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Selar crumenophthalmus, etc. photo by Kevin Bryant

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

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Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Micro-phytoplankton density and diversity at a pilot oyster culture barachois site of Mauritius Island

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Abstract. *Armance MEA, Mattan-Moorgawa S, Bhagooli R. 2019. Micro-phytoplankton density and diversity at a pilot oyster culture barachois site of Mauritius Island. Ocean Life 3: 1-12.* The density and diversity of marine micro-phytoplankton were studied at 4-6 stations from October to December 2014 within and around a pilot culture site of the oyster *Crassostrea cuculata* in the north-east of Mauritius Island to evaluate the water quality of the marine ecosystem suitable for oyster culture. Three micro-phytoplankton samples were taken at each station, and the physicochemical parameters such as temperature, pH, and salinity were measured *in situ* while turbidity was measured *ex-situ*. No bloom was observed, but a gradual increase in micro-phytoplankton density occurred from October to December 2014. In addition, the micro-phytoplankton density was positively correlated with temperature. A total of 20 genera of micro-phytoplankton were observed belonging to the classes of Baccillariophyta, Dinophyta, and Cyanophyta. The genera observed were *Bacteriastrum, Chaetoceros, Coscinodiscus, Leptocylindrus, Cylindrotheca, Nitzschia, Pseudo-nitzchia, Asterionellopsis, Licmophora, Striatella, Flagilariopsis, Navicula, Pleurosigma, Thalassionema, Dinophysis, Gymnodinium, Alexandrium, Peridinium, Nitzschia,* and *Pleurosigma* in the Bacillariophyta and Dynophyta, *Gymnodinium* and *Prorocentrum* were the dominant genera. In contrast, only *Lyngbya* was observed as Cyanophyta. Simpson's diversity index revealed an increase in species diversity from October to December 2014 at the studied stations. These findings indicate spatiotemporal variations in micro-phytoplankton density and diversity at the oyster culture site. Further long-term studies are warranted to identify the optimal stations for oyster culture.

Keywords: Baccillariophyta, *Crassostrea cuculata*, Cyanophyta, Dinophyta, micro-phytoplankton, physicochemical parameters, species diversity

INTRODUCTION

Phytoplankton is a large group of tiny drifting photosynthesizing organisms that comprises different subgroups according to their size ranges. Micro-phytoplankton size ranges from 20 µm to 200 µm (Reynolds 2006). There is a wide range of micro-phytoplankton classes around the globe, but the main ones are diatoms, dinoflagellates, and cyanobacteria. As the base of the marine food web, these microorganisms play a key ecological role in the marine environment. Moreover, the abundance of micro-phytoplankton can be used as a toolkit to evaluate the water quality of the marine ecosystem (Sagert 2008).

These photosynthesizing microorganisms depend on several factors for their growth, survival, and reproduction and directly affect their density and diversity. These factors can either be physical, chemical, or biological. The physical factors affecting the abundance of micro-phytoplankton are notably temperature, salinity, turbidity, currents, wind, light radiation, freshwater input from nearby rivers, or precipitation (Gilbes et al. 1996). The chemical factors are dissolved oxygen, pH, and nutrient availability, mainly phosphate, silicate, and nitrate (Gilbes et al. 1996). Biological factors are their interaction with other marine organisms, such as the grazing activities of zooplankton and oysters (Chung et al., 2012). Furthermore, it is known that micro-phytoplankton density differs spatially and temporally (Chandy et al. 1991; López-Flores et al. 2011; Sadally et al. 2012; 2014b).

Oysters are filter-feeding organisms that feed on phytoplankton, including micro-phytoplankton. Evidence has shown that oysters reduce the abundance of phytoplankton in the sea and thus increase water quality (Newell et al. 2007). Algal blooms are the rapid proliferation of phytoplankton on the sea surface resulting in the decrease of the penetration of light radiation to the hydrosphere. In the coastal waters of Poudre d'Or and Anse La Raie, located in the north of Mauritius, microalgal blooms have been reported, along with the death of corals (AFRC, 2009). The main impact of an algal bloom on the marine environment is that it depletes oxygen to a level that is insufficient to maintain the biodiversity of the marine ecosystem and may pose a threat to aquaculture activities and the surrounding ecological system (Kibria 2014).

Very few scientific studies have been published on the density and diversity of micro-phytoplankton at oyster culture sites in Mauritius. However, a few studies have been undertaken on micro-phytoplankton abundance and distribution within the coastal waters of Mauritius Island and at Poudre d'Or Barachois. These studies have shown that the dominant micro-phytoplankton type observed in

the Mauritius lagoon are diatoms (Modoosoodun et al., 2010; Sadally et al., 2012; Sadally et al. 2014a,b).

Both as a bioindicator and the foundation of the marine food web, it is important to analyze the abundance and diversity of this micro-phytoplankton at oyster culture sites. The abundance and diversity will result in a better understanding of the oysters' growing environment, ensuring that the oysters grown for commercial purposes have adequate food for their growth and development, resulting in a better yield. The study of micro-phytoplankton density and diversity along with the prevailing physicochemical parameters is important for comprehending the structure and dynamics of the marine biota and their trophic interactions. It is noteworthy that environmental parameters vary spatially in the islands' coastal waters. For instance, there are significant variations in seawater temperature on a coast-reef scale at some coastal sites around Mauritius Island (Bhagooli and Taleb-Hossenkhan 2012). The recurrence of high sea surface temperature anomalies will also recur more frequently around Mauritius Island (Bhagooli and Sheppard 2012). Sadally et al. (2012; 2014b) report variable micro-phytoplankton distributions at the coast-reef scale. However, spatiotemporal variations in micro-phytoplankton and environmental factors are yet to be thoroughly investigated in potential coastal barachois-based aquaculture sites around islands.

The aim of this study was to assess the effect of the physicochemical environment and spatial-temporal variations in micro-phytoplankton density and diversity within and around an oyster culture site at Poudre d'Or Barachois. The objectives of the study were to (i) measure the physicochemical parameters (seawater temperature, salinity, pH, and turbidity) at each station during each field sampling; (ii) determine the density of total micro-phytoplankton, diatoms, dinoflagellates, and cyanobacteria; (iii) analyze the spatial variation on micro-phytoplankton density (within and around the culture site); (iv) analyze the temporal variation on micro-phytoplankton density (October-December 2014); (v) correlate the physicochemical parameters and the biological parameters (total micro-phytoplankton, diatoms, dinoflagellates, and cyanobacteria densities); and (vi) analyze the taxonomic group composition of micro-phytoplankton up to generic level within and around the study site.

MATERIALS AND METHODS

Study site

Poudre d'Or is a coastal village located in the northeast of Mauritius Island, Western Indian Ocean (Figure 1A, B) (20º 4' 37" S, 57º 38' 52" E). The seawater of Poudre d'Or is usually muddy and brown throughout the year. The mouth of the river called 'Citronnier' (Figure 1A) in Poudre d'Or is quite near the sampling site. Since July 2014, a pilot project for culturing the oyster *Crassostrea cuculata* has been located in Poudre d'Or Barachois. Unfortunately, the Barachois was still under renovation when sampling was carried out, and due to this, the sample collection was restricted to only one part. Moreover, the oyster culture site was under environmental stress due to animal waste runoff from a nearby animal farm, especially during heavy rains. The GPS points and characteristics of the sampling stations are given in Table 1.

Measurement of physicochemical parameters

The temperature was monitored for three consecutive sampling months (October-December 2014). Therefore, seawater salinity, pH, and turbidity were monitored only in December 2014. The temperature was measured in situ during sample collection using a thermometer-Comark as the temperature fluctuates easily as soon as the sample is removed from its environment (Scheel 2008). The seawater temperature was measured at each station, and the mean temperature was then calculated. During this study, the pH of the seawater was measured in situ using a pH meter (Hanna H1 9024C). The pH meter was calibrated before going on the field for sample collection. pH values range from 1 to 14. Below pH 7, the water sample contains a greater number of H^+ ions compared to OH^- ions. The pH meter is a qualitative method to measure pH as it converts electrode voltage in the seawater sample to pH value. The pH value was taken five times at each station, and the mean pH value of each station was then calculated. Salinity was measured in situ using a refractometer. During the sample collection, a few drops of seawater were placed on the refractometer's glass prism. The latter measures the salinity of the seawater by refractive index. The Refractive index is the ratio of the speed of light through the vacuum to the speed of light through the seawater sample. The eyepiece of the refractometer was focused, and the refractive index was read through it (Scheel 2008).

Table 1. GPS coordinates and a brief description of the sampling stations

Stations	GPS coordinates		Brief description
	South	East	
S1	20° 03' 29.9	057° 41' 27.0	Open Coastal Sea-outside the oyster culture site (OS)
S2	20° 03' 31.0	057°41' 24.3	Flushing area between the oyster culture site and the coastal waters (FA1)
S3	20° 03' 28.5	057° 41' 24.0	Mangrove ecosystem inside the barachois of the oyster culture (ME1)
S4	20° 03’ 30.6	057° 41’ 21.6	Oyster culture site in the barachois (OC)
S5	20° 03' 28.7	057° 41' 17.7	Flushing area between the oyster culture site and another barachois (FA2)
S6	20° 03' 34.2	057° 41' 19.6	Mangrove ecosystem outside the barachois of the oyster culture (ME2)

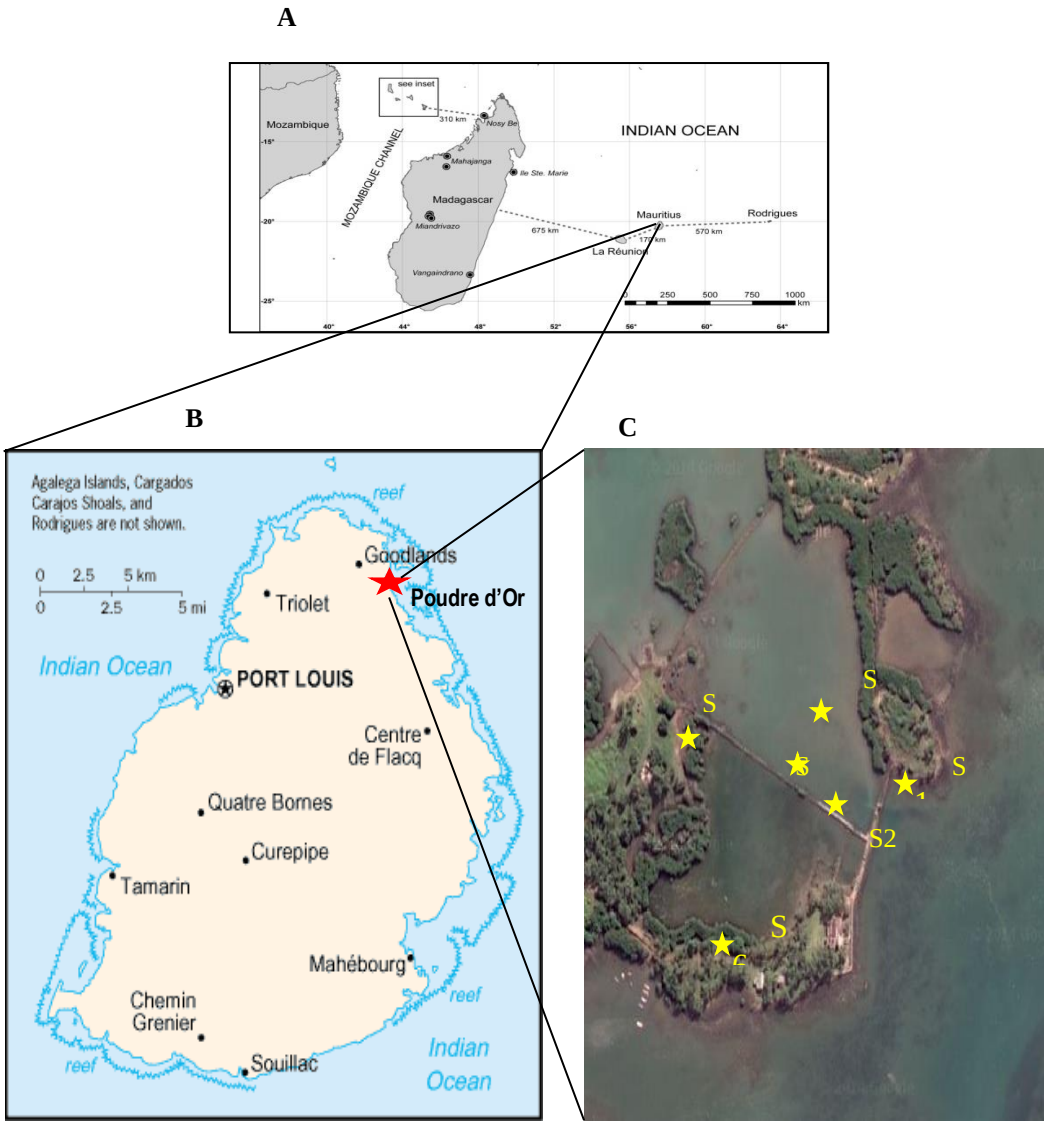

Figure 1. Map of Indian Ocean (A) (Source: Chan et al. 2011), Map of Mauritius where the red star indicates the location of Poudre d'Or (B) (Source: Exotic Mauritius 2005-2012) and the Barachois of Poudre d'Or (C) (Source: Google map 2014) where the yellow stars indicate the sampling stations. S1= Outside the oyster culture site (open sea), S2= the flushing area of the oyster culture site and the coastal waters, S3= Mangrove ecosystem inside the Barachois of the oyster culture site, S4= Oyster culture site, S5= Flushing area between the oyster culture site and another Barachois, S6= Mangrove ecosystem outside the Barachois of the oyster culture site

Five refractive indexes were taken at each station during December, and the mean indexes were then calculated. Turbidity was measured ex-situ by using a 2100A turbidimeter. In the laboratory, the turbidimeter was allowed to stabilize for 30 minutes before the water sample was put into it for reading. The water sample was poured into a small glass and then put in the turbidimeter, where the turbidity value was recorded. Turbidity values were recorded at each of the five stations during December, and the mean values were then calculated.

Micro-phytoplankton collection and processing

Micro-phytoplankton sampling was undertaken every month from October 2014 to December 2014 within and around Poudre d'Or Barachois. A plankton net was used to collect the samples. This type of net (< 20 µm; the size of micro phytoplankton) should collect plankton that is bigger than the mesh size (Verlencar and Desai 2004; Hötzel and Croome 1999). This study used a plankton net of mesh size 5 µm to collect micro-phytoplankton at the selected stations within and around the oyster culture site. Triplicate samples were taken at each station. Each micro-phytoplankton sample was obtained by filtering 10L of a seawater sample, which was taken within 0.5 m depth of the seawater surface. Lugols' solution fixed the samples (according to Hötzel and Croome 1999; Verlencar and Desai 2004) and kept in opaque 250 mL plastic bottles and stored immediately in a cold isotherm box to preserve the

samples during transportation before being taken to the laboratory for further analysis. The solution used was prepared one week before going on field sampling. The fixed phytoplankton samples were kept at 4 °C until further analysis.

Centrifugation is important to concentrate the micro-phytoplankton cells for enumeration and identification; a 250 mL sample of fixed micro-phytoplankton was divided into 5 centrifugation tubes of 50 mL. First, the tubes were centrifuged at 3500 rpm for 10 minutes using a REMI R-8CBL benchtop centrifuge). After 10 minutes, the supernatant water was decanted. After vortexing, the pellets formed from the 5 centrifugation tubes were put in a 15 mL centrifugation tube, and filtered seawater (0.22 µm) was added up to 15 mL. The samples were then allowed to centrifuge a second time at 5500 rpm for 15 minutes in a CENTROMIX P-SELECTA centrifuge. After 15 minutes, the supernatant water was decanted, and 1 mL of micro-phytoplankton was put in an aliquot tube (adapted from Sadally et al. 2014a). The aliquot tubes were then stored at 4 °C for further analysis.

Micro-phytoplankton identification was performed by using a Sedgewick rafter counting chamber of a dimension of 50 mm x 20 mm x 1 mm depth and an ocular microscope of eyepiece lens ×10 and objective lens of ×10, ×20, × 40, and ×100 (Verlencar and Desai 2004). First, 1 mL of the micro-phytoplankton sample was run from one corner of the Sedgewick rafter counting chamber using a micropipette of 1 mL. During this step, the coverslip was placed across the Sedgewick rafter counting chamber and moved to the whole chamber once the latter was filled (Hötzel and Croome 1999). Before identification, the micro-phytoplankton sample was allowed to settle into a single layer for 10 minutes in the Sedgewick rafter counting chamber. Then, the micro-phytoplankton samples were identified under the objective lens ×20 of the ocular microscope (Verlencar and Desai 2004). Finally, micro-phytoplankton were identified along the rows of the Sedgewick rafter counting chamber. Several phytoplankton manuals were used to identify the micro-phytoplankton genera (Verlencar and Desai 2004; Perry 2003; Botes 2003) of the three main micro-phytoplankton groups (diatoms, dinoflagellates, and cyanobacteria).

Micro-phytoplankton enumeration was done parallel to the micro-phytoplankton identification. The counting was done horizontally along the row of the Sedgewick rafter counting chamber from one corner to the next. This step was repeated for the second row until 100 micro-phytoplankton genera were identified (Huber 2012). The total number of quadrants was recorded to identify the 100 micro-phytoplankton genera. The average number of micro-phytoplankton cells in the 1 mL sample was calculated by multiplying the phytoplankton count (i.e., 100) with the ratio of the whole chamber of counted quadrants (modified from Verlencar and Desai 2004). The total number of phytoplankton present in 1 mL of seawater was calculated by using the following formula (Verlencar and Desai 2004):

$$N = [(n \times v)/V] \times 1000$$

Where,

N: total number of phytoplankton cells per liter of water filtered

n: average number of phytoplankton cells in 1 mL of plankton sample

v: volume of plankton concentrate (mL)

V: volume of total water filtered (L)

The diversity of the observed micro-phytoplankton genera at each sampling station for the three consecutive months was analyzed by using the following Simpson's diversity index (Oksanen 2015) as it takes into consideration both the richness and evenness of the micro-phytoplankton genera:

$$D = 1-\sum (Pi)^2 \; ; \; (Pi = Ni/N)$$

Where,

D: Simpson's diversity index

$\sum$: Means sum of all $(Pi)^2$

Pi: The proportion of individuals of genus 'i' in the community

Ni: Number of individuals of genus' i'

N: Total number of genera)

Statistical analysis

The micro-phytoplankton densities observed during this study were analyzed using the software SPSS 16.0 at a 5 % confidence interval. The data were first tested for normality by using the Shapiro-Wilk test. Then, the correlation between the physicochemical parameters and the micro-phytoplankton densities was analyzed using the Spearman correlation test due to the non-parametric data of the physicochemical parameters. Before conducting the two-way ANOVA analysis, the micro-phytoplankton densities were log-transformed. The two-way ANOVA analysis was followed by Tukey's Post hoc test for pairwise comparison of the means. Finally, a hierarchical cluster analysis (HCA) was used to explore and reveal the natural grouping of the micro-phytoplankton density among the stations each month (Ogbuagu and Ayoade, 2012).

RESULTS AND DISCUSSION

Variations in physicochemical factors

An increase in seawater temperature occurred from October to December 2014. In October 2014, the minimum seawater temperature was recorded at the OS Station (24.5 ± 0.0°C), and the maximum one was recorded at FA2 (28.2 ± 0.29°C). In November 2014, the minimum one was recorded at the ME1, and FA1 with mean temperature and SD of 28.0 ± 0.04°C and 28 ± 0.00°C, respectively, and the maximum one was recorded at OC (29.0 ± 0.00°C). In December, however, the minimum sea temperature was recorded at three stations, namely FA1, ME1, and OC, with 31.0 ± 0.23°C, 31.0 ± 0.21°C, and 31 ± 0.34, respectively. The maximum one was recorded at the other three stations: OS, FA2, and ME2, 32 ± 0.30°C, 32 ± 0.41°C, and 32 ± 0.22°C, respectively.

During the study, a narrow range of pH values was found at the sampling site. This narrow range was between 7.66± 0.05 to 7.87± 0.04. The lowest pH values were recorded at ME1 and ME2 with a pH and SD of 7.66 ± 0.05 and 7.68 ± 0.05, respectively. The highest one was recorded at the OS station with a pH and SD of 7.78 ± 0.07.

Salinities at all stations within the oyster culture site (FA1, ME1, OC, FA2) were all uniform (30 ± 0.00 ‰), while the salinities at the two stations outside the oyster culture site were different. At OS, the salinity of the seawater observed during December 2014 was 32 ± 0.35 ‰, and at ME1, the salinity in December 2014 was 28 ± 0.00 ‰. The lowest turbidity observed in December 2014 was at the OS station with 6 Nephelometric Turbidity Units (NTU) turbidity. The highest one was observed at the ME2 with a turbidity of 10 NTU.

Micro-phytoplankton density (TMPD)

A gradual increase in the TMPD was observed (Figure 2) from October to December 2014 at all stations except at OC station, where no visible differences were observed between November and December 2014. This increase in micro-phytoplankton density was apparent at the OS station, with a mean micro-phytoplankton density difference of 7.25 x 10^5 L^{-1} between October and December 2014.

The two-way ANOVA analysis (Table 2) revealed that there were significant differences in TMPD ($P < 0.001$), diatom density ($P < 0.001$), dinoflagellate density ($P < 0.001$) and cyanobacteria density ($P < 0.01$) from October to December 2014. However, only TMPD ($P < 0.05$) and dinoflagellate density ($P < 0.05$) had significant differences among stations. In addition to that, Tukey's Post hoc analysis supports the two-way ANOVA analysis by revealing that there were significant temporal differences from October 2014 to December 2014 for TMPD ($P < 0.001$), Diatom density ($P < 0.001$), and dinoflagellate density ($P < 0.001$ and $P < 0.01$). However, a significant temporal difference ($P < 0.05$) was recorded only between October and December for cyanobacteria density. Moreover, Tukey's Post hoc analysis also revealed statistical spatial variations of TMPD, diatoms density, dinoflagellates density, and cyanobacteria between certain sampling stations in October and December 2014. For example, in October spatial variation of TMPD was revealed between OS-ME1 ($P< 0.05$), FA1-ME1 ($P<0.05$) and FA2-ME2 ($P<0.05$) while in December 2014, the significant spatial variations for TMPD were between OS-OC (P 0.01), OS-FA2 ($P < 0.05$) and FA1-OC ($P < 0.01$). In addition, the hierarchical cluster dendrogram (HCD) (Figure 3) showed marked differences in micro-phytoplankton density at FA2 in October, OC in November, and CS in December 2014.

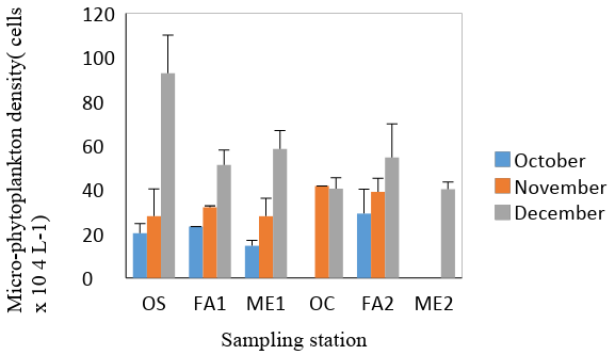

Figure 2. Total Micro-phytoplankton density (cells x 10^4 L^{-1}) for October, November, and December 2014. Data represent mean ± SD (n=3). OS= Open sea, FA1= Flushing area between the oyster culture site and the open coastal waters, ME1=Mangrove ecosystem inside the oyster culture site, OC= Oyster culture station, FA2= Flushing area between the oyster culture site to another Barachois, ME2= Mangrove ecosystem outside the oyster culture site. Data is unavailable for OC stations during October and ME2 during October and November 2014.

Table 2. Two-way ANOVA for temporal comparison (October-November 2014) and spatial comparison (S1-S6) of the micro-phytoplankton density. Asterisks indicate the significant differences at 5% level: *P< 0.05; **P<0.01; ***P<0.001

Parameters	Source	Df	SS	MS	F	P value
Total micro-phytoplankton density	Month	2	0.999	0.5	59.469	0***
	Station	5	0.119	0.024	2.827	0.036*
	Month * Station	7	0.34	0.049	5.788	0***
Diatom density	Month	2	0.89	0.445	43.633	0***
	Station	5	0.124	0.025	2.43	0.062
	Month * Station	7	0.305	0.044	4.273	0.003**
Dinoflagellate density	Month	2	3.653	1.825	37.285	0***
	Station	5	0.753	0.151	3.074	0.027*
	Month * Station	7	1.053	0.15	3.07	0.018*
Cyanobacteria density	Month	2	0.741	0.370	4.951	0.015*
	Station	5	0.407	0.081	1.087	0.391
	Month * Station	7	2.842	0.402	6.253	0.001**

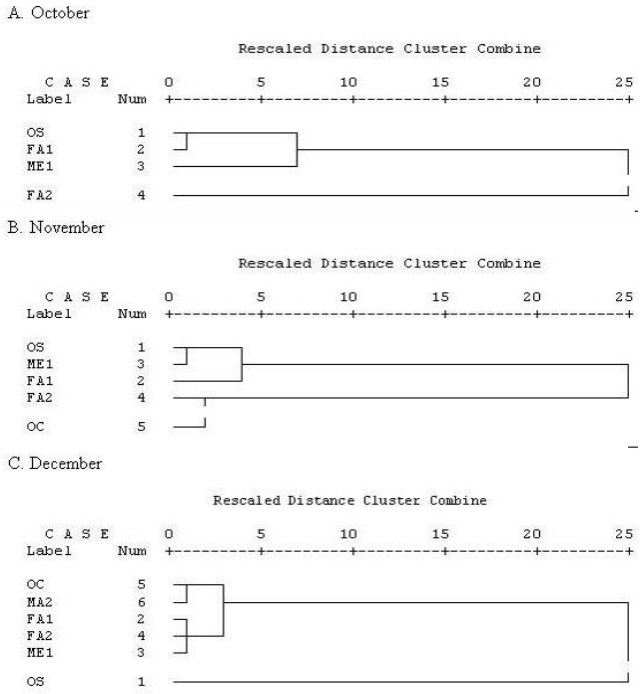

Figure 3. Hierarchical Cluster Dendrogram of the total micro-phytoplankton density from October 2014 to December 2014 at the sampling stations. OS = Open Coastal sea, FA1 = Flushing area between the oyster culture site and the open coastal waters, ME1 = Mangrove ecosystem inside the oyster culture site, OC = Oyster culture station, FA2 = Flushing area between the oyster culture site and the other Barachois, ME2 = Mangrove ecosystem outside the oyster culture site.

The dominant micro-phytoplankton observed during this study at all stations was diatom, with a percentage abundance of 87% in October 2014 (Figure 4A), 89 % in November 2014 (Figure 4B), and 83% in December 2014 (Figure 4C). Moreover, diatom density increased from October to December 2014 (Figure 5A). The lowest diatom density was observed in October at the stations ME1, OS, FA1, and FA2, respectively (Figure 5A). In addition, the highest diatom density in October and November was recorded at FA2 and December at the OS station (Figure 5A).

Low dinoflagellate densities (Figure 5B) were observed in October, November, and December 2014 at FA1 and FA2, FA1, and OC. In October and November, the lowest dinoflagellate density recorded was 1.2 x 10^4 $L^{-1,}$ whereas, in December, the lowest dinoflagellate density recorded was 3.3 x 10^4 L^{-1}. The highest dinoflagellate density for the three consecutive months was recorded at station OS with a mean density of 1.7 x 10^4 L^{-1} in October, 2.5 x 10^4 L^{-1} in November, and 11.6 x 10^4 L^{-1}in December 2014.

The lowest cyanobacteria density (Figure 5C) recorded in October, November, and December 2014 was at ME1, FA1, and OC stations with mean densities of 0.9 x 10^4 L^{-1}, 1.2 x 10^4 $L^{-1,}$ and 1.6 x 10^4 L^{-1}, respectively. On the other hand, the highest density of cyanobacteria for October, November, and December has been recorded at FA1, ME1, and OS, respectively. For example, in December, the cyanobacteria densities observed at FA1, ME1, and OS were 4.2 x 10^4 L^{-1}, 4.0 x 10^4 $L^{-1,}$ and 5.8 x 10^4 L^{-1}, respectively.

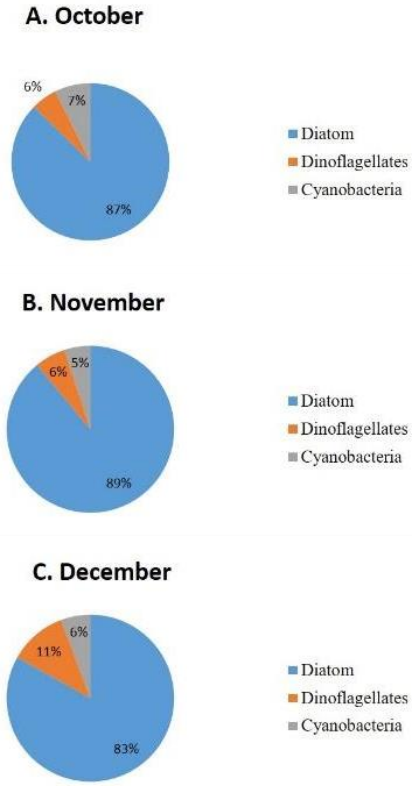

Figure 4. Percentage abundance of the three mains groups of micro-phytoplankton during October (A), November (B), and December 2014 (C)

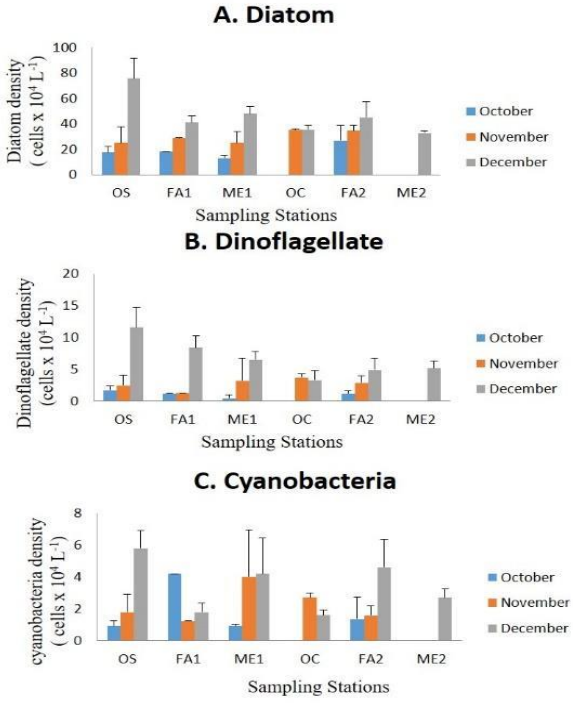

Figure 5. Distribution of the abundance of Diatom (A), Dinoflagellates (B), and Cyanobacteria (C) during October, November, and December 2014. Data represent mean ± SD (n=3). Data is unavailable for OC station during October, and for ME2, data is unavailable for October and November 2014.

Table 3. Spearman Correlation test, r-value, tested within and around the oyster culture site at Poudre d'Or Barachois from October 2014 to December 2014. Asterisks indicate significant differences at a 5% level of confidence. (*P< 0.05, ** P < 0.01, *** P < 0.001)

Parameters	Temp.	Salinity	pH	Turbidity
Total micro-phytoplankton density	0.874***	0.845*	0.319	-0.058
Diatoms	0.834**	0.845*	0.319	-0.058
Dinoflagellates	0.825**	0.507	0.464	0.029
Cyanobacteria	0.566*	0.507	0.203	0.551

Correlations between physic-chemical parameters and micro-phytoplankton density

The total micro-phytoplankton density (TMPD) ($P < 0.001$), diatom density ($P < 0.001$), dinoflagellate density ($P < 0.001$) and cyanobacteria density ($P < 0.05$) showed a strong positive correlation with seawater temperature during this study (Table 3). No significant relationship was recorded between the micro-phytoplankton densities and pH during December 2014. A significant correlation ($P < 0.05$) was recorded between the seawater salinity and the TMPD and diatom density. No significant correlation occurred between the micro-phytoplankton density and the seawater turbidity.

Micro-phytoplankton diversity

The micro-phytoplankton genera observed during this study belong to three main micro-phytoplankton groups: diatoms, dinoflagellates, and cyanobacteria. In total, 20 micro-phytoplankton genera were identified from October to December 2014 (Table 4). 14 genera of diatom were recorded, namely *Bacteriastrum, Chaetoceros, Coscinodiscus, Leptocylindrus, Cylindrotheca, Nitzschia, Pseudo-Nitzschia, Asterionellopsis, Licmophora, Striatella, Flagilariopsis, Navicula, Pleurosigma*, and *Thalassionema*. Dinophyceae, on the other hand, comprises 5 genera: *Dinophysis, Gymnodinium, Alexandrium, Prorocentrum*, and *Peridinium*. For Cyanophyceae, only one genus was detected-*Lyngbya*.

For diatoms, both pennate and centric diatoms were observed during this study. In October 2014, the dominant micro-phytoplankton genera at the OS and FA1 stations were *Pleurosigma* (21%) and *Thalassionema* (20%), respectively (Figure 6). However, at the ME1 and FA2 stations, the dominant micro-phytoplankton genus observed within that month was *Nitzschia*, with 12.5% and 29.5% abundance, respectively. In November 2014, the dominant micro-phytoplankton genus observed at the OS, FA1, and OC stations were *Nitzschia*, with 18.0%, 27.0%, and 28.5% abundance, respectively (Figure 7). At the ME1 and FA2 stations, the dominant micro-phytoplankton genera observed during that month were *Pleurosigma* (25.0%) and *Thalassionema* (22.5%). In December 2014, other micro-phytoplankton genera tended to be abundant (Figure 8). For example, the dominant micro-phytoplankton genera at the OS and OC stations were *Cylindrotheca* (13.0%) and *Bacteriastrum* (12.0%), respectively. The abundant genera observed at the ME1 and FA2 stations were *Thalassionema* (13.0%) and *Pleurosigma* (15.5%), whereas *Nitzschia* was abundant at the FA1 and ME2 stations during December 2014.

Table 5. Simpson's diversity index for micro-phytoplankton genera at the sampling stations from October to December 2014. ND represents no data available.

Month	Simpson's index					
	OS	FA1	ME1	OC	ME2	FA2
October	0.877	0.798	0.844	ND	0.847	ND
November	0.895	0.853	0.851	0.858	0.871	ND
December	0.907	0.904	0.902	0.911	0.917	0.907

Table 4. Taxonomic classification of the three main groups of micro-phytoplankton observed within and around the oyster culture site from October to December 2014

Micro-phytoplankton			
Class	**Order**	**Family**	**Genus**
Bacillariophyceae	Biddulphiales	Chaetocerotaceae	*Bacteriastrum*
			Chaetoceros
		Coscinodiscaceae	*Coscinodiscus*
		Leptocylindraceae	*Leptocylindrus*
	Bacillariales	Bacillariaceae	*Cylindrotheca*
			Nitzschia
			Pseudo-nitzschia
		Fragilariaceae	*Asterionellopsis*
			Licmophora
			Striatella
			Flagilariopsis
		Naviculaceae	*Navicula*
			Pleurosigma
		Thalassionemataceae	*Thalassionema*
			Lioloma
Dinophyceae	Dinophysiales	Dinophysiaceae	*Dinophysis*
	Gymnodiniales	Gymnodiniaceae	*Gymnodinium*
	Gonyaulocales	Goniodomataceae	*Alexandrium*
	Prorocentraceae	Prorocentraceae	*Prorocentrum*
Cyanophyceae	Oscillatoriales	Oscillatoriaceae	*Lyngbya*

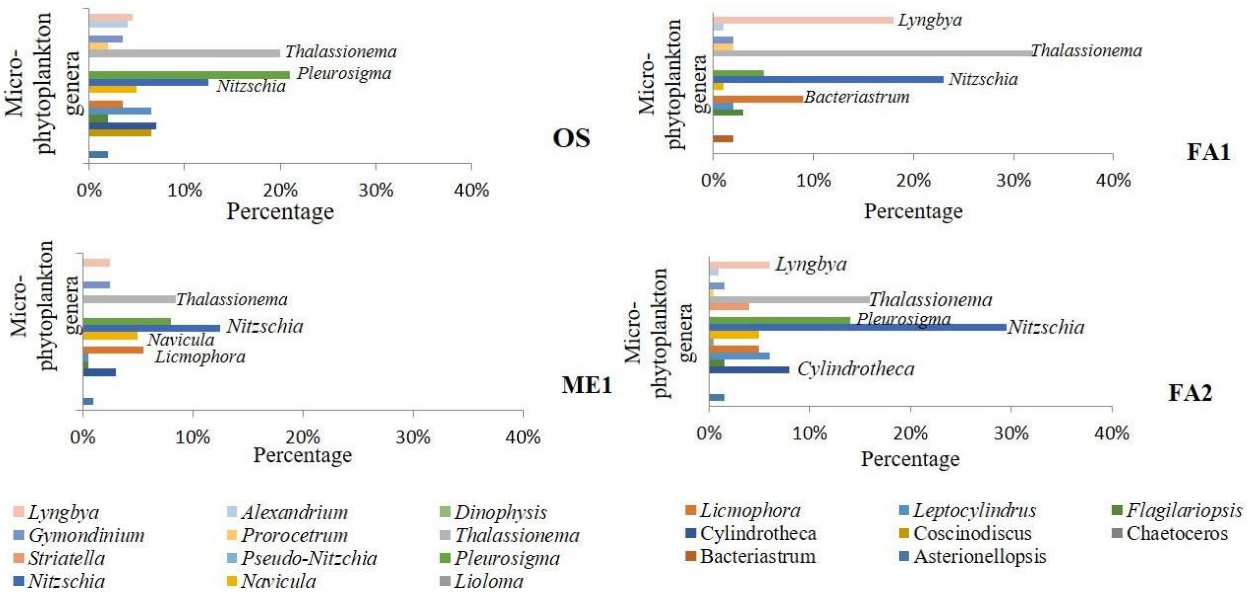

Figure 6. Micro-phytoplankton genera abundance within and outside the oyster culture site during October 2014. OS= Open Sea, FA1= Flushing area between the oyster culture site and the open sea, ME1= Mangrove ecosystem inside the oyster culture site, FA2= Flushing area between the oyster culture site and another Barachois

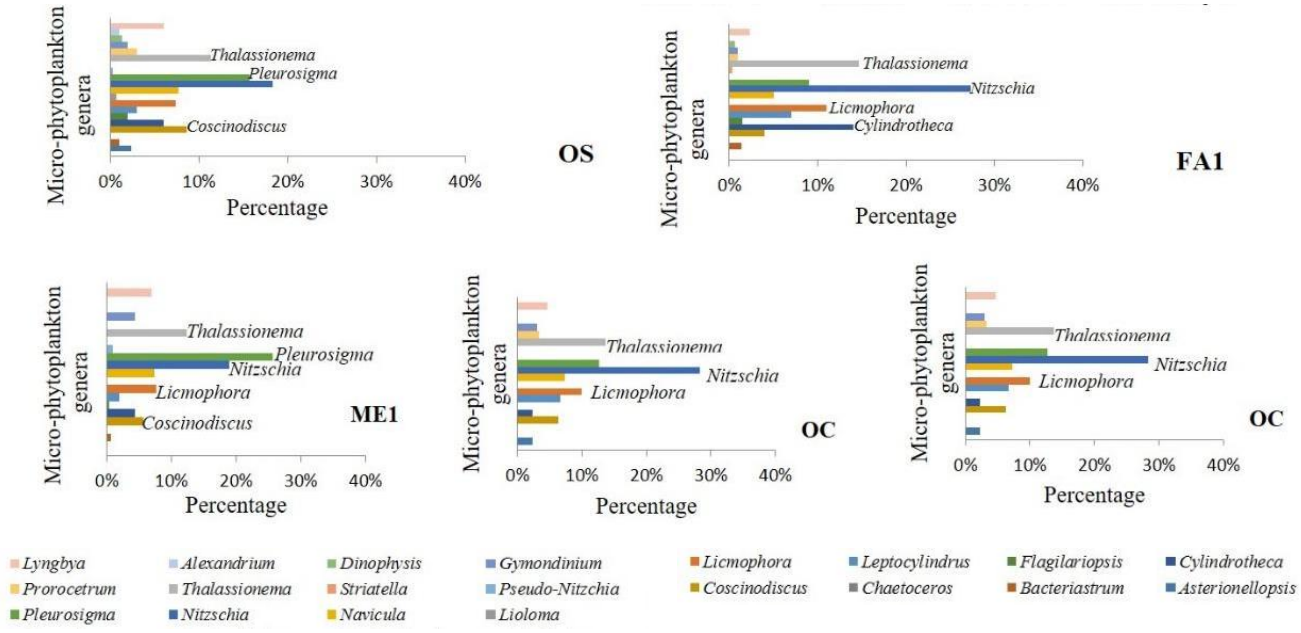

Figure 7. Micro-phytoplankton genera abundance within and outside the oyster culture site during November 2014. OS= Open Sea station, FA1= Flushing area between the oyster culture site and the open sea, ME1= Mangrove ecosystem inside the oyster culture site, D = Oyster culture station, FA2= Flushing area between the oyster culture site and another Barachois

Simpson's diversity index (Table 5) showed a high diversity of micro-phytoplankton genera during the study. It also revealed that micro-phytoplankton diversity increased slightly from October 2014 to December 2014. In October 2014, the lowest diversity was recorded at the FA1 station with a Simpson index of 0.798, and the highest during that month was recorded at the OS station with a Simpson index of 0.877. In December, however, the lowest diversity was recorded at the ME1 station with a Simpson index of 0.902, and the highest one (0.917) was recorded at the ME2 station.

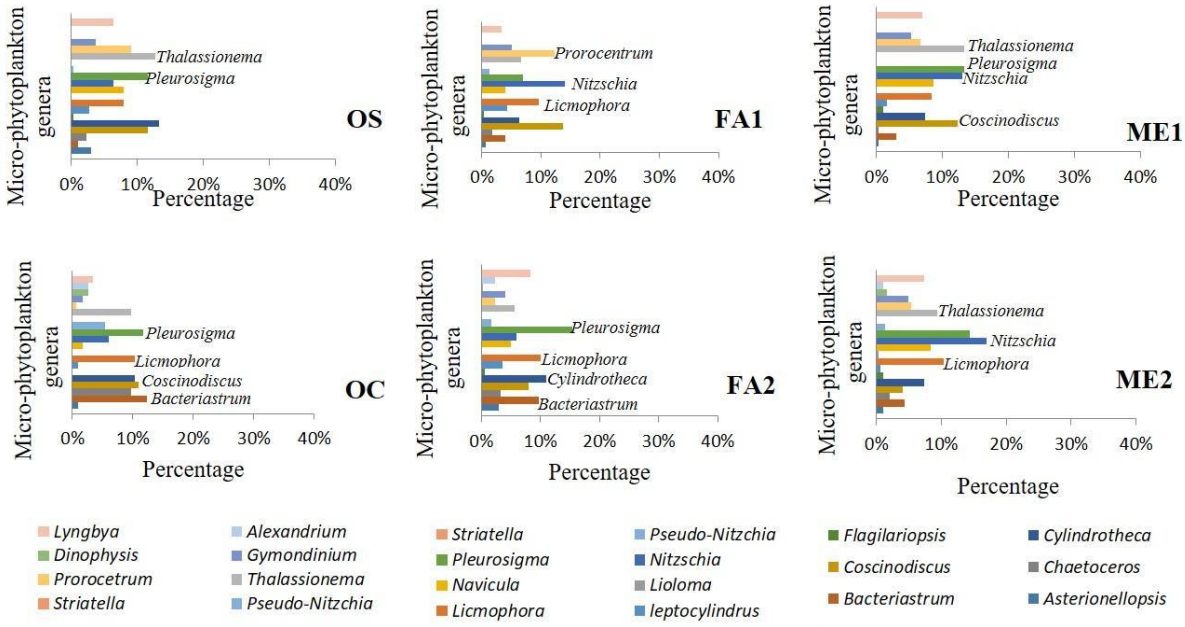

Figure 8. Micro-phytoplankton genera abundance within and outside the oyster culture site during December 2014. OS= Open Sea, FA1= Flushing area between the oyster culture site and the open sea, ME1= Mangrove ecosystem inside the oyster culture site, OC = Oyster culture, FA2= Flushing area between the oyster culture site and another Barachois, ME2 = Mangrove ecosystem outside the oyster culture site

Discussion

This study reports the first micro-phytoplankton density and diversity distribution in Mauritius's small-scale pilot oyster aquaculture farm. Both the density and diversity varied temporally among the studied months and spatially within the studied oyster farm. The implications of these findings concerning oyster farming and the surrounding marine environment are discussed.

Spatial variation of the micro-phytoplankton density

The significant spatial variation of the micro-phytoplankton density observed in October and December 2014 might result from combined factors such as nutrient availability from nearby coastal activities, seawater temperature, salinity, pH, grazing activities, and turbidity, which might differ among the different sampling stations. Previous studies have shown that variations in these parameters were significantly related to the variation of the micro-phytoplankton density (Gaul et al. 1999; Melo et al. 2007; Sahu et al. 2012; Sadally et al. 2014). Specifically, for each month (October-December 2014), the highest micro-phytoplankton density was recorded at the station exhibiting the highest seawater temperature. For example, in October 2014, the highest seawater temperature and micro-phytoplankton density were recorded at the FA2 station. Similar observations were made in November and December 2014 at the OC and OS stations. The Spearman correlation analysis further supported these observations, which revealed that the micro-phytoplankton density had a highly significant correlation with seawater temperature.

No significant spatial micro-phytoplankton variation was recorded in November 2014. The most likely explanation for this is the windy conditions in November which could have favored the mixing of the seawater. However, previous studies have shown that wind-induced the horizontal variation of micro-phytoplankton density (Moreno-Ostos et al. 2012). In addition, it was observed that during that month, the FA2 and OC stations showed a slight difference compared to the other studied stations. This observation might be since the FA2 and OC stations had a slightly higher micro-phytoplankton density than the other stations. Finally, in October 2014, a significant spatial difference in the micro-phytoplankton density between the ME1 station and the other sampling stations (OC, FA1, and FA2) was noted. This significant difference might result from the low micro-phytoplankton density at the ME1 station during that month. However, the HCD revealed FA2 was the most distant station in October 2014. The most probable reason for that is related to the micro-phytoplankton density, which was highest at the FA2 station during that month.

In contrast to the spatial variation of the micro-phytoplankton density in October, we observed that the OS station was significantly different compared to the OC and FA2 stations in December. This significant difference could be due to the high micro-phytoplankton density observed at the OS station during that month. Moreover, it was noted that OC was significantly different from the FA1 station due to the lower micro-phytoplankton density recorded at the OC station compared to the FA1 station in December.

The spatial variation of diatoms found between the ME1 and FA1 stations in October 2014 could result from a high nutrient influx from a nearby river causing increased phytoplankton density at the FA1 station compared to the ME1 station. Previous studies have found that diatoms are physiologically adapted to live in nutrient-rich environments (e.g., Wilkerson et al. 2006). The high diatom density observed at the OS station in December could be due to the oyster inside the barachois, which decreased the micro-phytoplankton abundance. The presence of oysters may have affected the micro-phytoplankton abundance (Ulanowicz and Tuttle 1992). The likely cause of the higher diatom density at the FA2 station compared to the OC and FA1 stations is the high nutrient influx from a nearby animal farm.

For dinoflagellates, the higher density at the ME1 station compared to the OS, FA1, and FA2 stations could be due to the lower abundance of diatoms observed at the ME1 station during October 2014. Previous studies have shown that heterotrophic dinoflagellates were more abundant in the presence of high diatom density (Hansen 1991; Archer et al. 2000; Verity 2002; Sherr and Sherr 2007). Therefore, the difference between some stations within the oyster culture site and the OS station in December 2014 could be related to the presence of oysters. It has been previously observed that dinoflagellate abundance was lower in the presence of oyster culture, and once the latter was removed, the dinoflagellate abundance increased (Huang et al. 2008).

The difference in cyanobacteria density recorded between the ME1 and FA1 stations could result from highly turbid water at the ME1 station. Previous studies have shown that cyanobacteria are more adapted to live in low turbid water (Karakassis et al. 1998). In addition, the spatial variation of the cyanobacteria abundance recorded in December 2014 could be due to variation in the nutrient availability. More nutrients might be available at the FA2 and OS stations during December 2014 and thus resulting in a higher abundance of cyanobacteria. Nutrient availability increases the abundance of benthic cyanobacteria (Armitage and Fong 2004).

Temporal variation of the micro-phytoplankton density

The significant temporal differences in the total micro-phytoplankton, diatom, and dinoflagellate densities observed during this study could be primarily due to an increase in seawater temperature from October to December 2014. The cause of this increase in seawater temperature might be a gradual increase in the atmospheric temperature, as seen in a previous study (Al-Banaa and Rakha 2009). Several studies have shown that temperature is directly proportional to phytoplankton abundance (Nowrouzi and Valavi, 2010; Dogiparti et al., 2013). Furthermore, evidence shows that an increase in seawater temperature also increases nutrient levels in the eutrophic zone by mixing the bottom seawater with the sea surface nutrients (Chandy et al., 1991; Huertas et al., 2011). It has also been suggested that an increase in seawater temperature promotes an increase in dissolved carbon dioxide in the marine ecosystem triggering an increase in the photosynthetic rate of the phytoplankton (Li et al., 2012).

Differences in diatom density could be due to a significant increase in the silica and iron availability each month from October to December 2014. Unfortunately, this study did not quantify iron levels. Previous studies have shown that diatom proliferation increased significantly in high silica and iron availability (Hutchins and Bruland 1997; Egge and Aknes 1992; Lasternas et al. 2008; Sadally et al. 2014b).

The most likely reason for the significant time difference observed for cyanobacteria between October and December 2014 is the significant increase in the seawater temperature and irradiance between these two months. Previous studies have shown that cyanobacteria proliferate in response to these factors (Pilkaityté and Razinkovas 2007; Blanco et al. 2008). However, no significant temporal differences were observed between October and November 2014, nor between November and December 2014. That could be due to similarity in the alkalinity (Sadally et al. 2014a,b), water turbulence (Blanco et al. 2008), turbidity (Karakassis et al. 1998), and nitrogen to phosphorus ratio (Vrede 2008) of the seawater in these months.

Patterns in micro-phytoplankton diversity

The pattern of dominance of the micro-phytoplankton groups observed in October 2014 was following the study done by Devassy and Goes (1991) in the EEZ of Mauritius. Diatom abundance was higher than cyanobacteria which were, in turn, more abundant than dinoflagellates. However, the observed micro-phytoplankton patterns in November and December 2014 (diatoms > dinoflagellates > cyanobacteria) were following recent studies done in Mauritius (Sadally et al. 2012; Sadally et al. 2014a,b).

The most likely reason for the high abundance of diatom during this study could be that only micro-phytoplankton consisting mainly of diatom and dinoflagellates (Kennish 2000) was taken into account during this study. The dominance of diatom over dinoflagellates could also result from the high light intensity at Poudre d'Or during this study. Studies have shown that dinoflagellates prefer to live in low light radiation (Jones and Gower 1990) compared to diatoms which are physiologically adapted to live in both low and high irradiance conditions (Post et al. 1985). In addition, it has been shown that dinoflagellates can move from the sea surface to the bottom zone during high light intensity due to their capability to sustain directed swimming (Jones and Gower 1990). Furthermore, the high abundance of diatoms could result from high silica and iron availability during the study. It has been shown that diatoms proliferate rapidly in the presence of these nutrients (Hutchins and Bruland 1997; Egge and Aknes 1992; Laternas et al. 2008; Sadally et al. 2014b). The low abundance of dinoflagellates could result from their lower affinity to nutrients than diatoms (Smayda 1997).

The high abundance of cyanobacteria in October 2014 compared to dinoflagellates could result from high alkalinity in October, increasing the proliferation of

cyanobacterial density. Sadally et al. (2014a) have proposed that cyanobacteria may fare better in alkaline conditions. However, in November 2014, the higher abundance of dinoflagellates compared to cyanobacteria could result from the windy conditions in November when the sample collection was carried out. The wind could have induced the upwelling of the water, thereby increasing the phosphate availability at the water surface. Previous studies have shown that phosphate is one of the main limiting factors for dinoflagellate abundance (Sadally et al. 2014a,b).

The high abundance of pennate diatoms (for example, *Nitzschia*, *Thalassionema*, and *Pleurosigma*) compared to centric diatoms in October, and November 2014 could be the result of high silicate concentration compared to nitrogen levels. Previous studies have shown that pennate diatoms can increase and compete with centric diatoms in a high silica environment (e.g., Pilkaityté and Razinkovas 2007). However, it is noteworthy that in December 2014, the centric diatoms *Coscinodiscus* and *Bacteriastrum* had quite a high abundance. This observation might be explained by an increase in the nitrogen level in December, as it has been shown previously that centric diatoms are more likely to proliferate in a nitrogen-rich environment (Pilkaityté and Razinkovas 2007).

The high abundance of the genus *Nitzschia* could also be a result of low alkalinity. *Nitzschia* has a maximum growth rate in low alkaline conditions (less than pH 8), whereas some species of *Navicula* have a maximum growth rate at pH 8 (Hinga 2012). It has also been reported that Nitzschia closterium can tolerate a wide array of salinities (Hulburt and Rodman 1963). This characteristic of the genus *Nitzchia* could be one factor that increased their abundance during this study. In addition, the increase in species diversity from October to December 2014 may be due to differences in seawater salinity as plankton diversity depends greatly on this (Kouwenberg 1994; Panda et al. 2012)

In conclusion, this study found that micro-phytoplankton density and diversity varied greatly on a short temporal (monthly) basis. Though occupying a small area, the study sites exhibited spatially variable micro-phytoplankton density and composition. Spatial variation in micro-phytoplankton density tended to be less than temporal variation. The main temporal factor affecting the micro-phytoplankton density was temperature and probably nutrient levels. In addition, the high percentage of pennate diatoms observed during this study indicated the presence of a high concentration of silica within and around the oyster culture sites and a low abundance of nitrogen. The absence of algal bloom suggested that the oyster culture sites may not be under direct bloom-causing environmental stresses. However, further in-depth quantitative and qualitative studies on the relationship of micro-phytoplankton abundance to a range of physicochemical parameters are needed to fully characterize and understand micro-phytoplankton dynamics and optimal stations for oyster farming in the North-East coastal Barachois of Poudre D'Or, Mauritius.

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Assessment of the *Holothuria atra* (Echinodermata: Holothurians) habitat based on the antibacterial effectiveness approach at Karimunjawa, Central Java Province, Indonesia

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Department of Aquatic Resources, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. Jl. Prof. H. Soedarto, SH, Tembalang, Semarang 50275, Central Java, Indonesia. Tel.: +62-24-7474698, Fax.: +62-24-7474698, ♥email: bambangsulardiono@gmail.com

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Abstract. *Sulardiono B, Anggoro S, Rudiyanti S, Rahman A. 2019. Assessment of the Holothuria atra (Echinodermata: Holothurians) habitat based on the antibacterial effectiveness approach at Karimunjawa, Central Java Province, Indonesia. Ocean Life 3: 11-17.* Sea cucumber *Holothuria atra* lives in shallow marine benthic habitats, and its life cycle depends on the quality of its environment. Bacteria in the body of sea cucumbers come from the bioaccumulation of bacteria in the sediment that they are exposed to through the absorption of sediments as a deposit feeder. While inhibiting bacterial growth in the body, sea cucumbers release bioactive compounds as antibacterial. The purpose of the study is to determine the effectiveness of sea cucumber inhibition ability of sea cucumber *H. atra* as an antibacterial in the waters of Karimunjawa. A total of 15 individual sea cucumbers were taken from the waters of Menjangan Besar and 20 individuals from the Alang-alang waters, then cleaned and dissected to take the part of the body wall. The body wall samples obtained from each observation station were as much as 1 kg, then sliced into small pieces and preserved in 96% ethanol. The bioactive compound was extracted by the maceration method. Antibacterial activity test was carried out by disc diffusion method on paper discs (10mm) and using the media of EMBA (Ethylene Methyl Blue Agar) (Merck) for *E. coli* bacteria and BPA (Baird Parker Agar) for *S. aureus* bacteria. The obtained antibacterial test results were based on the measurements of the formed clear zone diameter so that the effectiveness status of the antibacterial sea cucumbers of each observation station can be known. The study results show that the antibacterial effectiveness of sea cucumber *H. atra* in the Menjangan Besar waters is higher than in the Alang-alang waters. The increase of sea cucumber bioactive compounds in the waters of Menjangan Besar shows that the waters are suitable for the development zone of sea cucumber cultivation

Keywords: Bacteria, bioactive compound, *Holothuria atra*, Karimunjawa

INTRODUCTION

Menjangan Besar waters are included in the Karimunjawa National Park, Central Java Province, Indonesia. Based on the decision of the General Directorate of Forest Protection and Nature Conservation, No. SK.79/IV/Set-3/2005, the Menjangan Besar waters are included in the tourism and cultivation development. The Menjangan Besar waters have coral reef ecosystems and their associations and as a suitable habitat for the survival of sea cucumbers (Sulardiono et al. 2017). In addition, the Menjangan Besar Island area is a tourist destination with various activities, including floating hotels, fish cages, and sea transportation supporting tourism activities. The increasing intensity of activities in the Menjangan Besar waters has caused an increase in domestic waste so that the aquatic environment becomes vulnerable to pollution.

On the other hand, from 2013 to 2016, the waters of Menjangan Besar experienced a decline in coral reef ecosystems as a habitat of 7.92 ha (Januardi et al. 2016). Furthermore, Sulardiono et al. (2018) state that the Menjangan Besar water status is included in the oligotrophic category. Based on this, it is thought to influence the pattern of dynamics of sea cucumber life in the habitat of coral reef ecosystems. Furthermore, Sulardiono et al. (2017) state that the fertility status of the Menjangan Besar waters is oligotrophic, which means that the water fertility conditions are low to medium. Based on this, it is alleged that it will influence the pattern of the dynamics of sea cucumber life in the coral reef ecosystems' habitat in Menjangan Besar's waters. Sea cucumber *H. atra* then termed H. atra is a type of sea cucumber (Echinodermata: Holothurians) that can cope with extremely shallow aquatic environments. *H atra* sea cucumber likes habitats with various substrates. It can adapt to sand and fine sandy habitats to maintain heat by coating itself with fine sand on its entire body (Azis 1995). Dissanayake and Stefansson (2012) state that *H. atra* sea cucumbers live predominantly in shallow seagrass beds with a depth of 10 m. As they move towards the deeper sea, the *H. atra* density decreases.

Marine biota is a source of bioactive compounds with diverse natural functions, including antibacterial activity (Farjami et al., 2015). One type of marine biota containing bioactive compounds is *H. atra* sea cucumber (Lawrence et al., 2009). The same thing is said by Mayer et al. (2013) that marine biota (including sea cucumber) contains bioactive compounds with various functions, one of which is antibacterial. The study of bioactive compounds from sea cucumbers is usually widely used for developing the

pharmaceutical field, but not much for development in the ecological dimension. Sea cucumber *H. atra*, which functions as a deposit feeder that lives in shallow coastal habitats, has the potential to capture pathogenic bacteria that accumulate in sediments through its food chain system so that sediments are very good agents for microbes in the environment that enter the body of sea cucumbers. The concept is sea cucumber originating from potential waters against contamination of pathogenic bacteria is thought to affect biological activity in producing bioactive compounds, such as antibacterial, as a form of adaptation. The ecological function of bacterial-invertebrate interactions has not been widely studied. Still, microbial metabolites can play an important role in chemical defense associated with hosts and in forming symbiotic community structures. The metabolic and physiological changes experienced by these organisms in response to adaptation to extreme environmental conditions produce structural and functional production of bioactive compounds (Rizzo and Giudice 2018). Increased pollution and microbial effects on an environment are followed by the increased biological activity of sea cucumbers to produce bioactive compounds as antibacterial. Finally, the environmental quality of benthic ecosystems can be assessed from the antimicrobial properties of sea cucumbers that live in that environment. The purpose of the study is to determine the effectiveness of the inhibition ability of sea cucumber *H. atra* as an antibacterial in the waters of Karimunjawa. The principle of the antibacterial test is the formation of a clear inhibitory zone diameter, which measured the diameter of the formation of clear circles after reducing the diameter of the circle of disc paper.

Escherichia coli and *Staphylococcus aureus,* then called E. coli and S. aureus, are bacteria that can be used as indicators of the marine environment through the effectiveness test of antibacterial abilities of bioactive compounds produced from sea cucumber *H. atra*. *E. coli* bacteria is a normal flora in the human intestines that plays a role in health and lives normally. However, these bacteria can cause disease under certain conditions (Brooks et al., 2001). *E. coli* bacteria are included in the group of Gram-negative bacteria with thinner cell walls. Gram-negative bacteria cannot maintain the crystal violet dye during the Gram staining process, so that it will be red under a microscope. *E. coli* bacteria have a bacillary form with a length of about 2 micrometers and a diameter of 0.5 micrometers, are facultative anaerobes, move and live in the range temperature of 20-40 °C with an optimum 37 °C (Dwidjoseputro 1985). Under less clean environmental sanitation, these bacteria can transmit the diseases through direct contact (Radji 2011). Bacteria *S. eureus* are included in the group of gram-positive bacteria with thicker cell walls. Gram-positive bacteria can maintain methyl purple dyes during the gram coloring process. *S. aureus* bacteria have a round shape with a diameter of 0.7-1.2 micrometers, are facultative anaerobes, form no spores, and stay still most of the time (McCaig et al., 2006), growing optimally at a temperature of 37 ° C, with a room temperature of 20-25 °C. *S. aureus* bacteria can cause infectious wounds in humans (Welsh et al., 2010). Contamination is through direct contact with *S. aureus* in a wound or infection.

Experts previously regarded bioactive compounds as secondary metabolites as waste or useless things. Still, the advancing technology can turn secondary metabolism into something with many high benefits, such as antibacterial, antiviral, antioxidant, anti-inflammatory, and other benefits specifically related to pharmaceutical products. However, secondary metabolites about organisms and their environment have not been widely known, especially the effects of organisms in extreme environments related to their biological activities, which are exposed to extreme environmental effects, such as heavy metals, pathogenic bacteria, Harmful Algae Blooms (HABs) and disturbing substances others that can affect the metabolic compounds produced by an organism as an antibacterial to inhibit disturbing bacteria. Therefore, secondary metabolites that refer to microbial metabolites are defined by Bennett and Bentley (1989) as an intermediate or product that can be found in most living systems synthesized by several biochemical pathways.

Ecologically, benthic ecosystems play an important role in the life cycle of organisms. Various forms of association between microbes (including pathogenic bacteria) with other biotic and abiotic elements in one ecosystem will provide a natural balance that allows the life cycle of organisms to grow and develop naturally. Still, when the environment has begun to be contaminated by pollutants from anthropogenic sources, the organism's life cycle becomes disrupted, including the effect of sea cucumber biological activity. The principle of the antibacterial test is the formation of a clear inhibitory zone diameter, which measured the diameter of the formation of clear circles after reducing the diameter of the circle of disc paper. Usually, the study of sea cucumber biological resources was developed for pharmaceutical and health purposes, but its use for environmental aspects has not been done much, so in this study, a study of sea cucumber biological resources was developed for the use of sea cucumber habitat assessment, specifically about the effectiveness of sea cucumber antibacterial activity against *E. coli* and *S. aureus* in the Menjangan Besar waters.

MATERIALS AND METHODS

Study area

The research was conducted at Karimunjawa, Central Java Province (Figure 1), which consists of two stations, namely Menjangan Besar waters and Alang-alang waters, which are then denoted as station A and station B. The consideration of determining the two stations is based on the division of land use zones according to the Decree of the Directorate General of Forest Protection and Nature Conservation No. 79/IV/Set-3/2005 dated 30 June 2005. The Fisheries Product Technology laboratory and the Central Laboratory of Diponegoro University, Semarang, Indonesia, analyzed the test sample.

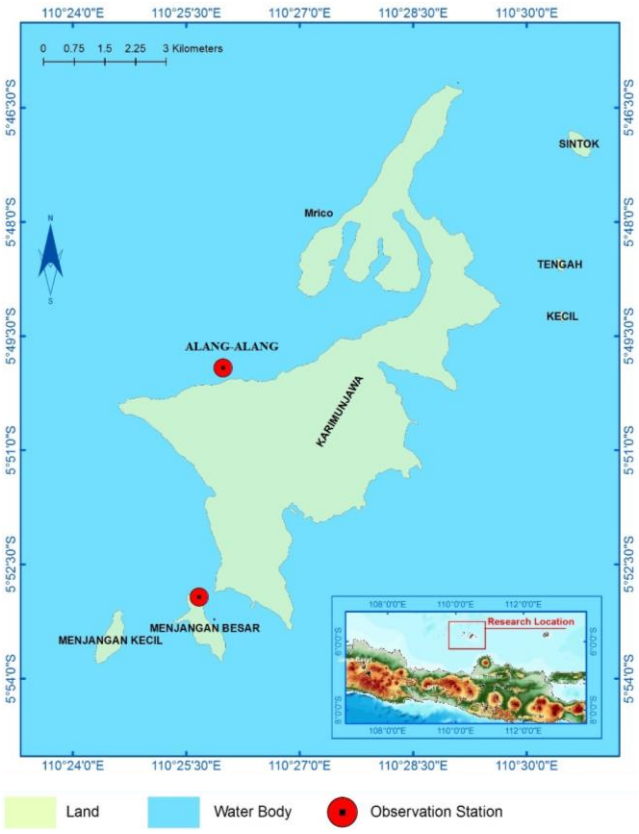

Figure 1. The observations stations in Karimunjawa, Central Java, Indonesia

The materials were samples of sea cucumber *H. atra* and chemical stuff, including aquades, ethanol 96% and 10% ammonia (Merck), chloroform (Merck), 2 N hydrochloric acid (Merck), Mayer reagents, 1 N Sodium hydroxide (Merck), Physiological Na Cl 0.9% sterile, distilled water distillate, Lieberman Buchard reagent, and spirit. The test bacteria were Escherichia coli and Staphylococcus aureus isolates, which were then referred to as *E. coli* and *S. aureus*. For bacterial growth media and the antibacterial activity test, Merck EMBA (Ethylene Methyl Blue Agar) (Merck) for *E. coli* bacteria and BPA (Baird Parker Agar) (Merck) for *S. aureus*, and Nutrient Broth are used. As a positive control, synthetic amoxicillin 500 mg antibacterial is used.

Procedures

Sea cucumber collection

The sea cucumber *H. atra* samples were obtained from 15 sea cucumbers of Menjangan Besar waters with a length of about 15-25 cm and from 20 sea cucumbers of Alang-Alang waters with a length of about 20-35 cm, which was then cleaned and inserted in a coolbox container. The samplings of sea cucumbers were by boat fishing and by diving. Habitat conditions in the waters of Menjangan Besar and Alang-alang at the time of sampling were coral reefs and their associations.

Extraction materials

Sea cucumber extraction used the maceration method with immersion in 96% ethanol solution. Using this ethanol solution is based on a study by Dwicahyani et al. (2018) informing that ethanol is the best solvent for extracting bioactive compounds in sea cucumber *H. atra*. A total of 15 individuals of sea cucumbers were taken from the waters of Menjangan Besar and 20 individuals from the Alang-alang waters. Then they were cleaned and dissected to take part of the body wall as samples were obtained. From each observation station, 1 kg of the samples were taken, sliced into small pieces, blended, and stored in 300 mL of ethanol for 72 hours. After being filtered with filter paper, they were evaporated with an evaporator at 40°C to separate the solution to obtain a crude extract.

Test of antibacterial activity

The antibacterial activity testing method uses the disc-diffusion method with 10 mm disc paper. The media used is modified EMBA (Ethylene Methyl Blue Agar) and BPA (Baird Parker Agar), taking into account that EMBA and BPA are solid media, where EMBA is selective for the type of *E. coli* bacteria by giving positive results, and BPA is more selective for *S. aureus.* Extract samples from each observation location were dissolved in distilled water with a ratio of 50 mg, 75 mg, 100 mg, and 150 mg/mL of extract material fractions. Then they were used for testing antibacterial activity against *E. coli* and *S. aureus.* Some parts were taken and put in a petri dish. In the ethanol solvent fraction, it was dripped in 25 µL into paper discs for each sea cucumber extract formula. The use of ethanol as a solvent was a negative control, each concentration was applied on *E. coli* and *S. aureus* bacterial replications, and sporicidal extract control against *E. coli* and *S. aureus* bacteria used synthetic antibacterial amoxicillin 500 mg with extract concentration.

Paper discs with a diameter of 10 mm were applied by immersing them in a solution of ethanol extract and pure isolates for ± 30 minutes. Then 50 ml of prepared solution was put into a sterile petri dish. Then the media is taken and poured into a petri dish filled with a pure culture solution of the test bacteria. The disc paper is placed on the dish's surface and pressed slowly using tweezers. Several papers are required according to prescribed treatments and replications. Next, they were incubated at 37°C for ± 24 hours. Then, the inhibitory zone was measured using the calipers. The area of the inhibitor zone is reduced by the diameter of the disc and the diameter of the solvent inhibition zone. Based on observations, the inhibitory zone is a clear distribution area of the inhibition. The data from the three replications were used as indicators of antibacterial effectiveness that show the strength of bacterial inhibition and refer to the criteria of Greenwood (1995), namely: Ø <5 mm = weak, Ø 5-10 mm = moderate, Ø 10-20 ml = strong, and Ø> 20 = very strong against antibacterial. As a comparison, the control of the positive extract against *E. coli* and *S. aureus* bacteria used synthetic antibacterial amoxicillin 500 mg with the same extract concentration.

The measurement results of the effectiveness of sea cucumber inhibitions between the observation stations of the Menjangan Besar waters and Alang-alang waters were compared, and the results of the measurement of inhibitory effectiveness between the Menjangan Besar waters and Alang-alang waters against positive control using synthetic antibiotics amoxicillin 500 mg were also compared. Thus it represented habitat conditions in Menjangan waters compared to other waters based on the criteria for environmental characteristics based on secondary data at the related location.

Data analysis

Data is compiled using Microsoft SPSS 17.0 software. ANOVA test was used to find out the difference in the effectiveness of H. atra sea cucumbers' antibacterial inhibitory at stations A and B by comparing the diameter of the disc paper used in each concentration in both *E coli* and *S. aureus* test bacteria on both observation stations A and B.

RESULTS AND DISCUSSION

Antibacterial activity

The results of the inhibitory test based on the measurement of the diameter of the inhibitory zone or clear zone on the disc paper can be seen in Table 1. Table 1 shows that the test for the extract of sea cucumber *H. atra* against *E. coli* and *S. aureus* in various concentrations at Station A showed the same inhibitory pattern, meaning that an increase in extract concentration tends to cause an increase in the inhibitory zone value. Likewise, treatment of *E. coli*, *S. aureus*, and positive controls with 500 mg synthetic antibacterial amoxicillin showed the same pattern of inhibitory ability.

Qualitatively, the value of measuring the inhibitory zone diameter of sea cucumber extract *H. atra* against *S. aureus* in all treatments was higher than the treatment against *E. coli* bacteria. Still, the diameter of the sea cucumber inhibition zone in Menjangan waters was greater than the diameter inhibitory zone in Alang-alang waters. Based on the criteria of Greenwood (1995), the measurement result at both stations shows the same category of inhibitory effectiveness, namely moderate. Suppose the treatment of sea cucumber H. atra extract at the two observation stations was compared with the treatment of positive control of amoxicillin 500 mg. In that case, the value of the diameter of the inhibitory zone in sea cucumbers from the Menjangan Besar waters and the Alang-alang waters appears to be lower than that in the positive control. The control treatment was compared with the optimal antibacterial effectiveness following the standards for pharmaceutical needs. The results obtained in the inhibitory effectiveness category were strong to very strong against E. coli bacteria and very strong against S. aureus.

Based on the two-way ANOVA test at a 99% confidence level (α = 0,001), and if the test results are less than 0.001, it can be concluded that there are very significant differences between treatments. Vice versa, if the P value < 0.001 is obtained between treatments, there was no significant difference. Between the inhibition zone diameter values at station A and station B at various concentrations, a very significant difference with the *P-value* = 8.1E-20 <0.001 was shown in Table 2.

Table 2. ANOVA test results for inhibition of *H. atra* sea cucumbers on *E. coli* and *S. eureus* bacteria in Menjangan Besar waters (Station A) and Alang-alang waters. (Station B), Karimunjawa, Indonesia

Treatment	*P value* (α = 0.001)
Station A against station B	8.1E-20
Station A against positive control	6.8E-27
Station B against positive control	6.3E-33

Table 1. Data on measuring the antibacterial activity of sea cucumber *H. atra* against *E. coli* and *S. eureus* at the Menjangan Besar waters (station A) and Alang-alang waters (station B).

Concentration (mg/mL)	The average diameter of the inhibitory zone extract and positive control					
	Station A		Station B		Positive control (amoxicillin 500 mg)	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
50	4.28 ± 0.12	5.22 ± 0.14	3.75 ± 0.10	4.2 ± 0.80	16.33 ± 0.25	19.42 ± 0.21
75	5.82 ± 0.15	7.28 ± 0.15	4,40 ± 0.13	6,15 ± 0.1	17.55 ± 0.22	21.23 ± 0.20
100	8.22 ± 0.15	10.20 ± 0.13	7.13 ± 0.10	8.85 ± 0.1	19.28 ± 0.22	22.68 ± 0.32
125	10.22 ± 0.15	12.19 ± 0.15	8.81 ± 0.15	10.78 ± 0.15	21.51 ± 0.21	24.98 ± 0.20
150	11.83 ± 0.13	13.85 ± 0.10	10.9 ± 0.13	12.26 ± 0.17	23.26 ±v0.22	27.20 ± 0.21
Inhibitory effectiveness (Greenwood 1995)	Moderate	Moderate	Moderate	Moderate	Strong to very strong	Very strong

Discussion

Although the antibacterial activity of sea cucumber *H. atra* against *E. coli* and *S. aureus* in both the Menjangan Besar waters and Alang-alang waters were classified as moderate in its inhibiting ability, statistically, there were significant differences in both locations, where the potential inhibition ability of sea cucumber from the Menjangan Besar waters is greater than the potential inhibition ability of sea cucumber from Alang-alang waters. The environmental conditions in which sea cucumbers live are important aspects that must be known. Ecologically, sea cucumbers are soft-bodied echinoderms that live in the waters as their habitat. In the life cycle of sea cucumbers as sedimentary animals, they use benthic biomass as a source of food and play an important role in recycling nutrient sediments (Schneider et al. 2011), which is known as a "bioturbation" process. According to Uthicke (1999), sea cucumber *H. atra* can recycle as many as 4,600 kg of dry sediment/year. Sediments absorbed by sea cucumbers as deposit feeders provide potential as agents of pathogenic bacteria originating from polluted environments. The accumulation of bacteria entering the sea cucumber's body provides physiological effects through its biological activity by releasing the bioactive compounds produced by them.

The differences in the ability of sea cucumber *H. atra* to inhibit the growth of *E. coli* and *S. aureus* in the Menjangan Besar waters and Alang-alang waters can be explained from several aspects, including the aspects of the aquatic environment and the biophysical-chemical processes that occurred in extreme and/or polluted water environment conditions, sea cucumber *H. atra* tends to carry out biological activities to produce bioactive compounds such as antibacterial. Bioactive compounds are a form of secondary metabolic compounds that defend themselves from unfavorable environmental conditions (Verpoorte and Alfermann 2000). Furthermore, it is said that secondary metabolites are characterized by very large chemical diversity, and each organism has its own set of secondary metabolites, some of which they can share with related or unrelated organisms anymore. For example, the bioactive compounds produced by sea cucumber organisms are a form of secondary compounds related to the physiological processes of sea cucumbers whose responses are related to the environmental effects. According to Giudice and Rizzo (2018), metabolic and physiological changes by organisms is an adaptation response to extreme conditions resulting in structural and functional bioactive compounds. According to Harper et al. (2001), secondary metabolites are adaptive and function as host defenses against pathogens, parasites, predators, competitors, and epibiota.

The results showed that the sea cucumber *H. atra* inhibition zone against *S. aureus* bacteria from both Menjangan Besar waters and Alang-alang waters was higher than the sea cucumber inhibition zone against *E. coli* bacteria, which indicates that the antibacterial ability of sea cucumber *H. atra* to *S. aureus bacteria* is stronger than that of *E. coli* bacteria. Bacteria *S. aureus*, as Gram-positive bacteria, are more powerful at attacking fish, including sea cucumbers in the benthic ecosystem. *S. aureus* bacteria are Gram-positive pathogenic bacteria (Woodford and Livermore 2009), which have the potential to attack sea cucumbers. That is because the *S. aureus* bacteria are Gram-positive bacteria which are more dangerous than *E. coli* as Gram-negative. The outer membrane on the cell wall of the *S. eureus* bacteria can protect the bacteria and host defense system so that it inhibits the bioactive compounds produced by sea cucumbers.

However, when viewed based on the observation station, the diameter size produced on sea cucumbers originating from the Menjangan Besar waters of sea cucumber *H. atra* extract against *E. coli* and *S. aureus* was higher than sea cucumber from Alang-alang waters. The high average value of sea cucumbers originating from the Menjangan Besar waters indicates that the bioactive compounds produced by sea cucumbers from the Menjangan Besar waters are higher than sea cucumbers from Alang-alang waters. The interesting thing from the results of this study is the antibacterial enhancement of sea cucumber *H. atra* in the Menjangan Besar marine habitat as a tourism development zone, and this cultivation is thought to be due to the presence of pathogenic bacteria accumulating in the body is more dominant than sea cucumbers from Alang-alang waters as a rehabilitation zone.

The effectiveness of antibacterial sea cucumber *H. atra* from Menjangan Besar waters relates to the environmental conditions of the sea cucumber habitat as a unit in the benthic ecosystem in which the sea cucumber habitat is located. The decrease in environmental quality in the Menjangan Besar waters is thought to be the effective cause of sea cucumbers producing antibacterial bioactive compounds as a form of sea cucumber adaptation in accepting these changes in environmental quality. As it is known that the Menjangan Besar waters are a tourism and marine culture development zone with various activities, these waters are thought to have deterioration in the quality of the aquatic environment. That is supported by a previous study by Sulardiono et al. (2018) on location in Menjangan Besar waters, in which water quality status tends to be an oligotrophic or low-quality category. Different things happen to sea cucumbers from the waters of Alang-alang, which the waters are included in the rehabilitation zone. That is supported by the results of statistical tests that there is a very significant difference in the effectiveness of the antibacterial inhibition of sea cucumber *H. atra* against the test bacteria in Menjangan Besar waters and Alang-alang waters. This significant difference also occurs in the Menjangan Besar and Alang-alang waters against the positive control of amoxicillin 500 mg.

The onset of the disease can be caused by the emergence of new pathogens or changes in the environment (Burge et al., 2014; Harvell et al., 2004). Sea cucumber *H. atra* living in the benthic ecosystem will certainly be affected by a natural balance system (Tjokrokusumo, 2006). The natural balance will be formed if all the elements involved mutually support one another. Microbes are one of the biotic elements in an ecosystem that can play a role in such a system, in which the type and

number of microbes are highly dependent on their environmental conditions, and microbes can play a positive or negative role. Decreasing environmental quality as a benthic ecosystem will change the life order of organisms, including changes in the pattern of biological activity of sea cucumber *H. atra* in their habitat, in which sea cucumbers make physiological adaptations to defend themselves against the attack of pathogenic bacteria that accumulate in their bodies by producing bioactive compounds as an antibacterial against pathogenic bacteria. It is known that pathogenic bacteria can appear in unhealthy or polluted aquatic environments. According to Slater and Jeffs (2010), the characteristics of the basic sediment are one of the important components that influence sea cucumber habitat preferences. Bioaccumulation of pollutants and/or pathogenic bacteria in sea cucumbers' bodies occurs through a consuming process as a deposit feeder. As benthic deposit feeder animals (Uthieke, 2001), sea cucumbers capture sediments as bacterial agents entering and accumulating in their intestines to grow and develop so that they can interfere with sea cucumber health. This increase in pathogenic bacteria was followed by an increase in the production of bioactive compounds by sea cucumber *H. atra*. Related to the relationship between bacteria and the production of this bioactive compound, Kamarudin, and Rehan (2018) compared the pathogenic bacteria in the intestine of sea cucumber *S. horrens* and *H. leucospilota*. The result showed that pathogenic bacteria in S. horrens were higher than in sea cucumber H. leucospilota. The high pathogenic bacteria in sea cucumber *S. horrens* pushed the potential for producing more bioactive compounds by sea cucumbers to a higher level.

In conclusion, based on the results of this study, it can be concluded that at the two observation sites, the antibacterial activity of sea cucumber *H. atra* against *S. eureus* bacteria was higher than that against *E. coli* bacteria. Therefore, statistically, there is a very significant difference in sea cucumber antibacterial activity between one the waters of Menjangan Besar and Alang-alang waters, in which antibacterial activity of sea cucumbers originating from the waters of Menjangan Besar is higher than sea cucumbers from the Alang-alang waters. Furthermore, it is also shown that sea cucumber *H. atra* from the Menjangan Besar waters gave a more effective response to produce bioactive compounds to inhibit *E. coli* and *S. aureus* bacteria. This condition is different from sea cucumbers originating from the Alang-alang aquatic environment. Given that the potential of sea cucumber bioactive compounds from the Menjangan Besar waters is higher than that of sea cucumbers from Alang-alang waters, the potential of sea cucumber *H. atra* in the Menjangan Besar waters can be used as an alternative in developing commercial sea cucumbers.

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Exploration of the collagen of non commercial sea cucumber *Holothuria atra* and commercial sea cucumber *Stichopus vastus* in the Karimunjawa Islands, Indonesia

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Abstract. *Yuniati R, Sulardiono B. 2019. Exploration of the collagen of non commercial sea cucumber* Holothuria *atra and commercial sea cucumber* Stichopus vastus *in the Karimunjawa Islands, Indonesia. Ocean Life 3: 18-23.* The commercial value of sea cucumber needs to be explored because of the availability of bioactive compounds that play an important role in biological activities, including collagen. However, based on the content of compounds, each species has a variety of values. Therefore, this study aims to determine the partial characteristics of collagen content of sea cucumber *Holothuria atra* as non-commercial sea cucumber and of sea cucumber *Stichopus vastus* as commercial sea cucumber in Karimunjawa, Central Java Province, Indonesia. The preparation stage includes sampling sea cucumbers H. atra and S. vastus, cleaning and washing, soaking in distilled water and alcohol, in a solution of Tris-HCl and ethylenediaminetetraacetate (EDTA) and, at last, in sodium hydroxide (NaOH) solution. Then, the extraction continued with the immersion of 0.5 M acetic acid solution and the isolation by precipitation in NaCl and dialysis solutions. In conclusion, the yield extract of *H. atra* and *S. vastus* had no different values, namely 0.88%, and 0.92%, and was classified as low. Furthermore, the whiteness degree of *H. atra* collagen was 61.60 ± 0.57%, which was higher than that of sea cucumber *S. vastus* which was 20 ± 1.00%. The functional group of sea cucumber *H. atra* consists of an amide with a wave number of 3,460.96 cm^{-1} and an alkene with a wave of 1,636.77 cm. In contrast, in the functional collagen group, sea cucumber *S. vastus* contains amide with a wave absorption number of 3,434.28 cm^{-1} and an alkene with a wave absorption number of 1639.46 cm-1 strong absorption peak at 1,033.85 $cm^{-1,}$ indicating the presence of free (C = O) bonds. The data and information obtained are used for the biological resource management plan in Karimunjawa.

Keywords: Collagen, *Holothuria atra*, Karimunjawa, *Stichopus vastus*

Abbreviations: FTIR: Fourier Transform Infra Red, EDTA: Trishcl Ethylenediamine Tetra Acetate, NaCl: Sodium chloride, NaOH: Sodium Hydroxide, KBr: Potassium Bromide, HCl: Hydrochloric Acid, TEMED: Tetra Methyl Ethylene Diamine, APS: Ammonium Persulfate, CBB: coomassie brilliant blue, BME: Betamercaptoethanol, PDA: Photodiode Array Detector, pH: The degree of acidity.

INTRODUCTION

Sea cucumbers are benthic that live in coral reef ecosystems and their associations in shallow waters to the deep sea, move slowly (Friedman et al. 2008), and have a relatively long life span (average 5-10 years) (Purcell 2009). Indonesia has 23 species of them that have been identified (Sendih and Gunawan 2006), and the ones with the highest economic values were found in the genera Holothuria, Muelleria, and Stichopus. Captured sea cucumbers are generally included in the aspidochirotida family, with 7 genera (Bruckner et al. 2003), including *S. vastus* and *H. atra*. Species *S. vastus* belong to the genera Stichopus (Setyastuti and Purwati 2015), and it is classified as a commercial species (Purcell et al. 2012), while *H. atra* species belong to Holothurian genera that live on shallow coastal coral reef ecosystems and muddy sand habitats. These species found in Karimunjawa are not used optimally and are classified as non-commercial. The species are also found in Palau, and in Palau, they are not used economically and belong to the group of non-commercial sea cucumbers (Pakoa et al. 2014).

Sea cucumbers are known to contain bioactive compounds that have benefits for health and pharmaceutical production. Various bioactive compounds from sea cucumbers explored in marine waters are widely developed, including collagen compounds. However, the development of integrated bioactive compounds as resource conservation and promising economic benefit has not been widely developed, especially in developing non-commercial sea cucumbers in Karimunjawa, Central Java Province. The Sea cucumber of *S. vastus* is exploited as a commercial sea cucumber because this species has useful bioactive compounds for health and pharmaceutical products, including collagen compounds. On the other hand, Karimunjawa sea cucumber *H. atra* is not exploited optimally even though Sea Cucumber (including *H. atra*) contains 80% collagen (Lubis et al. 2016), which has a consequence, namely, the decrease of the commercial sea cucumber catch target. Therefore, it is necessary to develop

non-commercial sea cucumbers; one type of non-commercial sea cucumber in Karimunjawa is *H. atra*. In this study, the function of sea cucumber *H. atra* is as a non-commercial sea cucumber. In contrast, *S. vastus* functions as a commercial sea cucumber in Karimunjawa so that the goal of sustainable management and conservation of resources can be achieved.

Collagen is a fibrous protein and is a major component of connective tissue (Lodish et al. 2000), which has the chemical structure $C_{102}H_{149}N_{31}O_{38}$ (Ogawa et al. 2004) and is a structural protein found in many marine resources (Silva et al. 2014). Sea cucumber collagen has the potential as a substitute for collagen from mammalian sources (Siddiqui et al., 2013), which is known to be harmful to humans due to the effects of infected diseases. Based on this, a preliminary study is needed to determine the qualitative characteristics of sea cucumber collagen *H. atra* as a non-commercial sea cucumber and, as a comparative study, also the characteristics of *S. vastus*, which were found in Karimunjawa. Several variables are used to see the partial characteristics of sea cucumber collagen, including pH, whiteness degree (color description), and functional groups. The study aimed to obtain information about the quality characteristics of sea cucumber collagen *H. atra* as a non-commercial group and sea cucumber *S. vastus* as a commercial group in Karimunjawa. The study's results were utilized as a database for managing sea cucumber resources.

MATERIALS AND METHODS

Materials and equipment

The material used in the study was sea cucumber *H. atra* and *S. vastus* taken from Karimunjawa waters. Sixty individuals of *H. atra* (non-commercial species) and 35 individuals of *S. vastus* (commercial species) were caught by fishermen from Karimunjawa waters. Equipment for the extraction and isolation of collagen were cutting boards, knives, scales, beaker glasses, magnetic stirrers, freeze dryers, refrigerated showcases, and refrigerated centrifuges, while for collagen testing/characterization were oven, furnace, kjeltec, soxhlet, electrophoresis, FTIR (Fourier transform infrared) spectrophotometers from Perkin Elmer, Spectrum One, pH meters, and Color Flex EZ Hunter Lab.

Analysis of collagen extract

Sea cucumber samples were dissected from Karimunjawa waters, and the visceral and body walls were separated. The body walls were taken, cleaned, and thawed, then inserted into the coolbox to be taken to the laboratory. Stages of extraction follow the method of Fawzya et al. (2012). Extraction was performed by immersing the sample in 10 parts volume of 0.5 M acetic acid for 2 days and then filtered using a calico cloth. To precipitate the filtrate containing collagen by adding NaCl salt to the final concentration of 1 M and stored overnight, then centrifuged for 60 minutes. Centrifuged pellets were dissolved in 0.5 M acetic acid. They removed salts in the process of preparation and extraction of collagen. Dialysis was carried out with 0.1 M acetate buffer for 1 night, which was replaced every 4 hours, in which at the last replacement, distilled water was used. By replacing it, the salt molecules diffuse out of the dialysis bag so that the collagen protein obtained is purer and is then dried through centrifugation. In the next step, the measurements of collagen extract yield, pH, whiteness degrees, and amino acid functional groups were analyzed. The measurement of extract yield percentage was intended to determine collagen levels based on the collagen weight produced by comparing the weight (gram) and dry weight of sea cucumber samples (grams). The percentage of extract yield was achieved by comparing the final results or the results of drying the frozen extract (after freeze-drying) with the initial weight of sea cucumber samples (%). The degree of acidity (pH) of the collagen solution was measured using a pH meter

Collagen isolation

Collagen isolation was carried out following the modification method by Fawzya et al. (2012). All extraction stages were carried out at 4°C. The steps were washing the sea cucumber, meat taking, cutting into small pieces, and soaking in aquades (1: 10 b / v) 2 times while stirring using a stirrer for 30 minutes. First, clean sea cucumber meat from the remains of stomach contents and other impurities. Next was the replacement of distilled water with 50% alcohol (1: 2), stirred for 30 minutes to remove fat; then the washing of it with distilled water until the pH was neutral. Subsequent immersion used 10 parts of the mixed volume of Tris-HCl 0.1 M and 4 mM EDTA for 1 night. Soaking was intended to maintain pH stability and reduce minerals. After washing with distilled water, the marinade solution was replaced with 10 parts volume of 0.1 M NaOH for 2 days to eliminate non-collagen proteins and then was washed again with distilled water. Soaking with NaOH was done until the solution marinade contained no protein, which was checked using the Biuret test.

The whiteness degree (color description) was measured through digital colorimetry using ColorFlex EZ Hunter-Lab, so the values of L, a, and b (%) were obtained. The whiteness degree classification (color description) used the CIE-L * a * b system, where the L * value showed the brightness of the color, where L * = 0 for black and L * = 100 for white. The CIE_a * dimension showed the type of green - red, where negative numbers a * indicated green and vice versa CIE_a * positive indicated red, the CIE_b * for blue - yellow, where negative numbers b * indicated blue and vice versa CIE_b * positive indicated yellow (Hutchings 1999). The color differences in both collagen *H. atra* and *S. vastus* extracts were analyzed by the following equation: $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$.

Characterize collagen functional groups using the Fourier Transform Infra Red (FTIR) method (Munyonga et al. 2004) with a spectrophotometer Perkin Elmer, spectrum one. First, the sample was added by KBr (1: 100), then smoothed until evenly mixed. The next step was pressing with a vacuum pump for 15 minutes and reading the absorbance at wave numbers 500-3000 cm^{-1}. Finally, based

on the formed curve, the type of bond and its functional group were determined using FTIR references.

RESULTS AND DISCUSSION

Identification of sea cucumber

The test sea cucumber sample was *H. atra* (Echinoderm: holothuridea). Based on the result of observations in the field, it is shown that this type of sea cucumber spreads on the shores of Karimunjawa waters, which morphologically has a cylindrical body shape. In general, sea cucumbers in Karimunjawa have a black body that turns red when they are held by hand in living conditions, and the walls are relatively thin. In Karimunjawa, sea cucumber *H. atra* is not classified as economically important and is commercially relatively inexpensive, so it is classified into non-commercial sea cucumbers. Meanwhile, the sea cucumber *S. vastus* (Echinoderm: stichopodidae) observed has a soft body, blackish white dorsal parts, wide papilla spread, and irregular fold formation. The tip of the papilla protrusion is clear white. The stomach part of the skin is white, and there are neatly arranged brown tube legs in 3 rows. When the sea cucumber of *S. vastus* is touched and/or disturbed, the sea cucumber removes all the contents of its innards being thrown out. Sea cucumbers *S. vastus* have been captured by many local people because the price is quite good, so sea cucumber *S. vastus* is grouped into commercial sea cucumbers in Karimunjawa and is known as *gametes.*

Extract analysis

The result of the extracted analysis showed that the extract yield of *H. atra* was 0.88%, with a moisture content of 0.12%, and with the appearance of blackish white. On the other hand, the yield analysis of *S. vastus* extracts obtained a value of 0.92% with a moisture content of 0.17%. The color appearance of *H. atra* is blackish white, and *S. vastus* is dull grayish white. The measurement result of acidity degree (pH) of collagen extract in both species showed the same value, equal to 6.00. The results of the study can be seen in Table 1.

The whiteness degree

The collagen analysis results of the degree of whiteness (color description) were 61.60 ± 0.57% in *H. atra* and 20 ± 1,00% in *S. vastus* (Table 2). The difference in whiteness degree between the two collagen extracts can be seen through the equation of $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$, so that the value of $\Delta E = 55.87$. Based on this equation, it could be seen that the difference in the color of collagen extract of *H.atra* and *S. vastus* is 55.87. Therefore, the analysis showed that *H. atra* collagen (61.60 ±0.57%) was brighter than *S. vastus* collagen (20.00± 1.00%).

Table 1. Yield content, Moisture content, pH, and color appearance of *H atra* and *S. vastus* from Karimunjawa, Central Java Province, Indonesia

Economic value	Species name	Extract yield content (%)	Moisture content (%)	pH	Color of extract appearance
Non commercial	*H. atra*	0.88	0.12	6.00	Blackish white
Commercial	*S. vastus*	0.92	0.17	6.00	Dull grayish white

Table 2. The whiteness degree (color description) of *H. atra* and *S. vastus* collagen from Karimunjawa, Central Java, Indonesia

Sample	Value (%)		
	L	a	b
H. atra	62.00	-16	17.0
	62.00	-15	16.0
	61.00	-15	16.0
Average	61.60	15.3	16.3
SD	0.57		
S. vastus	21.00	-7	15.0
	20.00	-7	13.0
	19.00	-7	13.0
Average	20.00	-7	13.6
SD	1.00		

Note: SD: Standard deviation

Functional group of collagen

The result of partial characterization of sea cucumber collagen compounds can be seen in Figure 1 for sea cucumber *H. atra* and Figure 2 for sea cucumber *S. vastus*. The interpretation of the data showed that the functional compound group of *H. atra* collagen consists of an amide group (N=H bonds) with a wave absorption number of 3,460.96 cm^{-1} and an alkene group (C= C bonds) with a wave absorption number of 1,636.77 cm^{-1}.

Figure 2 indicated that *S. vastus* functional compound group consists of an amide group (N = H bonds) with a wave absorption number of 3434.28 cm^{-1} and alkene groups (C=C) bonds with a wave absorption number of 1639.46 cm^{-1} and the strong absorption peak at 1033.85 cm^{-1} indicated the presence of C=O bonds.

From the results of the spectrum that appeared in both species, sea cucumbers have groups following the chemical structure of collagen in general, composed of amino acid sequences. When viewed from the first and second absorption peaks, collagen from the two types of sea cucumbers appears to have a similar functional compound group. Still, there is a noticeable difference in the fourth absorption area, which is a fingerprint, so these two compounds are actually not identical (Skoog 1998).

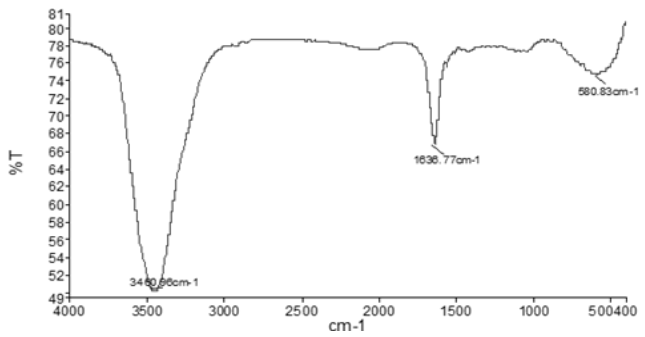

Figure 1. Graph of the infrared spectrum of collagen of sea cucumber *H. atra*

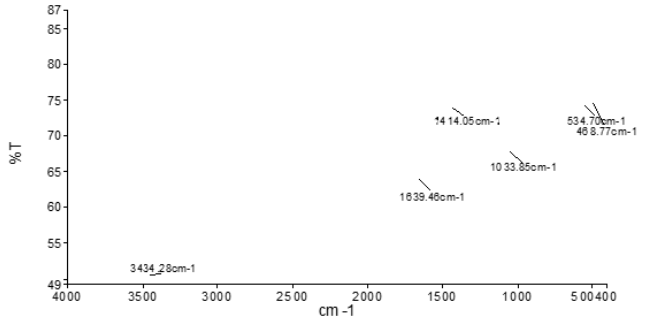

Figure 2. Graph of the infrared spectrum of collagen sea cucumber *S. vastus*

Discussion

Collagen contained in the body of sea cucumbers reaches 80% (Fawzya et al. 2016), and many are integumental (Abeidin et al. 2015). The quality of sea cucumber extract collagen can be used as an indicator of standard charts for the utilization of pharmaceutical and health products. Some of the used indicators are yield, pH, whiteness degree, and functional compound groups. Some roles of collagen are homeostasis, interactions with platelets, interactions with fibronectin, increasing fluid exudation, enhancing cellular components, increasing growth factors, and promoting fibroplasia and sometimes epidermal proliferation (Triyono, 2005). The extract yield is an important parameter in isolating sea cucumber collagen produced by the body tissue of sea cucumbers. Body wall collagen extract of sea cucumber *H. atra* is dull grayish white, while the *S. vastus* is rather blackish white. A freeze dryer does the drying process of sea cucumber collagen extract. Collagen extract yield was obtained by comparing the total powder of collagen extract resulting from drying with the initial weight of the sample before isolating the collagen in percentage.

The extract yield percentage of H. atra and S. vastus collagen calculation results were 0.88% and 0.92%, respectively. The value of *S. vastus* collagen in this research was lower than that of *S. variegatus*, which was 1.50% (Alhana et al., 2015), but higher than the yield value of *S. hermanii*, which was 0.66% (Safithri et al. 2018). The content of non commercial sea cucumber *H. atra* collagen was included in as low category (Lubis 2015). Water content can influence increased yield, in which extracts with low water content will produce high yield values. In this research, the Moisture content of the extract of non-commercial sea cucumber *H. atra* was 0.12% resulting in a yield of 0.88%, while the Moisture content of the extract of *S. vastus* was 0.17% resulting in a yield of 0.92%.

The results of pH measurement of *H. atra* and *S. vastus* extract were both 6.00. This pH value is lower than the value issued by the National Standardization Agency, which ranges from 6.5 to 8 (National Standardization Agency 2014). Suppose the pH value of the research results is compared with the pH value of Alhana et al. (2015) in the study of sea cucumber S. variagatus (pH value: 7.37). In that case, the results of this study are low pH values, but they are the same when compared to sea cucumber *S. hermanii* of 6.91 (Safithri et al. 2018). Although the material extracted collagen is the same, namely acetic acid, the concentration and extraction temperature applied in this study differ. Hence, it produces different pH characteristics of collagen. According to Peng et al. (2004), commercial collagen used for cosmetics is at pH 3.8-4, and the pH value is related to the salt/mineral content, which functions as a buffer for collagen solution. Based on this, if both sea cucumbers are applied to commercial cosmetic products, then both species need to be developed

The degree of whiteness (color description) can be used as an indicator of collagen quality based on the physical properties of collagen. Collagen could be categorized as good quality if it has a white base color with whiteness degrees close to 100% (Alhana et al., 2015). For example, the whiteness degree analysis (color description) on non-commercial sea cucumber *H atra* collagen was 61.60 ± 0.57%. As a comparison, the analysis of whiteness degree in the commercial sea cucumber *S. vastus* collagen was 20.0 ± 1,00%. Based on the analysis results, the degree of whiteness degree of non-commercial sea cucumber *H. atra* collagen was brighter than the collagen of commercial sea cucumber *S. vastus*, so it could be said that the collagen of non-commercial sea cucumber *H atra* had better quality than the one of commercial sea cucumber *S. vastus*.

On the other hand, the whiteness degree of *H. atra* was not much different from that of *S. variagatus*, which was 69.01% (Alhana et al., 2015). Therefore, the difference in the whiteness degree (color description) of sea cucumber collagen between species can be caused by the utilization of the extraction method, which affects the effectiveness of extraction in reducing pigment and increasing the whiteness degree. Thus, based on the value of the quality of the whiteness degree, the extract of sea cucumber *H atra* can be said to be still in the acceptable quality category for collagen products.

The functional group is the reaction of the reactive part of the organic molecule in which the results can be predicted. Functional groups contain atoms other than carbon atoms and have free electron pairs on these atoms. Based on the graph of the infrared spectrum of sea cucumber *H. atra* collagen, the amide group (N-H bonds) occurs at the wave absorption number of 3460.96 cm^{-1}. The alkene group (C=C bonds) is at the wave absorption

number of 1636.77 cm-1. In contrast, in the analysis of the infrared spectrum from sea cucumber *S. vastus* collagen, it can be seen that the presence of amide group (N=H bonds) is on the number of wave absorption of 3434.28 cm^{-1} and alkene groups (C=C) bonds with wave absorption number of 1639.46 cm^{-1} and the strong absorption peak is at 1033.85 cm^{-1}. According to Singh et al. (2011), a peak absorption strength at 1033.85 cm^{-1} indicates the presence of free (C = O) bonds and (N=H) bond vibrations. Of the two types of sea cucumbers, it generally can be said that collagen composition is structurally composed of amino acids.

Based on the measurement of pH values and the degree of leucorrhoea of non-commercial sea cucumber *H. atra* collagen and commercial sea cucumber *S. vastus* collagen, these two species had quite a good quality developed as a commercial products. Nevertheless, based on the assessment of the values of the two types of sea cucumber, the value of the percentage of collagen is low. But, on the other hand, That indicates the extraction quality in the sea cucumber *H. atra* and *S. vastus* in Karimunjawa is quite good. Furthermore, based on the functional group analysis of the sea cucumber *H. atra* and *S. vastus*, it can be seen that they are generally composed of amino acids with amide (N-H) and alkene (C-C) bonds.

Collagen from these two species of sea cucumber appears to have a similar functional group, based on the first and second absorption peaks. Still, there are noticeable differences in the area, namely fingerprinting that show no identical on them (Skoog, 1998). Given that, in this study, the character of functional groups is carried out qualitatively, so the results cannot be used in commercial product applications, and quantitative analysis is needed. Various functional groups contain atoms other than carbon atoms and have free electron pairs on these atoms. A functional group reference is a certain atom bound in a certain arrangement that gives a compound certain physical and chemical properties. According to Saraswati et al. (2016), functional groups are a group of atoms responsible for compounds' characteristic reactions, wherein these functional groups play an important role in controlling organic reactions.

As is known before, sea cucumber *H. atra* is rare, and sea cucumber *S. vastus* is not a catch target of Karimunjawa fishermen. That is supported by Abedin et al. (2015), who stated that sea cucumbers (*S. vastus*) are underutilized species in Malaysia, so their collagen benefits are wasted only. However, after the rare sea cucumber *H scabra* was found, the catch target of Karimunjawa fishermen switched to several other species that still had relatively good selling prices; among them was *S. vastus*. On the other hand, in the waters of Karimunjawa, there were abundant species of *H. atra* which had not been utilized optimally by local fishermen. Therefore, if the non-commercial species *H. atra* can be developed, it can assist in the efforts to conserve these resources (*H. atra and S. vastus*) to remain sustainable. Collagen is one of the compounds possessed by sea cucumbers that can be used commercially. That is because the benefits of sea cucumber collagen are very strategic for health products, especially in the use of the cosmetic product (Siahaan et al. 2017), and the other benefit is their use in hydrolyzing collagen as a functional ingredient in food and nutraceutical products (Abeidin et al. 2015). The first step to reaching this goal is the importance of spreading information about the quality of sea cucumbers, especially in *H. atra* and *S. vastus* species in Karimujawa. However, the quality of this collagen cannot be used as a comparison in its application; at least, this step could be used as a database before quantitative analysis is carried out.

It can be concluded that the whiteness degree analysis (color description) of *H. atra* collagen was 61.60 ± 0.57%; meanwhile, the *S. vastus* was 20.0 ± 1.00%. Therefore, it could be concluded that the quality of the whiteness in *H. atra* was better than that of the sea cucumber *S. vastus*. The FTIR spectrum of functional groups in the sea cucumber *H. atra* shows the presence of amides (N = H bonds) and alkene (C = C bonds); meanwhile, in sea cucumber *S. vastus*, it shows the presence of amides (N = H bonds) and alkene (C = C bonds) and strong absorption at the peak of 1033.85 cm^{-1} which indicates a free bond (C = O). The whiteness degree analysis (color description) on the collagen of non-commercial sea cucumber *H atra* was 61.60 ± 0.57%. In comparison, the analysis of the whiteness degree on the collagen of sea cucumber commercial *S. vastus* was 20.0 ± 1.00%, so it could be concluded that the degree of whiteness in *H. atra* is better than that of sea cucumber *S. vastus*. The FTIR spectrum of functional groups contained in sea cucumber *H. atra* shows the presence of amides (N = H bonds) and alkene (C = C bonds); meanwhile, in sea cucumber *S. vastus*, it shows the presence of amides (N = H bonds) and alkene (C = C bonds) and strong absorption at the peak of 1033.85 cm-1 which indicates a free bond (C = O).

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Meiofauna and nematode community assemblage as sediment disturbance indicator in Mida Creek, Kenya

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Abstract. *Wafula M, Muthumbi A, Wang'ondu V. 2019. Meiofauna and nematode community assemblage as sediment disturbance indicator in Mida Creek, Kenya. Ocean Life 3: 24-37.* Meiofauna is a group of benthic fauna mostly found in the sediments' upper centimeters (cm), including Copepoda, Polychaeta, and Nematoda. This study focused on determining nematodes' potential as bio-indicators of sediment disturbance caused by bait harvesting. Furthermore, bait harvesting was common, albeit with different intensities, on the different beaches of Mida Creek, Kenya, on November 2013. Sediment samples were collected along two transects within the mud flat running from the mangrove forest edge to the edge of the subtidal zone. Along the transects, 1 m² was placed at 20 m intervals. A replicate of 2 sediments core (3.4 cm diameter, 10 cm deep) was taken from each quadrat. Next, samples were fixed using 5% formalin, stored in plastic bottles, and then transported to the laboratory for processing and identification. The nematodes were used because it could bring out a clear difference in their distribution, community assemblage, diversity, and abundance due to sediment disturbance compared to other meiofauna. Of the total meiofauna, 3,988 individual nematodes were identified to the genus level. In addition, Nematoda was the most abundant taxon of all other meiofauna taxa. The bait harvesting through digging was more pronounced in Mayonda due to its wider mud flat width. The result showed a clear difference in the distribution, diversity, and community assemblage of the nematodes seen in all the sites. A sum of 83 nematode genera was encountered in Mida Creek. Kirepwe had 58 genera, Dabaso had 46 and Mayonda had 35. Mayonda had the highest nematode diversity, then followed by Kirepwe and Dabaso. On the contrary, Dabaso had the highest nematode density, then followed by Mayonda and Kirepwe. The meiofauna had a high community similarity, while nematode community similarity was lower at different levels of sediment disturbances. That makes the nematodes better bio-indicators as compared to meiofauna as a group. Therefore, this study points out that digging out polychaetes causes sediment disturbance. The disturbance affects the benthic fauna distribution, community assemblage, diversity, and abundance. Moreover, *Terschellingia* and *Spirinia* were distributed across all sites, even though in disturbed quadrats the numbers reduced significantly. On the contrary in disturbed quadrats, *Viscosia*, *Pontonema*, *Synochium*, *Haliplectus*, and *Pheronus* increased in numbers. To measures such as educating the fishermen on the effects of digging polychaete should be emphasized to safeguard marine biodiversity and its ecosystem.

Keywords: Bait, meiofauna, nematode, polychaete, sediments disturbance

INTRODUCTION

The Benthic communities are organisms that live in and/or on the sediments of oceans, lakes, and rivers, also referred to as benthos (Graf 1989, Hadi et al. 2018). These include both flora and fauna (Graf 1989). Benthic endofauna is divided into; infauna - those living in the sediments, and the epifauna - those that live on the sediments (Fredrik et al. 2011). Both benthic infauna and epifauna could be classified into microfauna, meiofauna, macrofauna, and megafauna. Microfauna is organisms that are lessthan 32 μm, while meiofauna range between 32 μm and 1,000 μm in size. The macrofauna range between 500 μm and 2 mm (Eggleston et al. 1999), while megafauna is organisms larger than2 mm in size (Lalli and Persons 1997; Johnson and Michael 2009). Sometimes juveniles of macrofauna could be classified as meiofauna, whereas some mature adult microfauna can be classified as meiofauna, too (Funch and Kristensen 2002).

In the marine ecosystem, the benthic communities can be found from the highest intertidal levels to the deepest trenches making the sediment the second largest habitat on earth (Nascimento et al. 2010). Due to the difference in depth, the organisms that are found at different depth zones are affected differently by environmental factors such as light, pressure, salinity, temperature, prey and predator interactions, organic matter, and sediment granulometry (Johnson and Michael 2009). For instance, shallow water benthic communities are bigger and larger than in deep water due to the high input of food in the form of decomposed organic matter in shallow waters compared to deep waters (Heip et al. 1985). On the contrary, these shallow water benthic fauna found near the coastal zone are more prone to human interference than deep communities (National Ocean and Atmospheric Administration Marine Debris Program, 2016). However, in understanding the benthic community, it is hard to generalize how these communities are structured because of the complexity of the soft aquatic bottom (Wilson 1990). Therefore, to understand the benthic community, the benthic invertebrates are determined by how individuals acquire nutrients. Furthermore, most ocean bottoms are situated

below the photic zone, meaning they depend on organic matter for food from the phytoplankton bloom (Graf 1989). Therefore, determining the organic matter is important in determining the benthic community (Graf 1992).

Meiofauna includestaxa like the Nematoda, Copepoda, and Polychaeta, among others (Renaud-Debyser 1963), and are mostly found in the upper centimeters (0-50 cm)of the sediments in abundance due to food availability, sediment type, and oxygen content (Grant 1981). Due to the semi-sessile nature of infauna and their close association with sediments, benthic fauna,specifically the nematodes, can be used as indicators of different parameters affecting the environment, such as oxygen deficiency, salinity change, pollution, seabed disturbances, among others hence referred to as bio-indicators (Finch et al. 1998).

Benthic fauna is affected by both environmental and human factors (Finch et al. 1998). For example, the most common baits used for fishing by fishermen around Mida Creek are hermit crabs, gastropods, and polychaetes (choo). However, they prefer the polychaetes over the hermit crabs and other gastropods because the polychaetes are easily captured, causing sediment disturbances. Several studies have assessed sediment disturbances, heavy metal contamination, and oil and petroleum spillage. However, the use of nematodes as bio-indicators can be an alternative method. This alternative method can be used to test and assess contamination due to heavy metals, oil spillage, and sediment disturbance. This study finding can show that nematodes can be used as bio-indicators and as an alternative assessment method of sediment disturbances. Furthermore, this is because nematodes as bio-indicator are cost-effective compared to the different available chemical methods.

This study aimed to detail as follows (i) to assess the potential value of meiofauna and the nematode community assemblages as an indicator of sediment disturbance following polychaete harvesting at Mida Creek, Kenya, (ii) to determine the distribution of the nematodes genera due to sediment disturbance, (iii) to determine the diversity of meiofauna and nematodes genera due to sediment disturbance. Finally, (iv) to determine the community assemblage of meiofauna and nematode genera due to sediment disturbance.

MATERIALS AND METHODS

Description of the study area

The Kenyan coastline is approximately 536 km long, bordering the countries of Somalia and Tanzania to the north and south, respectively (Ochieng and Erftemeijer 2003; UNEP 2006). Towards the North of Mombasa, about 111 km, the 32 km^2 Mida Creek stretches inland from the Indian Ocean into the Arabuko Sokoke Forest along the Kenyan coastline. It is a broad water tidal creek that is lined with palm trees surrounded by mangrove forest that support about 500 species of birds, different species of crabs, and monkeys (Kennedy 1990; Dahdouh-Guebas et al. 1999; Kairo et al. 2002; IUCN 2015) with which the three sampling sites are within the Mida Creek.

At Mida Creek, harvesting of polychaetes happens everywhere, especially in all three mud flat sites but at different intensities due to the width of the sites, from the land to the lowest tide of the ocean. Three sampling sites within the creek were identified as follows; Kirepwe (Kir), Dabaso (Dab), and Mayonda (May). Kirepwe (S03°27.28': E039°58.490') is an island in the creek; Dabaso is locatedon the eastern edge of the creek (S 15°03 20.53´: E 039°59.23´) and Mayonda to the west of the creek (S03°19.274': E039°59.098') (Figure 1).

Sampling design

This study was part of a larger project investigating the impacts of bait harvesting on thebenthic invertebrates in the mud flats in Mida Creek, Kenya. From that study, sediment sampleswere collected to analyze sediment organic matter, grain size, and meiofauna from the three sites (Kirepwe, Dabaso, and Mayonda).

Two line transects located 200 m apart at each site were identified within the intertidal zone (from the shoreline to the low water mark). Quadrats of 1 m x 1 m were laid out at 20 m intervals along theline transect, with quadrats A and B being in the mangrove forest zone, quadrat C was at the edgeof the forest, while the rest of the quadrats D, E, F, and G were in the mud flat. The number of quadrats identified along the transect length was dependent on the width of the intertidal zone, where Kirepwe had only 4 quadrats (QA, QB, QC, and QD), Dabaso had 6 quadrats (QA, QB, QC, QD, QE, and QF) and Mayonda had 7 quadrats (QA, QB, QC, QD, QE, QF, and QG) (Figure 2).

Sample collection

Two replicate sediment samples were collected from the mangrove forest edge (QC) towards the mudflat (QD, QE, QF, QG). A hand corer of 3.4 cm diameter was used to collect the sediment samples for analysis of meiofauna, grain size, and sediment organic matter up to a depth of 10cm. The samples for sediment organic matter analysis were placed in ziplock bags, carried in a cooler box, and put in the freezer on arrival at the laboratory. Samples for sediment grain size analysis were carried in plastic bottles without preservation. The samples for meiofauna were immediately fixed in a 5% formaldehyde solution for preservation in plastic bottles. All samples were well labeled with the date, site, transect, and replicate and transported to the University of Nairobi, Kenya, hydrobiology laboratory for processing and analysis.

Laboratory analysis

Determination of sediment grain size

In the laboratory, a sub-sample of sediment was weighed and dried in the oven at 70ºC until there was no change in weight. The dried block sample was then loosened gently not to tamper with the original sediment particles and sieved on different sieve sizes on a mechanical shaker {2.00mm to 63 µm, i.e., 2.00 mm, 1.00 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.063 mm (from ASTM D6913-04, 2009)}. The proportion of sediment collected on each of the sieves was weighed and calculated as a percentage of the total sample weight.

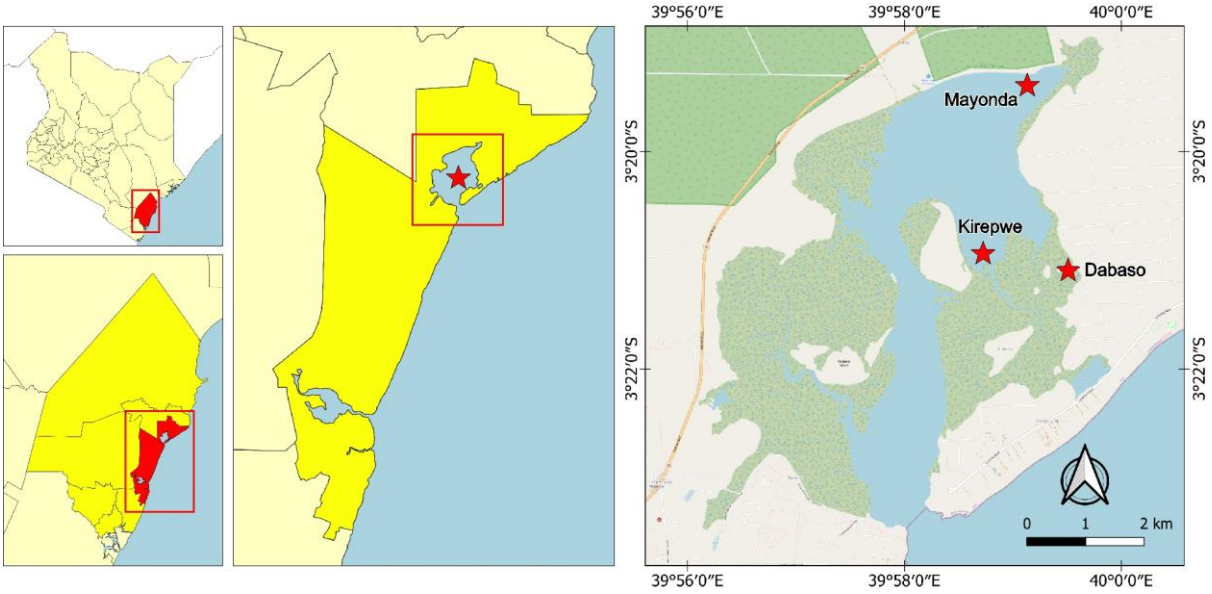

Figure 1. Map of Mida Creek, Kenya, showing sampling sites

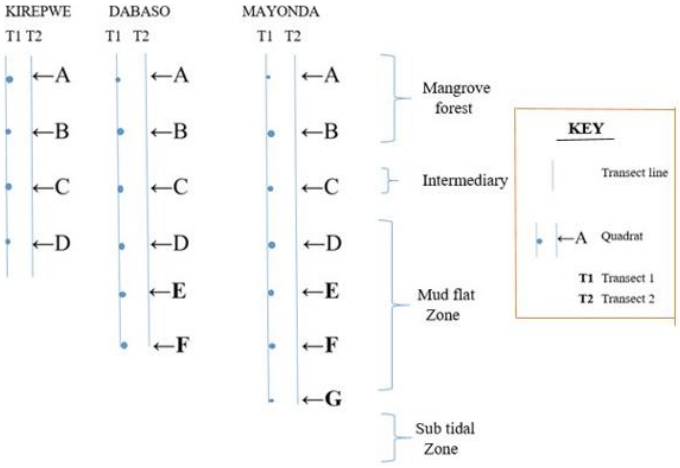

Figure 2. Sketch of the sampling design concerning sites, transects, and quadrats

This study re-calculated sediment grain size data from one transect (Transect 1) in each site (Kirepwe, Dabaso, and Mayonda). Quadrats QC and QD at Kirepwe, quadrat QC, QD, QE,and QF for Dabaso, and Mayonda quadrat QC, QD, QE, QF, and QG were considered because these were the quadrats considered for nematode identification.

Determination of sediment organic matter

Sediment samples were dried in the oven and weighed before ashing at 600ºC using a muffle furnace for six hours. The difference between the dried and ash weights was calculated and expressed as a percentage proportion of the total sediment weight to represent the sediment organic matter.

This study re-calculated sediment organic matter data from one transect (Transect 1) in each site (Kirepwe, Dabaso, and Mayonda). Quadrats QC and QD at Kirepwe, quadrat QC, QD, QE, and QF for Dabaso, and Mayonda quadrat QC, QD, QE, QF, and QG were considered because these were the quadrats considered for nematode identification.

Meiofauna analysis

The sediment samples for meiofauna analysis were rinsed with normal tap water over a 1 mm sieveto exclude macrofauna and any other debris and collected on a 38µm sieve. Magnesium Sulphate ($MgSO_4$) with a specific density of 1.28 g/cm³ was used to wash the fraction retained on the 38 µm sieve into a centrifugation tube. $MgSO_4$ was added to the three-quarter mark of each centrifugation tube with different samples and balanced. The samples were centrifuged 3 times for 10 minutes at 6,000 rpm. After each centrifugation, the supernatant was decanted onto a 38 µm sieve, rinsed thoroughly with water, and put back into well-labeled sample bottles using water and 4% formaldehyde solution. Three drops of Rose Bengal were added to the

bottle and left overnight for staining before sorting. Before the analysis of meiofauna was done, the samples were washed through a 38 µm sieve with tap water to remove traces of formaldehyde solution.

Enumeration of meiofauna

The sample was shaken well, and a small amount was poured into a counting chamber (Counting dish) for enumeration. Using a dissecting microscope at magnification X100, the sample was observed from the top left square across to the top right square. In the row below, observation was done from the right to the left side of the counting chamber to the end. This process was repeated until all samples were analyzed. All the meiofauna organisms were picked, identified, enumerated, recorded, stored in sample bottles, and kept safe in the laboratory.

Nematode analysis

For this study, meiofauna samples from only one transect (Transect 1) in each site (Kirepwe, Mayonda, and Dabaso). Therefore, Quadrats QC and QD at Kirepwe, quadrat QC, QD, QE, and QF for Dabaso, and Mayonda quadrat QC, QD, QE, QF, and QG were considered for nematode identification.

Two hundred nematodes were picked from each replicate sample and processed to make permanent slides. That was done first by picking the nematodes and placing them in embryo dishes containing De grise 1 (99 parts formaldehyde {4%} + 1-part glycerine) solution while covering it with no time restrictions. Then, in the evening, the embryo dishes were uncovered, placed in a desiccator with ethanol in the lower chamber, and placed in an oven set at 45ºC overnight.

In the morning, the embryo dishes were removed from the desiccator, half covered, and returned to the oven set at 45ºC. Three drops of De grise 2 (95 parts ethanol {96%} + 5-part glycerine) solution were added using a dropper along the sides of the embryo dish. The process was repeated after every 2-3 hours. In the evening, De grise 3 (50 parts ethanol {96%} + 50-part glycerine) solution was added to the embryo dishes in the oven and set at 45ºC overnight. This procedure was done to replace the water in the nematode with glycerine. The next morning, the embryo dishes were removed from the oven and placed in another desiccator with blue copper (ii) sulphate crystals to dry the samples overnight. Slides and coverslips were placed in ethanol overnight to clean up any oils. The slides and coverslips were removed from ethanol the next morning and dried using clean tissue paper.

Mounting of nematodes onto slides

Clean solid wax was placed in the glass petri dish and left in the oven overnight to liquefy, after which it was allowed to solidify. Next, a wax ring was made on the clean slide using a heated brass rod. A small droplet of pure glycerine was placed inside the wax ring using a needle. With a fine needle, at least ten nematodes of the nearly same diameter were placed in the glycerine, one at a time, ensuring that they did not lie on each other. A cover slip was placed on top of the slide, ensuring no air bubbles were trapped in the glycerine. The slides with the nematodes were heated slowly by placing them at one end of a bridge while the burner heated the bridge from the other end until the wax melted slowly. The slides with nematodes were then removed from the bridge and left to cool and labeled appropriately depending on the sample from which the nematodes were picked.

Nematode identification

Identification of the nematodes to the genus level under the stereo microscope. The immersion oil objective lens was used to watch each nematode, and the key to Platt and Warwick's (1988) identification of the genus level was made. Several characteristics were used to identify the different nematode genera, including the type of cuticle, either punctuated or annulated, the type of buccal cavity and size of teeth, reproductive systems, length and size of the nematode, and the tail form.

Data analysis

The data analyzed was from Kirepwe T1: QC and QD, Dabaso T1: QC, QD, QE, and QF, while Mayonda T1: QC, QD, QE, QF, and QG. Sediment organic matter and sediment grain sizes were re-calculated. The data identified genus categories and diversity, abundance, distribution, and densities were calculated and analyzed. All the genera of nematodes encountered were tabulated on the MS EXCEL and analyzed for relative abundance and distribution. The multidimensional scaling (MDS) and TWINSPAN for community analysis were done using PRIMER 5. The diversity indices (Dominance and Shannon diversity) were analyzed using the PAST. Bar graphs were drawn using MS EXCEL and SPSS. Significance tests for sediment organic matter, densities, relative abundance, distribution, and diversity between the sites were done using MS EXCEL and PAST.

RESULTS AND DISCUSSION

Sediment organic matter (SOM) content

The percentage of Sediment organic matter at Kirepwe was 4.1±2.48. The sediment organic matter percentage mean was highest at Dabaso (8.1±7.01), while Mayonda had the least percentage mean of 2.6±0.64. One-way ANOVA indicated that sediment organic matter was not significantly different across the sites ($F= 4.00$; $df=2$; $P=0.13$) (Figure 3).

Sediment grain size distribution

The silt was the least in mean proportion in all the sites. However, Kirepwe had a mean proportion of 4%, making it the highest in silt, while Dabaso had 3%. Mayonda had only 1%. One-way ANOVA indicated that silt had a significant difference across the sites. That played an important role in the results for meiofauna and nematode diversity, distribution, and community assemblages ($F= 14.4$; $df= 2$; $P= 0.000156$).

Very Fine sand was second least across all the sites. Kirepwe had a mean proportion of 5%, Dabaso had 7%,

and Mayonda (2%) had the least mean proportion of very fine sand, like silt.The very fine sand also played a key role in the meiofauna and nematode diversity, distribution, and community assemblages. However, one-way ANOVA indicated that very fine sand was not significantly different across the sites (F= 0.69; df= 2; P= 0.513).

Kirepwe had a mean proportion of Medium sand of 29%, while Dabaso had the least mean proportion of medium sand of 22%. Mayonda had the highest mean proportion of medium sand at34%. Medium sand plays an important role in the colonization of other meiofaunal taxa. One-way ANOVA indicated that medium sand was not significantly different across the sites (F= 1.77; df= 2; P= 0.193) (Figure 4).

Meiofauna and nematode density

The mean density of meiofauna ranged between 1183±613.7-1429±492 individuals/10 cm^2. Mayonda had the lowest mean of meiofaunal densities of 1183±613.7 individuals/10 cm^2, Dabaso had the highest meiofaunal density of 1429±492 individuals/10 cm^2 while Kirepwe's meiofaunal density was 1309±220.9 individuals/10 cm^2 (Figure 5).

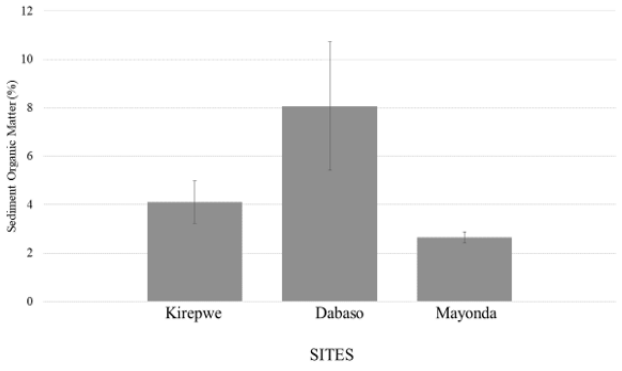

Figure 3. Sediment organic matter percentage mean in Kirepwe, Dabaso, and Mayonda (Kenya). The errorbars represent standard error

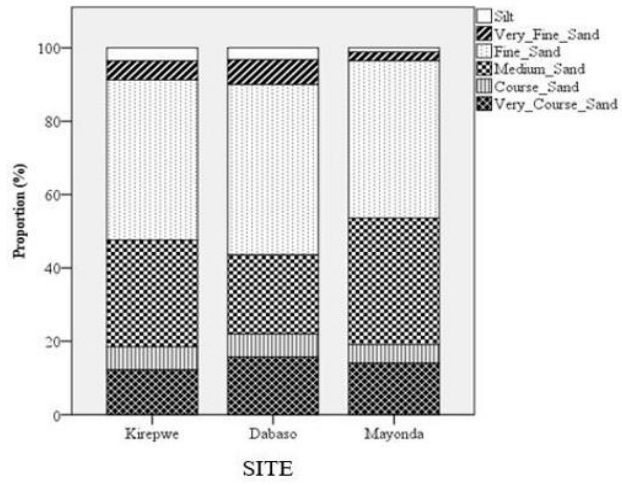

Figure 4. Sediment grain size mean proportion (%) at Kirepwe, Dabaso, and Mayonda (Kenya)

Nematode density ranged between 734±153.9-1042±374.1 individuals/10 cm^2. Kirepwe had the lowest nematode density of 734±153.9 individuals/10 cm^2, while Mayonda had a medium density of 782±385.1 individuals/10 cm^2. Dabaso had the highest nematode densities of 1042±374.1 individuals/10 cm^2.

One-way ANOVA indicated that the mean meiofaunal and nematode densities did not differ significantly across the sites (F= 0.09; df= 2, P= 0.91) and (F= 1.14; df= 2, P= 0.33), respectively (Figure 5).

Relative abundance of nematodes and the other meiofauna

Some of the 12 taxa of meiofauna were observed in the three sites sampled, with the Nematoda taxon being the highest in relative abundance across all the sites. The meiofauna taxa encountered included the Nematoda, Oligochaeta, Isopoda, Cladoceran, Amphipoda, Gastrotricha, Insecta, Rotifera, Polychaeta, Thermobanaceae, and Tardigrada. The relative nematode abundance was lowest at Kirepwe with 56%, while the relative abundance of other meiofauna taxa against the nematode wasthe highest (44%). The highest nematode relative abundance was at Dabaso (71%), while the other meiofaunal relative abundance was the lowest (29%). Mayonda nematode relative abundance was 59%, while the other meiofauna taxa relative abundance was 41%. One-way ANOVA indicated that the nematode abundance did not differ significantly across the sites (F= 1.14; Df= 2, P= 0.33) (Figure 6).

Distribution of nematode taxa

Some of the 3,985 individual nematodes from 83 genera were identified across the three sites (Kirepwe, Dabaso, and Mayonda). However, the distribution of nematode genera differed across the sites, with Kirepwe having the highest number of genera (58), followed by Dabaso (46), while the least number of genera was at Mayonda (35).

However, most genera were thinly distributed (below 6.3%) across all the quadrats. For example, only 2 genera were above 6.3%, that is, *Spirinia* (18.8%) and *Terschellingia* (13.5%), while 18 genera had an overall distribution of between 1.2% and 6.3% across all the quadrats (Table 1).

Nematode genera, *Spirinia*, *Terschellingia*, and *Daptonema*, were observed across all the sites; however, they were not recorded in some quadrats in Mayonda. *Pheronus* was restricted to a single quadrat in Dabaso (QC) but was widely distributed in Mayonda. *Dorylaimid* (plant-parasitic nematode), *Viscosia*, *Pontonema*, *Synonchium*, and *Haliplectus* were widely distributed in the quadrats and restricted at Mayonda. (Table 1).

One-way ANOVA indicated that the nematode distribution did not differ significantly across the sites (F= 0.9; df= 2; P= 0.99).

Meiofauna and nematodes diversity

The meiofaunal and nematode diversity H' was highest at Mayonda with 1.3 and 2.7, respectively. The lowest diversity of both meiofauna and nematode was at Dabaso,

with 0.9 and 2.5, respectively. Meiofauna and nematode diversity at Kirepwe was 1.2 and 2.6, respectively (Figure 7).

The meiofaunal diversity (Shannon Wiener H') in Dabaso and Kirepwe differed significantly from that of Mayonda ($P<0.05$), ($t= -22.501_{(21740)}$) and ($t= 9.7601_{(21316)}$) respectively. In addition, the meiofaunal diversity (H') between Dabaso and Kirepwe also differed significantly ($P<0.05$) ($t= -13.14_{(19635)}$). In summary, the meiofaunal diversity H' differed significantly across all sites.

The nematode diversity (Shannon Wiener H') indicated that Kirepwe and Dabaso differed significantly from that Mayonda. ($P<0.05$), ($t=-3.8212_{(2772.8)}$) and ($P<0.05$), ($t= -4.347_{(2312.3)}$) respectively. On the other hand, the *t*-test indicated that Kirepwe nematode diversity H' did not differ significantly from that of Dabaso ($P>0.05$) ($t= 0.8721_{(2777.9)}$).

The highest nematode dominance (d) was in Dabaso (0.14), while nematode evenness was the second highest (0.27). Kirepwe's nematode dominance was second highest (0.13), with the least nematode evenness (0.23). Mayonda had the lowest nematode dominance (0.09) and the highest nematode evenness (0.43) (Figure 8).

The calculated probability at a 95% confidence interval of both dominance and evenness of nematodes between the three sites was ($0.0765>0.05$) and ($0.27>0.05$), respectively, showing no significant difference.

Meiofauna and nematodes community assemblage

Generally, the meiofauna communities from the three sites had a very high similarity of at least 75%. As a result, the different sites and quadrats were mixed out in the cluster analysis, as shown in the dendrogram. The higher the similarity in the community, the lower the chance to conclude whether the community has undergone any disturbances. Therefore, the meiofauna community is not ideal to be used as a bio-indicator in this study (Figure 9).

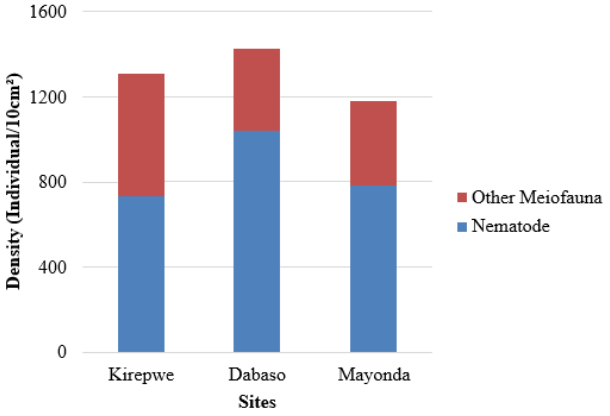

Figure 5. Other meiofauna and nematodes densities (individuals/10 cm^2) at Kirepwe, Dabaso, and Mayonda (Kenya)

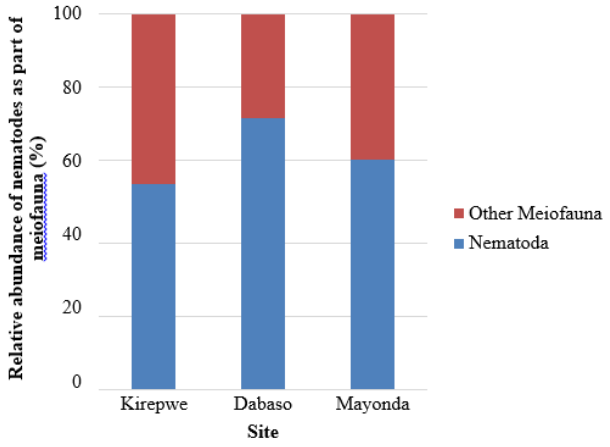

Figure 6. Relative abundance of nematodes and other meiofauna taxa (%) at Kirepwe, Dabaso, and Mayonda (Kenya)

Table 1. Distribution of nematode genera across the sites in Mida Creek, Kenya

Genera	Kirepwe		Dabaso				Mayonda					Mean
	QC	QD	QC	QD	QE	QF	QC	QD	QE	QF	QG	
Spirinia	19.8	28.5	7.8	41.1	12.9	43.4	16.3	30.7	2.6	2.7	0.7	18.8
Terschellingia	9.7	22.4	46.5	5.1	21.6	19.1	19.3	3.6	1.0	0.0	0.0	13.5
Pheronus	0.0	0.0	1.7	0.0	0.0	0.0	0.7	0.3	12.9	4.1	49.6	6.3
Daptonema	8.9	11.5	1.9	5.1	3.8	0.5	18.6	12.2	3.6	0.0	0.0	6.0
Dorylaimid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6	46.6	4.3	5.0
Viscosia	2.5	0.1	1.7	1.5	0.0	0.0	9.0	29.9	4.6	0.0	0.7	4.6
Pontonema	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	42.5	0.0	3.9
Paracomesoma	1.8	0.0	0.0	24.9	6.2	7.5	0.7	0.0	0.0	0.0	0.0	3.7
Synonchium	0.2	0.0	0.8	0.0	0.3	0.0	0.0	0.0	7.7	0.0	29.1	3.5
Dorylaimopsis	0.5	14.7	0.0	2.5	11.6	7.2	0.0	0.0	0.0	0.0	0.0	3.3
Haliplectus	0.2	0.0	0.6	0.0	0.0	0.0	0.0	0.0	28.4	0.0	7.1	3.3
Dichromadora	14.1	1.4	1.9	0.0	0.8	0.3	12.3	0.8	3.1	0.0	0.0	3.2
Paracanthonchus	0.8	0.0	0.3	0.0	0.0	0.0	4.3	19.8	2.6	0.0	0.7	2.6
Gomphionema	6.9	2.7	0.0	7.1	0.3	1.8	7.0	1.0	0.0	0.0	0.0	2.4
Chromadorita	10.7	2.3	0.0	0.0	2.2	1.3	0.3	0.3	7.2	0.0	0.7	2.3
Marylynnia	2.7	4.1	0.0	0.0	4.9	12.4	0.0	0.0	0.0	0.0	0.0	2.2
Longicyatholaimus	0.3	0.0	0.0	0.0	20.8	0.0	0.3	0.0	0.0	0.0	0.0	1.9
Stylotheristus	0.0	0.1	0.3	1.5	6.7	1.8	3.3	1.0	0.0	0.0	0.0	1.3
Spilophorella	1.0	0.8	5.3	0.0	0.5	0.3	5.3	0.0	0.0	0.0	0.7	1.3
Megadesmolaimus	0.0	0.0	12.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.2

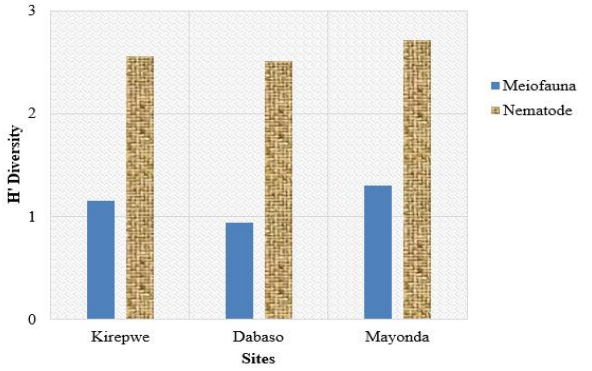

Figure 7. Diversity index H' of meiofauna and nematodes at Kirepwe, Dabaso, and Mayonda (Kenya)

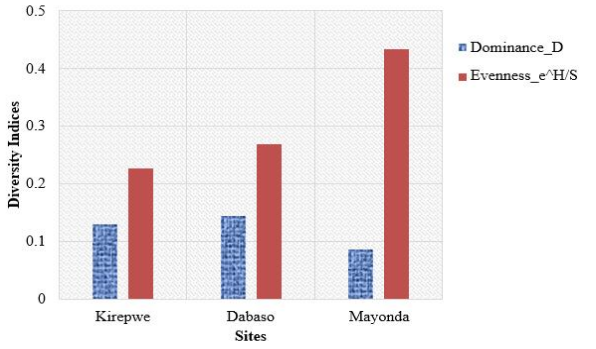

Figure 8. Nematode dominance and evenness at Kirepwe, Dabaso, and Mayonda (Kenya)

On the other hand, the nematode community showed generally low similarity in samples from the different sites and quadrats. The above dendrogram shows that the nematode community at the quadrat in Mayonda QF, QE, and QG were all clustered together, which showed that these quadrats had a higher nematode community similarity.

Furthermore, the Dabaso QD nematode community was clustered together with Mayonda QC and QD. Which also showed that these quadrats had a higher nematode community similarity. On the other hand, Dabaso QC, QE, and QF were clustered with Kirepwe QC and QD, showing these quadrats had a higher nematode community similarity. However, Kirepwe QC and QD were clustered together, showing a high nematode similarity within the quadrats. It is important to note that the Mayonda QC and QD nematode community is closely related to that of Dabaso QD. Despite the overall similarity of the nematode community in the three studied sites, there was apparent segregation of the Mayonda nematode community from the nematode communities at Dabaso and Kirepwe.

Discussion

Sediment organic matter and sediment grain size

Mangroves forest contributes a large percentage of organic matter to the sediment through the litterin the marine ecosystem (Odum and Heald 1972; Alongi 1990). In addition, these mangrove forests play an important role in holding and compacting the sediments. That, in turn, helps protect the shoreline against all kinds of erosion (both wind and waves) (Odum and Heald 1972; Alongi 1990). Mayonda had the least amount of the mean sediment organic matter, and one-way ANOVA indicated that silt was significantly different across all sites. That could be attributedto the low proportion of silt. Tyson (2012), in his book on sedimentary organic matter, and Secrieru and Oaie (2009), reported that sediment organic matter is influenced by grain size distribution in an area. They also suggested that a small proportion of finer particles promotes a lower percentage of sediment organic matter, as seen at Mayonda compared to Kirepwe and Dabaso.

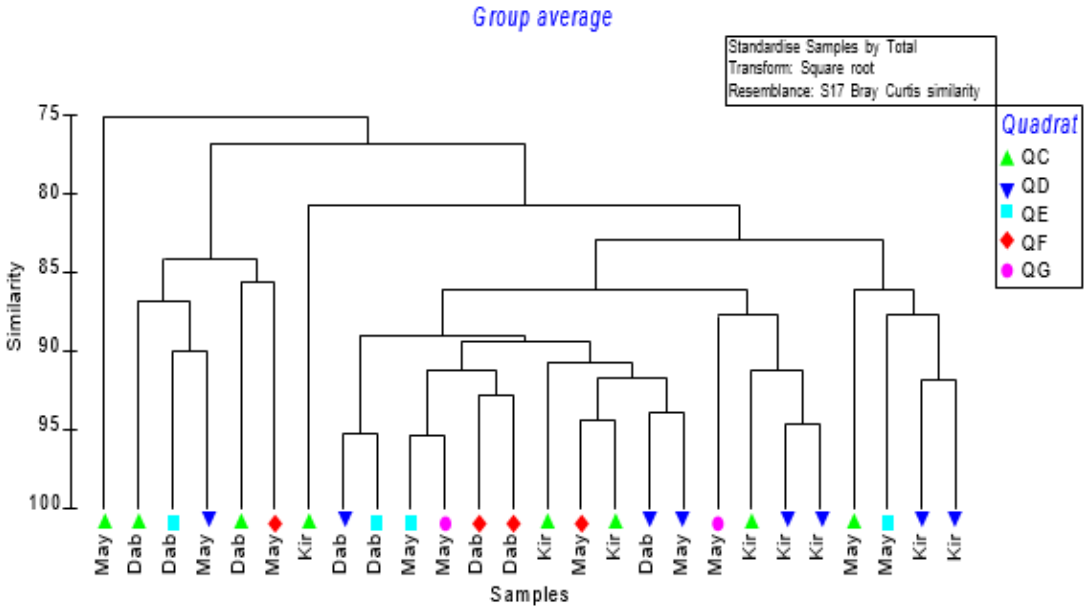

Figure 9. Dendrogram showing the cluster analysis for meiofauna at Mida Creek, Kenya

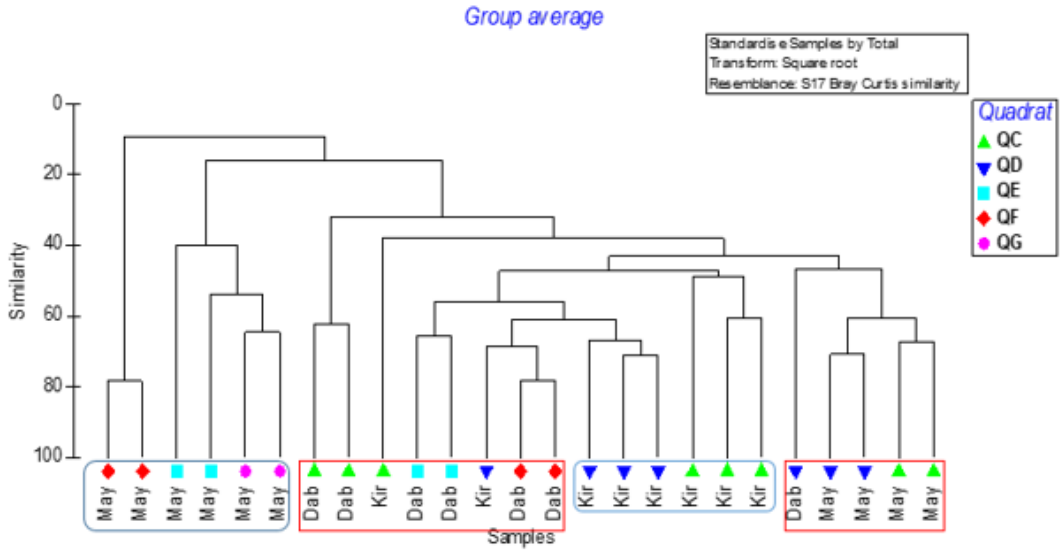

Figure 10. Dendrogram showing the cluster analysis for nematodes in Mida Creek, Kenya

There was a lot of sediment disturbance caused by the fisherfolk at Mayonda. The disturbances were caused by the ever digging and churning of the sediment. The main aim was to capture the large polychaetes, which contributed greatly to the disintegration of sediments through mechanical breakdown (Secrieru and Oaie 2009; and Tyson 2012). Odum and Heald (1972) and Alongi (1990) reported the importance of mangrove forest cover along the coastline. They reported that mangrove forest cover helps sediment organic matter formation through leaf fall, and mangrove protects the sediments by compacting and holding soil sediments together. In Mayonda, silt and very fine sand were easily eroded since the sediments were exposed to the agents of erosion due to little mangrove cover compared to Dabaso and Kirepwe. Apart from the low silt amounts that hindered the decomposition of organic matter at Mayonda, the little mangrove forest cover also contributed greatly to the low sediment organic matter. Secrieru and Oaie (2009) and Tyson (2012) reported that sediment organic matter is influenced by the distribution of grain sizes found in an area when they noted that a small proportion of finer particles promotes a lower percentage of sediment organic matter. This study confirms that silt in Mayonda was significantly different from other sites that play a key role in the decomposition and presence of sediment organic matter in an area (Secrieru and Oaie, 2009; Tyson, 2012). Wieser (1953) noted that genera such as *Spirinia* and *Terschellingia* are higher in concentration in areas with high sediment organic matter. That can be used to infer that Mayonda had the least concentration of sediment organic matter due to the drastic drop of existing genera such as *Spirinia* and *Terschellingia*, which are non-selective feeders, and selective feeders of sediment organic matter, respectively. Wieser (1953) and Moens and Vincx (1997) also noted that a drop in selective and non-selective feeders gives room to an increase of other opportunistic and scavengers genera such as *Viscosia*, *Pontonema*, *Synochium*, and *Pheronus*.

In Kirepwe, the mean sediment organic matter was less than that of Dabaso. It was evident that the forest cover at Dabaso was higher than the cover at Kirepwe, which is a major source of sediment organic matter through leaf fall (Odum and Heald 1972 and Alongi 1990). In addition, the same phenomenon of grain size by Secrieru and Oaie (2009) and Tyson (2012) comfortably explains the difference in the mean sediment organic matter present in both areas of Kirepwe and Dabaso. The lower mean sediment organic matter at Kirepwe can be attributed to the high proportion of fine and medium sand compared to Dabaso. High silt at Dabaso, compared to Kirepwe, can be used to explain the difference in mean sediment organic matter. Fine and medium sand does not promote sediment organic matter compared to silt and very fine sand, as documented by Secrieru and Oaie 2009; Tyson 2012. Heip et al. (1985) documented that nematode genera such as *Daptonema* and *Chromadorita* are mostly found on sandy beaches. Due to the presence of *Daptonema* at Kirepwe, this is evident that the area was sandier than Dabaso.

Furthermore, there was a high concentration of *Terschellingia* at Kirepwe. Alongi (1987) recorded that *Terschellingia* survives better in the mangrove forest because it can feed not only on the sediment organic matter since it is a selective deposit feeder but also does well in mangrove-derived tannin. He also documented that tannin from mangroves is more in sandy areas because it binds more easily with sand than fine sand and very fine sand, as observed at Kirepwe; that evidence is sufficient to show that there was more fine and medium sand at Kirepwe compared to Dabaso leading to the low sediment organic matter.

Gheskiere et al. (2005) and Maria et al. (2012) reported that high amounts of silt with fewer disturbances promote a high amount of sediment organic matter. Therefore, the high mean sediment organic matter in both Kirepwe and Dabaso can be attributed to the fewer sediment disturbances happening in the area compared to Mayonda. At the same

time, high amounts of sediment organic matter favor the presence of nematodes due to great amounts of food (Wieser 1953).

Less amount of silt and sediment disturbances caused by fisherfolk contributed greatly to the low sediment organic matter experienced at Mayonda. Tyson (2012) observed that sediment organic matter is influenced by the distribution of grain sizes found in an area when he noted that asmall proportion of finer particles promotes a lower percentage of sediment organic matter. In addition, Giere (2009) observed that low proportions of silt, very fine sand, and fine sand, as experienced in Mayonda, favor the existence of other meiofaunal groups. Moreover, these groups will include Oligochaeta, Polychaeta, Copepoda, Thermobanacea, Gnathostomulida, Cladocera, Turbellaria, Arachnida, Ostracoda, tanaidacea, cnidarian, Insecta, and Rotifera, which is clearly demonstrated at Mayonda where different meiofaunal taxa are thriving more across the quadrats as compared to Dabaso and Kirepwe. Therefore, as observed in this study, sediment disturbances experienced at Mayonda affect the decomposition of organic matter, affecting the distribution of nematode genera. Furthermore, sediment grain sizessuch as silt play a major role in sediment organic matter decomposition, with which the sediment organic matter also influences the distribution and existence of nematode and other meiofaunal taxa.

Meiofauna density

Medium sand was higher in proportion at Mayonda than at Dabaso and Kirepwe. Giere (2009) documented that low silt, very fine sand, and fine sand, with high proportions of medium sand, favor the survival of other meiofaunal groups. This information can explain the high meiofaunal density at Mayonda since the site had lower finer sands and greater proportions of medium sand sizes compared to Dabaso and Kirepwe, which caused the high meiofaunal density. However, we cannot use the meiofauna in general to give a conclusive and concrete reason that it is due to the sediment disturbances happening at Mayonda that caused the high meiofaunal densities.

Nematode density

The study showed no significant difference in the population densities of nematodes between Kirepwe, Dabaso, and Mayonda. Gheskiere et al. (2005) on meiofauna as a descriptor of tourism-induced changes at sandy beaches. Maria et al. (2012) on the relationship between sandy beach nematodes and environmental characteristics in two Brazilian sandy beaches. These researchers all documented that high amounts of silt and fine sands caused a high sediment organic matter with less disturbance. These conditions enhance high nematode density because of the high food content provided by the availability of high sediment organic matter. This phenomenonis replicated in Dabaso since the site had high amounts of silt, very fine sand, and high sediment organic matter with less disturbance leading to high food content. As a result, the area supported the nematode population comfortably, leading to high densities. Kirepwe had the least nematode density, although it had fewer disturbances with low sediment organic matter content than Mayonda. This rear phenomenon can be researched further to explain these results.

The silt and very fine sand were relatively higher in proportion in all the sites favoring nematode existence and survival. A similar study done by Renaud-Debyser (1963) concluded that the nematode assemblages reach up to a depth of 50cm. Therefore, the high amounts of silt and very fine sand played a major role in the high-density occurrence of the nematodes (Gheskiere et al. 2005; Maria et al. 2012), which was supported too, by Secrieru and Oaie (2009) and Tyson (2012). Therefore, this phenomenon can be related to the high nematode density at Dabaso. Furthermore, silt, very fine sand, and fine sand were high in proportion at Dabaso and Kirepwe, causing the high nematode densities as compared to Mayonda, which had low silt, very fine sand, and fine sand proportion.

Relative abundance of nematode within the meiofauna

Research by Giere (2009) indicates that a high proportion of medium sand promotes the survival of other meiofaunal groups. That can explain the high meiofaunal relative abundance at Kirepwe compared to Dabaso and Mayonda. Such meiofaunal taxa include oligochaeta, cladocera, insecta, rotifera, copepoda, polychaeta, thermobanaceae, turbellaria, ostracoda, and tanaidacea. Similar results of an increase in medium sand caused an increase in other meiofaunaltaxa (Maria et al. 2012).

The relative abundance of nematode taxon compared to other meiofauna taxa was very high in all sites. These findings were similar to research by Giere (2009), who recorded that the nematode community dominates any area in relative abundance. Moreover, they are always among the first living organisms to colonize any area, which can be attributed to the presence and availability of silt, very fine sand, and fine sand that promotes the decomposition of organic matter, which is food to the nematodes (Secrieru and Oaie 2009; Tyson 2012).

Despite nematode dominating all sites in relative abundance compared to other meiofaunal taxa, Dabaso had the highest proportions of silt that enhanced high sediment organic matter, which promoted the survival and existence of a high relative abundance of the nematode as compared to Kirepwe and Mayonda. Moens and Vincx (1997) and Moens et al. (2013) indicated that high sediment organic matter favors nematode colonization and specialization due to the availability of food and well-physical conditions (Gheskiere et al. 2005; Maria et al. (2012). That can still explain the high relative abundance of nematode and Dabaso compared to Kirepwe and Mayonda.

On the other hand, Mayonda had little proportions of silt, very fine sand, and fine sand compared to Dabaso and Kirepwe, which caused a decrease in sediment organic matter decomposition, which usually affects nematode abundance. In addition, Mayonda was the site that experienced a lot of sediment disturbances from the fishfolker through digging and churning. Therefore, it was expected that relative nematode abundance should be much less than in Kirepwe. Still, the relative abundance of

nematodes in Mayonda (59%) was higher than in Kirepwe (56%). These can be attributed to the continuous sediment disturbances at Mayonda, which do not allow nematode species specialization. Instead, those give room for more opportunistic and hardy nematode genera that can survive heavy sediment disturbances very well to migrate and occupy the area. Such nematode genera include *Viscosia, Pontonema, Synochium, Haliplectus,* and *Pheronus,* as stated by Moens and Vincx (1997). These genera played a key role in pushing the relative abundance of nematodes at Mayonda compared to Kirepwe and Dabaso.

Distribution of nematode taxa at Mida Creek

Feeding in marine nematodes plays an important role in their distribution (Wieser 1953). Wieser (1953) came up with 4 trophic groups: first, the selective deposit feeder, mainly characterizedby having a small buccal cavity with no tooth. A few examples are *Tershellingia, Paracanthochus, Bathylaimus, and Haliplectus,* among others. The second trophic group is non-selective deposit feeders. Their major characteristic is that they possess a large buccal cavity but without teeth. Some non-selective deposit feeders include *Spirinia, Daptonema, Sabatiera, and Theristu*s. The third trophic feeder is the epistratum feeder, which feeds on diatoms. Some examples include *Gomphionema, Marylynnia, Dichromadora, Chromadorita, Spilophorella, Longicytholaimus, and Stylotheristu*s. The final trophic group is the omnivore-predator. Their major characteristic is the possession of a rather big strong tooth. They include *Synochium, Pheronus, Viscosia, Pontonema, and Paracomesoma*. However, this group is not understood much. Moens and Vincx (1997) reported that *Viscosia* and *Pontonema* could be described as scavenger predators because of their ability to feed on dead foraminiferans.

From this study, One-way ANOVA indicated that the nematode distribution across the quadrats was not significantly different across the sites. However, *Spirinia, Terschellingia,* and *Daptonema* were widely distributed across all the sites though reduced in numbers in some quadrats of Mayondabecause such genera are greatly affected by heavy sediment disturbances. The sediment disturbance affects their feeding grounds since they are all deposit feeders, impacting their distribution. As explained before, sediment disturbance greatly affects sediment organic matter decomposition and deposition, which is the deposit feeders' main food source. Such sediment disturbances were more pronounced at Mayonda than at Kirepwe and Dabaso. The disturbances caused a reduced sediment organic matter concentration, affecting the distribution of deposit feeders at Mayonda: *Synochium, Pheronus, Viscosia, Pontonema, Viscosia,* and *Pontonema* increased in number due to their opportunistic characteristics.

Terschellingia is a selective deposit feeder, whereas *Daptonema* and *Spirinia* are non-selective feeders feeding mostly on deposited foods such as organic matter (Wieser 1953; Moens and Vincx1997; Schratzberger et al. 2006). In addition, these genera dominate every habitat (Alongi 1990).*Spirinia* was more in quadrats D, located at the start of the mudflat region across all sites, while Terschellingia was more in quadrats C, where mangrove cover was across all sites. Alongi (1987) documented that *Terschellingia* survives better in the mangrove forest because it could feed not only on the organic matter since it is a deposit feeder but also does well in mangrove-derived tannin. On the other hand, *Spirinia* was more in the quadrats located at the mudflats, which is because the mudflats have substantial sediment organic matter deposits best suited for survival (Alongi 1987). In addition, *Spirinia* is a non-selective deposit feeder; hence it is not deprived of its feeds (Alongi 1987). However, with the disturbances happening at Mayonda, both *Spirinia* and *Terschellingia* drastically reduced, especially towards the mudflats. Furthermore, polychaetes survive best in the mudflats where frequent sea water is coming in and moving out (Heip et al. 1985), giving Mayonda a good ground for polychaetes to thrive. The digging of the polychaetes towards the mudflat of Mayonda by the fisherfolk caused a drastic drop in both *Spirinia* and *Terschellingia* due to the reduced sediment organic matter, which is food for such nematodes.

Heip et al. (1985) noted that genera such as *Daptonema* and *Chromadorita* (Epistrate feeder) are found on sandy beaches. *Daptonema* and *Chromadorita* were more in Kirepwe, and this is because fine sand and medium sand were higher in Kirepwe as compared to Dabaso and Mayonda. More of *Terschellingia* was present in Kirepwe as compared to other sites. Alongi (1987) recorded that *Terschellingia* survives better in the mangrove forest because it could feed not only on the organic matter since it is a deposit feeder but also does well in mangrove-derived tannin. He also documented that tannin from mangroves is more in sandier areas because it binds more easily with sand than fine sandand very fine sand, as observed at Kirepwe.

Despite *Spirinia* and *Terschellingia* being widely distributed across all the sites, in Mayonda, the genera were low in distribution. Those are due to the physical disturbances that destroy their feeding habitat (Alongi 1990). They are non-selective and selective deposit feeders, respectively. The frequent disturbances prevented them from having a specific niche while taxa such as *Viscosia, Pontonema, Synochium,* and *Pheronus* increased in number (Moens and Vincx 1997). In addition, the sediment disturbance interfered with the decomposition of sediment organic matter, which is the main source of food for *Spirinia* and *Terschellingia,* causing a drastic drop at Mayonda as comparedto Kirepwe and Dabaso.

Terschellingia, Daptonema, and *Spirinia* are the genera among the nematode assemblages that are best known to occur in muddy and organically rich sediments (Schratzberger et al. 2006), just like in Dabaso and Kirepwe. These genera are not only found in undisturbed areas that allow the utilization of the dissolved sediment organic matter content by the specific genera of nematodes. They can also adapt and change during disturbances (Vanreusel 1990;), which explains why these three genera were found in Mayonda despite the serious sediment disturbances in small numbers.

Pheronus, Viscosia, Pontonema, and Synonchium were highly distributed in Mayonda, which is attributed to their predatory feeding habit (Moens and Vincx 1997). They are

also termed opportunistic nematodes that colonize disturbed areas just because other nematode genera cannot thrive in areas such as Mayonda (Moens and Vincx 1997). It is important to note that *Pontonema* was able to thrive at Mayonda better due to its ability to scavenge for its food. From the general observation, these predators were more concentrated in the quadrats of Mayonda, where oligochaetes were concentrated. These can be used to postulate a relation between such oligochaetesand predatory marine nematodes though further research to prove this can be conducted. *Sabatieria*was not present in Mayonda despite its ability to tolerate both hypoxic and anoxic conditions. Fromthe research findings by Jensen (1987) and Fonseca et al. (2018), *Sabatieria* and *Dorylaimopsis* thrive best under reduced sediments, as found in Kirepwe and Dabaso.

Diversity of meiofauna and nematode

Frequent moderate disturbance of sediments reduces dominance and increases evenness and diversity of nematodes Giere (2009). Communities that are diverse biologically most likely contain resilient species. That is made possible by the ability of these species to adapt to the changing environment because they have a higher chance of getting traits that help them survive (Yachi and Loreau 1999). The meiofauna diversity H' did not differ significantly across all the sites. However, Mayonda had a slightly higher meiofaunal diversity than Kirepwe and Dabaso. That was because the site had very little silt, similar to fine sand and fine sand from the results. This promoted the existence of other meiofaunal taxa such as Oligochaeta, Polychaeta, Copepoda, Thermobanaceae, Gnathostomulida, Cladocera, Turbellaria, Arachnida, Ostracoda, Tanaidacea, Cnidaria, Insecta, and Rotiferas documented by Giere (2009). As mentioned, sediment disturbances at Mayonda caused the meiofauna taxa not to occupy a specificniche; hence new taxa colonizing the area pushed the meiofauna diversity up (Moens and Vincx 1997).

Furthermore, in the meiofaunal group, Nematoda, Oligochaeta, and Copepoda will always recolonize an area after disturbances. However, the Nematoda taxon recolonization is faster and more immediate (Ingole et al. 2000) because nematodes quickly adapt to any disturbance over a very short period (Sherman and Coull 1980). Thus, after the fisherfolk's physical sediment disturbance at Mayonda by fisherfolk, nematodes recolonized the area almost immediately, pushing the meiofaunal diversity up as compared to Kirepwe and Dabaso. That is because nematodes recolonize the disturbed area almost immediately and fast. They also have the ability to easily adapt to changes in the environment due to their hardy nature as organisms suggesting that they can be used as indicator organisms in recolonization experiments (Ingole et al. 2000). Therefore, Oligochaeta and Copepoda recolonized the disturbed area by pushing the meiofaunal diversity up (Moens and Vincx 1997).

Mayonda had the least number of nematode individuals while Kirepwe had the highest, whereas Mayonda recorded the highest nematode diversity, followed by Kirepwe while Dabaso had the lowest. The nematode diversity in Dabaso and Kirepwe differed significantly from that of Mayonda. Yachi and Loreau (1999) concluded from their research that increasing diversity confers a greater chance of destabilizing the system. That is because there is a limit to the number of individuals packed in a particular community, confirming that as the number of community species increases, the average community population decreases. That means that the smaller the population size is, the higher the chances of extinction and species richness levels.

Therefore, from the data at Mayonda, diversity is high, which, as Yachi and Loreau (1999) explain, can cause species extinction due to the ever-increasing disturbances caused by fisherfolk, which can also be attributed to the difference in the intensity of sediment disturbances that affected the sediment organic matter in an area. Grain size proportion also played a big role in the nematode diversity. A lesser proportion of finer particles enhances a lesser percentage of sediment organic matter. The same phenomenon of sediment disturbances affecting the concentration of sediment organic matter (Secrieru and Oaie 2009; Tyson 2012) in comfortably explains the difference in the mean total sediment organic matter that affects the presence of nematodes in Mayonda. Moreover, Mayonda had the least proportion of finer sand that played a major role in the slow decomposition and presence of the mean total sediment organic matter in the area. That gave Mayonda a unique nematode diversity. Netto et al. (1999) noted that sediment stability is a very important variable in controlling nematode diversity and abundance at the studied sites, which is replicated at Kirpwe, Dabaso, and Mayonda.

Moreover, the low diversity observed in Dabaso and Kirepwe was due to nematode species specialization. At the same time, Mayonda had the highest diversity due to a lack of nematode species specialization, which caused a rise of opportunistic nematode species that came to colonize the areabecause of the sediment disturbances that occurred (Moens et al., 2013). That is because frequent sediment disturbances do not allow nematodes to occupy a specific niche. Those because, as the nematodestry to occupy a niche in the environment, there is sediment disturbance that causes others to migrateor die while providing room for predatory species to colonize the area (Moens and Vincx 1997; Moens et al. 2013), pushing the nematode diversity up as recorded in Mayonda.

Dabaso diversity and abundance of nematodes were slightly lower than Kirepwe's nematode species diversity and abundance. Those can be attributed to the fact that all kind of nematodes inhabits Kirepwe, that is, selective and non-selective deposit feeders (*Spirinia* and *Terschellingia*), predators (*Pheronus, Viscosia, Pontonema, and Synonchium*) to species that can also survive extreme *(Daptonema)* physical disturbances, epistratum feeders (*Chromadorita, Marylynnia, Dorylaimopsis, Dichromadora, Gompionema,* and *Longicytholaimus*) (Jensen 1987). Therefore, a unique specialization did not exist. On the other hand, various studies show that nematode abundance varies greatly among habitats, free-living nematodes in the marine environment (Heip et al.

1985). This variability is caused by the complex interactions among biotic and abiotic factors such as food and disturbances. For instance, Heip et al. (1985) reported that muddy estuarine sediments are characterized by high densities of more than 3,000 individuals/10cm^2 and a few dominant species per sample.

The results of the diversity indices of nematode showed significant differences among the three sites; Dabaso, Mayonda, and Kirepwe. According to the Shannon diversity index and Simpson's diversity index, Mayonda had higher species diversity (richness) than Dabaso and Kirepwe. That was brought majorly about by the frequent sediment disturbances at Mayonda, hindering species specialization and giving rise to more opportunistic species. In addition, sediment disturbance brought the differences in sediment texture of sampling sites that played an important role in influencing the diversity and distribution pattern of these nematodes, as indicated by Bertness et al. (2001) and Nabavi et al. (2008). The high diversity reduces dominance in anyecosystem; hence, species dominance at Mayonda was hindered since no species can come up strongly to dominate the area with frequent physical sediment disturbances. Low dominance automatically leads to the high evenness of nematodes, which is seen in Mayonda compared to Dabaso and Kirepwe; therefore, nematodes cannot be allowed to occupy a specific niche (Bertness et al. 2001; Nabavi et al. 2008).

Meiofauna and nematode community assemblage

Despite having different Physico-chemical parameters, Kirepwe, Dabaso, and Mayonda meiofaunal community assemblage did have a high percentage similarity from the cluster analysis. Furthermore, despite Mayonda experiencing a lot of sediment disturbance by the fisherfolk, from the community cluster analysis, the meiofaunal community did not differ from Dabaso and Kirepwe. Which shows that the meiofaunal community from the studied sites cannot be used as bio-indicators because they do not bring out a clear difference between them.

On the other hand, the nematode community assemblage at Kirepwe and Dabaso had a close similarity to each other compared to that of Mayonda nematode community assemblages. That can be attributed to the physical disturbances caused by the fisherfolk during the harvesting of polychaetes, sediment sizes, sediment organic matter decomposition, and feeding habits of the nematodes. Digging out of the polychaete causes a shift of the nematode community through death and migration away or into the area (Shine 2005; Anasari et al. 2012).

Dabaso and Kirepwe had substantial amounts of sediment organic matter content compared to Mayonda, which recorded the least amounts of the mean sediment organic matter. Odum and Heald (1972) and Alongi's (1990) indicated that mangroves forest contribute a large percentage of organic matter to the sediment through litter fall in the marine ecosystem. The low sediment organic matter at Mayonda could be attributed to the existence of very little mangrove forest cover. This sediment organic matter concentration promotes the survival of different nematode genera, as seen in Kirepwe and Dabaso. Apart from the low sediment organic matter caused by the little mangrove cover, Mayonda had low silt and very fine sand concentration, which play a key role in sediment organic matter decomposition, as reported by (Giere 2009).

Furthermore, the sediment disturbances greatly affected the decomposition of the organic matter, as Arntz et al. (1994) indicated, which is also seen at Mayonda. These factors played a major role in contributing to the low sediment organic matter at Mayonda. The low sediment organic matter simultaneously caused a drastic drop in the selective and non-selective deposit feeders such as *Spirinia* and *Terschellingia*. In contrast, more opportunistic genera such as *Viscosia, Pontonema, Synochium*, and *Pheronus* increased in number (Moens and Vincx 1997).

A similar study by Francisco et al. (2012) noted that frequent physical disturbances affect the decomposition of matter to sediment organic matter. This disturbance caused Mayonda to have a very little occurrence of sediment organic matter. As mentioned, sediment organic matter is a major food source for the nematodes (Arntz et al. 1994). That meant that sediment organic matter is critical in the distribution of nematode communities (Shine 2005; Anasari et al. 2012), just as observed during this study in all sites. Apart from sediment disturbance and sediment organic matter, grain size also affects the community structure of nematodes. Giere (2009) indicated that very small amounts of silt, very fine sand, and fine sand hinder the decomposition of organic matter. Mayonda had very small proportions of silt, very fine sand, and fine sand as compared to Kirepwe and Dabaso, which caused the nematodes community assemblage in Mayonda to differ from that of Dabaso and Kirepwe greatly. That was because of the continuous physical sediment disturbances at Mayonda that caused epistratum, selective and non-selective feeders such as *Spirinia, Terschellingia, Paracanthochus, Bathylaimus, and Haliplectus*, among others, to decrease in number. In contrast, more predatory feeders such as *Viscosia, Synochium, Pheronus, and Pontenema* increased.

Due to the mechanical weathering of soil sediments, more sand was recorded in Mayonda. In contrast, silt and very fine sand were eroded due to sediment disturbance and the little mangrove forest (root to compact and the soil sediments together) available. Therefore, *Paracanthocus* and *Chromadorita* increased in number due to their ability to survive in areas with sandier conditions (Alongi 1997), while *Sabateria* and *Dorylaimopsis* were absent (Jensen 1987; Moens and Vincx 1997; and Fonseca et al. 2018) as observed during their studies, which was also observed in Mayonda. At the same time, Dorylaimid, a plant parasite nematode, was widely distributed at Mayonda, which needs further research to explain these findings.

Spirinia, *Terschellingia*, and *Daptonema* were widely distributed across all the sites, though not observed in some quadrats of Mayonda. These nematode genera are all deposit feeders. *Terschellingia* is a selective deposit feeder, whereas *Daptonema* and *Spirinia* are non-selective feeders feeding mostly on deposited foods such as organic matter (Wieser 1953; Moens and Vincx 1997; Schratzberger et al.

2006). They also dominate every habitat (Alongi 1990). Spirinia was more in quadrats D, located at the start of the mudflat region across all sites, while *Terschellingia* was more in quadrats C, where the mangrove was across all sites. Alongi (1987) documented that *Terschellingia* survives better in the mangrove forest because itcould feed not only on the sediment organic matter since it is a deposit feeder but also does well in mangrove-derived tannin. *Spirinia* was more in the quadrats located at the mudflats. That is because the mudflats have substantial sediment organic matter deposits best for their survival, as reported by theresearch done by Alongi (1987). In addition, Alongi (1987) reported that *Spirinia* is a non-selective deposit feeder; hence it is not deprived of its feeds. However, with the sediment disturbances happening at Mayonda, both *Spirinia* and *Terschellingia* drastically reduced, especially towards the mudflats. That is because the sediment disturbance hindered organic matter decomposition caused by digging out the polychaetes at the mudflat of Mayonda, which played a majorrole in the drastic drop of both *Spirinia* and *Terschellingia*. After all, they have been deprived of their food.

Heip et al. (1985) documented that genera such as *Daptonema* and *Chromadorita* (Epistrate feeder) are found on sandy beaches. *Daptonema* and *Chromadorita* were more in Kirepwe, and this is because fine sand and medium sand were higher in Kirepwe as compared to Dabaso and Mayonda. Therefore, more Terschellingia were present in Kirepwe compared to other sites. Alongi (1987) noted that *Terschellingia* survives better in the mangrove forest because it could feed not only on the organic matter since it is a deposit feeder but also does well in mangrove-derived tannin. Alongi (1987) recorded that tannin from mangroves is more in sandier areas because it binds more easily with sand than fine sand and very fine sand, as observed at Kirepwe.

Spirinia, Terschellingia, and *Daptonema* were not distributed in some quadrats of Mayonda compared to Dabaso and Kirepwe. That is because the genera are among the nematode assemblages best known to occur in muddy and organically rich sediments (Schratzberger et al. 2006). Furthermore, these genera are not only found in undisturbed sediments in high numbers due to the availability of sediment organic matter, which acts as food, but they can also adapt and change during disturbances (Vanreusel 1990). Their ability to quickly adapt to a change in environment can be used to explain why these three genera were found in Mayonda despite the serious sediment disturbances.Therefore, the reduced number of such genera at Mayonda, compared to Kirepwe and Dabaso, played an important role in relaying information about the nematode community during the community analysis.

Pheronus, Pontonema, and *Viscosia* were restricted to a single quadrat of Dabaso (QC) while widely distributed in Mayonda, which was because of the predatory nature that enabled themto colonize disturbed areas (Moens and Vincx 1997). These genera contributed greatly to relaying important information about the nematode community assemblage during the community analysis between the three sites (Kirepwe, Dabaso, and Mayonda). *Dorylaimopsis, Marylynnia, Sabatiera,* and *Pontonema* were absent at Mayonda completely. *Dorylaimopsis, Sabatiera,* and *Pontonema* did not survive in Mayonda mainly because of the different proportions of the grain size. Such genera thrive best in areas with a high proportion of silt and very fine sand (Jensen 1987; Fonseca et al. 2018), as seen in Kirepwe and Dabaso.

Marylynnia is an epistratum feeder not seen in Mayonda alone (Wieser 1959). Giere (2009) confirmed that frequent physical sediment disturbances cause a shift in the nematode community from epistratum and deposit feeding to predatory feeding. That can explain why there were more predators at Mayonda compared to Kirepwe and Dabaso. During the community analysis, the presence of more predators and the absence of some epistratum, such as Marylynnia in Mayonda, caused the difference in the nematode community. Therefore, compared to the meiofauna from the studied sites, the nematodes can be used as bio-indicators of sediment disturbance to create a clear distinction between sites.

Despite *Spirinia* and *Terschellingia* being widely distributed in all the sites, in Mayonda, the genera had a low distribution. That is because of the physical sediment disturbances that destroy their feeding habitat (Alongi 1990). They are non-selective and selective deposit feeders, respectively. Therefore, the frequent disturbances prevented them from having a specific niche. At the same time, genera such as *Pheronus, Viscosia, Pontonema,* and *Synonchium* were widely distributed in Mayonda, which is attributed to their predatory feeding habit (Moens and Vincx 1997). They also noted that these nematodes are term opportunistic nematodes that colonize sediment disturbed areas just because other nematode genera cannot thrive in sediment disturbed areas such as Mayonda. It is important to note that *Pontonema* was able to thrive at Mayonda better due to its ability to scavenge for its food. *Sabatieria,* on the other hand, was not present in Mayonda despite its ability to tolerate both hypoxic and anoxic conditions. That is because *Sabatieria* and *Dorylaimopsis* thrive best under a high proportion of finer sediments size such as silt and very fine sand (Jensen 1987; Fonseca et al. 2018), as recorded in Kirepwe and Dabaso.

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Thermal tolerance, density, and distribution of mangrove crabs, *Perisesarma guttatum* and *Uca urvillei* at Gazi Bay, Kenya

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Abstract. *Kipyegon JK, Obudho PA, Kairo JG. 2019. Thermal tolerance, density, and distribution of mangrove crabs,* Perisesarma guttatum *and* Uca urvillei *at Gazi Bay, Kenya. Indo Pac J Ocean Life 3: 38-50.* Mangrove crabs are fundamental for the viability of mangrove forests. Therefore, the effects of climate change can impact mangrove ecosystems. The aim of this study was to determine the thermaltolerance, density, and distribution of the mangrove crabs *Perisesarma guttatum* (A.Milne-Edwards, 1869) and *Uca urvillei* (H.Milne Edwards, 1852) within Gazi Bay, Kenya. Field activities included collecting data on the density and distribution of the two crab species and environmental variables in the *Rhizophora mucronata* Lam., *Ceriops tagal* (Perr.) C.B.Rob. and *Avicennia marina* (Forssk.) Vierh. monospecific stands. The crabs were maintained at temperatures between 17-37°C in the laboratory, and respiration rate measurements were performed in closed chamber systems after the crabs were acclimated for 8 hours at a temperature of 27°C. The results indicate the temperature ranges for *P. guttatum* and *U. urvillei* adult crabs were 27-31°C and 27-33°C, respectively, beyond which the crabs got stressed as indicated by increased metabolism. Findings suggest that *P. guttatum* is more sensitive to temperature variation than *U. urvillei*. At the highest average densities of *U. urvillei* and *P.guttatum* crabs were recorded at 66.25±7.7/m² and 11.75±4.1/m², respectively, in the *R. mucronata* zone. The densities of *U. urvillei* and *P. guttatum* were strongly related to the temperature, fine sand, and organic matter using stepwise regression analysis ($P<0.05$). This study has increased the understanding of mangrove crab populations' physiological responses and possible distribution patterns to the expected impacts of climate change on these species.

Keywords: Density, distribution, Gazi Bay, mangrove crabs, *Perisesarma guttatum,* thermal tolerance, *Uca urvillei*

INTRODUCTION

Mangroves support distinctive fauna and flora, dominated by crabs and mollusks. There are 275 species of mangrove crabs globally, with 35 in Kenyan mangroves (Cannicci et al. 2009). In addition, mangrove crabs play an important role in the mangrove ecosystem as a food source (Gillikin et al. 2001; Gita et al. 2015). Thus, their diversity and relative abundance are crucial for the viability of mangrove forests, which in turn support coastal community livelihoods.

Uca urvillei (H.Milne Edwards, 1852) (Family: Ocypodidae) and *Perisesarma guttatum* (A.Milne-Edwards, 1869) (Family: Sesarmidae) are intertidal semi-terrestrial crabs. The *U. urvillei* is detritivorous and digs burrows for shelter. The *P. guttatum* is mainly omnivorous and uses other crabs' burrows and shade in mangroves to minimize thermal stress (Skov et al. 2002). In Gazi Bay, Kenya, both *P. guttatum* and *U. urvillei* occur in the *Rhizophora mucronata* Lam. and *Avicennia marina* (Forssk.) Vierh. zone (Cannicci et al. 2009). Few studies have described the densities of these crabs in the *Ceriops tagal* (Perr.) C.B.Rob. pure stands (Dahdouh-Guebas et al. 2002).

Climate change can threaten mangroves through temperature, sea level, salinity, low pH value, changes in precipitation, and frequency of storms located at the land-sea margins (Macintosh and Ashton 2004). These changes will soon affect the abundance and distribution of aquatic fauna, including crabs, causing local extinctions (Perry et al. 2008). Portner and Knust (2007) reported that thermal changes are the key drivers of ongoing ecosystem changes co-occurring with increasing hypoxia and carbon dioxide accumulation in a climate context. Portner (2010) observed that ecosystem changes have brought out the need to understand the mechanical background underlying physiological responses of aquatic ectotherms to thermal stress. Temperature determines distribution ranges and boundaries for marine and terrestrial species (Gaston 2003; Sanford et al. 2006). Portner et al. (2000) proposed the concept of oxygen and capacity-limited thermal tolerance in aquatic ectotherms. They successfully explained the climate-induced effects of rising temperatures on species abundance in the field (Portner and Knust 2007). However, there is a lack of documented studies on the thermal tolerance of mangrove crabs, *P. guttatum,* and *U. urvillei* in African mangroves.

It is still unknown if there is a link between mangrove crab distribution and thermal determinants (Tewksbury et al. 2008). Meta-analyses of species distribution patterns haveexplained climate-driven effects that have emphasized discovering large-scale patterns without necessarily understanding the mechanisms underlying the physiological processes and their response to abiotic stress. In addition, the abundance of crabs in some mangrove zones, such as the *C. tagal* pure stands, is yet to be fully documented.

The results from this study will allow an appraisal of possible differences in thermal tolerances of crab species that may help explain species distribution patterns within mangroves. The specific objectives were: (i) To establish the optimal thermal tolerance of male adults of *P. guttatum* and *U. urvillei* crabs at Gazi Bay; (ii) To determine the density of *P. guttatum* and *U. urvillei* crabs within the *R. mucronata, C. tagal* and *A. marina* mangrove zones at Gazi Bay; (iii). To establish the variation in the distribution of *P. guttatum* and *U. Urvillei*, concerning the environmental factors in the *R. mucronata, C. tagal*, and *A. marina* mangrove zones in Gazi Bay, Kenya.

MATERIALS AND METHODS

Study area

Gazi Bay is situated along the south coast of Kenya, between (4°25'S and 39°30'E) (Figure 1). Mangroves of Gazi Bay cover an estimated area of 615 ha (Kairo et al. 2008). That bay is sheltered from strong waves by the presence of the Chale peninsula to the east and a fringing coral reef to the south.

Monsoon winds principally influence the climate in Gazi Bay with two rainy seasons. The rains are heavier during the southeasterly monsoons and fall around April/August, whereas the northeasterly Monsoon winds bring light rains around October/November (Kairo 2001; Bosire et al. 2004). As a result, it is normally hot and humid with an average annual air temperature of about 28°C, with little seasonal variation and a temperature range between 24.8-39°C (Bosire et al. 2006). The seaward mangroves are inundated by tides twice a day, with the highest tidal range of spring tide being about 4.0 m (Bosire et al. 2006).

In Kenya, mangrove forests are facing increasing threats exacerbated by climate change leading to loss of biodiversity; 70% of the mangroves of Gazi Bay are degraded (Dahdouh-Guebas et al. 2004) by several factors, including the effects of 1997/8 El-Niño rains. Trial mangrove plantations were initiated in degraded intertidal areas in 1991 To enhance regeneration (Kairo 2001), and monospecific mangrove stands were planted in denuded mudflats between 1994 and 2000 (Bosire et al. 2004). There are more than 35 species of brachyurans belonging to six families and 4 anomurans associated with the Kenyan mangroves, including Gazi Bay (Cannicci et al. 1997). The two species were chosen because they are abundant and have easy collection and maintenance under laboratory conditions and wide geographic distribution. Further, the two species are strict residents of mangroves throughout their adult life and occupy different niches (Skov et al. 2002).

Sampling design

For crab density data, the sampling design adopted was stratified random sampling. The sampling stations, referred to as zones, were carried out in natural monospecific stands of *R. mucronata*, *C. tagal*, and *A. marina*. Two transects approximately 100-500 m apart, perpendicular to the waterline, were set randomly, cutting across the three mangrove zones to ensure the independence of samples. Four plots per zone of 10×10 m² and at least 20 m apart were randomly selected from a pool of eight plots for each zone, and their positions were marked with GPS. Density surveys were carried out in these plots during October and November 2011 and repeated in December 2011 and January 2012. Each plot was divided into four quarters, and four 1-m² sub-quadrats were randomly selected for the survey from each quarter, making a total of 48 sub-quadrat samples per month. Stratified random sampling protocols were adapted during this study (Chapmanand Tolhurst 2004; Cannicci et al. 2009). Visual observation and burrow counts provide crude estimates of population density at best, even where a single species is concerned (Macia et al. 2001; Skov and Hartnoll 2001).

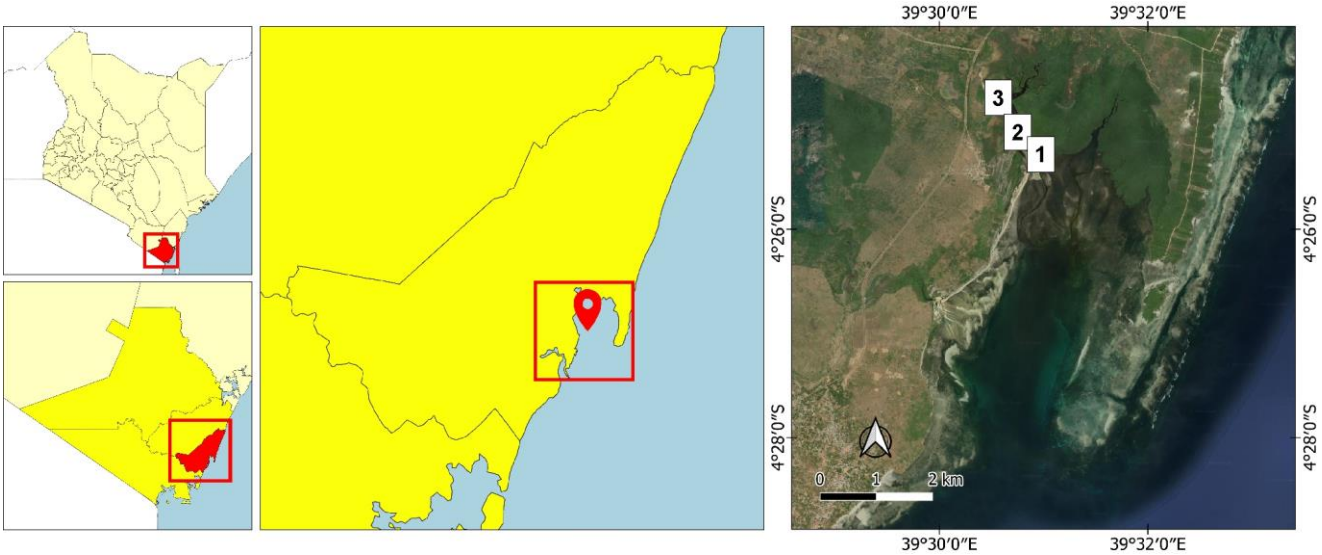

Figure 1. Map of the Kenyan coast showing the study area, Gazi Bay. Adopted from Bosire et al. (2004). Sampling stations: 1. *Rhizophora mucronata* 2. *Ceriops tagal* 3. *Avicennia marina* zones

Figure 2. One (1) m² quadrat (enclosed with a piece of rope round) used to sample crabs in the *Ceriops tagal* zone

Data collection

Determination of density of Perisesarma guttatum *and* Uca urvillei *crabs*

Crab density sampling was carried out across the intertidal area in the threemangrove zones using binoculars in the four 1-m² sub-quadrats per plot (Figure 2) for five days during spring high tides. For *U. urvillei*, density was estimated using visual and burrow counts (Skov et al. 2002; Cannicci et al. 2009). Visual counts of all active crabs were done using binoculars (8x40 magnification) when standing at about 3.5 meters from each quadrat, and crabs were enumerated after the observer remained motionless for 15 minutes to provide sufficient time for resumption of full activity. In addition, crab burrow openings were counted to avoid underestimation of crabs not active on the surface during visual counts (Hartnoll et al. 2002). The density of *U. urvillei* was estimated by the burrow count data and calibrated with the species ratio (all species counted that make burrows observed within the quadrat) obtained from visual counting. For *P. guttatum*, they were counted visually using binocularsthroughout the 1-m² sub-quadrats, as no burrow counts were used for *P. guttatum* because they do not make burrows (Skov et al. 2002).

Measurement of abiotic factors

At each sampling station, data for the following parameters were collected to characterize the crab's habitat: Sediment temperature on the sub-surface was recorded using a digital probe thermometer, and pore water salinity of sediment (determined from centrifuge-extracted interstitial water) was measured using an optical refractometer (Atago brand), in situ data on Redox potential (Eh) and PH was measured using standard electrodes and a combination millivolt/pH meter. Redox potential (Eh) is a quantitative measure of reducing the power that provides a diagnostic index of the degree of anaerobiosis or anoxia (Patrick and Delaune 1977). The pH was measured with fresh samples of sediments to avoid oxidation of iron pyrites to sulfuric acid to avoid giving a much lower pH value than normally occurs. The above samples were taken at 10 cm and 40 cm depths along the core.

Sediment characteristics sampled were; fine and coarse sand, silt and clay, and organic matter of the sediment. In the sediment sampling, 4 cores of 5 cm deep by 2.5 cm wide per plot per sub-quadrat in each zone were taken for granulometric analysis. Sediments were measured by placing a known weight of sediment of about 100-150 grams into an oven at 80ºC for about 24 hours until the constant dry weight was obtained for granulometric analysis. About 25 g for each dry sample from the oven were then weighed and transferred into pre-labeled beakers with 250 mL water and 10ml of aqueous sodium hexametaphosphate $(NaPO_3)_6$) and stirred for 10 minutes and leftfor a minimum of four hours. Afterward, they were subjected to a series of sieves ranging from <63 to 500 µm mesh sizes for wet-sieving to measure cumulative percent weights of soil particle sizes. The organic matter in the samples was obtained by ashing about 20 g of the remaining dry sample from the oven to 450ºC for four and half hours in the furnace and then cooling and weighing. The difference in weight gave an estimate of organic matter (Wartel et al. 1995) as:

$$\% \text{ Organic matter} = \text{Initial weight(g)} - \text{Final weight(g)} \times 100\%$$

Collection of crab specimens for laboratory experiments

The *P. guttatum* and *U. urvillei* specimens for thermal tolerance experiments were collected by hand capture at low tide. At least ten male adults per species per experiment were collected. They were kept and maintained in the laboratory for at least 24 hours (Vernberg 1959) at ambient temperature before the experiments. They were kept in plastic tanks with 5 cm mangrove soil sediment from their habitat area and were not fed. The health of the crabs was determined using the Righting Response Time method (Hogarth 1999), and only those crabs that quickly righted themselves up were used.

Optimal thermal tolerance experiments for male adults of Perisesarma guttatum *and* Uca urvillei

In temperature-controlled rooms, adults of *P. guttatum* and *U. urvillei* were maintained separately at different experimental temperatures (17-37ºC). Respiration rate measurements were performed using a closed chamber system consisting of five partially darkened respiration chambers (using aluminum foil to avoid visual stresses) and connected to single-channel, temperature-compensated oxygen meters (Fibox 3-Presents de), as shown in Figure 3. The Fibox 3 was connected to a PC desktop that recorded all measurements. Each respiration chamber had a single crab, except the control, and was submersed in a constant temperature recirculating water bath heated to the appropriate trial set-point temperature. The control chamber with no crab inside was used to correct bacterial oxygen consumption.

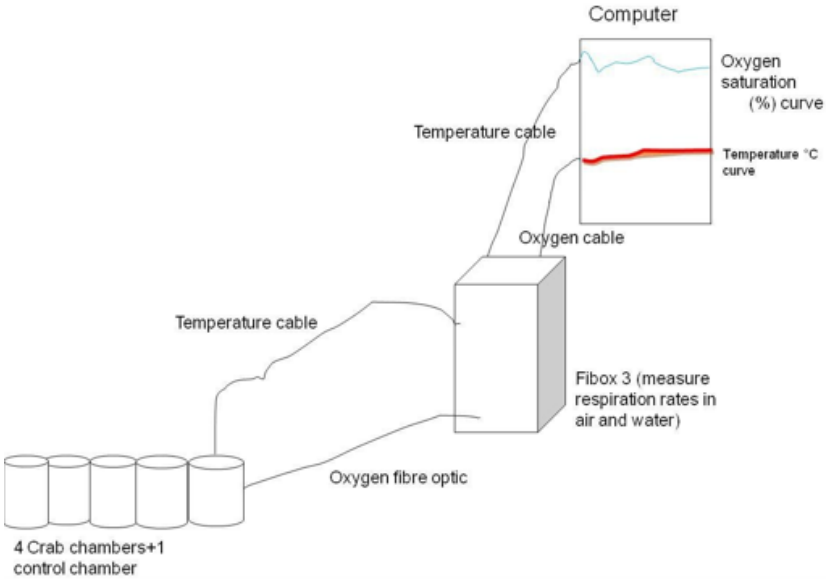

Figure 3. Laboratory crab experimental set-up to measure respiration rates

The air and water experiments were performed separately for each species. For water experiments, caution was taken within the crab chambers to ensure no bubbles were introduced while putting the crab inside the chamber. Sea water was used that had been filtered to remove particulate matter (0.2 µm filter). The water flow from each respirometer was controlled with a screw pinch clamp. The crab chambers were made of glass with removable airtight lids and a screw fitting for the oxygen fiber optic cable, and they had a capacity volume of 450 mL.

The experiments were divided into two parts; (a) increasing temperature from 27 to 37°C in air media per species separately and increasing temperature from 27 to 37°C in water media per species separately (b) decreasing temperature from 27 to 17°C in water media only for each species separately. During each experiment, the crabs were acclimated for eight (8) hours in the respirometry chambers at 27°C, the ambient temperature at Gazi Bay during the study period. The valves were then switched off using a screw pinch valve/clamps to cut off atmospheric oxygen for air experiments and external dissolved oxygen for water experiments, as well as the control chambers to record oxygen consumption rate inside the chambers only. The respiration rate of each crab in the closed chamber was determined by measuring the decline in oxygen saturation (initial 100%) in the known volume of water and the air surrounding the crab in the chamber and was recorded each lasting 5 minutes making a total of 25 minutes for 5 chambers and used as their basal metabolism (Hervant et al. 1998; Frederich and Pörtner 2000). At least two measurements/replicates of oxygen consumption rates per experiment were made for each crab's respiration rates per temperature step to ensure a strong regression line of declining oxygen saturation.

Measurements of oxygen consumption rates were conducted every 20 minutes between each of the two replicate measurements (one replicate here refers to one full measurement in 5 chambers per temperature step) at each experimental temperature per experiment. The declining rate of oxygen content/percent saturation (Y-axis) versus time (X-axis) was calculated as the slope of the regression line fitting the oxygen consumption rates data versus time from the two pooled replicate measurements per temperature level was automatically recorded by the computer. The oxygen content of the water was never allowed to fall below 80-90% saturation to avoid hypoxic stress during recording. All measurements lasted between 4 hours for air experiments and water experiments up to 2 hours. In the case of air experiments, the volume of the chambers was too great to allow feasible measurement of the consumption of 10-20% of the available oxygen, so the volume was decreased by adding glass marbles to the chamber. The volume of glass marbles was recorded after the experiment, and the new volume of the chamber was calculated. The crabswere used only once in each experiment, and water was not changed between replicate measurements. After the respiration rate at the acclimation temperature at 27°C was recorded, the valves were removed, and the crab chambers reaerated again. The temperature was then increased gradually at 2°C intervals every 2 hours to the next experimental temperature. At the same time, oxygen consumption rates were recorded to the maximum and minimum experimental temperatures of 37°C and 17°C, respectively.

At every experimental test temperature, the crabs were left to acclimate to the experimental temperature for a further 30 minutes. The experiment was then repeated at the new temperature, and the respiration rate was recorded. Each experiment lasted about 33 hours. Moreover, 12 and 10 specimens or replicates of *P. guttatum* adult males were used in water and air respiration experiments, respectively. In comparison, for *U. urvillei*, 15 and 12 specimens/replicates of males for water and air respiration experiments were used, respectively. At least two temperature measurements were performed at each temperature level. At the end of each experiment, the mass

and volume of the crabs were recorded and subtracted from the empty chamber to determine the exact amount of respiratory medium of each crab per experiment to get mean weight-specific oxygen consumption rates (µmol). All crabs were released 24 hours post experiment back to their area of collection (Eshky 1999; Addo-Bediako et al. 2000; Jimenez and Bennet 2005). Three incidences of mortalities of *P. guttatum* were reported during the experiments, with two at 33°C and one at 37°C may be due to oxygen bubbles in the chambers. In almost all instances in water experiments, therefore, measurements were made in 2°C increments over a total temperature range of 10°C above and below 27°C, which for every species bracketed its preferred temperature. Assumptions were made that the comparatively brief period spent at each measurement temperature (≤2 hours) provided little or no temperature acclimation. Moreover, the considerable care taken to avoid disturbance during sampling or flushing of the respirometer probably prevented any cumulative stress effects during the experiment.

Oxygen consumption rate recordings were carried out in constant darkness to tryto eliminate the effects of diel changes in oxygen consumption rate. In addition, the decreasing temperature in water experiments was performed to understand the physiological responses/cold tolerance ofthese animals in cold water as these two crab species are naturally known to occur up to their southern latitudinal limit range in subtropical regions on the north coast of South Africa where they are exposed seasonally to chilly weather (Lee 2008).

Only male crabs were used in this study following previous similar crab physiological studies, which showed no differences in responses of either sex (Frederich and Pörtner 2000; Jimenez and Bennett 2005) and thus was a representative model of these crab populations.

Data analysis

General Linear Model (GLM) ANOVA was used to test for the effect of increasing temperature (independent variable) on oxygen consumption rate (dependent variable) for each species. Tukey test was used to show which paired temperatures significantly affected oxygen consumption rates. GLM was also used to test for the effect of temperature, species, and media (independent variables) on oxygen consumption rates (dependent variable). GLM 3-factor ANOVA with species (two levels), zones (3 levels), transects (2 levels), and season (2 levels) as the factors and their interactions was used to test for significant effectsof the factors on the densities of the two species. Differences in environmental parameters between zones and season and between zones and transects were tested using a two-way ANOVA with zones, season, and transects as the factors. The relationship between crab density (dependent variable) and environmental parameters (independent variables) was carried out using Backward stepwise multiple regression analysis. Percentage data were arc-sine transformed prior to analysis. For the ANOVAs, the dependent variable crab density was square-root transformed prior to analysis to stabilize variance and ensure normality. All P- Values less than 0.05 ($P<0.05$) were significantly different. All analyses were carried out using Minitab Version14 software.

Q_{10} is the factor by which an ectothermic physiological rate increases over a 10°C interval but also T_1 and T_2 do not need to be exactly 10 degrees apart to use this equation as shown in the formula:

$$Q_{10} = (R_2/R_1^{10/(T_2-T_1)}$$

Where R_2: Final Respiration rate; Respiration rate; R_1: Initial Respiration rate; $^{T}_{2}$**:** Temperature at R_2; ^{T}: Temperature at R_1 (Jimenez and Bennett 2005).

RESULTS AND DISCUSSION

Determination of thermal tolerance of adult males of *Perisesarma guttatum* and *Uca urvillei*

The *P. guttatum* and *U. urvillei* displayed similar respiratory response patterns to increasing temperature in both air and water media (Figure 4). The results show a general increase in oxygen consumption rate with increasing temperature in both media, but not constantly at the range of temperatures tested.

Oxygen consumption rate at an initial temperature of 27°C differed for each species per media. At 27°C in water, *P. guttatum* and *U. urvillei* had 0.08±0.04 and 0.06±0.02 µmol/gmin, respectively, while at 27°C in air, *P. guttatum* and *U. urvillei* had 0.14±0.03 and 0.51±0.02 µmol/gmin respectively. At 37°C in water, oxygen consumption rates for *P. guttatum* and *U. urvillei* were 0.14±0.02 µmol/gmin and 0.09±0.01 µmol/gmin, respectively. While at 37°C in air 0.23±0.11 µmol/gmin and 0.13±0.04 µmol/gmin for *P. guttatum* and *U. urvillei*, respectively.

Results in Figure 5 indicate oxygen consumption rates increased with increasing temperature in water media from 17-37°C in both species, but not constantly at the range of temperatures tested. The graphs showed three major levels giving astaircase pattern; the lower temperature level of 17-27°C and middle level of 27-31°C for *P. guttatum* and of 27-33°C for *U. urvillei*, where there was an apparent regulation of respiration rate and lastly the high-level temperature 31-37°C. In the low-level temperature, very low oxygen consumption rates with an increase in temperature between 17-23°C were recorded, indicating low metabolism for both species. Then oxygen consumption rate increased exponentially with temperature rise from 25-27°C. At the middle level, oxygen consumption rates did not increase with increasing temperature (oxygen consumption rate is temperature independent). In contrast, for *P. guttatum*, this temperature range was 27-31°C while that of *U. urvillei* range was 27-33°C. Finally, the third level indicates increased oxygen consumption rates by both species with increasing temperature, with *P. guttatum* rates nearly more than doubling that at 37°C.

The average rate of oxygen consumption of *P. guttatum* was 0.012±0.007 µmol/gmin, half that for *U. urvillei* of 0.02±0.008 µmol/gmin at 17°C. On the hand, oxygen consumption rates for *P. guttatum* and *U. urvillei* at 37°C

were 0.14±0.02 µmol/gmin and 0.08±0.01 µmol/gmin, respectively, which suggests that at low temperatures *U. urvillei* tend to have higher metabolic rates than *P. guttatum. In contrast*, at high temperatures, it is the opposite.

Temperature variation significantly affected oxygen consumption rates (F5,244=15.95, P<0.05) of P. guttatum and U. urvillei in air and water media. In addition, oxygen consumption rates were significantly different between the two species ($F_{1,244}$=155.39, P<0.05) and significantly different between air and water media ($F_{1,244}$=27.64, P<0.05).

Temperature variation showed a significant effect on oxygen consumption rates ($F_{5,125}$=10.81, P<0.05) in *P. guttatum* and *U. urvillei* ($F_{5,134}$=9.32, P<0.05) in both air and water. There was a significant difference in oxygen consumption rates between air and water in *P. guttatum* ($F_{1,125}$=61.35, P<0.05). At the same time, there was no significant difference in oxygen consumptionrates between air and water in *U. urvillei* ($F_{1,134}$=0.12, P>0.0.05). The *P. guttatum* consumed higher oxygen consumption rates in the air than in water throughout the tested temperatures, while it is the opposite for *U. urvillei*, with higher oxygen uptake in water than in air except for between 33-35°C, where there was a drop and then it rose again from 35-37°C (Figure 5).

For the respiration rate of *P. guttatum* in air and water, temperature variation showed a significant effect on oxygen consumption rates ($F_{5,125}$=10.81, P<0.05). Further analysis, by the Tukey test, a low oxygen consumption rate which did not vary with increasing temperature was observed between the temperature range 27-31°C, where temperature variation had no significant effect on oxygen consumption rates(P>0.05) in both air and water (Figure 4 and 5). However, temperature variation showed a significant effect on oxygen consumption rates between 33-37°C (P<0.05) and between 17-27°C (P<0.05) (Figures 4 and 5).

For the respiration rate of U. urvillei in air and water media, temperature variation significantly affected oxygen consumption rates ($F_{5,134}$=9.32, P<0.05). The temperature range between 27-33°C did not show any significant effect on oxygen consumption rate (P>0.05) in both air andwater (Figures 4 and 5). However, temperature variation showed a significant effect on oxygen consumption rates between 33-37°C (P<0.05) and between 17-27°C (P<0.05).

Q_{10} over the whole temperature range of 17-37°C for *P. guttatum* was 3.31, while for *U. urvillei* was 2.02. Aquatic oxygen consumption rates of *P. guttatum* and *U. urvillei* increased from 17-27°C with a Q_{10} value of 6.58 and 3.11, respectively, thus clearly showing that Q_{10} of *P. guttatum* was higher than the latter, in fact, by double value. Over the temperature range of 27-37°C in water media, the Q_{10} of oxygen uptake was not similar for the two species, with *P. guttatum* having 1.7 while that of *U. urvillei* was 1.3. Q_{10} for *P. guttatum* and *U. urvillei* in the air over 27- 37°C was 1.6 and 2.6, respectively, opposite for *U. urvillei* to that recorded in water. Within the optimal temperature range for *P. guttatum* of 27-31°C, the Q_{10} was 1.5, while for *U. urvillei*, at the optimum temperature range of 27-33°C, Q_{10} was 1.4.

In summary, the optimum temperature range for *P. guttatum* was 27-31°C, while that of *U. urvillei* was 27-33°C, where the temperature had no significant effect on oxygen consumption rates (P>0.05). The optimal thermal ranges of the two species are significantly different ($F_{3, 161}$=4.02, P<0.05).

Crab density and distribution in mangroves

Figure 6 generally indicates a higher density of U. urvillei than *P. guttatum* in the *R. mucronata* zone compared to other zones. The *P. guttatum* density was highest in the *R. mucronata* zone, followed closely by the *C. tagal* zone, and lowest in the *A. marina* zone. The highest mean density (Number (No)/m²±SE) of *U. urvillei* and *P. guttatum* was 66.25±7.7 and 11.75±4.1, respectively, in the *R. mucronata* zone. The *A. marina* zone recorded the lowest mean density (No/m²±SE) of the two species, *U. urvillei* was 0.18±0.7, and that of *P. guttatum* was 1.9±1.8. The density (No/m²±SE) of *U. urvillei* and *P. guttatum* in the *C. tagal* zone was 0.47±0.28 and 8.03±1.8, respectively.

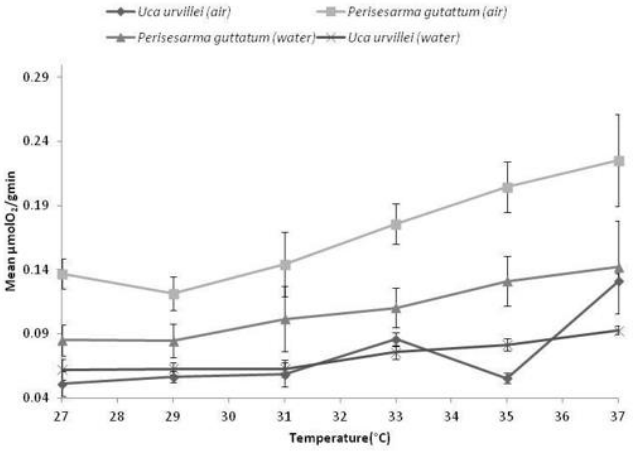

Figure 4. Relationship between temperature (mean±SE) and oxygen consumption rates of *P. guttatum* and *U. urvillei* crabs in air and water media

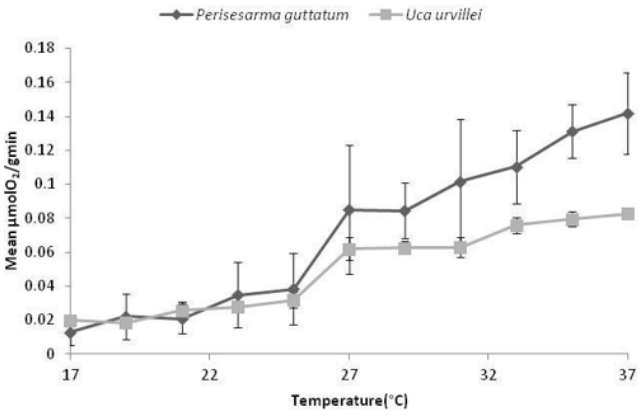

Figure 5. Relationship between temperature (mean±SE) and oxygen consumption rates of *P. guttatum* and *U. urvillei* crabs in water media

There were significant differences in the density of the two species between the three mangrove zones ($F_{2,47}$=402.95, P<0.05) for *U. urvillei* and ($F_{2,47}$=65.04, P<0.05) for *P. guttatum*. Further analysis showed that *U. urvillei* density was significantly different between; *R. mucronata* and *A. marina* zones (T=-28.75, P<0.05), *R. mucronata*, and *C. tagal* (T=-27.66, P<0.05) but was not significantly different between *A. marina* and *C. tagal* zones (T=1.088, P>0.05). For *P. guttatum*, there was a significant difference indensity between; *R. mucronata* and *A. marina* zone (T=-9.996, P<0.05), *C. tagal* and *A. marina* zones (T=7.994, P<0.05) but was not significantly different between *R. mucronata* and *C. tagal* zone (T= -2.003, P>0.05). The season transects and neither interaction between zones, transects, and seasons did not show any significant effect on the density of these crabs (P>0.05) (Figure 6).

Environmental variables

The results revealed significant environmental parameters and soil particle characteristics differences between the three mangrove zones (P<0.05). However, the environmental factors did not significantly vary with seasons and transects (P>0.05).

Salinity

The highest porewater salinity recorded during the study period was 63‰ in the *A.marina* zone, and the lowest was 21‰ in *R. mucronata*. The results in Figure 7 indicate mean porewater salinity in *A. marina* zone ranged from 42.25 to 51.75‰, for *R. mucronata*, it ranged from 27.152 to 34.13‰ while for *C. tagal*, it ranged from 34.53 to 44.75‰. There were significant differences in salinity between the three mangrove zones ($F_{2,47}$ =31.40, P<0.05) but not between seasons ($F_{1,47}$=4.73, P>0.05), and neither interaction between season nor zones ($F_{2,47}$=0.72, P>0.05). There was no significant difference in salinity between transects ($F_{1,47}$=0.07, P>0.0.5) and neither interaction between transects and zones ($F_{2,47}$=0.29, P>0.05).

Temperature

The highest temperature recorded during the study period was 36.8°C in December in the *A. marina* zone, while the lowest temperature recorded was 29.0°C in the *R. mucronata* zone in November (Figure 8). There were significant differences in temperature between the three mangrove zones ($F_{2,47}$=6.11, P<0.05). Analysis revealed a significant difference between *R. mucronata* and *A. marina* zones (T=2.548, P<0.05) and between *R. mucronata* and *C. tagal* (T=3.371, P<0.05), while the temperature was not significantly different between *A. marina* and *C. tagal* zones (T=0.8225, P>0.05). There was no significant difference in temperature between seasons ($F_{1,47}$=0.72, P>0.0.05) and neither interaction between season and zone ($F_{2,47}$=1.21, P>0.05). There was no significant effect of temperature on transects ($F_{1,47}$=,4.95, P>0.05) and neither interaction between zone nor transects ($F_{2,47}$= 1, P>0.05).

pH

The mean PH value ranged between 5.6-6.67 in the *R. mucronata* zone. In the *A. marina*, the pH ranged from 5.6 to 6.4, while in C. tagal, it ranged from 6.0 to 6.3 (Figure 9). There was no significant difference in pH between the three mangrove zones ($F_{2,47}$=0.43, P>0.05) nor the interaction between season and zones ($F_{2,47}$=0.32, P>0.05). Likewise, there was no significant difference in PH between zones and transects ($F_{1,47}$ =,0.24 P>0.0.05) and neither interaction between zones nor transects ($F_{2,47}$=,0.30, P>0.05).

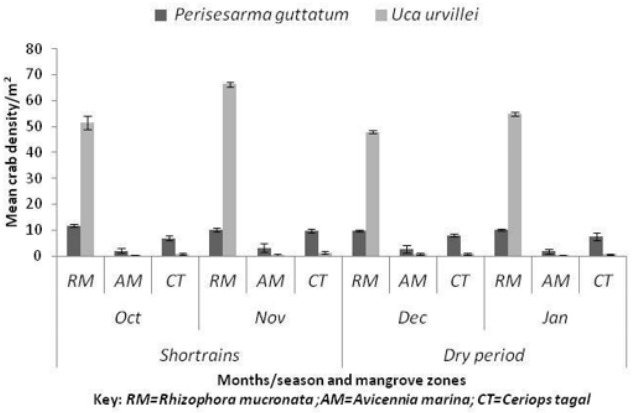

Figure 6. Mean density (±SE) of *P. guttatum* and *U. urvillei* in mangroves zones

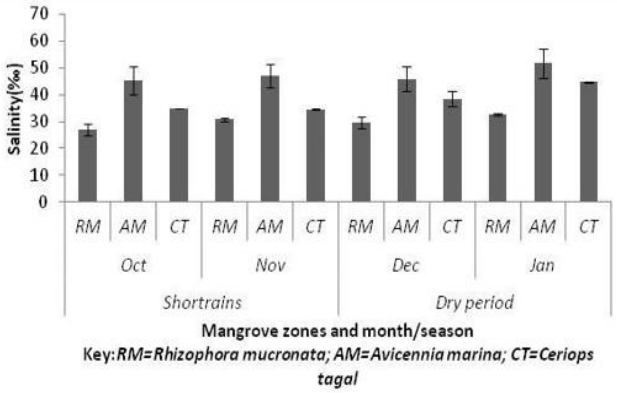

Figure 7. Mean (±SE) monthly salinity in mangrove zones

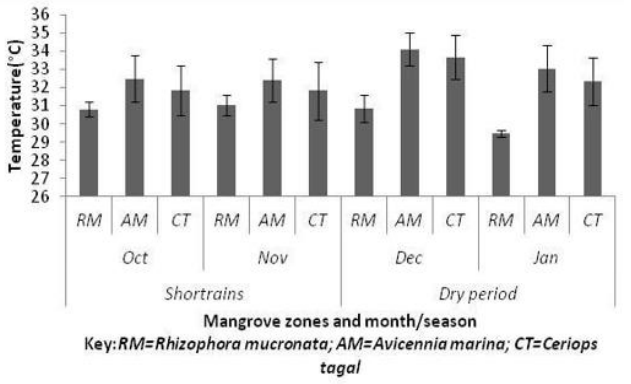

Figure 8. Mean (±SE) monthly temperature in different mangrove zones pH

Redox potential (eH)

The highest redox potential recorded during the study period was 155.6 in *A. marina*, and the lowest was -229.9 in the *R. mucronata* zone (Figure 10). There was a significant difference in Redox potential between the three mangrove zones ($F_{2,47}$=28.3, P<0.05), and analysis revealed a significant difference in redox between; *R. mucronata* and *A. marina* (T=7.176, P<0.05), *A. marina* and *C. tagal* (T=-5.546, P<0.05) while redox potential was not significantly different between

The *R. mucronata* and *C. tagal* zones (T=1.630, P>0.05). Redox potential did not show any significant difference between seasons ($F_{1,47}$=4.01, P>0.05), neither interaction between zone nor seasons ($F_{2,47}$=3.21, P>0.05). Likewise, there was no significant difference in Redox potential between transects ($F_{1,47}$=1.78, P>0.05) and neither interaction between transects and zones ($F_{2,47}$=1.81, P>0.05).

Soil characteristics

The *C. tagal, R. mucronata,* and *A. marina* recorded the highest percent silt of 68.2±8.02%, 52.7±4.05%, and 31.3±18.8%, respectively (Table 1). There were significant differences in silt between the three zones ($F_{2,47}$=125, P<0.05), and analysis revealed significant differences between; *R. mucronata* and *A. marina*, *R. mucronata*, and *C. tagal* and between *C. tagal* and *A. marina* zones (P<0.05). There was no significant difference in silt between transects ($F_{1,47}$=0.01, P>0.05) nor the interaction between transects and zone ($F_{2,47}$=0.01 P>0.05).

The *A. marina* zone generally had higher fine sand compared to the other two zones (Table 1). There were significant differences in fine sand between the three mangrove zones ($F_{2,47}$=27.37, P<0.05). In further analysis, fine sand showed significant differences between *R. mucronata* and *A. marina* zones (T=2.552, P<0.05), between *R. mucronata* and *C. tagal* zones (T=3.372, P<0.05), while there was no significant difference in fine sand between *C. tagal* and *A. marina* zones (T=0.8198, P>0.05). There was no significant difference in fine sand between transects ($F_{1,47}$=0.72, P>0.5) nor the interaction between transect and zone ($F_{2,47}$=0.15,P>0.05).

The *A. marina* generally had the coarsest mean sediment or sand and recorded the highest value of 19.3±7.0%, followed by *R. mucronata* 12.6±7.4% and finally *C. tagal* 8.4±0.6% (Table 1). There were significant differences in percent coarse sand between the three mangrove zones ($F_{2,47}$=71.00, P<0.05). Analysis revealed significant differences in coarse sand between *R. mucronata* and *A. marina* (T=3.331, P<0.05), *C. tagal*, and *A. marina* zones (T=-3.41, P<0.05), while no significant difference was observed between *R. mucronata* and *C. tagal* zone (T=-1.307, P>0.05). There was no significant difference in coarse sand between transects ($F_{1,47}$=0.63, P>0.05) nor the interaction between transects and zones ($F_{2,47}$=0.52, P>0.05).

The highest percent organic matter content was recorded in the *R. mucronata* zone at 24.2±2.4%, followed by *C. tagal* at 14.7±11.4% and *A. marina* zone at 12.3±8.1% (Table 1). The organic matter content revealed significant differences between the three mangrove zones ($F_{2,47}$=152.60, P<0.05) and the interaction between zones and transects ($F_{2,47}$=150.70, P<0.05) but not between transects ($F_{1,47}$=62.53, P>0.05). Tukey test revealed significant differences in percent organic matter between; *R. mucronata* and *C. tagal* (P<0.05), *R. mucronata* and *A. marina* (P<0.05), but there was no significant difference in the organic matter between *A. marina* and *C. tagal* zones (P>0.05).

Environmental variables and crab density

Table 2 indicates that temperature, salinity, silt, fine sand, and organic matter significantly affected *U. urvillei* density (P<0.05) and accounted for 69.82% of the variations in density. The lowest mean temperature, salinity, and higher organic matter content, silt, and fine sand were recorded in the *R. mucronata* zone, which also recorded the highest densities of this species.

Table 3 indicates that temperature, fine sand, and organic matter significantly affected *P. guttatum* density (P<0.05) and accounted for 67.92% of the variations in density. However, silt could have a marginal effect on the density of the species (P>0.074).

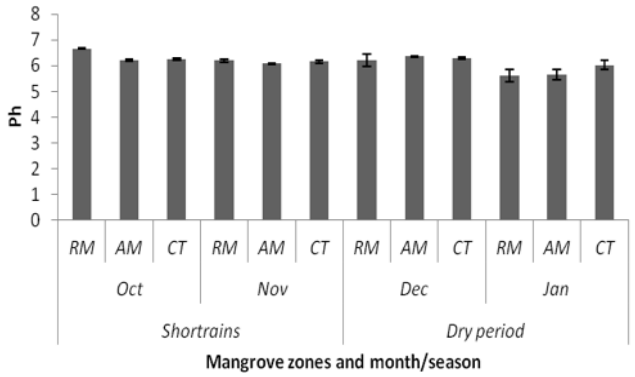

Figure 9. Mean (±SE) monthly pH in different mangrove zones. Note: RM: *Rhizophora mucronata*; AM: *Avicennia marina*; CT: *Ceriops tagal*

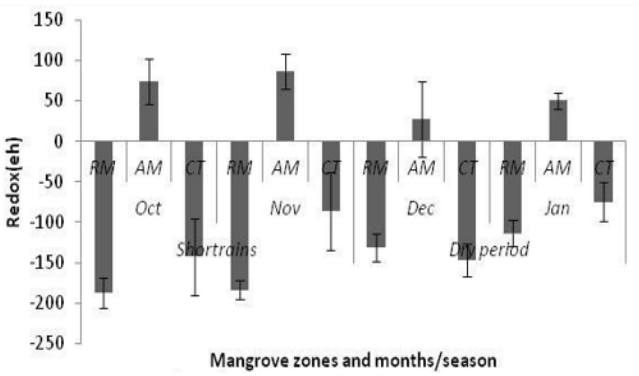

Figure 10. Mean (±SE) monthly Redox potential (eH) in different mangrove zones. Note: RM: *Rhizophora mucronata*; AM: *Avicennia marina*; CT: *Ceriops tagal*

Table 1. Particle sizes (%) variation over months/seasons and mangrove zones

Season	Month	Zone	Silt %	Fine sand (>63µm)%)	Coarse sand (>500µm)%	Organic matter (%)
Short-rains	OCT	RM	52.7±4.05	36.3±4.1	10.95±4.5	22.7±2.6
		AM	20.1±8.15	63.2±14.7	16.7±6.6	12.3±8.1
		CT	68.2±8.02	24.8±5.8	6.9±2.3	14.6±10.7
	NOV	RM	34.6±10.7	52.8±6.7	12.6±7.4	24.2±2.4
		AM	23.8±4.6	56.9±4.2	19.3±7.0	10.9±5.3
		CT	54.27±5.1	37.3±12	8.4±0.6	13.8±10.2
Dry-period	DEC	RM	51.2±20.9	39.4±15.6	9.3±6.7	24.1±0.9
		AM	31.3±18.8	53.9±17.8	14.8±9.1	9.7±5.7
		CT	60.3±15.4	32.9±12.8	6.8±2.7	14.7±11.4
	JAN	RM	42.4±25.2	51.1±1.3	6.5±23.9	23.6±1.6
		AM	13.1±3.3	73.6±7.5	13.4±10.2	11.8±6.4
		CT	48.8±17.4	43.6±2.4	7.6±15.0	12.6±9.1

Note: RM: *Rhizophora mucronata*; AM: *Avicennia marina*; CT: *Ceriops tagal*

Table 2. Stepwise regression analysis (Backward) of the effects of dependent variable *Uca urvillei* density on the independent variables

Variable	Constant	T-value	P-value
Silt (<63 µm)	-27.4	-4.0	0.000
Fine sand (>63µm)	-26	-4.13	0.000
Organic matter	0.155	-4.16	0.001
Temperature	-0.433	-3.66	0.001
Salinity	-0.086	5.01	0.000

Note: S=0.442, R^2=69.82, R-Sq(adj)=66.23, Mallows C-p=4.1, r^2=69.82%, P<0.05, *U. urvillei* density (Y)=35.9(C), -27.4 Silt, –26 Fine sand, +0.155 Organic matter, -0.433 Temperature, -0.086 Salinity

Table 3. Stepwise regression analysis (Backward) results in the effects of dependent variable *P. guttatum* density on independent variables

Variable	Constant	T-value	P-value
Fine sand (>63µm)	1.96	-2.05	0.047
Organic matter	4.3	3.80	0.000
Temperature	-0.074	-2.53	0.015

Note: S=0.200, R^2=67.92, R-Sq(adj)=64.10, Mallows C-p=3.4, r^2=67.92%, P<0.05. The *P. guttatum* density (Y)=2.221(C), +1.96 Fine sand, +4.3 Organic matter, -0.074 Temperature

Discussion

Determination of thermal tolerance of adult males of Perisesarma guttatum *and* Uca urvillei

The study indicated that the metabolic rates of *P. guttatum* and *U. urvillei* increased over the temperature range of 17-37°C indicating the animals were displaying standard metabolic rates (Bennett, 1988). However, within a certain temperature range of each species in both media, the oxygen consumption rate was less independent of temperature, suggesting the optimum temperature range. This temperature range of no metabolic change with increasing temperature for *U. urvillei* was 27-33°C, while that of *P. guttatum* was 27-31°C, suggesting the latter's thermal tolerance window is narrower, thus indicating that the two species have different optimal thermal ranges. That clearly showed a difference in the thermalsensitivity of the two species, which agrees with the general observation by Angilletta et al. (2009).

These results are like Frederich and Pörtner (2000), who determined the optimum temperature range for spider crab, *Maja squinado* (Herbst, 1788), to be between 8-17°C for maximum aerobic scope. Other similar studies by Teal (1959) for seven *Uca* species observed that oxygen consumption rates were relatively temperature-insensitive within a given optimum temperature range; for example, *Uca minax* (Le Conte, 1855) and *Uca pugilator* (Bosc, 1801) consumed oxygen at the same rate between 11.1-15.9°C and 13.2- 19.4°C respectively, and the temperature had little effect on their metabolism. A discontinuous metabolic response was observed at certain points in the range despite the increasing temperature. In this case, *P. guttatum* corresponds tothe temperature range of 27-31°C with a Q_{10} of 1.5, while that for *U. urvillei* was at 27-33°C with a Q_{10} of 1.4. These Q_{10} values are closer to 1, which indicates thermal insensitivity. Relatively low Q_{10} values indicate wide thermal tolerance of the species (Katsanevakis et al. 2007). Within the normal environmental temperature range, the Q_{10} values for oxygen consumption rates are consistently less than or equal to 2. Q_{10} over the whole temperature range of 17-37°C for *P. guttatum* was 3.31, while for *U. urvillei* was 2.02. That agrees with studies by Katsanevakis et al. (2007), who reported that most crustaceans have a Q_{10} of between 2 and 3.

These studies have shown that, for both species, values of Q_{10} are lower in the temperature range normally experienced but increase at temperatures outside this range. This reduction in the sensitivity of metabolic rate to changing temperature has been interpreted as a mechanism by which energy could be conserved despite the increase in environmental temperature. Newell (1969) reported that the metabolic rate in response to acute temperature change varies very little over a wide portion of the physiological temperature range in intertidal animals. The adaptive significance of this is that the standard rate of respiration of intertidal invertebrates is relatively

independent of fluctuations in temperature and is not only lower but often has a low-temperature coefficient. Some organisms may temporarily decrease metabolism below these levels or even enter ametabolic states in response to unfavorable environmental conditions. However, they are not fully functional organisms under these conditions and must return to normal metabolic levels to proceed with normal processes of life (Bennett 1988). Thus, a comparatively stable oxygen consumption rate over that range of temperature fluctuation encountered daily is of significant value to these intertidal organisms. As a result, adaptation to intertidal life in these crabs entails high metabolic flexibility and efficient regulation of metabolism in a wide range of environmental temperatures. The difference in Q_{10} values for oxygen consumption rates between the two species *P. guttatum* and *U. urvillei*, may reflect differences in the range of temperatures that they normally experience in the field. Although both species occur at the same location, differences in their lifestyle in occupying different niches and behavior may result in the two species experiencing somewhat different temperature regimes (Eshky 1999).

The temperature ranges of 27-31°C (*P. guttatum)*and 27-33°C (*U. urvillei)* infer the temperature window of maximum scope for aerobic activity, which suggests the range of optimum performance supporting successful survival where availability of aerobic energy is maximal for all physiological functions in the natural environment. That is in agreement with studies by Weinstein (1998) on the ghost crab *Ocypode quadrata* (Fabricius, 1787), who reported a temperature range of 24-30°C, which did not display a significant change in resting metabolic rate suggesting the maximal rate for aerobic scope. However, further studies on the measurement of oxygen tension in crab hemolymph, heart rate, anaerobic products, and LT_{50} (Lethal Temperature at which 50% of animals experimented die) need to be done to validate optimum temperature range and establish critical temperature ranges.

The results suggest that *P. guttatum* is more sensitive to temperature variation than *U. urvillei*. That could be interpreted to mean that excessive oxygen demand causes insufficient oxygen levels in the body fluids to compensate for increased metabolic rates at high temperatures. Higher oxygen demand is limited by oxygen availability as the solubility of oxygen in warm water decreases with increasing temperature leading to stress in the crabs. The limited capacity of the circulatory and ventilatory systems to keep pace with the increased oxygen demands of basal metabolism at higher temperatures causes a reduction in aerobic scope, allowing less energy to be devoted to, for example, feeding, growth, and reproduction. Portner and Knust (2007) suggested that a reduced aerobic scope is the key physiological mechanism determining ectotherms' response to increased ocean temperature. Oxygen consumption rates decreased in the decreasing temperature from 27 to 17°C for both animals, which could be interpreted to mean that at low temperatures, even in fully aerated waters, there is limited capacity by the ventilatory and circulatory systems to match oxygen demand for basal metabolism. Also, the aerobic capacity of mitochondria may become limiting and could cause a reduction in aerobic scope where the animals could resort to anaerobic metabolism.

The rate of metabolism of both species in water and air displayed different patterns, whereby the oxygen consumption rate for *P. guttatum* was consistently lower in water compared to the rate in the air. In comparison, for *U. urvillei,* the rate in water was higher than in air media. This observation in *P. guttatum* agrees with studies by Jimenez and Bennett (2005) on the fiddler crabs *U. vocans* (Linnaeus, 1758), *U. tetragonon* (Herbst, 1790), and *U. crassipes* (White, 1847) who reported similar patterns. That further suggests that *P. guttatum* uses more energy in air than in water to meet metabolic costs and hence must spend more time in the air as compared to *U. urvillei,* as evidenced by the more than twice the oxygen consumption rates by *P. guttatum* than *U. urvillei*, which could mean a better tolerance to temperature change in the air than in water by *P. guttatum* and is less dependent on anaerobic respiration. It may not need to pay back oxygen debt rapidly. The *U. urvillei* showed that oxygen uptake in the air was lower concerning water (except at temperatures 33-35°C), suggesting that *U. urvillei* is more sensitive in the air than in water. This low oxygen uptake in the air was interpreted to be the direct consequence of the lower metabolic cost of extracting oxygen from the air than from the water. This observation on

Eshky et al. (1990) and Jimenez and Bennett (2005) reported that the difference in relative oxygen uptake in water and air by these semi-terrestrial crabs exhibiting bimodal respiration is often used to ascertain how these organisms are adapted to the aquatic or terrestrial environment. Due to low dissolved oxygen tension, crabs have adopted several methods to maintain a constant oxygen uptake rate (Eshky et al. 1990). Skov et al. (2002) noted that in their natural environmental conditions, *P. guttatum* is a free-roaming species found in shaded mangrove areas and not often in open areas and is active throughout the day and night at low tide to feed and utilize other crab burrows or crevices during high tide and only burrows when shelter such as root systems is unavailable for protection from temperature fluctuations and predators. On the hand, *U. urvillei* is a known burrower and active during daytime only at low tide, emerging from its burrow to feed, and can be found in both exposed and shaded areas in the mangroves. That implies that it can withstand wide temperature variation. Thus *P. guttatum* spends more time in the air than *U. urvillei.*

Most activities of these crabs take place in the air, and the oxygen concentration of air is 20% higher than in water (Hogarth 1999), even at saturation levels. Because *P. guttatum* spends most of its time in the air, it is less dependent on anaerobic respirationand may not need to pay back oxygen debt rapidly. Portner (2002) and Cannicci et al. (2011) reported that the leading advantage of evolving a terrestrial development is the thirty-fold abundance and 10,000 times higher diffusiveness of oxygen in the air than in water. Therefore, adopting aerial respiration could reduce the problems described for marine species due to the limiting oxygen demand in water as it warms due to

increased temperatures. The temperature tolerances of this *P. guttatum* and *U. urvillei* could reflect their distribution in the mangrove forests as the laboratory optimal temperature range of 27-33°C established falls within the normal field environmental temperature ranges of these animals observed**.** Therefore, small changes in temperature of a few degrees Celsius from the current optimal limits suggest it could influence these crabs' physiological condition, developmental rate, growth rate, and reproductive performance. If the environmental temperature rises beyond these optimal ranges, *P. guttatum*, which is more thermally sensitive than *U. urvillei*, will be more vulnerable to thermal stress. That is because they live in constant shade, are not generally adapted to the high temperatures found in warmer open habitats, and have few behavioral options available to evade rising temperatures. Consequently, any climate-induced increase in operative temperature could cause thermal performance and fitness declines.

When conditions change rapidly, resistance mechanisms are important, and oxygen limitation has been demonstrated in the present study to be the mechanism dictating survival limits which agrees with Pery et al. (2008) under adaptational limitation. Although death may not be the immediate response, sublethal effects such as the decrease in the number of offspring may contribute to the species' eventual decline or demise.

Determination of density and distribution of Perisesarma guttatum *and* Uca urvillei *crabs*

The current study recorded almost similar densities of these crabs to those recorded by Cannicci et al. (2009). The results clearly show that the *U. urvillei* and *P. guttatum* reported high densities in the *R. mucronata* zone, suggesting it is a preferred habitat. However, densities of *P. guttatum* in the *R. mucronata* and *C. tagal* zone were comparable, but very low densities were recorded in the *A. marina* zone. The results further indicated that the *P. guttatum* and *U. urvillei* were not evenly distributed within the three mangrove zones and varied widely from very low densities in the *A. marina* and *C. tagal* zones to very high densities in the *R. mucronata* zones. On the other hand, *P. guttatum*, despite having a much lower density than *U. urvillei*, was found in all three mangrove zones in shaded areas.

The very low densities of *U. urvillei* in the *A. marina* and *C. tagal* zone could be attributed to high temperatures, high salinities, high coarse sand particles, and low organic matter content. These factors could also contribute to the stunted trees, which were observed during the study as the landward *A. marina* and *C. tagal* trees are stunted and have a less dense canopy. In addition, hypersalinity has been shown to induce stunted growth of *A. marina* (Kathiresan and Bingham 2001).

Salinity was highest in the landward *A. marina* zone and lowest in the lower shores of *R. mucronata.* The high salinity in the landward *A. marina* and *C. tagal* zone could be attributed to a less dense shade canopy which is more open, leading to high water evaporation due to high-temperature rates resulting in concentration of salts, thus high soil salinity. There is also the restricted exchange between tidal and interstitial water as these areas are rarely inundated except only during high spring tides. Mangrove vegetation is more luxuriant in lower salinities (Kathiresan and Bingham 2001) which is true for *R. mucronata* compared to the other two mangrove species. That clearly indicated that *U. urvillei* is intolerant to higher salinities, which pose serious physiological problems.

There was no variation in pH between the three mangrove zones, and Middelburg et al. (1996) reported similar results. Kathiresan and Bingham (2001) observed that mangroves achieve maximum root growth at an acidic pH of 6, indicating normal mangrove soil pH. Acidic conditions can occur in mangrove sediments that are regularly flooded, and mangroves are known to affect the acid-base balance of their sediments (Middelburg et al. 1996) which could explain the similarity in pH in the three mangrove zones during this study in spring high tides. The *U. urvillei* are the least advanced toward a terrestrial existence and are mostly confined to firmer mud (Crane 1975). Similar distribution patterns were observed for this species in this study. Thus, the presence of vegetation is an important factor affecting the distribution of *U. urvillei* (Edney 1962). The results of this study demonstrated a strong relationship between the density of *U. urvillei* and environmental variables such as temperature, salinity, and soil characteristics such as organic matter, silt, and fine sand.

The high density of *U. urvillei* in the *R. mucronata* zone could be attributed to low sediment temperature and salinity compared to the other two zones, as these factors are known to affect their physiological responses and behavior, which concurs with past studies by Hartnoll et al. (2002), Ashton et al. (2003), Bosire et al. (2004) and Cannicci et al. (2009) who reported the existence of a correlation between abundance and distribution of mangrove crabs with soil salinity, temperature, and soil organic carbon. However, Ashton et al. (2003) indicated that environmental measurements should be treated with caution as they only give a general indication of conditions because they vary with the time of day and concerning tidal inundation, seasons, and weather. During the study period, sediment temperature within the mangrove forest varied from 29.01 to 36.8°C and was significantly different between the three mangrove zones. However, there was no clear pattern of temperature effect on these crabs' density over the season. *U. urvillei* require fine grain size to sort their food; thus, it strongly relates to the densities of these crabs. Therefore, substrate characteristics may be very important in influencing crab distribution (Seiple 1979). However, silt was also abundant in the *C. tagal* zone, which recorded very low densities of *U. urvillei*, suggesting that silt may not be the only variable explaining their abundance but could be occurring in combination with the other factors. That agrees with Frith and Brunenmeister (1980), who observed the absence of *U. urvillei* from non-mangrove shores around Phuket Island.

Bosire et al. (2004) observed that organic matter content varies concerning substrate type; thus, the finer substrates of silt and fine sand observed in *R. mucronata*,

and *C. tagal* zone contained more organic material than the coarser substrates of landward *A. marina* zone. The correlation of particle size with organic matter is appropriate for a deposit feeder with a sorting mechanism attuned to a certain particle grade (Cannicci et al., 2009). Thus sediment quality with high organic matter content is more relevant to *U. urvillei*. Further, the high organic matter content encourages the growth of algae and diatoms, serving as the food for these detritivore fiddler crabs. The presence of *P. guttatum* species in the *C. tagal* pure zone in almost equal densities as in the *R. mucronata* zone in the present study, clearly indicates that this zone is an equally important preferred habitat for this species, hence explaining their abundance patterns.

The stunted *C. tagal* and *A. marina* zones have less dense shade canopy. The *P. guttatum* density was significantly related to temperature, organic matter content, and fine sand. Their low densities in the *A. marina* zone could have been affected by the high temperature and the low organic matter content, as these crabs are known to occupy shady areas to avoid thermal stress. The *P. guttatum* like other *Perisesarma* spp. supplement their diet with mangrove leaves in addition to sediment with high organic matter (Skov and Hartnoll 2002), which was quite low in the *A. marina* zone to sustain *P. guttatum* populations. *P. guttatum* is an omnivore, although most of its diet through stomach content analysis (Hogarth 1999) indicates it is mostly an herbivore and its ability to collect fallen leaves is very dependent on prolonged exposure to air within the shaded mangrove areas inundated by tides most of the time with high organic matter content. The high densities of *P. guttatum* in the *C. tagal* and *R. mucronata* zone suggest it plays a big role in these mangrove zones.

An important observation from these results is that the optimum temperature rangeestablished for *P. guttatum* and *U. urvillei* crabs were 27-31ºC and 27-33ºC, respectively. The results indicate that *P. guttatum* is more sensitive to temperature variation and displayed a slightly narrower thermal tolerance window, probably to minimize maintenance or energetic costs. Thus, the null hypothesis that the two species have no preferred optimum thermal range was rejected. The alternative hypothesis accepted that the two species have a preferred optimal thermal range. Given the prevailing global warming trends as projected by the IPCC, the results suggest that thermal extremes will affect the performance of the two species differently, as indicated by the metabolic rates and the difference in thermal tolerance between the two species. As a result, *P. guttatum* will be more vulnerable to an increase in temperature, while *U. urvillei* are likely to persist at higher temperatures.

This study showed that *U. urvillei* and *P. guttatum* were not evenly distributed in the three mangrove zones. Temperature, fine sand, and organic matter strongly influenced the density and distribution of these crabs. The landward shores of *A. marina* arerarely inundated, except during spring high tides, and have higher temperatures, salinity, and low organic matter content. That could explain these two species' observed low abundance patterns in this zone.

Temperature showed significant influence, as supported by laboratory experiments. Information on thermal tolerance levels is important for conserving crabs to ensure the right community structure of mangrove trees. In addition, monitoring these animals will increase the knowledge and understanding of mangrove ecosystems' structure, dynamics, and resilience, particularly in climate change scenarios.

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