

# Diversity of cyanobacteria in shipwrecks in the shallow water of New Calabar River, Nigeria

## Abstract

The diversity of shipwrecks cyanobacteria in shallow water of New Calabar River, in River State – Nigeria was examined. Bio-concretions from three shipwrecks located at the estuary of New Calabar River were collected and the visual examination of the bio-concretions revealed 3 types of rusticles: Brown rusticles (braided structures attached on the wreck surfaces), Dendritic concretion (layered coatings of different concretions) and Biofilm (slimy coatings). The 16S rRNA gene sequences from the rusticles was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of cyanobacteria present in the rusticle samples using automated Illumina Miseq analyser. The results revealed a diversity of cyanobacteria in the rusticle samples. The cyanobacteria composition showed different species of diazotrophic filamentous genus *Trichodesmium*, it dominated the bio-concretion, having in abundance 4 of its species; *T. erythraeum* (8.75%), *T. hildebrandtii* (1.08%), *T. contortum* (1.04%) and *T. tenue* (1.02%). The availability of iron on the bio-concretions could explain the reason for the presence of the *Trichodesmium* clades present. Phormidiaceae, cyanobacteriaceae, we can associate the formation of rusticles by cyanobacteria as one of their eroding characteristics on shipwrecks. This study attempts to validate the role of mat-matrix forming cyanobacteria in aerobic corrosion in shallow water shipwrecks.

**Keywords:** shipwrecks, rusticles, cyanobacteria, illumina, *Trichodesmium*

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## Introduction

Cyanobacteria are a group of microorganisms that can perform oxygenic photosynthesis with the ability of fixing atmospheric nitrogen and, carbon.<sup>1</sup> There about 15 genera of cyanobacteria with more than 2000 species with remarkable diversity in their morphology. Cyanobacteria are unicellular, with species growing as colonies or filaments. These are large enough to be visible to the naked eye.<sup>2</sup> Being ubiquitous in nature explains the existence of cyanobacteria in diverse habitat ranging from freshwater, soil, biological soil, crust, and rocks. These prokaryotes can tolerate extreme conditions such as hot springs, hypersaline water, freezing environments, and deserts.<sup>3</sup> Even in extremely stressed conditions such as volcanic ash and anthropogenically disturbed areas,<sup>1</sup> a minimum requirement of light, Carbon dioxide and water,<sup>4</sup> a temperature range of 45°C- 75°C have been reported. With these remarkable adaptabilities of Cyanobacteria to different environmental conditions, they are reported to inhabit extremely lithic habitats such as rocks and walls of monuments and buildings.<sup>5</sup> The lithobionic cyanobacteria cause an unacceptable discolouration and bio-deterioration of coloured surfaces of walls of monuments and buildings.

Cyanobacteria have also been reported on natural reefs.<sup>6</sup> Wagner<sup>7</sup> hypothesized the presence of cyanobacteria on artificial reef such as shipwreck and a relationship between cyanobacteria and other organisms around the shipwreck have been reported. He posited that life especially corals in direct contact with shipwreck will have to contend with invading cyanobacteria that colonize the damaged reef. When a ship goes aground, cyanobacteria which are primary

colonizers or pioneer microorganisms of lithic, substrates begin to colonize and in turn promote the growth of other heterotrophic bacteria. Ship grounding can have significant adverse effects on life in water, especially, coral reefs. Shipwreck's physical impact in the marine environment can change species composition and competitive dynamics within the benthic assemblages.<sup>8</sup> Marshall and Edgar (2003) reported the potential risk of a shipwreck introducing a non-native species that may subsequently colonize the natural reef. It is remarkable to note that the presence of a wreck alters the water chemistry as the ship continues to rust away. During the course of time, the change in water chemistry can promote the colonization of certain species of benthic organism especially species whose growth is fascinated by the leached iron from the wreck.

In a marine environment (ocean) primary production is limited by the low concentration of iron (0.1-1nm),<sup>9</sup> although, this is not the case in Niger Delta region where iron concentration is naturally high. And as such the competition for the bio-available iron is high. Cyanobacteria a pioneer colonizer of shipwreck usually is in high demand of iron due to its involvement in the function of a variety of crucial enzymes. Despite the deleterious effects of shipwreck on the water body, cyanobacteria with their siderophores are adapted to scavenging and solubilizing iron from shipwrecks. To this effect, researchers had hypothesized that there would be bio-available iron in the vicinity of shipwrecks that encourages the growth of cyanobacteria. In a certain extreme environment like salt marshes, cyanobacteria play a key ecological role in binding sediments by forming densely layered mats and these mats are referred to as stromatolites.<sup>2</sup>

## Materials and methods

### General description of the study site

Three different shipwrecks situated at Rumuolumeni axis, popularly known as Iwofe Jetty along the New Calabar River in Rivers State were used for this research. As at the time of study only one of the wrecks still had its name visible on the wreck hull, -the Endurance Shipwreck. The names of the other two had been eaten off by rust, and are considered for the purpose of this work as

Unknown shipwreck I and Unknown shipwreck II. All the wrecks differ in regards to orientation and position on the bottom, therefore, the exposures of the wrecks to sunlight and water circulations varies, The Unknown shipwreck I is almost buried in the sediments with a few of the hull iron protruding upwards. Endurance Shipwreck rests upright with half the hull submerged and the unknown Shipwreck II rest horizontally at the bottom. The criterion for choosing each wreck is having been sunken for over 20 years; the exact time of wreck is unknown. The study site is brackish water and is predominantly mangrove, comprising mud flat when the tide is low (Figure 1 & 2).

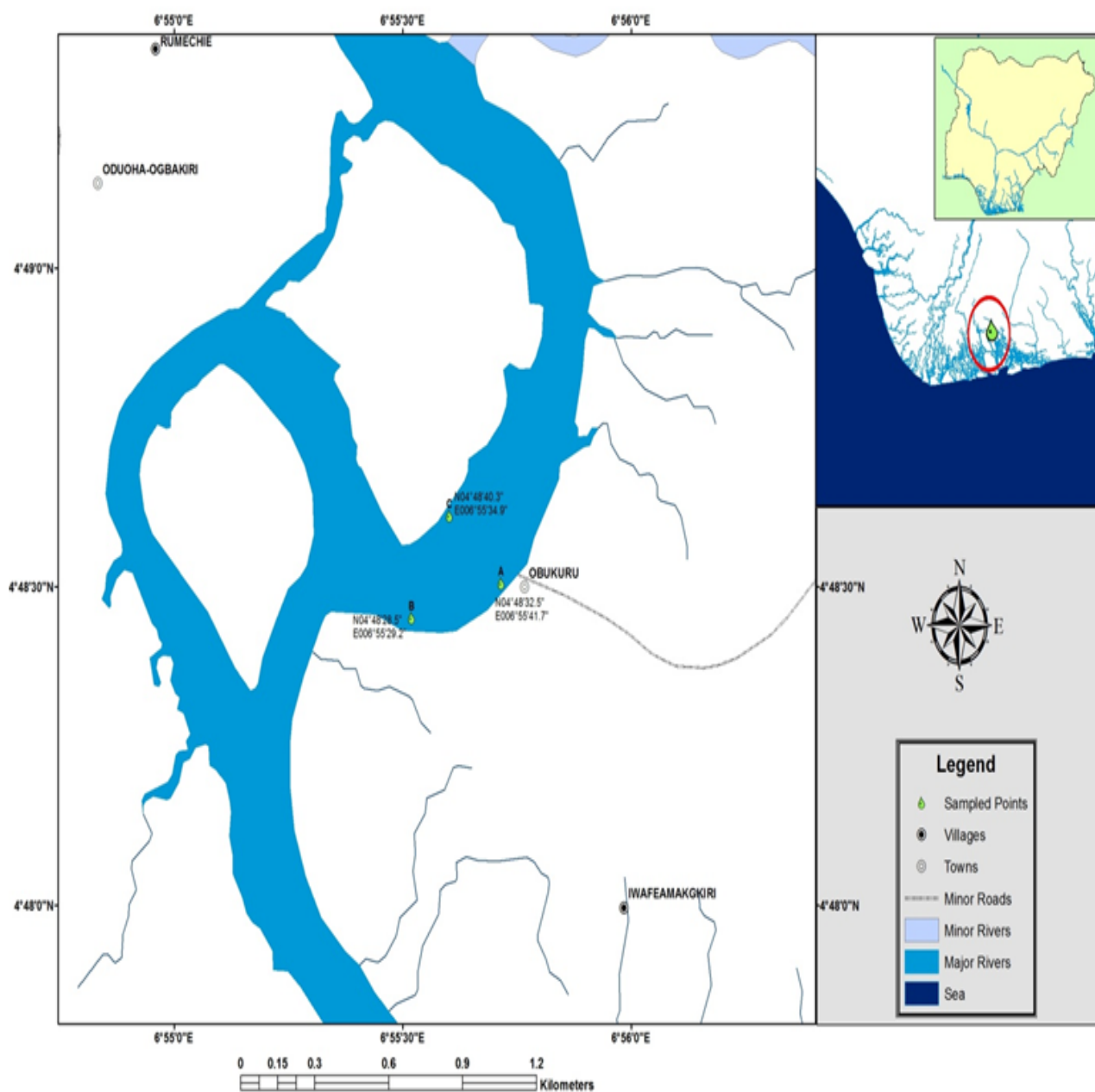


Figure 1 Study area location in New Calabar River.



**Figure 2** Study locations plotted on Google Earth image Scale 1:100 000.

•  Sampled points

Key

Point A, unknown ship designated as SH-D;

Point B, Endurance Vessel designated as SH-E;

Point C, Unknown ship designated as SH-F.

### Collection and study of biological samples (rusticles)

The study of rusticles was done by direct sampling followed by DNA analysis. The documentation of the microbiological communities (rusticles) growing on the wrecks was conducted. The colour and texture of rusticles were assessed. All rusticle samples were collected in sterile polythene bags and were placed in a sample cooler and carried to the laboratory where they were analysed.

### Molecular microbial diversity study

#### DNA extraction

Total genomic DNA was extracted from rusticles sample using Zymo Soil Microbe DNA extraction Kit according to the manufactures. 0.25g of rusticles sample was added into a ZR bashing bead™ lysis tube followed by the addition of 750µl lysis solution to the tube. The content in the ZR bashing bead™ was secured in a bead



beater fitted with a 2ml tube holder and was disrupted in a vortex mixer at maximum speed for 5 minutes. The ZR bashing bead™ lysis tube was centrifuge in a micro centrifuge at 10,000 x g for 1 minute. 400µl of the filtrate was added to a Zymo-Spin™ IV spin filter in a collection tube and centrifuge at 7,000 rpm (7,000 x g) for 1 minutes.

This was followed by the addition of 1,200µl of soil DNA Binding Buffer to the filtrate in the Collection Tube. 800µl of the mixture from above was added to a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 x g for 1 minute. The flow through from the collection tube was discarded and this particular step was repeated with the remaining filtrate. 200 µl of DNA pre-wash buffer was thereafter added to the Zymo-Spin™ IIC column in a new collection tube and centrifuge at 10,000 x g for 1 minute after which 500 soil DNA wash buffer was added to the Zymo-Spin™ IIC column and centrifuge at 10,000 x g for 1 minute. The Zymo-Spin™ IIC column was transferred into a clean 1.5 ml micro centrifuge tube and 100µl DNA elution buffer was added directly to the column matrix. This was centrifuge 10,000 x g for 30 seconds to elude the DNA. The eluted DNA was transferred to a Zymo-spin™ IV-HRC spin filter in a clean 1.5 microcentrifuge tube and centrifuge at 8000x g for 1 minute. The filtered DNA is now suitable for PCR applications.

#### DNA amplification by polymerase chain reaction (PCR)

PCR amplifications were performed, although not with a cyanobacteria specific primer. A standard 50µl PCR reaction solution contained 25µl Dream Taq Master Mix, 1µM of each primer, and 10µl of metagenomic DNA as the template. Amplification of a part of the 16S rRNA gene was done using a universal bacteria primer 27F with adapter and 518R with adapter, this was to determine both the cyanobacteria and other classes of bacteria present in the environmental samples. After an initial heating step at 95°C for 3 min, a total of 35 PCR cycles were run under the following conditions: denaturation at 95°C for 30s, annealing temperature at 52°C for 30s and extension 72°C for 1 min 30s and a final extension at 72°C for 10 min. The primer pair used (forward primer, 27F); reverse primer, 518R, with adapter (Baker et al., 2003). Amplified products were detected on 1% agarose gels electrophoresed in 1x TBE buffer, stained with ethidium bromide and photographed on an UV trans-illuminator.

#### DNA sequencing

##### 16S rRNA gene amplification and Illumina Miseq sequencing

An aliquot (50 ng) of purified DNA from each sample were used as template for amplification. The V1 and V3 hyper variable regions of bacterial 16S rRNAs (*Escherichia coli* positions 27-518) were amplified using a unique 7-bp barcode sequence contained the Illumina Miseq sequencing adapter at the 5' end of each primer, respectively. The targeted gene region has been shown to be the most appropriate for the accurate phylogenetic reconstruction of bacteria (Biddle et al., 2008).

##### Processing of Illumina Miseq data

Metagenomics analysis was done by MiSeq Reporter, in-built software on Illumina' Miseq platform was used for the 16S metagenomics analysis. The version of the software used is Analysis software version: 2.4.60.8.

## Results

The physical examination of bio-concretions collected from the shipwrecks revealed 3 types of rusticles: Brown rusticles (braided

structures attached on the wreck surfaces), Dendritic concretion (layered coatings of different concretions) and Biofilm (slimy coatings). The colour of rusticles observed on our site had a shade of brown through white with sparingly orange on it. We also notice a black colour inside the rusticles. The term rusticle is used as a collective name to describe the bio-sconcretions.<sup>8</sup>

The relationship of elements within the bio-concretitious structure with dominant atom first is;

**SH-RD:** Fe>Ca>Mn>Mg

**SH-RE:** Fe>Ca>Mn>Mg

**SH-RF:** Fe>Ca> Mn>Mg

The concentration of the metal found on the bio-concretions of the three shipwrecks followed the same pattern. Fe was the dominant metal and was as high as 9250 mg/kg. On the three wrecks the Fe content obtained was very high, 8950mg/kg, 9250mg/kg & 9050mg/kg respectively. The high concentration of iron on the rusticles is likely due to bio-extraction of iron from the steel of shipwreck which aggravates the weakening of the steel and this suggest that with time there will be a complete collapse of the frame of the ship. Calcium content was high on the rusticles observed on the wrecks. Cullimore & Johnston<sup>10</sup> reported low calcium content on rusticles collected from Titanic. There is considerable variation in the elemental composition of the rusticles tested; however, this reflects the heterogeneous nature of the structures themselves. This is also consistent with Cullimore & Johnston.<sup>10</sup> When the Fe content is high on the rusticles it is said to be more of complex ferric oxides and hydroxides.<sup>10</sup>

The result of the 16SrDNA obtained from the rusticle samples revealed the presence of cyanobacteria from the phylum-level down to the species-level in the shipwreck's samples investigated. The percentage total reads of cyanobacteria in the three shipwrecks are 34.72%, 21% and 24.07% for SH-RD, SH-RE and SH-RF respectively. The Oscillatoriothycidae (32.7%) is the only class of cyanobacteria found in the rusticles of shipwreck SH-RD.

From the rusticle samples from shipwreck SH-RE, the total reads generated was 51,156. The percentage of the unclassified at kingdom level is relatively higher than the total percentage of cyanobacteria and other classes of bacteria, 66.44%. Three classes of cyanobacteria were identified in the shipwreck SH-E Oscillatoriothycidae, Nostocophycidae and Synechococophycidae, while the class Oscillatoriothycidae showed the most dominant of the three with percentage total read of 14.78%. The total number of reads in rusticle samples in SH-F location is 670,372. The percentage quality reads are 82.1%. The percentage bacteria identified at kingdom level is 87.55% and the unclassified at this level is 12.41%. Two clusters of Cyanobacteria were also identified in rusticle samples in shipwreck SH-RF, Oscillatoriothycidae and Nostocophycidae. Again, the class Oscillatoriothycidae makes up the most abundance of the two groups. Of the three wrecks studied the class Oscillatoriothycidae was the most dominant (Figure 3).

Although a universal bacteria primer (27F- 518R) was used for the amplification of the 16S rRNA genes obtained from the rusticle environmental DNA in this study, it is interesting to note that cyanobacteria species dominated other bacterial species found. The species identified are *Crocospaera watsonii*, *Phorimidium murrayi*, *Nostoc microscopium*, *Trichodesium contortum*, *Oscillatoria coralline*, *Arthronema africanum*, *Hydrocoleum lynghyaceum* (Figure 4), (Table 1& 2).

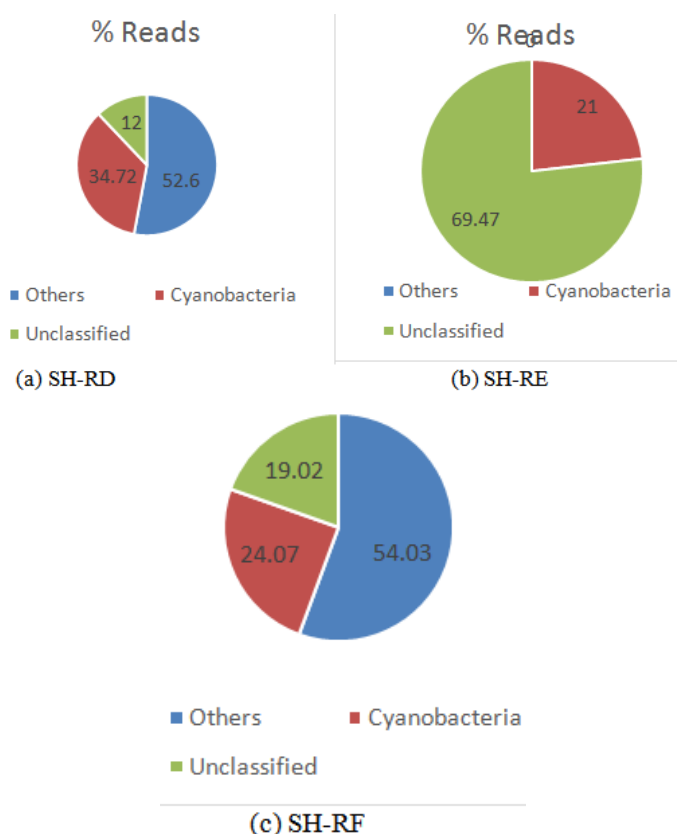


Figure 3 Percentage read of cyanobacteria in the three Shipwrecks (a-c).

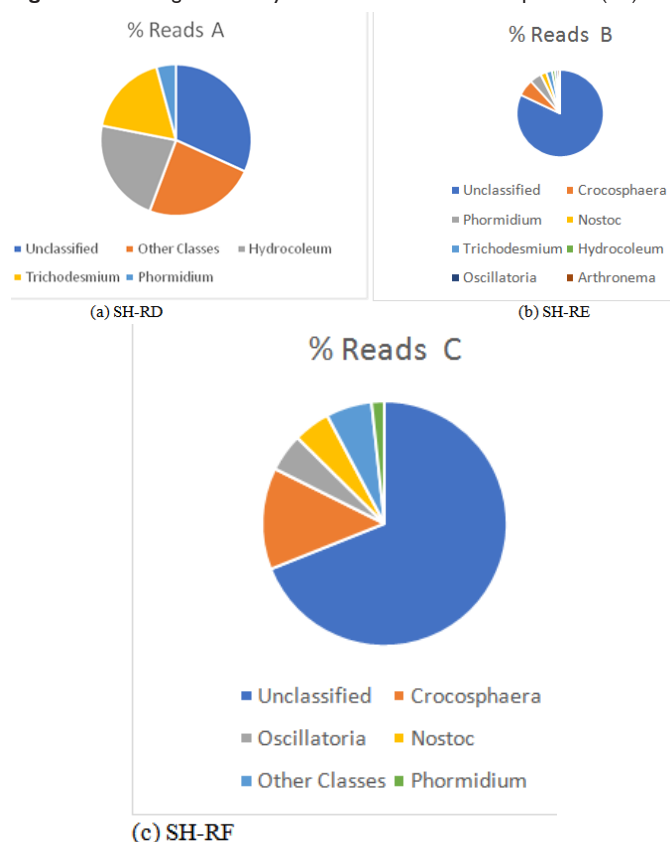


Figure 4 Percentage read of cyanobacteria Genera in the three Shipwrecks (a-c).

Table 1 Summary of the bio-concretion growths on the Shipwrecks exterior surfaces

Shipwrecks	Brown rusticle	Dendritic concretions	Biofilms
SH-RD	**	***	***
SH-RE	***	***	**
SH-RF	***	***	***

Keys: \*\*\*, abundance; \*\*, moderate

Table 2 Concentrations of metal on the rusticle samples

Elements	SH-RD (Mg/Kg)	SH-RE (Mg/Kg)	SH-RF (Mg/Kg)
Fe	8950	9250	9050
Ca	210	385	285
Mn	162	256.5	162.5
Mg	110	170	140

Cyanobacteria genera were the most dominant bacteria found on the rusticle samples in all the three shipwreck sites, particularly, *Phormidium* was detected in the 3 rusticle samples. The SH-RD shipwreck site was characterized by a mixed pattern, three Cyanobacteria genera, three Actinobacteria genera and one alpha-Proteobacteria genus. *Hydrocoleum* (15.75%) and *Trichodesmium* (12.47%) being the most dominate genera. While the total genus level taxonomy for SH-RE shipwreck showed a one-directional pattern, only cyanobacteria genera were found, with *Crocospaera* (5.66%) as the most dominate genus. On the other hand, SH-RF shipwreck had a slightly different pattern, Cyanobacteria was in abundance and also very low number of Alpha-proteobacteria was recorded. The most dominate genus in SH-RF is also the *Crocospaera* (8.12%). The cyanobacterial genera present in SH-RD and SH-RE are *Hydrocoleum*, *Trichodesmium*, and *Phormidium*. While the genera found common to SH-RE and SH-RF were, *Crocospaera*, *Oscillatoria*, *Phormidium* and *Nostoc*. On the other hand, the genus common to both SH-RD and SH-RF was *Phormidium*.

## Discussion

Shipwrecks are one of the numerous surfaces with distinct physical and chemical properties found submerged in water. They have been referred to as ‘hot spot’ diversity for organisms<sup>11</sup> and are known to serve for microbially catalysed, biogeochemically important activities.<sup>12</sup> Predictably, the nature of its surface plays important role in the microbial adaptation and biogeochemical functioning in marine environments. Surface associated microorganisms play important roles in numerous critical marine processes, including organic matter remineralization nutrient regeneration and element cycling.<sup>12</sup> Surfaces once submerged in marine water are rapidly colonized<sup>13</sup> and subsequent biofilm formation follows a sequence of chemical and biological occurrences.

In our study, cyanobacteria were observed as the dominance colonizers of the shipwreck surfaces. The question is why did the cyanobacteria dominate the rusticle samples and what possible roles are they playing on the shipwrecks? From the state of the art, there is little or no information on cyanobacteria diversity of shipwreck ecosystem. The attempted answer on the possible role of cyanobacteria on the shipwreck surface were compared with the presence and activities of cyanobacteria on biogenic surfaces such as rock submerged in water and also on natural reefs. Larouche<sup>14</sup>

observed high abundance of cyanobacteria exclusively on rock biofilms, which is in accordance with our findings. In another study by Hullar<sup>15</sup> cyanobacteria clones comprise the majority (40%) of the sediments. Charpy et al.,<sup>6</sup> reported that cyanobacteria have always dominated marine environments and been known as reef builders on Earth. In his review, he stated that all limestone surfaces have a layer of boring algae in which cyanobacteria often play a dominant role. This suggested role, played by cyanobacteria was further explained by the study of Walker,<sup>16</sup> that the boring activity of euendolithic cyanobacteria (living within or penetrating deeply into stony substances as rocks, and corals) results in biological corrosion and disintegration of carbonate surfaces. This gives an insight into the possible role of cyanobacteria on the shipwreck surface, maybe similar biological corrosion processes and the disintegration of the metal surfaces. This could suggest that cyanobacteria could possibly have a part to play in metal corrosion in shallow water and mostly in brackish water. This fact could explain the predominance of cyanobacteria in our rusticle samples. Indeed, according to Charpy et al.,<sup>6</sup> we can associate the formation of rusticles by bacteria and especially by cyanobacteria as one of their eroding characteristics in shipwrecks. The photosynthetic activity of cyanobacteria, their extracellular polymeric substances, and possibly also the adherent heterotrophic bacteria are responsible for the construction of various carbonate structures and the ability to penetrate shipwreck hull.<sup>6</sup>

Grazing organisms on carbonate surfaces colonized by epi- and endolithic cyanobacteria also produce specific biokarst forms and specific grains that can contribute to near-shore sedimentation, biological corrosion and abrasion altogether constitute bioerosion.

Having established the general roles of cyanobacteria on the shipwreck surface, insight into the specific genera that make up the community can further reveal their individual roles in the shipwreck ecosystem. *Phormidium murrayi* was the only species common to the three wrecks although in relatively low abundance on the three shipwrecks (2.93%, 3.78% and 1.03% respectively). The presence of *Phormidium* on the rusticle samples is substantiated with the report from other researchers about the ubiquity of this genus, *Phormidium*. And that their presence is not surface-specific, that means that *Phormidium* are not strictly related to a specific lithic substratum. Although, Macedo et al.,<sup>17</sup> portend that this genus prefer siliceous substratum. The genus *Phormidium* is an endolithic cyanobacteria which has gelatinous sheet that play an important role in adhesion to the substratum. As endolithic organism, they have been reported to penetrate deep down rocks.<sup>16</sup> This then suggest that, they have potential to penetrate the shipwreck metals. With the activities of the bacteria consortia (sulphate reducing bacteria (SRB), Iron related Bacteria (IRB) and acid producing bacteria (APB)) held together in a biofilm<sup>13</sup> and the release of metabolic substances that could corrode and form pitting as a result of the H<sub>2</sub>S released on metal surfaces by the SRB.

There are two genera of cyanobacteria that dominated the rusticle samples, *Trichodesmium* and *Crocospaera*. There was an abundance of *Trichodesmium* in SH-RD and SH-RE and an abundance of *Crocospaera watsonii* in SH-RE and SH-RF.

Four species of *Trichodesmium* were identified in the rusticle samples; *T. erythraeum*, *T. hildebrandtii*, *T. tenue* and *T. contortum*. According to Janson et al.,<sup>18</sup> a genetic characterization of *Trichodesmium* revealed two distinct clades; Clades I and II. There was a good representation of these two clades in the species of *Trichodesmium* found in our study.

According to Janson et al.,<sup>18</sup> characterisation, Clade I – contains: *T. erythraeum* (8.75%) and *T. contortum* (1.04%) while Clade II – contains: *T. tenue* (1.02%) and *T. hildebrandtii* (1.08%). *T. erythraeum*, *T. hildebrandtii* and *T. tenue* predominated the rusticle samples from the Unnamed shipwreck 1 (SH-RD) while *T. contortum* was observed in rusticle samples from Endurance shipwreck (SH-RE). The Unnamed shipwreck 2 (SH-RF) had no representation of the *Trichodesmium* species. In classical grouping based on morphology, *T. tenue* always was clustered with *T. erythraeum*.<sup>18</sup> These two species were noticed clustering together in the same pattern in our study in SH-RD samples. *Trichodesmium* thrives in stratified, warm water with high light<sup>19</sup> and this can explain the abundance of *Trichodesmium* in the rusticle samples, the wrecks are submerged in water though but in shallow water where sunlight could easily reach these phototrophic bacteria.

It has been reported that nutrients such as phosphorus (P) and iron (Fe) affects *Trichodesmium* growth and nitrogen fixation rates.<sup>20</sup> The high availability of Fe in our rusticle could possibly explain the high abundance of cyanobacteria. The rich presence of *Trichodesmium* among the species of bacteria found under cyanobacteria revealed that the bioavailability of Fe can encourage the abundance and activity of *Trichodesmium* in the rusticle samples.

*Trichodesmium* species often form extensive blooms that are visible from space. Although, Hynes et al.,<sup>21</sup> argued that the presence of *Trichodesmium* clade I cells in deep region does not necessarily indicate growth under those conditions, but that cells may have been carried there by water current. The low temperature and depth may suggest that clade I has higher tolerance for low light and cold conditions than clade II.

In our study, *T. erythraeuma* member of clade I dominated shipwreck SH-RD which is almost completely submerged in sediments at an abundance level of 8.25%. While members of clade II were low in abundance. This is in agreement with the findings of Hynes,<sup>21</sup> who observed that clade I was more in colder and deeper areas in Pacific than clade II. The clade II members were observed very low in the samples from the Unknown shipwreck I and Endurance shipwreck. *T. contortum* a member of clade I was observed in SH-RE rusticle, this wreck is located where the water is shallow and warmer, and was not completely submerged in the water but the hulls were usually covered when the water tide was high and exposed in low tide.

Our study observed a great diversity of cyanobacteria in the shipwreck ecosystem and much more the diversity that existed within the genus *Trichodesmium*. The two major clades present in our study have been reported to exist in tropical and subtropical waters.<sup>22</sup> The ecological role of *Trichodesmium* is Nitrogen fixation, an ability that enables them to grow successfully where little or no combined nitrogen is available.<sup>3</sup> *Trichodesmium* species is a dominant oxygenic marine diazotroph that differs from *Crocospaera* in that it fixes both nitrogen and carbon during the day (Inomura et al., 2019). In view of this, *Trichodesmium* species does not make use of iron conservation strategy to the extent used by *Crocospaera*.

*Crocospaera watsonii* richly dominated two of our shipwrecks, SH-RE (5.62%) and SH-RF (8.12%). *Crocospaera watsonii* belongs to a novel genus of marine unicellular diazotrophic cyanobacteria that occur in marine waters warmer than 24.5°C. The size, abundance and rapid growth rate of *Crocospaera watsonii* indicate that the diazotrophic cyanobacteria is capable of contributing significantly to oceanic carbon and nitrogen budgets in the tropical regions of



the world's oceans and are predicated to be limited by iron in most marine environments (Inomura *et al.*, 2019). This is consistent with our report, since the abundance of *Crocospaera watsonii* could be attributed to the richness of iron on the steel of the shipwreck surface.

*Phormidium murrayi*, *Oscillatoria coralline* and *Nostoc Microscopium* were present in relatively low abundance. Macedo *et al.*,<sup>17</sup> reported the ubiquity of the genus *Phormidium*, are commonly reported on cultural heritage and that their presence is not related to a specific lithic substratum. This seems to be in agreement with our findings since this genus was observed on the three shipwrecks. Many genera of *Oscillatoria* are known to produce wide variety of toxins have also been related to neuro-muscular organ distress as well as external contact irritation both in fresh and marine habitat and can be a threat to marine habitat. Therefore, monitoring of cyanobacteria in marine environments is very important and needed.<sup>23</sup> All species of *Oscillatoria* grow in mats on different substrates ranging from (mud, sand bottom, stones and any anthropogenic surface as shipwrecks or any other derelicts) mainly in shallow water habitats.

Could any potential risk exist as cyanobacteria inhabit shipwrecks? From our study, a great deal of cyanobacteria-diversity has been observed on the shipwreck surfaces. These shipwrecks have also been reported as hot spot for marine biodiversity,<sup>11</sup> as artificial reef it forms an extra habitat for fish<sup>24</sup> and are also susceptible for the settlement of a wide range of benthic invertebrates.<sup>11</sup> Zebra Mussels a marine invertebrate that attaches to hard surfaces was reported to have substantially covered the six shipwrecks on Lake Champlain.<sup>25</sup> The concentration of the Zebra mussel was found around metal fittings and fastenings and also on both the vertical and horizontal surfaces of the wrecks. Interestingly, in our study colonies of Mussels were found in patches on the both horizontal and vertical surfaces of our three shipwrecks although more were found on the vertical surface. The point is this, shipwreck as hot spot for marine diversity (habitat) and also being richly colonized by cyanobacteria, can expose these marine invertebrates to the risk of ingesting toxic cyanobacteria. And if this is possible, these fish and other marine invertebrates that feeds on toxic cyanobacteria could be in turn eaten by man which can pose a high public health risk.

Microcystins, a toxin from cyanobacteria accumulate in marine invertebrates such as mussels (Prepas *et al.*, 1997). In Mussels the highest concentration of microcystin is found in hepatopancreas, although, it is still uncertain whether the levels of microcystin accumulation are sufficient to pose a risk to humans. This may also depend on the levels of consumption.<sup>26</sup>

## Conclusion

A lot of studies have discussed the biodiversity of cyanobacteria on natural habitats. This study attempted to investigate the biodiversity of cyanobacteria on artificial habitat such as shipwreck. These accidental artificial reefs have provided opportunity to evaluate the cyanobacteria species inhabiting shipwreck structures and their possible role on the wreck. This study serves as a baseline data, the first to characterize the cyanobacteria inhabiting shipwrecks. Difference cyanobacteria found on our shipwreck substratum may not necessarily imply high bioreceptivity of the substratum since many other environmental parameters (temperature, presence of water, and sunlight) play an important role in a successful colonization. Cyanobacteria diversity and abundance is likely dependent on the availability of water that allows them form biofilm on any surface.<sup>27</sup> Future studies should aim at metagenomics characterization of *Trichodesmium* species in

shipwrecks ecosystem, defining the differences among the species and identifying the mechanisms which enables them to coexist.<sup>28-35</sup>

## Author contributions

CGD collected samples, performed experiments, analysed data, prepared figures; GCO, supervised the project and read the paper.

## Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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