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Variation of pH, salinity and temperature for the establishment of *Vibrio cholerae* in the city of Rio de Janeiro (Brazil)

Variação de pH, salinidade e temperatura viabilizantes para estabelecimento do *Vibrio cholerae* nas águas portuárias da cidade do Rio de Janeiro, Brasil

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ABSTRACT

Introduction: The toxigenic *Vibrio cholerae*, which causes Cholera, have a defined environmental tolerance regarding pH, temperature and salinity which does not prevent it from entering viable-but-nonculturable (VBNC) state, when the ranges of these parameters are not favorable. Port areas are vulnerable places for pathogen introduction, due to the contribution of urban effluents and the disposal of ballast water. **Objective:** The work evaluated whether the port of Rio de Janeiro present pH, salinity and temperature conditions that allow the microorganism establishment. **Method:** Twenty-two monitoring campaigns were carried out in 9 points, from March 2017 to February 2018, always searching for different climatic and tidal conditions. **Results:** The tide had a slight influence on the salinity, while the climate interacted with all the factors. The temperature and pH remained within the optimal range for the pathogen establishment, while the salinity was outside the ideal range most of the times. **Conclusions:** Analyzing only these factors, we can suggest that, if these standards are maintained, the region could present a risk of the microorganism survival.

KEYWORDS: Invasive Species; Port Water; *Vibrio cholerae*; Ballast Water

RESUMO

Introdução: O *Vibrio cholerae* toxigênico, causador da cólera, possui tolerância ambiental definida quanto ao pH, temperatura e salinidade, o que não o impede de entrar em estado viável, mas não cultivável (VNC), quando as faixas destes parâmetros não lhe são propícias. As zonas portuárias são áreas vulneráveis de introdução do patógeno, devido ao aporte de efluentes urbanos e descarte de água de lastro. **Objetivo:** O trabalho avaliou se as águas portuárias da cidade do Rio de Janeiro apresentam condições de pH, salinidade e temperatura que possibilitam o estabelecimento do microrganismo. **Método:** Foram realizadas 22 campanhas de monitoramento em nove pontos, no período de março de 2017 a fevereiro de 2018, buscando sempre diferentes condições climáticas e de maré. **Resultados:** A maré apresentou ligeira influência na salinidade, enquanto o clima interagiu com todos os fatores. A temperatura e o pH se mantiveram dentro da faixa considerada ótima para o estabelecimento do patógeno, enquanto a salinidade se apresentou, na maioria das vezes, fora da variação ideal. **Conclusões:** Analisando somente estes fatores, podemos sugerir que, caso estes padrões se mantenham, a região apresenta um risco de sobrevida do microrganismo.

PALAVRAS-CHAVE: Espécie Invasora; Água Portuária; *Vibrio cholerae*; Água de Lastro

INTRODUCTION

Over the course of human history, diseases have always been closely related to questions of life in society and the environment[1]. In this way, the causality of harm goes beyond mechanistic, descriptive and analytical analysis, based on causes and linearity of the classical host-parasite interaction medicine[2], and acquires a much more complex and multifactor perspective of reality, involving social and environmental conditions in which this construction is directly linked to the current political context[3]. This perspective goes opposite ways to a health policy based on the correction of harm and makes way to a broader, prevention-centered perspective: Health Surveillance that monitors and prepares contingency measures in cases of health problems[4].

Attention to diseases, mainly the infectious ones, should not only be directed to corrective actions, but also to preventive practices and improvement in social and environmental issues. Cholera clearly fits into this scenario, since it is a neglected disease related to basic sanitation[5]. This disease presents severe enteric symptoms and is caused by the toxigenic strains of the O1 and O139 serogroups of *Vibrio cholerae*. According to data from the World Health Organization[6] and the World Bank[7], there is a negative relation between the HDI/basic sanitation access indicators and its occurrence, especially in Africa.

The organisms of the Vibrionacea family are abundant in aquatic environments, either suspended in the water column as free living organisms or parasitizing plankton or the tissue of several marine organisms[8,9]. They play the ecological role of degrading the chitin present in the carapace of most zooplankton organisms abundant in port areas, and may be pathogenic for both invertebrates and vertebrates[10].

Chitin, present in these hosts, may protect against the acids of the gastric juice of the digestive tract of a human feeding on crustaceans[11] and cause greater microbial resistance to chemical agents like chlorine and aluminum salts[8].

Some bacteria, like *V. cholerae*, can survive in unfavorable environments for some time in, for example, the *viable but nonculturable* (VBNC) state, which is a phenomenon of adaptation to the environment, where factors like salinity, temperature and pH can influence the process[12]. According to Mai et al.[13], in laboratory studies, cells in the VBNC state may remain viable for years and are usually found on the surface of copepods; however, when in contact with the human intestine, they pass on to the viable state (VC).

The organism has well defined environmental tolerance. Miller et al.[14] suggest that environments with salinity between 0.25‰ and 3‰, typically hot, with temperatures exceeding 5° C for long periods and pH between 7 and 9, are likely natural reservoirs of *V. cholerae* O1, producer of cholera toxin. Huq et al.[15] examined strains under alternating conditions of salinity, temperature and pH onsite and indicated an ideal salinity range of up to 15% (with a higher tolerance - about 20% - when associated with copepods) and temperatures above 30° C. However, Silva et al.[5] identified non-toxigenic strains in Guanabara Bay waters (city of Rio de Janeiro, Brazil) at salinity conditions above 25%, temperatures between 20° C to 30° C and pH ranging from 7 to 9. In estuarine environments, these parameters may vary depending on the sediment input, climate and tide.

According to Gerolomo and Penna[16], until 1991, cholera was not a problem in Brazil. However, the epidemic plagued the country. It originated from the seventh pandemic that began in 1961, whose epicenter was in Indonesia and spread to other countries of Asia, Middle East, Africa and Europe, with occasional cases in the United States since 1970. This epidemic reached South America through the coast of Peru in 1991 and, later on, fourteen other Latin American countries, including Brazil, where it entered through the Amazonian region of Alto Solimões and progressively spread over the North Region[17].

In Brazil, the first cases related to this event were recorded in April 1991 in the cities of Benjamin Constant and Tabatinga (state of Amazonas)[18]. At the time, two distinct strains of the *El tor* serotype were identified, as well as nontoxigenic, but pathogenic, variant forms of the Amazonian strain[19].

After its introduction, the disease became an outbreak in the Northeast Region, mainly due to poor basic sanitation conditions and quality of life of the population[18].

Like any large body of water, the Guanabara Bay is subject to tides, which are the main force for the circulation of water in the region. Thus, they influence the renewal of the bay water. The tide there is mixed. There is a semidiurnal predominance (two high and two low tides during the day) and diurnal variations, with a high and a low tide during the day[20]. As the tide rises, seawater enters and increases the salinity of the water of the bay. As the tide lowers, the salinity decreases and reaches its lowest values. This shows the interference of the tides on the salinity[21].

The entrance of seawater coming from the Atlantic Ocean into the Guanabara Bay reaches first the east margin and propagates preferentially with the same orientation of the navigation channel, where the largest amplitudes are observed in the inner eastern portion of the bay. This process brings changes not only to the vertical oscillation of the water level but also to physical and chemical conditions, according to the tidal regime[20].

In addition to marine influences, the Guanabara Bay also receives fresh water from rivers belonging to its watershed, with an average of 200 thousand liters/sec[21, 22, 23]. There are about 55 rivers that flow toward it, the most important of which are Iguaçu, Caceribu, Macacu, Guapimirim, Estrela, Sarapuí and São João de Meriti[24]. This influence of fresh water reduces the average salinity of the ocean, which may facilitate the permanence of the vibrio[5].

According to Valentin et al.[25], the salinity of the Guanabara Bay is lower in the internal areas due to the interference of the continental effluents (increased during the rainy season). However, there is a positive relation with depth due to the input of water from the continental shelf during high tides, which causes a strong vertical stratification in the water column. The same authors also showed that for the temperature, the pattern is the opposite and varies according to the seasons of the year.

Ports are located in sheltered coastal areas, often estuaries that have high biological productivity most of the times and are threatened by the intense influx of sediment from surrounding urban areas. This requires periodic dredging, cleaning, clearing, removal, dragging and excavation of sediment[26], which in turn end up causing serious environmental problems, like changes in the water flow and in the hydraulic, sedimentological, chemical, physical and biological properties of the site[27]. Therefore, intense and constant movements done by the port activity disrupt the environment and require more effective port management mechanisms[26].

As trade demand increases and, with that, the need to transport goods, naval transportation stands out as the main mean for this purpose. Without shipping, intercontinental trade, bulk transportation of raw materials, imports and exports of affordable food and manufactured goods would simply not be possible. Ships are responsible for about 90% of all world commercial transportation and carry around 600 million tons/year[28].

Consequently, with increased sea traffic, the movement of ballast water also increases. Ballast water is stored in tanks throughout the vessel to improve its stability, balance and hull penetration in the water, thus making navigation safer[29].

Every year, about ten billion cubic meters of ballast water are transported around the world. About 7,000 species are carried daily[30] between large (continents) and small distances within the same country, for example[31].

Ballast water is considered worldwide as the main way through which aquatic species, both animal and plant, are transported between coastal environments[32]. Here, we highlight *V. cholerae*[33] favoring antibiotics resistance[34], horizontal gene transfer[35] and the formation of cells in a viable but nonculturable state[36].

Rio de Janeiro port is located in the Guanabara Bay coastal region, one of the most important regions of the Brazilian coast. This region is very important for Brazilian economy because of its high fishing activity and intense port movement[37]. The water of the region has a history of intense environmental degradation due to frequent earth filling, drainage and reception of urban effluents, thus making the system prone to harbor pathogenic microorganisms[5,38,39]. This water can affect human populations who use it for recreation, eating or drinking[40].

Regarding the port, in the first half of 2017, about 2.7 million tons were transported (an increase of 9.4% compared to the same period of the previous year). About 5% of the vessels in this period came from places with cholera from 2010 to 2017[6].

Routine monitoring of environmental factors that are important for the survival and presence of the organism itself is a primary measure for establishing an active surveillance process that results in a network system that is prepared for a possible cholera emergency.

According to the "Technical guidelines for environmental monitoring of *Vibrio cholerae*" of the Brazilian Ministry of Health[41], cholera monitoring and prevention measures aim to: (i) map the sites with identification, isolation and detection of *V. cholerae* O1 and O139 in the environment; (ii) identify and monitor areas vulnerable to disease transmission and (iii) subsidize epidemiological surveillance actions in risk areas.

Accordingly, this work aims to detect whether the variation of pH, salinity and temperature of the port area of Rio de Janeiro is within the ideal ranges of survival of the organism, in case it is introduced in the study site.

METHODS

We monitored the region in 22 campaigns from March 2017 to February 2018. During this period we sought the greatest alternation between tide and climatic conditions (Table). We chose the sites P1 (22° 51' 54.55" S/43° 12' 33.70" W/average depth: 8 m) and P3 (22° 53' 49.04" S/43° 12' 36.04" W/average depth: 5 m) because there is urban effluents outflow there; P2 (22° 53' 20.84" S/43° 11' 37.90" W/average depth: 12 m), P4 (22° 53' 20.84" S/43° 11' 37.90" W/average depth: 8 m), P5 (22° 52' 49.91" S/43° 12' 16.83" W/average depth: 10 m) and P9 (22° 53› 14.53» S/43° 12› 44.80» W/average depth: 9 m) because they cover all the port area; P8 (22° 49' 52.41" S/43° 9' 5.95" W/average depth: 15 m) because it is an external anchoring area; P7 (22° 52' 6.61" S/43° 9' 20.69" W/average depth: 14 m) because it is a sea route area and P6 (22° 53' 21.50" S/43° 8' 22.50" W/average depth: 12 m) to close a quadrant of Guanabara Bay (Figure 1).

We collected the samples using Niskin bottles, at a depth of 1 meter from the surface and 1 meter from the substrate. Then we measured pH, temperature (Multiprocessed pH meter AT-315 ALFAKIT®) and salinity (Conductivity meter 8306 AZ®). We statistically and graphically analyzed the data with spreadsheet editor software (Microsoft Excel 2007®). For the comparison of the parameters with the tide, we classified the data by tide type (flood or ebb) and adopted the minimum and maximum values of each parameter detected (per site/monitoring). We compared the data from the surface and the bottom in every parameter and site.

RESULTS AND DISCUSSION

During the period of this study, we noticed a small influence of the tide regime on the monitored factors, thus indicating well defined characteristics about the maximum and minimum

Table. Dates of the monitoring campaigns.

Camp	Date	Temp	RH	TV	Weather conditions	Wind
C01	20.03.2016	***	***	0.4-1.1	Sunny	***
C02	02.05.2016	***	***	0.4-1.0	Cloudy	NE 1.6km/h
C03	24.06.2016	18° C-21° C	82%	1.1-0.3	Partially cloudy	SW 3.3 km/h
C04	22.07.2016	16° C-21° C	75%	1.1-0.2	Partially cloudy	NE 9 km/h
C05	30.08.2016	20° C-29° C	62%	0.3-0.9	Partially cloudy	SW 1.1 km/h
C06	29.09.2016	15° C-31° C	49%	1.9-0.2	Partially cloudy	SW 11 km/h
C07	21.10.2016	22° C-28° C	81%	0.6-0.8	Partially cloudy	SW 12 km/h
C08	23.11.2016	22° C-26° C	65%	1.2-0.4	Cloudy	ESE 2.9 km/h
C09	21.12.2016	27° C-37° C	49%	0.2-0.8	Sunny	***
C10	23.01.2017	22° C-35° C	53%	0.5-0.4	Partially cloudy	NNE 10km/h
C11	22.02.2017	20° C-37° C	32%	0.4-1.0	Cloudy	NE 8.7 km/h
C12	21.03.2017	21° C-27° C	71%	0.8-0.6	Rainy	W 11 km/h
C13	26.04.2017	21° C-34° C	88%	0.3-0.9	Partially cloudy	NNE 10.2 km/h
C14	24.05.2017	18° C-26° C	94%	0.1-1.1	Partially cloudy	NE 8.3 km/h
C15	21.06.2017	17° C-21° C	70%	0.2-1.2	Cloudy	ESE 2.5 km/h
C16	20.06.2017	12° C-23° C	94%	0.1-1.1	Rainy	NE 5 km/h
C17	19.09.2017	20° C-30° C	82%	1.2-0.0	Cloudy	N 10 km/h
C18	25.10.2017	18° C-30° C	93%	1.0-0.6	Cloudy	E 6.2 km/h
C19	28.11.2017	15° C-26° C	96%	0.3-1.0	Cloudy	S 6.9 km/h
C20	20.12.2017	21° C-36° C	98%	1.2-0.4	Cloudy	SE 8 km/h
C21	30.01.2018	22° C-27° C	89%	0.8-0.7	Cloudy	SW 6.7 km/h
C22	28.02.2018	24° C-29° C	74%	0.3-1.0	Sunny	N 3.2 km/h

Camp: campaign; Temp: minimum and maximum temperature of the day; RH: relative air humidity: TV: tide variation from start to end of the campaign; Wind: with direction and speed; SW: southwest; ESE: east-southeast; NNE: north-northeast; NE: northeast; W: west; N: north; E: east; S: south and SE: southeast.
(***) Data not collected

values of these elements. However, we found a variation of these parameters with relative air humidity, especially when it rained during the days before the monitoring.

The temperature (Figure 2) ranged from 20° C to 33° C, the ideal conditions for the organism. We recorded small variations between surface and bottom conditions during the monitoring. We found higher values between the samples eight to 15 (November 2016 to June 2017). We noticed a rising trend starting in sample 17 (September 2017).

As for pH, it also remained within the ideal range (between 7‰ and 9‰) during the monitoring. We found few variations between the values found at the surface and at the bottom (Figure 3).

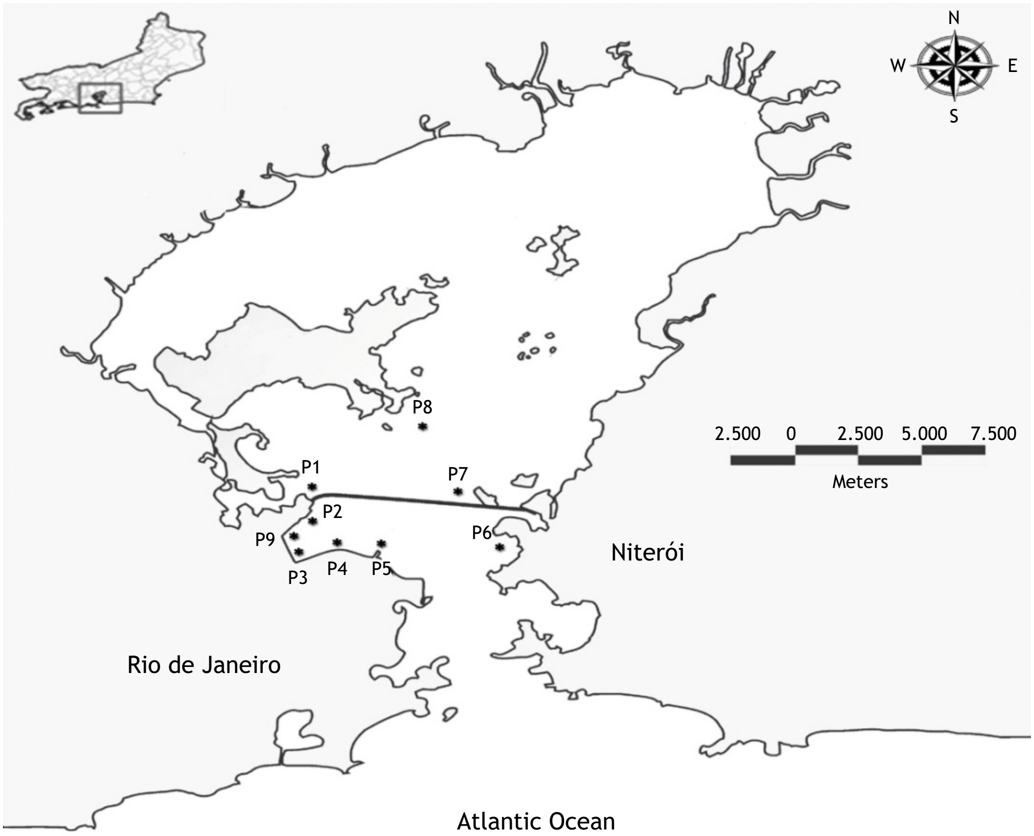
Salinity was the only factor outside the ideal range (always above, with the exception of surface samples of the campaigns 21 and 22). We found significant differences between depths and collections: salinity drops after rainy days and ebb tides (Figure 4), in line with the results obtained by Kjerfve et al.[42] and Ribeiro and Kjerfve[43], which indicate values from 20 to 35 ups. The variation ranged from 17.7% to 35% and showed a declining trend starting in campaign 20 (December 2017 to February 2018).

Although the organism has a well defined environmental tolerance regarding the analyzed parameters, Rivera et al.[44] isolated O1 and non-O1 strains in samples of ballast tanks with salinity higher than 30‰, which shows a greater resistance of the organism to environmental limitations.

In addition to the living chitinous reservoirs, abundant in the port region, other important reservoirs are the chitinous substrates forming the so-called benthic biofilms. This is an ecological advantage, since in these environments there is a great amount of nutrients, enabling them to survive for long periods[45], providing protection and nutrients[46]. It also favors their lifecycle, especially in estuarine environments, where nutrients can emerge due to port dredging processes[47].

Knowledge of water dynamics of a coastal environment is also essential for the management of urban structures, like the port of Rio de Janeiro. This knowledge helps to design measures that aim to minimize the environmental and social impact on this environment.

Since the region receives a lot of urban effluents, seawater inflow and vessel traffic, which interact directly with these



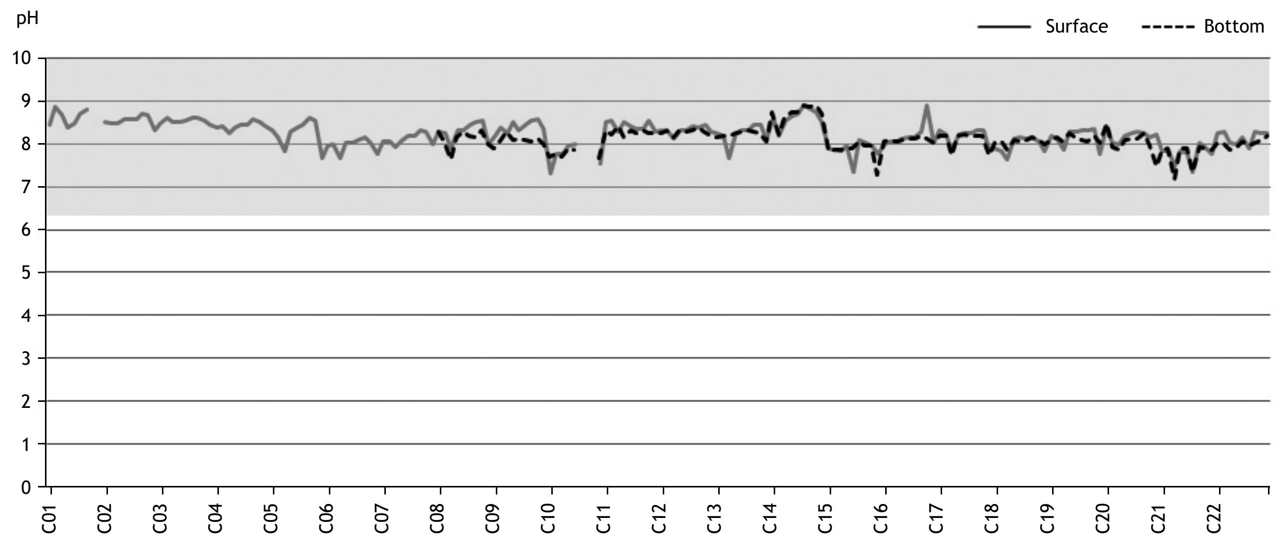
Source: Author.

Figure 1. Location of monitoring points: Guanabara Bay (Rio de Janeiro, Brazil).

Source: Author.

Figure 2. Temperature variation (°C) in the sites/campaigns: surface and bottom. The bottom sampling only started in campaign 8 (C08). In campaign 1 (C01) we did not collect data at sites 8 and 9. The hachured area shows the ideal variation for the survival of the vibrio.

factors, permanent monitoring becomes an important and indispensable tool for the preparation of measures to prevent the introduction of *V. cholerae* and, thus, future outbreaks of the disease, ensuring human safety.



Source: Author.

Figure 3. pH variation (%) in the sites/campaigns. The bottom sampling only started in campaign 8 (C08). In campaign 1 (C01) we did not collect data at sites 8 and 9. We also did not collect data at sites 6, 7 and 8 in campaign 10 (C10). The hachured area shows the ideal variation for the survival of the vibrio.

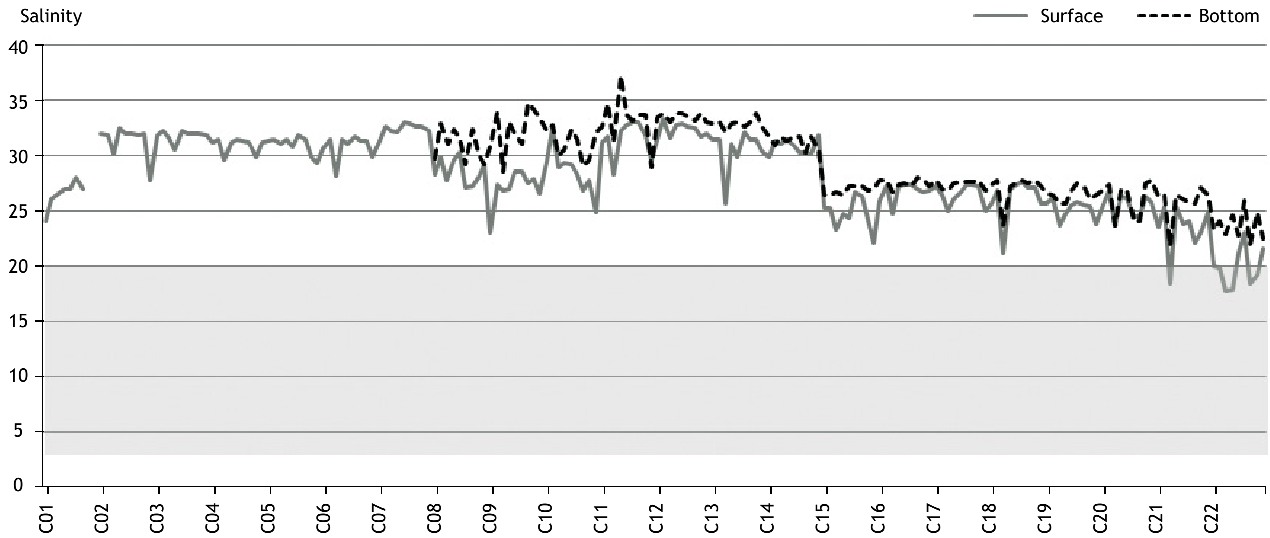


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Figure 4. Salinity variation (%) in the sites/collections: surface and bottom. The bottom sampling only started in campaign 8 (C08). In campaign 1 (C01) we did not collect data at sites 8 and 9. The hachured area shows the ideal variation for the survival of the vibrio.

CONCLUSIONS

Our data (pH, salinity and temperature) indicate that there is a risk of successful establishment of the organism if it is introduced via ballast water, with possible human health problems.

The results show a concern in other Brazilian port regions, which present the analyzed factors closer to the ideal range (mainly places with low salinity). This fact requires greater attention, since maritime transport (cabotage) accounts for about 90% of all goods movement in Brazil.

For this reason, more detailed studies that recognize and monitor all the important factors are necessary as primary measures to design public policies to prevent and control future disease outbreaks in Brazil.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.