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Bioaccumulation and depuration of *Escherichia coli* in the tropical clam *Anomalocardia brasiliana* at different salinities

[*Bioacumulação e depuração de* Escherichia coli *no molusco de areia Anomalocardia brasiliana em diferentes salinidades*]

F.J.S. *Lagreze*[1], S. *Sühnel*[2], R.J. *Ramos*[2], M. *Miotto*[2], M.C.P. *Albuquerque*[2], C.R.W. *Vieira*[2], C.M.R. de *Melo*[2]

[1]Universidade Federal do Paraná, Centro de Estudos do Mar, Pontal do Paraná, PR, Brasil
[2]Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil

ABSTRACT

Anomalocardia brasiliana is an intertidal filter-feeding clam that can accumulate enterobacteria, such as *Escherichia coli*, and consequently affect human health. Shellfish depuration is a procedure which reduces microbiological contaminants; however, salinity and depuration time can vary across species to adequately reduce bacteria load. To analyze the effect of salinity on the bioaccumulation and depuration of *E. coli* by *A. brasiliana*, this study evaluated salinity and depuration time in animals artificially contaminated with *E. coli*. Each experimental group of clams were acclimated for 6 hours in a recirculating aquaculture system (RAS) and then exposed to *E. coli* for 18 hours. Following exposure, clams were then held at one of four salinities (35, 30, 25 e 20) for a period of one of four depuration times (0, 12, 24, 36 and 48h). The highest bioaccumulation of *E. coli* in *A. brasiliana* was observed in clams held at salinities of 35, 30 and 25. The greatest reduction of *E. coli* in *A. brasiliana* was observed in clams held at 25 for 48 hours. A salinity of 20 showed low bioaccumulation and depuration of *E. coli*. The results of this study will contribute to developing a protocol for depurating *A. brasiliana* to mitigate human health concerns.

Keyword: Shellfish Aquaculture, microbial contamination, *E. coli*, depuration

RESUMO

Anomalocardia brasiliana é um molusco de areia filtrador que habita entremarés, o qual pode acumular enterobactérias como E. coli *e, consequentemente, afetar o ser humano. A depuração de moluscos é o procedimento para reduzir a contaminação; para isso, é necessária uma adequada qualidade da água. A fim de analisar o efeito da salinidade na bioacumulação e na depuração de E. coli por* A. brasiliana, *o presente estudo avaliou quatro salinidades (35, 30, 25 e 20) e quatro tempos de depuração (0, 12, 24, 36 e 48h) em animais contaminados artificialmente com* E. coli. *Todos os moluscos foram aclimatados por seis horas e posteriormente expostos a* E. coli *por 18h no sistema de depuração. O experimento de depuração foi realizado em um sistema de recirculação de água (RAS). A maior bioacumulação de* E. coli *em* A. brasiliana *foi observada nas salinidades de 35, 30 e 25, e a maior redução de E. coli nos animais foi observada na salinidade de 25, após 48h de depuração. A salinidade de 20 apresentou uma baixa bioacumulação de* E. coli. *A maior redução de* E. coli *em* A. brasiliana *foi observada na salinidade 25 depois de 48h de depuração. Os resultados do presente estudo podem contribuir para o desenvolvimento de um protocolo de depuração para essa espécie.*

Palavras-chave: berbigão, depuração, coliformes

INTRODUCTION

The clam *Anomalocardia brasiliana* (Gmelin, 1791) has a distribution from the Antilles (West Indies) to Uruguay, occurring all along the Brazilian coast (Rios, 2009), inhabiting calm waters in intertidal zones (Narchi, 1972), including mangroves and unvegetated shallows with sandy or sandy-muddy sediment.

The clam *Anomalocardia brasiliana*, is a marine bivalve with socio-economic importance, being

Corresponding author: lagreze@ufpr.br
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marketed at different scales along the Brazilian coast, mainly by coastal communities, where it can be the main protein source for impoverished families. Currently, *A. brasiliana* market is based on the harvest from wild stocks. Clams are organisms that feed on suspended particles in the water column (Navarro, 2001) and could accumulate, and concentrate pathogenic microorganisms present in seawater. Food safety risks of contaminated clams are dependent on the sanitary condition of the growing water. Bays and estuarine areas, where surrounding population settlements occur, may be exposed to anthropogenic pressures, which contribute to increased microbiological and chemical contamination of these ecosystems. The presence of pathogenic organisms, such as *Vibrio* spp., *Salmonella* sp., pathogenic *E. coli* (El-Shenawy, 2004; Ramos *et al*., 2014; Miotto *et al*., 2018), hepatitis A and adenovirus (Rigotto *et al*., 2010; Souza *et al.*, 2018), may represent a risk to human health and compromise the consumption of shellfish and food safety.

Escherichia coli is a Gram-negative bacterium, present in the gastrointestinal tract of humans and warm-blooded animals. Most of the commensal *E. coli* strains are harmless, however some pathogenic strains may cause diseases in humans (Kaper *et al*., 2004) and have been widely implicated in food-borne outbreaks around the world (Foodborne, 2017). *E. coli* is traditionally recognized as an indicator of fecal contamination in water and seafood (Kumaran *et al*., 2010) and the presence of *E. coli* in seafood is considered a food safety concern, representing a risk to the consumers when pathogenic strains are present (Costa, 2013). The European Union (2004) and Brazil (Brazil, 2012) have established regulatory limits and monitoring programs using *E. coli* counts in shellfish, and the microbiological monitoring results are used to classify shellfish production areas, in some cases conditioned to depuration prior to consumption.

Research to understand the bioaccumulation of *E. coli* in clams and the dynamics of microbial depuration are important to determine the appropriate depuration parameters to meet the legislation requirements.

Microbial bioaccumulation and depuration in oysters have been described by Ramos *et al*. (2012) and Ballesteros *et al*. (2016) and contamination of oysters with *E. coli* by Vásquez-García *et al*. (2019) and Vásquez-García *et al*. (2020); however, there is a lack of studies reporting contamination, bioaccumulation, and depuration of *E. coli* in *A. brasiliana*.

Depuration processes in the clam *A. brasiliana* has been studied for trace metals Cu, Zn, Cd and Pb, by Wallner-Kersanach *et al.* (1994), total mercury, by Silva-Cavalcante *et al*. (2016) and for the dinoflagellate *Prorocentrum lima* by Leite *et al.* (2021); however, there has been no published data related to *E. coli* depuration by *A. brasiliana*.

The present study evaluated depuration time of *A. brasiliana* artificially contaminated with *E. coli* in four salinities 35, 30, 25 and 20 in a RAS (recirculation aquaculture system) depuration system. This is the first report of bacterial bioaccumulation and depuration by the clam *A. brasiliana* exposed to different salinities.

MATERIAL AND METHODS

Escherichia coli bioaccumulation and depuration were tested separately, by the clam *Anomalocardia brasiliana* in four salinities 35, 30, 25 and 20. All the salinities were prepared mixing seawater at 35 and dechlorinated fresh water and measured with a refractometer. Four depuration times (12, 24, 36 and 48h) at salinities 30, 25 and 20 were tested, and two depuration times (12 and 24h) at salinity 35. Bioaccumulation and depuration experiments were performed in a RAS depuration system located at the Laboratory of Marine Molluscs (LMM) hatchery of Federal University of Santa Catarina (UFSC).

Adults of the clam *A. brasiliana*, 25-35 mm shell length were collected March 2011 at Daniela Beach, Florianópolis, Brazil (27º27'32.46"S and 48º32'37.16"W) and transported to the Laboratory of Marine Molluscs (LMM) hatchery of Federal University of Santa Catarina (UFSC) in insulated coolers (Fig. 1). At the laboratory, animals (n=400) were acclimated in each tested salinity for 6h in a tank (250 L) containing treated seawater (filtered to 1 µm and sterilized with UV), at temperature of 24 ± 0.5°C and oxygen saturation of 6.5 ± 0.5 mg L^{-1}, monitored with a multiparameter sensor (model YSI-550A, YSI, Inc., Yellow Springs, OH).

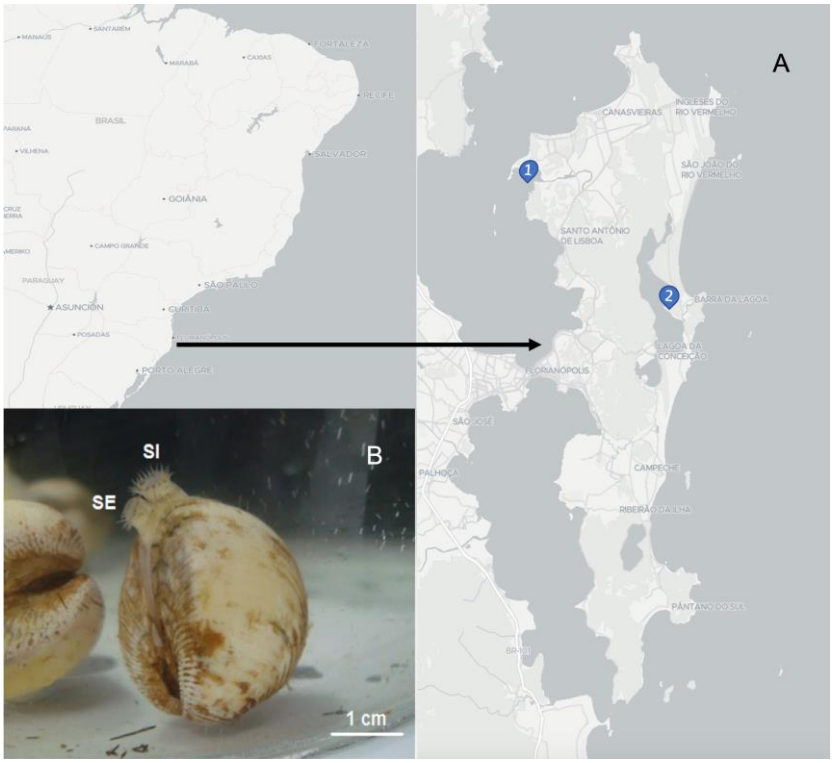

Figure 1. Map of collection point, Daniela beach (1; 27º27'32"S and 48º32'37"W), and depuration point, Laboratory of Marine Molluscs (2; 27°34'59''S and 48°26'31''W) (Fig. 1A). Picture of an adult of *A. brasiliana* with both siphon, exhalant (SE) and inhalant (SI) (Fig. 1B).

The RAS depuration system (Fig. 2) consisted in a depuration tank (350L fiber glass tank, water volume of 250L), two sequential polypropylene filters (5 and 3µm; 3M/Brazil), centrifugal pump (1/4CV), aeration system (venturi tube) and two 16W UV lamps (model Q884-23, Quimis) working in parallel. Depuration was carried out at a flow rate of 7L min^{-1} using a spray bar over the water surface. The clams were placed on a perforated plastic basket, that was suspended 90mm above the bottom of the depuration tank to minimize possible recontamination.

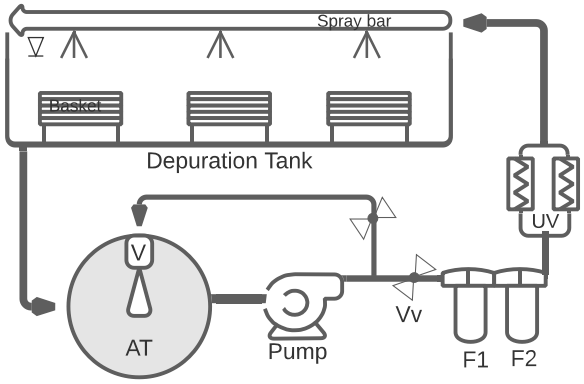

Figure 2. Schematic diagram for the Recirculation Aquaculture System (RAS) depuration system consisting of depuration tank, baskets, aeration tank (AT), venturi injector (V), ¼ CV centrifugal pump, globe valve (Vv) for flow adjustment, 5 µm polypropylene filter (F1), 3 µm polypropylene filter (F2), two UV of 16W in parallel and spray bar. Arrows show direction of seawater flow.

After acclimatization (Ti), clams were divided into two groups, the exposed group (animals exposed to *E. coli*, *n*=300) and the control group (negative control, animals unexposed to *E. coli*, *n*=100).

Infection of clams in the exposed group was performed by adding the *E. coli* suspension at final concentration of 10^3 to 10^4 CFU mL^{-1}, in the depuration tank. Exposure time for bioaccumulation of bacteria was fixed in 18h.

Animals of control group, marked with red dot paint to differ from those not exposed, were also submitted to the same procedure, but without bacterial, using separated tank. After 18 hours of exposure to the *E coli* suspension or the control treatment, the exposed control groups, were placed in the same tank for the beginning of the depuration experiment, following methodology described by Ramos *et al*. (2012).

After the exposure period, clams from exposed and control groups, were washed, adhered materials (feces and pseudo feces) were removed, and the shells cleaned in sodium hypochlorite solution (0.5%) prior to the depuration trials. Before transferring the animals to the depuration tank, the tank was cleaned with freshwater, sanitized with sodium hypochlorite (0.5%), rinsed with freshwater, and finally rinsed with treated seawater. In the depuration tanks, animals from exposed and control groups were divided into 3 plastic baskets per group.

Depuration treatments at each salinity was carried out with treated seawater, at temperature of 24.3±1.1°C, oxygen saturation of 6.5±0.5mg L^{-1} and pH of seawater in 8.0±0.1. Temperature and oxygen saturation were monitored during the depuration experiments with the multiparameter sensor.

Clams and seawater from the depuration tank were sampled for *E. coli* analysis before bioaccumulation exposure (Ti), after exposure (T0; start of depuration) and again at the end of each depuration period.

For *E. coli* analysis in clam meat, three pools (*n*=3) of 15 animals each from exposed group, and one pool, of 15 clams for control group were used. Individual clams from exposed and control groups stored in separately in sterile bags prior to the analysis. A seawater sample of 100mL from the depuration tank was stored in a (1L) sterile polypropylene container (1L) prior to testing for *E.* coli levels in the seawater. Samples (clams and seawater) for each depuration treatment were transported to the Laboratory of Food Microbiology (LMA/UFSC) in coolers with frozen gel-packs to maintain temperature at approximately 7°C.

Escherichia coli (ATCC 25922), a surrogate for *E. coli* O157:H7, was grown in tryptone soy broth (TSB, Oxoid, Basingstoke, UK) at 35ºC for 18 to 24h. The enriched cultures were streaked individually onto tryptic soy agar (TSA, Oxoid, Basingstoke, UK) and incubated at 35ºC for 18 to 24h. A single colony from a TSA-plate was selected and cultured in 50 mL of TSB at 35ºC for 18h. Enriched culture of *E. coli* was transferred to a sterile centrifuge tube and centrifuged at 3,000 x *g* for 15 min (MCD HT-2000, Formosa, China). Pelleted cells were resuspended in 50 mL of sterile salt solution (0.9%) to produce a cell suspension of approximately 4 $x10^8$ CFU mL^{-1}, according to the 0.5 McFarland standard (bioMe'rieux, Marcy l'Etoile, France).

Microbiological analysis of *E. coli* in clam meat samples was performed according to the methodology described by the American Public Health Association (APHA, 2001) using the most probable number method. For analysis of seawater from the depuration tanks, five multiple-tube technique, described in the Standards Examination of Water and Wastewater (APHA, 2005), was used.

At LMA/UFSC the clams were immediately washed with a brush under running tap water and dried outdoors in plastic trays, disinfected with 70% alcohol. Clam samples were collected, transported, and examined according to recommended procedures described by the APHA (APHA, 1970) as follows: after drying, the valves were opened with a sterile knife and the tissue meat and the intervalvar liquid were transferred aseptically to sterile bag and pooled samples were formed (15 animals). Samples (25 g of tissue) from each pool was diluted into 225 mL of peptone water (0.1%) and, subsequently disintegrated and homogenized. From this dilution (1/10), 1 mL was transferred from dilutions (10^{-1}, 10^{-2}, 10^{-3}) to each tube (series of 3 tubes for each dilution) containing Lauryl Sulfate

Tryptose broth (LST, Himedia, Mumbai, India) and incubated at 35°C for 48h. 100 µL were transferred from LST tubes which showed turbidity and gas production, to tubes containing *E. coli* broth (EC, Himedia, Mumbai, India) and incubated in a water-bath (45°C) for 48h. The ECs tubes that showed turbidity and gas production were inoculated in Eosin Methylene Blue agar-plates (EMB, Oxoid, Hampshire, UK) and inoculated at 35°C for 24h. For confirmation of *E. coli*, typical colonies were subjected to biochemical tests (Indole, Methyl Red, Voges Proskauer and Citrate - IMViC). The *E. coli* count was performed using the table of Most Probable Number (MPN).

The analysis of *E. coli* in seawater was conducted by incubating the samples in a series of 5 tubes containing LST doubly concentrated, followed by two series of 5 tubes containing LST in normal concentration. To each 5 tubes series, 10, 1 and 0.1 mL of seawater cultivation were added respectively, and the tubes were then incubated at 35°C for 48h. Tubes that showed turbidity and gas production were transferred to tubes with EC and incubated in a water-bath at 45.5°C for 24h. From this stage, the analysis follows the same sequence as described above, until the biochemical confirmation of *E. coli*. Counts were performed through the MPN table for series of five tubes per dilution (10, 1 and 0.1 mL), section 9221C (APHA, 2005).

The results of microbiological tests were transformed into base-10 logarithm (log_{10}) prior analysis. Data were tested for basic assumptions for analyses of variance (ANOVA) using Shapiro-Wilk and Levene tests for normality of errors and homogeneity of variances, respectively. Bacterial populations in shellfish and in the seawater from depuration tank (Log_{10} MPN) in each treatment from the different sampling times were analyzed with ANOVA and mean separation Tukey's test with $p<0.05$. To analyze the reduction of *E. coli* (in MPN) in *A. brasiliana*, a paired *t* test was applied to the logarithmic reduction between depuration times tested. All statistical analyses were performed using SAS software.

RESULTS

After the acclimation period and before the *A. brasiliana* exposure to *E. coli* bacteria (Ti), the clams showed the same bacterial concentrations for salinities 30, 25 and 20 and 2.7 more *E. coli* for salinity 35 (Table 1).

Table 1. *Escherichia coli* in clams before bioaccumulation (Ti) and in the seawater of the depuration tank after bioaccumulation (T0)

Salinity treatment	*E. coli* in the clams at Ti (Log_{10} MPN g^{-1})	*E. coli* in the seawater at T0 (log_{10} MPN 100 mL^{-1})
S35	1.30	0.67 ± 0.26
S30	0.48	0.42 ± 0.24
S25	0.48	0.24 ± 0.01
S20	0.48	0.42 ± 0.20

Bioaccumulation of *E. coli* in the clam samples was observed for all tested salinities (T0; Fig. 2). The salinity 35 showed higher values of *E. coli* (5.32 ± 0.76 log_{10} MPN g^{-1}), significantly different ($p<0.05$) from 20, that presented lower value of *E. coli* (2.42 ± 0.23 log_{10} MPN g^{-1}) after contamination. The salinity 20 bioaccumulation was significantly different ($p<0.05$) from salinities 30 and 25.

Initial *E. coli* populations in the unexposed clams (control group; T0) were low for salinities 35, 30 and 20 and higher for 25 (Fig. 2).

After depuration started, for the salinity 35, the lowest *E. coli* bacterial load of exposed animals was observed after 24h (T24) of depuration, significantly different ($p<0.05$) from T0 (Figure 3). For the salinity 30, the lowest bacterial load of clams was observed after 36h (T36) of depuration, different ($p<0.05$) from T0 (Fig. 3).

In contrast, clams depurated at salinity 25 showed the lowest bacterial load after 48h, different ($p<0.05$) from T0. The *E. coli* depuration by clams after T12, T24 and T36 were different ($p<0.05$) from T0 for the salinity 25 (Fig. 3).

Clams exposed to salinity 20 showed the lowest value of bacterial load after 48h. The bacterial loads in the clams at T12 and at T48 were different ($p<0.05$) from T0 (Fig. 3).

Analyzing salinity effects on bacterial load, significant differences ($p<0.05$) were observed after 12h (T12), with 20 lower than 30 and after 48h of depuration (T48), where 20 and 25, were lower than 30 (Fig. 3).

Clams that have not been exposed to artificial contamination (control group) and depurated at salinities 35, 30 and 25 showed increasing in *E. coli* population in the tissue during the depuration period, in relation to T0 (Fig. 3) until T24 decreasing in T48 for salinities 30 and 25. In the animals at salinity 20, the *E. coli* counts remained low over the tested time depuration processes (Fig. 3).

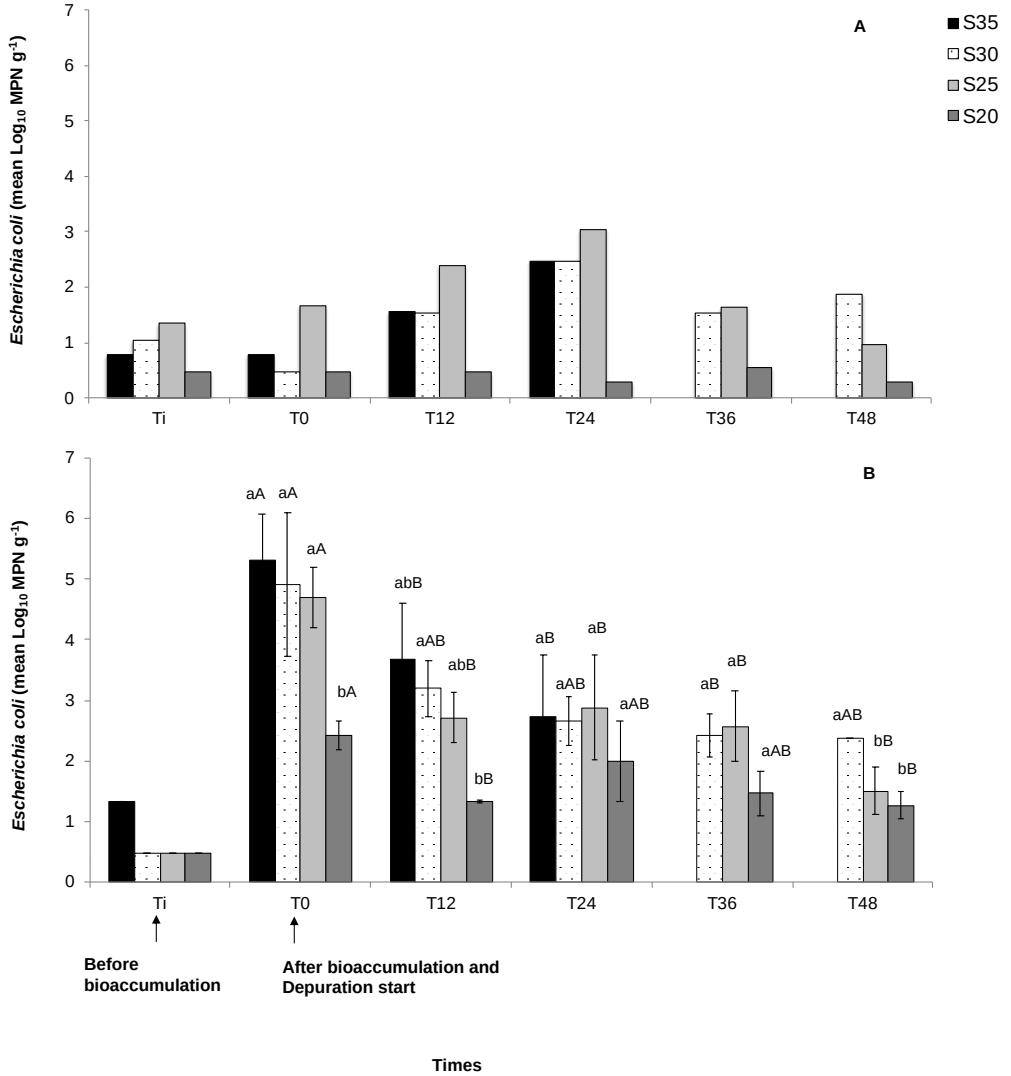

Figure 3**.** Mean and standard deviation of *Escherichia coli* amount (MPN in logarithm at base 10) in the clam *Anomalocardia brasiliana* over the time of control group (A) and exposed group (B). Where: Ti = before bioaccumulation start; T0 = after bioaccumulation and depuration start; T12 = 12h after depuration start; T24 = 24h after depuration start; T36 = after depuration start, and T48 = 48h after depuration start for the four tested salinities S35, S30, S25 and S20. Lower case letters above the histogram bars indicate differences between salinities in each time (T0; T12; T24; T36 and T48) (Tukey-HSD; ANOVA; $p<0.05$); and capital letters above the histogram bars indicate significantly different between times in each salinity (Tukey-HSD; ANOVA; $p<0.05$).

In the analysis of *E. coli* counts reduction, presented in logarithm cycles, the salinity 35 showed a significant ($p<0.05$) reduction between T0 and T24 (2.59 log_{10} MPN g^{-1}), however T12 (1.65 log_{10} MPN g^{-1}) did not show difference from T0. For salinity 30, no significant reduction was observed among the tested time (T12: 1.71 log_{10} MPN g^{-1}, T24: 2.25 log_{10} MPN g^{-1}, T36: 2.49 log_{10} MPN g^{-1} and T48: 2.53 log_{10} MPN g^{-1}) and with respect to T0. Yet, for salinity 25 was observed significant difference ($p<0.05$) between T0 and all tested depuration times (T12: 1.99 log_{10} MPN g^{-1}, T24: 1.83 log_{10} MPN g^{-1}, T36: 2.13 log_{10} MPN g^{-1} and T48: 3.20 log_{10} MPN g^{-1}), and between T12 and T48 (1.20 log_{10} MPN g^{-1}). For salinity 20, a significant reduction ($p<0.05$) was observed only between T0 and T12, T36 and T48 (1.09, 0.95 and 1.16 log_{10} MPN g^{-1}, respectively).

Escherichia coli in the seawater from depuration tank at T0 for salinities 35, 30, 25 and 20 was below zero (Table 1), and from T12 until the end of the depuration period (T48), no *E. coli* growth was observed in the water samples for all tested salinities (*E. coli* MPN was lower that method quantification limit 1.8 MPN 100 mL^{-1}).

DISCUSSION

This is the first study that evaluates the ability of the clam *A. brasiliana* to bioaccumulate and depurate *E. coli* at different salinities. This study is important because the depuration process is mentioned in the Brazilian legislation. Souza *et al*. (2018) suggested that raw clams to be sold to consumer should be depurated. In this case, depuration works to increase the safety of these species when they are cooked by the consumers at home.

The microbiological contamination of clams is a great concern in relation to other bivalves, such as oysters and mussels, since the clams are grown on the intertidal sea bottom where are contamination, especially from fecal origin, could be greater.

The presence of *E. coli*, even non-pathogenic strains in mollusks, should alert the public health as these bacteria is recognized as an indicator of fecal contamination in growing waters. Enteropathogenic and enteroaggregative *E. coli* serogroups were isolated from bivalves cultivated in South region of Brazil (Miotto *et al.*, 2018). This reinforces the importance to establish the depuration parameters to prevent contaminated clams from reaching final consumers and causing food safety issues.

The temperature, pH and oxygen parameters used of in this study are within the values suggested by FAO (Lee *et al*., 2008) for the bivalve mollusks depuration process.

The initial presence of *E. coli* bacteria in the clam and the inoculum concentration did not affect the bioaccumulation. Other factors can affect the bioaccumulation, such as the number of suspended particles in water, as observed for mussels (Plusquellec *et al*., 1990) and for clams (Cabelli and Heffernan, 1970), and salinity, which was observed in the present study.

In the present study, the lowest bioaccumulation was observed for the salinity 20, which is near the limit of salinity tolerance for this species (salinity 17). In the present study, higher salinities (25, 30 and 35), an increased bioaccumulation of *E. coli* in the animals was observed. According to Leonel *et al*. (1983), *A. brasiliana* survives in salinities between 17 to 42.5 during 29 days of exposure but does not mean that species has the same physiological response in these salinities. Lower bioaccumulation of *E. coli* observed in *A. brasiliana* depurated in salinity 20 could be related to a lower clearance rate.

Several factors are believed to be important to establish an effective depuration process. These include the system design, initial water quality, oxygenation and flow rates, salinity, temperature, removal and settlement of fecal material and the period of purification (Lee and Younger, 2002). The depuration RAS system used in this study showed constant water quality parameters for each tested salinity, using a venturi injector allowed a high oxygen saturation due to the large flow of micro bubble. The effectiveness of our depuration RAS system in decontaminating the water was proven in this study from T12 until the end of the depuration period (T48), no growth of *E. coli* in the water was observed for any of the tested salinities.

Salinity is a parameter that can affect filtration rates in mollusks since they are osmoconformers with little or no capacity for extracellular osmotic regulation (Shumway, 1996). In the present study, salinity affected the depuration process, with a

significant reduction of *E. col*i in all tested times for the salinity 25. The greatest reduction in *E. coli* counts for salinity 25, after 48h of depuration, suggests that this salinity could represent a physiological comfort zone for *A. brasiliana*. At salinity of 20, the lower reductions after 12, 36 and 48h of depuration, could indicate decreased filtration processes. Further physiological studies for this species would help to better understand responses the salinities examined during the depuration trials.

Recently, studies with the pacific oyster *Crassostrea gigas* developed by Silvestre *et al.* (2021), founded that salinity did not affect depuration of *E. coli* by the oyster, but affect tested biomarkers at different cell level, suggesting that the best salinity for depuration with this species is between 25 and 30.

Ekanem and Adegoke (1995) observed that 48h wase sufficient to reduce *E. coli* to levels in the clam *Egeria radiates* fit for human consumption in Nigeria. Rowse and Fleet (1984) observed for the oyster *Crassostrea commercialis* that the *E. coli* elimination from the tissue is slow and inconsistent in salinities of 16 and 20 when compared to salinities of 32-36 or 43-47. Love *et al.* (2010) observed for *M. mercenaria* that the best salinity for depuration was 28.

Results from the present study suggest that the salinity of 25 provided to the clam *A. brasiliana* a greater physiological comfort through improved bioaccumulation observed and a more efficient purification with greater reduction of *E. coli* in the clams. Holding clams at a salinity 20 suggested that clams may undergo physiological stress and they possibly need a longer period of acclimation prior to bioaccumulation and depuration processes.

Recontamination was observed in the clams after 24h at the salinity 20 and this may be a factor on the low bacterial reduction at this salinity of the bacteria *E. coli* in the clams from control group, depurated at higher salinities (25, 30 and 35), can be related to the contact with the biodeposits (feces and pseudo feces) from the exposed animals. Similar recontamination with biodeposits was observed by Ramos *et al*. (2012), testing depuration of *Crassostrea gigas* oysters contaminated with *Vibrio parahaemolyticus* and *Vibrio vulnifucus*. Timoney and Abston (1984) observed *E. coli* adhered by physical connection and not by ionic bonding to the feces and pseudo feces of the clam *M. mercenaria*. This suggests that it is important to ensure that depuration plant design and operation avoid biodeposits resuspension or mixing of treatment cohorts. Additional studies should be performed to evaluate the effectiveness of *A. brasiliana* depuration in different temperatures and higher salinities (e.g., 40-45), such as those recorded in Northeastern Brazil during the dry season, where this resource is highly exploited.

In conclusion, highest bioaccumulation of *E. coli* in the clams *A. brasiliana* were observed at salinity 35, 30 and 25, and the greatest reduction of *E. coli* in the clams were observed for the salinity 25, after 48h. Therefore, it can be concluded that salinity influences the bioaccumulation and depuration of *E. coli* by *A. brasiliana*. The results of this study can be used as a recommendation and guidance for bivalve molluscs producers to conduct the depuration process in clams.

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