



## A Comparative Assessment of Morphometrics and Bacterial Assemblages of *Crassostrea gasar* (Oyster) from Riparian Swampy Areas of the Lagos Lagoon, Nigeria

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### ABSTRACT

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The *Crassostrea gasar* (*C. gasar*) are economically important bivalves. The study investigated the physico-chemistry, the morphometrics and microbial profiles of *C. gasar*, water, and sediment from the Lagos Lagoon. The physico-chemistry and morphometry of *C. gasar* were determined using standard methods while the microbial were identified by Vitek 2 automated system. The mean Total Heterotrophic Bacterial Counts (Log CFU/mL) of *C. gasar* flesh and shells ranged from  $5.28 \pm 0.3$  to  $5.41 \pm 0.3$  and  $5.49 \pm 0.1$  to  $5.62 \pm 0.3$  respectively. The mean total coliform counts of sediment ranged from  $4.34 \pm 0.2$  to  $4.39 \pm 0.2$  Log CFU/mL, while the highest mean faecal coliform counts and total fungal counts of water samples were  $2.41 \pm 0.3$  and  $2.41 \pm 0.1$  Log CFU/mL, respectively. *Vibrio* spp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* spp, *Streptococcus faecalis*, *Escherichia coli* and *Aspergillus niger* were the species found in all samples in varying percentages. The median-sized *C. gasar* had the highest values of 7.5 – 12.4 cm (TL); 4.5 – 7.4 cm (MW); and 80.5 – 160.4 g (TW). The highest mean values for surface water temperature, pH, salinity, Chemical Oxygen Demand and Phosphate were  $27.3 \pm 1.51^\circ\text{C}$ ,  $29.8 \pm 1.94$ ,  $16.5 \pm 0.39$  ‰,  $7.26 \pm 0.01$  mg/L and  $0.39 \pm 0.01$  mg/L respectively. This study revealed that *C. gasar* from the riparian swampy areas harboured some potential pathogenic organisms of significant public health concern and can contribute to the spread of illnesses when consumed.

**Keywords:** *Crassostrea gasar*, Physicochemical properties, Morphometric, Microbial, Lagoon

### Introduction

Seafoods (fin and shellfish) are an important constituent of food rich in protein, minerals, and essential vitamins and, most importantly, serve as a means of livelihood for inhabitants of coastal zones.<sup>1,2</sup> Recently, the south-west riverine regions in Nigeria have continuously been exposed to improper human waste disposal (soap lather, food wastes, excreted); oil spillages (from canoes, boats, and ships) and industrial effluents (trace metals, heavy metals, and other non-degradable matters) which are bio-accumulated, and bio-magnified in the ecosystem through the organisms, the water column and sediments.<sup>3,4</sup>

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The presence, type and quantity of these microbes on freshly harvested sea-foods are majorly determined by the season (dry or wet), type of water (freshwater, brackish or marine), location (proximity to point of release of contaminants), source of pollution (human or industrial), and the water physico-chemistry (temperature, salinity, turbidity, hydrogen ion, etc.) parameters, while the abundance of bacteria and/or microbes found in aquatic entities depends majorly on fish genera, feeding practices, internal and external factors, including effects from seasonal and ecological properties.<sup>5</sup>

Shellfishes obtain microbial organisms in their gastrointestinal bands from their environment through ingested food and that are infested by microorganisms.<sup>6</sup> Microbial species isolated from majority of the fish gastrointestinal tracts are documented to be aerobic or facultative anaerobic species such as *Staphylococcus aureus*, *Escherichia coli*, *Aeromonas* sp., *Salmonella* sp., *Enterococcus faecalis*, *Yersinia* sp. and *Vibrio cholerae*.<sup>6,7,8</sup> Nonetheless, an accurate grasp of the movement of these micro-organisms within the food chains and webs is crucial to envisage the experience(s) of final consumers of these seafoods to the possible and potential well-being allied with their ingestion. These pathogenic organisms are found attached to different parts of these animals (flesh, buccal cavity, gill/gill rakers) that are wholly or subliminally interacting with the aquatic column and sediments.<sup>9,6,10</sup>

Consequently, elevated pathogenic bacteria have been isolated from fins and shellfish, which can cause diseases in fish as well as in the final consumer.<sup>11</sup> Mangrove oysters are sedentary aquatic organisms that are reliant on filtering water from their environment to obtain nutrients.<sup>12</sup> As filter feeders, oysters sip in micro-organisms along with their

nutrient (phytoplankton) from their environment, which by accumulation cannot always be detached by distillation<sup>13</sup> subsequently, leading to the concentration of infective microorganisms (bacteria and viruses) polluted or contaminated waters.<sup>14</sup> Even though the sea food is healthy, it acts as a source of pathogenic bacteria occurring naturally in contaminated lagoons.<sup>15</sup>

Final consumers (humans and animals) of these oysters are exposed to microbes and pollutants/contaminants from the ecosystem. Since the harvested oysters in the long run, ends up on the tables as delicacies for man and animals, it is therefore, very significant to assess the bacterial loads they harbor as they are documented to be sedentary organisms that are good monitors of accumulated toxins either from metals or bacteria. Lots of studies on the seafoods from Lagos Lagoon are available in literatures but most times, only emphasized on fin fishes. The few documented studies on mangrove oysters from Lagos Lagoon to the best of my knowledge are<sup>16,17,14,2,18</sup> It is pertinent to note that in all these documented literatures on mangrove oysters, none of these researches focused on the bacterial loads in the mangrove oysters found in the Lagos Lagoon. Consequently, it is of utmost necessity that studies are carried out on a regular basis across the Lagos lagoon to ascertain the existence and level of concentration of micro-organisms in the *Crassostrea gasar* (*C. gasar*). The study investigated the morphometric characteristics of *C. gasar*, the physicochemical composition of the lagoon water, and the microbial assemblages of the *C. gasar*, water, and sediment from the Lagos Lagoon, Nigeria.

## Materials and Methods

### Sampling site

The Lagos Lagoon (Figure 1) occupies latitude 6° 26' and 6° 37' N, and longitude 3° 23' and 4° 20' E in the South-West of Nigeria, with a land mass area of 208 km<sup>2</sup>.<sup>1</sup> The Lagos Lagoon is the largest in size compared out of the ten coastal lagoons in the South-West of Nigeria<sup>19</sup> with seasonal salinity changes.<sup>20</sup> The *C. gasar* (mangrove oyster) samples (Figure 2) were collected from three stations in the Lagos Lagoon: Ebute-Oko, Tomaro, and Agala with coordinates (Table 1) and receive domestic and industrial wastes as well as oil leaks as they are surrounded by suburban and industrialized blueprints with the presence of diesel-powered vessels and boats at the jetties.

### Sample collection and handling

Six hundred (600) *Crassostrea gasar* - *C. gasar* (Figure 2) with total lengths values of 1.5 to 18.4 cm, maximum widths values of 1.5 to 10.4 cm, and total weights values of 17.50 and 251.0 g were collected monthly for six (6) months between April and September, 2021, from the riparian swampy areas of the Lagos Lagoon, Lagos State, Nigeria. The *C. gasar* were randomly sampled from the roots of the mangrove trees, which were exposed at low tide, using a sharp cutlass.<sup>4</sup> The samples (water, sediment, and *C. gasar*) were collected using sterile screw-capped sampling bottles and polyethylene bags respectively. They were kept in ice boxes and taken to the Post graduate wet laboratory of the Department of Marine Science, University of Lagos, Nigeria.

### Physicochemical measurement

The physicochemical parameters were determined once-a-month between 6.00 a.m. and 8.00 a.m. (water and air temperatures, hydrogen ion concentration and salinity were determined at the point of collection (*in situ*) while Total Dissolved Solid (TDS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate and Phosphate were analysed *ex-situ* using the methods and instruments as described by.<sup>21</sup> Each evaluation was carried out in triplicates, and the results were expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD).

### Morphometric characteristics

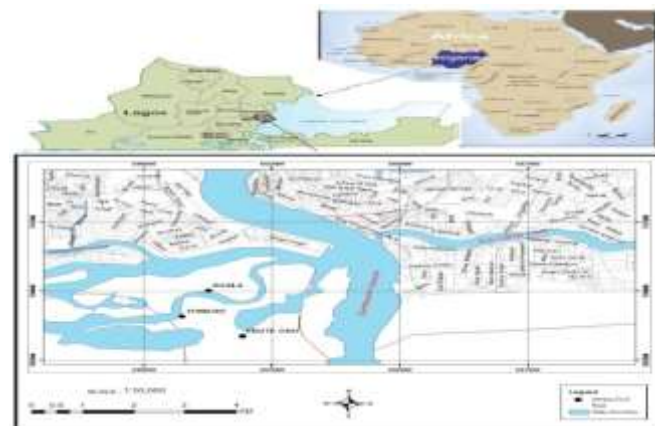
Three (3) measurable morphometric characteristics according to<sup>18</sup> were recorded in this study. The total length, maximum width, and total weight of the *C. gasar* (n = 600) were measured using a sterile Grip Vernier Caliper calibrated in centimeters (cm) with a precision of 0.5

cm (Figure 3), and the total weight was measured using an Electronic Compact Scale Atoms (-110°C) calibrated to the nearest 0.01 gram (g).

### Microbiological Analysis

#### Isolation of bacteria

One milliliter (1 mL) of sterile water and 1g of *C. gasar* flesh, shell and sediment sample were added into test tubes containing peptone water (10 mL), the test tubes were shaken strongly to dislodge stuck bacteria/fungi. One (1) mL of each serial dilution made was transferred to plates containing Nutrient Agar (NA), MacConkey Agar (MCA), Eosin Methylene Blue (EMB) agar and Sabouraud Dextrose agar (SDA). The inoculated NA, MCA, and EMB plates were incubated using CO<sub>2</sub> incubator (ICB – 170, Scitex, China for 24 hours at 37°C, while the inoculated SDA plates were incubated at 28°C  $\pm$  2 for 72 hours.



**Figure 1:** The riparian swampy buffer showing the sampling sites (onset: Map of Nigeria, indicating Lagos State)

Source:<sup>1</sup>.



**Figure 2:** *Crassostrea gasar* (mangrove oyster) X 90

Source:<sup>18</sup>

**Table 1:** The GPS coordinate descriptions of the sampling areas in the Lagos Lagoon, Lagos State, Nigeria

Sites	Latitude	Longitude
Agala	N05° 41'. 2° 66'	E07° 09'. 9° 01'
Ebute-Oko	N05° 41'. 9° 28'	E07° 08'. 3° 68'
Tomaro	N05° 40'. 7° 57'	E07° 09'. 5° 81'

Source:<sup>1</sup>



**Figure 3:** *Crassostrea gasar* with the Vernier caliper Mg X 8.0MP  
Source: <sup>18</sup>

After incubation, colonies on plates with bacterial and fungal growth were enumerated and the results expressed as CFU/g and/or CFU/mL for the Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC), Faecal Coliform Counts (FCC), and Total Fungal Counts (TFC), respectively.<sup>6</sup> Thereafter, colonies were sub-cultured onto NA and SDA plates, and incubated for 24 hours at 37°C (bacteria) and 28°C ± 2 for 72 hours (fungi). The pure cultures were streaked onto NA and SDA slants, incubated for 24 hours at 37°C and 28°C ± 2 for 72 hours, and thereafter deposited in the freezer at 4°C for morphology identification and characterization. The tests were repeated thrice and the results obtained were expressed as mean ± Standard Deviation (mean ± SD).

#### Identification and characterization of bacterial isolates

Bacterial isolates from the *Crassostrea gasar* flesh and shell, water, and sediment samples were characterized and identified using Gram staining and conventional biochemical tests such as catalase, coagulase, Vogues Proskauer, methyl red, indole, urease, motility, citrate, hydrogen sulphide, oxidase, spore, fructose, mannitol, maltose, galactose, lactose, glucose, and sucrose. The characteristics of the bacteria were evaluated using *Bergey's Manual of Determinative Bacteriology*.<sup>22</sup> The bacterial isolates were further classified to species level using the Vitek 2 automated system (Biomeriux Inc., France).

#### Identification and characterization of fungal isolates

Fungal isolates from the *C. gasar* flesh and shell, water, and sediment samples were characterized and identified based on their colonial growth pattern and morphological features such as soma, nature of hyphae, pseudo-mycelium, and asexual reproductive. A few drops of lactophenol cotton blue were placed at the center of clean microscope

slides. A small portion of mycelium of each isolate was picked with a sterile mounting needle and placed on cotton blue-in-lactophenol on a slide; emulsified and cover slip was gently placed at the center of the slide and viewed under a light microscope with × 10 and × 40 objective lenses.<sup>23</sup>

#### Data analysis

The Statistical Package for the Social Sciences (SPSS, version 22.0) was used to analyze the data collected. Some heat map correlation coefficients between the *in situ* and *ex situ* physicochemical parameters were determined. The Duncan Multiple Range Test (DMRT) was used to statistically analyze the data, with a significance level of 0.05 corresponding to 95% confidence intervals.

## Results and Discussion

#### Physicochemical parameters of the sampling sites

The mean (x ± S.D) monthly variations of air and water temperatures (°C), hydrogen ion concentration (pH) and salinity (‰) of Ebute-Oko, Tomaro and Agala in Lagos Lagoon are presented in Table 2. The mean (x ± S.D) monthly variations of air and water temperatures (°C) of the sampling sites varied, ranging from 27.3 ± 0.64 to 27.5 ± 0.37°C and 28.5 ± 0.76°C to 29.2 ± 1.18°C. The pH values were between 7.15 ± 0.03 and 7.26 ± 0.01, while salinity regimes for the three sampling sites were at 15.1 ± 0.15 ‰ to 16.5 ± 0.23 ‰. Statistically, there was no significant difference in the mean pH and water temperature values of water samples from the sampling sites at p ≤ 0.05. The results of the mean (x ± S.D) of nitrate, phosphate, total dissolved solid, dissolved oxygen, biochemical oxygen demand, and chemical oxygen demand concentration of the water samples from the three sampling sites are shown in Table 2. The highest mean values (mg/L) for nitrate, phosphate, TDS, DO, BOD, and COD (1.94 ± 0.04; 0.39 ± 0.01; 196 ± 1.00; 4.79 ± 0.02; 10.8 ± 0.15 and 17.8 ± 0.26), respectively, were all obtained from the water samples collected from Ebute-Oko, while second place variations were interchanged between Tomaro and Agala. Tomaro had higher mean values (mg/L) of nitrate (1.56 ± 0.02), TDS (176 ± 1.53) and DO (4.61 ± 0.02) than Agala, while values for phosphates (0.29 ± 0.02 mg/L), BOD (9.12 ± 0.03 mg/L) and COD (14.7 ± 0.15 mg/L) were higher in Agala compared to values of the same parameters obtained in Tomaro. There was a statistically significant difference in the mean values of phosphate and TDS of water samples from the sampling sites at p ≤ 0.05 (Table 2).

**Table 2:** Mean values of physicochemical parameters of the riparian swampy areas

Parameters	Mean Values (x ± S.D) / Sampling Sites		
	Ebute-Oko	Tomaro	Agala
Air temperature (°C)	27.3 ± 1.51 <sup>a</sup>	27.5 ± 0.37 <sup>a</sup>	27.3 ± 0.64 <sup>a</sup>
Water temperature (°C)	29.2 ± 1.18 <sup>a</sup>	28.9 ± 0.49 <sup>a</sup>	28.5 ± 0.76 <sup>a</sup>
Hydrogen ion (pH)	7.26 ± 0.01 <sup>b</sup>	7.18 ± 0.03 <sup>a</sup>	7.15 ± 0.03 <sup>a</sup>
Salinity (‰)	16.5 ± 0.23 <sup>b</sup>	15.1 ± 0.15 <sup>a</sup>	15.1 ± 0.21 <sup>a</sup>
Nitrate	1.94 ± 0.04 <sup>b</sup>	1.56 ± 0.02 <sup>a</sup>	1.5 ± 0.01 <sup>a</sup>
Phosphate	0.39 ± 0.01 <sup>c</sup>	0.21 ± 0.01 <sup>a</sup>	0.29 ± 0.02 <sup>b</sup>
TDS	196 ± 1.00 <sup>c</sup>	176 ± 1.53 <sup>b</sup>	158.6 ± 0.58 <sup>a</sup>
DO	4.79 ± 0.02 <sup>b</sup>	4.61 ± 0.02 <sup>b</sup>	4.31 ± 0.03 <sup>a</sup>
BOD	10.8 ± 0.15 <sup>b</sup>	7.68 ± 0.03 <sup>a</sup>	9.12 ± 0.03 <sup>b</sup>
COD	17.8 ± 0.26 <sup>b</sup>	13.3 ± 0.25 <sup>a</sup>	14.7 ± 0.15 <sup>a</sup>

Keys: x: mean; SD: Standard Deviation; TDS: Total Dissolved Solid; DO: Dissolved Oxygen; BOD: Biological Oxygen Demand and COD: Chemical Oxygen Demand; mean within the column followed by the different superscript letters are significant as determined by Duncan Multiple Range Test (P < 0.05).

*Correlation co-efficient among physicochemical parameters of water in sampling sites*

In water samples from Ebute-Oko, there was a strong positive relationship between air temperature and phosphate ( $r^2 = 0.8675$ ), TDS ( $r^2 = 0.9998$ ), while nitrate exhibited a weak negative correlation with dissolved oxygen with a correlation coefficient ( $r^2$ ) of  $-0.2402$  ( $p < 0.05$ ). The pH was observed to have a negative correlation with salinity ( $r^2 = -0.8863$ ) and dissolved oxygen ( $r = 0.5001$ ) (Table 3). In water samples from Tomaro, a highly positive relationship was exhibited between air temperature and water temperature ( $r^2 = 0.9999$ ) at  $p < 0.05$ . Phosphate had a very strong negative relationship with dissolved oxygen ( $r^2 = -0.9819$ ) and biochemical oxygen demand ( $r^2 = -0.9964$ ), while salinity had a weak negative relationship with nitrate ( $r^2 = 0.1428$ ) at the 0.05 level (Table 4). In water samples from Agala, the water temperature was found to have a very strong negative relationship with pH ( $r^2 = -0.9985$ ), salinity ( $r^2 = -0.8386$ ), phosphate ( $r^2 = -0.8946$ ), and COD ( $r^2 = -0.7857$ ). The TDS and COD showed a strong positive relationship ( $r^2 = 0.9228$ ), while air temperature exhibited a weak positive relationship with biochemical oxygen demand ( $r^2 = -0.1555$ ) and dissolved oxygen ( $r^2 = -0.1275$ ) at the 0.05 level (Table 5).

Aquatic ecological parameters have been identified as drivers of mangrove oyster distributions.<sup>24</sup> The air and surface water temperature ranges obtained in this study were expected because *C. gasar* has been reported to have a wide temperature tolerance and can be cultured at

temperatures as low as  $15^\circ\text{C}$ .<sup>25</sup> The results were similar to those obtained by,<sup>26</sup> who worked on the Ogun State water (Omni water body), and,<sup>27</sup> but differed slightly from those reported by,<sup>28</sup> on the physicochemical parameters in Niger-Delta waters. The values obtained for both air and surface temperatures were within the permissible limit of the WHO ( $40^\circ\text{C}$ ).

The pH levels fluctuated between  $7.14 \pm 0.03$  and  $7.26 \pm 0.01$ , and this is attributable to the influx of freshwater from adjacent water bodies and rainwater percolation, which might cause low pH in the rainy season while littoral waters' impact on the mangrove swamps may upsurge estuarine pH in the dry season.<sup>29</sup> The variant pH values recorded in this research conforms with the findings of<sup>28</sup> in Bonny River, Niger Delta. Salinity is an important ecological factor that has a great effect on the biotic life in aquatic ecosystems because each fish species has a salinity range where they can operate maximally. It has long been considered a major factor affecting the survival and growth of oysters.<sup>30</sup> The salinity of the Lagos Lagoon varied between  $15.2 \pm 0.21\%$  and  $16.6 \pm 0.23\%$ . This could be due to seasonal influx, tidal variations, and sediments.<sup>20</sup> The salinity values recorded in this experimental research were slightly lesser than the  $16.75 \pm 0.01\%$  recorded by.<sup>31</sup> The concentrations of nitrate ranged from  $1.50 \pm 0.0$  mg/L to  $1.94 \pm 0.0$  mg/L, and those of phosphate ranged from  $0.21 \pm 0.01$  mg/L to  $0.39 \pm 0.01$  mg/L in this study. These findings could be attributed to non-point source overflow from agronomic watersheds, domestic waste, and ship effluent.<sup>32</sup>

**Table 3:** Heat map correlation co-efficient among the physicochemical parameters of water in Ebute-Oko

	Air Temp.	Water Temp.	pH	Salinity	N	P	TDS	DO	BOD	COD
Air Temp.	1									
Water Temp.	-0.0846	1								
pH	0.5	-0.9052	1							
Salinity	-0.8662	0.5717	-0.8863	1						
N	0.2402	-0.9875	0.9608	-0.6934	1					
P	0.8675	-0.5715	0.8691	-0.9975	0.6933	1				
TDS	0.9998	-0.2846	0.4997	-0.8889	0.2402	0.8241	1			
DO	-0.9985	0.1121	-0.5001	0.8667	-0.2401	-0.8442	-0.9981	1		
BOD	0.3273	-0.9698	0.9819	-0.7559	0.9959	0.7566	0.3993	-0.3535	1	
COD	0.3812	-0.9534	0.9912	-0.7924	0.9889	0.7922	0.3111	-0.3812	0.9953	1

Keys: N – Nitrogen; P – Phosphorus; TDS – Total Dissolved Solid; DO – Dissolved Oxygen; BOD – Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

**Table 4:** Heat map correlation co-efficient among the physicochemical parameters of water in Tomaro

	Air Temp.	Water Temp.	pH	Salinity	N	P	TDS	DO	BOD	COD
Air Temp.	1									
Water Temp.	0.9999	1								
pH	0.7293	-0.7302	1							
Salinity	0.1751	-0.1764	0.7371	1						
N	-0.9762	0.9759	-0.5636	0.1428	1					
P	0.1147	-0.1197	-0.5926	-0.9819	-0.3273	1				
TDS	0.9011	-0.9058	0.9538	0.5005	-0.7857	-0.3273	1			
DO	-0.3704	0.2997	0.4335	0.9285	0.4998	-0.9819	0.1428	1		
BOD	-0.3004	0.2775	0.5549	0.9424	0.5001	-0.9964	0.1132	0.9979	1	
COD	0.8033	-0.8037	0.9979	0.6546	-0.6546	-0.5002	0.9865	0.3273	0.3981	1

Keys: N – Nitrogen; P – Phosphorus; TDS – Total Dissolved Solid; DO – Dissolved Oxygen; BOD – Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

The TDS ranged from  $158 \pm 1.73$  mg/L to  $196 \pm 2.00$  mg/L during the study. This was result of water dilution from rainfall, watershed sources, impurity point bases, and residue re-suspension influx into the lagoon. The concentrations of DO, which ranged from  $4.31 \pm 0.03$  mg/L to  $4.78 \pm 0.02$  mg/L, were low in this study compared with the least scale level of 5.0 mg/L oxygen content for the appropriate existence of aquatic biota.<sup>31</sup> The reduction in DO during the study might be associated with increased runoff and higher turbidity, which resulted in lower photosynthetic action because estuarine dissolved oxygen and chlorophyll-a concentrations are interrelated.<sup>29</sup> The BOD values recorded at the three sampling sites were higher than the stipulated values of 5 mg/L for unpolluted water<sup>33,31</sup> and also exceeded the 4 mg/L by FEPA standard.<sup>34</sup> The high BOD values at the three sampling sites were attributed to a variety of anthropogenic activities and pollutant discharges into the sampled environment (water) through sewage contamination, and oil leakage run-off which could result in a potentially significant public health risk.<sup>35</sup> The concentrations of COD at the three sampling sites agreed with the values reported by Shaikh & Mandre.<sup>36</sup> This might be due to the flow of chemical resources discarded into surface water and its infiltration into the groundwater through precipitation.

#### Morphometric parameters

The results of summary of morphometric and frequency distribution (by size groups) of *Crassostrea gasar* from the sampling stations are represented in Table 6. The TL (1.5 cm – 18.4 cm), MW (1.5 cm – 10.4 cm) and TW (17.5 g – 254.4 g) were measurements of *C. gasar* from the three sites. The total length frequency of *C. gasar* showed that size group 7.5 – 12.4 cm was the most abundant, having a total sum of 330 (55%), followed by size group 12.5 – 18.4 cm with 180 (30%), and the least size group represented in the sampling was size group 1.5 – 7.4 cm with 90 (15%). The maximum width frequency polygon of *C. gasar* showed that size group 4.5 – 7.4 cm, with 54% (324), was the most abundant, followed by size group 7.5 – 10.4 cm with 26.8% (161) and 19.2% (115) in size group 1.5 – 4.4 cm (Table 6).

The Length-Weight Relationship (LWR) is one of the universal techniques that yields convincing biological markers. The Length-Width Frequency (LWF) relationship showed that size groups 7.5–12.4 cm (TL) and 4.5–7.4 cm (MW), with 55 % and 54 %, correspondingly, were more abundant in the three sampling sites, revealing that medium-sized mangrove oysters were the largest in the sites at the time of this study. These observations of dominant sizes could be ascribed to the environment, nutrients from food, or water attribute of the region where these populations were present, as cited by.<sup>37,38</sup> The oysters' body weights of 80.5-160.4 g (55%) increased with body length, indicating positive growth. Likewise, positive allometric growth forms have been documented by<sup>39</sup> and<sup>18</sup>. Unlike this study, negative allometric growth was reported by<sup>40</sup> and,<sup>41</sup> who worked on samples of finfish and shellfish, respectively.

#### Microbial counts in *Crassostrea gasar*, sediment, and water from sampling sites

The Total Heterotrophic Bacterial Counts (THBC), Total Coliform Counts (TCC), Faecal Coliform Counts (FCC), and Total Fungal Counts (TFC) of *C. gasar* (flesh and shell), water, and sediment from Ebute-Okò, Tomaro, and Agala are shown in Table 7. The THBC of *C. gasar* flesh and shells from the three sites ranged from  $5.28 \pm 0.3$  log CFU/g in Agala to  $5.41 \pm 0.3$  log CFU/g in Tomaro and  $5.49 \pm 0.1$  log CFU/g in Agala to  $5.62 \pm 0.3$  log CFU/g in Tomato, respectively (Table 7). The highest THBC, TCC, and FCC were found in the water samples from Tomaro, while the lowest THBC, TCC, and FCC were recorded from the water samples from Agala. The ranges of microbial loads of sediment samples from Ebute-Okò, Tomato, and Agala were as follows: THBC  $6.32 \pm 0.1$  to  $6.46 \pm 0.4$  log CFU/g; TCC  $4.34 \pm 0.2$  to  $4.39 \pm 0.2$  log CFU/g; FCC  $3.00 \pm 0.1$  to  $3.04 \pm 0.3$  log CFU/g and TFC  $2.36 \pm 0.4$  to  $2.41 \pm 0.1$  log CFU/g (Table 7).

**Table 5:** Heat map correlation co-efficient among the physicochemical parameters of water in Agala

	Air Temp.	Water Temp.	pH	Salinity	N	P	TDS	DO	BOD	COD
Air Temp.	1									
Water Temp.	-0.9849	1								
pH	0.9086	-0.9985	1							
Salinity	0.9215	-0.8386	0.8386	1						
N	-0.6712	0.7559	-0.7469	-0.2773	1					
P	0.6286	-0.8946	0.7559	0.2616	-0.9981	1				
TDS	0.6449	-0.5006	0.4998	0.891	0.1889	-0.1504	1			
DO	-0.1275	0.3539	-0.3017	0.2401	0.8661	-0.8143	0.6671	1		
BOD	-0.1555	0.3273	-0.3226	0.2613	0.8444	-0.8247	0.6964	0.9798	1	
COD	0.8824	-0.7857	0.7142	0.9923	-0.1616	0.1616	0.9228	0.3273	0.3397	1

Keys: N – Nitrogen; P – Phosphorus; TDS – Total Dissolved Solid; DO – Dissolved Oxygen; BOD – Biochemical Oxygen Demand; COD – Chemical Oxygen Demand

**Table 6:** Ranges of morphometric of *Crassostrea gasar* from sampling sites

Parameters	Ranges (Sizes, cm)			Total No (%)
	Small	Medium	Large	
Total Length (cm)	1.5 – 7.4	7.5 – 12.4	12.5 – 18.4	
N (%)	90 (15%)	330 (55%)	180 (30%)	600 (100%)
Maximum Width (cm)	1.5 – 4.4	4.5 – 7.4	7.5 – 10.4	
N (%)	115 (19.2%)	324 (54%)	161 (26.8%)	600 (100%)
Total Wet Weight (g)	17.5 – 80.4	80.5 – 160.4	160.5 – 254.4	
N (%)	90 (15%)	330 (55%)	180 (30%)	600 (100%)

**Table 7:** Microbial counts from *Crassostrea gasar* (flesh and shell), water and sediment

Stations	Samples	Mean Microbial Counts (Log CFU/g / CFU/mL)			
		THBC	TCC	FCC	TFC
Ebute-Oko	Flesh	5.34 ± 0.1 <sup>c</sup>	3.60 ± 0.2 <sup>b</sup>	2.28 ± 0.3 <sup>a</sup>	2.11 ± 0.2 <sup>a</sup>
	Shell	5.53 ± 0.2 <sup>c</sup>	3.71 ± 0.2 <sup>b</sup>	2.34 ± 0.1 <sup>a</sup>	2.11 ± 0.2 <sup>a</sup>
	Water	5.81 ± 0.1 <sup>c</sup>	4.28 ± 0.3 <sup>b</sup>	2.41 ± 0.2 <sup>a</sup>	2.38 ± 0.2 <sup>a</sup>
	Sediment	6.41 ± 0.2 <sup>d</sup>	4.39 ± 0.1 <sup>c</sup>	3.00 ± 0.1 <sup>b</sup>	2.41 ± 0.1 <sup>a</sup>
Tomaro	Flesh	5.41 ± 0.3 <sup>c</sup>	3.62 ± 0.2 <sup>b</sup>	2.30 ± 0.2 <sup>a</sup>	2.11 ± 0.3 <sup>a</sup>
	Shell	5.62 ± 0.3 <sup>c</sup>	3.74 ± 0.6 <sup>b</sup>	2.36 ± 0.4 <sup>a</sup>	2.20 ± 0.3 <sup>a</sup>
	Water	5.85 ± 0.7 <sup>c</sup>	4.30 ± 0.4 <sup>b</sup>	2.41 ± 0.3 <sup>a</sup>	2.30 ± 0.2 <sup>a</sup>
	Sediment	6.46 ± 0.4 <sup>d</sup>	4.39 ± 0.2 <sup>c</sup>	3.04 ± 0.3 <sup>b</sup>	2.36 ± 0.4 <sup>a</sup>
Agala	Flesh	5.28 ± 0.3 <sup>c</sup>	3.57 ± 0.2 <sup>b</sup>	2.26 ± 0.2 <sup>a</sup>	2.00 ± 0.2 <sup>a</sup>
	Shell	5.49 ± 0.1 <sup>c</sup>	3.69 ± 0.2 <sup>b</sup>	2.32 ± 0.2 <sup>a</sup>	2.20 ± 0.2 <sup>a</sup>
	Water	5.76 ± 0.2 <sup>c</sup>	4.26 ± 0.2 <sup>b</sup>	2.38 ± 0.2 <sup>a</sup>	2.30 ± 0.2 <sup>a</sup>
	Sediment	6.32 ± 0.1 <sup>d</sup>	4.34 ± 0.2 <sup>c</sup>	3.00 ± 0.2 <sup>b</sup>	2.38 ± 0.2 <sup>a</sup>

Keys: THBC: Total Heterotrophic Bacterial Counts; TCC: Total Coliform Counts; FCC: Faecal Coliforms Counts and TFC: Total Fungal counts. CFU: Coliform Forming Units. mean within the column followed by the different superscript letters are significant as determined by Duncan Multiple Range Test ( $p < 0.05$ ).

**Table 8:** Morphological, biochemical and enzymatic characteristics of bacterial isolates from *Crassostrea gasar*, sediment, and water

Gram reaction	COA	CAT	STA	VP	MR	NIT	IND	URE	MOT	CIT	H <sub>2</sub> S	OXI	SPORE	GAS	DCE	ARD	ODX	ONPG	HYA	FRU	RAF	MAN	MAL	GAL	LAC	GLU	SUC	Probable Bacteria
+	+	+	-	+	+	+	-	+	-	+	-	+	-	-	-	+	+	-	+	-	+	+	+	+	+	+	+	<i>S. aureus</i>
+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	-	+	-	+	+	+	+	+	+	<i>S. faecalis</i>
-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	<i>Vibrio</i> spp.
-	-	+	-	-	+	+	+	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	+	+	+	<i>E. coli</i>
-	-	+	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	+	-	+	-	+	+	+	+	+	+	<i>C. freundii</i>
-	-	+	-	-	+	+	-	-	+	+	-	+	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	<i>P. aeruginosa</i>
+	-	+	+	+	-	+	-	-	+	+	-	+	-	-	+	-	-	+	+	-	+	+	+	-	-	+	+	<i>Bacillus</i> spp.
-	-	+	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+	-	+	-	+	-	<i>Shigella</i> spp.
-	-	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+	+	-	+	-	<i>S. typhi</i>
-	-	+	-	-	+	+	-	-	+	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	-	+	-	<i>C. spp.</i>
-	-	+	-	-	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	<i>P. spp.</i>
-	-	+	-	+	-	+	-	-	+	+	-	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	<i>E. aerogenes</i>
+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	+	-	-	+	+	+	-	+	-	-	-	+	-	<i>Clostridium</i> spp.

Note: C-rod: Curved Rod; COA: Coagulase; CAT: Catalase; STA: Starch; VP: Vogues Proskauer; MR: Methyl red; NIT: Nitrate; IND: Indole; URE: Urease; MOT: Motility; CIT: Citrate; H<sub>2</sub>S: Hydrogen sulphide; OXI: Oxidase; DCE: D-Cellobiose; ARD: Arginine Dehydrolyase; ODX: Ornithine Decarboxylase; ONPG: Beta-galactosidase; HYA: Hyaluronidase. FRU: Fructose; RAF: Raffinose; MAN: Mannitol; MAL: Maltose; GAL: Galactose; LAC: Lactose; GLU: Glucose; SUC: Sucrose; +: Positive; -: Negative; CoN: Coagulase negative.

The high microbial load of 5.41 ± 0.3 log CFU/g found in oyster flesh is in agreement with the reports of <sup>42</sup> in their study on bio-Indices of bacterial loads in water and *C. gasar* of Woji / Trans-Amadi Creek, Port Harcourt, Nigeria.

#### Morphological and biochemical characteristics and enzymatic reactions of bacterial isolates from *Crassostrea gasar*, sediment, and water

The results of the morphological (rod, curved rod and cocci) and biochemical characteristics (spore, coagulase, catalase, starch, Vogues Proskauer, methyl red, nitrate, indole, urease, motility, citrate, hydrogen sulphide, oxidase, fructose, raffinose, mannitol, maltose, galactose, lactose, glucose, and sucrose), and enzymatic reactions (D-cellobiose, arginine dehydrolyase, ornithine decarboxylase, β-galactosidase, and hyaluronidase) of bacterial isolates from *C. gasar*, sediment, and water are presented in Table 8. It is significant to note that only *Vibrio* spp. was detected as a gram-negative curved rod species; *Staphylococcus aureus* and *Streptococcus faecalis* were gram positive cocci, *Bacillus* spp. and *Clostridium* spp. were gram positive rods while others isolates

were gram negative rods. The isolates detected are known to be latent moribific genera and poison producing organisms.

The occurrences of micro-organisms from water, sediments, flesh and shell of *C. gasar* from the three different sites of Lagos Lagoon are presented in Figure 4 a, b, c, and d. Sixteen (16) micro-organisms comprising thirteen (13) bacterial (*Bacillus*, *Clostridium*, *Vibrio*, *Streptococcus*, *Citrobacter*, *Shigella*, *Salmonella*, *Chromobacterium*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Escherichia*) and three (3) fungal isolates were obtained from the flesh (Figure 4a) and shell (Figure 4b) of *C. gasar*, water, and sediments, respectively, using seven different culture media (n = 7) and the Vitek 2 automated system. The occurrences of micro-organisms from water and sediments from the sampling sites are presented in Figures 4c and d. Six (6) bacterial isolates (*P. aeruginosa*, *S. faecalis*, *Vibrio* spp., *Bacillus* spp., *E. coli*, and *S. aureus*) and one (1) fungal isolate (*A. niger*) were found in all samples (flesh and shell of *C. gasar*, water, and sediments) in varying percentages ranging from 1 (14.3%) to 5 (71.5%). Common isolates found in water (Figure 4c), flesh (Figure 4a) and shell (Figure 4b) of *C. gasar* was *Proteus* spp.; in the sediment (Figure 4d), flesh (Figure 4a) and shell (Figure 4b) of *C. gasar* was *Cladosporium* spp.

while isolates detected in the shells (Figure 4b) of *C. gasar* and sediments (Figure 4d) only were *Clostridium* spp. and *Chromobacterium* spp.; isolates exclusively found in the flesh (Figure 4a) of *C. gasar* and water (Figure 4c) was *E. aerogenes*, while isolates found in the shell (Figure 4b) of *C. gasar* and water (Figure 4c) only was *Fusarium* spp. *S. typhi* was only isolated in the flesh (Figure 4a) of *C. gasar*, while *C. freundii* and *Shigella* spp. were obtained from the water samples (Figure 4c) only.

*Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Vibrio* spp., *Bacillus* spp., *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger* were isolated from the flesh and shell of *C. gasar*, water, and sediments in our study (Figure 4a, b, c and d). The isolation of *Vibrio* spp. and *E. coli* in the oyster (*C. gasar*) corroborated the findings of <sup>43</sup> on pathogenic microorganisms linked with oysters and estuarine water along the south coast of Brazil. Similarly, the isolation of *Vibrio* spp. from oysters substantiated the study on the seasonal abundance of *Vibrio* spp. from oysters in the Mandinga Lagoon System, Veracruz, Mexico. <sup>44</sup>

The detection of *Escherichia coli* (*E. coli*), an indicator organism, in the water suggested faecal pollution as well as human interference. *E. coli*, *Salmonella typhi*, *Shigella* spp., and other pathogenic microorganisms can survive and multiply in the tissue or flesh of oysters, making it a potential vector of human diseases such as typhoid fever, cholera, food poisoning, diarrhoea, dysentery, septicemia, and meningitis. <sup>6</sup> The presence of microorganisms in the samples (water, sediment, flesh and shell of oysters) used for this study suggests that they survive in the brackish water habitats (sediments and water) and in the oysters (flesh and shell). *Clostridium* spp. and *Chromobacterium* spp. were isolated in the shell of *C. gasar* and sediments. The presence of *Clostridium* spp. in the sediment is a potential pointer to the existence of sewage entities in the sediment. <sup>45</sup> In this research, *Salmonella typhi* was collected from the flesh of *C. gasar*, while *C. freundii* and *Shigella* spp. were obtained from the water samples. The isolation of *Salmonella* spp. from oysters in this study corresponds with the findings of <sup>46</sup> in their study on the microbial variety and dietary profile of processed and unprocessed *C. gasar* (oyster).

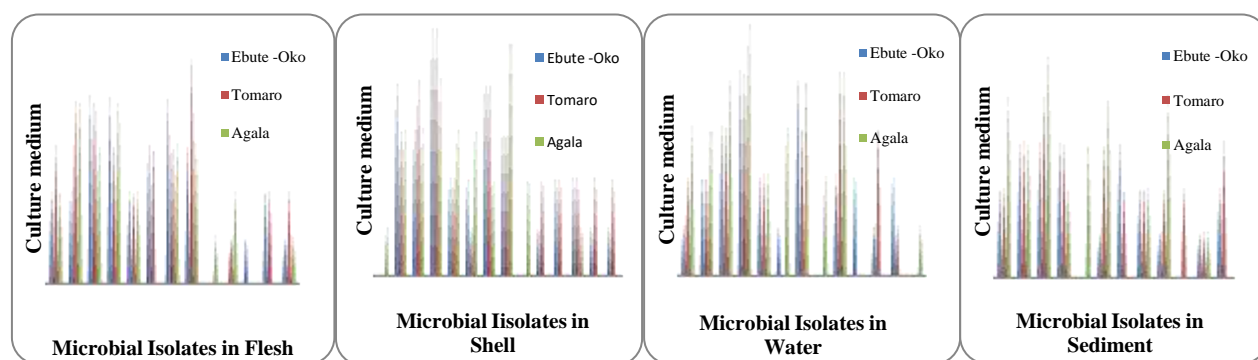


Figure 4a, b, c, d: Percentage occurrences of micro-organisms in the flesh and shell of *Crassostrea gasar* and in the water and sediments from the study sites

## Conclusion

This study revealed the physico-chemical status of Lagos Lagoon, and the morphometrics of *C. gasar* and showed that *C. gasar* from the riparian swampy areas harboured some potential pathogenic organisms of significant public health concern in the flesh of *C. gasar* and can contribute to the spread of illnesses when consumed. These results may be useful in the evaluation of toxic consequences of infective bacterial on shellfishes.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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