNQUEIRA, AOR. and SILVA, SHG., 2005. Succession in rocky intertidal benthic communities in areas with different pollution levels at Guanabara Bay (RJ-Brazil). *Brazilian Archives of Biology and Technology*, vol. 48, no. 6, p. 951-965.

BURKHARDT III, W. and CALCI, RC., 2000. Selective accumulation may account for shellfish-associated viral illness. *Applied and Environmental Microbiology*, vol. 66, no. 4, p. 1375-1378.

CALCAGNO, JA., LÓPEZ GAPPA, J. and TABLADO, A., 1998. Population dynamics of the barnacle *Balanus amphitrite* in an intertidal area affected by sewage pollution. *Journal of Crustacean Biology,* vol. 18, no. 1, p. 128-137.

CASTRO, FJV., BEZERRA, TNC., SILVA, MC. and FONSÊCA-GENEVOIS, V., 2006. *Spirinia elongata*, sp. nov. (Nematoda, Desmodoridae) from Pina Basin, Pernambuco, Brazil. *Zootaxa*, vol. 1121, p. 53-68.

CASTRO, IB., ALVES DE LIMA, AF., BRAGA, ARC. and ROCHA-BARREIRA, CA., 2007. Imposex in two muricid species (Mollusca: Gastropoda) from the northeastern Brazilian coast. *Journal of the Brazilian Society of Ecotoxicology*, vol. 2, no. 1, p. 81-91.

CERUTTI, RL. and BARBOSA, TCP., 1991. Flora bacteriana heterotrófica em ostras (*Crassostrea rhizophorae*) e águas da Baía Norte, Ilha de Santa Catarina, Brasil. *Revista de Microbiologia*, vol. 22, p. 330-334.

CHIGBU, P., GORDON, S. and STRANGE, TR., 2005. Fecal coliform bacteria disappearance rates in a north-central Gulf of Mexico estuary. *Estuarine Coastal and Shelf Science*, vol. 65, p. 309-318.

CHIROLES-RUBALCABA, S., GONZÁLEZ-GONZÁLEZ, MI., TORRES-ROJAS, T., VALDÉS-ÁGUILA, M. and DOMÍNGUEZ-MARTÍNEZ, I., 2007. Bacterias indicadoras de contaminación fecal en aguas del río Almendares (Cuba). *Higiene y Sanidad Ambiental*, vol. 7, p. 222-227.

Conselho Nacional do Meio Ambiente – CONAMA, 2005. *Resolução nº 357, de 17 de março de 2005:* dispõe sobre a classificação dos corpos de água e diretrizes ambientais para seu enquadramento, bem como estabelece condições de padrões de lançamento de efluentes. Brasília: CONAMA.

CROSS, I. and REBORDINOS, L., 2003. Efecto de la contaminación marina sobre la estructura genética poblacional del bivalvo *Crassostrea angulata*. *Ciencias Marinas*, vol. 29, no. 2, p. 239-250.

DESAI, DC. and ANIL, AC., 2004. The impact of food type, temperature and starvation on larval development of *Balanus amphitrite* Darwin (Cirripedia: Thoracica). *Journal of Experimental Marine Biology and Ecology*, vol. 306, no. 1, p. 113-137.

DESAI, DV., ANIL, AC. and VENKAT, K., 2006. Reproduction in *Balanus amphitrite* Darwin (Cirripedia: Thoracica): influence of temperature and food concentration. *Marine Biology*, vol. 149, no. 6, p. 1431-1441.

ESCOBAR-NIEVES, A., 1988. Condición bacteriológica de las ostras del manglar (*Crassostrea rhizophorae* Guilding) en la Cienaga Grande de Santa Marta, Caribe Colombiano. *Anales del Instituto de Investigaciones Marinas de Punta de Betin*, vol. 18, p. 137-151.

FARRAPEIRA, CMR., 2006. Barnacles (Cirripedia Balanomorpha) of the estuarine region of Recife, Pernambuco. *Brazilian Journal of Oceanography*, vol. 34, no. 2, p. 100-119.

FARRAPEIRA, CMR., 2008. Cirripedia Balanomorpha en el estuario del río Paripe (Isla de Itamaracá-Pernambuco-Brasil). *Biota Neotropica*, vol. 8, no. 3, p. 31-39.

FARRAPEIRA, CMR., TENÓRIO, DO. and MELO, AVOM., 2009. Comunidade bentônica de lagunas costeiras impactadas de Recife e Olinda, Pernambuco - possível grupamento biológico bioindicador de poluição orgânica. *Arquivos de Ciências do Mar*, vol. 42, no. 1, p. 1-11.

FENG, P., WEAGANT, SD. and GRANT, MA., 1998. Enumeration of *Escherichia coli* and the coliform bacteria, s.pp. In FOOD AND DRUG AMNINISTRATION - FDA. *Bacteriological analytical manual*. 8 ed. Rockville: FDA/Center for Food Safety & Applied Nutrition. p. 14-18.

FOSTER, BA., 1978. The marine fauna of New Zealand: Barnacles (Cirripedia: Thoracica). *New Zealand Oceanographic Institute Memoir*, vol. 69, p. 3-160.

GESTEL, CAM. and BRUMMELEN, TC., 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. *Ecotoxicology*, vol. 5, p. 217-225.

GOROSTIAGA, JM., BORJA, Á., DÍEZ, I., FRANCÉS, G., PAGOLA-CARTE, S. and SÁIZ-SALINAS, JI., 2004. Recovery of benthic communities in polluted systems. In BORJA, MA. and COLLINS, M. (Eds.). *Oceanography and marine environment of the Basque Country*. Amsterdam: Elsevier. p. 549-578. Elsevier Oceanography Series, 70.

GOSSELIN, LA. and QIAN, PY., 1997. Can bacterivory alone sustain larval development in the polychaete *Hydroides elegans* and the barnacle *Balanus amphitrite? Marine Ecology Progress Series,* vol. 161, p. 93-101.

GRIMONT, PA., FARMER, JJ., GRIMONT, F., ASBURY, MA., BRENNER, DJ. and DEVAL, C., 1983. *Ewingella americana* gen. nov., sp. nov., a new Enterobacteriaceae isolated from clinical specimens. *Annales de Microbiologie*, vol. 134, p. 39-52.

GRISI, SJFE., KIMURA, HM., BELLIZIA-NETO, L., EJZENBERG, B., RODRIGUES-NETO, AJ. and HORITA, SM., 1983. Pneumonia por *Klebsiella pneumoniae*. *Pediatria*, vol. 5, p. 376-378.

HOOD, MA. and NESS, GE., 1982. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. *Applied and Environmental Microbiology*, vol. 43, no. 3, p. 578-584.

HOOD, MA., NESS, GE. and BLAKE, NJ., 1983. Relationship among fecal coliforms, *Escherichia coli*, and *Salmonella* spp. in shellfish. *Applied and Environmental Microbiology*, vol. 45, no. 1, p. 122-126.

JENG, HAC., ENGLANDE, AJ., BAKEER, RM., HENRY, B. and BRADFORD, HB., 2005. Impact of urban stormwater runoff on estuarine environmental quality. *Estuarine Coastal And Shelf Science*, vol. 63, p. 513-526.

JORGE, LC., GARCIA, LM., MARTINS, VB., KOSAWA, A. and PAULS, E., 2002. Interações dos processos sócio-ambientais nas bacias das enseadas de Icaraí e São Francisco, Niterói (RJ). 2. Organismos aquáticos como bioindicadores da qualidade ambiental com enfoque no mexilhão *Perna perna* (Linnaeus, 1798), em Niterói-RJ. *Mundo & Vida*, vol. 3, no. 2, p. 108-116. Available from: <http://www.uff.br/cienciaambiental/mv/mv3/MV3(2)108-116.pdf>.

JUNQUEIRA, AOR., FALCÃO, APC., MAYER-PINTO, M., VIANA, MS. and SILVA, SHG., 2000. Spatial and temporal variations on intertidal barnacle abundance in a tropical bay. *Nauplius*, vol. 8, no. 2, p. 195-204.

KANDHAI, MCM., REIJ, W., VAN PUYVELDE, K., GUILLAUME-GENTIL, O., BEUMEF, RR. and VAN SCOTHORST, M., 2004. A new protocol for the detection of *Enterobacter sakazakii* applied to environment samples. *Journal of Food Protection*, vol. 67, p. 1267-1270.

KOLM, HE. and ABSHER, TM., 2008. Bacterial density and coliform organisms in waters and oysters of Paranaguá estuarine complex, Paraná, Brazil. *Boletim do Instituto de Pesca*, vol. 34, no. 1, p. 49-59.

LACOMBE, D. and MONTEIRO, W., 1986. Balanídeos como indicadores de poluição na Baía de Guanabara. *Brazilian Jornal of Biology = Revista Brasileira de Biologia*, vol. 34, no. 4, p. 633-644.

LECLERC, H., GAVINI, F. and OGER, C., 1981. Les indicateurs bacteriens dans le controle bacteriologique de l'eau: exigences et limites. *Journal of Hydrology*, vol. 12, p. 213-228.

LUCENA, F., LASOBRAS, J., McINTOSH, D., FORCADELL, M. and JOFRE, J., 1994. Effect of distance from the polluting focus on relative concentrations of *Bacteroides fragilis* phages and coliphages in mussels. *Applied and Environmental Microbiology*, vol. 60, no. 7, p. 2272-2277.

NEWMAN, WA., 1967. On physiology and behavior of estuarine barnacles. *Journal of the Marine Biological Association of India*, vol. 2, no. 3, p. 1038-1066.

NEWMAN, WA. and ROSS, A., 1976. Revision of the balanomorph barnacles; including a catalog of the species. *Memoirs of the San Diego Society of Natural History*, vol. 9, p. 1-108.

PAGOLA-CARTE, S. and SAIZ-SALINAS, JI., 2001. Changes in the sublittoral faunal biomass induced by the discharge of a polluted river along the adjacent rocky coast (N. Spain). *Marine Ecology. Progress Series*, vol. 212, p. 13-27.

PATARNELLO, T., GUINEZ, R. and BATTAGLIA, B., 1991. Effects of pollution on heterozygosity in the barnacle *Balanus amphitrite* (Cirripedia: Thoracica). *Marine Ecology. Progress Series*, vol. 70, p. 237-243.

PEARSON, TH. and ROSENBERG, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology: An Annual Review*, vol. 16, p. 229-311.

POMMEPUY, M., UTIN, MB., DERRIER, A., GOURMELON, M., COLWELL, RR. and CORMIER, M., 1996. Retention of enteropathogenicity by variable but nonculturable *Escherichia coli* exposed to seawater and sunlight. *Applied and Environmental Microbiology*, vol. 62, no. 12, p. 4621-4626.

POUND, MW., TART, SB. and OKOYE, O., 2007. Multidrug-resistant *Ewingella americana*: a case report and review of the literature. *The Annals of Pharmacotherapy*, vol. 41, no. 12, p. 2066-2070.

RECIFE. Prefeitura da Cidade do Recife, 1995. *Recuperação ambiental das bacias dos rios Jordão e Pina- Concepção geral*. Recife. 148 p.

RINDERHAGEN, M., RITTERHOFF, J. and ZAUKE, GP., 2000. Crustaceans as bioindicators. In GERHARDT, A. (Ed.). *Biomonitoring of polluted water*: reviews on actual topics. Zürich: Switzerland: TransTech Publ. p. 161-194. Trans Tech Publications Environmental Research Forum, v. 9.

RIVERO, MS., ELÍAS, R. and VALLARINO, EA., 2005. First survey of macroinfauna in the Mar del Plata Harbor (Argentina), and the use of polychaetes as pollution indicators. *Revista de Biologia Marina y Oceanografia*, vol. 40, no. 2, p. 101-108.

SILVA, AIM., VIEIRA, RHSF., MENEZES, FGR., FONTELES-FILHO, AA., TORRES, RCO. and SANT'ANNA, ES., 2003. Bacteria of fecal origin in mangrove oysters (*Crassostrea rhizophorae*) in the Cocó River estuary, Ceará State, Brazil. *Brazilian Journal of Microbiology*, vol. 34, p. 126-130.

SILVA, JJA., 2004. Diretrizes para usos dos manguezais do Pina, Recife: uma análise crítica. In *Proceedings of the 4th Congresso Brasileiro de Geógrafos*. Rio de Janeiro: UERJ, 2004. p. 385-396.

SILVA, N., JUNQUEIRA, VCA. and SILVEIRA, NFA., 1997. *Manual de métodos de análise microbiológica de alimentos*. São Paulo: Varela. 295p.

SOMERFIELD, PJ., FONSÊCA-GENEVOIS, VG., RODRIGUES, ACL., CASTRO, FJV. and SANTOS, GAP., 2003. Factors affecting meiofaunal community structure in the Pina Basin, an urbanized embayment on the coast of Pernambuco, Brazil. *Journal of the Marine Biological Association of the United Kingdon*, vol. 83, no. 6, p. 1209-1213.

SUWANSRINON, K., WILDE, H., SITPRIJA, V. and HANVESAKUL, R., 2005. Enteric fever-like illness caused by infection with *Citrobacter amalonaticus*. *Journal of the Medical Association of Thailand*, vol. 88, no. 6, p. 837-840.

THE WORLD BANK, 2003. *Project appraisal document on a proposed loan in the amount of US$ 46.0 million to the state of Pernambuco, Brazil*. Recife: The World Bank – Finance. 116 p. Report No: 23331-BR.

TOMMASI, LR., 1987. *Poluição marinha no Brasil*: síntese do conhecimento. Sao Paulo: Instituto Oceanográfico. 30 p. Publicaçao especial do Instituto Oceanográfico, 5.

TROUSSELLIER, MJ., 1998. Response of enteric bacteria to environmental stresses in seawater. *Oceanologica Acta*, vol. 21, p. 965-981.

VIEIRA, RHSF., VASCONCELOS, RF. and CARVALHO, EMR., 2007. Quantificação de víbrios, de coliformes totais e termotolerantes em ostra nativa *Crassostrea rhizophorae*, e na água do estuário do Rio Jaguaribe, Fortim-CE. *Revista Brasileira de Higiene e Sanidade Animal*, vol. 1, p. 1-13.

WALDICHUK, M, 1989. The state of pollution in the marine environment. *Marine Pollution Bulletin*, vol. 20, no. 2, p. 598-602.

WALKER, G., RAINBOW, PS., FOSTER, P. and CRISP, DJ., 1975. Barnacles: possible indicators of zinc pollution? *Marine Biology*, vol. 30, no. 1, p. 57-66.

WESTMAN, WE, 1985. Ecotoxicology: assessing impacts of pollutants on biota. In WESTMAN, WE., *Ecology, impact assessment and environmental planning*. New York: John Wiley & Sons. p. 364-408.

WATSON, D., FOSTER, P. and WALKER, G., 1995. Barnacle shells as biomonitoring material. *Marine Pollution Bulletin*, vol. 31, p. 111-115.

ZAUKE, GP., RITTERHOFF, J. and RINDERHAGEN, M., 1988. *Concepts and applications in aquatic biomonitoring - Internal Review Paper*. [updated version: november 2005]. Oldenburg, Germany: Aquatic Ecology Group, ICBM, CvO Univ. Oldenburg. 38 p.