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Assessment of contamination of the beach clam *Tivela mactroides*: implications for food safety of a recreational and subsistence marine resource in Caraguatatuba Bay, Brazil

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Abstract

Background: The clam *Tivela mactroides* is an important sandy-beach resource along the western Atlantic coast, and is widely harvested by both tourists and residents for recreational, subsistence and/or economic purposes. These clams are intensively exploited in Caraguatatuba Bay on the southeastern Brazilian coast. Similarly to most coastal areas around the world, this bay is subject to a variety of environmental threats derived from human occupation (e.g., sewage) and economic activities (e.g., oil spills). Considering the history of changes in this area and current plans for development, environmental pressures are expected to increase. This prospect raises concerns regarding food safety of members of the public, including clam harvesters, who consume local seafood. In order to provide baseline information to compare with future situations, this study analyzed the contamination of clam meat by microorganisms (fecal coliforms, *Salmonella* sp., *Vibrio cholerae*, and *Staphylococcus aureus*) and Polycyclic Aromatic Hydrocarbons (PAHs).

Results: Preliminary evaluations revealed microorganism contamination levels above the maximum limits allowed under Brazilian legislation; with higher levels in the central portion of the bay. Temporal evaluations at three sampling points in this area revealed year-round contamination by all microorganisms, i.e., a continuous risk for clam consumers. Although the effectiveness of thermal processing used by consumers could not be formally tested in this study, it has the potential to reduce the contamination by fecal coliforms, *Salmonella* sp., and *S. aureus* to safe levels, as demonstrated in the two samples analyzed. However, although *S. aureus* can be totally eliminated, its heat-tolerant toxins may still affect consumers. Concentrations of individual compounds (congeners) and total PAHs were recorded, indicating contamination derived from oil spills.

Conclusions: The results raise concerns regarding traditional small-scale fisheries, which can be threatened by the intensification of human activities in the coastal region, thus requiring continuous monitoring of the quality of seafoods, in addition to effective communication of the risks to consumers, and efficient measures to reduce both sewage and industrial pollution.

Keywords: Clams; Coliforms; *Salmonella* sp; *Vibrio cholerae*; *Staphylococcus aureus*; Hydrocarbons; Caraguatatuba Bay

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Background

Seafood is an important source of protein, but the stocks and safety of fish and shellfish are being impacted by overfishing and marine pollution. Coastal areas are subject to a variety of conflicting interests and activities (Cicin-Sain and Knecht 1998), which reduce water quality and food safety. Sewage outfalls and nutrient enrichment as well as oil spills (Islam and Tanaka 2004; Diaz and Rosemberg 2008) are among the factors responsible for worldwide impacts in the sea (Halpern *et al.* 2008) and a general reduction in ocean health (Halpern *et al.* 2012). This situation is expected to worsen in developing countries, where monitoring and control policies are insufficient to mitigate coastal pollution (Wingqvist *et al.* 2012). This is a challenge even in developed countries such as the USA, where 6.1% (589,310 outbreaks) of the 9,638,301 foodborne outbreaks, between 1998 and 2008, were caused by consumption of contaminated aquatic animals (Painter *et al.* 2013), with bacteria identified as the etiological agent in 24% of the cases.

Food safety emerged as one of the central themes of the United Nations Conference on Sustainable Development, held in 2012 in Rio de Janeiro (UNCSD United Nations Conference on Sustainable Development 2012), in addition to ocean sustainability and poverty reduction. These themes interact in the context of environmental degradation of coastal zones, where impaired food safety may increase poverty and compromise human well-being. This is especially applicable to developing countries (Andrew *et al*. 2007) where traditional small-scale fisheries comprise a fragile socioecological system, prone to collapse under environmental degradation.

One type of small-scale fishery that might be especially endangered by coastal impacts is beach (or estuarine) clam harvesting (McLachlan *et al*. 1996; Castilla and Defeo 2001; Defeo *et al*. 2009). This fishery is affected not only by habitat alterations that reduce clam stocks, such as beach erosion, but also by the risks to humans consuming clams from polluted areas. According to Martinez and Oliveira (2010), counts of coliform bacteria are always higher in mollusks than in the water, due to their filtering habit and bioaccumulation, which increases the risks of consuming them. Consumption of mollusks was responsible for 3% of the cases of all foodborne disease recorded in the United States from 1998 to 2008 (Painter *et al*. 2013). Mollusks are related with 42.5% of seafood-associated outbreaks (Iwamoto *et al.* 2010), raising concerns for areas where mollusk consumption is intense.

According to Gelli *et al.* (1979), microbiological and chemical analyses of bivalve mollusks can indicate the microbiota and the contaminants that are present in the marine environment. This is the background for the worldwide Mussel Watch Program (Kimbrough *et al.* 2008), which uses this kind of information as a sentinel for public health, to control foodborne infections (Dias *et al*. 2003). Accordingly, concerns have increased and consequently new studies are being conducted on monitoring and assessment of microbiological contamination in marine mollusks, especially cultivated species (Pinheiro Jr 2000; Barardi *et al*. 2001; Garcia 2005), leaving a gap in information on the natural stocks.

The risks involved in beach-clam harvesting depend on the amount of clams collected and consumed, but also on their contamination levels, which are expected to increase in developing countries due to intense coastal development. One example of this situation is the southeastern coast of the states of São Paulo and Rio de Janeiro in Brazil, where most marine extraction of oil and natural gas in the country takes place. Between 1978 and 2004, 268 environmental accidents involving ships and oil pipelines were recorded near the São Sebastião Channel on the northern coast of São Paulo (São Paulo 2004), where more than half of the country's oil is transferred to the continent. The prospective negative environmental impacts of exploitation of the pre-salt oil reserves (Oxford Analytica 2010) may impact environmental quality in this region, especially due to its complex geomorphology and relatively low hydrodynamics, which result in areas that are more likely to retain the oil (Gherardi and Cabral 2007).

In addition to the pre-salt oil exploitation, several projects are being planned or implemented on the northern coast of São Paulo that will have individual and cumulative impacts (Legaspe 2012; Teixeira 2013). Specifically, the prospect for increased population density in this area is expected to put pressure on the existing infrastructure for collection and treatment of domestic sewage. This is a significant bottleneck in the region, since the present facilities are inadequate to the demand, and cause contamination that makes bathing at most beaches inadvisable (São Paulo 2005a). The number of acute diarrhea cases in the region increased from 1,007 to 8,046 between 1998 and 2003 (São Paulo 2005b). The local bays, which have low hydrodynamics, receive large amounts of fresh water and urban effluents from rivers that flow through the urban zones, as in Caraguatatuba Bay, where there is traditional and intense harvesting of a bivalve mollusk, the clam *Tivela mactroides* (Born 1778). The "berbigão", as it is locally named, can be found buried in the surface sediment layers in the intertidal and shallow subtidal zones (Denadai *et al*. 2005) and is an important resource for recreation, subsistence and economic input for tourists and residents (Denadai et al. in press). In 2005, an estimated 27 tons of clams were consumed in Caraguatatuba Bay by around 590 families or 2,183 individuals (Denadai et al. in press).

This study addressed the food safety of the *T. mactroides* fishery in Caraguatatuba Bay, as a baseline to understand the eventual impacts of future coastal changes. We focused on the two most important threats, microbiological contamination due to sewage outfalls, and chemical toxicity due to potential oil spills. The spatial and temporal occurrence of pathogenic bacteria was evaluated, as well as the capacity of thermal treatment to reduce contamination to safe levels before consumption. We selected the bacteria that are traditionally considered indicative of fecal contamination (fecal coliforms; Huss 1997), that are historically recorded or have high pathogenicity in estuarine environments (*Staphylococcus aureus* and *Salmonella* sp.; Huss 1997), and that are typical of marine environments (the indigenous *Vibrio cholerae*; Huss 1997). Polycyclic Aromatic Hydrocarbons (PAHs) are important organic pollutants, mostly from anthropogenic (pyrolytic or petrogenic) activities (Woodhead *et al.* 1999), and merit special attention due to their potential negative effects and continuous input into the environment (Yunker *et al.* 2002). As PAHs may serve as indicators of oil spills in the region, and in order to improve understanding of their potential risks to humans, their concentrations were evaluated in different areas in Caraguatatuba Bay.

Methods

Study area and sampling procedure

The study was conducted in Caraguatatuba Bay on the northern coast of São Paulo state, Brazil, from 2003 through 2005. This bay is bounded by a 16 km-long sandy beach, which is crossed by four rivers that collect and transport continental effluents into the bay (Figure 1).

Initially, three collection expeditions were conducted, on March 30, 2003 and January 10 and February 26, 2004, to screen for the presence of the selected bacteria in clam meat. Six samples were collected in each period at different sites along the shore, in order to cover the clam harvesting area (Figure 1; points 1–6).

Subsequently to this pilot sampling, year-round samples were collected to determine the temporal variation in levels of bacterial contamination. Three new sampling sites (A, B, and C) were defined in the area where both clam harvesting intensity (Denadai et al. in press) and the preliminary results on contamination were highest (Figure 1). At each site, samples of beach clams were collected on April 22, May 19 and September 2, 2004, and December 2, 2005. The contamination levels for fecal coliforms and *S. aureus* were compared along the

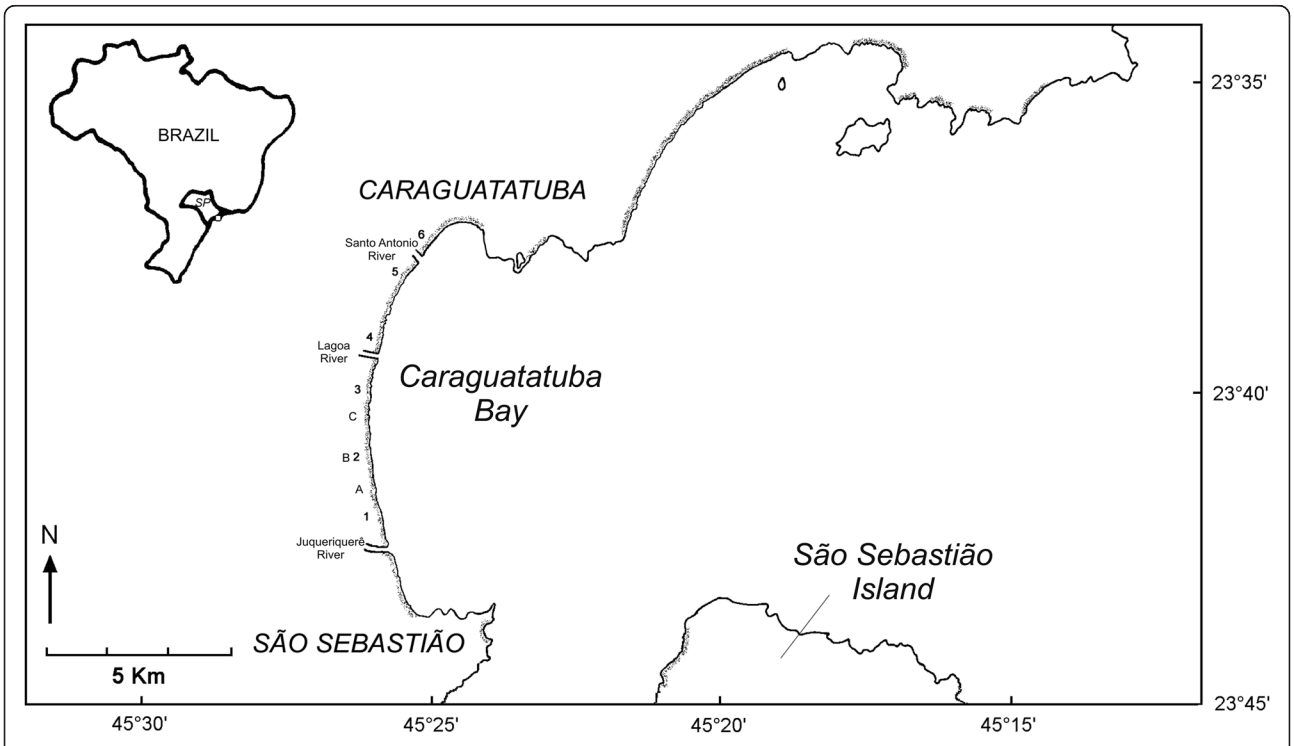

Figure 1 Map of Caraguatatuba Bay showing the sampling sites. Site 1 - 23°41′43.5″S 45°25′43.7″W; 2 - 23°40′53.5″S 45°25′49.9″W; 3 - 23°39′57.9″S 45°25′46.2″W; 4 - 23°39′12.9″S 45°25′36.0″W; 5 - 23°38′15.5″S 45°25′11.8″W; 6 - 23°37′48.4″S 45°24′52.9″W; Sample A - 23°41′26.8″S 45°25′47.4″W; B - 23°40′51.7″S 45°25′51.2″W; C - 23°40′04.6″S 45°25′49.0″W.

time series with a one-factor analysis of variance (ANOVA) followed by a Student–Newman–Keuls (SNK) test for pairwise comparisons (Underwood 1997). The results for *V. cholerae* and *Salmonella* sp. were not analyzed quantitatively, since the analysis is based on their detection (presence/absence). The mere presence of these bacteria makes seafood unsafe for human consumption, under current legislation (see below).

At the same time, we analyzed the potential effect of thermal processing in reducing the microbiological contamination. The samples from sites 2 and 5, collected on February 26, 2004, were analyzed before and after the heating procedure, which consisted basically of the caiçaras' (traditional coastal residents of southeastern Brazil) practice of cooking the clams in boiling water for 10 minutes (Denadai et al. in press).

Each sample for microbial-contamination analysis consisted of 25 g of clam meat, which is equivalent to eight individual clams approximately 30 mm in shell length. The clams were collected during low tide and immediately refrigerated (<10°C for 24 h). In the laboratory, the shells were cleaned with 70% ethanol in a laminar-flow hood and their soft tissues were extracted with a sterile spatula. This pooled sample size was based on the regulation of the Brazilian Health Surveillance Agency (ANVISA) for detection of *Salmonella* sp. (Brasil, Agência Nacional de Vigilância Sanitária 2001). The only specified regulation for raw samples established at the time of the sample collection was for *Salmonella* sp., requiring 25 g of clam meat. For this reason, we used 25-g samples for the analyses of all other contaminants.

We followed the methods of Downes and Ito (2001) for all microbiological analyses. The samples of clam meat were aseptically taken and homogenized with 225 mL of sterile 0.2% peptone water using a stomacher for 2 minutes. Samples were used for fecal coliforms analyses by Most Probable Number (MPN/g) method specified in ISO 21528–1:2004 (Thermo Fisher 2013). For the isolation of *Vibrio cholerae* we utilized TCBS Agar (HiMedia Laboratories, Mumbai, India) that was developed by Kobayashi *et al.* (1963). TCBS Agar is also recommended by the American Public Health Association (APHA) for the selective isolation of *V. cholerae* that shows yellow color colonies on the TCBS agar surface. For the isolation of *Salmonella* species we used HiCrome *Salmonella* Agar (Sigma-Aldrich, St. Louis, MO, USA), a selective medium used for simultaneous detection of *Escherichia coli* and *Salmonella* from food and water. *Escherichia coli* and *Salmonella* are distinguishable due to the colony characteristics since *Salmonella* give light purple colonies with halo. For the isolation of *Staphylococcus aureus* we used Baird-Parker Agar Base (BD Difco, Franklin Lakes, NJ, USA) with Egg Yolk Tellurite Enrichment in the preparation for selective isolation and enumeration (CFU/g; Colony-Forming Units) of coagulase-positive staphylococci from food. The typical colonies of *S. aureus* are black, shiny, convex and surrounded by clear zones of approximately 2–5 mm.

Concomitantly with the microbiological approach, we analyzed contamination by PAHs. For this baseline characterization, two samples of 200 g each of beach clams were collected on March 30, 2004. The first sample consisted of specimens from the northern part of the bay (sites 2 and 3) and the second sample of specimens from the southern part (sites 4 and 5) (Figure 1). The analyses were performed by CQA (Centro de Qualidade Analítica Ltda., Campinas, SP, Brazil) using methods 3550B and 8310 as recommended by the United States Environmental Protection Agency (EPA United States Environmental Protection Agency 1986, 1996). Clam tissues were dried and homogenized with sodium sulfate and extracted three times using an ultrasonic disruptor with a mixture of acetone/methylene chloride (1:1, v/v). The extract was purified in a silica gel chromatographic column, and PAHs were quantitatively analyzed by high-performance liquid chromatography (HPLC) coupled with fluorescence detection and excitation at 280 nm and emission at 389 nm. A reverse-phase column HC-ODS Sil-X, 5-micron particle size diameter, in a 250-mm × 2.6-mm I.D. stainless steel column was used in an isocratic elution for 5 min using acetonitrile/water (4:6)(v/v), then a linear gradient elution to 100% acetonitrile over 25 min at 0.5 mL/min flow rate. Certified PAH standards at five different concentrations were used to construct the analytical curve.

Results and discussion

The pilot study of microbiological contamination on March 30, 2003 and January 10 and February 26, 2004 showed that all the bacterial groups analyzed were present in the clam tissue (Table 1).

According to Brazilian regulations (Brasil, Agência Nacional de Vigilância Sanitária 2001), In order to be considered safe for consumption, clam tissues must contain no *Salmonella* sp. A single sample from each site was sufficient to detect *Salmonella* sp. in the samples collected on February 26, 2004. Its absence from the two samples collected in 2003 does not mean that the area was safe for shellfish harvesting at that time, since, according to Brasil, Agência Nacional de Vigilância Sanitária (2001) and FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization (2008), five samples from each site must be negative for an area to be considered free of *Salmonella* sp.

Brazilian legislation establishes the maximum acceptable levels of fecal coliforms as follows: for cooked bivalve mollusks, spiced or not, factory-chilled or frozen, the ANVISA established that for five samples of 200 g of

Table 1 Microbiological contamination of meat of the clam *Tivela mactroides* sampled in 2003–2004 along the shore of Caraguatatuba Bay, southeastern Brazil

Agents	Unit	Ref. val.		March 30, 2003						January 10, 2004						February 26, 2004					
				Samples						Samples						Samples					
		Brasil, Agência Nacional de Vigilância Sanitária (2001)	FAO/WHO Food and Agriculture Organization of United Nations/ World Health Organization (2008)	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Fecal coliforms	MPN/g	50	700	920	170	110	8	13	8	460	14	2400	110	43	11	43	≥2400	1100	93	9	460
Staphylococcus aureus	CFU/g	1000		-	-	1700	3000	-	-	-	-	-	-	-	-	7000	12000	57000	3500	4000	5000
Salmonella sp.		Abs.		-	-	Abs.	Abs.	-	-	-	-	-	-	-	-	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.
Vibrio cholerae		Abs.		-	-	-	-	-	-	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.

Ref. Val. = maximum reference limits for human consumption, MPN = most probable number, CFU = colony-forming units, Abs. = absence, Pres. = presence, – = No data.

the edible part, none of the samples may contain more than 50 MPN/g coliforms at 45°C and only two samples may contain between 10 and 50 MPN/g (Brasil, Agência Nacional de Vigilância Sanitária 2001). In 2008, after our sampling and processing had been completed, the FAO/WHO Commission established a regulation in the CODEX Alimentarius for live or raw bivalve mollusks in which, for five 100 g samples of edible parts, none may contain more than 700 *Escherichia coli* and no more than one sample may contain between 230 and 700 *E. coli*. However, the maximum acceptable levels of the CODEX Alimentarius were defined specifically for *E. coli* because it is a broader indicator of fecal contamination. Because our study evaluated fecal coliforms as a whole (not specifically *E. coli*) and also because we used 25 g of fresh meat, instead of the non-fresh 100/200 g mentioned in the previous resolutions, we decided to present as a reference limit for interpreting the results for fecal coliforms, both the values of Brasil, Agência Nacional de Vigilância Sanitária (2001) and also the values of the CODEX Alimentarius (FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008). Even though our pooled sample size was smaller than the ANVISA recommendation of 200 g for fecal-coliform analysis, the samples with 25 g were sufficient to detect a high level of contamination. On March 30, 2003, fecal coliform counts above the reference limits were recorded in three (according to Brasil, Agência Nacional de Vigilância Sanitária 2001) and one (according to FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008) of six samples. On January 10, 2004, in the summer vacation period, fecal coliform counts above the reference limits were recorded in three (Brasil, Agência Nacional de Vigilância Sanitária 2001) and one (FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008) of six samples. On February 26, 2004, just after the summer vacation period of 2004, fecal coliforms showed levels higher than reference values in four (Brasil, Agência Nacional de Vigilância Sanitária 2001) and two (FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008) of the six sites.

For raw, chilled, or frozen bivalve mollusks that will not be consumed raw, the maximum acceptable level of *Staphylococcus*-positive coagulase (= *S. aureus*) is 10^3 CFU/g (Brasil, Agência Nacional de Vigilância Sanitária 2001, FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2012). In March 30, 2003, counts of *S. aureus* above the limits were recorded in each of two samples analyzed, and in February 26, 2004, *S. aureus* occurred above the limits at all six sites.

For *V. cholerae*, no reference values were found in the current legislation, so we considered absence as an acceptable quality indicator. *V. cholerae* was recorded in all samples collected in January 10, 2004 and February 26, 2004.

The most-contaminated sites in all periods (1 to 4) were those that were most used by clammers (Denadai et al. in press). Therefore, a new and more-detailed sampling strategy was implemented in the southern part of the study area, in four different periods year-round, with three samples (sites) per period.

In this new sampling series, *V. cholerae* and *Salmonella* sp. were present in all 12 samples analyzed (Table 2). The level of contamination with fecal coliforms varied among sampling periods (ANOVA, $F = 524.46$, $df = 3$, $p < 0.001$), with the highest values recorded on 22 April, 2004 (SNK, $p < 0.001$ for all comparisons) (Table 2), during a long national holiday, when the population increased significantly along the coast. Fecal-coliform contamination levels exceeded the reference limits in six (Brasil, Agência Nacional de Vigilância Sanitária 2001) and three (FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008) of the 12 samples. *S. aureus* did not show significant temporal variation (ANOVA, $F = 2.75$, $df = 3$, $p = 0.113$), but the highest individual values (3,900 $CFU.g^{-1}$) were found in April 2004, and the lowest (300 $CFU.g^{-1}$) in February 2005. Contamination of *S. aureus* above the limits was found in six of the 12 samples, and only the samples taken on February 16, 2005 showed no value above the limits.

Although no published records of beach-clam contamination in Brazil were found, contamination with pathogenic bacteria has been an important factor in Brazilian mariculture. Studies with cultivated mollusks have revealed considerable levels of contamination. Pereira *et al*. (2006) detected the frequent presence of fecal coliforms in the cultivated oyster *Crassostrea gigas* sold in markets in the state of Santa Catarina. *E. coli* was present in 9% of the cultivated samples and in 35.5% of the market samples, and although Pereira *et al*. (2006) did not detect *Salmonella* sp. or *Vibrio* sp., *S. aureus* was present in only one sample, with 80 CFU/g, while the remaining samples contained <10 CFU/g. On the Ceará coast, Vieira *et al*. (2008) found that contamination of the cultivation water by fecal coliforms remained within the allowed limits. Only one of 15 samples showed high levels of total coliforms (2,800 MPN/100 mL) and thermotolerant coliforms (3,500 MPN/100 mL). These results indicate contamination of the beach clams from Caraguatatuba Bay, since the frequency and the absolute scores for fecal-coliform contamination were higher than those found by other investigators in cultivated mollusks (Pereira *et al.*

Table 2 Microbiological contamination of meat of the clam *Tivela mactroides* sampled in 2004–2005 along the shore of Caraguatatuba Bay, southeastern Brazil

Agents	Unit	Ref. val.		April 22, 2004			May 19, 2004			September 2, 2004			February 16, 2005		
		Brasil, Agência Nacional de Vigilância Sanitária (2001)	FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization (2008)	Sampling sites			Sampling sites			Sampling sites			Sampling sites		
				A	B	C	A	B	C	A	B	C	A	B	C
Fecal coliforms	MPN/g	50	700	≥2400	≥2400	≥2400	210	240	15	9	4	9	240	39	9
Staphylococcus aureus	CFU/g	1000		3500	610	3900	2400	1500	500	1400	1200	800	300	300	300
Salmonella sp.		Abs.		Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.
Vibrio cholerae		Abs.		Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.	Pres.

Ref. Val. = maximum reference limits for human consumption, MPN = most probable number, CFU = colony-forming units, Abs. = absence, Pres. = presence.

Table 3 Microbiological contamination of meat from the clam *Tivela mactroides,* before and after thermal processing, sampled in Caraguatatuba Bay, southeastern Brazil

Agents	Unit	Ref. val.		Thermal processing			
		Brasil, Agência Nacional de Vigilância Sanitária (2001)	FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization (2008)	Site 2		Site 5	
				Before	After	Before	After
Fecal coliforms	MPN/g	50	700	≥2400	<3	9	9
Staphylococcus aureus	CFU/g	1000		12000	Abs.	4000	Abs.
Salmonella sp.		Abs.		Pres.	Abs.	Pres.	Abs.
Vibrio cholerae		Abs.		Pres.	Abs.	Pres.	Pres.

Ref. Val. = maximum reference limits for human consumption, MPN = most probable number, CFU = colony-forming units, Abs. = absence, Pres. = presence.

2006; Vieira *et al*. 2008). We consider that the different factors such as the physical structure of the embayment, with its low hydrodynamics, and the large and diffuse sewage runoff in the region are responsible for these findings.

According to FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization (2008), environmental parameters such as temperature and salinity are not predictive for *Salmonella* sp. contamination in areas of mollusk harvesting, and data that could quantify the probability of growth of this bacterium in mollusk tissues after harvesting are not available. A relationship exists between the fecal coliform concentration in the tissues and the probability of *Salmonella* sp. presence, but may vary according to the location.

Brazilian legislation mandates, for the natural or intensive farming of species destined for human consumption and that will be consumed raw, that the mean concentration of fecal coliforms in the water must not exceed 14 MPN/100 mL, and 10% of the samples must not exceed 43 MPN/100 mL (CONAMA Resolution No. 20/1986) (Brasil, Conselho Nacional de Meio Ambiente 1986). The beaches of Caraguatatuba Bay, monitored by the Companhia de Tecnologia de Saneamento Ambiental (CETESB), had a mean concentration of 81.8 MPN/100 mL during the study period (Paulo 2005a). Only 24% of the 260 water samples analyzed in 2004 had fecal coliform levels below 14 MPN/100 mL, and 48.5% had levels above 43 MPN/100 mL (Paulo 2005a). These data agree with the bacterial levels found in the beach-clam meat, and indicate that the sanitation conditions of the bay waters represent a high risk for harvesters and consumers of clams.

The most contaminated samples of clam meat were from the central part of the bay, where clamming is most common (Paulo 2005a, Denadai et al. in press). The data suggest an effect of summer holiday periods, when the increase in population may overload the inadequate sewage system. As rain and thus continental drainage (including non-point pollution sources) may increase the input of microbial contaminants, and as tourism is higher in summer months, which is the rainy season in southeastern Brazil, this period is considered the most risky, when clam collecting and consumption should be avoided and communication of this potential hazard to the public should be intensified.

We also applied the thermal treatment commonly used by locals (10 min in boiling water) to two of the samples. Even though this procedure was not conducted to formally evaluate the thermal treatment effectiveness, the results demonstrated that this cooking time totally eliminated *S. aureus* and *Salmonella* sp. and reduced fecal coliforms to acceptable levels in both samples (Brasil, Agência Nacional de Vigilância Sanitária 2001; FAO/WHO Food and Agriculture Organization of United Nations/World Health Organization 2008). However, this cooking method did not eliminate *V. cholerae* in one of the samples (Table 3). This indicates that even though *V. cholerae* is very sensitive to high temperatures (D_{50} of 0.30 min at 71°C) (Shultz *et al*. 1984), the simple boiling procedure may not suffice to totally eliminate the microorganism. Makukutu and Guthrie (1986) found that *V. cholerae* can survive and remain viable after one hour exposed to 60°C.

The incomplete elimination of fecal coliforms can be explained by their known high heat tolerance (D_{50} of 45 min at 60°C) (Padhye and Doyle 1992). *Salmonella* sp. and *S. aureus* are more sensitive to heat, with D_{50} of 10 min at 60°C (Thomas *et al*. 1966) and 3 min at 55°C (Halpin-Dohnalek and Marth 1989). Even though *S. aureus* was completely eliminated, the toxins produced by this species are very heat-resistant (D_{50} of 68.5 min at 98.9°C) (Read and Bradshaw 1966) and might not be completely eliminated by cooking, thus posing an additional threat to consumers.

Brazilian legislation has no established criteria for the maximum PAHs allowed in foods. We used the limit from European Regulation 1881/2006/EC (ER) of 10 μg kg^{-1} dw in bivalve mollusks for benzo[a]pyrene, used as a reference for toxicity of individual PAHs because it is one of the most toxic compounds in this group (Wenzl *et al.* 2006). Only low-molecular-weight compounds (2 or 3 fused aromatic rings), such as naphthalene, acenaphthylene, anthracene, fluorene and phenanthrene were found (Table 4). They are more

Table 4 Concentrations of PAHs in meat from the clam *Tivela mactroides* sampled in Caraguatatuba Bay, southeastern Brazil

Compounds	South area (μg/kg)	North area (μg/kg)
Naphthalene	1.90	1.80
Acenaphthylene	0.20	0.20
Fluorene	0.40	<0.01
Anthracene	0.90	<0.01
Pyrene	<0.01	<0.01
Benz[a]anthracene	<0.01	<0.01
Chrysene	<0.01	<0.01
Benzo[k]fluoranthene	<0.01	<0.01
Benzo[a]pyrene	<0.005	<0.005
Dibenzo[a,h]anthracene	<0.01	<0.01
Benzo[g,h,i,]perylene	<0.01	<0.01
Indeno[1,2,3]pyrene	<0.01	<0.01
Phenanthrene	1.00	1.40
Fluoranthene	<0.01	<0.01
Total PAH	4.40	3.40

soluble in water, and their presence, even in relatively low concentrations, can be indicative of petroleum-derivative inputs at the two sampling sites (Villeneuve *et al*. 1999). Based on the limit in the European Regulation (Wenzl *et al.* 2006), no compounds in this study, even the total PAHs, showed concentrations above 10 μg kg^{-1} dw, and thus would not put consumers at risk. Particularly in view of the single sampling point and also the small sample size, the presence of oil compounds in the clam meat indicates the presence of pollution in the region, which is threatened by the presence of petroleum industry facilities and activities. As these compounds are bioaccumulated, it is important to analyze their levels periodically in clams over 25 mm, which require seven to ten months to reach this size, depending on the year (Turra *et al*. 2014); as well as after oil spills in the region, to assess the possible risks to consumers.

Conclusions

With the sample size and strategy employed here it was not possible to estimate the true prevalence of contamination in Caraguatatuba Bay, but it was possible to detect the presence of the pathogenic agents and contaminants investigated. The results provide evidence that in the 2003–2005 period, the health conditions of Caraguatatuba Bay were unsatisfactory, causing bacterial contamination of the clam meat, including at higher levels than allowed under Brazilian health regulations and possibly related to the sites that are most used by clammers. Complementarily, recent reports on bathing conditions in Caraguatatuba Bay have shown no improvement in water quality since the study period.

Based on this case study of *T. mactroides*, socioecological systems based on beach-clam fisheries, which have recreational, subsistence and economic importance, may be considered at high risk worldwide. Education about public health and adequate sewage treatment must be provided by the public sector, to guarantee safer consumption and environmental health. Thus, in addition to the education measures required to adequately inform the general public about the risks of consuming clams and the best way to prepare them in order to reduce or eliminate possible infectious agents, additional efforts are needed to reduce the risks of consuming non-regulated seafood. Extensive expansion of the sewage system is urgent in order to reduce the runoff of household waste, as well as to improve sewage treatment for safe disposal in the sea. This requires attention from the public sector and investment in sanitation facilities and equipment, mainly due to the recent Caraguatatuba population increase (19% between 2004 and 2013; São Paulo 2013) and the expected future increase due to the different industrial projects that are being implemented in the region. During this period (2004–2013) the sanitation conditions of the beaches and rivers entering Caraguatatuba Bay oscillated, with recent worsening of water quality (Paulo 2013); from 2011 to 2013, the water quality was within acceptable limits ≤ 25% of the time. A report by CETESB on thermotolerant coliforms, *E. coli*, and enterococcus, indicated that the beaches in Caraguatatuba Bay were classified as acceptable (Pan Brasil, Palmeiras, and Porto Novo), poor (Centro) or very poor (Indaiá). Considering that the criteria used to classify water quality for bathing are less stringent than those used to classify zones for food production, the results of this study are probably consistent with the current situation in Caraguatatuba Bay. In addition to the various threats to the habitats and stocks of the clams themselves, increasing concern regarding food safety may lead to interdictions on clamming, which is a very important economic activity for low-income fishing communities in developing countries.

The development of a regulated surveillance program based on continuous monitoring of both biological and chemical contaminants in the water (continuously evaluated by CETESB) and in *T. mactroides* is strongly recommended to detect possible risks to humans who consume these clams. Beach-clam fisheries appear to be an important socioecological indicator of coastal resilience and health, and should be incorporated into future assessments of ocean quality.

Abbreviations

ANOVA: Analysis of variance; ANVISA: Agência Nacional de Vigilância Sanitária; CETESB: Companhia de Tecnologia de Saneamento Ambiental; CFU: Colony-forming units; MPN: Most probable number; PAHs: Polycyclic Aromatic Hydrocarbons; SNK: Student–Newman–Keuls.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MRD planed the sample and analytical strategies, carried out the samples, analyzed the data and revised the manuscript in all the phases. DFCJ carried out the microbiological analysis and revised the manuscript. IF prepared the final version of the manuscript and incorporated the reviewers suggestions. ST prepared the text on HPA contamination and revised the manuscript. AT planed the sample and analytical strategies, carried out the samples, analyzed the data and revised the manuscript in all the phases. All authors read and approved the final manuscript.

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