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Cover image: The mudskipper *Periophthalmus argentilineatus* on the mangrove of Ulo Beach, Cabo Delgado, North Mozambique. © José Paula, 2006

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Impact of a ring net fishery in the inshore marine waters of Kilifi on the reproductive biology of six pelagic fish species

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Abstract

Ring nets are modified purse seines used for pelagic fishing along the Kenya coast. This method is however associated with potential negative environmental implications in inshore and shallow fishing grounds. Biological impacts on selected pelagic species caught in the marine waters of Kilifi were investigated during the Southeast Monsoon season (SEM) in April, May and September 2014, and the Northeast Monsoon season (NEM) in October and November 2014, and March 2015. *Amblygaster sirm, Rastrelliger brachysoma*, *Sardinella gibbosa, Rastrelliger kanagurta*, *Hemiramphus far* and *Sphyraena obtusata* were the most abundant species caught by the ring nets and comprised 68.9 % of fish catch composition. Analysis of ring net impacts on the fish sizes and maturity status were conducted for the NEM and SEM seasons. The catch composition for *A. sirm* in October comprised of 53.3 % juveniles and 53.4 % juveniles for *S. obtusata* in November. High percentages of *H. far* (78.3%) and *S. obtusata* (58.9 %) were captured in September. Incidences of undersize fish for *A. sirm*, *S. gibbosa*, *R. kanagurta* and *R. brachysoma* were observed across the seasons. Sex ratios for *A. sirm*, *R. brachysoma*, *R. kanagurta* and *S. obtusata* in the NEM season deviated from a normal ratio of 1:1 with more males observed. Females were dominant in the catch for *H. far* (1: 1.28) but the ratio was not different for *S. gibbosa* (χ^2 = 5.564, df = 1, p = 0.21). Males were dominant for *A. sirm* (1: 0.8) and *S. gibbosa* (1: 0.7) in the SEM season but the ratios were not different for *H. far*, *S. obtusata*, *R. brachysoma* and *R. kanagurta*. Differences in sex ratios were attributed to fish migrations and reproductive processes. Ring nets fished relatively offshore during the NEM season and targeted mainly spawning aggregations in stage IV. Immature fish in stage I and II which comprised of juveniles were harvested within sheltered inshore waters in the SEM season. Use of ring nets to target juveniles and spawning aggregations may disrupt recruitment processes. To enhance sustainable management of the ring net fishery, there is a need to develop harvesting strategies based on the information on stock status of the target fish.

Keywords: ring nets, reproductive biology, impacts, Kilifi

Introduction

Ring nets are modified purse seines used in coastal fisheries to target small pelagic fish, mostly in the families Carangidae, Clupeidae, Scombridae and Sphyraenidae (Halland Roman, 2013). In the Philippines, ring nets are used in conjunction with Fish Aggregating Devices (FADs) to improve productivity but are associated with capture of undersize fish (Malig *et al.*, 1991). Ring net fishing was introduced in Kenya in the 1990s from Pemba, Tanzania and embraced by the State Department of Fisheries, Aquaculture and Blue Economy (SDFA&BE) as a means to access offshore fish resources (Government of Kenya, 2005). However, lack of a regulatory framework to guide its operations has since raised concerns about environmental degradation and overfishing (Government of Kenya, 2005; Okemwa *et al.*, 2017). The fishery is associated with fishing in the inshore waters, targeting spawning aggregations, fishing of immature undersize fish and causing physical damage to the benthic habitats (Maina, 2012; Samoilys *et al.*, 2011). Although capture of spawning and immature fish has been reported, no comprehensive studies have been undertaken on the Kenyan coastline and particularly in the inshore marine waters of Kilifi to evaluate impacts of ring nets on target pelagic stocks which form 73%

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of the landings (Okemwa *et al.,* 2017). The purpose of the present study was therefore to determine the impact of ring nets on spawning and juvenile fish of selected target species with high catch composition in the NEM and SEM seasons. This will provide scientific data to inform management decisions on the ring net fishery and enhance environmental conservation and ensure sustainable utilization of the target fish stocks.

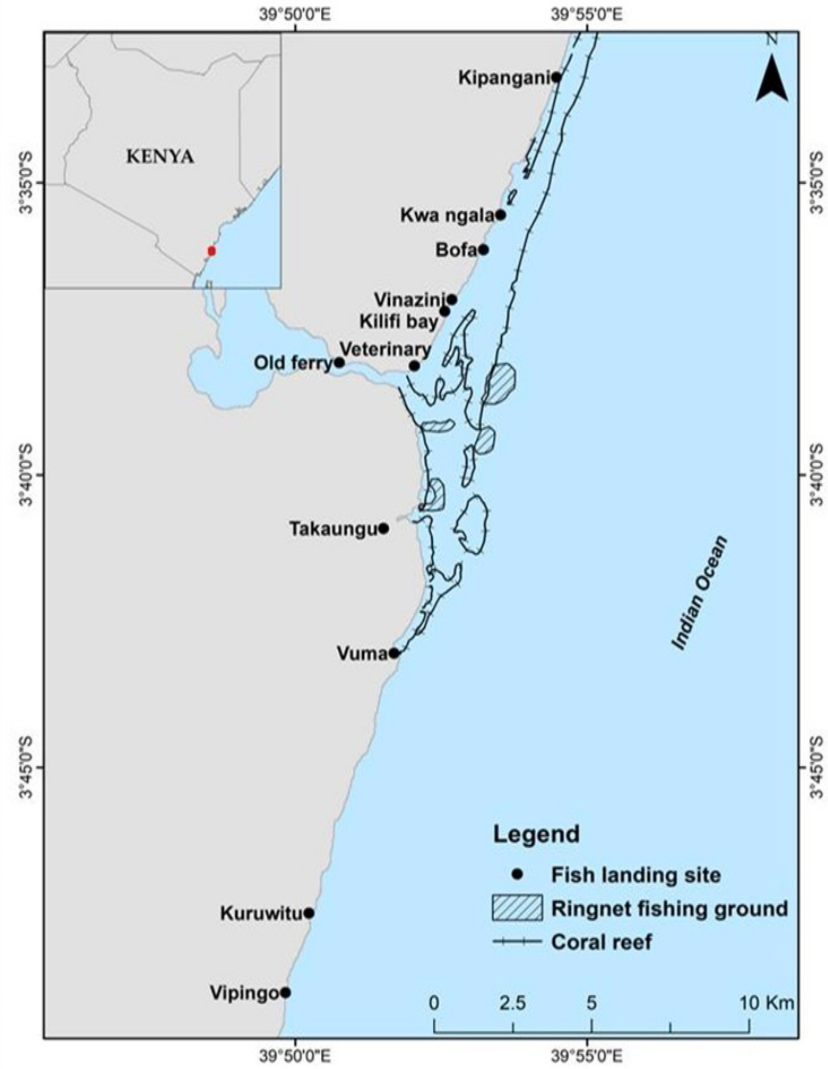

Figure 1. Map of Kenya showing fish landing sites and ring net fishing grounds in Kilifi, Kenya.

Materials and methods

The study area

The study was located in the inshore marine waters of Kilifi County located along the Kenya coastline. These waters are administratively managed by the Beach Management Units (BMUs) of Kuruwitu, Takaungu, Kilifi Central and Bofa (Fig. 1). The fishing grounds comprised of Kilifi - Mlangoni, Bofa, Kwa-Ngala, Takaungu - Mlangoni, Takaungu and Vuma. The area experiences two seasons; the Northeast Monsoon(-NEM) which runs from October to March each year and is characterized by calm, sunny and dry weather conditions, and the Southeast Monsoon (SEM) covering April to September and dominated by strong winds, rough sea conditions and heavy rains(McClanahan, 1988; Munga, 2008).

Data collection

Biological data and information on ring net operations were collected at the landing sites and fishing grounds. The data were collected in the NEM season in October, November 2014 and March 2015, and September 2014, April and May 2015 in the SEM season. Fish landed by three vessels using ring nets were sampled during the study period based on preliminary data from fisheries surveys (Government of Kenya, 2016). Fish species were identified using published literature (Anam and Mostrada, 2012) and the online database, Fishbase (2018) (http//www.fishbase.org). Samples of six species were used to determine size structure by measuring individual total length (TL) to the nearest 0.1 cm on a measuring board (Kahn *et al*., 2004).

Individual fish were dissected to determine their sex and gonad maturity status following standard procedure. Gonads were categorized into 5 stages; Stage I (Immature), II (Maturing), III (Mature), IV (Ripe) and V (Spent) according to West (1990). The proportion of juveniles of each species was determined by the number of individuals below their respective length at first maturity (L_{mat}) as per information in Fishbase.

Fishing crew were accompanied to the fishing grounds to obtain data on fishing locations and distances to the nearest shoreline as recommended by Munga *et al.* (2010) in a study of ring net fishing at Kipini fishing grounds. Deployment points of selected ring nets in the common sites were captured using a Geographical Positioning System (GPS) device and latitudes and longitudes recorded.

Data analysis

Seasonal (NEM and SEM) data on fish size structure, sex ratios and gonad maturity status were analyzed using MS Excel® and Statistica8.0 software. Percentage (%) species composition of fish caught in the NEM and SEM season were determined. The proportions of stage I-V for each species for both the NEM and SEM season were determined for seasonal comparisons. The number of males and females of each species were used to compute sex ratios which were tested for significant difference from the expected 1:1 ratio using the Chi-squire (χ^2) test (Zar, 1999). Significance was determined at $\alpha = 0.05$ for all statistical tests.

Results

Fishing grounds description, gear use and operation

Deployment points of ring nets were randomly referenced for common fishing grounds: Kilifi - Mlangoni (03°39.17’S and 039°52.51’E; 03°39.24’S and 039°52.38’E); Takaungu - Mlangoni (03°40.37’S and 039°52.28’E); Takaungu (03°39.06’S and 039°53.23’E; 03°39.14’S and 039°53.20’E); and Bofa (03°38.07’S and 039°52.513’E; 03°38.50’S and 039°53.32’E). Kipangani, Kwa-Ngala, Vuma and Vipingo grounds were fished during calm conditions. The ring nets measured 160 - 280 m long and18 - 28 min depth with net mesh sizes of 0.5 inches (12.7 mm) and fished at depths of between 30.0 ± 9.0 m in the NEM season and 15.0 ± 3.0 m in the SEM season. Plastic containers filled with beach sand were used as sinkers with floats attached to the top rope to prevent the net from being in contact with the sea bottom. The ropes passed through rings attached at the lower part of the net and were pulled together to close the bottom before the catch was hauled onto the boats.

Species composition and structure of sampled fish

Seasonal and average catch composition for *A. sirm, R. brachysoma*, *S. gibbosa, R. kanagurta*, *S. obtusata* and *H. far* are presented in Table 1. The catch composition of the selected species comprised 69.0 % of the total ring net landings. *S. obtusata and R. kanagurta* comprised

Table 1. Seasonal and average % species composition of selected species.

	Seasonal species composition (%)		
Species	**NEM**	**SEM**	**Average landings**
S. obtusata	25.1	28.8	27.0
R. kanagurta	19.6	13.6	16.6
R. brachysoma	6.1	14.2	10.2
H. far	5.0	1.8	3.4
S. gibbosa	2.8	13.8	8.3
A. sirm	1.6	5.3	3.5
Others*	39.8	22.5	31.0

Others:* Represents percentage combination of pelagic and demersal fish landed in small proportions by the ring nets for both seasons.

Table 2. Seasonal mean lengths, numbers, estimated size at maturity and % proportion below L_{mat} of targeted pelagic species.

Species	**Seasonal mean sizes (cm)**					**% proportion below L_{mat}**	
	NEM	**n**	**SEM**	**n**	**L_{mat}, cm**	**NEM**	**SEM**
A. sirm	18.8 ± 4.7	92	19.0 ± 3.7	219	15.0	10.8	5.7
R. brachysoma	23.2 ± 2.3	342	19.7 ± 2.7	537	17.0	0.4	2.0
S. gibbosa	16.3 ±1.65	150	15.8 ± 2.2	552	12.8	1.4	3.1
R. kanagurta	24.1 ± 2.6	791	21.9 ± 2.6	670	19.9	4.1	4.3
H. far	31.0 ± 1.4	72	22.7 ± 4.5	69	26.5	0	78.3
S. obtusata	23.0 ± 4.1	1329	24.7 ± 4.5	1327	22.9	37.6	29.8

a high proportion in the landings with 27.0 % and 16.6 % respectively. A smaller proportion of catches grouped under other species constituted 31.0 % and comprised a mixture of both pelagic and demersal species.

Table 2 shows seasonal (NEM and SEM) average sizes and percentages of fish individuals for *A. sirm, R. brachysoma*, *S. gibbosa, R. kanagurta, H. far* and *S. obtusata* captured below L_{mat} by ring nets. The proportions (%) of the species captured below L_{mat} during the months in each season are presented in Fig. 2.

The results for *A. sirm* showed more individuals caught below L_{mat} during the NEM season in the months of October (53.3 %) and a few in November (7.4 %), and during SEM season, a small proportion were captured in April (11.7 %). Based on the average lengths in the NEM season (18.8 ± 4.7 cm), 10.8 % of the individuals were below L_{mat} and the SEM season (19.0 ± 3.7 cm) had 5.7 % below L_{mat}. The fish sizes landed in both seasons were not significantly different (ANOVA, F = 0.14; p = 0.706).

The majority of *R. brachysoma* individuals attained a mature size at harvest except a few below the L_{mat} in March (1.2 %) and April (6.0 %). The sizes of fish caught in the NEM season (23.2 ± 2.3 cm) and SEM season (19.7 ± 2.7 cm) were different.

A few individuals for *S. gibbosa* were below L_{mat} (12.8 cm) in March (1.6 %), September (9.4 %) and November

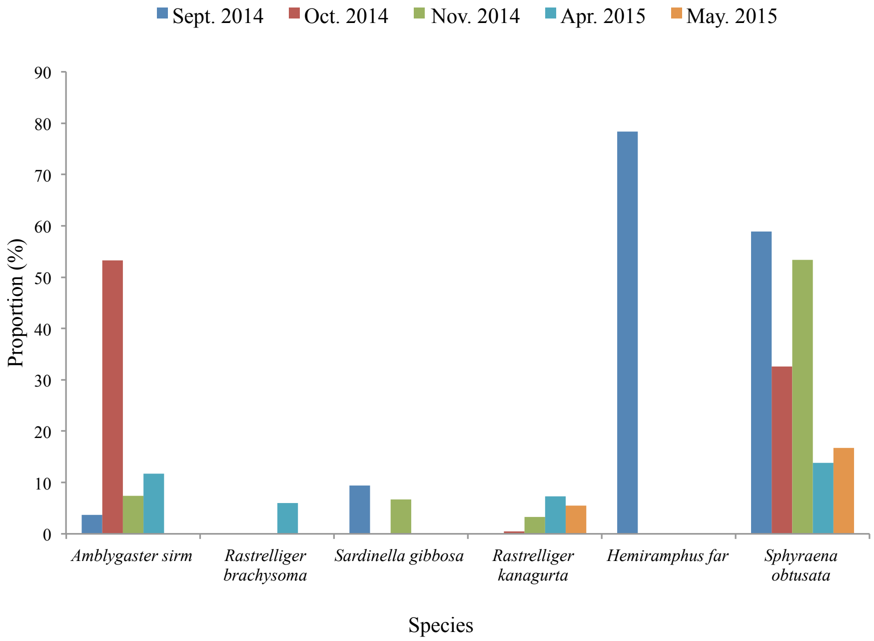

Figure 2. Proportion (%) of individuals below size at maturity for various species in different months.

Table 3. Proportion (%) of individuals in various gonad maturity stages (I-V) in the NEM and SEM seasons (NEM = Northeast monsoon; SEM = Southeast monsoon).

Species	Season	% proportion of gonad maturity					n
		I	II	III	IV	V	
A. sirm	NEM	9.0	2.8	11.7	19.0	57.5	89
	SEM	35.2	16.8	6.4	7.5	34.1	219
R. brachysoma	NEM	1.4	0.7	2.1	49.5	46.3	287
	SEM	31.7	5.7	4.7	12.2	45.7	385
S. gibbosa	NEM	20.5	0.0	2.4	28.5	48.6	139
	SEM	23.2	44.8	6.4	10.4	15.2	464
R. kanagurta	NEM	0.5	0.6	1.7	58.3	38.9	673
	SEM	31.6	23.5	15.3	10.4	19.2	539
H. far	NEM	0.0	13.6	21.5	35.4	29.5	73
	SEM	47.7	18.7	9.0	23.2	1.4	69
S. obtusata	NEM	67.2	16.5	2.1	6.2	8.0	943
	SEM	53.7	23.4	10.4	7.4	5.1	1240

(6.7 %). The results show that a small proportion of individuals in the NEM season (1.4 %) and SEM season (3.1 %) were caught below size at maturity. Based on the results, it is apparent that the species was harvested at different sizes across the seasons (ANOVA, F=5.98; $p < 0.05$).

R. kanagurta individuals captured during both seasons were mostly above the L_{mat} but a few were below this size in October (0.5 %), November (3.3 %), March (8.6 %), April (7.3 %) and May (5.5 %). The findings showed that a few individuals were captured below size at maturity in the NEM season (4.1 %) and SEM season (4.3 %). According to the results, the species was fished at different sizes in the NEM season (24.1 ± 2.6 cm) and SEM season (21.9 ± 2.6 cm).

H. far individuals assessed in the NEM season attained size at maturity at capture but in the SEM season, 78.3 % of the individuals were captured below L_{mat} in the month of September. Based on the results, the species was fished at different sizes in the NEM season (31.0 ± 1.4 cm) and SEM season (22.7 ± 4.5 cm).

The majority of *S. obtusata* were captured below size at maturity during the NEM season (37.6 %) during the months of October (32.6 %), November (53.4 %) and March (26.9 %). In the SEM season, 29.8 % were captured below L_{mat} with a majority being observed in September (58.9 %). The mean sizes of fish individuals landed in the NEM season (23.0 ± 4.1 cm) and SEM season (24.7 ± 4.5 cm) indicated fishing vulnerability of the species at various sizes.

Sex ratios

Males were dominant in the NEM for *A. sirm* with a sex ratio of 1: 0.4 which deviated from the normal 1: 1.The males were also dominant in the landings in the SEM season ($\chi^2 = 2.215$, df = 1, p = 0.137, n = 163). Male *R. brachysoma* were more common during the NEM season (1: 0.8; $\chi^2 = 4.77$, df = 1, $p < 0.05$, n = 287), but both sexes were equally captured in the SEM season (1: 1.1).

An equal number of males and females were landed for *S. gibbosa* with 1: 0.8 ratio in the NEM season ($\chi^2 = 5.564$, df = 1, p = 0.21, n = 143) but males were dominant in the SEM season at a ratio of 1: 0.7($\chi^2 = 10.515$, df = 1, $p < 0.05$, n = 309). Landings for *R. kanagurta* had more males in the NEM season at a ratio of 1: 0.7 ($\chi^2 = 29.672$, df = 1, $p < 0.05$, n = 974) but both sexes were equally captured with a 1: 1.1 ratio in the SEM season.

Females were observed to be more frequent for *H. far* in the NEM season with a ratio of 1: 2.8 but were equally harvested in the SEM season at a ratio of 1: 1.0. Samples of *S. obtusata,* were dominated by males

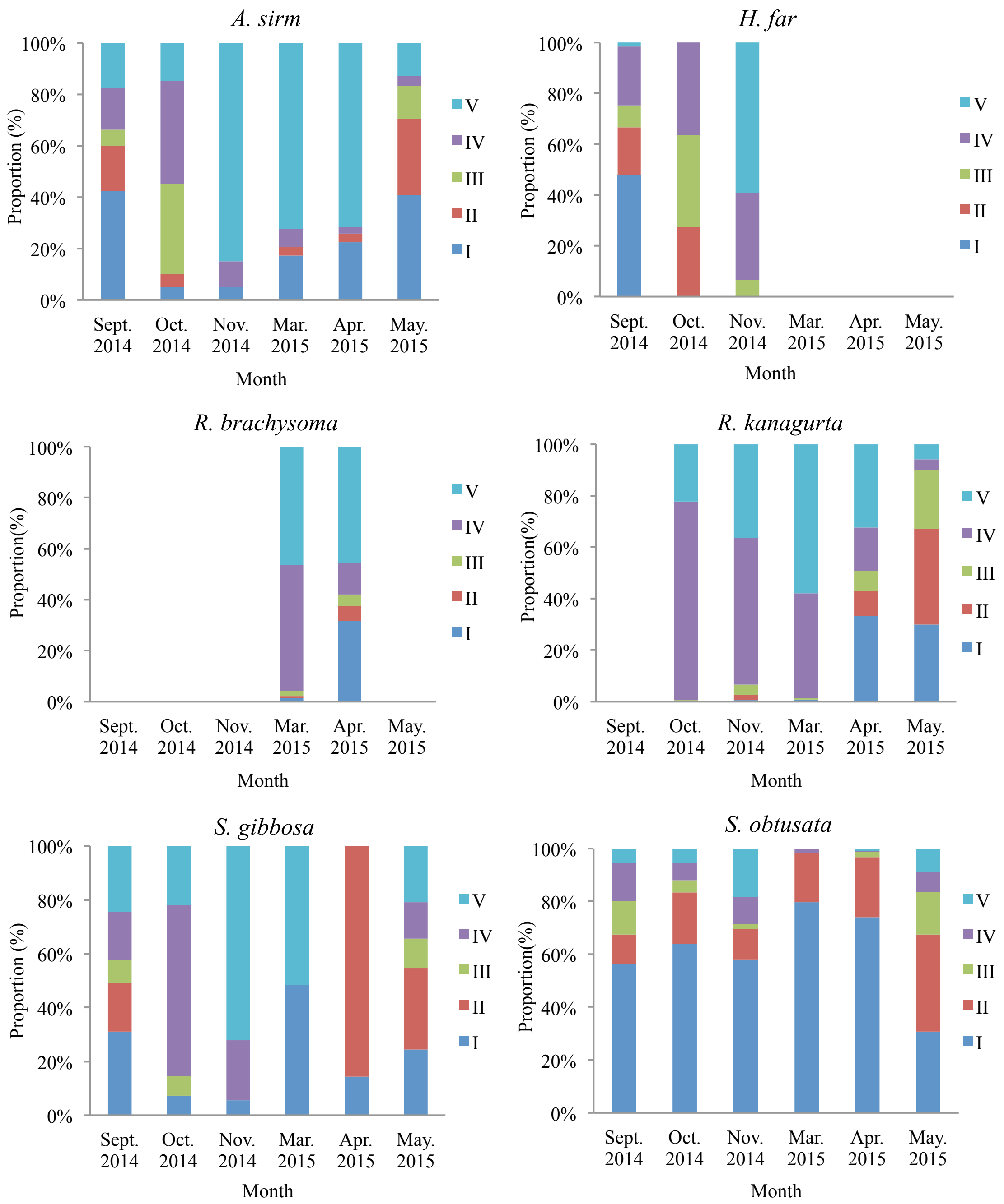

Figure 3. Proportion (%) of individuals in various gonad maturity stages (I-V) in different months.

in the NEM season at a ratio of 1: 0.86 (χ^2 = 5.564, df = 1, $p < 0.05$, n = 1011) but equal numbers of males and females were harvested in the SEM season at a ratio of 1: 1.0.

Gonad maturity status

The gonad status of various species collected during the NEM and SEM season are presented in Table 3, whereas proportional trends across months are shown in Fig. 3. Most *A. sirm* captured in the NEM season were in stage V (57.5 %) while in SEM season most were in stage V (34.1%) and I (35.2 %). High proportions of stage V individuals were observed in March (72.4 %), November (85.0 %) and April (71.8 %). More individuals were also observed in stage I (42.5 %) in September, and stage III (35.0 %) and IV (40.0 %) in October.

R. brachysoma in the NEM season had more individuals in stage IV (49.5 %) and V (46.3 %) in March. The observations made in the SEM season indicated more individuals in stage I (31.7 %) and V (45.7 %) landed in April.

Gonad assessment for *S. gibbosa* in the NEM season comprised mostly of individuals in stages IV (28.5 %), V (48.6 %) and I (20.5 %).The majority of the individuals in stages I (48.4%) and V (51.6%) were observed in March, and stage IV (63.4%) in October. The assessment carried out in the SEM season comprised mostly of individuals in stage I (44.8 %) and II (23.2 %). During this season, the months of April and September had the highest proportions of individuals captured in stages II (85.7%) and I (31.1%) respectively.

The assessment of gonads for *R. kanagurta* in the NEM season showed more individuals in stages IV (58.3 %) and V (38.9 %). During this season, a high proportion of individuals in stage IV were observed in March (40.6 %), October (77.1 %) and November (57.1 %). The majority of individuals in stage V were observed in March (57.9 % and November (36.3 %). The assessment carried out in the SEM season showed more individuals in stages I (31.6 %) and II (23.5 %). The majority of the individuals in stages I (33.4 %) and V (32.4 %) were captured during the month of April.

Assessment of *H. far* gonads in the NEM season indicated most individuals in stage IV (35.4 %) and V (30.0 %) which occurred during the months of October and November, respectively. The results in the SEM season indicated more individuals in stage I (47.8 %) and IV (23.2 %) which were captured in September.

The assessment of gonads for *S. obtusata* in the NEM season indicated the majority of the individuals in stage I (67.2 %). The individuals were captured mostly in the months of October (64.0 %) and November (58.0 %). High proportions of individuals observed in the SEM season were in stages I (53.7 %) and II (23.4 %). The majority of the individuals in stage I (74.0 %) were captured in April, and stage II (53.7 %) in September.

Discussion

The results of the current study show that ring nets were used in shallow grounds near the coral reef areas during the SEM season and in slightly deeper coastal waters in the NEM season. The coral reef areas serve as feeding, breeding and nursery grounds for most tropical fishes and fishing with ring nets within these areas are likely to impact on fish recruitment (McClanahan, 1988; Robinson *et al*., 2008). Harvests of undersize, immature and hydrated fish were observed during the months fished during the NEM and SEM seasons.

Fish catches in the NEM season included fish captured before they attained maturity sizes (Fishbase, 2018). Catch composition of *A. sirm* and *S. obtusata* comprised had more than 50 % below L_{mat} in October and November respectively. Juveniles are reported to aggregate to feed as calm conditions in the sea prevail (Morais *et al.,* 2010). Schools of juveniles were likely to have been targeted and harvested by the ring nets as they move from the nursery grounds into the open waters. A small percentage of undersize fish were recorded for *R. brachysoma*, *S. gibbosa* and *R. kanagurta* in March, October and November. The incidental catches of the juveniles probably occurred as they accompanied aggregations of adult fish to the feeding grounds or while in transit to spawning sites (Robinson *et al.,* 2008). The fishermen were observed to target aggregating fishes which were surrounded and harvested irrespective of sizes (pers. obs. first author; Samoilys *et al*., 2011). The calm sea conditions in the NEM season made it possible for ring net fishers to access and operate in the offshore waters (pers. obs. first author; Munga *et al*., 2010).

The rough and windy conditions in open waters in the SEM season pushed ring net fishing into the shallow and sheltered grounds. The operations of the ring nets at the sites impacted greatly on *H. far* and *S. obtusata* with more than 50 % of the catches below their respective maturity sizes. The aggregations of juveniles were likely to have been surrounded and harvested by the ring nets while transiting to feeding grounds (Robinson *et al.,* 2008). Small percentages of undersize fish were also observed in this season for *A. sirm*, *R. brachysoma*, *S. gibbosa* and *R. kanagurta*. The incidental catches of the juveniles are predicted to have occurred while aggregating together with shoals of adult fish moving to feeding grounds or spawning sites (Morais *et al.,* 2010). The sheltered grounds fished in the SEM season also serve as breeding and nursery grounds for most reef and reef-associated fish where young fish are subjected to fishing mortalities (McClanahan, 1988). Harvest of juveniles in the SEM season probably occurred as a result of fishing within the nursery grounds using small mesh size nets.

Ring nets used in both the NEM and SEM season harvested both adult and juvenile fish as a result of mesh size dimensions of the nets which measured 0.5 inches (12.7 mm). The sizes are designed to maximize fish catch and allow easy offloading onto the vessels, but they prevent escape of young fish. Although the ring nets increase fishing efficiency and increase catch, they are non-size selective and capture all sizes of fish (Okemwa *et al.,* 2017).

The study on biological and socio-economic aspects by Munga *et al.* (2010) on ring net fishing off Kipini fishing grounds on the Kenyan coast also reported the harvesting of juvenile fish. Despite government recommendations for 2-inch (50.8 mm) mesh size nets for use by the ring nets to curb the harvest of undersize fish, the fishermen are yet to adopt this requirement (Government of Kenya, 2012). Post-harvest losses, low fish quality of gillnetted fish and reduced catch due to the escape of small but mature pelagic fish are some of the reasons given for opposing the use of 2-inch mesh size nets (Okemwa *et al*., 2017).

Though the majority of species harvested comprised mostly of adult fish across the seasons, the small proportion of juveniles impacts on fish recruitment. The extent of juvenile mortality in the total stock could cause disruption of fish recruitment to the spawning stock (Robinson *et al*., 2004; Prince *et al.,* 2015). To enhance the sustainability of ring nets and reduce harvest of juveniles, the introduction of a 'closed season' in October - November is proposed as a result of this study. A ban on ring net fishing within shallow and sheltered grounds in the SEM season is also proposed to protect young fish in the nursery grounds.

However, fishers would have to be engaged in alternative fisheries or occupations to cushion them from the loss of livelihoods likely to occur as a result of changes in marine fisheries management regulations. There is need to enhance sustainable livelihoods options for the fishermen through capacity and skills development, value addition, access to capital assets and credit facilities, all of which depend on an understanding of the socioeconomic context of artisanal fisheries (Cinner *et al*., 2009; Morara *et al.,* 2015).

Sex ratios in the NEM season for *A. sirm*, *R. brachysoma*, *R. Kanagurta* and *S. obtusata* deviated from the natural ratio of 1: 1 with more males being harvested. Males and females were equally captured for *S. gibbosa* but more females for *H. far*. In the SEM season, more males were harvested for *A. sirm* and *S. gibbosa* but the ratios for *H. far*, *S. obtusata*, *R. brachysoma* and *R. kanagurta* were not significantly different.

The high catches of males observed for the majority of selected target species in the NEM season were probably as a result of aggregation within the feeding grounds while the females were in transit to spawning sites (Robinson *et al.,* 2004). Fishing with ring nets within sheltered grounds would likely have subjected males and females equally to fishing mortality as they both participate in reproduction processes within spawning sites. The difference in the numbers of males and females captured in the seasons could be associated with spawning activities, time spent at spawning sites and migrations to feeding grounds (Robichaud and Rose, 2003; Hamilton *et al.,* 2007).

Gonad assessment results showed a high proportion of mature and heavily hydrated fish in stage IV and individuals in stage V (spent) in the NEM season. Spawning fish in stage IV were high in October and November for *A. sirm*, *R. brachysoma*, *R. Kanagurta*, *H. far* and *S. gibbosa*. Some individuals observed in March, November and October had spawned before they were captured. According to the results, the majority of the species spawned in the NEM season which peaks in October - November, except for *S. obtusata* which is likely to spawn from April - July (Robinson *et al.,* 2004). The shoals of fish which aggregated for the purpose of spawning during this season became susceptible to ring nets that actively fished in the open waters. The temporal and spatial spawning activities for most fishes are reported to occur in NEM season and are known to be fished intensively by the fishermen as they aggregate to spawn in reef areas (Robinson *et al.,* 2004; Robinson *et al.,* 2008). The high catches of hydrated and translucent (spent) fish occurred as a result of prevailing stable sea conditions in the NEM season that allowed accessibility and operation of ring nets in the open waters.

A high proportion of immature (stage I) specimens of *S. obtusata* was harvested in October and November. During this period ring net fishers actively fished in the open waters and were likely to have targeted the aggregations of juveniles moving to the feeding grounds (Robinson *et al.,* 2004).The findings of the study on maturity status of the target fish are in agreement with the study undertaken by Munga *et al*. (2010) which assessed the maturity status of fish landed by a ring net off Kipini fishing grounds.

Gonads observed in the SEM season comprised mostly of immature (stage I) and maturing (stage II) fish in April and September among all the selected species, especially for *S. obtusata*. The catches of immature fish are attributed to ring net fishing within the nursery grounds to shelter against windy conditions in the open waters (Okemwa *et al.*, 2017). The immature fish were likely to have been harvested while aggregating in the nursery grounds or on transit to feeding grounds (Robinson *et al.,* 2004).

The majority of immature fish were caught within the nursery grounds before attaining sexual maturity and participating in the spawning process (Johannes, 1988). Ring net fishing on the immature fish denied them the opportunity to participate in fish recruitment and rebuilding of stocks. Exploitation of hydrated and immature fish is likely to cause negative implications on fish population growth and recruitment leading to unsustainable stocks (Johannes, 1988; Johannes *et al.,* 1999; Prince *et al.,* 2015). The Ring Net Fishery Management Plan (RFMP) proposed only one ring net on the Kilifi fishing grounds for sustainable management of the fishery, but according to the present study, eight ring nets fished during both the NEM and SEM season. The increase in fishing effort coupled with intensive fishing on spawning aggregations and the capture of immature specimens may not be sustainable and is likely to impair the target stock. However, little is known about the distribution and abundance of these stocks offshore, outside the reach of this artisanal fishery (Okemwa *et al.*, 2017).

Though ring net fishing continues to be adopted to increase fish catches from Kenya's inshore marine waters, the findings of the present study indicated the need for management measures to control exploitation of juveniles and spawning aggregations. It is suggested that the local BMUs integrate ring net-specific fishing zones and closed seasons in their by-laws to enhance sustainable management and utilization of the fish resources. Fishing communities have played an active role in sustainable management of fisheries resources through the establishment of community conservation areas where fishing activities are restricted (Maina *et al.*, 2011). These approaches have positively enhanced community responsibility and ownership towards conservation and management of the resources.

In Kenya, non-governmental organizations (NGOs) and researchers create awareness on resource management among the fishing community through participatory monitoring and research approaches (Alidina, 2005). Kuruwitu Community Managed Conservation Area (KCMCA) is an example of a marine conserved area established by the fishing community to promote sustainable utilization and management of coastal marine resources.

More research is needed to establish the stock status, spawning potential and fishing mortalities of ring net target fish. The findings will provide information on whether the fished stocks have the potential to spawn and rebuild based on the current fishing pressure. This will form a basis for formulation and implementation of sustainable management measures for ring net fishing in Kilifi marine waters and along the Kenyan coastline.

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Local people and mangroves: Ecosystem perception and valuation on the south west coast of Mauritius

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Abstract

Mangrove ecosystem services are multi-dimensional (ecological, socio-cultural and economic), and their valuation is complex as not all of these dimensions are quantifiable in terms of monetary value. The main goal of this study was to determine the perception of local residents on the economic values of the mangrove ecosystem along the south west coast of Mauritius (Savanne and Black River Districts). A survey questionnaire (Savanne district, N=142; Black River district, N=126) was designed electronically in English and Mauritian Creole language using dichotomous, multiple choices and closed ended questions. The mean value for the awareness score was 52.3 and the standard deviation was 4.8 for the district of Savanne as compared to Black River district, where the mean value was 53.8 and the standard deviation was 9.0. The mangrove products harvested by locals from the surveyed sites were fish, crabs and oysters. The respondents were less willing to contribute to mangrove conservation activities both in terms of money (Savanne district, 4.2 %; Black River district, 3.9 %), and time (Savanne district, 7 %; Black River district, 4 %). Hence, campaigns and education programmes are critical to raise the awareness and concern of local residents to achieve Sustainable Development Goal (SDG) 14 in Small Island Developing States (SIDS) like Mauritius.

Keywords: ecosystem services, Mauritian Creole, survey questionnaire, awareness score

Introduction

The worldwide distribution of mangrove forests coincides with the 20 °C summer isothermal line (Duke *et al.*, 1992). The global coverage of mangroves is estimated to be between 13,800 ha (Giri *et al.*, 2011) to 15,300 ha (Spalding, 2010). Approximately two thirds of the total coverage of mangroves in the world is located mostly in eighteen countries - Indonesia, Brazil, Australia, Mexico, Nigeria, Malaysia, Myanmar, Bangladesh, Cuba, India, Papua New Guinea, Colombia, Guinea Bissau, Mozambique, Madagascar, the Philippines, Thailand and Vietnam (Giri *et al.*, 2011).

On the African continent, mangrove cover represents approximately 20 % of the world's mangroves (Giri et *al.*, 2011). The greatest mangrove cover occurs in Nigeria and Mozambique (Fatoyinbo and Simard, 2013). In the Western Indian Ocean (WIO), mangroves occur along the coasts of Madagascar, Mauritius, Seychelles, Kenya, Tanzania, north and central Mozambique and South Africa. The South African mangroves at the Mgazana estuary mark the southern limit for mangroves in Africa.

In Mauritius, mangroves are found in estuarine conditions providing shelter to the coastline. The narrow belt of mangroves is found mostly in the regions of Poste la Fayette, Roches Noires, Trou d'Eau Douce, Poste de Flacq, Vieux Grand Port and Black River. However, other major areas of mangroves are found along the shores of small islets such as Ile aux Cerfs and Ile D'Ambre which also form part of the Republic of Mauritius. Some other small patches are found in the south and south-west regions, namely in Maconde and Tamarin (FAO, 2005). *Rhizophora mucronata* and *Bruguiera gymnorrhiza* are the only species of mangrove found in Mauritius.

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Within the last three decades deforestation and degradation of mangrove ecosystems has become widespread all around the world (Alongi, 2002). Unfortunately, in the same period, 30 % of mangrove forest area in Mauritius has undergone exploitation or alteration through 1) clearing for settlement, 2) infrastructural development, 3) provision for boat passages, 4) firewood, and 5) vandalism (Gopala, 1980). Consequently, policy makers have reacted by promulgating the first Fisheries and Marine Resources Act of 1998, which was later amended in 2007. The Fisheries and Marine Resources Act of 2007 stipulates that 'No person shall, except with the written approval of the permanent secretary, cut, take, remove or damage a mangrove plant'. In order to restore the mangrove ecosystem, the Government of Mauritius in collaboration with non-governmental organizations initiated several mangrove restoration programmes (Gopala,1980). A total of 50,000 mangrove seedlings were planted at Le Morne, and 10,000 at Quatre Soeurs and Case Noyale. As a result, this has boosted the declining mangrove area from 45 ha in the year 1980 to 145 ha in 2013 (Bosire *et al.*, 2016).

The major role of mangrove trees lies in the provisioning of socioeconomic benefits, such as timber, fish, tourism opportunities, and environmental services (Sarhan *et al.*, 2018). In addition to providing forest dependent livelihoods, mangrove forests greatly influence the local and national economy (Udhin *et al.*, 2013). Several studies have attempted to quantify the economic value of mangrove ecosystems. The study conducted by Costanza and Folke (1997) estimated the average value of mangrove ecosystem services worldwide to be USD9,990 per hectare per year which is well above tropical forests, estimated to be USD2,007 per hectare per year (Chow, 2015). In contrast to quantitative economic valuation, mangroves exhibit a social value, which is critical for the proper functioning of society and human well-being (Chiesura and de Groot, 2003). Such social value is understood to be the source of well-being and is closely related to historical, communal, ethical, religious and spiritual values (James *et al.*, 2013).

Within the context of the current study, social value is based on the view of local residents of mangroves for their value and the perceived tangible and non-tangible benefits which are provided by the mangrove forests in the south-west coast region of Mauritius. Until now, no studies on the social valuation of mangroves have been undertaken in Mauritius. In this modern era, with the emergence of the blue economy and sustainable development goal (SDG) targets, policy makers are committed to prioritize the conservation and management of coastal and marine living resources. This study sheds light on how to achieve the proper management of the mangrove ecosystem for future generations with respect to SDG 14 which calls for conservation and sustainable use of the oceans, seas and marine resources.

Methodology

Study site description

The study was carried out along the south west coast of Mauritius (Fig. 1) which spans a total area of 10 km^2 and comprises the districts of Savanne (20.4740° S, 57.4854° E) and Black River (20.3708° S, 57.3949° E) with a total population of 31,228 (Statistics Mauritius, 2011). The mangrove areas selected for this study occur in the following areas: 1) Pointe Koenig – Tamarin (5 km^2); 2) Case Noyale (2.6 km^2); 3) Maconde area (1.2 km^2); and 4) St Martin area (1.2 km^2). The study areas are mostly dominated by large patches of *Rhizophora mucronata* with few patches of *Brugueira gymnorrhiza*. Mangroves in the south west of Mauritius are utilized by some local people as fishing grounds and most of the houses are located adjacent to the coastal road where the mangrove ecosystems and the livelihood of the inhabitants are directly linked to the sea.

Survey design and structure

The survey questionnaires were designed in electronic format using the Adobe Acrobat X Pro software in English and Mauritian Creole. By using a PC tablet to minimize the administrative cost of printing questionnaires, a pre-test survey was conducted on a sample of 15 randomly chosen households to ensure questions were not too lengthy and understandable to the respondents. The questionnaires were arranged in three sections: information and household views about the mangroves (Section A); socioeconomic background and household characteristics (Section B); and how local residents value the tangible and non-tangible benefits derived from the mangrove ecosystem services (Section C).

Section A of the questionnaire focused on the details of the goods and services derived from the mangrove ecosystem which are regrouped into seventeen criteria as presented by Costanza and Folke (1997) and Rönnbäck *et al.* (2007). These ecosystem services include 1) Gas regulation, 2) Climate regulation, 3) Disturbance regulation, 4) Water regulation, 5) Water supply, 6) Erosion control and Sediment retention, 7) Soil formation, 8) Nutrient cycling, 9) Waste treatment, 10) Pollination, 11) Biological control, 12) Refugia, 13) Food production, 14) Raw materials, 15) Genetic resources, 16) Recreation, and 17) Cultural Value. Using the Likert scale, values ranging from 5- for strongly agree, 4- for agree, 3- for neutral, 2- for disagree and 1- for strongly disagree, were recorded for each of the seventeen criteria (Vo and Kuenzer, 2012).

interview (Otieno, 2015). Respondents were selected in each district by probability sampling. This procedure is strongly recommended to be used in household surveys (United Nations, 2008). It consists of inferring the sample estimates to represent the total population from which the sample was drawn. This ensured that the number of respondents per *sitio* was proportional to their respective population.

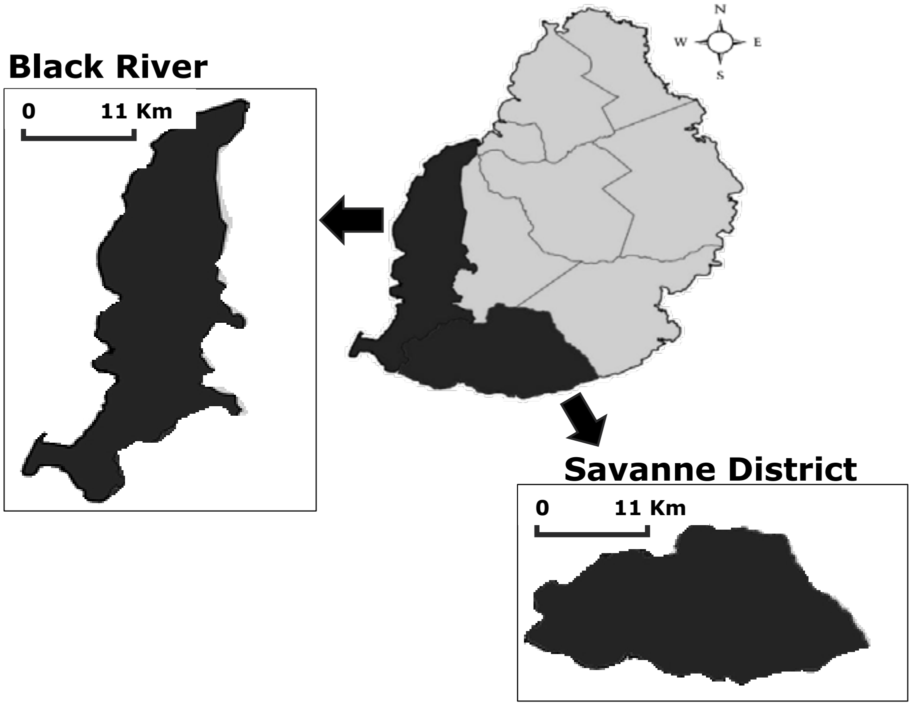

Figure 1. The south west region of Mauritius.

Section B of the questionnaire was designed to seek information on socio-economic characteristics such as age, gender, education level, income, occupation, number of household members, and resident status. Section C of the questionnaire required information on how local residents value the tangible and non-tangible benefits derived from the mangrove ecosystem services. Respondents were asked whether they would be willing to invest some of their money, time or both to help in the conservation of the goods and services of the mangrove ecosystems. Those who responded positively were asked how much money, time or both they would be willing to contribute per month (O'Garra, 2007).

Survey procedure and data collection

The study used Slovin's formula with 7 % margin of error to determine the number of household respondents to

Data were collected from households in the south west region of Mauritius using a PC tablet. The data were stored in the device with a specific reference code prior to analysis. A sample of 269 households in the Savanne district were approached for the survey and 142 responded favourably. Eighty-one respondents chose to fill the questionnaires in Mauritian Creole language. In the Black River district, 268 households were approached and 126 responded favourably. Out of the 126 respondents 93 chose Mauritian Creole as the medium to fill in the survey questionnaire and the rest chose English.

Data analysis

Descriptive statistics were used to summarize and describe the primary data collected from the household survey through tables and discussion of the results using Microsoft Excel 2016 software and the statistical software SPSS v21.

For section A of the questionnaire, the computation of awareness score of respondents was carried out by adding up the response (using the Likert scale, in SPSS Software) to seventeen statements which depicted the details of goods and services from the mangrove ecosystem. The awareness score was computed in three distinct categories: 1) low awareness (17-33.5), 2) moderate awareness (33.5-68.5), and 3) high awareness (68.5-85).

Results

Socio-demographic profile of local residents

The socio-demographic profile of respondents as detailed in Table 1 showed that 69 % of respondents in Savanne and 72 % in Black River districts were male. The percentage of married respondents in the district of Savanne was 47.9 % as compared to that of Black River district which was 65.9 %. The majority of respondents in both Savanne (81 %) and Black River districts (86 %) belonged to the age group 31-60 (Table 1).

Table 1. Socio-economic characteristics of respondents in Savanne and Black River Districts. (Rs1 = USD0.026).

Socio-economic characteristics	Savanne District		Black River District	
	Frequency	Per cent	Frequency	Per cent
Household Size				
< 4	56	39.4	50	40
≥ 4	86	60.6	76	60
Age				
< 30	14	9.9	6	4
31 - 60	115	81	108	86
> 61	13	9.1	12	10
Gender				
Male	98	69	72	57
Female	44	31	54	43
Education				
Literate	7	95.1	125	99.2
Illiterate	135	4.9	1	0.8
Marital Status				
Single	46	32.4	15	11.9
Married	68	47.9	83	65.9
Widowed	16	11.3	11	8.7
Divorced	3	2.1	4	3.2
Cohabitant	9	6.3	13	10.3
Occupation				
Fishing	17	12	3	2
Crop Farming	14	9.9	12	10
Business	7	4.9	7	6
Salary	49	34.5	49	39
Wage	11	7.7	44	35
Remittance	44	31	11	9
Income (MUR)				
< 5000	8	5.6	13	10
5000 - 25000	82	57.7	81	65
> 25001	52	36.7	31	25

Table 2. Valuation of mangrove products.

Mangrove Products	Frequency of respondents Savanne District (n=142)			Frequency of respondents Black River District (n=126)				
	0 USD	>14 USD	14 – 55 USD	0 USD	15 – 55 USD	56 – 96 USD	97 – 136 USD	137 – 178 USD
Fish	10.6	2.1	0.7	3.2	4.0	0.8	0.8	0.8
Crabs	9.2	2.8	1.4	4.8	1.6	0.8	2.4	0
Oyster	12.0	1.4	0	8.7	0.8	0	0	0

The overall number of residents per household in both districts were four, or more than four (60.6 % in Savanne and 60 % in Black River districts). Education levels were quite high among the respondents. On average, most of the respondents had basic primary level education (99.2 %), with a small proportion not completing primary education, or having no formal education (0.8 %).

Livelihoods of local communities

As shown in Table 1, formal employment provided the major source of income along the south western region of Mauritius (34.5 % of respondents for Savanne district and 39 % for Black River district). Besides formal employment, some respondents were engaged in other income generating activities such as crop farming, entrepreneurship, and fishing. The yearly income of 57.7 % of respondents in Savanne and 65 % of respondents in Black River district varied from USD135 to USD680. In contrast, 5.6 % and 10 % of respondents in Savanne and Black River districts earned less than USD135.

Household source of income derived from the mangrove ecosystems

Fish, crabs and oysters, as shown in Table 2, were those products which generated a monthly income in both Black River and Savanne district. Of these, fish were the highest income generator, which ranged from 0 to USD55 in the Savanne district as compared to that of Black River which was 0 to USD178. The second highest income generator was crabs, which generated income in the range of 0 to USD55 in the Savanne district and 0 to USD136 in the Black River district. Oysters were the lowest income generator, which varied from 0 to USD14 in both districts.

Awareness level of local residents

The results in Table 3 show that the awareness score ranged from 44 to 69, with a mean value of 52.3 and a standard deviation of 4.8 for the district of Savanne. Similarly, in Black River, the awareness score varied from 29 to 69, with a mean value of 53.8 and a standard deviation of 9.0. A higher frequency value for the “moderate awareness” category (99.3 %) was noted in the district of Savanne as compared to the “high awareness” category (0.7 %). On the other hand, in the Black River district 96.1 % respondents were under the category “moderate awareness”, 0.8 % respondents in the category of “high awareness” and 2.4 % respondents in the “low awareness” category. This confirmed that in Mauritius the great majority of local residents were aware to a certain extent of the goods and services provided by the mangrove ecosystems.

Moreover, as illustrated in Table 4, the perception of respondents on the mangrove ecosystems were recorded in the Savanne and Black River districts. Respondents with the opinion “I have no idea” for both Savanne (24 % Male, 11 % Female) and Black River (11 % Male, 13 % Female) districts were significant. In contrast, respondents who felt that “It is an eyesore

Table 3. Awareness Score, and descriptive statistics of respondents in the districts of Savanne and Black River.

Districts	Awareness Score		Mean	Standard Deviation
	Minimum	Maximum		
Savanne	44	69	52.3	4.8
Black River	29	69	53.8	9.0

Table 4. Percentage of respondents according to their views in the districts of Savanne and Black River.

Respondent's Views	Savanne		Black River	
	Male	Female	Male	Female
"It is an eyesore obstructing the beautiful scenery of the sea"	12%	8%	24%	10%
"It is nothing more than a group of trees growing in the coastal area of the sea"	5%	8%	11%	5%
"It has great economic values to the local people"	15%	3%	16%	8%
"It provides a beautiful coastal scenery"	14%	1%	1%	7%
"I have no idea"	24%	11%	11%	13%

obstructing the beautiful scenery of the sea" (12 % Male, 8 % Female respondents for Savanne district; and 24 % Male, 10 % Female respondents for Black River district) were relatively common. However, respondents also felt that "It has great economic value to the local people" (15 % Male, 3 % Female respondents for Savanne district; and 16% Male, 8% Female for respondents for Black River district), and "It provides a beautiful coastal scenery" (14 % Male, 1 % Female respondents for Savanne district; and 1 % Male, 7 % Female for Black River district).

Investment for conservation of mangroves

A majority of 88.1 % of local residents in the district of Savanne and 92.1 % in the Black River district as detailed in Table 4 were not in favour of any form of investment to protect and conserve the mangrove ecosystems. However, 3.9 % (Black River) and 4.2 % (Savanne) of respondents were in favour of investing for conservation of mangroves in terms of money. Moreover, 4 % of respondents in the district of Black River and 7 % in the district of Savanne were in favour of dedicating some of their time for conservation activities. Only 0.7 % of respondents in the district of Savanne agreed to contribute both forms of investment (money and time).

As illustrated in Table 5, the mean monthly investment in terms of money was estimated to the nearest Rupee value and found to be USD0.14 for the district of Black River and USD0.30 for the district of Savanne, respectively. The mean monthly time which respondents were agreeable to dedicate for the conservation of the mangrove ecosystems were 0.8 hours (Savanne District) and 0.7 hours (Black River District) respectively.

Discussion

Socio-demographic profile of local residents

The socio-economic and demographic results of this study indicated that people living along the south west coast of Mauritius were composed of nuclear families with two adults and one child on average and an average household size of 3.9. The local communities were mostly middle class, with the majority of head of households in the age group 41-50 years old, and had completed post-secondary education.

Table 5. Investment mode for conservation of mangroves.

Type of Investment	Savanne District		Black River District	
	Percentage of Respondents	Mean Monthly Contribution	Percentage of Respondents	Mean Monthly Contribution
Money	4.2	0.30 USD	3.9	0.14 USD
Time	7.0	0.8 Hours	3.9	0.7 Hours
Both	0.7	0	0	0

The majority of family heads were the sole bread winner and also obtained their monthly income through formal employment in different sectors. Therefore, in this study poverty was insignificant as compared to a similar study in Kenya where local communities were found to be highly dependent on the mangroves as a means to diversify their source of income due to lack of other opportunities and low levels of education (Okello, 2019).

This study revealed that the local communities generally displayed a unique attitude towards the protection of natural resources. As in other developing-economy studies (Whittington, 2004), local respondents were willing to invest only limited resources (USD0.14 for the district of Black River and USD0.30 for the district of Savanne) towards the protection of natural resources on a monthly basis. This represents approximately 0.05 % to 2.5 % of the respondent's monthly household income. However, the direct willingness to contribute (WTC) either money or time was a new concept to respondents and was not viewed as a priority or duty. The current belief was that protection and conservation of the mangrove ecosystem should be ensured by the authorities and not by them. The island of Mauritius is a welfare state with a different culture from other developed countries where it is common practice to contribute to the goods and services of the mangrove ecosystems. Hence, in the local context respondents were less likely to participate or contribute in conservation activities for the protection of mangroves at the study sites. This however does not imply that they are not concerned about the mangroves especially during a moment of crisis. The strong involvement and mobilization of Mauritian communities was clearly demarcated when mangroves on south east coasts were affected by a recent oil spill and several people contributed to fund raising and donations to NGOs for mitigating the impacts (Lee, 2020).

Utilisation of mangrove goods and services by local residents

In past decades, people who lived adjacent to the mangrove ecosystems were highly dependent on them for various fishery and forestry products for domestic and commercial purposes (Malik, *et al.*, 2015). This was a way to diversify their source of income (Cinner *et al.*, 2009). In a study conducted by Okello (2019) in Kenya, a high reliance on mangroves for livelihoods was noted. This was mainly because 62 % of the Kenyan coastal population were living below the poverty threshold of USD1.25 per day (Okello, 2019). However, in the present study, the great majority of respondents living along the south west coast of Mauritius were in the middle or low-income class who obtained their monthly income through formal employment in different sectors. Hence, they are economically independent on the mangrove ecosystem.

Fish, crabs and oysters were considered to be making significant economic contribution to the welfare of a small proportion of local communities living along the south west coast of Mauritius. The socio-economic survey carried out in this study showed that maximum earnings of USD178 for fish, USD136 for crabs and USD55 for oysters were collected per head of household when selling those mangrove products on a monthly basis. This revealed the economic value associated with the extractive uses of the mangrove ecosystems in Mauritius. To date this study is among the first to investigate the direct use value of the mangrove ecosystems in Mauritius. Local communities living adjacent to the study area were able to describe some of the goods and services of the mangrove ecosystem. This is due to their frequent interaction with the mangroves for either subsistence needs or recreational purposes.

Perception of local residents towards the goods and services of the mangroves

The mangrove ecosystem provides an enormous array of ecological and economic benefits to the local community at large. Nevertheless, the extent to which the local residents are aware about those benefits has never been studied before in Mauritius. The awareness level of the local community towards the goods and services provided by mangroves was moderate. The local communities were not fully aware of all the benefits that a mangrove ecosystem can provide. Of the 17 proposed goods and services of the mangroves, only 6 services were positively recognized. These were 1) climate regulation, 2) disturbance regulation, 3) waste treatment, 4) erosion control/sediment retention, 5) recreational, and 6) cultural value. The awareness level was quantified and the result showed a moderate level of awareness. However, the opinions of male and female respondents varied significantly (Table 4) at the study sites. This indicated that with more awareness, the high frequency of respondents who opined “I have no idea” and “It is an eyesore obstructing the beautiful scenery of the sea” would decrease. Consequently, the findings of this study are interesting and unique in the sense that studies from other counties (Okello, 2019; Naylor *et al.*, 1998) reported that local communities who lived adjacent to mangrove ecosystems had an adequate level of knowledge on their goods and services. Thus, it is apparent that public understanding and awareness with respect to the mangrove biological and economical values can be improved if the local community is made aware of the full potential benefits that could be derived from the mangrove forest (Rahman and Asmawi, 2016). As proposed by Sayers (2006), this can be achieved by educating and informing people in order to raise their awareness on all the goods and services of the mangrove ecosystems. Ultimately, this will ensure the proper management of the mangrove ecosystem for the benefit of future generations in Mauritius.

Conclusion

This study demonstrated that mangroves along the south west coast of Mauritius have economic and ecological significance to the local communities. However, knowledge of the goods and services of the mangroves at the study sites were moderate amongst local residents. This implies that conservation campaigns and education programmes are essential to raise local concern towards the protection and conservation of the mangrove ecosystems in order to guarantee future generations of the same benefits that they currently provide. To achieve both sustainable blue growth and SDG-14, a comprehensive and strategic plan is required to educate coastal communities on the need for the protection and conservation of mangroves in Mauritius.

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Seasonal variability of vertical patterns in chlorophyll-*a* fluorescence in the coastal waters off Kimbiji, Tanzania

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Abstract

A study on the vertical pattern of chlorophyll-*a* (Chl-*a*) fluorescence was undertaken in the Mafia Channel off Kimbiji, Tanzania. Data was collected during the Southeast Monsoon (SEM) and Northeast Monsoon (NEM) seasons. There was higher Chl-*a* concentration of 0.1 to 1.1 mgm^{-3} in the surface layer off Kimbiji to about 50 m depth due to the presence of mixed layer depth (MLD) which allowed water mixing in the layer. A deep Chl-*a* maximum was recorded at around 40 m depth during the NEM and between 40 and 70 m in the SEM. Surface water between longitude 39.9°E and 40.2°E had low Chl-a from the surface to about 50 m depth due to poor nutrient input. The NEM had an insignificantly higher Chl-*a* value than the SEM ($p > 0.05$) which differed from other studies in which Chl-a was higher during the SEM than the NEM, than, the Chl-a concentration was higher at the surface during the SEM than during the NEM. Satellite data showed higher Chl-*a* in the SEM than NEM, localized along the Mafia Channel. During the SEM season the wind pushes higher Chl-*a* water from the Mafia Channel towards the north and leads to a higher concentration at Kimbiji.

Keywords: Chlorophyll-*a*, CTD, Kimbiji, Mafia Channel, monsoon season

Introduction

Phytoplankton are microscopic free–floating aquatic plants. They possess chlorophyll-*a,* a green pigment that absorbs light from the sun and converts it into energy through the photosynthetic process (Peter, 2013; Peter *et al.*, 2018; Semba *et al.*, 2016). The concentration of organic carbon associated with phytoplankton (usually expressed as mgm^{-3}) is commonly used to express the amount of phytoplankton (Cullen, 1982). Phytoplankton account for about half of global primary production (Boyce *et al.*, 2010). They also serve as primary producers in marine environments and form the base of most aquatic food webs (Kyewalyanga *et al.*, 2007; Semba *et al.*, 2016). Phytoplankton provides food for herbivorous zooplankton and other invertebrates, fishes and large mammals, including whales (Roy, 2009). Further, phytoplankton regulates climate processes and bio-geochemical cycles through the carbon cycle (IPCC, 2014).

Chl-*a* found in phytoplankton is used as a proxy to determine the concentration of these microscopic plants in coastal and marine waters. Normally, surface waters have high Chl-*a* concentrations and thus contribute significantly to ocean productivity. However, high Chl-*a* concentration is not always found in the surface waters; sometimes they are subsurface, at greater depths, especially in areas with strong thermal stratification (Cullen, 1982). This type of Chl-*a* peak is referred to as the deep chlorophyll maximum (DCM) or subsurface Chl-*a* maximum. In the Western Indian Ocean, the DCM is reported to occur at around 50-75

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m depth (Conkright *et al.*, 1998; Owens *et al.*, 1993; Wiggert *et al.*, 2006).

The DCM is very important in ecology since it contains much of the world's primary production and facilitates nutrient cycling. This layer is an important food source for consumers as it has a higher concentration of primary producers at one location in the water column. In nutrient poor waters, the DCM accounts for more than half of the overall primary production due to phytoplankton growth (Macías *et al.*, 2014; Weston *et al.*, 2005). A high rate of primary production in the DCM facilitates nutrient cycling to higher trophic levels in the mixed layer. Studies shows that the DCM occurs at the same depth as the nutricline (Estrada *et al.*, 1993), thus phytoplankton at this layer can obtain nutrients coming up from deep waters. Phytoplankton in the DCM can then cycle back up the water column providing nutrients for other organisms in the mixed layer (Cullen, 2015). The DCM is therefore associated with predator-prey interactions, energy and biomass flow and biogeochemical cycles within the ecosystem (Leach *et al.*, 2018). Since most parts of Tanzanian coastal waters are nutrient poor and have oligotrophic characteristics, primary production might be concentrated in the subsurface waters, at the DCM. Thus, studies on vertical distribution of Chl-*a* and the dynamic of the DCM are important in understanding productivity potential in Tanzania coastal waters.

Until recently, most studies conducted in the coastal waters of Tanzania focused mainly on the abundance of phytoplankton in surface waters. For example (Lugomela *et al.*, 2002) examined biomass of colony forming *Trichodesmium* species and found higher biomass during the NE than the SE monsoon season. A study by Peter (2013) found higher Chl-*a* concentrations during the rainy season as compared to the dry season in the Zanzibar and Pemba Channels. Another study by Semba *et al.* (2016) assessed how the decrease in phytoplankton biomass in the Rufiji-Mafia Channel impacts on prawn catches. They found that the decline in Chl-*a* concentration was associated with the decrease in prawn catch in the Rufiji-Mafia Channel. Also, a study by Peter *et al.* (2018) showed how physical-chemical variables influence the spatial and seasonal variation of Chl-*a* in the coastal waters of Unguja, Zanzibar. They revealed that ammonia and nitrate were the major drivers of high Chl-*a* during the SE monsoon as compared to the NE monsoon season. Other studies used ocean colour satellite images and found that Chl-*a* levels are usually below 1 mgm^{-3} (Barlow *et al.*, 2011) and there is considerable variability with seasons in Tanzania waters (Semba *et al.*, 2016).

These previous studies widen our understanding of the dynamics of phytoplankton and Chl-*a* in the coastal and marine waters of Tanzania. However, these studies focused on phytoplankton at the surface of the ocean. Knowledge about vertical distribution and seasonal variability of Chl-*a* in coastal waters of Tanzania is lacking. Also, there is paucity of knowledge on forces that control phytoplankton abundances. Preliminary investigations of the vertical structure of Chl-*a* and environmental variables (temperature, salinity) were undertaken during the Tanzanian leg of the Second International Indian Ocean Expedition (IIOE-2) in early November 2017 and June 2018. This study intended to investigate: (1) vertical variation in Chl-*a* concentration off Kimbiji during the two monsoon seasons; (2) seasonal variation in the mixed layer depth; and (3) environmental factors influencing seasonal variation in Chl-*a* concentration off Kimbiji.

Materials and methods

Study area

This study was conducted in the coastal water off Kimbiji, located between longitude 39.3°E and 40.3°E and latitude 8°S and 6.8°S (Fig. 1). The area is about 40 km southeast of Dar es Salaam's city center and bordered by the Mafia Channel in the south. The area was selected because there were no phytoplankton records for this site. The other reason for picking this area was because the area contains two transect with hydrographic profile data of temperature, oxygen, salinity, chlorophyll fluorescence and turbidity recorded during the 2017 and 2018 International Indian Ocean Expedition 2 (IIOE-2). The first transect has four sampling stations recorded during the SE monsoon season of 2018, while the second transect has eight sampling stations with similar profiles as the first transect recorded during the NE monsoon season of 2017 (Fig. 1).

The monsoon season influences the climate of the study area. The NE monsoon season runs from November to March and the SE spans from May to September (Semba *et al.*, 2019). The monsoon seasons have a marked effect on air and water temperature, winds and rainfall (Mahongo *et al.*, 2011; Richmond, 2011). Winds are a particularly important feature of the Western Indian Ocean, driving water circulation and affecting wave action, local climate, biological processes and human activities (Richmond, 2011). The trade winds are steady and light, blowing from the northeast towards the southwest at about 5 ms^{-1} during NE monsoon season. The NE monsoon is associated with the short rainy period (October–December). The long rainy season begins after the NE monsoon season from March to June. Trade winds reverse during the SE monsoon and blow from the southeast towards the northwest at a relatively strong speed of about 9 ms^{-1}, associated with cooler temperature and rough ocean. During April and October the ocean is calm, with moderate temperatures forming during the inter-monsoon seasons. The seasonal reversal of monsoon winds influences phytoplankton distribution in this region (George *et al.*, 2013; Mahongo *et al.*, 2011; Richmond, 2011). The reverse of the monsoon winds regulates mixing in the ocean that affects nutrient flux across the thermocline and the distribution of Chl-*a* concentration. Air temperature in the study area rarely fall below 20 °C and the seawater temperature reaches a minimum of 25 °C in September and maximum of 29 °C in March (McClanahan, 1988; Peter, 2013).

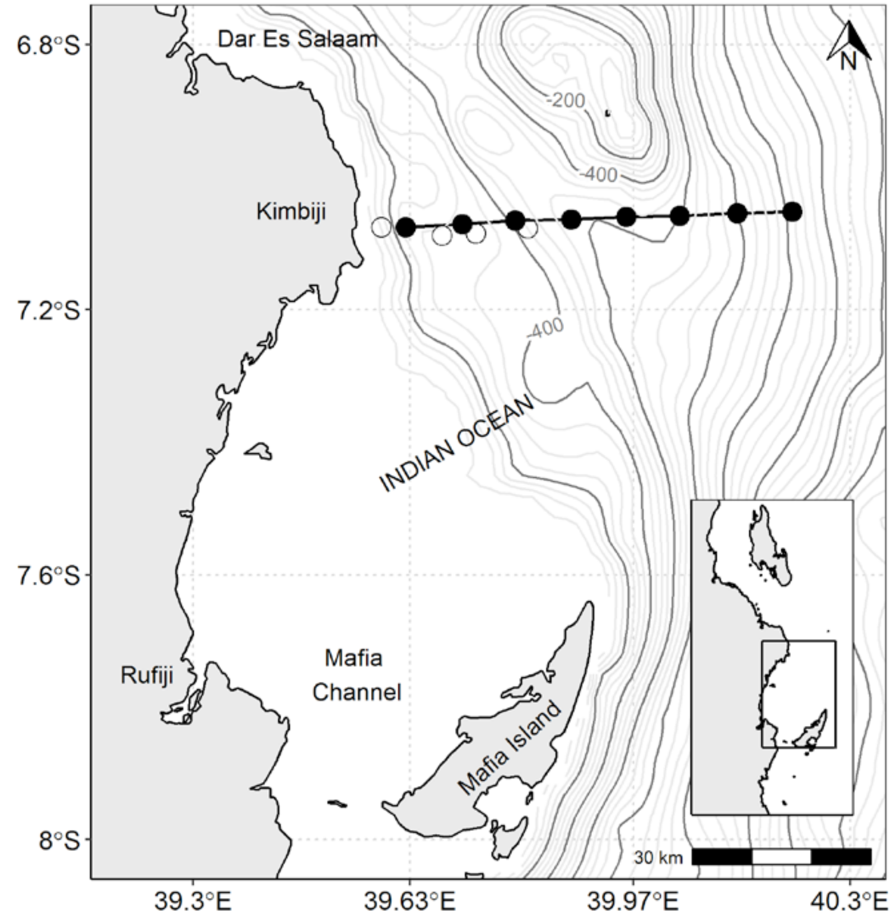

Figure 1. Map showing the location of the transect off Kimbiji located on the northern side of the Mafia Channel. The grey solid lines are contours at 50 m interval and solid black lines are contours at 200 m. The black filled points show the stations for the NE monsoon and the black hollowed points show stations for the SE monsoon season. The inset map shows the location of the study area in the coastal waters of Tanzania.

Data collection

Conductivity–temperature–depth (CTD)

Data for this study was collected during the 2017 and 2018 International Indian Ocean Expedition 2 (IIOE-2) onboard Agulhas II, respectively for the NE and SE monsoon seasons. Data for the NE and SE monsoon seasons were collected in November 2017 and June 2018, respectively, with all samples taken from the coastal waters of Tanzania. The vertical profile of temperature and fluorescence were recorded with a CTD (Seabird 11 plus, Seabird Electronics, USA). The spatial information of the sampling locations is shown in Figure 1 and Table 1.

Table 1. Table showing location of the CTD cast off the Kimbiji transect.

Date	Time	Longitude	Latitude
2017-11-05	23:29:37	40.2117	-7.0525
2017-11-06	03:57:19	40.1283	-7.0543
2017-11-06	05:26:03	40.0407	-7.0587
2017-11-06	08:00:43	39.9592	-7.0600
2017-11-06	09:25:07	39.8750	-7.0642
2017-11-06	14:52:20	39.7897	-7.0658
2017-11-06	16:07:12	39.7095	-7.0710
2017-11-06	18:47:34	39.6238	-7.0755

Surface drifters

Drifter data was used to assess the influence of ocean currents on the seasonal and spatial distribution of Chl-*a* fluorescence. Drifter data was obtained from the Global Drifter Program as a text file and was used to compute surface current speed and direction in the study area. The ocean currents were then grouped and averaged between the NE and SE monsoon.

Satellite data

To complement distribution of Chl-*a* both in space and season, satellite derived chlorophyll data was used. The level 3 monthly Chl-*a* concentrations were downloaded from MODIS with a spatial resolution of 4 km from September 2017 to August 2018. To understand the association between Chl-*a* and sea surface temperature (SST), sea surface temperature with the same resolution and time period was downloaded from MODIS. Surface wind data was also downloaded from QuikSCAT.

Data processing

The raw CTD data was converted to the *.cnv* file format using the Sea Bird (SBE) software and then processed in R-language using the **oce** package (Kelly and Richards, 2018). Because of the large number of profiles, the processing of CTD data was iterated. The process involved several steps. First, a list of all CTD files in the working directory was created in the R-working directory using the *dir()* function. Second, a dummy file that stored CTD files was then constructed using the *list()* function. After that, each file in the working directory was imported with the *read.ctd()* function. Only the downcast values of the CTD profiles were used for analysis.

To determine the mixed layer depth (MLD), the method described by (Chu and Fan, 2010) was applied. Here, successively deeper data points in each of the profile potential temperatures is examined until one is found with a potential temperature value differing from the value at the 10 m reference depth by more than the threshold value (δT) of ± 0.5 °C. 0.5 °C. To understand the influence of vertical structure, fluorescence profiles were divided into stratum of 50 meter intervals from the surface to 200 meter depth. The difference in mean Chl-*a* concentration between seasons and among strata was computed with general linear modelling and the multiple comparison between the seasons was calculated with Tukey's HSD test.

The monthly satellite sea surface temperature (SST) and Chl-*a* as well as wind speed and direction were converted into tabular data and averaged for the NE and SE monsoon seasons. The average SST and Chl-*a* were used to map distribution patterns of temperature and Chl-*a* both in space and season in the study area. The surface current, wind speed and direction were overlaid into seasonal maps of temperature and Chl-*a* to examine their influence on the distribution of temperature and Chl-*a* within the study area. Drifters were also aligned to the NE and SE monsoon season and mapped against the Kimbiji transect to examine the pathway of surface water across a transect. The data processing, analysis, plotting and mapping was done in the R environment.

Results

Vertical profiles of temperature and Chl-*a*

The vertical profiles of Chl-*a* and temperature during the NE and SE monsoon seasons off the Kimbiji

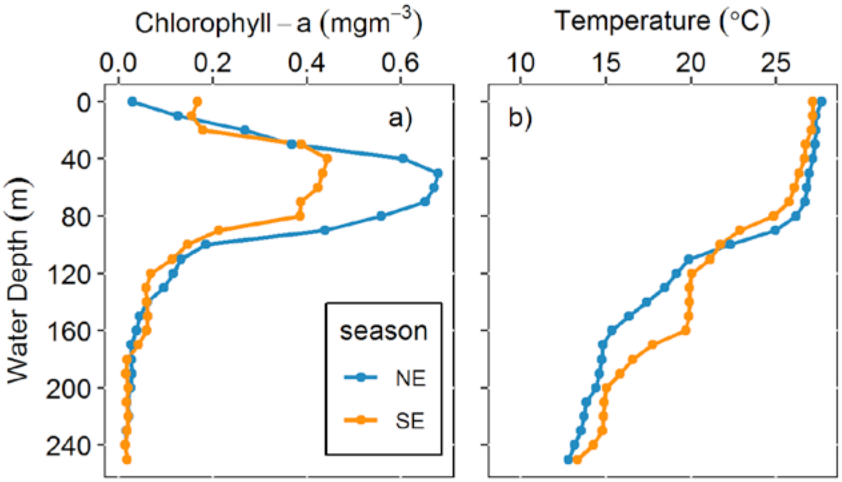

Figure 2. Vertical profiles of *in-situ* a) chlorophyll-*a*, and b) temperature off the Kimbiji coast measured in November 2017 (NE) and June 2018 (SE).

coast near longitude 39.7095°E and latitude7.0755°S are shown in Figure 2a and 2b. Chl-*a* profiles showed a marked difference during the two monsoon seasons (Fig. 2a). Figure 2 indicates that while the surface water has almost uniform temperature, Chl-*a* values differ with season. Chl-*a* profiles at Kimbiji show lower values at the surface with concentration values of less than 0.1 mgm^{-3} during the NE monsoon season and about 0.17 mgm^{-3} in the SE monsoon season (Fig. 2a). These values were uniform to about 20 m depth, where they started to increase sharply and reached a maximum concentration of 0.44 mgm^{-3} at 40 m depth during the SE monsoon season. There was a slow decline in concentration from 40 m to 80 m depth where Chl-*a* started to decline sharply reaching nearly 0 mgm^{-3} at the bottom in the SE monsoon season. During the NE monsoon, Chl-*a* concentration increased sharply from about 0.02 mgm^{-3} at the surface to a maximum concentration of about 0.7 mgm^{-3} at 50 m depth at Kimbiji (Fig. 2a). Chl-*a* then decreased sharply from 0.7 mgm^{-3} at 50 m to about 0.19 mgm^{-3} at 100 m depth and remained relatively low and constant with increasing depth, reaching nearly 0 mgm^{-3} at the bottom in the NE monsoon. Chl-*a* was slightly higher in the upper 30 m during the SE monsoon (mean ± SE = 0.222 ± 0.0555) than during the NE monsoon (0.198 ± 0.075) (t = -0.26, df = 5.53, p = 0.80; Fig. 2a). However, in the 30-80 m water layer, Chl-*a* values were significantly higher during the NE monsoon season (0.59 ± 0.05) than during the SE monsoon

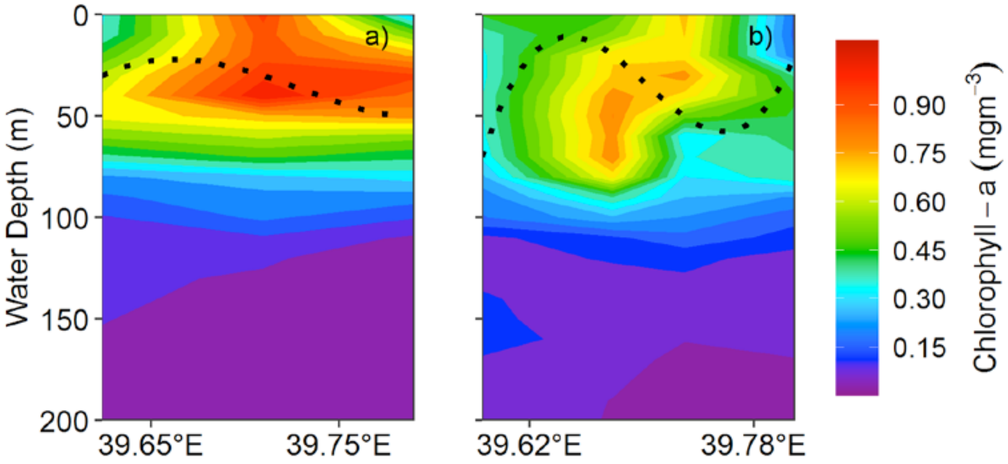

Figure 3. Longitudinal cross-section of chlorophyll-a within a 200 meter water column off Kimbiji during a) the NE and b) SE monsoon season. The black dotted lines show the location of the MLD for each season.

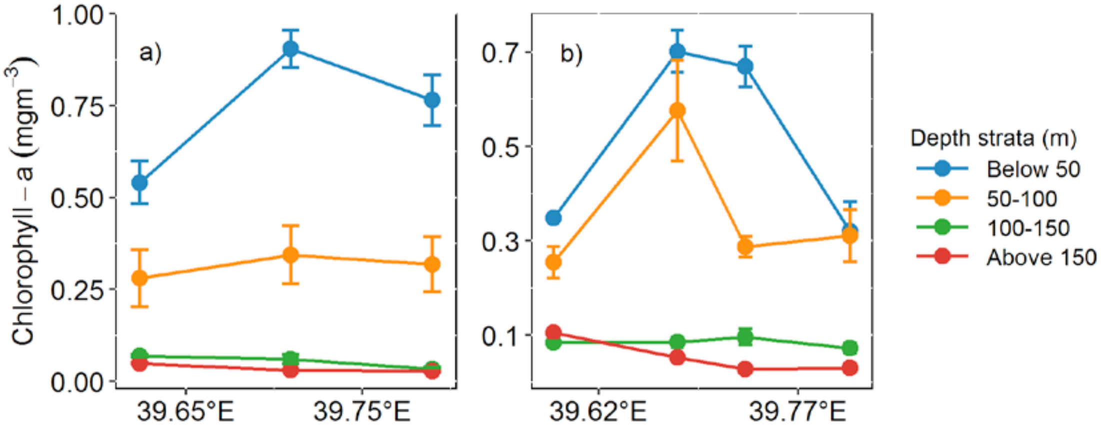

Figure 4. Chlorophyll-a distribution along the Kimbiji transect stratified into depth groups during a) the NE and b) SE monsoon seasons.

(0.41 ±0.01) (t = 3.63, df = 5.51, *p* = 0.01). The temperature profiles indicate that water temperatures were slightly higher during the NE monsoon season with nearly uniform water temperatures from the surface to about 80 m depth (Fig. 2b). Water temperature decreased gradually from ~28 °C near the surface to about 25 °C at about 80 m depth and then decreased sharply to about 14 °C at 120 m, followed by a gradual decrease to the lowest temperature at 240 m.

Seasonal cross-sections of Chl-*a*

To address the effect of physical processes on Chl-*a* distribution off Kimbiji, the vertical distribution of Chl-*a* in the upper 200 m of the water column and the mixed layer depth were analyzed during the SE and NE monsoon season. Figure 3a and b show that the vertical structure of Chl-*a* concentration varies with monsoon season and depth. During the NE monsoon, the water from the surface to about 75 m depth has relatively higher Chl-*a* concentrations ranging between 0.1 and 1.1 mgm^{-3} (Fig. 3a). The values for Chl-*a* concentration then increased with depth from the coastline toward offshore (Fig. 3a). The bottom water above 90 m was relatively low in Chl-*a* concentration and ranged between 0.01 to 0.2 mgm^{-1} (Fig. 3a). Similarly, during the SE monsoon season, water had a Chl-*a* concentration ranging between 0.5–0.8 mgm^{-3} along the transect from the surface to about 75 m depth (Fig. 3b), with peak values near longitude 39.68°E. The concentration of Chl-*a* was below 0.3 mgm^{-3} for water above 90 m depth (Fig. 3b). The mixed layer depth (MLD) pattern varies between the NE (Fig. 3a) and SE (Fig. 3b) monsoon seasons. The MLD during the NE monsoon ranged between 22.2 and 49.8 m depth with the MLD of about 42 m with a maximum Chl-*a* concentration (~1.03 mgm^{-3}) (Fig. 3a). During the SE monsoon, the MLD ranged between 20 to 70 m and there was an unclear pattern between MLD and Chl-*a* concentration (Fig. 3b).

Seasonal and depth variation of Chl-*a*

Figure 4a and b show the Chl-*a* concentration along the transect divided into four depth groups during the two monsoon seasons. During the NE monsoon season, the stratum below 50 m depth had relatively higher Chl-*a* concentration (0.5–0.9 mgm^{-3}) followed by the 50-100 m strata (0.28 and 0.35 mgm^{-3}). The remaining two water layers (100-150 and 150-200 m depth) had low concentrations below 0.2 mgm^{-3} (Fig. 4a). Similarly, the SE monsoon had higher Chl-*a* concentrations in water below 50 m depth, which ranged between 0.35 and 0.7 mgm^{-3} compared to the other depth strata (Fig. 4b). The 50-100 m stratum had slightly lower concentrations than the surface stratum. The 100–150 and 150–200 m strata had concentrations below 0.1 mgm^{-3}.

Figure 5a and b show seasonal variation of Chl-*a* concentrations within the upper 50 m (Fig. 5a) and 50–100 m water layers (Fig. 5b). Both strata had insignificantly higher Chl-*a* concentration during the NE as compared to the SE monsoon season (t = -1.053, *p* = 0.3). However, the concentration of Chl-*a* at station 2 of the 50–100 m water layer was relatively higher during the SE monsoon (~0.58 mgm^{-3}) than during the NE monsoon season (~0.34 mgm^{-3}).

Seasonal temperature cross section

Figure 6a and b show the vertical structure of temperature off the Kimbiji transect in relation to monsoon seasons. In both the NE and SE monsoon season, the

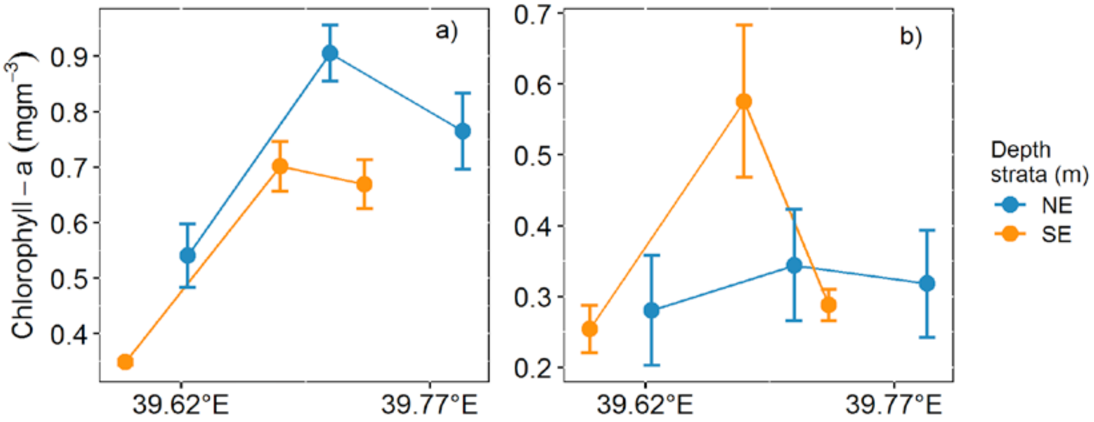

Figure 5. Mean and standard error of chlorophyll-a concentration at Kimbiji divided into strata of a) surface to 50 meter depth, b) 50-100 meter for the NE and SE monsoon seasons.

surface water layer below 75 m depth was very warm (26–28 °C) compared to bottom waters (Fig. 6). However, surface water temperature during the NE monsoon (Fig. 6a), was slightly higher (~28 °C) than that of the SE (~27°C) monsoon season (Fig. 6b). The thermocline during the NE monsoon was shallower at around 80 m depth indicated by a sharp decrease in temperature from 24 to 18 °C. Below the thermocline, temperature decreased gradually from 18 °C to 14 °C at 200 m. For the SE monsoon season, the thermocline was present at about 100 m depth (Fig. 6b), below which temperature decreased to the lowest (16 °C) at 200 m.

Seasonal and spatial variation of surface Chl-*a* and SST

The MODIS satellite data was used to map seasonal distribution patterns of surface SST and Chl-*a* within the study area. Figure 7 shows that the SE monsoon had a relatively higher Chl-*a* concentration (Fig. 7b) than the NE monsoon season (Fig. 7a). The nearshore waters within the Mafia Channel had higher Chl-*a* concentrations than those further from the coastline (Fig. 7a and b). The wind vector arrows show a north-westerly direction during the SE monsoon which pulls the channel water northwards spreading high Chl-*a* water in the channel and even further north off Kimbiji (Fig. 7b). The reversal of wind direction to south-westerly during the NE monsoon prevents the productive waters in the Mafia channel from moving further north, thus making the water off Kimbiji less productive at this time (Fig. 7a).

Figure 8a and b show the sea surface temperature patterns over season and space, indicating that the NE season has warmer temperatures (Fig. 8a) than the SE monsoon season (Fig. 8b). During the NE, SST ranged between 28 and 31° C, which was relatively higher compared to the 26 to 28° C observed during the SE

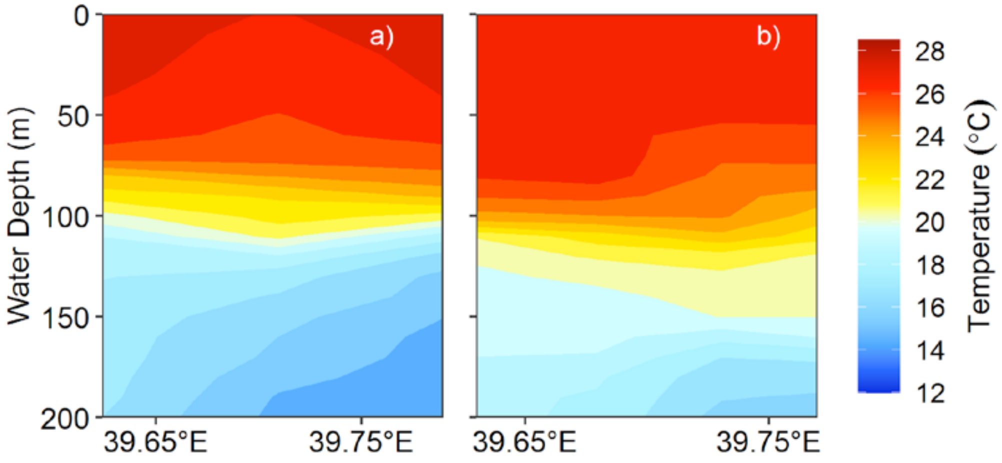

Figure 6. The hydrographic section of temperature at Kimbiji during a) NE and b) SE monsoon seasons.

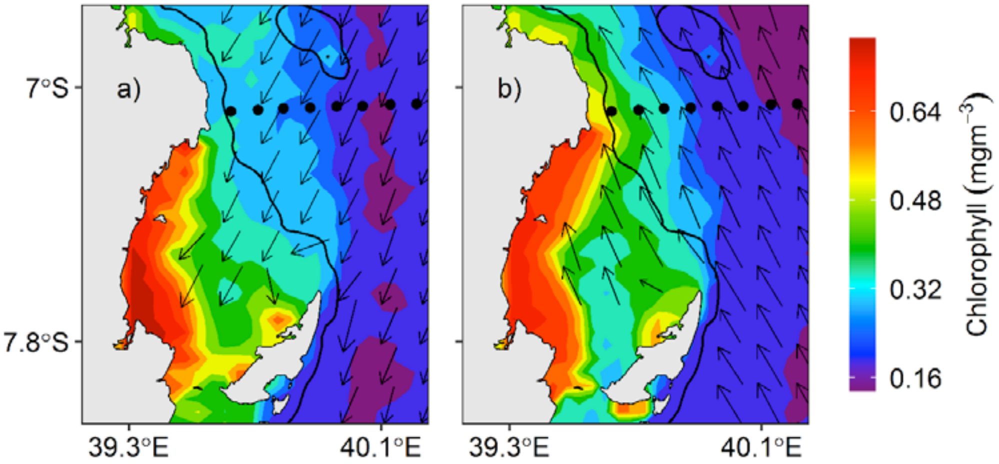

Figure 7. The seasonal surface chlorophyll-a overlaid on wind vectors of trade winds during a) the NE and b) SE monsoon seasons. The black solid line represents the isobaths at 200 m.

monsoon season. Furthermore, patches of very high temperature of about 31ºC was observed off thet Rufiji River delta during the NE monsoon season (Fig. 8a).

Figure 9a and b show the seasonal averaged surface currents of the East Africa Coastal Current (EACC). The surface current shows northward propagation during the NE (Fig. 9a) and SE monsoon seasons (Fig. 9b). The pathways of the EACC align well with water of low Chl-*a* concentration and mark a clear distinction between the high and low Chl-*a* waters during the NE and SE monsoon season (Fig. 9a and b), respectively.

The vertical section in Figure 10 shows a cross–section of Chl-*a* along the transect presented in Figure 9. It shows that surface water within the path of the EACC (between longitude 39.8°E and 40.2°E) has relatively low Chl-*a* values compared to the coastal waters (longitude below 39.8°E).

Discussion

This study provides information on the vertical distribution of Chl-*a* in coastal marine waters of Tanzania. The study reports for the first time on the presence of a seasonal subsurface peak of Chl-*a* concentration in these waters. High surface Chl-*a* values were measured during the SE monsoon season unlike during the NE season, where the Chl-*a* concentration was slightly low (Fig. 2). Also, for the first time, this study relates Chl-*a* concentrations to the effect of the EACC where it was found that Chl-*a* was appreciably lower within the limits of this important current, regardless of

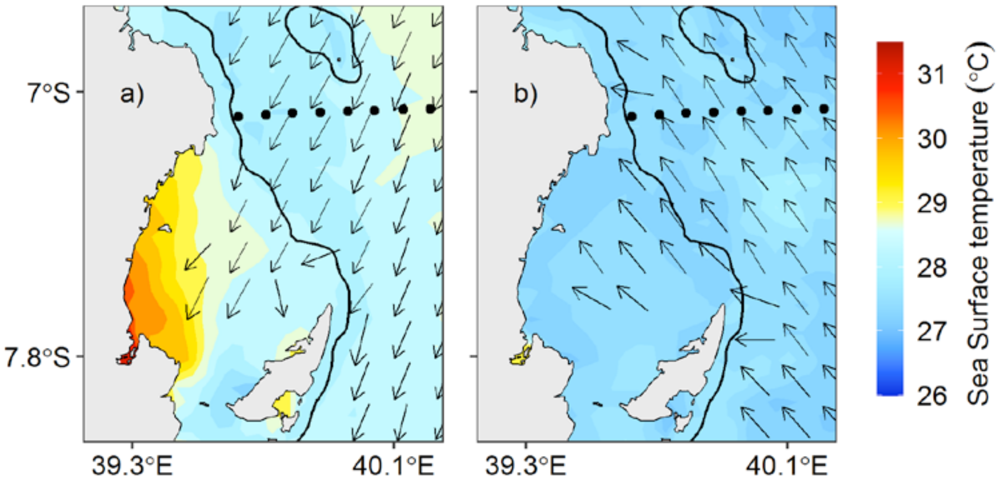

Figure 8. The seasonal surface temperature overlaid on wind vectors of trade winds during a) the NE and b) SE monsoon seasons. The black solid line represents the isobaths at 200 m.

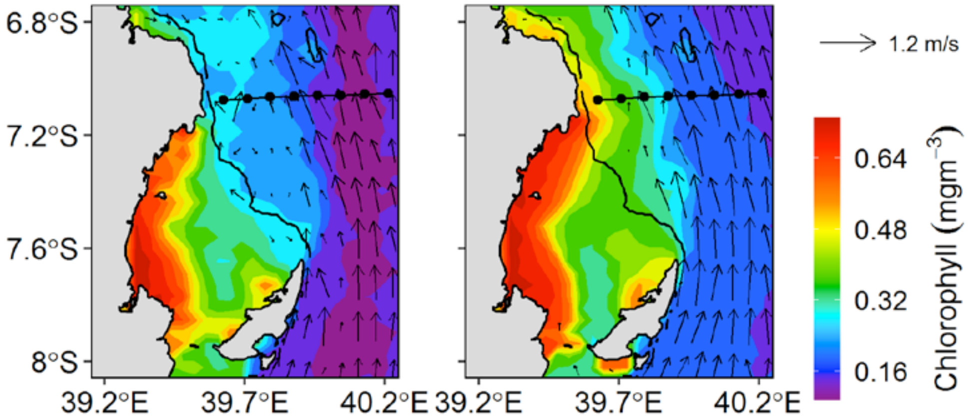

Figure 9. Vector arrows showing the speed and direction of East African Coastal Current (EACC) superimposed on surface chlorophyll-a during a) the NE and b) SE monsoon season. The black solid line represents the isobaths at 50 m.

season. In contrast, however, the nearshore areas had relatively higher Chl-*a* concentrations, attesting to the oligotrophic nature of the EACC, which is known to transport nutrient-poor water throughout the year (Mahongo and Shaghude, 2014).

The subsurface waters below 75 m depth off Kimbiji had relatively higher Chl-*a* values, which ranged between 0.1 and 1.1 mgm^{-3} during the NE and SE monsoon seasons (see Fig. 3). However, Chl-*a* values were relatively lower during the SE monsoon than during the NE monsoon season. Vertical distribution of Chl-*a* is governed by the thermal structure of the water column (Fig. 2) and other factors facilitating vertical mixing (Valenti *et al.*, 2015) such as vertical diffusivity which allows nutrients across the thermocline, light penetration or availability, nutrients availability in the productive layer (Cullen, 1982), and the variability of the mixed layer (see Fig. 3; Cullen ,1982; Valenti *et al.*, 2015). Nevertheless, the Chl-*a* maximum (DCM) was found close to 40 m depth and between 40 and 80 m during the NE and SE monsoon seasons, respectively. This is similar to the DCM depth reported in subtropical waters of the Western Indian Ocean (Conkright *et al.*, 1998; Owens *et al.*, 1993; Wiggert *et al.*, 2006). However, shallower DCM depths ranging between 25 and 47 m have been reported in the Bering Sea, and that currents, rather than nutrients, were the main driver of Chl-*a* spatial distribution (Cullen, 1982).

While nutrient concentration and light intensity may govern the vertical distribution of Chl-*a* in the marine environment, internal wave oscillations and Langmuir circulations have been shown to determine the dynamics of Chl-*a* within the thermocline and in the upper 5 m of the water column, respectively (George *et al.*, 2013). Also, Valenti *et al.* (2015) observed that while light is plentiful in surface waters, nutrients become a limiting factor for phytoplankton growth because of poor mixing. Similarly, at depths, light

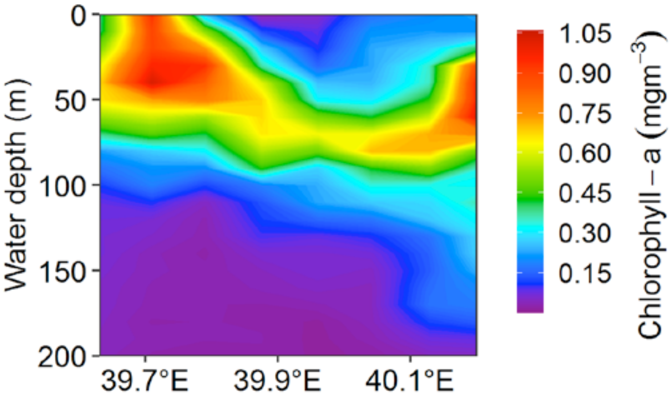

Figure 10. Hydrographic section showing chlorophyll-a concentration in the water column along the transect off Kimbiji.

becomes limiting while nutrient concentrations are high maintaining an increase in Chl-*a* within the photic layer (Cullen, 1982; Valenti *et al.*, 2015).

Chl-*a* concentration showed significant seasonal variation in different water layers (Fig. 4). The surface water layer (below 50 m) had the highest Chl-*a* concentration, followed by the water layer within 50–100 m (Fig. 4a and b), while water above 100 m depth had the lowest Chl-*a* values (Fig. 4). The reason for higher Chl-*a* concentration at these two surface strata is because the MLD is located within these strata; that is at 22.2 to 49.8 m and 20 to 70 m depth during NE (Fig. 3a) and in SE monsoon season (Fig. 3b), respectively. The MLD is important as it helps nutrient enrichment to the surface thereby increasing Chl-*a* concentration and also allows phytoplankton distribution in the mixed layer (George *et al.*, 2013; Vinayachandran and Saji, 2008; Waliser *et al.*, 2005)

It was surprising that the NE had an insignificantly higher Chl-*a* value than the SE monsoon season for water below 50 m and between 50 and 100 m (Fig. 5a and b; $p > 0.05$). This finding is opposite to previous studies, which found a higher Chl-*a* value during the SE as compared to the NE monsoon season (Limbu and Kyewalyanga, 2015; Peter *et al.*, 2018; Semba *et al.*, 2016). While these previous studies focused on Chl-*a* variation at the surface of the ocean (less than 5 m depth), the present study dealt with Chl-*a* throughout the water column which had higher subsurface Chl-*a* concentration in the NE than SE monsoon season. This might account for the difference observed (Fig. 2).

Contrary to the hydrographic section of Chl-*a*, the surface Chl-*a* value from the satellite clearly showed the SE season had relatively higher Chl-*a* value than the NE monsoon season (Fig. 7). The Chl-*a* pattern over the study area indicates a relatively higher value in the Mafia Channel, which is confined within shallow waters below 200 m depth (Fig. 7). The relatively colder waters and northwesterly wind (Fig. 8b) during the SE monsoon season provides sufficient surface mixing which is conducive for elevated productivity in this area. This productive water is pushed northwards by the trade winds during SE monsoon season and transports high Chl-*a* concentration waters towards the Kimbiji segment (Fig. 7b). However, the reversing wind during the NE monsoon season prevents transport of high Chl-*a* value water toward the Kimbiji segment, leading to a low value of surface Chl-*a* at this time of the year (Fig. 7a). The Mafia Channel is unaffected by the EACC, which passes in waters deeper than 200 m, just offshore of Mafia Island. A previous study in the Mafia Channel indicated similar findings and found nutrient input from the Rufiji River to be important in this area (Semba *et al.*, 2016).

Although the surface water is well lit, the water between longitude 39.9°E and 40.2°E has relatively low Chl-*a* value from the surface to nearly 50 m depth (Fig. 10). This indicates that less–productive surface water along this section is mainly caused by the flushing of nutrient–poor, high salinity, and relatively warm water (Figure A1), which is dragged across the area by the EACC (Fig. 9). However, high Chl-*a* values recorded at stations close to the coast are supported by both the hydrographic section (Fig. 10) and surface Chl-*a* from satellite data (Fig. 7).

Information on the vertical distribution pattern of Chl-*a* in Tanzanian coastal and marine waters is limited, partly due to the absence of reliable data from within the water column. Missions such as the RV Algoa cruise and the second International Indian Ocean Expedition conducted in the Western Indian Ocean provided the CTD profile data used in this study. The results show that the EACC affects Chl-*a* values in the area between longitude 39.9°E and 40.2°E. These low Chl-*a* values are caused by flushing of nutrient-poor, high salinity, and high temperature water pulled across this area by the EACC. The study also found that the NE had higher Chl-*a* value than the SE monsoon season which was different from other studies in Tanzania. But when considering only the surface of the ocean, as was done in previous studies, then the SE had higher concentrations than NE. Also, the satellite surface Chl-*a* showed higher concentrations in the SE as compared to the NE monsoon season. Although the CTD data from different missions have helped research into the study of physical, chemical and biological variability of the water column in the ocean, continuous data collection is missing. This information could help in understanding short and long-term changes of the ocean throughout the water column.

The upper ocean is characterized by a quasi-homogeneous layer where temperature, salinity and density are almost constant with increasing depth (Costoya *et al.*, 2014). This homogeneity is caused by turbulent vertical mixing that is driven by heat loss from the ocean to the atmosphere, as well as by wind stress (Stranne *et al.*, 2018). The deepest layer affected by this turbulent mixing is called the mixed layer depth

(MLD), which marks the section of the upper ocean that interacts with the atmosphere (Kelly and Richards, 2018). The MLD influences the exchange of heat and gases between the atmosphere and the ocean and constitutes one of the major factors controlling ocean primary production as it affects the vertical distribution of biological and chemical components in near-surface waters. Estimation of the MLD are often made by means of conductivity, temperature and depth (CTD) casts (Stranne *et al.*, 2018). However, different techniques are used and there is little agreement on which technique is the best for estimating MLD (Kelly and Richards, 2018).

Acknowledgements

We are grateful to the second International Indian Ocean Expedition and the RV Algoa cruise for giving us permission to use their data. We are thankful to the MODIS for providing the satellite SST and chlorophyll-*a* data used in this study. We are also grateful to the Global Drifter Program for allowing us access to the drifter data that was used to calculate the ocean surface currents. The QuikSCAT is highly acknowledged for the permission to access the surface wind data.

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Appendix

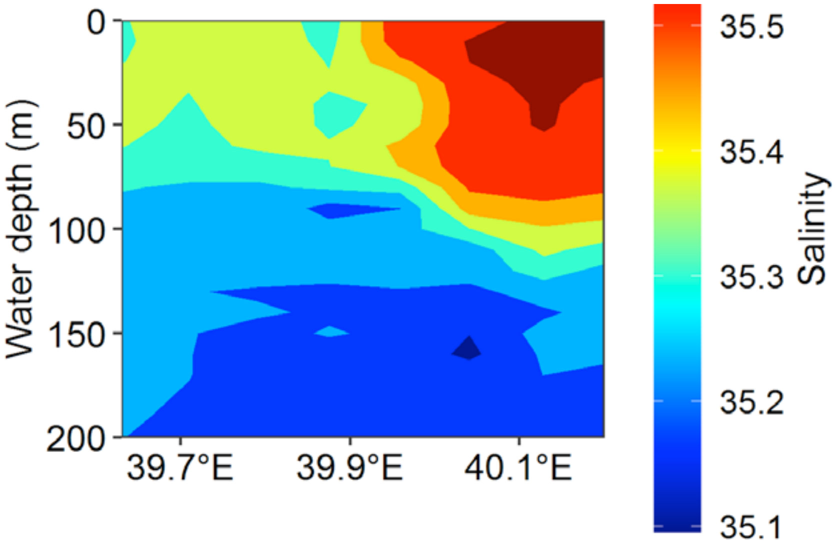

Figure A1. Hydrographic section showing the salinity in the water column along the transect off Kimbiji.

Efficacy of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on growth improvement of hybrid Nile and Rufiji tilapia populations

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Abstract

An investigation of the role of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on growth performance of hybrids from *Oreochromis niloticus* and *Oreochromis urolepis urolepis* was carried out in plastic tanks at 10 fish/m^3 density and a salinity of 25 units. The probiotic treatments were 2, 4 and 6 g/kg feed while the control feed did not include any probiotics. Growth parameters were measured fortnightly and water quality parameters monitored every day. Survival rate and water quality factors revealed non-significant variations ($p > 0.05$). Final weight differed significantly among the treatments ($p < 0.05$). Furthermore, Food Conversion Ratio (FCR), Specific Growth Rates (SGR), and weight gain did not differ significantly ($p > 0.05$) between the control and 2 g/kg treatments. However, the 4 and 6 g/kg feed treatments showed significant differences ($p < 0.05$) from the control diet and 2 g/kg for both *L. plantarum* and *S. cerevisiae*. The condition factor did not show any significant difference ($p > 0.05$) among the treatments. Results of this study indicate that increasing the quantity of commercial probiotics in feeds improved growth rates. Condition factor observed in the treatments could be due to the role of the tested probiotics in improving the water quality of tanks. Therefore, probiotics could potentially be used to enhance coastal aquaculture development.

Keywords: probiotics, growth performance, salinity, hybrids, *Oreochromis* species

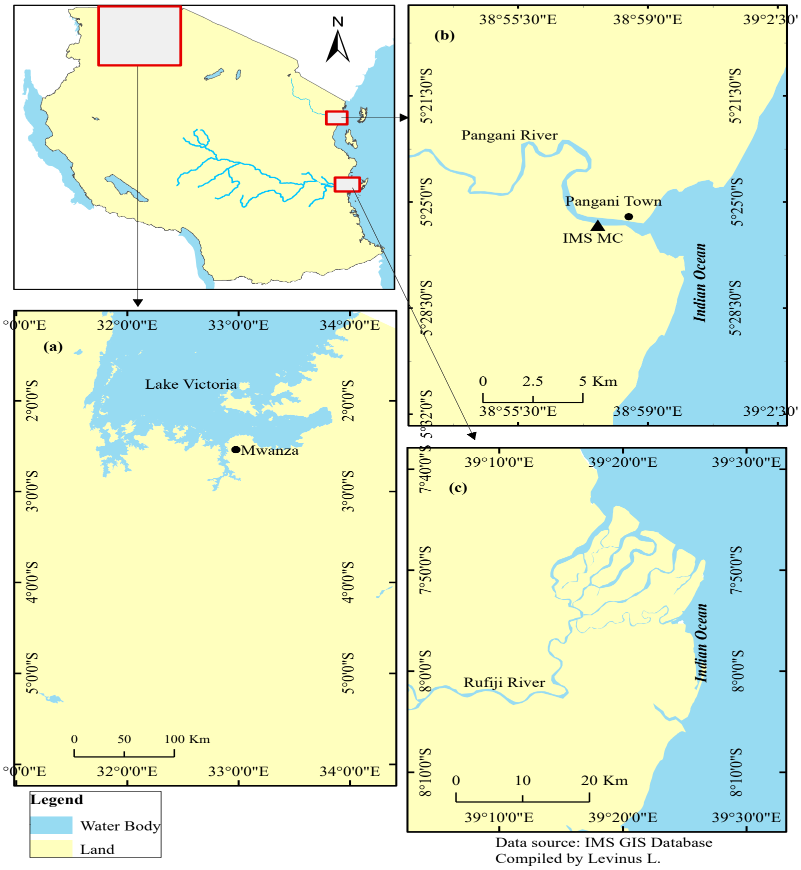
Introduction

Global fish production from aquaculture requires different farming techniques to achieve the goal of high productivity. One of the important aspects recently considered to improve aquaculture production is the application of food additives, particularly probiotics. The contribution of probiotics to global aquaculture production was noticed in the culture of Mozambique tilapia and Nile tilapia (Gobi *et al.*, 2018; Al-Deriny *et al.*, 2020). These authors reported improvement of growth performance and immunity when tilapia were fed probiotics. Involvement of probiotics in aquaculture production in Africa is based on experiences from Egypt. Ibrahem (2015) reported on the status of probiotic use in Egypt's aquaculture. An investigation on the effect of probiotics on growth performance, feed efficiency and immune response in Nile tilapia culture was also conducted in Egypt (Dawood *et al.*, 2020). Little work has been carried out to improve the understanding and use of probiotics to enhance aquaculture production in East Africa, including in Tanzania.

Probiotic additives are live microbes that are fed to fish and thought to improve intestinal microbial balance and fish growth (Wang *et al.*, 2005). When these live microorganisms are properly provided in recommended volumes they may result in a healthier host

http://dx.doi.org/10.4314/wiojms.v20i1.4

(FAO and WHO, 2002). Probiotics have the ability to inhibit pathogenic bacteria, and improve both water quality and immune response of host species (Verschuere *et al.*, 2000; Carnevali *et al.*, 2006), although a study by Khatun and Saha (2017) indicated that probiotics have no significant influence on water quality. Furthermore, probiotics produce digestive enzymes which improve fish nutrition (Carnevali *et al.*, 2006), provide disease resistance and anti-carcinogenic activity (Wang, 2007). The influence of probiotics on growth and disease resistance in *Oreochromis niloticus* has been discussed by several authors (e.g. Verschuere *et al.*, 2000; Carnevali *et al.*, 2006; Kim *et al.*, 2010). Probiotics differ from pre-biotics in that they add new microbes to the gut whereas the latter act as fertilizer to the important bacteria in the gut. *L. plantarum* and *S. cerevisiae* showed improved growth, enhanced enzymatic activity, increased stress resistance and feed utilization in *O. niloticus* (Maurilio *et al.*, 2002; Essa *et al.* 2010). It has been documented that probiotics can be important for pathogenic control in aquaculture systems (Cruz *et al.*, 2012). Therefore, probiotics are non-pathogenic and non-toxic (Kherraz *et al.*, 2012).



Data source: IMS GIS Database
Compiled by Levinus L.

Figure 1. Map of Tanzania showing (a) Lake Victoria, (b) Pangani, and (c) Rufiji River. The study site was at Pangani, while broodstock were collected from Lake Victoria and the Rufiji River.

Tilapia production faces challenges in a mixed culture condition of male and female fish. All-male fish populations are believed to have better growth performance than mixed sex culture (Banerjee and Chakraborty, 2010). Also, they control unnecessary reproduction (Mensah *et al.*, 2013), prevent stunted growth and produce fish with reliably uniform market size. Manual sexing, genetic manipulation, temperature influence in rearing facilities, hormonal reversal (Silva, 2013) and hybridization have been used in monosex tilapia production. Testosterone hormone is widely used for sex reversal of tilapia fry. Yet, both hormonal reversal and use of antibiotics have raised public worries. Dethlefsen and Relman (2011) argued that antibiotics may kill beneficial microbes in the gastrointestinal tract and have hence received injunctions in many countries. Alternatively, application of probiotics and natural all-male hybrids are encouraged in fish farming. However, little is known on the influence of commercial *L. plantarum* and *S. cerevisiae* to growth performance in tilapia hybrids. Therefore, this study was conducted to determine the influence of commercial probiotic feed supplements on the growth of all-male tilapia hybrids in brackish waters. The probiotics used were *L. plantarum* (bacteria) and *S. cerevisiae* (yeast). The hybrids were progenies of the hybridization between female Nile and male Rufiji tilapia populations. The hybridization between Nile and Rufiji tilapia populations produces 100 % male fish (Mapenzi and Mmochi, 2016).

Materials and methods

This study was conducted at the Institute of Marine Sciences Mariculture Center (IMS-MC) of the University of Dar es Salaam, located at Pangani, Tanga Region in Tanzania. Two experiments with two probiotics and three levels of diet treatments were conducted at IMS-MC. A total of 210 fish hybrids were stocked into 1000 L white plastic tanks at densities of 10 fish/m^3. In the first and second experiments, the symbols SC$_{1\text{-}3}$ and LP$_{1\text{-}3}$ were used to denote *S. cerevisiae* and *L. plantarum* at three treatments 2, 4 and 6 g/kg feed, respectively. The hybrids were raised at a salinity of 25 units which is considered optimum for hybrid growth (Mapenzi and Mmochi, 2016b). They were acclimated to the salinity for 13 days with an increase of 2 units per day and 1 unit on the last day. Each treatment was replicated three times making a total of 21 tanks including controls. *L. plantarum* and *S. cerevisiae* were added to basal feeds at 2, 4 and 6 g/kg feed respectively. The 40 % crude protein feed consisted of cassava flour, maize flour, shrimp and fish meals, sunflower seed cake and premix. Feed formulation was carried out by weighing each component followed by thorough mixing. Feed pellets were prepared using a meat mincer machine. Hybrids were fed at 5 % body weight twice a day. Feeding adjustment was effected depending on fish biomass increment. Control groups were not fed diets with probiotic additives.

Fish sampling using a locally made scoop net was carried out by taking any five individuals from each tank after every two weeks. The fish were sedated using a 2 ml clove oil dose in 20 L of water prior to measurements, and were returned into tanks soon after measurements that took a maximum of 10 minutes. Total weight and length of each hybrid were measured using a BOECO BEB 61 digital balance and a wooden measuring board respectively, for determination of the length-weight relationship. The hybrids were kept unfed for 24 hours prior to sampling to reduce metabolic activities that would increase stress on the fish. Weekly replacement of 20 % of the water and siphoning of water sediments twice a week were practiced to control water quality in tanks. The pH and temperature were measured using a HI8424 pH meter, salinity with a DMT-10 Digital Hand-Held Marine Tester, and Dissolved Oxygen (DO) with a PDO-520 Dissolved Oxygen Meter each morning and evening. This study took 63 days for completion.

Analytical design

Growth performance of hybrids

The following fish growth parameters were established to determine the growth performance of the hybrids:

Specific Growth Rate (% day^{-1}) = $\left[\frac{ln\,Wf - ln\,Wi}{T}\right]$ x 100.......1

Food Conversion Ratio (FCR) = $\frac{Total\ feed\ intake\ by\ fish\ (grammes)}{Total\ weight\ gain\ by\ fish\ (grammes)}$ 2

Survival Rate (%) = $\left[\frac{Nf}{Ni}\right]$x 100...3

Weight Gain (g) = $W_f - W_i$..4

Where T is the number of days of the experiment, W_i and W_f are the initial and final mean body weights, and N_f, N_i are the numbers of harvested and stocked fish, respectively.

The coefficient of condition $(K) = \frac{W}{L^3}$ x 100 (Pauly, 1983 5

Where W = Weight of individual fish (g), L = Total length of individual fish, K = condition factor

Length-weight relationship was calculated as $W = a\,L^b$ (Ricker, 1978..6

which was transformed into common logarithm as $\log W = \log a + b.\log L$ 7

Where W = Weight of fish in gram (g), L = Total length of fish in centimeters (cm), a = proportionality constant, b = the value obtained from the length-weight equation/coefficient of regression.

Statistical analysis

Data on fish growth were pooled for each probiotic treatment. The homogeneity of variance and distribution of data were tested using Levene's and Kolmogorov–Smirnov tests respectively. The data were normally

Table 1. Average water quality parameters (SC$_{1-3}$) denotes *S. cerevisiae* supplemented feeds at 2, 4 and 6 g/kg levels respectively).

	Probiotic Levels g/kg Feed			
Parameters	**Control**	**SC1**	**SC2**	**SC3**
Temp (°C)	27.28±0.12	27.23±0.12	27.21±0.12	27.25±0.12
DO (mg/L)	6.33±0.05	6.29±0.05	6.2±0.05	6.27±0.05
pH	7.29±0.05	7.2±0.03	7.18±0.03	7.2±0.04

Table 2. Average water quality parameters (LP$_{1-3}$) represents *L. plantarum* supplemented feeds at 2, 4 and 6 g/kg levels respectively).

	Probiotic Levels g/kg Feed			
Parameters	**Control**	**LP1**	**LP2**	**LP3**
Temp (°C)	27.28±0.12	27.17±0.12	27.24±0.12	27.31±0.12
DO (mg/L)	6.33±0.05	6.45 ±0.05	6.39±0.03	6.26 ±0.06
pH	7.29±0.05	7.27±0.03	7.31±0.03	7.2±0.04

distributed and showed similarity of variance. Therefore, one-way ANOVA was conducted to analyze the data in Origin 9 software. The probiotic experiments were conducted separately hence the need for one-way ANOVA analysis. The Tukey test ($p < 0.05$) was used to identify the groups that showed significant differences. A t-test was conducted to determine the conformation of hybrids to the cube law. The length-weight relationship and condition factor (K) were calculated, and feed conversion efficiency determined.

Results

Water quality parameters

There was no significant difference in temperature observed among all treatments ($p > 0.05$, Tables 1 and 2). Furthermore, DO and pH did not differ significantly among treatments ($p > 0.05$, Tables 1 and 2) despite a slightly higher DO at LP$_1$. Water quality in the controls did not show any significant difference. In that regard, all water quality parameters were in acceptable ranges for fish farming.

Table 3. Results of one-way ANOVA for growth parameters of hybrids treated with *S. cerevisiae*.
^{2}Superscripts "*a, b* and *c*" in a row indicate significant variations among treatments with control inclusive ($p < 0.05$, Tukey test). ^{1}The SC$_{1-3}$ levels refer to 2, 4 and 6 g/kg of *S. cerevisiae*.

	Probiotic levels g/kg Feed				
Parameters	**Control**	**SC1**	**SC2**	**SC3**	**P**
Initial mean weight (g)	16.85±0.15^{a}	15.61±0.16^{a}	15.37±0.15^{a}	17.17±0.18^{a}	0.07
Final mean weight (g)	58.03±2.9^{a}	58.5±2^{a}	68.05±2.5^{b}	72.85±1.9^{c}	0.01
Weight Gain (g)	42.11±4.36^{a}	42.26±4.45^{a}	51.58±4.79^{b}	53.86±4.18^{b}	<0.001
Survival rate (%)	90±0.2^{a}	91±0.8^{a}	93±0.8^{a}	94±1.7^{a}	0.1
FCR	1.2±0.08^{a}	1.2±0.08^{a}	0.73±0.06^{b}	0.68±0.04^{b}	0.02
SGR (%/day)	2.0±0.14^{a}	2.1±0.14^{a}	3.2± 0.13^{b}	3.5± 0.11^{b}	0.02

Table 4. One-way ANOVA hybrid growth parameters results when treated with *L. plantarum.* [2]The superscripts "*a*, *b* and *c*" across rows specify significant differences among treatments ($p < 0.05$, Tukey test). [1]The levels LP$_{1-3}$ refer to of 2, 4 and 6 g/kg treatments.

	Probiotic levels g/kg Feed				
Parameters	**Control**	**LP$_1$**	**LP$_2$**	**LP$_3$**	**p**
Initial mean weight(g)	16.85±0.15^{a}	15.99±0.16^{a}	16.47±0.19^{a}	18.99±0.17^{a}	0.06
Final mean weight (g)	58.03±2.9^{a}	59.19±1.7^{a}	69.52±2.4^{b}	75.03±1.7^{c}	<0.001
Weight Gain(g)	42.11±4.36^{a}	41.59±3.82^{a}	54.15±4.7^{b}	57.86±4^{b}	0.03
Survival rate (%)	90±0.2^{a}	92±1.6^{a}	93±1.7^{a}	93±0.8^{a}	0.06
FCR	1.2±0.08^{a}	1.9±0.07^{a}	0.94±0.63^{b}	0.66±0.03^{b}	0.01
SGR (%/day)	2.0±0.14^{a}	2.08±0.12^{a}	3.44±0.13^{b}	4.2±0.1^{b}	0.02

Growth performance

Initial mean weight did not differ significantly among the tested feeds ($p > 0.05$, Tables 3 and 4). Final average weight showed a significant variation in both probiotics ($p < 0.05$, Table 3, 4). The *S. cerevisiae* 2 g/kg feed final weight and the control differed significantly from the 4 and 6 g/kg feed, respectively (Table 3). Similarly, for *L. plantarum* the 4 and 6 g/kg treatments showed significant deviation from the 2 g/kg and control (without probiotics) treatments in terms of average final weights (Table 4). The weight gains at 4 and 6 g/kg treatments were observed to differ significantly from 2 g/kg and the control in *S. cerevisiae,* respectively (Table 3). Despite slightly higher values in survival rates for the 4 and 6 g/kg treatments in *S. cerevisiae* and *L. plantarum,* survival was not significantly affected by treatment diets, including control (Table 3, 4). Hybrid weight increment after treatment with *S. cerevisiae* and *L. planatrum* are shown in Figures 2 and 3 respectively.

Length-weight relationship (LWR)

The hybrid length-weight relationship (LWR) showed negative allometric growth among all treatments (Figs. 4 and 5). Results of the present study indicate that hybrids fed on *S. cerevisiae* and *L. plantarum* probiotics showed allometric growth. Their "b" values were 2.4 for all *S. cerevisiae* treatments while the values ranged from 0.4-2.4 across *L. plantarum* treatments.

Condition factor (K)

The average "K" values were similar across all *S. cereviasae* treatments, ranging from 2.1-2.2. The values ranged from 2.2-2.5 for *L. plantarum* treatments. The control experiment showed clear similarity to *S. cereviasae* for average "K" values. None of the values

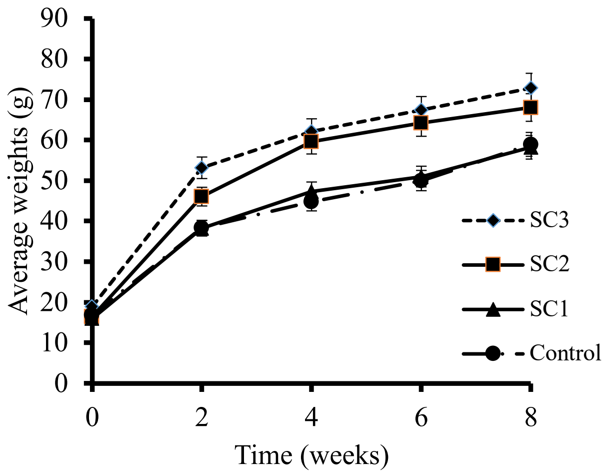


Figure 2. Weight increase of hybrids treated with *S. cerevisiae* probiotics. SC1-SC3 refers to 2, 4 and 6 g/kg, while Control denotes 0 g/kg.

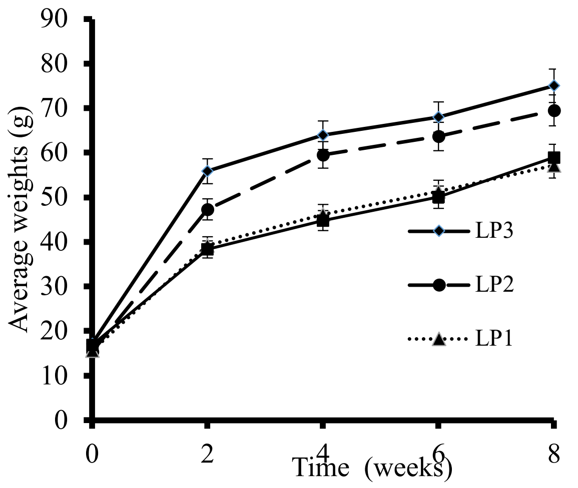


Figure 3. Weight increase in hybrids treated with *L. plantarum* probiotics. LP1-LP3 refers to 2, 4 and 6 g/kg, while Control denotes 0 g/kg.

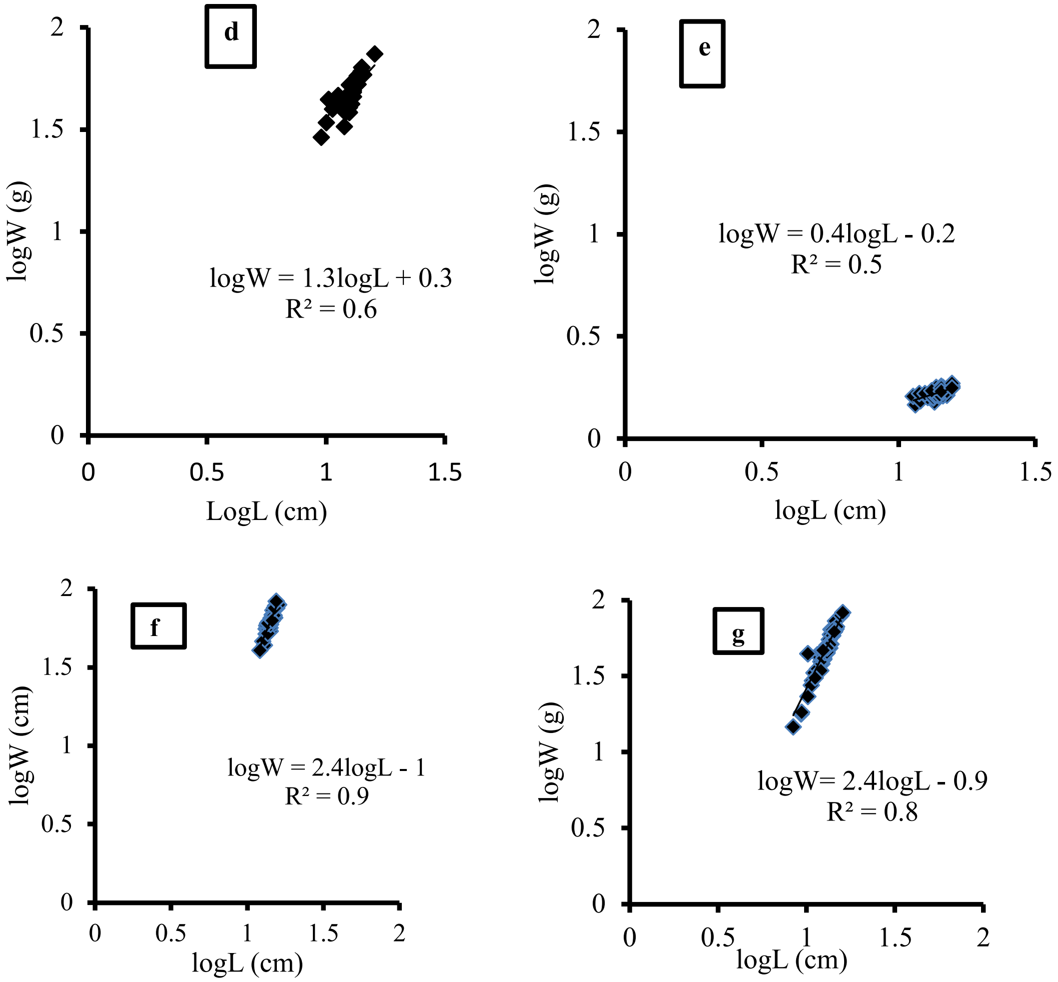


Figure 4. LWR of hybrids treated with (d) 2g, e 4g, and (g) 6 g/kg of *L. plantarum*, and (f) control, respectively.

showed any significant variation within and among treatments (Table 3). The "b" values were not significantly different from the isometric 3 value ($p > 0.05$, t-test).

Discussion

Administration of probiotics as a fish dietary supplement may be a strategy to improve immune response and growth performance. The present study has investigated the latter, which seems to vary with both probiotic and target species. A study on the influence of *S. cerevisiae* on *O. niloticus* has, for example, shown improved growth performance (Osman *et al.*, 2010; Welker and Lim, 2011; Ozório *et al.*, 2012).

However, Abraham *et al.* (2008) reported a negative influence of commercial probiotics on the ornamental fish, *Carassius auratus*. Results of the present study agree with Osman *et al.* (2010) who similarly reported a significant increase in growth performance (de Rodrigáñez *et al.*, 2009) and stimulation of FCR and weight gain in *O. niloticus* fed on higher levels of *S. cerevisiae*. In this study, the best final mean weight, specific growth rates (SGR), weight gain, survival rates and FCR were observed at 6 g/kg of both *S. cerevisiae* and *L. plantarum* while control groups had the lowest growth. Regarding probiotic roles in influencing growth, Tovar *et al.* (2002) associated yeast inclusion in fish feeds with enhancement of amylase secretion in the fish gut. Tawwab *et al.* (2008) links the live yeast addition to fish feeds with improved protein and diet digestion as well as feed utilization.

Probiotics seem to improve digestion through production of digestive enzymes (Welker and Lim, 2011). It is possible that *L. plantarum* and *S. cerevisiae* in the present study enhanced the hybrid growth performance through similar mechanisms. Intestinal digestion and absorption enhancement depend on provision of digestive enzymes like proteases and lipases (Ramirez and Dixon, 2003). Therefore, enhanced FCR and fish growth may be due to increased appetite and improved feed digestibility (Osman *et al.*, 2010). The same may have

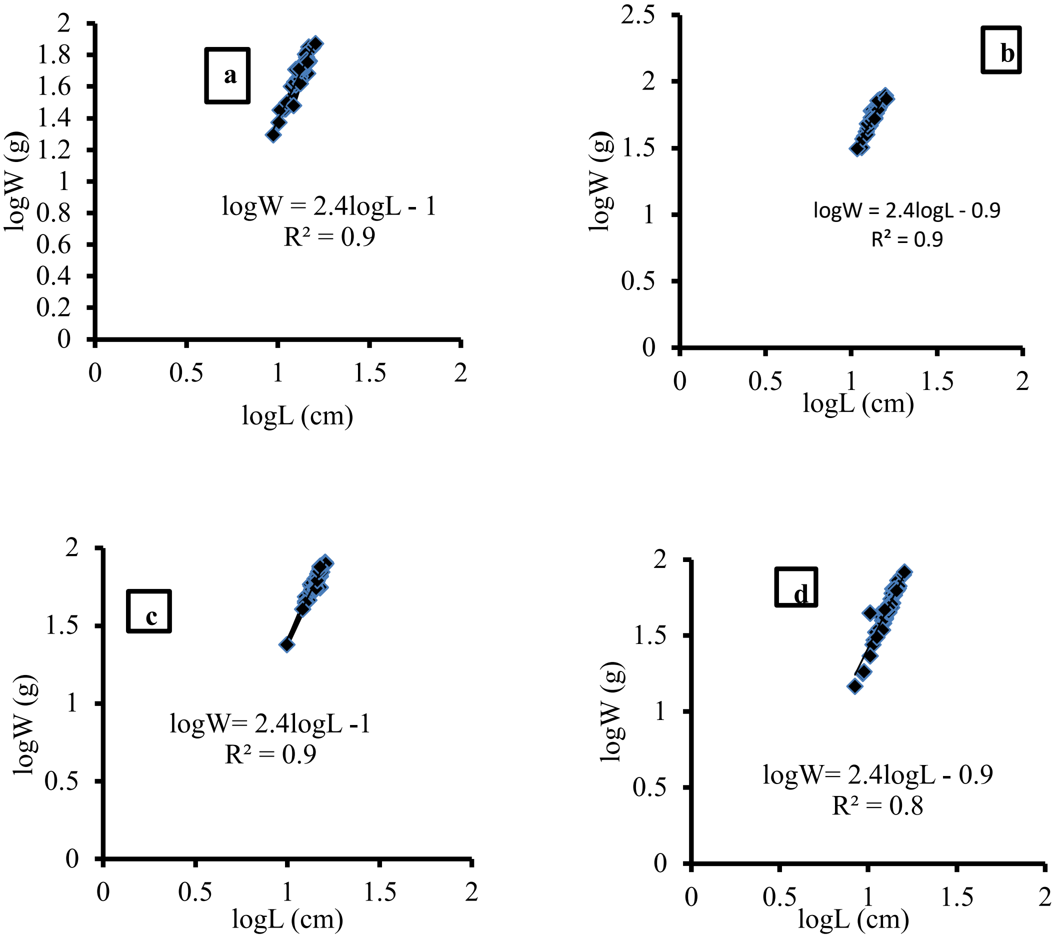


Figure 5. LWR of hybrids treated with (a) 2g, (b) 4g, (d) 6 g/kg of *S. cereviasie*, and (c) control, respectively.

accounted for the results of the present study where hybrids where fed on *L. plantarum and S. cerevisiae.* Improved hybrid growth may also be due to production of vitamins, dietary detoxification and breakdown of complex feed components (Irianto and Austin, 2002).

Talpur *et al.* (2013) showed that *L. plantarum* improves water quality in crab farms. The findings concur with the present results in which water quality parameters were in supportive ranges for fish growth, perhaps as a consequence of the presence of *L. plantarum* and *S. cerevisiae* probiotics. Nevertheless, probiotics did not lead to significant environmental quality improvement in brackish water used to rear monosex Nile tilapia (Khatun and Saha, 2017). The inhibitory capacity of *Bacillus* species (Maia *et al.*, 2013) and yeast (Lukwambe *et al.*, 2015) probiotics to harmful bacteria and algae development have been shown in shrimp ponds. The same might have led to superior growth performance of hybrids fed on *L. plantarum and S. cerevisiae* probiotics in tanks in this study.

A significant influence of both *L. plantarum* and *S. cerevisiae* was realized in SGR, FCR, weight gain and average final weight among the treatments. These results correspond to Tawwab *et al.* (2008) who reported the significant effect of *S. cerevisiae* supplemented feeds on weight gain and SGR in tilapia. Conversely, the treatments did not vary significantly from each other at 4 and 6 g/kg feed levels. SGR rates improved as *L. plantarum* and *S. cerevisiae* levels were raised in the feeds. This suggests that the SGR of hybrids was dependent on levels of probiotic supplements in feeds. Therefore, both *L. plantarum* and *S. cerevisiae* may have provided similar growth promoting factors to hybrids in this study. Consequently, *S. cerevisiae* can be used as a replacement to both live food and fish meal (Oliva-Teles and Gonçalves, 2001) to improve SGR of the hybrids.

FCR varied significantly between controls, and the 4 and 6 g/kg feed levels for both *L. plantarum* and *S. cerevisiae* respectively. Similar results were documented for tilapia fed on 40 % crude protein diets supplemented with *S. cerevisiae* (Flores *et al.*, 2010). Similarity in protein content of the basal feed in this study may also explain significance in FCR for the hybrids. Live yeast supplementation enhances food utilization and nutrient decomposition in the gastro-intestinal tract (Wang and Xu, 2006; Tawwab *et al.*, 2008), and improves protein digestion (Flores *et al.*, 2003) in tilapia. Furthermore, dietary probiotic additives supply essential nutrients (Welker and Lim, 2011). All these factors could explain the better FCR obtained for hybrids in the present study, as *L. plantarum* could be working through similar mechanisms as *S. cerevisiae*. However, there was no significant influence of the 2 g/kg feed which indicates that this level may be too low to have any effect.

Studies by Flores *et al.* (2003) and Mohammadi *et al.* (2016) showed that yeast probiotics improve survival in both hybrid striped bass and Nile tilapia. The findings corresponded with the results of the present study where survival increased with an increase in *L. plantarum* and *S. cerevisiae* levels. Similar survival rates (90.67±1.15 %) were reported for *Penaeus monodon* in Bangladesh (Hossain *et al.*, 2013).

LWR studies are used to indicate fish wellbeing and how the fish interacts with its environment (Mansor *et al.,* 2012). In addition, these studies are widely used in fisheries management because they can contribute to explaining stock condition. Further, the "b" value in the regression equation specifies heavy, light and isometric fish growth (Ricker, 1973; Mansor *et al.*, 2010) where "b" < 3 and "b" > 3 experience both negative and positive allometric growth (Weatherley and Gill, 1987; Mansor *et al.*, 2010), respectively. According to Mansor *et al. (*2012), the condition factor "K" indicates favourability of the environment in which the fish reside. Therefore, higher "K" values indicate that the environment is more favourable for fish rearing.

Results of the "b" values reported for Nile tilapia (Offem and Omoniyi, 2007) in Nigeria were similar to results from the present study, which showed negative allometric growth (Ricker, 1973; Silva *et al.*, 2015). However, they differ from the values of 2.908 (Britton and Harper, 2008) and 3.415 (Ahmed *et al.,* 2011) for Nile tilapia from Atbara River and Khashm El-Girba reservoir. The difference may be due to geographical location variances. In addition, hybrids were reared in saline water tanks in the present study. Hybrids of Nile tilapia♀ and Rufiji tilapia♂ grow well in all water salinities ranging from 0-35 units with the best growth rates between 15-25 units (Mapenzi and Mmochi, 2016b). Negative allometric growth means their bodies did not grow proportionally (Silva *et al.*, 2015). The allometry may also be affected by differences in oxygen supply in tanks. Moreover, fish with b < 3 are slender (Silva *et al.*, 2015) and have extended bodies (Otieno *et al.*, 2014). This was observed for the hybrids in the present study in which the "b" values for fish fed with *S. cerevisiae* and *L. plantarum* were below the isometric value of 3. Therefore, the cube law was not obeyed by the hybrids; similar to the results of Otieno *et al.* (2014).

The "K" values of the hybrids in this study are similar to the ranges of 1.97-2.38 for males and 2.02-2.63 for females found in Nile tilapia in Lake Naivasha, Kenya (Otieno *et al.*, 2014). Ighwela and Ahmed, (2011) recommended that "K" values greater than 1 indicate that the fish are in good condition. Furthermore, good "K" values can be attributed to better feeding intensity (Ndimele *et al.*, 2010) and water quality parameters (Musa *et al.*, 2016). This can also explain better "K" values for hybrids obtained in this study.

Conclusions

The inclusion of *L. plantarum* and *S. cerevisiae* had a significant influence on growth performance of the hybrids in the present study. The probiotic dietary supplementation may constitute a valuable approach for enhancing better fish growth in both semi-intensive and intensive farming systems where stress related diseases and fish losses are prevalent.

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First evaluation of coral recruitment in Madagascar

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Abstract

The distribution of larvae and recruitment of scleractinians in the southwest region of Madagascar were evaluated for the first time between October 2013 and September 2014 at 3 sites. The presence of coral larvae (planulae) was monitored through weekly sampling using a plankton net and the recruitment rate evaluated by monthly sampling of the newly settled corals (<1 year stage) on recruitment tiles and by a monitoring of the recruitment of juveniles (1< Juveniles < 2 years) using the quadrat method. Planulae were present in the plankton for 9 months during the survey. The recorded mean annual density of planula varied from 0.43 ± 0.41 larvae m^{-3} to 3.23 ± 5.72 larvae m^{-3} depending on site, with a peak in larval density towards the end of November and the beginning of December. The variability in the occurrence of planula is very high and implied that the density observed in the year does not present a significant difference between the sites (p_{KW}=0.33). The average density of total recruits was 620.13 ± 621.30 recruits m^{-2}, 40.28 ± 50.97 recruits m^{-2} and 36.34 ± 33.82 recruits m^{-2}, respectively at the sites of Nosy Tafara, Grande Vasque and Rose Garden. Seasonal distribution of coral recruitment was different between the sites. The mean annual density of newly settled recruits (< 1 month stage) was significantly higher at Nosy Tafara with 94.91±101.08 recruits m^{-2} compared to Grande Vasque and Rose Garden with 18.75±34.32 recruits m^{-2} and 11.57±18.47 recruits m^{-2} ($p_{KW}<0.001$), respectively. The highest density of newly settled recruits was observed between October to December. Higher density of recruits was also observed in March at Nosy Tafara and in May at all three sites. Results of juvenile monitoring showed high rates (> 10 juveniles m^{-2}) compared to other regions and the threshold, but it revealed high mortality among recruits. Coral recruitment in the southwest region of Madagascar was found to be high and could result in increased resilience of the coral reef assemblages.

Keywords: recruitment, planula, recruits, juveniles, scleractinians, Madagascar

Introduction

Madagascar is part of the tropical Indo-Pacific Region where coral reefs constitute the typical coastal marine ecosystem (Pichon, 1978). The eastern part of the island, exposed to the Indian Ocean, is much less rich in coral reefs than the west, near the Mozambique Channel. In the west, coral reefs are mostly developed from Androka (in the south) bounded by the Linta River, to Antsiranana in the north. In the east, the coral reefs are distributed from Amber Cape (Antsiranana) to Toamasina. In total, coral reefs extend over approximately 1,400 km of coastline, in addition to coral banks and offshore shoals. Together, they cover an estimated area of 2,400 km² (Cook *et al.*, 2000).

The southwest (SW) region of the island (between the Manombo river in the north - latitude 22 ° 58 'S - and the Onilahy river - latitude 23 ° 34' S - in the south) is characterized by the presence of several types of coral reefs, which are divided into three zones from north to south: (1) Ranobe Bay which groups together barrier reefs and coral banks; (2) the area between the village of Ifaty and the Fiherenana river which is characterized by a long fringing reef; and (3) the Tulear Bay which is distinguished by the presence of the largest coral reef in the Indian Ocean, the Grand Récif de Tulear (GRT) behind which are several coral banks and the fringing reef of Sarodrano (Clausade *et al.,* 1971). In total, the region contains more than

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20 coral reefs; more than 10 in the Bay of Ranobe, one between Ifaty and the Fiherenana river, and 10 in the Bay of Tulear (Clausade *et al.,* 1971).

Coral reefs provide goods and services that are essential for the economy (tourism, fishing), society, ecology (protects coasts from damage caused by storms) and aesthetics (Woodhead *et al.*, 2019). This is important for coastal populations in the tropical Indian Ocean region (Moberg and Folke, 1999), including the coastal communities in SW Madagascar, who are particularly dependant to the reef resources (Mahafina, 2011). However, coral mortality has increased in recent decades (Prada *et al.*, 2017). The long-term survival of reef ecosystems is threatened by various anthropogenic stressors and global climate change (Hoegh-Guldberg, 1999). Over 50 % of the structure of coral reefs is directly threatened by human pressure (Wilkinson, 1999; Nyström *et al.*, 2000). Anthropogenic activities in coastal areas include overfishing, pollution of the sea and land runoff (Hughes *et al.*, 2003; Wilkinson, 2008), which greatly affects the reefs and causes change in the coral communities in Ranobe Bay and in Toliara Bay in SW Madagascar (Belle *et al.*, 2009, Todinanahary *et al.*, 2018). In addition to these pressures, climate change and associated warming also increases the vulnerability of the reefs (Hughes *et al.*, 2003; Hoegh-Guldberg *et al.*, 2007). In particular, the rise in seawater temperature is the main cause of coral bleaching events which lead to the degrading of the structure of the coral community (Hoegh-Guldberg, 1999).

Moreover, bleaching also reduces the productive capacity of coral populations (Szmant, 1991). Ward et al., (2000) mentioned that even corals that have recovered from bleaching no longer contained gametes and did not reproduce during the normal season. It should also be noted that juveniles are more sensitive to environmental disturbances than adults (Ward and Harrison, 1997). It is therefore particularly important to protect and restore this ecosystem and improve management before degrading effects cause its loss (Woodhead *et al.*, 2019). The resilience of the coral reefs is attributed to their ability to continue producing larvae (Richmond, 1997). Thus, understanding the ecological processes that influence this resilience is particularly essential and has become a high priority (Hughes and Tanner, 2000). Information on recruitment processes is important to understand the dynamics and resilience of coral reefs (Adjeroud *et al.*, 2016).

Scleractinian coral spawning is well documented in several regions of the world and is known to occur a few days after the full moons of the warm season. Mass spawnings were observed between October and November (spring) on the Great Barrier Reef and between March to April (autumn) on the Western Australian coast facing the Eastern Indian Ocean (Rosser and Baird, 2008). In the Western Indian Ocean (WIO), the first spawning studies were performed more recently. In South Africa and in La Réunion *Acropora austera* and *Platygyra daedalea* spawnings were observed in February (Massé, 2014). Monthly assessment of their sexual reproduction suggested an extended spawning period between September and March, with a recruitment period that covers the summer and winter seasons (Massé, 2014). In Madagascar, only the spawning of *Acropora* species were recorded from the coral reefs of Andavadoaka, in the SW region (Gress *et al.*, 2015).

Notable differences in spatial distribution of coral recruitment have been recorded at a regional scale, notably between Indo-Pacific and Atlantic reefs (Ritson-Williams *et al.*, 2010), and at a local scale within reefs or between sites of the same region. In the Pacific, the recruitment rate is much lower (e.g., ~40 recruits m^{-2} $year^{-1}$ at Mo'orea in French Polynesia (Adjeroud, 2007; Pénin, 2007) than in the Western Pacific reefs where ~200 to 700 recruits m^{-2} $year^{-1}$ and up to 4,590 recruits m^{-2} $year^{-1}$ were recorded (Hughes *et al.,* 1999). The recorded recruitment rates in the WIO are comparable to the higher rates, but have been proven to be higher in subtropical latitudes (548 recruits m^{-2} $year^{-1}$ in South African reefs) than in tropical latitudes (305 recruits m^{-2} $year^{-1}$, La Réunion Island) (Massé, 2014).

To date, few papers have reported on the distribution patterns of coral juveniles, despite the relatively developed observation methods (Baird *et al.*, 2006). For instance, Zahir *et al.* (2002) reported a juvenile (< 10 cm size) recruitment rate of 30 – 49 m^{-2} in the Maldives Islands. In Mayotte and the Glorieuses Islands, the observed juvenile recruitment rate was < 30 juveniles m^{-2} (< 5 cm size).

Limited resolution in the identification of recruits is one of the main constraints in coral recruitment monitoring. The methods used generally depend on the survey level. At the level of newly settled recruits, unglazed terracotta and ceramic tiles are the most widely used, while for the juveniles that are observable *in situ* (mostly < 5 cm size), the method of *in situ* quadrat is usual (Hill and Wilkinson, 2004). Based on morphological characteristics, recruits are only observable to the level of the 3 families Acroporidae, Pocilloporidae and Poritidae (Babcock *et al.,* 2003).

The present paper is the first to record scleractinians recruitment in Madagascar. It aims at characterizing the spatial and temporal distribution of coral larvae, recruits and juveniles off the coral reefs of the SW region, using the most recent survey methods and recruit identification techniques.

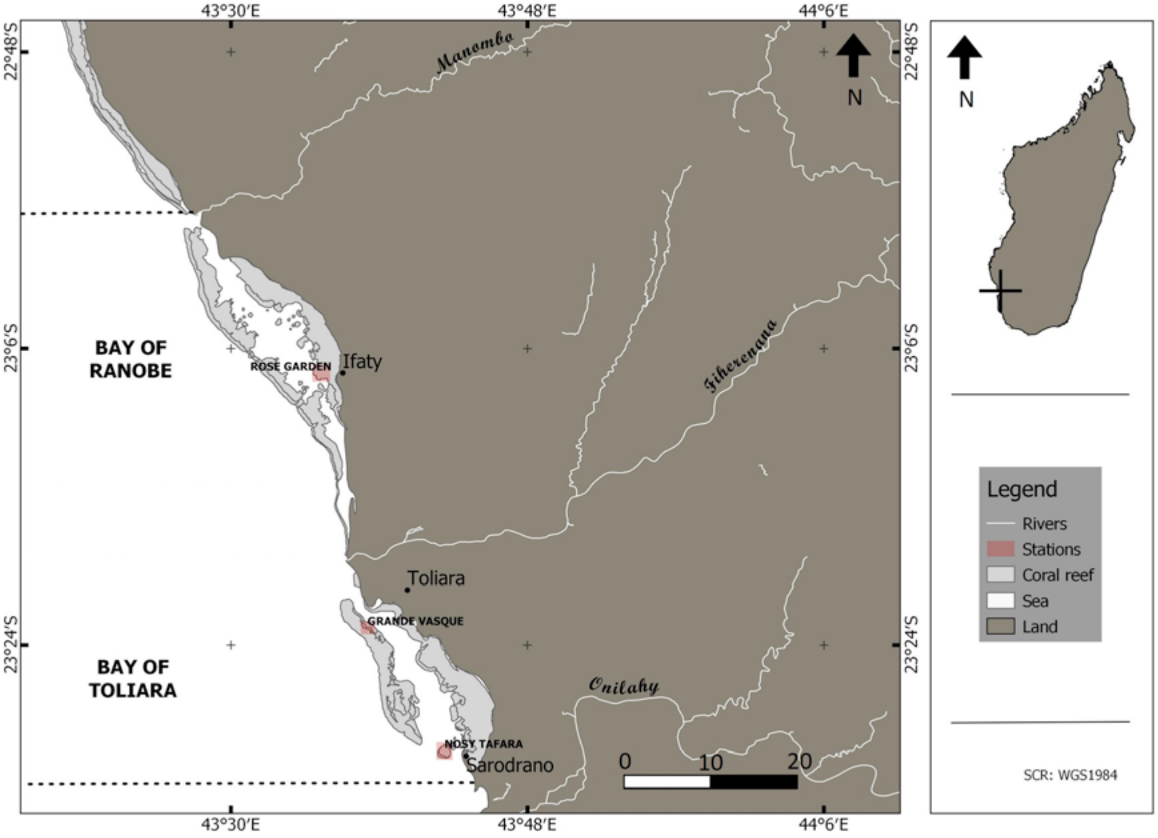

Figure 1. Location of the studied sites.

Materials and methods

Study area

The present study was performed on the coral reefs of SW Madagascar, between the latitude 22°58'S in the north and the latitude 23°34'S in the south (Fig. 1). Three distinct sites were chosen. The first site was located on the coral reef of Nosy Tafara (latitude 23°30'S), which is a complex of patch reefs located between the southern tip of the GRT and the sandy coast of Sarodrano village. The second site was located in the "Grande Vasque" (latitude 23°22' S), a basin of 1 km diameter situated in the flat of the GRT. These two reefs are located in the Bay of Toliara. The third site was located at the Rose Garden reef, in the Bay of Ranobe (latitude 23° 8' S), which is a reef patch dominated by *Montipora* species and is a locally managed protected area.

Monitoring of physicochemical parameters of the water

At each site, temperature of the bottom water, at the same depth that the recruitment tiles were situated (see *Recruitment monitoring* below) was measured every hour with a HOBO pendant temperature logger (©ONSET) from which data were downloaded and calculated using the ONSET HOBOware Pro version 3.7.0 (Onset Computer Corporation, 2002-2014). Salinity and water visibility were measured weekly using a refractometer and Secchi disc respectively.

Planula records

Weekly sampling of zooplankton was performed to record the planula larvae for one year from October 2013 to September 2014 at the 3 sites. Sampling was performed every week at the surface (< 1 m depth),

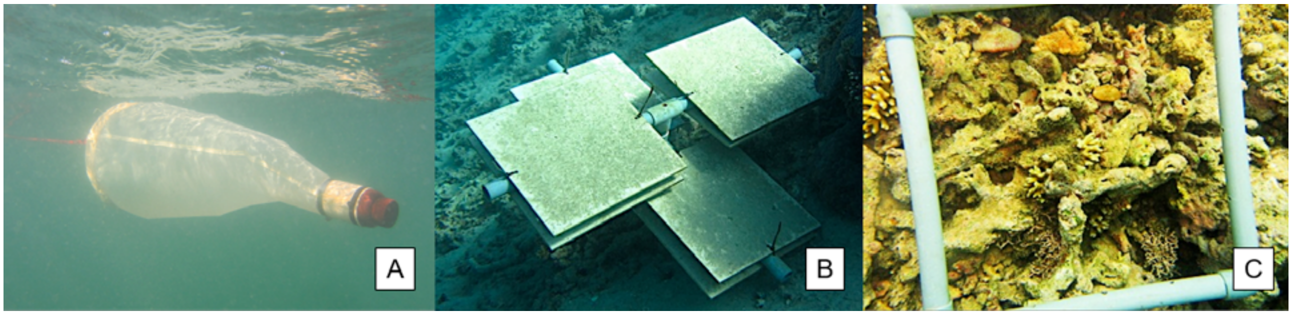

Figure 2. Equipment used for planula, recruit and juvenile sampling: A. 63 µm mesh plankton net with a 25 cm diameter mouth; B. Recruitment tiles setup on PVC cross-system, each tile measuring 30 cm x 30 cm; C. Recruitment quadrat of 25 cm x 25 cm.

between 6 am and 8 am, successively for the 3 sites. A 63 µm mesh plankton net was used with a 25 cm diameter mouth that was dragged by a small boat (7 m long) for 15 minutes, covering a distance of approximately 900 m. For each sample, the volume of filtered seawater was 44 m^3. Samples were filtered to separate the plankton of different sizes using sieves (63 µm, 80 µm, 100 µm and 250 µm). The filtration aimed at facilitating observation and counting of larvae within the sampled zooplankton. Eight replicates of 50 µl for each sieve size and each site were observed and coral larvae were identified and counted using an Euromex Oxion photonic microscope.

Recruitment survey

A total of 144 ceramic tiles measuring 30 cm x 30 cm (48 per site) were immersed to estimate the distribution of newly settled recruits. The tiles were installed in July 2013 over a total surface area of 4.32 m^2 at each site. About 3 months of conditioning are needed to allow the substratum to become suitable for settlement (Erwin *et al.,* 2008; Massé, 2014). Therefore, the one-year experiment started in October 2013 until September 2014. Four conditioned tiles were collected per site per month. The tiles were fixed horizontally, unglazed face down on a Polyvinyl chloride (PVC) cross-system (Fig. 2) after pre-research experiments that revealed the resistance of the system against waves and other conditions at the sites. Each system was fixed to the substrate with an iron rod treated with antirust, which was planted into the substrate.

In addition, *in situ* observation was performed to estimate coral juveniles at two of the three sites (Nosy Tafara and Grande Vasque). The survey was performed in September and October 2013 at Nosy Tafara, while at Grande Vasque it was performed in January 2014. Juveniles were not recorded at Rose Garden as it was considered a monospecific site with a dominance of *Montipora* species. Juveniles observable *in situ* are colonies of <5cm size, which are typically aged at least 1 year (Adjeroud *et al.*, 2016). The method described below provided a reliable estimation of future coral species assemblages and allowed estimation of the post-settlement rates of mortality that are often very high during the first weeks following the settlement, mainly due to predation, competition, sedimentation and stress (Adjeroud *et al.*, 2016). The recruitment quadrat method of Atlantic and Gulf Rapid Reef Assessment (AGRRA) (Hill and Wilkinson, 2004) was used and adapted for the characteristics of the studied sites. The length of the 50 x 2 meters transect was swum, and the 25 cm x 25 cm quadrats were placed on the substratum in areas lacking large (> 25 cm diameter) sessile invertebrates. Eighty quadrats per site were observed. All small scleractinians were counted (two classes: early juveniles with < 2 cm diameter and older juveniles with 2 cm < diameter < 5 cm) within the quadrats separated into four groups (Acroporidae, Pocilloporidae, Fungidae and "others"), grouping the families that were not reliably distinguishable during *in situ* observations.

Identification method

Once in the laboratory, each newly settled recruit was photographed directly on the tiles using a trinocular stereoscopic Novex microscope (Euromex microscopes). Based on the morphology of the first skeleton, recruits were identified to the level of the 3 distinguishable families of Acroporidae, Pocilloporidae and Poritidae using the scleractinian recruit identification key proposed by Babcock *et al.* (2003) and summarised in Table 1, and the unidentified recruits grouped as "others". In addition, recruits were classified by age: less than a month and more than a month, on the basis of the same criteria developed by Babcock *et al.* (2003). This allowed the recruitment rate per month to be assessed, which is difficult to perform with most of the recruitment counting methods considering the conditioning period of tiles (Erwin *et al.*, 2008; Massé, 2014). The morphological

Table 1. Summary of the morphological description of early stage recruits for 3 distinguishable families (after Babcock *et al.*, 2003).

	Acroporidae	*Pocilloporidae*	*Poritidae*
0-1 month	Basal plate,	Basal plate,	Basal plate,
	12 basal ridges in a single cycle,	Corallite wall formed through the growth and fusion of lateral outgrowths of the basal ridges,	Epitheca,
	Corallum grown by extension of the basal plate,	After 1 week: solid coenosteum,	6 primary septa thickened,
	Lateral processes evident on the inner end of basal ridges,		Prominent vertical toot
	Prominent laminar septa in 2 cycles,		
	A porous coenusteum,		
	Absence of columella,		
	Secondary corallites appeared within 3 weeks		
>1 month	Secondary corallites developed	Solid coenosteum	Corallite beyond the epitheca,
	Mound and small juvenile had yet to develop adult colony morphology	Prominent septa	Primary septa grown
		Prominent columella, Secondary corallites developed	2nd cycle of septa originated at the perimeter of the basal plate,
			Fusion of the secondary and the primary septa to form 4 pairs of laterals and a triplet leaving the directive independent
			Epitheca still visible within corallite,
			Presence of 2 corallites,
			Epitheca no longer visible,
			10 to 12 corallites.

characteristics of the observed early stages of corals are presented in the supplementary material.

Statistical analysis

All the statistical analyses were performed using the R software (R Core Team, 2017). Descriptive statistics were calculated first. For physicochemical parameters, the annual mean, the mean per season and mean per site, as well as the minimum and maximum values were calculated. For planula, recruits and juvenile records, annual and/or monthly mean density were calculated per site. Normality of the data was determined using a Shapiro-Wallis test and homogeneity of the variance was calculated using Levene's test. The significance (or not) of the difference in means was determined using ANOVA, the t-test or the Kruskal-Wallis test (KW), and the Nemenyi test, at a level of 5 %.

Results

Physicochemical parameters of the water

The water temperature varied from 17.8 °C during the dry-cold season to 32.4 °C during the wet-warm season. The highest temperature was recorded in January and February while the lowest occurred in July and

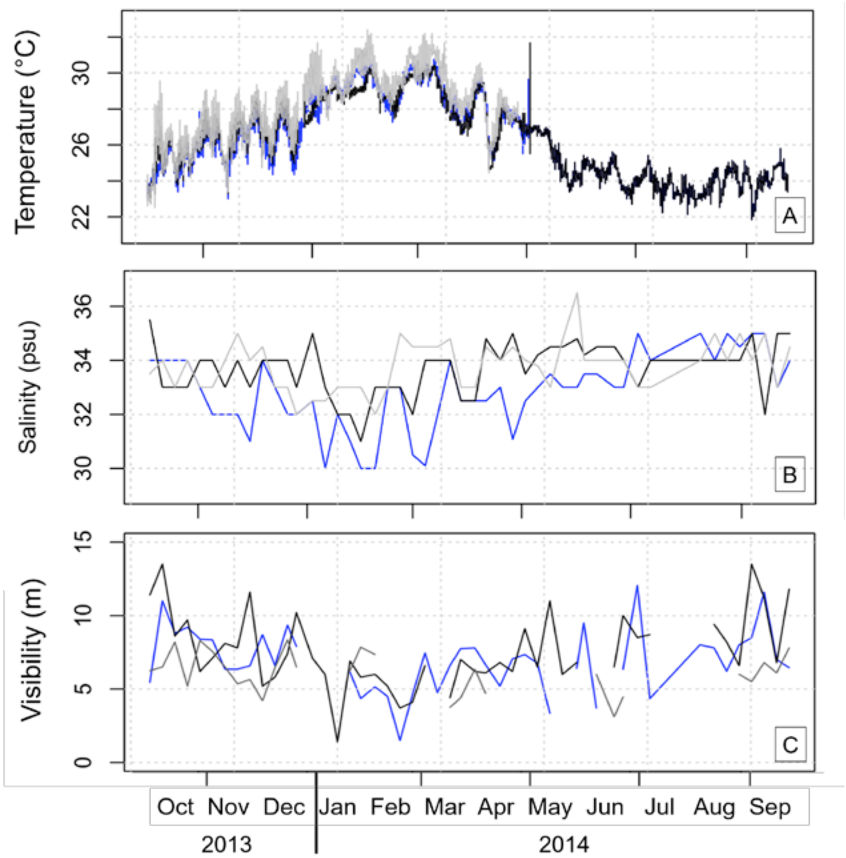

Figure 3. Variation of temperature, salinity and visibility (Secchi depth, in meter) in the water at the studied sites. A. Temperature at the bottom; B. Salinity; C. Weekly variation in visibility. Blue line: Nosy Tafara; Black line: Grande Vasque; Grey line: Rose Garden.

August (Fig. 3A). The mean annual temperature was 25.5 ± 2.3 °C. The mean temperature was significantly lower during the dry-cold season at 24.7 ± 1.3 °C compared to wet-warm season at 27.9 ± 1.6 °C (ANOVA, $p<0.001$). No significant difference in temperature was observed between the 3 sites (ANOVA, $p=0.793$).

The salinity varied from 30 psu to 36.5 psu with an average of 33 ± 1.3 psu in the wet-warm season and 34±0.8 psu in the dry-cold season. The salinity was significantly higher during the dry-cold season (ANOVA, $p<0.001$). The mean annual salinity was 33.4 ± 1.2 psu. The mean annual salinity was significantly lower at Nosy Tafara compared to the other sites (ANOVA, $p<0.001$) with 32.8 ± 1.4 psu at Nosy Tafara, and 33.7 ± 1 psu and 33.8 ± 0.9 psu respectively at Grande Vasque and Rose Garden. The lowest salinity occurred in the wet-warm season (Fig. 3B).

The water visibility varied from 1.4 m (lowest during wet-warm season) to 13.5 m (highest during dry-cold season). Results showed that the visibility was significantly lower

Table 2. Annual occurrence and density of planula (larvae m-3). Dark grey shading corresponds to occurrence but where the number could not be counted due to very low density. Cases filled with the light grey shading correspond to weeks where sampling could not occur due to bad weather or where samples were not usable.

	October 2013	November 2013	December 2013	January 2014	February 2014	March 2014	April 2014	May 2014	June 2014	July 2014	August 2014	September 2014
Nosy Tafara	■	13.4				0.37	0.26	0.34			■	1.80
Grande Vasque		0.43				0.24	0.02	0.34			■	1.10
Rose Garden		1.30										■

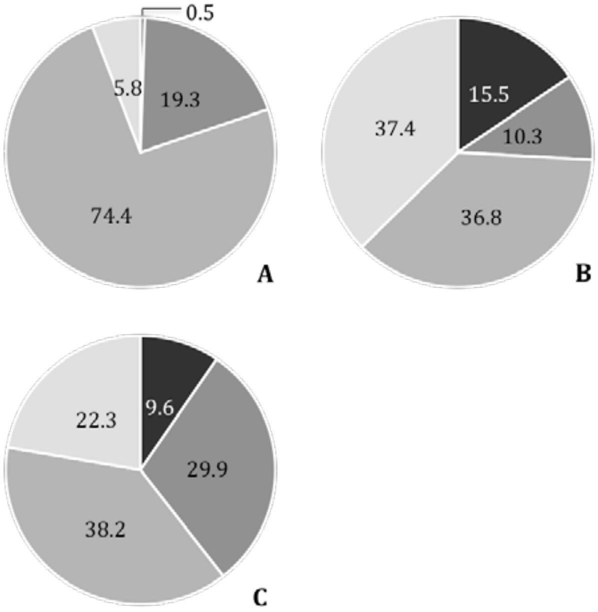

Figure 4. Dominance (%) of recruit families (all ages combined). A. Nosy Tafara, B. Grande Vasque, C. Rose Garden. Colours represent families. Black: Acroporidae; Dark Grey: Pocilloporidae; Grey: Poritidae; Light Grey: Others.

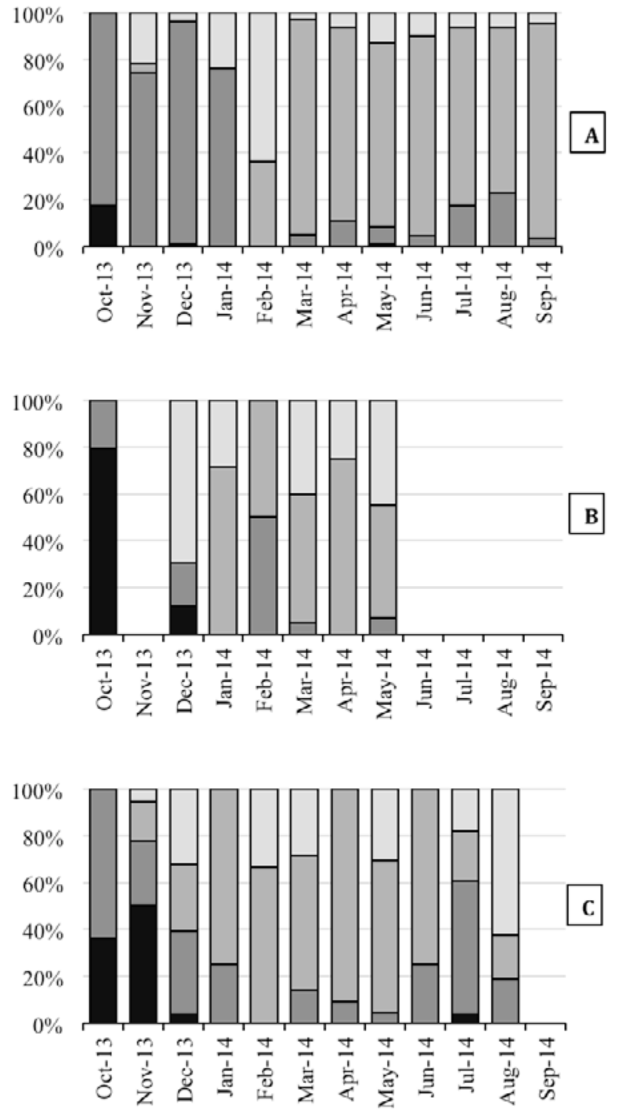

Figure 5. Dominance (%) of recruit families per month (all ages combined). A. Nosy Tafara, B. Grande Vasque, C. Rose Garden. Colours represent families. Black: Acroporidae; Dark Grey: Pocilloporidae; Grey: Poritidae; Light Grey: Others

during the wet-warm season (6.3 ± 1.6 m) compared to the dry-cold season (7.6 ± 2.2 m) (ANOVA, $p<0.001$). The highest values of water visibility were observed from September to December, during which it reached > 8 m (Fig. 3C). The highest mean annual visibility was observed at Grande Vasque (7.6 ± 2.5 m) and the lowest at Rose Garden (6.3 ± 1.2 m) with a significant difference observed between sites (Tukey, $p=0.002$). The visibility observed on Nosy Tafara was not significantly different from the 2 other sites (Tukey, $p>0.1$).

Planula records

The variability in the occurrence of planula is very high and implied that the density observed in the year does not present a significant difference between the sites (Kruskal-Wallis, $p=0.33$). The maximum occurrence of planula was during 9 months of the year, from August to December and from February to May. Planula occurred most often at Nosy Tafara (9 months) compared to Grande Vasque and Rose Garden where planula were observed in 8 and 3 months of the year, respectively. During 7 weeks between December and February, samples were not useable due to the high levels of non-biological material in suspension, or because sampling was interrupted due to bad weather (Table 2). For the rest of the year, samples from successive weeks with the occurrence of planula were mixed to maximize the efficiency of larval observation. The highest density of planula was observed in the last week of November and the first week of December (Table 2). Nosy Tafara presented a relative higher mean annual density of planula (3.23 ± 5.72 larvae m^{-3}) compared to the other sites, with 0.43 ± 0.41 larvae m^{-3} and 0.65 ± 0.92 larvae m^{-3} for Grande Vasque and Rose Garden, respectively.

Recruit records

Total recruitment

The first recruits were observed in October 2013. Observation from 20 tiles out of 144 were not taken into account due to *in situ* breakages, lowering the reliability of the obtained results. A total of 3010 recruits were observed from all the sites: 2679, 174 and 157 recruits were identified respectively on 48 tiles from

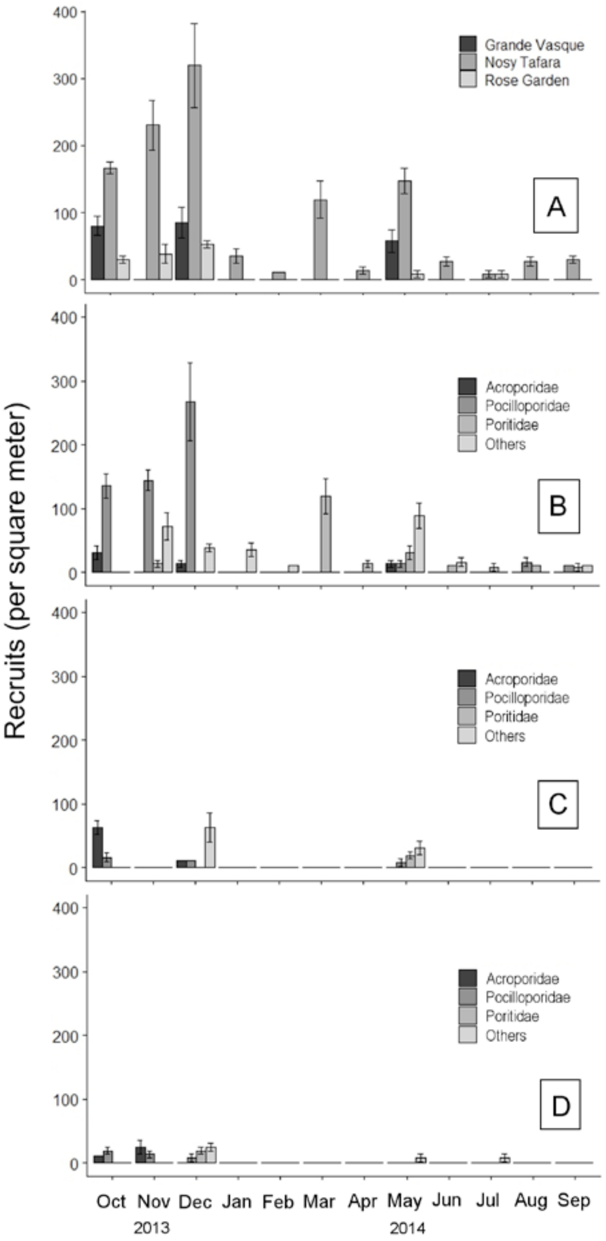

Figure 6. Average density of recruits per site per month (A), and monthly distribution of recruit families. (B) Nosy Tafara; (C) Grande Vasque; (D) Rose Garden. The bars represent standard deviation.

Nosy Tafara, on 32 tiles from Grande Vasque and 44 tiles from Rose Garden. The number of recruits per tile varied from 0 (Grande Vasque in November 2013) to 218 (Nosy Tafara in August 2014). The mean density of total coral recruits was significantly higher at Nosy Tafara compared to Grande Vasque and Rose Garden, with 620.13 ± 621.30 recruits m-^{2}, 40.28 ± 50.97 recruits m-^{2} and 36.34 ± 33.82 recruits m-^{2} (KW, $p<0.001$), respectively. The Post hoc Nemenyi test confirmed a significant difference between Nosy Tafara and Grande Vasque ($p<0.001$) and between Nosy Tafara and Rose Garden ($p<0.001$), and no difference between Grande Vasque and Rose Garden ($p=0.88$).

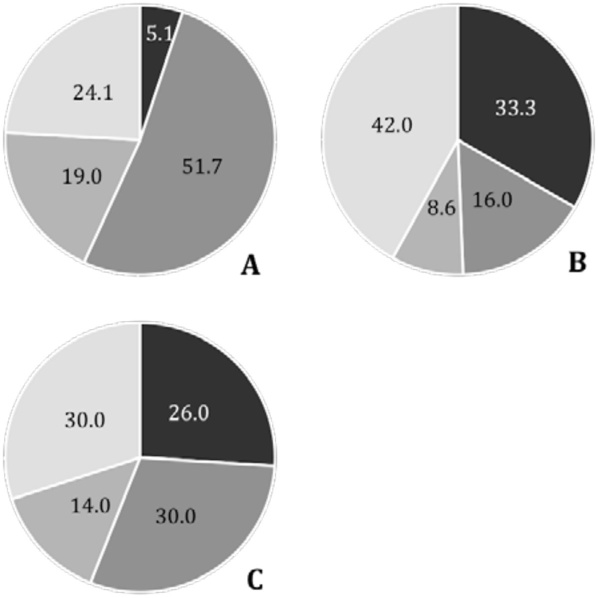

Figure 7. Dominance (%) of recruit families (age < 1 month). A. Nosy Tafara, B. Grande Vasque, C. Rose Garden. Colours represent families. Black: Acroporidae; Dark Grey: Pocilloporidae; Grey: Poritidae; Light Grey: Others.

During the year, the overall recruitment rate (all ages included) was significantly dominated by Poritidae species that represented 49.8 % of the total recruits from all the sites. The site at Nosy Tafara presented the most important dominance of Poritidae with 74.4 % of the total recruits observed on the tiles (Fig. 4A), followed by Pocilloporidae with 19.3% of the total recruits. Acroporidae species are poorly represented at this site (0.5 %, Fig. 4A). At Grande Vasque and Rose Garden, Poritidae represented 36.8 % and 38.2 %, Pocilloporidae 10.3 % and 29.9 %, and Acroporidae 15.5 % and 9.6 %, respectively (Fig. 4). Monthly observation showed that at Nosy Tafara, the Pocilloporidae species represented more than 75 % of the composition of the recruits from October 2013 to January 2014 (Fig. 5A). Four months after immersion of the tiles, from February, recruitment is dominated by Poritidae species. At the site Grande Vasque and Rose Garden, no obvious dominance of any family was observed from October to December, but Poritidae also represented more than 50 % of the composition of the recruits from January at both sites (Fig. 5B and 5C).

Seasonal distribution of newly settled corals

Seasonal distribution of coral recruitment was different between the sites (KW, $p<0.001$). Newly settled corals (<1 month age) were observed at Nosy Tafara every month during the survey, while they were observed

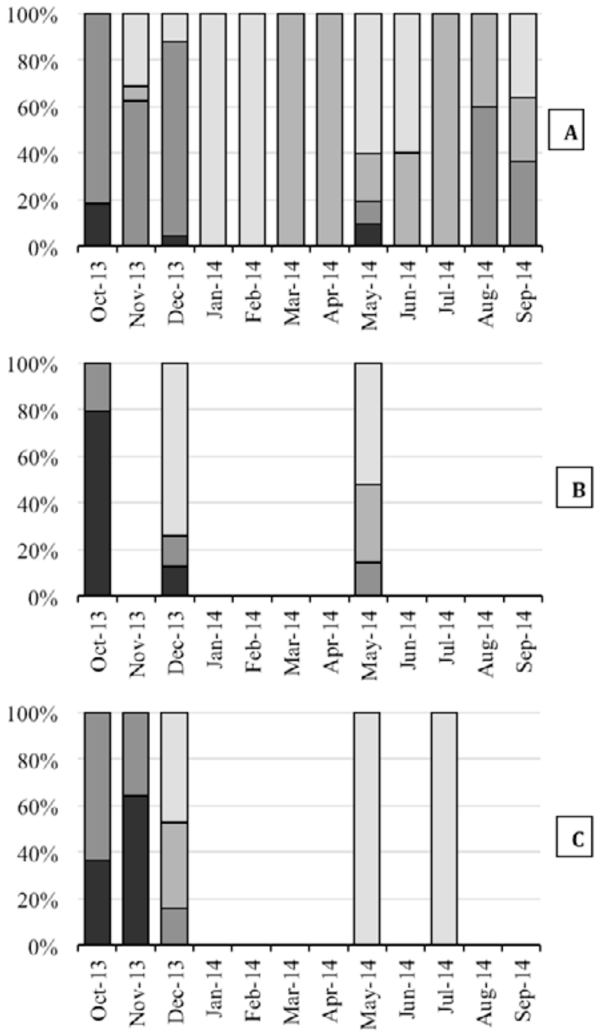

Figure 8. Dominance (%) of recruit families per month (age < 1 month). A. Nosy Tafara, B. Grande Vasque, C. Rose Garden. Colours represent families. Black: Acroporidae; Dark Grey: Pocilloporidae; Grey: Poritidae; Light Grey: Others.

only during 3 months at Grande Vasque and during 5 months at Rose Garden (Fig. 6). The annual mean density of newly settled recruits was significantly higher at Nosy Tafara with 94.91±101.08 recruits m^{-2} compared to Grande Vasque and Rose Garden with 18.75±34.32 recruits m^{-2} and 11.57±18.47 recruits m^{-2}, respectively (Nemenyi test, $p<0.001$). The two last sites presented no significant difference between them (Nemenyi test, $p=0.98$). The highest density of newly settled recruits was observed for all the sites during the period of October to December (Fig. 6A). Higher density of recruits was also observed in March at Nosy Tafara and in May at all three sites.

The newly settled coral recruitment rate at Nosy Tafara is dominated by Pocilloporidae species that represented 51.7 % of the total newly settled recruits (Fig. 7A). A peak of Pocilloporidae recruits was observed in December, but high rates were also recorded in October and November (Fig. 8A). Recruits belonging to the family Poritidae were also abundant at this site with a peak observed in March (Fig. 8B). Recruits of the other families were distributed with approximately the same values. At Grande Vasque, recruits of Acroporidae were the most dominant, representing 33.3 % of the total recruitment rate with a peak recorded in October (Fig. 8C), followed by the Pocilloporidae that represented 16 %. At Rose Garden, a dominance of Pocilloporidae recruits (30 %) compared to Acroporidae (26 %) and Poritidae (14 %) was recorded in December. The newly settled recruits recorded in May and July were all unidentified (Fig. 8C).

Records of coral juveniles

The mean density of early coral juveniles (< 2 cm) at Nosy Tafara was significantly higher with 105 ± 61.7 juveniles m^{-2}, compared to Grande Vasque with 52.8 ± 34.1 juveniles m^{-2} (KW, $p<0.001$). The mean density of older juveniles (2 – 5 cm diameter) was 47 ± 47 juveniles m^{-2} at Nosy Tafara and 32.4 ± 19.1 juveniles m^{-2} at Grande Vasque. No significant difference was observed (KW, $p=0.352$).

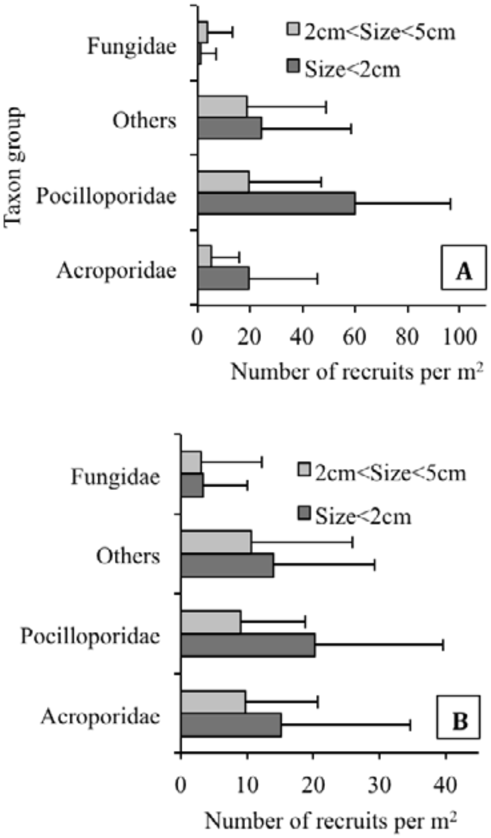

Figure 9. Density of coral juveniles recorded at Nosy Tafara (A) and Grande Vasque (B).

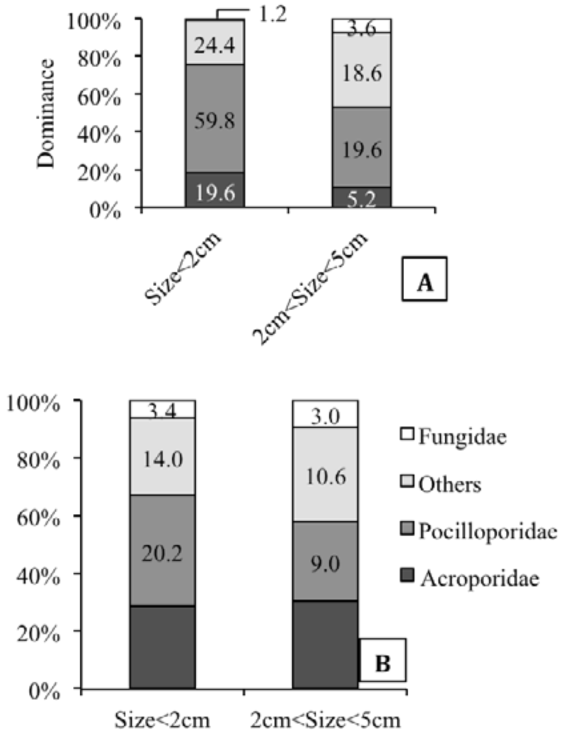

Figure 10. Dominance of juvenile families. A. Nosy Tafara; B. Grande Vasque.

Records from Nosy Tafara and Grande Vasque showed that Pocilloporidae early juveniles dominated the recorded juveniles with 57 % and 38.3 % of the total abundance, respectively. The density of Pocilloporidae older juveniles was lower with 41.7 % at Nosy Tafara and only 27.8 % at Grande Vasque (Fig. 9). At both sites, juveniles of Pocilloporidae and Acroporidae dominated more than the half of the total rate (Fig. 10).

Estimation of post-settlement mortalities

The results showed that the recruits had higher mortality than early juveniles. The density of early juveniles (< 2 cm) represented 74 % of the density of recruits and the density of older juveniles (2 – 5 cm diameter) represented 53.1 % of the density of early juveniles. The density of older juveniles represented 44 % of the density of recruits, with a lower value for Nosy Tafara (7.6 %) compared to Grande Vasque (80.4 %).

Discussion

The present study reports on the first records and analysis of recruitment and early development of corals in Madagascar. It covers a period of one year and includes larval observation records and cover estimations of coral recruits and juveniles. To date, the only record of coral spawning in Madagascar indicates a spawning event that occurred on the 9th and 24th September 2015 on the coral reefs of Andavadoake located 50 km from the northern coral reefs studied in the present work (Gress *et al.*, 2015). The spawning happened 4 to 6 nights after the new and the full moon and concerned only *Acropora* species. In the present paper, the observation of high planula densities in the last week of November and the first week of December combined with the increasing densities of recruits from October to December suggests the occurrence of massive coral spawning during this period of the year. Also, the extended presence of planula and recruits during February, March, April and August indicates that punctual spawning occurs regularly. This phenomenon is not an isolated case but has been observed in several regions where scleractinian reproduction and spawning occurred over several months of the year with mass spawning following the full moons from September to March (e.g., Guest *et al.,* 2002; Carroll *et al.*, 2006; Rosser and Baird, 2008; Massé, 2014). Recently, Terrana and Eeckhaut (2019) observed male and female gametes of the antipatharian species *Cirrhipathes anguina* in Toliara, throughout the year, suggesting a massive spawning event and irregular minor spawning events that may occur during the rest of the year. The phenomenon seems to be common to the Hexacorallia taxon.

The peaks of recruitment recorded from October to December in SW Madagascar correlated with planula observed during the same period. Knowing that planula can survive several weeks before settlement (Baird *et al.*, 2012), the observed newly settled recruits may result from spawning that occurred from September. The weak recruitment in January and February suggests the end of massive spawning in December. A difference of recruit and juvenile records between the sites, notably between Nosy Tafara and the two other sites, Grande Vasque and Rose Garden was also observed. Nosy Tafara showed a higher recruitment rate which occurs during each month of the year. At Grande Vasque and Rose Garden, the highest recruitment rate was observed from October to November and was followed by a lower recruitment rate in May (Grande Vasque) to July (Rose Garden). The Nosy Tafara site is more exposed to the open sea compared to Grande Vasque (being a basin in a reef flat) and to Rose Garden (a patch reef in a protected lagoon).

The recruitment rates recorded in Madagascar are comparable to recruitment rates observed in the tropical Pacific reefs (Hughes *et al.,* 1999) and most of the Indo-Pacific countries, including in the WIO such as in La Réunion island and South Africa (Massé, 2014), where the recruitment rates do not exceed 100 recruits m^{-2} $year^{-1}$ (Adjeroud *et al.,* 2007). However, differences between the methods and materials used (tile surface, depth and tile nature) may lead to error in estimating and comparing results of research from different regions. These differences should be analysed by performing recruitment monitoring with all the methods used and comparing the results from the same site and same period. The observed recruitment rates of juveniles at each site were classified as very high compared to the theoretical assessment scale of the scleractinian recruitment rates proposed by Engelhardt (2003). This theoretical scale considers the recruitment rate as very high if the absolute abundance of branching corals (Acroporidae and Pocilloporidae) of 2 – 5 cm size is > 10 juveniles m^{-2}.

The temperature and visibility of the water at the three sites are not significantly different. Salinity in Nosy Tafara is lower than in Grande Vasque and Rose Garden. This might be due to the proximity of the Onilahy River a few kilometres from Nosy Tafara. In Madagascar, the period from September to December corresponds to the transition between the dry-cold season and wet-warm season, during which the water temperature slowly rises to reach the highest values in January and February. During the same period, the light irradiance is at its highest level. Solar light cycles are amongst the main conditions that determine coral spawning (Brady *et al.,* 2009) and the abundance of coral recruitment. The period from January to February 2014 was particularly rainy and strong influences of tropical cyclones were noticed at the sites affecting the salinity and the water visibility. The second period of larval occurrence and recruits recorded (March – May) corresponds to the transition between the wet-warm season and the dry-cold season during which the water temperature was decreasing. Water currents are also known to be one of the main dispersal agents for planula (Adjeroud *et al.*, 2016). The high density of larvae and the higher recruitment rate recorded at Nosy Tafara may be explained by the water current regime. The existence of the Southwest Madagascar Coastal Current (SMACC) running along the SW coast of the island may explain the high level of coral recruitment in this area (Ramanantsoa *et al.*, 2018). These authors reported that the SMACC is likely to influence local fisheries and larval transport patterns. Terrana *et al.* (2021) hypothesized after genetical connectivity analysis, that this current influences the settlement the black coral species *Stichopathes cf. maldivensis*, which has a high occurrence in the area compared to other locations in the SW of Madagascar. The high density of coral larvae and high rate of settlement observed at this site may originate from local spawning but also from spawning of corals located to the north of the region.

The post-settlement processes are one of the important keys to characterize the dynamics and resilience of coral reef assemblages. Results presented in the present paper show an important post-settlement mortality, but the abundance of juvenile that survived still represents a higher rate compared to other regions (Wending, 2003; Adjeroud *et al*., 2007). The earlier the coral stage, the higher the mortality, and the major sources of high mortality of early recruits are unsuitable environmental conditions, competition, and predation (Adjeroud *et al*., 2016). Environmental conditions are strongly influenced by the seasons and sedimentation at the studied sites. During the rainy season, upstream factors such as river flow particularly increase and result in erosion of the soils from the watershed of Fiherenana (Payet *et al*., 2011) and of Onilahy (Rakotondralambo, 2008). Sheridan *et al.* (2014a, 2014b) reported that sedimentation induces coral disease and mortality on coral reefs in the SW of Madagascar. In addition, anthropogenic pressures due to destructive fishing techniques (Mahafina, 2011; Bruggemann *et al.,* 2012; Andréfouët *et al.,* 2013) are particularly high on the studied coral reefs where coral trampling is practiced (Salimo, 1997). Competition with algal assemblages is also important, especially on the degraded reef flat (Harris *et al*., 2010, Bruggemann *et al*., 2012).

In conclusion, the coral recruitment patterns observed in the SW of Madagascar could result in increased resilience of the coral reef assemblages, although several post-settlement factors may induce important mortality of coral recruits and juveniles. Further studies should be performed to identify the main causes of this mortality and the implications for coral recruitment.

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Appendix

Morphological characteristics of the observed early stages of corals.

Most of the early stages of the coral life-cycle have been observed during the present study. Figure 1 presents the stages observed from the 3 stations: (A) a ciliated planula showing endoderm with dense endosymbionts (end), an epidermis that lacks symbionts (ect), the blastopore (blast) and the cilia (cil); (B) newly settled recruit (see description on Fig. 3); (C) early juvenile of *Pocillopora*; and (D) older juveniles of *Acropora* (Acr) and *Stylophora* (Sty).

The morphological characteristics could be differentiated for the newly settled recruits of the families Acroporidae, Pocilloporidae and Poritidae (Fig. 2). Each observed recruit has also been grouped into 2 age categories: <1 month and 1 month to 12 months with the criteria described by Babcock *et al.* (2003) as a reference. The characteristics specific to the Acroporidae family have been noted. The basal plate, septa in 2 cycles, the porous coenosteum and especially the absence of columella are clearly observed on <1 month recruits (Fig. 2A). From 1 month age, secondary corallites are developed (Fig. 2B). For the Pocilloporidae of <1 month recruits, the basal plate and prominent septa are also clearly apparent (Fig. 2C). The solid coenosteum and the prominent columella permit this family to be distinquished. >1 month Pocilloporidae recruits have developed secondary corallites, each with prominent columella (Fig. 2D). The coral family Poritidae presents epitheca and 6 thickened primary septa and prominent vertical toot few days after settlement (Fig. 2E). The main characteristic of the Poritidae recruits after a few weeks is the development of the corallite beyond the epitheca, which remain visible within corallite after the secondary and primary septa fusion (Fig. 2F).

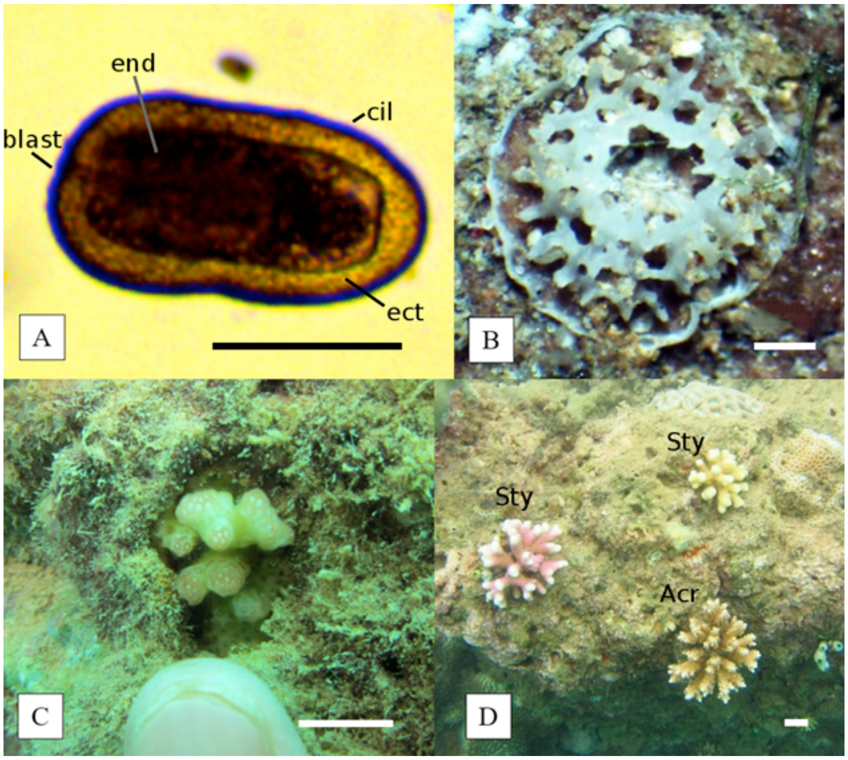

Figure 1. Photographs of scleractinian at the different development stages: A. planula; B. Recruit (Acroporidae); C. Early juvenile of *Pocillopora* (< 2 cm); D. Juveniles of *Acropora* and *Stylophora* (2 – 5 cm). A, B and D were sampled from Nosy Tafara and C from Grande Vasque. Scale bar: A and B = 500 µm; C and D = 1 cm.

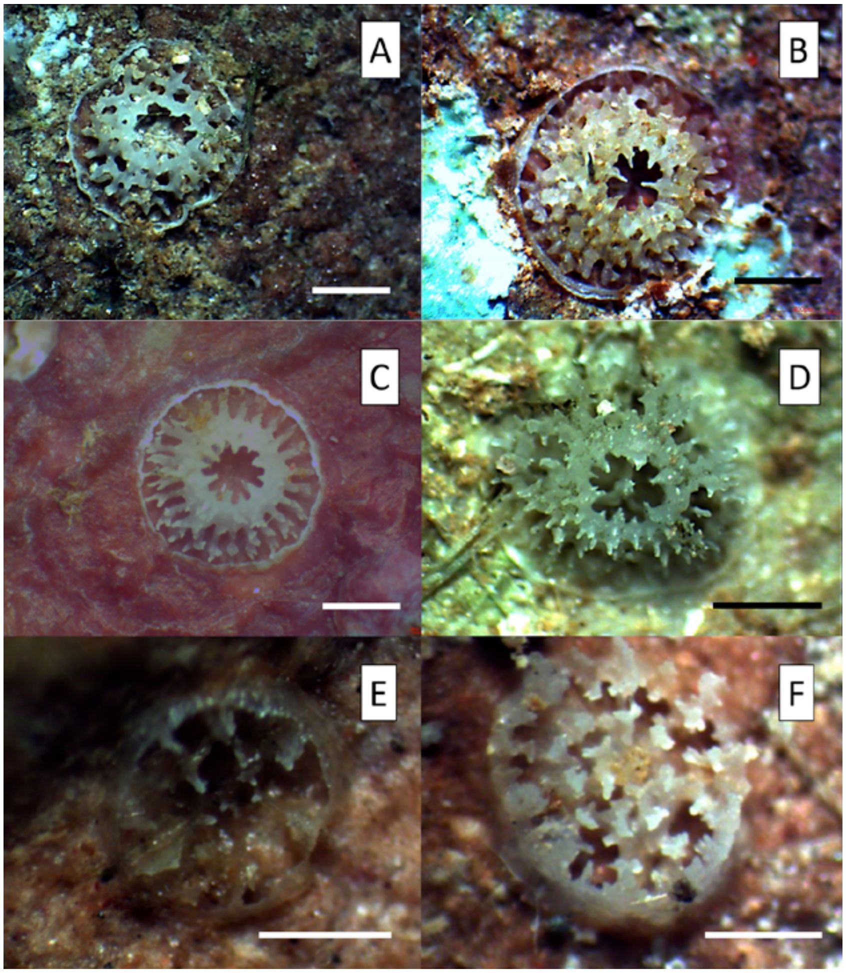

Figure 2. Recruits of the families Acroporidae (A: <1 month, B: >1 month), Pocilloporidae (C: <1 month, D: >1 month) and Poritidae (E: <1 month, F: >1 month). Scale bar: 500 µm.

Quick and cost-effective mapping of subsistence and artisanal fishing areas within and adjacent to a marine protected area

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Abstract

Spatial information on artisanal fisheries is largely lacking, making subsequent management and threat or impact assessments difficult. Local knowledge, current and readily available mapping software and Global Positioning System (GPS) techniques were used to map small-scale (subsistence and artisanal) fishing areas within and adjacent to the western boundary of the Ponta do Ouro Partial Marine Reserve in southern Mozambique. Data on habitat types were collected in the field and combined with available literature. Thirty-one fishing areas were identified and mapped resulting in a total area of 293.1 km^2 with a perimeter of 396.7 km, from the Inhaca Island lighthouse in the north to the Maputo River mouth in the south. Habitats within the fishing areas included isolated intertidal sand banks, seagrass beds, estuarine channels, as well as intertidal muddy areas adjacent to the coast. The fishing areas covered on average 9.5 km^2 (SD=12.0) and varied immensely in size (0.3–50.1 km^2). Overall, 58.4 % (171.2 km^2) of the total fishing area mapped was located inside the reserve. Costs associated with data collection equated to an average of U$D 3.61 per mapped km^2 or U$D 2.63 per km tracked. The method was found to be quick, cost-effective and easily replicable elsewhere.

Keywords: artisanal and subsistence fishing areas, local knowledge, Maputo Bay, Ponta do Ouro Partial Marine Reserve, small-scale fisheries, spatial mapping

Introduction

The small-scale (subsistence and artisanal) sub-sectors constitute the backbone of marine fisheries in most developing countries, especially in the Western Indian Ocean (WIO) region (van der Elst *et al.*, 2005; Groeneveld, 2015). In Mozambique, more than 315 000 people are directly involved in artisanal and subsistence fisheries (Ochiewo, 2015), with reported catches of 314 470 tonnes in 2017, whereas the combined catches reported for the industrial (15 100 tonnes) and semi-industrial (8 806 tonnes) sub-sectors represented less than 10 %, in the same year (MIMAIP, 2019).

Common challenges associated with the management of small-scale fisheries include lack of baseline data, limited/over-exploited resources, high numbers of fishers, weak governance and political will, poor or ineffective enforcement and management regulations, and open access rights (Béné *et al.*, 2004; Salas *et al.*, 2007; Batista *et al.*, 2014). Several authors have advocated that these challenges in conjunction with the socio-economic importance of small-scale fisheries for developing countries make user participation essential (Léopold *et al.*, 2014; Corral and de Lara, 2017). The application of local knowledge to support planning and management of artisanal fisheries has thus gained widespread acceptance and use (Hele, 2007; de Freitas and Tagliani, 2009; Ratsimbazafy *et al.*, 2016; Thiault *et al.*, 2017).

One of the simplest ways to acquire and make use of local knowledge is the mapping of fishing areas – a data collection method used to develop a participatory geographic information system (Dunn, 2007). Within the WIO, participatory mapping of fishing areas has been achieved in Madagascar for

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the octopus fishery (Ratsimbazafy *et al.*, 2016), in Kenya for the ringnet fishery (Thoya *et al.*, 2014) and main fishing grounds of migrant fishers (Wanyonyi *et al.*, 2018). Hele (2007) and Book (2012) mapped hand-lining and gillnet fishing areas in northern and southern Mozambique, respectively. Primarily, these studies involved participatory drawing of habitat maps and fishing areas, which where later digitised into GoogleEarth maps and then validated by the fishers. While this technique is largely advocated as being cost effective, the accuracy is generally poor. Wanyonyi *et al.* (2018) presented an alternative, yet slightly more costly approach; eleven handheld GPS units were provided to fishers for tracking of fishing activities which were complemented with vessel logbook records.

This paper reports on a quick, easy and cost-effective participatory mapping method used to characterize fishing areas of artisanal and subsistence fishers within and adjacent to the Ponta do Ouro Partial Marine Reserve (POPMR) in southern Mozambique. Here, the two general approaches used in previous studies in the WIO are combined. The method entailed tracking the actual perimeters of the fishing areas, using a readily available GPS unit and open source mapping software followed by validation by the fishers. This constitutes the first baseline for spatial small-scale fisheries data for a marine protected area (MPA) in Mozambique.

Materials and methods

Study area

The POPMR is located in southern Mozambique and is administratively part of two coastal districts of Maputo Province – the Matutuíne District and the KaNyaka Municipal District (which in turn falls under the Maputo Municipality). The geographical limits of the POPMR are from the border with South Africa to the south, and Cabo da Inhaca to the north, to the Maputo river mouth in Maputo Bay (Fig. 1). The reserve covers 678 km^2 and stretches from the highwater mark, following the contour of the base of the coastal dunes, to three nautical miles into the Indian Ocean, and one nautical mile into Maputo Bay (DNAC, 2011).

The study was performed on the western side of the reserve (i.e. within Maputo Bay), from the Inhaca lighthouse to the Maputo river mouth, a stretch of coast of about 70 km. The general area is described in detail in Bandeira and Paula (2014). The area is very shallow, with the deepest points within channels attaining about 20 m. At low tide, the average depth ranges from the intertidal zone to about 10 m. The study area consists of a series of seagrass and muddy intertidal sand banks which are almost continuously fringed by mangroves. The area is subjected to multi-gear and multi-species fisheries, including beach and boat seining, gill netting, hand-lining, traps, spear-fishing, as well as invertebrate collection (Louro *et al.*, 2017).

Data collection and analysis

The data were collected over four working days (total of 27 h and 20 min) between June and July 2017. The area was divided into three main strata following the reserve's subsistence and artisanal catch monitoring system, namely: Inhaca Island, Santa Maria, and Mabuluco (Louro *et al.*, 2017). For each stratum, a Google Earth map of the area was printed on A3 paper and discussed with experienced fishers (n=3) familiar with the area, usually the leader of the local community fishing council. The names of the fishing areas were previously obtained from the artisanal catch monitoring system and the approximate boundaries of each area were identified and confirmed by the fisher.

A shallow draft, 19-foot catamaran ski-boat, powered by twin 90 HP four-stroke motors was used to travel within the perimeter of each area. The local fishers joined the surveying team on board to validate the areas that were mapped. The boat was equipped with a Garmin GPS/fish-finder combo (Garmin echoMap CHIRP 94SV), which tracked the perimeters of the areas. Notes on the general characteristics of the area, including the bathymetry and bottom type were collected. Bottom type was determined by using a combination of information from the fish-finder on board, the local knowledge of the fisher and a GoPro camera, lowered to the bottom with weights and set in a time-lapse photo mode. These data were then combined with information from available literature (Kalk, 1995; Bandeira *et al.*, 2014; Ferreira and Bandeira, 2014).

The tracks of each day's work were transferred to Garmin's free mapping software (Homeport 2.2.10) from which the coordinates of the perimeters were extracted and transferred into GoogleEarth to snap vertices and edges of the polygons. Preliminary maps were produced using QGIS 2.18, and then discussed with the fishers for amendments and validation. The shapefiles of the Mozambican coastline, Maputo Bay and the POPMR were downloaded from free online sources (Biofund, 2019; Flanders Marine Institute, 2020).

Results and discussion

Thirty-one fishing areas were identified and mapped (Fig. 1, Appendix 1), representing a total surface area of 293.1 km^2 and a perimeter of 396.7 km. The fishing areas included isolated intertidal sand banks, seagrass beds, sandy/muddy areas, estuarine channels, as well as intertidal muddy areas adjacent to the coast. The fishing areas covered on average 9.5 km^2 (SD=12.0) and varied greatly in size (0.3–50.1 km^2). A few areas (n=6; totalling 47.3 km^2, 16.4 %) were located outside the limits of the POPMR, whilst the majority were located inside the reserve (n=15), although relatively small and thus representing only 19.4 % of the total surface of the fishing areas mapped (56.8 km^2). Some large fishing areas (n=10) overlapped the reserve's boundaries, representing 64.5 % (189 km^2). Overall, 58.4 % (171.2 km^2) of the total fishing area mapped was located inside the reserve.

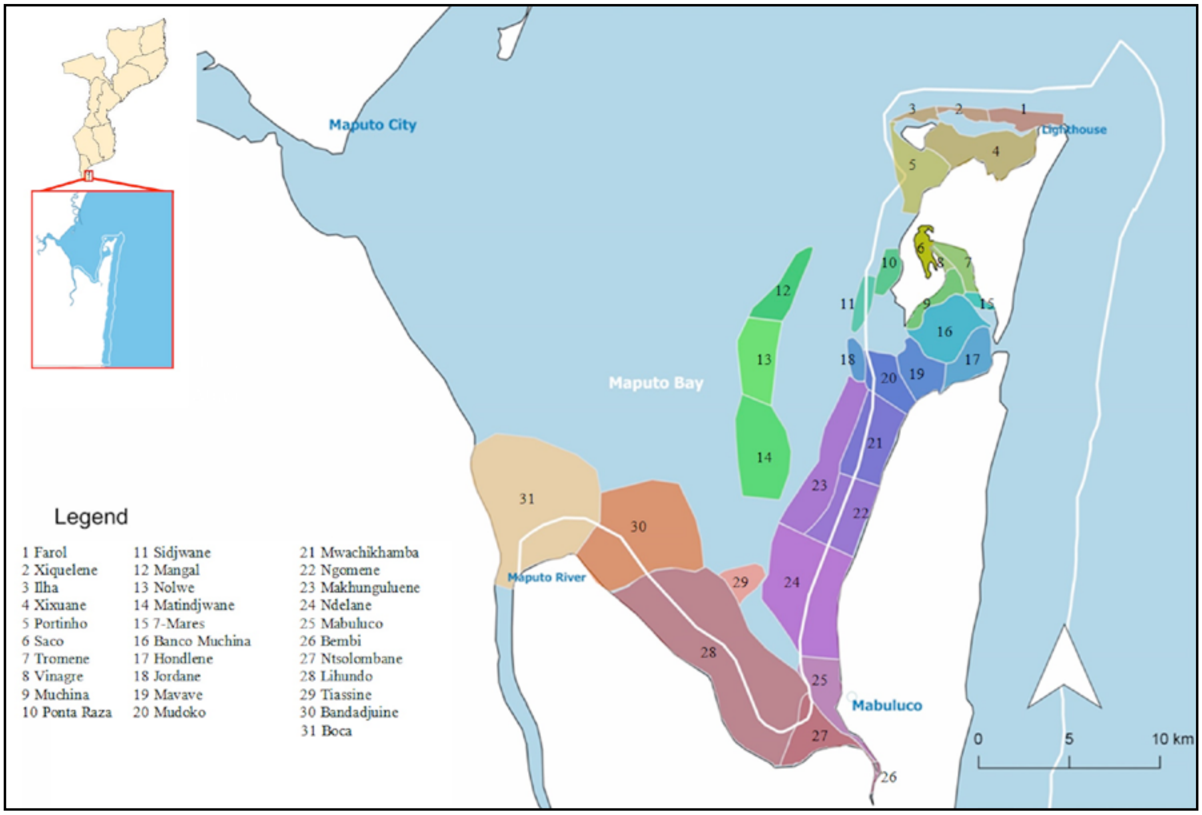

Figure 1. Map showing the location of the Ponta do Ouro Partial Marine Reserve (inset) and the subsistence and artisanal fishing areas (n = 31) mapped in this study. White perimeter defines the POPMR boundary area.

Some previous mapping of this study area has been conducted but the results from the present study differ in relation to accuracy and the total area covered. The most probable explanations for these differences are as follows. The study by Book (2012) reported 23 fishing areas used by fishers from Inhaca Island, of which 47 % were located inside the reserve, 28 % outside the reserve's boundaries and the remaining 25 areas were trans-boundary. These discrepancies probably result from the fact that the present study covered a larger area inside the Bay and was not restricted to the area surrounding Inhaca Island or only used by fishers from the island. Book (2012) mentioned fishing grounds in the open ocean (e.g. Baixo Danae), which were not covered by the present study. Additionally, Book (2012) did not quantify areas or perimeters of the fishing areas, which constitutes a significant shortcoming and makes any comparison difficult. Also, the study was based solely on identification of areas used by the fishers from a printed map, so the accuracy is indeed questionable (due to the fishers' difficulty in interpreting and scaling maps).

Only three fishers were interviewed and participated in the present study. Notwithstanding their local knowledge and experience (all three are affiliated with local fishing councils), it is recognized that the accuracy of the limits and location of the fishing areas could definitely be improved with more participants. This would be of value in areas where conflicts may exist amongst fishers, and the participation of a larger number of fishers to verify and validate the maps produced both in a workshop and *in situ*, would certainly contribute to strengthen the relationship between fishers, managers and researchers as reported by Ratsimbazafy *et al.* (2016).

The overall costs associated with data collection in the field (including fuel, boat use, *per diems* and fishers' time) totalled about U$D 1 045.00, which equates to an average of U$D 3.61 per km^2 of mapped area or U$D 2.63 U$D per km tracked. Unfortunately, there are no estimates of the costs involved in mapping fishing areas from previously published works in the WIO (cf. Hele, 2007; Book, 2012; Thoya *et al.*, 2014; Ratsimbazafy *et al.*, 2016; Wanyonyi *et al.*, 2018), which makes any comparison difficult, if not impossible. However, taking into account that for a given MPA or locally managed marine area this would be a one-off cost, and given how quickly the information is generated with substantial accuracy, the method is considered to be very cost-effective.

This mapping method could prove useful in environmental impact assessments, marine spatial planning initiatives, endangered species or ecosystems conservation, and the participatory drafting and implementation of conservation and fisheries management plans. In fact, the map of fishing areas generated has been shared with the reserve's authorities and relevant stakeholders and is being used in the planning, monitoring and management of coastal resource use within the reserve (Williams *et al.*, 2018). It is worth mentioning the need for ground truthing and collecting data *in situ* when conducting similar mapping exercises, which results in more accurate, quantitative mapping data. In conclusion, the method is simple, quick and cost-effective and, given the readily available techniques and resources used, it can be easily replicated in developing countries.

Acknowledgements

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Appendix

Table A1. Summary description of artisanal and subsistence fishing areas mapped, within and adjacent of the Ponta do Ouro Partial Marine Reserve. Ecological Information was collected during the present study and compiled from the literature (Kalk, 1995; Bandeira *et al.*, 2014; Ferreira & Bandeira, 2014).

Nr.	Strata	Fishing Area	Perimeter (km)	Surface (km^2)	General description
1	Inhaca	Farol	11.3	3.6	Sandy substrate. Channel with an intertidal area interspersed with sand banks influenced directly by the Indian Ocean. The shore encompasses sandy beaches and high vegetated dunes
2	Inhaca	Xiquelene	7.2	1.4	These areas are essentially a continuous channel with sandy substrate. The shoreline encompasses a continuous and slightly elevated sandy bar with pioneer dune vegetation
3	Inhaca	Ilha	6.7	1.3	
4	Inhaca	Xixuane	18.5	11.3	Intertidal sandy to muddy substrate covered by two main seagrass communities: *Thalassia hemprichii* and *Halodule uninervis*. Between Portuguese Island and Portinho, at Inhaca Island, there's a seagrass community of *Thalassodendron ciliatum* and *Cymodacea serrulata*. At low tide most of the area is exposed. On land, the Sangala Bay non-estuarine mangrove forests are still in good condition
5	Inhaca	Portinho	14.4	8	The area is mostly submerged and shares the same seagrass community (*T. ciliatum* and *C. serrulata*) with Xixuane. The shoreline is mainly composed of red sand cliffs (Barreira Vermelha) and sandy beaches with coral rubble
6	Inhaca	Saco	9.5	1.9	Intertidal sandy to muddy substrate covered by three main seagrass communities: *T. ciliatum* /*C. serrulata*, *T. hemprichii*/*H. uninervis* and small patches of *Zostera capensis*. Adjacent to the seagrass beds, there are non-estuarine mangrove forests composed mostly by fringing *Avicenna marina* and *Rizophora mucronata* (mostly on creeks)
7	Inhaca	Tromene	4.4	0.6	Sandy to muddy substrate covered by four seagrass species (*T. ciliatum*, *C. serrulata*, *T. hemprichii* and *H. uninervis*)
8	Inhaca	Vinagre	8.6	2.8	
9	Inhaca	Muchina	10.7	3.1	Thsomene channel (sandy/muddy substrate) bordered by intertidal sand and muddy banks with seagrass communities (*T. ciliatum* /*C. serrulata*, *T. hemprichii*/ *H. uninervis* and small patches of *Z. capensis*). The shoreline is fringed by *A. marina* and *R. mucronata* (mostly on creeks)
10	Inhaca	Ponta Raza	6.9	2.6	Intertidal mostly sandy substrate, with two main seagrass communities (*T. ciliatum* and *C. serrulata*). The shoreline encompasses sandy beaches and pioneer dune vegetation

Nr.	Strata	Fishing Area	Perimeter (km)	Surface (km^2)	General description
11	Inhaca	Sidjwane	7.5	2.6	An isolated and intertidal sand bank bordered by a channel and covered by non-continuous seagrass bed communities of *T. ciliatum* and *C. serrulada*. The shoreline encompasses sandy beaches, rocky outcrops and pioneer dune vegetation
12	Santa Maria	Mangal	11.9	5.5	Sandy substrate with *T. ciliatum* and algae. An isolated intertidal bank bordered by a deep channel and covered by non-continuous seagrass bed communities
13	Santa Maria	Nolwe	12.7	9	Sandy substrate with *T. ciliatum* and algae. An isolated and intertidal sand bank bordered by a channel and covered by scattered seagrasses
14	Santa Maria	Matindjwane	15	14	Sandy substrate with *T. ciliatum* and algae. An isolated and intertidal sand bank bordered by a channel and covered by non-continuous seagrass beds
15	Inhaca	7-Mares	4.5	0.9	Sandy to muddy substrate covered by three seagrass species (*T. hemprichii*, *H. uninervis* and *C. rotundata)*
16	Inhaca	Banco Muchina	13.3	9.4	A large intertidal sand and muddy bank covered mainly by a large patch of *Z. capensis* and smaller patches of seagrass communities of *T. ciliatum / C. serrulata*, *T. hemprichii / H. uninervis*. The bank is bordered by the deep Thsomene channel
17	Santa Maria	Hondlene	10.7	5.2	Sandy substrate with a seagrass bed composed mainly by *T. ciliatum.* The sand bank is borderd by the Sisse channel. The shoreline is fringed by mangroves (*A. marina*). Vegetated coastal dunes, sandy beaches with rocky outcrops also characterize the shoreline
18	Santa Maria	Jordane	5.9	1.9	Isolated, intertidal sandy bank with seagrass beds composed mainly by *T. ciliatum, C. serrulata* and macroalgae. Borderd by a shallow channel
19	Santa Maria	Mavave	10.3	5.7	Sandy substrate with seagrass bed composed mainly by *T. ciliatum* and bordered by the Xihogo channel. The shoreline is fringed by mangroves, mainly *A. marina*
20	Santa Maria	Mudoko	10.3	5.8	Sandy substrate with macroalgae and deposits of broken mollusc shells. Fringed by *A. marina* on the seaward margins and *R. mucronata* along the creeks. Extensive muddy intertidal area, adjacent to the mangroves
21	Santa Maria	Mwachikhamba	14	10.8	Sandy and mostly muddy substrate. Fringed by mangroves, mostly *A. marina* and *R. mucronata* along the creeks. Extensive muddy intertidal area, adjacent to the mangroves
22	Santa Maria	Ngomene	13.6	9.7	

Nr.	Strata	Fishing Area	Perimeter (km)	Surface (km^2)	General description
23	Santa Maria	Makhunguluene	21	13.7	Sandy substrate with rocky and seagrass patches composed mainly by *T. ciliatum* and algae. Gradient between the estuarine ecosystem and the channel. Composed of an array of small intertwined channels and sand banks
24	Mabuluco	Ndelane	20.7	24.5	Sandy and mostly muddy substrate. Fringed by *A. marina* on the seaward margins and *R. mucronata* along the creeks. Extensive muddy intertidal area, adjacent to the mangroves
25	Mabuluco	Mabuluco	16.5	6.7	Adjacent to the mouth of the Bembi esturary. Muddy/sandy substrate with a scattered algae covered banks and deposits of broken mollusc shells. The shoreline encompasses red sand dunes, with sparse mangrove trees and rocky outcrops. Fish traps commonly known as "gamboas" were commonly observed
26	Mabuluco	Bembi	5.5	0.3	At the mouth of the Bembi estuary. Muddy substrate. High turbidity. Fringed by mangroves, mostly *A. marina* and *R. mucronata*. On the eastern bank (Mabuluco side), there were signs of mangrove clearance. On the western bank, vigorous natural mangrove regeneration is taking place. Along the fringing mangrove forest, several creeks are used as pathways and anchoring sites
27	Mabuluco	Ntsolombane	16.4	7.1	Sandy to muddy substrate with scattered deposits of broken mollusc shells. Fringed by *A. marina* on the seaward margins and *R. mucronata* along the creeks. Extensive muddy intertidal area, adjacent to the mangroves. Obvious natural mortality of mangroves (tall dead trees). Fencing nets (gamboas) were commonly observed in this area
28	Mabuluco	Lihundo	33.6	50.1	Muddy substrate. The shoreline is fringed by *A. marina* on the seaward margins and *R. mucronata* along the creeks. Extensive muddy intertidal area, adjacent to the mangroves. Fencing nets (gamboas) were commonly observed in this area
29	Mabuluco	Tiassine	7.5	3.2	Deep channel with muddy substrate. Important hand-lining area
30	Mabuluco	Bandadjwine	21.6	29.4	At the mouth of the Maputo river. Muddy substrate with intertidal muddy/ sandy banks with extensive deposits of broken mollusc shells. Very turbid water. Estuarine ecosystem fringed by *A. marina* on the banks and *R. mucronata* along the creeks. Fishing traps (gamboas) were commonly observed in these areas
31	Mabuluco	Boca	26	41	

Growth, mortality, exploitation rate and recruitment pattern of *Octopus cyanea* (Mollusca: Cephalopoda) in the WIO region: A case study from the Mafia Archipelago, Tanzania

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Abstract

Octopus cyanea is a commercially important cephalopod in the Western Indian Ocean (WIO) region, but scientific information to inform management strategies for the species is limited. A study was conducted in 2014, 2015, 2017 and 2018 to investigate biological parameters including growth, mortality, exploitation rates and recruitment patterns in the sea around Mafia Archipelago, Tanzania. Virtual population analysis (VPA) indicated differential mortality between two sampling sites; the lowest and highest fishing mortality of $F = 1.5yr^{-1}$ and $F = 2.7yr^{-1}$ were observed in Bwejuu (Dorsal mantle length, DMT = 18-20 cm) and Jibondo (DMT = 8-12 cm) fishing villages, respectively. The maximum exploitation rate (E_{max}), which gives the maximum relative yield per recruit, was estimated at 0.380 and 0.379 for Jibondo and Bwejuu, respectively. The exploitation rates $E_{0.5}$, which corresponded to 50% of the unexploited stock relative biomass per recruit, were estimated at 0.248 for Jibondo and 0.247 for Bwejuu. These values differ greatly from the exploitation rates of 0.53 and 0.41 for Jibondo and Bwejuu, respectively, suggesting that the stock of *O. cyanea* is probably being overfished both in terms of yield per recruit and biomass per recruit. The stock-recruit pattern was observed to be continuous year-round, with the peak being between May and July. Since the peak in recruitment of both areas coincides with the south-east monsoon (SE Monsoon) and the level of maximum sustainable yield has been overshot, it is recommended that management plans are implemented that will reduce effort while increasing biomass, for example, implementing temporal octopus fishery closures at a village level.

Keywords: *Octopus cyanea*, growth, mortality, exploitation rate, recruitment, Western Indian Ocean Reserve, small-scale fisheries, spatial mapping

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Introduction

Octopus is an important food item among local coastal communities in tropical and temperate regions. The small scale fishery for *Octopus cyanea* is among the important commercial cephalopod fisheries and contributes to foreign currency earnings in the Western Indian Ocean (WIO) (Rocliffe and Harris, 2014). This fishery is an important economic activity for local coastal communities in the WIO and Tanzania, where *O. cyanea* dominates landed catches (Guard and Mgaya, 2002; TAFIRI, 2017). For instance, in the Mafia Archipelago of Tanzania mainland (Fig.1), the octopus fishery serves as an important economic activity for the local people on the island. Octopus also plays an essential ecological role in the marine ecosystem where they act as predators and potential prey to larger fishes such as sharks and some neritic tuna (Forsythe and Hanlon, 1997; Guard, 2009). Despite the importance of octopus catches in providing food and income to the people of the region, and their ecological function, this species has recently experienced alarming over-exploitation. Most previous studies indicate that the mean harvestable sizes and weights of the landed catches of octopuses in the region has declined (Rocliffe and Harris, 2014; Sauer *et al.*, 2011; TAFIRI, 2017; Guard, 2003; Guard and Mgaya, 2002).

The reported declines in octopus catches have been linked to several factors, including over-exploitation, seasonal change in sea temperatures, habitat degradation, disease outbreaks, pollution and predation (Katsanevakis and Verriopoulos, 2006; Heukelem, 1973; Sparre, 1998). To further understand the observed decline in octopus catches, it is necessary to obtain information on mortality, recruitment and exploitation patterns, particularly in data-poor fisheries (Hordyk *et al.*, 2014). To address this information gap in the *O. cyanea* fishery, a comprehensive study was designed to: (i) understand factors that cause octopus demise; whether natural or as a result of fishing pressure; (ii) understand octopus exploitation ratios (E) to better describe octopus stock status; and (iii) determine octopus recruitment periods, and/or age at which the octopus can recruit as a critical factor triggering catch fluctuations.

Material and methods

The study area

This study was conducted in the Mafia Archipelago, which is located between longitudes 39.33°E and 39.95°E and latitudes 7.60°S and 8.15°S (Fig. 1). The island has an estimated land area of 435 km^2 and is home to around 46,850 people (NBS, 2012). About 60 % of the inhabitants are fishermen (NBS, 2012). The village on Jibondo Island represents a typical fishing village of the WIO (McClanahan and Cinner, 2012; Cinner *et al.*, 2012). The weather and climate are both influenced by the monsoon trade winds, which reverse on an annual basis to form two alternating seasons: the northeast (NE) and southeast (SE) monsoons. The SE monsoon season occurs between May and September and is dominated by southerly trade winds of relatively high speeds (Semba *et al.,* 2019). This season affects mostly the southern part of the Mafia Archipelago. The reverse of the trade winds forms a NE monsoon, which occurs from November to March and is dominated by winds of relatively low magnitude blowing from the north. The NE monsoon season mostly affects the northern side (windward side) of the Mafia Archipelago. The change in monsoon seasons affects sea surface temperature (SST) and rainfall patterns on the island (Bryceson *et al.*, 2006), which also affects catches of major marine fish (McClanahan and Cinner, 2012; Meynecke *et al.,* 2006). The two islands of Bwejuu and Jibondo in the Mafia Archipelago were selected as sites (Fig.1). These islands were selected because of the high number of active octopus fishers. The number of fishers in the villages on these two islands differs greatly with around 94 active fishers on Bwejuu Island and 500 on Jibondo Island. The study sites fall within the boundary of the Mafia Marine Protected Area (MPA), where fishers are required to abide by Fisheries Regulation of 59 (1) d (MLFD, 2009) and capture octopuses that are above half a kilogram (0.5 kg). The sites also have local government by-laws limiting fishing activities during spring tides (Fisheries officer, personal communication).

Data collection

Octopus samples for morphometric data were obtained from the artisanal fishers at the selected landing sites between August 2014 and July 2015, and between November 2017 and March 2018. Data were collected from foot fishers and those using vessels. The two study sites had permanent localised fishers, who ensured availability and consistency of data. Sampling and measurement of the length and weight of individual octopus was done for 15 days during the low tide period. Data collection was conducted during spring tides (low tides) because octopus fishing occurs when the water is low in the intertidal reefs, and reefs are exposed, thus leaving a total of 16 fishing days a month. The morphometric data collected included the total length (TL, cm), dorsal mantle length (DML, cm) and weights (kg) of

O. *cyanea* individuals per site per day. DML and TL of individuals were measured to the nearest 1 mm using a 100 cm measuring board, and the individual weights of octopuses were measured using a 50 kg digital scale to the nearest 100 g. The data records were kept on paper forms. The information recorded in these forms was later entered into an electronic Catch Assessment Survey (eCas) system using a mobile phone application. This *(eCas)* is hosted by the Tanzania Fisheries Research Institute (TAFIRI). It is a server system that facilitates data entry and access in a consistent way for fisheries management.

Data analysis

Length-weight relationships

The individual DML and weights of the octopuses were grouped according to each site and analysed with the power curve equation:

$$W = qL^b$$

Where 'W' represents wet weight, 'L' is the DML in centimetres, 'b' is the slope of the regression line and 'q' is the intercept of the y-axis. The parameter 'b' is used to determine the growth pattern of octopus. When the 'b' value of the length-weight relationship at 95 % confidence level is 3, it indicates that the octopus population has an isometric growth. When the value is less or greater than 3, it means an allometric growth pattern.

Total mortality coefficient (Z)

The annual total mortality coefficient (Z) was estimated by the length-converted catch curve using the ELEFAN package as fitted in the FiSAT II programme (Gayanilo Jr *et al.*, 1996; Pauly, 1980; Sparre and Venema, 1992). In this analysis, the percentage of samples in length classes were grouped to mimic a steady-state population. The data for the catch curve was obtained by grouping monthly length frequencies.

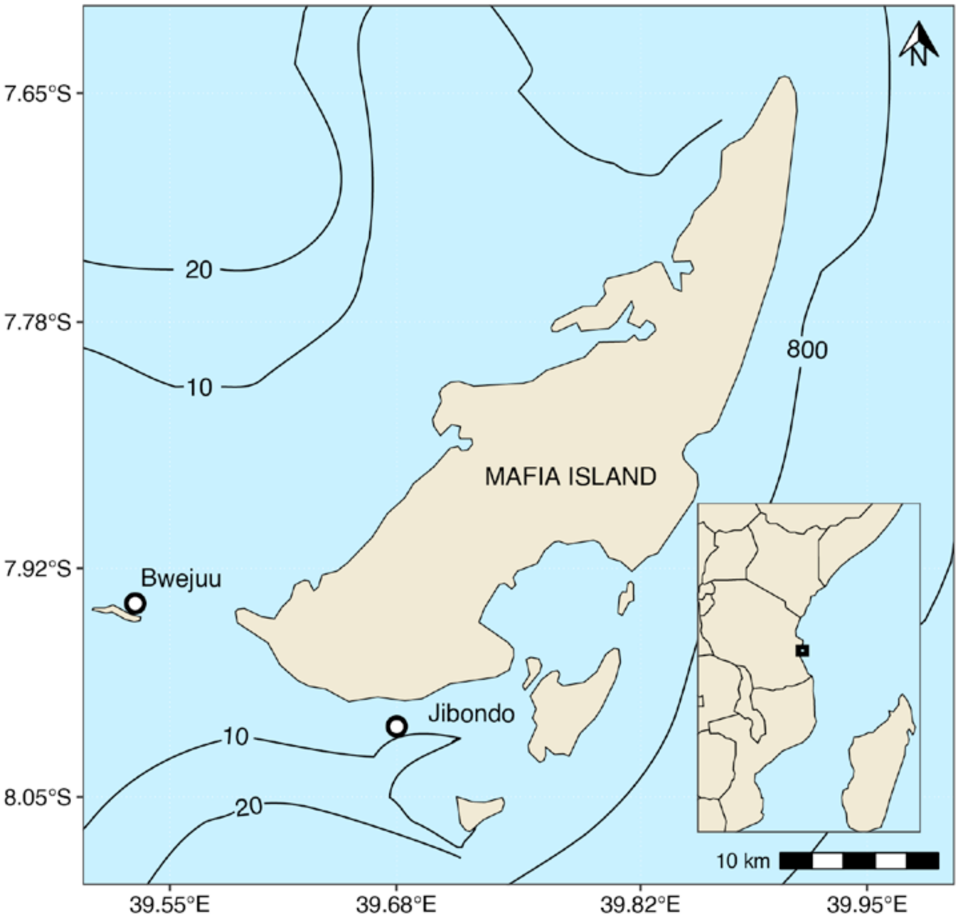

Figure 1. Map of Mafia Archipelago with the location of the study sites. An inset map of the western Indian Ocean locating the position of Mafia in the region. The label lines are depth isobars.

Instantaneous natural mortality' and fishing mortality 'F'

The instantaneous natural mortality of octopuses (M) was estimated with the empirical formula of Pauly (1980, 1983).

$$Log_{10}M = 0.0066 - 0.279Log_{10}L + 0.654Log_{10} + 0.4634\log_{10}T$$

Where *L* and *K* were derived from the Von Bertalanffy Growth function and T is the average sea surface temperature (SST) for the study area. Fishing mortality (F) was then calculated from the function $F = Z - F$

Exploitation ratio

The state of exploitation of *O. cyanea* (E) was calculated from the equation F/Z (Appeldoorn, 1988), which is the fraction of the total mortality (Z) caused by fishing mortality (F).

Octopus recruitment pattern

The octopus relative yield-per-recruit (Y/R) and relative biomass-per-recruit (B/R) was determined by the knife-edge recruitment method, which is described as a yield-per-recruit by Beverton and Holt (1959) and has been integrated into the FiSAT programme to assess recruitment (Pauly, 1983; Gayanilo Jr *et al.*, 1996). Analysis of the recruitment patterns was carried out in FiSAT using the maximum likelihood approach of the separation of the normally distributed components of the sampled length-frequency (NORSMSEP) to fit the Gaussian assumptions for the length-frequency data for the years grouped (Pauly, 1983; Gayanilo Jr *et al.*, 1996).

Results

Length-weight relationships

The regression analysis of the *O. cyanea* length-weight relationship at Jibondo and Bwejuu sites, respectively, were computed by the equations: $W = 0.0032DML^{2.2809}$ ($r^2 = 0.7615$); and $W = 0.0058DML^{2.1623}$ ($r^2 = 0.6838$). The value of *b* at Jibondo was 2.28 compared to that of Bwejuu, which was 2.16, and both sites had relatively lower *b* values compared to a typical value of 3. The lower *b* value suggests an increase in the size of different organs or parts of the octopus at various rates during growth (Fig. 2).

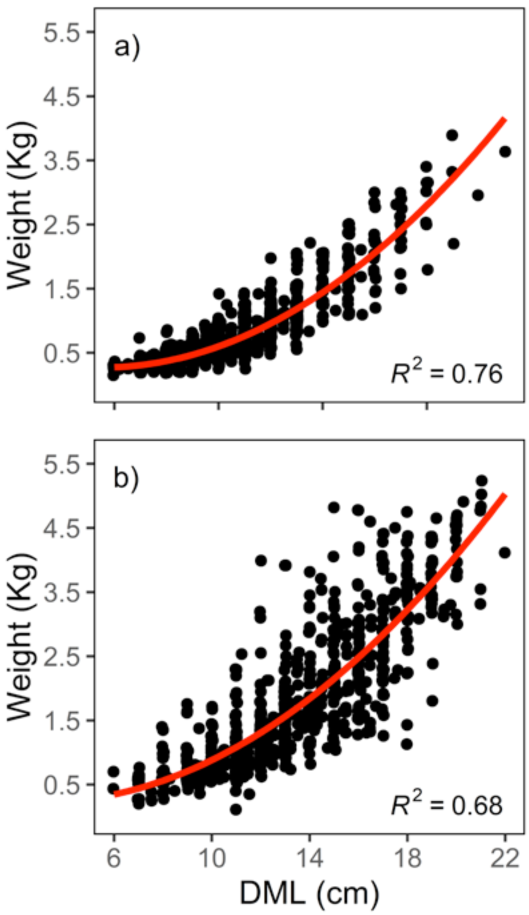

Figure 2. Length-weight relationship of *Octopus cyanea* sampled at a) Jibondo and b) Bwejuu islands in Mafia Archipelago.

Mortality coefficient (Z, M and F) and exploitation rate (E)

The result of the mortality (Z, M, and F) and exploitation rate (*E*) of *O. cyanea* at Jibondo and Bwejuu is presented in Table 1. Total mortality, natural mortality, fishing mortality and exploitation rate are all higher at Jibondo than Bwejuu. In addition, this study showed higher fishing mortality at Jibondo at a relative smaller

Table 1. Estimated mortality parameters for *Octopus cyanea* in Mafia Archipelago.

Site	Z	M	F	E	F – Class (DML)
Jibondo	5.24	2.48	2.76	0.53	08 - 12
Bwejuu	3.63	2.14	1.49	0.41	18 - 20

Z = Coefficient of total mortality, M = Coefficient of natural mortality, F = Coefficient of fishing mortality and E = Exploitation ratio. F – Class = Octopus size class with higher fishing mortality

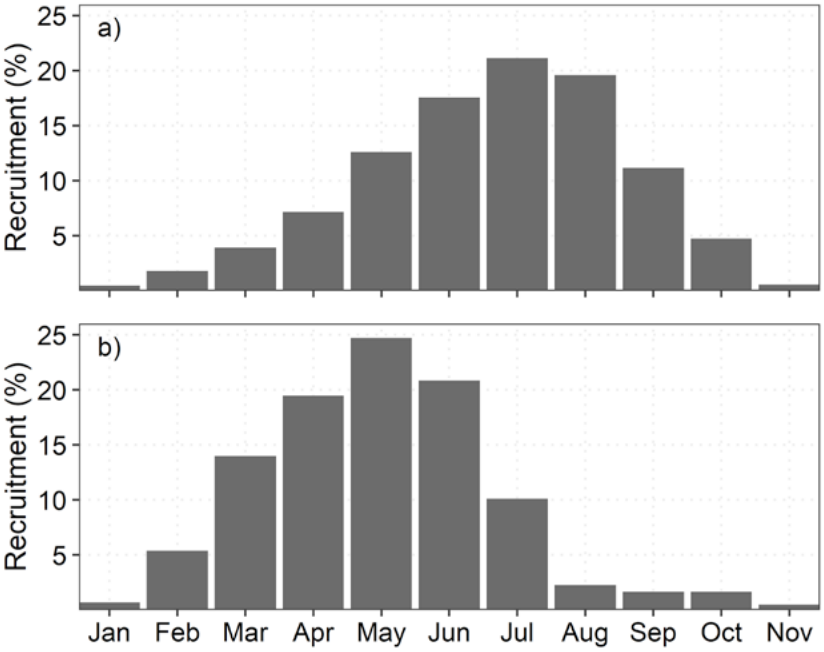

Figure 3. Relative yield-per- recruit and relative biomass-per-recruit of *Octopus cyanea* using the knife-edge recruitment method for Jibondo (a) and Bwejuu (b) fishing areas in the Mafia Archipelago (Green = $E_{0.1}$, Red = $E_{0.5}$ and Yellow = E_{max}).

octopus size class (8-12 cm, DML) while lower fishing mortality at Bwejuu at a relative higher size class (18-20 cm, DML).

Exploitation rate and relative coefficient rates

The estimated optimum exploitation rates from relative yield-per-recruit (Y/R) and related coefficient rates from relative biomass-per-recruit (B/R) analysis by the knife-edge selection for Jibondo and Bwejuu are presented in Figure 3. The exploitation rate 'E_{max}' that provides maximum relative yield-per-recruit was 0.380 and 0.379 for Jibondo and Bwejuu, respectively. The exploitation rate at which marginal increase occurred in the relative yield-per-recruit was similar for both sites, where $E_{0.1}$ was at 0.307. The exploitation rate $E_{0.5}$, which corresponded to 50 % of the unexploited stock relative biomass-per-recruit, was almost the same for Jibondo and Bwejuu; 0.248 and 0.247, respectively. The mean ratio of length at first capture L_c and asymptotic length L_∞ was 0.41 for Jibondo and Bwejuu, while that of natural mortality and growth rate was 2.06 and 1.42 K yr^1 for Jibondo and Bwejuu, respectively (Table 2).

Octopus recruitment pattern

The recruitment pattern of *O. cyanea* for Jibondo and Bwejuu is presented in Figure 4. Recruitment was year-round with one major peak. Length at infinity (L∞) was 23.1 for both sites, while the K values were 1.2 and 1.5 for Jibondo and Bwejuu, respectively. In the analysis, C = 0, WP = 0, and t_o = 0, were set similarly for both sites. The percentage recruitment values for Jibondo and Bwejuu respectively for the different months were: January (0.67 and 0.27 %); February (5.17 and 1.61 %), March (13.74 and 3.93 %); April (19.47 and 6.99 %); May (24.67 and 12.57 %); June (20.92 and 17.60 %); July (9.98 and 21.09 %); August (2.12 and 19.73 %); September (1.49 and 11.04 %); October (1.37 and 4.72 %); November (0.39 and 0.44 %); and December (0.00 and 0.00 %), as shown in the Figure 4.

Table 2. Estimated optimum exploitation rates and relative coefficient rates of *Octopus cyanea* at Mafia Archipelago.

Site	E_{max}	$E_{0.1}$	$E_{0.5}$	L_c	L∞	L_c /L∞	K	M/K
Jibondo	0.38	0.307	0.248	9.54	23.1	0.41	1.2	2.06
Bwejuu	0.379	0.307	0.247	9.5	23.1	0.41	1.5	1.42

E_{max} = Exploitation rate that gives maximum yield-per-recruit, $E_{0.1}$ = Exploitation rate at which relative yield-per-recruit was 10 %, $E_{0.5}$ = 50 % of unexploited stock, L_c = Length-at-first capture, L_∞ = asymptotic length, M/K = Ratio of natural mortality (m) and growth rate (K)

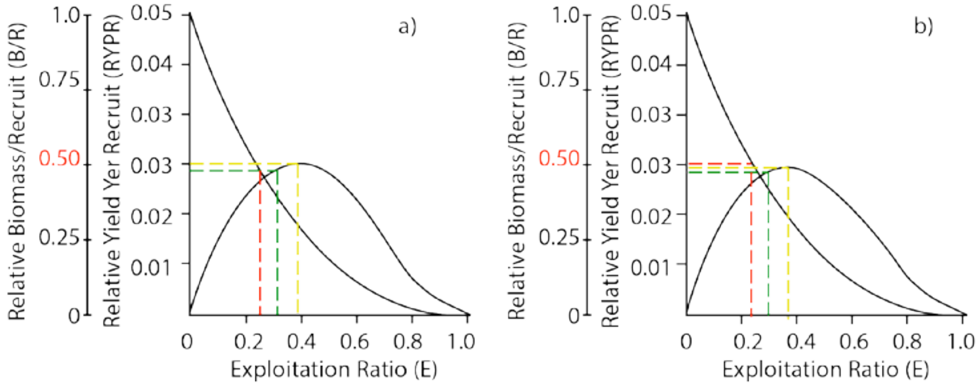

Figure 4. Recruitment pattern of *Octopus cyanea* of Mafia Archipelago. Jibondo L_α = 23.1, K = 1.5, WP = 0, t0 = 0 (a) and Bwejuu (b) L_α = 23.1, K = 1.2, WP = 0, t0 = 0.

Discussion

The octopus fishery in the Mafia Archipelago is an important economic and subsistence activity for local coastal communities. Octopus fishing dominates small-scale fishing effort at the islands which have a shallow continental shelf with coral reefs, and is a particularly important economic activity for women as well as men. Octopus provides food and income that support livelihoods for the local communities and forms an important element of the local ecology. However, despite its economic and ecological importance, exploitation ratios and recruitment periods of octopus in the Mafia Archipelago is poorly understood. This study is the first to reveal important information on the exploitation and recruitment patterns of octopuses at Bwejuu and Jibondo islands.

This study found that exploitation of octopus as expressed by yield-per-recruit and biomass-per-recruit has increased in the study area in recent years. The increased fishing pressure has resulted in the landed catches of octopus being dominated by small size classes. The maximum exploitation rate (Emax), which is expected to contribute to the maximum relative yield-per- recruit, was estimated at 0.380 and 0.379 for Jibondo and Bwejuu, respectively (Fig. 3 and Table. 2). Interestingly, these estimated exploitation values were lower compared to 0.53 for Jibondo and 0.41 for Bwejuu found in this study (Table. 1). The high exploitation values suggest that the *O. cyanea* stocks are overfished in terms of both yield- and biomass-per-recruit, as described by Gulland (1971).

On the other hand, the exploitation rate for Jibondo, which is characteristic of numerous fishing villages with many fishers in Tanzania (McClanahan and Cinner, 2012), was higher with E = 0.53. The high values of 'E' indicate a high fishing rate. Furthermore, the higher 'E' value corresponded with a relatively small size class 8-12 cm DML. In comparison, the exploitation rate at Bwejuu was lower (0.41), and the octopus were of a much larger size-class of18-20 cm DML. Based on the assumption that in an optimally exploited stock, natural mortality (M) and fishing mortality (F) should balance ('E' = 0.5 (E = F/Z = 0.5)) as suggested by Gulland (1971), then it is evident that small-sized octopus at Jibondo are exposed to high fishing pressure.

The link between high exploitation pressure and smaller fish sizes has been reported for the crab *Callinectes amnicola* in West Africa (Abowei *et al.,* 2010), the garfish *Belone euxini* in the Black Sea (Ceyhan *et al.,* 2019) and *O. cyanea* in the Indian Ocean (Guard and Mgaya, 2002; Jhangeer-Khan *et al.,* 2015). In the Fernando de Noronha Archipelago (tropical oceanic island of Brazil), however, the variation of the octopus size was caused by depth gradient between sites rather than fishing pressure. Small-sized octopuses preferred shallow habitats, and large-sized octopus were likely to be found in deeper water habitats (Leite *et al.,* 2009). This was not the case for the two sites accessed by fishers in the Mafia Archipelago where the depth difference between them was negligible.

Despite estimates made in this study, additional scientific information is needed to adjust the mortality values since the method used (length at age) assumed natural mortality of the octopus is constant throughout its life span. It is however known that adult females die after spawning, which results in changes from a female-dominated sex ratio in all seasons to equally represented sex rations after spawning has occurred (Herwig *et al.,* 2012; Arreguín-Sánchez *et al.,* 2000). However, mortality values obtained give a better understanding of how fishing pressure is likely to define octopus sizes that are caught, which has been found to be important for the management of the fishery.

Like previous studies which found that octopus spawn throughout the year, this study also found that *O. cyanea* recruits all year round at Mafia, but with a peak between May and July. This peak coincides with the SE monsoon that occurs between May and September (Semba *et al.,* 2019). The low sea surface temperature and high magnitude of wind with nutrient enrichment from the land after the long rain season during the SE monsoon season provides the ideal conditions for octopus spawning, growth and recruitment. These findings are similar to those from previous studies conducted in Rodrigues Island (Sauer *et al.,* 2011) and Madagascar (Raberinary and Benbow, 2012) in the WIO, where recruitment peaks occurred around May and July. In Madagascar, the recruits of octopus showed an additional lower peak in October and November (Raberinary and Benbow, 2012). The peaks of octopus recruitment in Madagascar are contrary to the findings of this study where only one peak was evident. In general, year-round recruitment is perhaps a compensation for the high octopus mortalities found in this and some other studies (O'Dor, 1998; Raberinary and Benbow, 2012).

This study also used the M/K ratio to examine the accuracy of growth parameter indices, which are constant for a group of species or closely related families of taxa (Chakraborty, 2001; Charnov *et al.,* 1993). The M/K ratio for the octopus in this study was 1.42 for Bwejuu and 2.06 for Jibondo, and the values are within the recommended range indicating good environmental state (Beverton and Holt, 1959; Silas *et al.,* 1985; Mohamed, 1996). In addition, the 'b' value from the fitted power equation suggested an increase in the size of different organs or parts of the octopus at various rates, as has been described previously for the WIO octopus (Guard and Mgaya, 2002).

During this study, *O. cyanea* was found to be overexploited, which was evident from the higher exploitation levels found in areas where there are many fishers. This suggests that fishing mortality could be controlled to attain the maximum yield and biomass per recruit that has already been overshot. However, fishing mortality estimated in the study needs to be adjusted based on additional information because larvae and post-spawning mortalities are higher than for the adult octopuses. This analysis assumed octopus mortality to be constant across age. Recruitment was found to be occurring all year round, with one major peak in the SE monsoon season. However, further research is needed to confirm the recruitment pattern, as in some other parts of WIO, recruitment peaks occur twice a year. Management measures could include better control over the issue of fishing licences, spatial rotation of fishing activities and voluntary octopus fishing closures to address the current excessive fishing pressure which has resulted in the small-sized octopuses that are landed.

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Effects of different types of manure on the culture of marine plankton as a potential source of food for mariculture hatcheries

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The study examined different types of organic manure on the culture of marine plankton as a potential source of food for rabbitfish, *Siganus stellatus*, larvae. Cow dung showed significantly higher species abundance and diversity of cultured marine plankton followed by chicken and finally mixed media manure ($p<0.05$). A total of 36 genera of phytoplankton (21) and zooplankton (15) were identified in all culture media. Class Bacillariophyta was the most abundant and diverse group which accounted for 41.3 % of the total phytoplankton. Calanoida was the dominant group of the identified zooplankton, accounting for 51.7 %. It was observed that the organic manure used favoured the growth of commercially important species of phytoplankton such as *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp. and *Spirulina* sp., and zooplankton such as *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods. The growth performance and survival rate of early stage rabbitfish larvae fed with live marine zooplankton performed better compared to those fed with *Artemia* spp. and commercial dry feed. This indicates that zooplankton have the potential to enhance growth performance and survival rate, hence increasing productivity and the development of mariculture.

Keywords: marine plankton, organic manure, cow dung, chicken droppings, mariculture

Introduction

There is continued and growing demand for alternative and proper nutritive food for commercial marine finfish and shellfish species, especially during larval stages in captivity (Evjemo *et al.,* 2003; Iyiola, 2014; Delbos, 2019). Marine zooplankton are appropriate food for them due to their nutritious quality, and the newly hatched nauplii are rich in essential fatty acids (EFA), polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA), and omega-3 fatty acids in the most adequate ratios (Delbos, 2019). Other common food (Rotifers and *Artemia* sp.) are deficient in highly unsaturated fatty acids (HUFA) that are essential for better growth performance and survival rate of marine fish larvae (Evjemo *et al.,* 2003; Delbos, 2019). There are various technologies developed worldwide to culture plankton, especially microalgae and a few species of zooplankton such as rotifers, *Atermia* spp., and copepods (Creswell, 2010; Dhont *et al.,* 2013; Delbos, 2019; Mtaki *et al.,* 2021). Most of these advanced technologies have been used for freshwater phytoplankton and zooplankton while there is limited information for marine plankton culture for coastal marine fish hatcheries. These efforts have also not considered small fish farmers who would struggle to afford the technology and materials for further development and improved production. Furthermore, of marine fish nursery production and commercial

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hatcheries in coastal areas are not fully integrated with the production of live food in large commercial facilities to meet the demand of small fish farmers.

Mariculture hatcheries in coastal regions are facing challenges with rearing early stages of marine fish species. In addition, commercial and local dry diets are used which are not ideal for better growth and survival rate of larvae and fry stages due to their small size and under developed digestive system. They are unable to provide easily digest dry feed compared to live food which has been shown excellent quality nutrients for marine fish species (Evjemo *et al.,* 2003; Olurin *et al*., 2012, Delbos, 2019). Commercial *Artemia* spp. and other genera of calanoid, harpacticoid and cyclopoid have been reported as ideal live feed in several geographic areas (Dhont *et al.,* 2013; Delbos, 2019). Despite these positive findings, rotifers and *Artemia* continue to be the live feeds of choice in commercial hatcheries, because copepods are not currently cultured at sufficient densities to be economically efficient on a commercial scale (Dhont *et al.,* 2013). Furthermore, there is limited technology for culture especially using saltwater for marine hatcheries. Also, the use of single species for fish culture which is most practiced is not recommended because mixed species of live food are more nutritious than using single species. There is a need to provide a nutritive variety of live food to larvae and fry stages, hence improving their growth, survival rate, and immune system to resist diseases. Furthermore, the collection and use of plankton directly from the wild is not advisable due to susceptibility to pathogens and parasites. Because of this, the plankton should first be obtained from the wild as seed, isolated and pure strains cultured as potential live food in the hatchery to avoid infections from the wild and harmful toxin-producing species.

Previous studies have reported that plankton culture and its production depends on the type of manure used (whether organic or inorganic) to facilitate their growth in the water medium (Wurtz, 2009; Abu Hena and Hishamuddin, 2012). A wide variety of organic materials have been used to promote the growth of plankton as well as stimulate the development of invertebrates and other micro-organisms from aquatic systems (Iyiola, 2014). Manures from chickens, goats, rabbits, sheep, cattle and horses have been reported as excellent fertilizers for culturing plankton (Iyiola, 2014; Wurtz, 2009). Most of these studies were based on freshwater plankton and there is limited information for marine plankton culture. The present study was conducted to evaluate the effect of using different types of manure on plankton abundance, to conduct a qualitative and quantitative analysis of cultured marine phytoplankton and zooplankton, to determine nutrient of media culture used, and the potential of live marine zooplankton as a source of food for rabbitfish *S. stellatus* larvae. Furthermore, environmental parameters for culturing marine plankton were determined.

Materials and methods

Collection of manure and culture media preparation

Cow dung and chicken droppings were collected from the local farming villages of Muungoni, Bambi and Muyuni in Unguja Island, Zanzibar as culture media for marine plankton. Precaution was taken to ensure that the manure was not contaminated with treatments that are sometimes used for livestock to control flies and which may inhibit the growth of some zooplankton, especially cladocerans (Oladele and Omitogun, 2016). The manure was transported in dry, clean zip bags to the wet laboratory at the Institute of Marine Sciences, University of Dar es Salaam. The manure was allowed to dry in sunlight because moisture content can affect manure quality (Bocek and Gray, 2002). According to Oladele and Omitogun (2016) the recommended dosage rate for culture using organic manure is 1.5 g/L. For this study 150 g of manure was added in 10 L of salty water to obtain culture media for each treatment. The culture media was allowed to remain in water for 3 days for nutrients to mineralize into the water. Thereafter, filtration was conducted using a 1 mm mesh size net to eliminate unwanted particles and organisms, followed by autoclaving using an HV-85 Top-Loading 85L Autoclave (Amerrex Instruments, Inc. United States) at a temperature range 105-135 °C. The culture media were then allowed to cool and inoculated into each polyethylene bag culture system treatment (cow dung, chicken droppings and mixed, separately in replicates) and left for 4 days with sufficient light (50-watt bulbs) for phytoplankton growth at a room temperature of 27 °C maintained with air conditioners, while each culture system was equipped with an aeration tube for oxygenation.

Sampling procedure and experimental design

The collection of marine phytoplankton and zooplankton was conducted by using nets with mesh sizes of 30 μm and 80 μm respectively, from the wild near the shore of Buyu, Unguja Island, Zanzibar. 50 L of water was concentrated in a phytoplankton net while zooplankton were collected by towing the net behind the boat on the surface at very low speeds for 15 minutes to concentrate the samples for zooplankton culture. The concentrates of phytoplankton and zooplankton were kept alive separately in 10 L plastic containers containing autoclaved sea water aerated with a battery-operated pump, and thereafter placed in the prepared polyethylene culture systems (with volume of 100 dm^3). A total of 24 (12 for phytoplankton and 12 zooplankton) polyethylene bag culture systems were used for marine plankton culture experiment (Fig. 1). Four treatments were used (sea water only as a control; cow dung media; chicken droppings media; and a mixture of cow dung and chicken droppings media) at the ratio of 50:50 by dry weight, respectively. Live concentrated marine plankton was added to each culture treatment (100 dm^3 culture system contained sea water with media culture) in equal amounts of 1 L. All experimental treatments were triplicated.

Nutrient determination of media culture used

500 ml of water from the culture media was prepared by filtering through 0.45 µm millipore filter paper in a filtration pump unit. A SHIMADZU Spectrophotometer UV-1201-Japan was used to determine nitrate and phosphate concentrations according to Parsons *et al.* (1984). Nitrate was determined by taking a 100 ml sample in an Erlenmeyer flask and adding 2 ml of ammonium chloride (NH_4Cl). 5 ml of the mixture was poured onto the top cadmium-copper column and allowed to pass through. The remaining sample was added into the column after which the Erlenmeyer flask was used to collect 50 ml of the sample at the bottom of the collection tube. 1.0 ml of sulphanilamide solution was added in the 50 ml sample using a micro-pipette and allowed to react for 5 minutes. 1.0 ml of naphthyl ethylenediamine solution was then added and the contents mixed immediately. The extinction of a solution in a 10 cm cuvette was measured at 543 nm wavelength. Nitrate was determined using the equation:

$$\mu\text{g-at N/l} = (\text{corrected extinction x F}) - 0.95C \;\ldots\ldots\ldots\ldots(1)$$

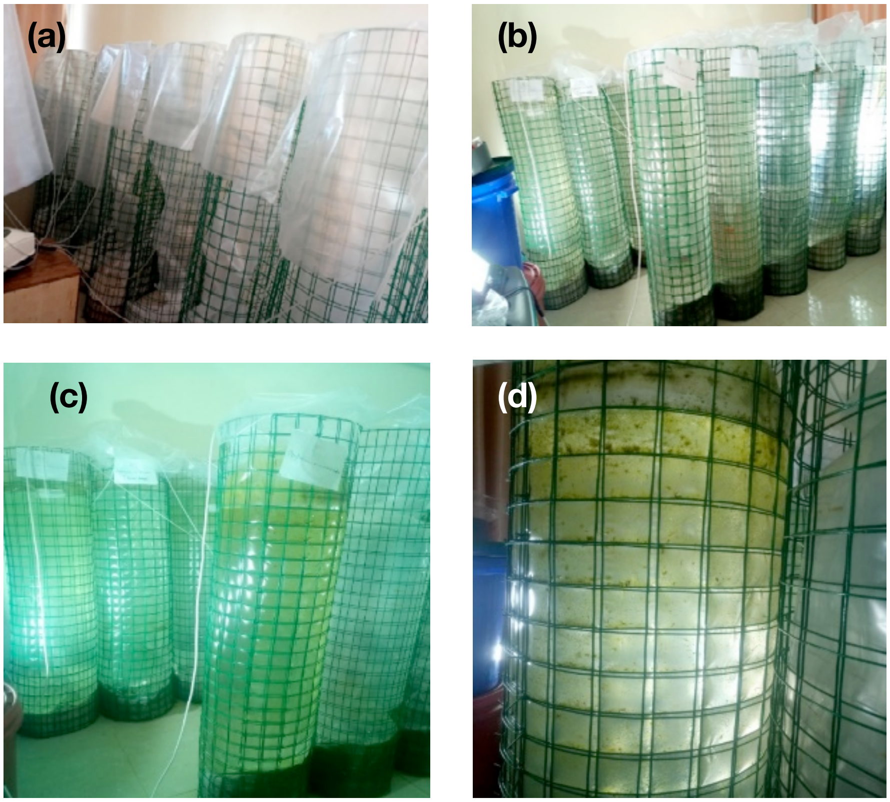

Figure 1. (a) Experimental setup before the polyethylene bags culture system was filled with sea water; (b) Setup after polyethylene bags culture system was filled with sea water and media inoculation; (c) colour changes started to be observed in a third week; and (d) complete colour change (greenish and brown) after sixth week with filamentous algae observed.

Where: C = Concentration of nitrite in the sample in µg-at N/l.

Phosphate was determined by taking a 10 ml sample (in duplicate) and adding 1 ml of mixed reagent (Ammonium molybdate solution, Sulphuric acid, Ascorbic acid and Potassium antimonyl-tartrate at a ratio 2:5:2:1, respectively) using a micropipette and mixed. After 5 minutes the extinction of a solution was measured in a 10-cm cuvette against distilled water at 885 nm wavelength. Phosphate concentration was then determined using the equation:

µg-at P/l = corrected extinction x F.............................((2)

Where F is a factor of a standard solution. The standard curves for nitrates and phosphates were determined through serial dilutions of analytical grade Potassium Nitrate (KNO_3) and analytical grade Potassium di-hydrogen phosphate (KH_2PO_4) for nitrate and phosphate respectively.

Qualitative and quantitative analysis of cultured marine plankton

Population abundance and diversity of cultured marine plankton was determined by measuring 3 ml out of a concentrated 20 liters of water from the culture system after stirring thoroughly to ensure even distribution of organisms in the beaker. Identification and counting of specimens in each sample was conducted by using a Sedgewick-rafter cell and inverted microscope using the guide provided by Conway *et al.* (2003). The phytoplankton cells were identified using a guide by Bryceson (1977).

Feeding and sampling of rabbitfish larvae for comparison of growth and survival rate

A total of 270 larvae and fry (with an average weight of 0.25 g and length of 1.32 cm) of *S. stellatus* were collected from Chwaka Bay in Unguja Island using a trap and seine nets. Nine aquaria (dimension of 3600 cm^2 and volume 80 dm^3) were used to examine the growth and survival rate when fed with the live cultured marine zooplankton *Artemia* spp. and a commercial dry feed. All experimental setups were triplicated. Commercial dry powder feed (35 % crude proteins) was bought from Eden Agri-Fish Farm Limited together with *Artemia* spp. cysts. One gram of dry cysts was added to 1000 ml of aerated saline water (35 practical salinity units) followed by treatment with sodium hypochloride to decapsulate cysts and left for 24 hours for eggs to hatch (Asem, 2011). Phytoplankton in this study were harvested at an interval of one week after observing clear greenish/brownish colour in the polyethylene culture system. 1.5 L of phytoplankton was used in the zooplankton culture systems to ensure continuous production of zooplankton in each culture system for feeding rabbitfish larvae and fry. Each aquarium had 30 individual fish larvae and fry. Weight differences between them were taken into consideration. Larvae and fry were fed four times a day (9:00 am, 12:00 pm, 3:00 pm and 5:00 pm). Mean weight and length measurements were taken at an interval of 7 days after recording initial weight and length. Length measurements were taken with a transparent ruler (mm/cm) and weight (mg/g) measurements were recorded with a very sensitive electronic balance.

Determination of growth performance indices of S. stellatus, and water quality parameters

Growth performance indices including final body weight (FBW), body weight gain (BWG, %), specific growth rate (SGR, %/day), feed utilization in terms of feed conversion ratio (FCR), and survival rate were measured according to Ridha (2006). In all culture systems and aquaria, water quality parameters such as pH, dissolved oxygen, temperature and salinity were measured twice a day at 10:00 pm and 4:00 pm using a pH meter, DO meter and refractometer, respectively.

Data analysis

All data were tested for normality and homogeneity using Levene's test at a significance level of $p<0.05$. Population abundance, species diversity and similarity of marine plankton cultured with different types of manure were determined with the Shannon-Wienner index of diversity by using Plymouth Routines in Multivariate Ecological Research (PRIMER) 6.1.2 version software. Paleontological Statistics (PAST) 3 version software was used for Analysis of Variance (ANOVA) to compare the means and state the significant differences followed by pairwise-comparison. Constrained ordination analyses of plankton cultured with different media were performed where abundance data were square root transformed to reduce the contribution of most proportion species. The Bray-Curtis similarity matrix was then generated followed by clustering analysis. A complete linkage dendrogram plot and Multidimensional Scaling Map (MDS) was generated. Growth performance, survival rates, means body weight of rabbit fish larvae and fry were compared using ANOVA followed by Turkey post hoc test.

Table 1. Mean values plus the standard error of the mean of water quality throughout the experimental period.

Water quality parameters	Media culture used (Mean value ± SE)			
	Cow dung	Chicken droppings	Mixed media	Sea water only
Temperature (°C)	27.3 ± 0.27	27.0 ± 0.28	26.9 ± 0.28	27.1 ± 0.28
pH	8.54 ± 0.02	8.63 ± 0.09	8.54 ± 0.04	8.63 ± 0.01
Oxygen saturation (%)	97.55 ± 0.21	94.3 ± 4.62	97.9 ± 0.99	94.15 ± 2.61
Dissolved oxygen (mg/l)	6.25 ± 0.19	6.15 ± 0.54	6.54 ± 0.16	6.21 ± 0.15
Salinity (psu)	34.8 ± 0.14	34.65 ± 0.07	34.85 ± 0.35	34.75 ± 0.35

Results

Water quality parameters in marine plankton cultures

In all treatments water temperature ranged from 25.65 °C to 29.05 °C, dissolved oxygen varied from 4.05 to 8.45 mg/L, pH varied from 7.3 to 8.4, and salinity ranged from 33.9-35.8. The mean of all water quality parameters monitored are presented in Table 1. No significant differences were observed among all treatments ($p > 0.05$) in all water quality parameters. All water quality parameters were within the safe range for the growth of phytoplankton and zooplankton.

Nutrient concentration in media culture used

Nutrient analysis of all manure and sea water (not fertilized) was carried out for nitrates and phosphates, and the results are presented in Table 2. There was a significant difference ($p<0.05$) in nutrient concentration in all culture media used, where cow dung had the highest concentrations of phosphates followed by chicken droppings, and mixed media having the lowest. Nitrate concentration was highest in chicken droppings followed by cow dung and mixed media respectively.

Community structure of marine plankton cultured in the different media

The results showed that there was a significant difference among all treatments in both abundance and species diversity of marine plankton ($p<0.05$). The species diversity was significantly higher in the cow dung culture medium followed by chicken droppings and mixed media culture. Poor results were observed in sea water medium culture as a control and all results were positively correlated with nutrient concentration.

A total of 36 genera of marine plankton were morphologically identified in all culture media, with 21 species of phytoplankton belonging to the Bacillariophyta (diatoms), Dinophyta (dinoflagellates), Mediophyceae, Chlorophyta (green algae), Cyanophyta (cyanobacteria) and Dictyochophyceae (Table 7 and Fig. 3). Overall, the class Bacillariophyta was the most abundant and diverse group and accounted for 41.3 % of the total phytoplankton in the culture experiment with 9 species. Cyanophyceae ranked second in terms of abundance (29.9 %) and this group was dominated by 7 species, followed by Mediophyceae (18.5 %). Other classes had less than 10 % and were each represented by single species. The first week of the phytoplankton culture system was dominated by *Guinardia delicatulata, Chaetoceros* sp.*, *Chlorella vulgaris***, Chlorophyta (green algae) and *Eucampia zodiacus*, and were followed by other commercial species after two weeks such as *Nannochloropsis* sp. and *Spirulina subsalsa*** and other filamentous algae.

Table 2. Nutrients concentration (µg. atom/L) of manure used during experimental culture.

Culture media	Nitrate (NO_3^-)	Phosphate $(PO_4)^{3-}$
Cow dung manure	2.1571 ± 0.01	0.6812 ± 0.02
Chicken droppings	2.1648 ± 0.03	0.64645 ± 0.01
Mixed manure	2.0052 ± 0.02	0.58755 ± 0.01
Sea water	0.0543 ± 0.0	0.0632 ± 0.00

Table 3. The presence (X) and absence () of phytoplankton species cultured from different manure.

Phytoplankton (Taxa group)	Species identified	Sea water only	Cow dung manure	Chicken manure	Mixed manure
Class Bacillariophyceae (Diatoms)	*Guinardia delicatulata*	X	X	X	X
	Melosira sp.			X	X
	,		X	X	X
	Leptocylindricus sp.		X	X	X
	Rhizosolenia alata		X	X	X
	Synedra formosa		X	X	X
	*Skeletonema costatum***		X	X	X
	Pleurosigma/Gyrosigma		X	X	X
	Amphisolenia sp.				X
Class Chlorophyceae	*Chlorella vulgaris***			X	X
Class Prymnesiophyceae	*Isochrysis* sp.**	X	X		
	Nannochloropsis sp.**				X
Class Mediophyceae	*Eucampia zodiacus*	X	X	X	X
Class Cyanophyceae	*Schizothrix arenaria*		X	X	X
	*Spirulina subsalsa***		X	X	X
	Johannesbaptistia pellucida			X	X
	Prorocentrum sp.		X	X	X
	Leptocylindricus sp.		X	X	X
	Rhizosolenia alata		X	X	X
	Synedra formosa		X	X	X
Class Dinophyceae	*Phalacroma rotundatum*			X	

**Commercially important species for mariculture development.

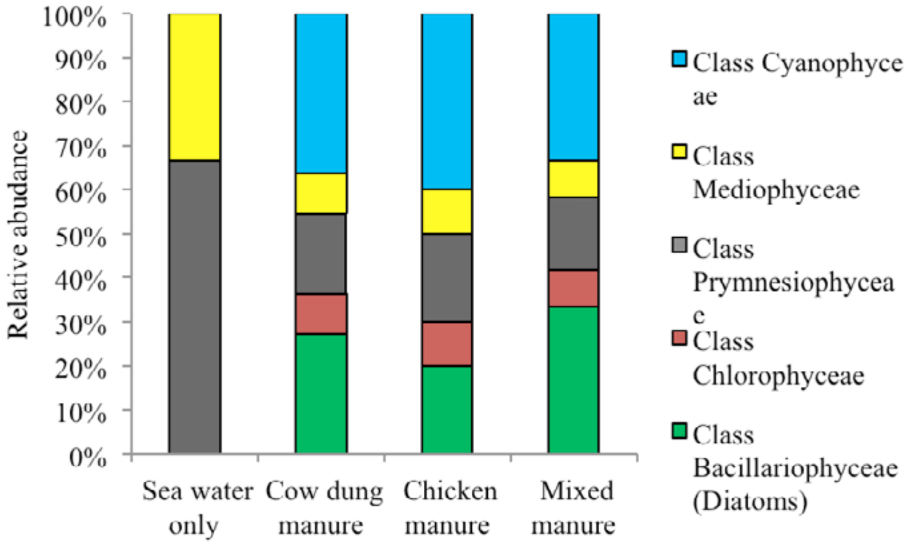

Figure 2. Relative abundance of phytoplankton species in the culture media treatments.

Table 4. The presence (X) and absence () of zooplankton species cultured from different manure.

Zooplankton (Taxa group)	Species identified	Sea water only	Cow dung manure	Chicken manure	Mixed manure
Calanoida	*Eurytemora affinis*		X	X	X
	*Canthocalanus pauper***		X	X	X
	Pseudocalanus sp.		X		X
Cyclopoid	*Oithona helgolandica***		X	X	X
	Centropages furcatus		X		X
Harpaticoda	*Microsetella* sp.		X	X	X
	Corycaeus agilis	X	X	X	X
Euphausiidae	*Paracalanus parvus*		X	X	X
Cirripedia	*Nauplii*	X	X	X	X
Rotifera	*Brachionus* sp.**		X		X
Cladocera	*Daphnia sp. (Moina micrura)* **		X		X
Cnidaria	*Hydroid medusae*		X	X	X
Ostracoda	*Ostracods* sp.**		X		X
Decapoda	*Caridina nilotica***				X

**Commercially important genera/species for mariculture development

Fifteen genera of zooplankton were identified belonging to major groups such as Calanoida, Euphausiidea, Cirripedia, Rotifera, Cyclopoida, Harpaticoida, Cladocera, Ostracoda, Decapoda and Cnidaria (Table 4 and Fig. 3). Of these, the Calanoida was the dominant group which accounted for 51.7 % followed by Cyclopoida (32.6 %) of all zooplankton identified (Fig. 4). In the first week of the zooplankton culture the system was found to be dominated by the calanoid *Canthocalanus pauper*, the cladoceran *Daphnia* sp. and the harpaticoid *Corycaeus* sp.

Cluster analysis and multidimensional scaling (MDS)

Cluster analysis and multidimensional scaling (MDS) were performed to find out the degree of similarity of the species composition of marine plankton. The hierarchical cluster analysis revealed the similar nature between the species composition of the individual culture media. The similarity was observed to be strong between cow dung and chicken manure at about 90 %, followed by 65 % for mixed manure compared with other culture media, while lowest

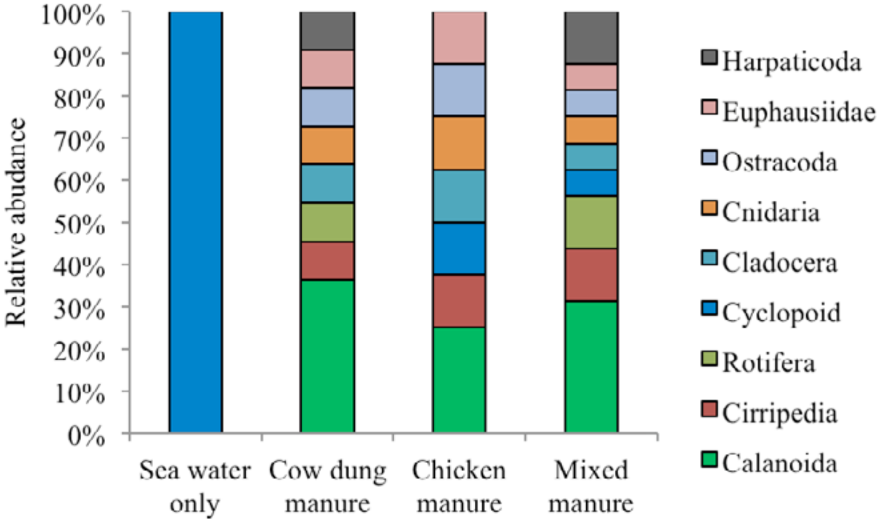

Figure 3. Relative abundance of zooplankton species in the culture media treatments.

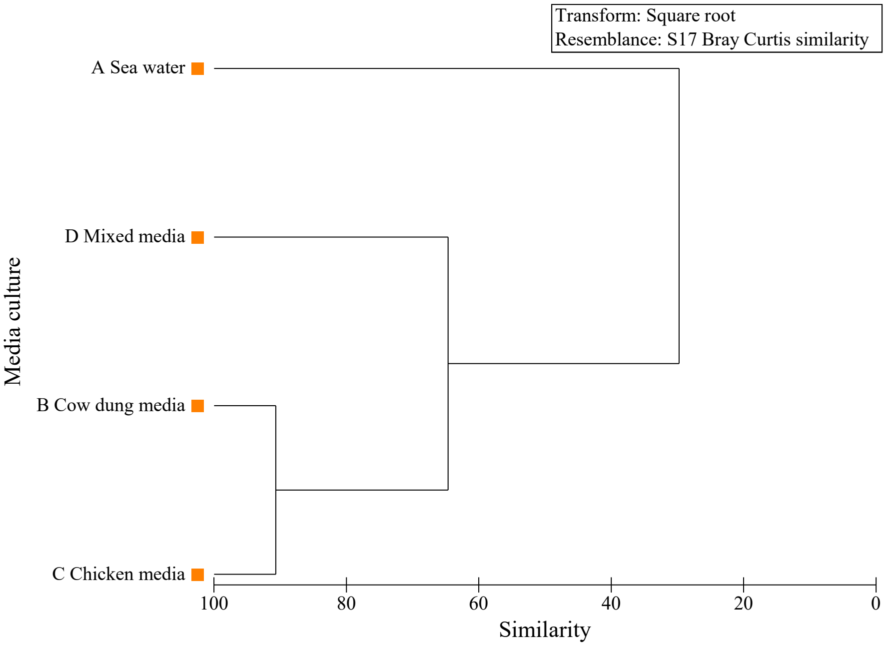

Figure 4. Species diversity percentage similarity of marine plankton (phytoplankton and zooplankton) grown in different manure treatments.

similarity of about 20 % was observed in marine plankton cultured in sea water only (Fig. 4). This was further confirmed by the MDS map (Fig. 5). The stress value was found to be less than 0.01 which is an excellent ordinance pattern that indicates distances among culture media are perfect and are a good representation of the observed data.

Growth performance and survival rate of rabbitfish larvae and fry

There was a significant difference in the growth performance and survival rate of *S. stellatus* larvae and fry fed with live *Artemia* spp. and commercial dry feed ($p<0.05$). The highest growth performance of rabbitfish larvae and fry in terms of final body weight (FBW),

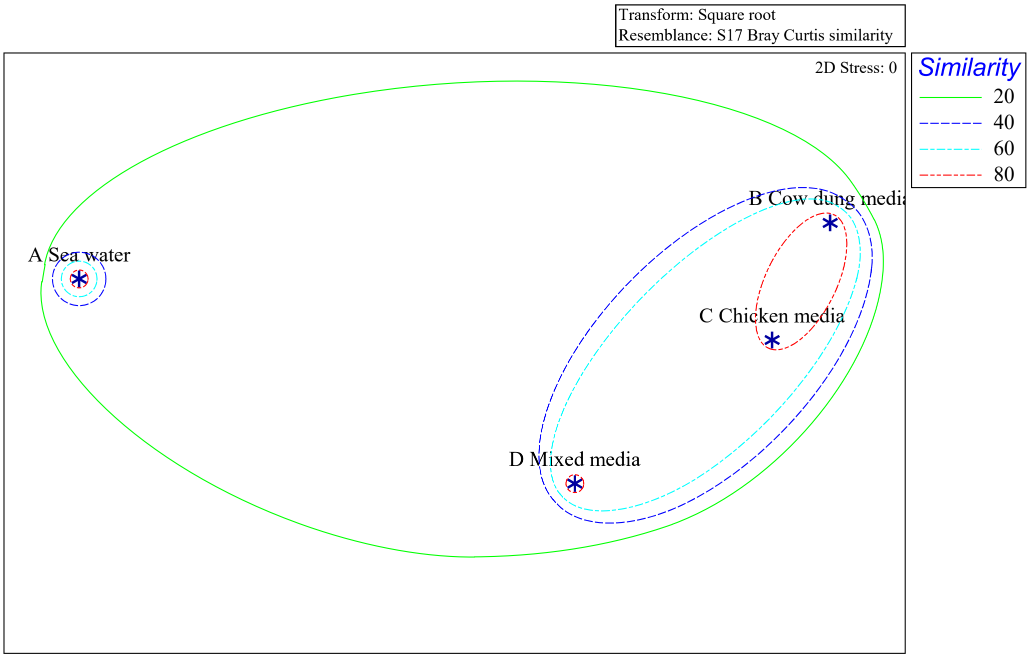

Figure 5. Multidimensional Scaling (MDS) for marine plankton composition of media culture used.

Table 5. The mean values of growth performance parameters of the rabbitfish *S. stellatus* larvae and fry.

Growth parameters	**Diet treatments**		
	Marine-live zooplankton	***Artemia* spp.**	**Commercial dry feed**
Initial mean body weight (g fish^{-1})	0.22 ± 0.03	0.23 ± 0.01	0.23 ± 0.05
Initial body length (cm)	0.6 ± 0.12	0.5 ± 0.02	0.5 ± 0.04
Final mean body weight (g fish^{-1})	6.05 ± 0.19	5.97 ± 0.23	7.22 ±0.32
Mean body weight gain (g fish^{-1})	5.83 ± 0.21	5.74 ± 0.23	6.99 ± 0.31
Final body length (cm)	2.4 ± 0.11	2.3 ± 0.11	3.9 ± 0.21
Specific growth rate (% day^{-1})	1.806 ± 0.00	1.789 ± 0.00	1.986 ± 0.00
Feed conversion ratio	2.08 ± 0.05	2.26 ± 0.05	1.72 ± 0.03
Survival rate (%)	96 ± 0.00	93 ± 0.00	86 ± 0.00

body weight gain (BWG) and specific growth rate (SGR) was observed in the fish fed with live marine zooplankton compared with other diet treatments (F (2,444) = 3.12, p = 0.04). This was followed by fish fed with *Artemi*a spp. while poor performance and low survival rate was recorded in the early stages with commercial dry feed. The best feed utilization efficiency of larvae and fry in terms of feed conversion ratio (FCR) was observed in fish fed with live marine zooplankton, followed by those fed with *Artemia* spp.. In both cases, slightly lower growth performance and the feed utilization efficiency was observed in the fish fed commercial dry feed. The survival rate in all treatments varied significantly at $p<0.05$. The mean values of growth performance, feed utilization efficiency, and survival rate of the rabbitfish larvae and fry reared in the 9 aquaria by feeding them 3 experimental diets are summarized in Table 5.

From Figure 6 it can be seen that the live marine zooplankton and *Artemi*a spp. performed better than commercial dry feed at the early stages. However, this trend changed after 21 days when good performance was observed for the fish fed commercial dry feed in terms of survival rate and body weight gain. These results imply that commercial dry feed shows better performance during the grow-out stage of rabbitfish as compared to the early larval stages.

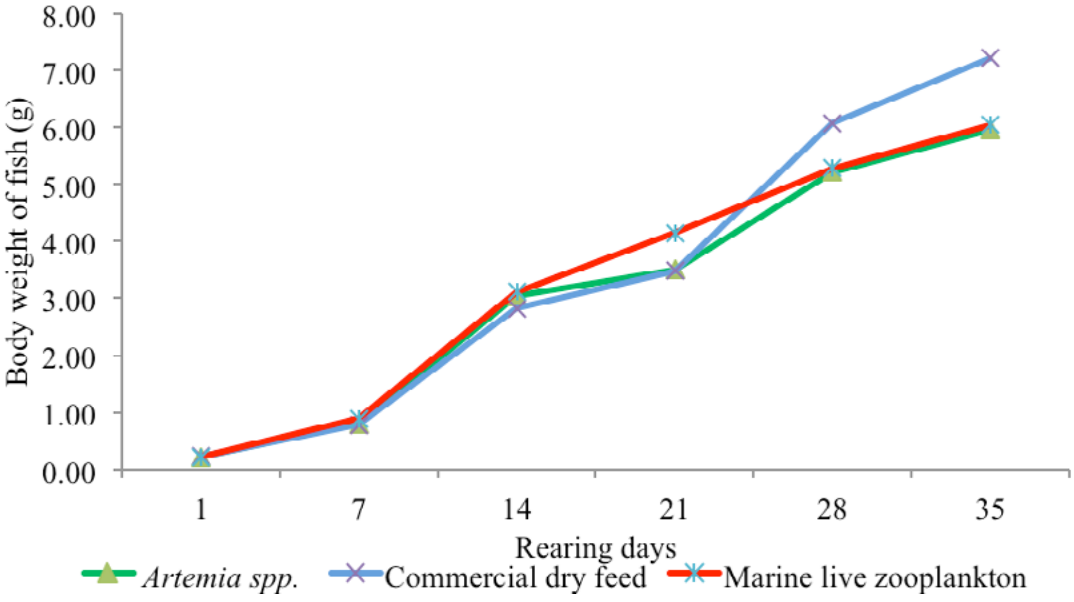

Figure 6. Growth performance of fingerlings of *S. stellatus* fed different types of diets.

Discussion

Water quality parameters in the plankton culture system

The culture of marine plankton (phytoplankton and zooplankton) is affected by different environmental factors such as water quality, including water temperature, salinity, pH, dissolved oxygen, and nutrient (phosphate, PO_4 and nitrates, NO_3) concentration. Zooplankton are totally dependent upon the water to breathe, feed, grow, excrete wastes, maintain salt balance and reproduce (Rottmann *et al.* (2003). However, phytoplankton depend mainly on the amount of nutrients and light available in order to grow. Therefore, ensuring adequate water quality parameters including light penetration is critical to successful culture of phytoplankton and zooplankton for aquaculture production (Olurin and Aderibigbe, 2006). In the current study, water quality parameters monitored included pH, oxygen, temperature and salinity.

All water quality parameters for culturing marine plankton were within the range for survival and growth of plankton. As recommended by Bocek and Gray (2002), the optimum temperature for culture of tropical aquatic organisms is between 25 – 32 °C; pH should range between 6.5 – 9.0, and the recommended level of dissolved oxygen in the culture system must not be less than 5.0 mg/L. The observed temperature values in this study fall within acceptable ranges for culturing plankton as recorded by Rottmann *et al.* (2003). Average dissolved oxygen (DO) was 6.31 ± 0.39 mg/L and the results of the present study are consistent with DeLong *et al.* (2009) and Bocek and Gray (2002), who recommended 5 mg/L as the minimum DO requirements for survival of aquatic organism in captivity. This was achieved through continuous aeration through the aerator pump.

Community structure of marine plankton in relation to nutrient concentration

The growth of phytoplankton depends mostly on the availability of nutrients such as phosphates and nitrates. In turn the phytoplankton becomes a source of food to zooplankton, fish larvae and other larger organisms. In the present study, adequate nutrient concentrations were observed in the cow dung and chicken droppings media, moderate levels in the mixed media, and poor levels in sea water. A linear relationship between nutrient concentration and the amount of phytoplankton and zooplankton was observed. The amount of nutrients in the manures used were slightly different from that reported in Ipinmoroti and Iyiola (2011) where the percentage nitrogen and phosphate in the form of dry weight of was 2.34 % and 0.24 % respectively; perhaps due to different geographical conditions and sources . The least amount of nutrients was recorded in the mixed medium and sea water control. The sea water only control experiment had the lowest concentration of phosphates and nitrates (Table 2) and had poor phytoplankton and zooplankton growth. The control produced low levels of zooplankton because of low algal/phytoplankton concentrations. This emphasizes that culture systems for raising phytoplankton and zooplankton must be adequately fertilized.

The cow dung culture media accounted for the highest plankton abundance and diversity followed by chicken droppings, and this was largely due to the high nutrient content (both nitrates and phosphates) available as shown in Table 2. This was similar with the research findings of Ipinmoroti and Iyiola (2011), and Wurtz (2009) where high nutrient composition in organic media was also important for the growth of plankton in captivity. However, according to their study based on freshwater zooplankton, poultry (mean mixed manure from chicken, birds and others) showed the best results for the growth of zooplankton. In this study the mixed growth medium had slightly lower nutrient concentrations, possibly due to dilution. A high level of similarity in marine plankton was observed between media with cow dung and chicken droppings, while a low similarity was observed with mixed manure media and sea water only. This might be due to differences in nutrient composition. Iyiola (2014) explained that the two important components of algal nutrients are phosphates and nitrates and both have a linear relationship with the amount and diversity of plankton production. Furthermore, it has been observed that the organic culture media with cow dung, chicken droppings and mixed media favour the growth of commercially important species of marine plankton such as the phytoplankton *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp, and *Spirulina* sp., and the zooplankton *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods, that can be used in mariculture. This study is supported by the results obtained by Creswell (2010), Conceição *et al.* (2010), Iyiola (2014), and Oladele and Omitogun (2016) who reported that some species such as *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Brachionus* sp., *Daphnia* spp., *Moina* sp. and cyclopods favour manure such as cow dung and chicken droppings and are widely used in hatcheries as startup food for fish larvae and fry stages.

Growth performance and survival rate of rabbitfish larvae and fry

Growth performance, feed utilization efficiency and survival rate are impacted by differences in the quality of the feeds in terms of nutrient composition and other environmental stress factors (Yousif *et al.,* 2005; Ogunji *et al.*, 2008). In this study the performance of three diets (live marine zooplankton, *Artemia* spp., and commercial dry-powder feeds) were tested on the growth, feed utilization and survival rate of rabbitfish larvae and fry. The highest growth performance of rabbitfish larvae and fry in the early stages of rearing in terms of FBW, BWG and SGR was observed in the fish fed with live marine zooplankton, followed by fish fed with *Artemia* spp. (as shown in Fig. 6). It is possible that the mixed species of zooplankton provide a wider diversity and richness of nutrients suitable for growth of rabbitfish. Studies by Delbos (2019) and Giri *et al.* (2002) show that mixed species of zooplankton are richer in proteins (approximately 42-65 crude protein), carbohydrates, minerals and essential fatty acids to promote growth compared to a single species and therefore promote better growth. Further, protein quality and quantity determine growth and survival of larvae and fry of the reared fish. Also, the studies of Furuya *et al.* (2004) and Owodeinde and Ndimele (2011) reported that the difference in crude protein content between feeds or food causes the differences in growth performances of the fish in captivity. Besides the protein content, ideal culture conditions could also contribute to faster growth and high survival rate of larvae and fry.

In support of the above, a study by Yousif *et al.* (2005) and Yousif *et al.* (2014) reported that protein content and ideal culture conditions including good water quality parameters, promote faster growth of rabbitfish in captivity. In this study, the mixed species of zooplankton that were cultured in different manures provided good protein sources for fish larvae and fry. Previous studies by Refstie *et al.* (1998), Conceição *et al.* (2010) and Shaheen (2013) have shown that zooplankton are the preferred prey for fish larvae due to their good nutrition, flavour, texture, digestibility, palatability and attraction to predators. In this study, mixed live zooplankton such as daphnia, copepods, rotifers and *Artemia* spp. were present in culture experiments and were used in growth performance comparison. The low growth performance, survival rate and slightly lower value of feed utilization efficiency of the fish fed on commercial feed could be due to the higher fiber content in feed used (7.3 %). A study by Ulloa and Verreth (2002) supported the fact that high dietary fiber levels could reduce growth performance, feed and protein utilization in fish and other domestic animals, regardless of amount of protein present. Also, low palatability and digestibility are considered possible causes for low growth performance, and this is more important for the first period of fish feeding, as reported by Ahmad *et al.* (2012). There was a slight difference between fish fed with live marine zooplankton and *Artemia* spp. Using mixed species of live zooplankton is crucial for fast growth, improved immunity to resist diseases and increased production due to higher survival rate of fish in the culture system. The intercepts of the graph in Fig. 6 has commercial implications in that dry feed can be used in the grow-out stage of rabbit fish culture. Commercial dry feeds promote healthy growth in juveniles, perhaps because zooplankton are very small to be fed by fish of this size. This was observed after 21 days in the present study where larvae and fry started to show a good response to commercial dry feed. This is similar to the results of the first study of this nature in the western Indian Ocean carried out by Bwathondi (1982) who reported that rabbitfish showed a good response when fed with artificial commercial dry feed, and grew better with these feeds during the juvenile and mature stages compared to other feeds.

In this study, the feed utilization efficiency recorded was within acceptable ranges for the growth of rabbitfish larvae and fry and other fishes such as Nile tilapia. Similar results were provided by Yousif *et al.* (2005) who reported an average value for FCR of 2.01 for rabbitfish fingerings fed with commercial dry ARASCO (Saudi Arabia) sinking feed. Also, studies by Ogunji and Wirth (2000) and Yousif *et al.* (2005) reported FCRs ranging from 1.19 to 3.5. Therefore, all FCRs obtained in this study (1.72-2.26) were within the acceptable ranges for the growth of rabbit fish larvae and fry.

Conclusion

The study revealed that there is good potential for culturing marine plankton (phytoplankton and zooplankton) by using media fertilized by manure and that the cultured marine plankton were a better source of food for rabbitfish larvae and fry than artificial feed during the onset of the feeding. It was observed that the types of manure used favour the growth of commercially important species of plankton such as the phytoplankton *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp., and *Spirulina* sp., and the zooplankton *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods that can be isolated, cultured as pure strains and used in mariculture. The study suggested that the use of cow dung provides excellent results and can be an alternative to chicken manure. However, other nutrients such as silica need be measured in the cow dung that are important for the growth of some genera such as diatoms. Phytoplankton culture can be used to ensure sustainable production of zooplankton in captivity for feeding larval stages of fish. For the growth and survival rate of rabbitfish *S. stellatus*, the study suggested the use of mixed species of zooplankton instead of single species in larvae and fry rearing, to improve growth and immunity of fish to resist diseases and further enhance the quality of fingerlings and development of mariculture hatcheries. The good response of the rabbit fish to commercial dry feed makes the species very suitable for culture in the western Indian Ocean region.

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Population characteristics and exploitation of yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788) in the coastal waters of Kenya

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Yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788) is a highly migratory and important commercial fishery species. Data on length-frequency, growth parameters and mortality rates of yellowfin tuna in the coastal waters of Kenya is limited. We assessed Catch Per Unit Effort (CPUE), size distribution, growth parameters and mortality rates based on length-frequency and catch data collected from August 2015 to December 2016 at five fish landing sites along the Kenyan coast. The sample comprised of 1281 individuals of yellowfin tuna weighing 12,671 kg. Highest CPUE was recorded in October 2015 (10.8 kg·Fisher^{-1}Trip^{-1}) and lowest CPUE, 2.6 kg·Fisher^{-1}Trip^{-1} in December 2016. Yellowfin tuna was more abundant in the South-East Monsoon (SEM), from May to October, with an average CPUE of 7.3 kg Fisher^{-1}Trip^{-1} compared to North East Monsoon (NEM), from November to April, with a CPUE of 7.0 kg Fisher^{-1}Trip^{-1}. Spatial variation of CPUE was evident. Old Town recorded the highest CPUE of 54.2 kgTrip^{-1}, while Mnarani the lowest of 19.4 kgTrip^{-1}. At least 91 % of the fish sampled were < 100 cm FL and hence not yet mature. The asymptotic Length (L∞) was found to be 195 cm FL, the Von Bertalanffy growth constant (K) 0.43 year^{-1}, t_0 - 0.82 and the Growth Performance Index (ϕ) 4.21. The total mortality coefficient (Z) was 2.59 year^{-1}, Natural Mortality (M) 0.59 year^{-1}, Fishing Mortality (F) 2.00 year^{-1} and Exploitation Rate (E) 0.77 year^{-1}. Results show that mortality and exploitation rates were above the optimal, indicating high fishing pressure on coastal yellowfin tuna. The information generated by this study provides some further insights on the scientific knowledge of the coastal yellowfin tuna to inform policy for sustainable management and development of this fishery in Kenya and the entire South West Indian Ocean (SWIO) region.

Keywords: yellowfin tuna, length-frequency, mortality, growth parameters

Introduction

Tunas are highly migratory species that are distributed globally, occurring mainly in tropical and sub-tropical waters in the Indian, Pacific and Atlantic Oceans (ISSF, 2020; Chassot *et al.,* 2019; Prathibha *et al.,* 2012). Yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788) is a fast growing tropical species which belongs to the Scombridae family. Yellowfin tuna is one of the principal tropical tunas that contributes significantly to commercial harvests of many countries in the world including Kenya (Chassot *et al.,* 2019; Pillai and Palanisamy, 2012). The management of tunas in the Indian Ocean falls under the responsibility of the Indian Ocean Tuna Commission (IOTC) (Kaplan *et al.,* 2014; Pillai and Palanisamy, 2012). The Kenya Fisheries Service (KeFS) is responsible for the management of fisheries including yellowfin tuna in Kenyan waters (Kimani *et al.*, 2018).

An estimated catch of 427,240 metric tons (Mt) was landed in the Indian Ocean in 2019 (IOTC, 2020). The average catch for yellowfin tuna in the IOTC area of competence for the period 2015 – 2019 was estimated at 424,103 Mt compared to the Maximum Sustainable Yield (MSY) of 403, 000 Mt (IOTC, 2020). The European Union, mostly Spain and France, accounted for 21 % of the yellowfin tuna catches from 2014 – 2018, closely followed by Maldives, Iran, Seychelles and Sri Lanka with 13 %, 12 %, 10 % and 9 %, respectively (IOTC, 2019). Other countries contributed to the remaining 35

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% of the catch to a lesser extent, with Kenya only contributing some 108 Mt of the industrial yellowfin tuna to the total catch reported in 2018 (IOTC, 2018).

In Kenya, marine fisheries are one of the most important resources that contribute to the national economy, food security and livelihoods, though its potential is not fully maximized (Mueni *et al*., 2019; Ministry of Agriculture, Livestock, Fisheries and Irrigation, 2019; Kimani *et al.,* 2018). Kenya has an Exclusive Economic Zone (EEZ) of 200 nautical miles and 12 nautical miles of territorial waters (Republic of Kenya, 1989) which is not fully exploited (Ministry of Agriculture, Livestock and Fisheries, 2016). The country's coast is also located within the rich tuna migratory route in the SWIO (Campling, 2012). Yellow fin tuna (*Thunnus albacares*) is one of the most important commercial tuna species occurring in Kenya's coastal and EEZ waters.

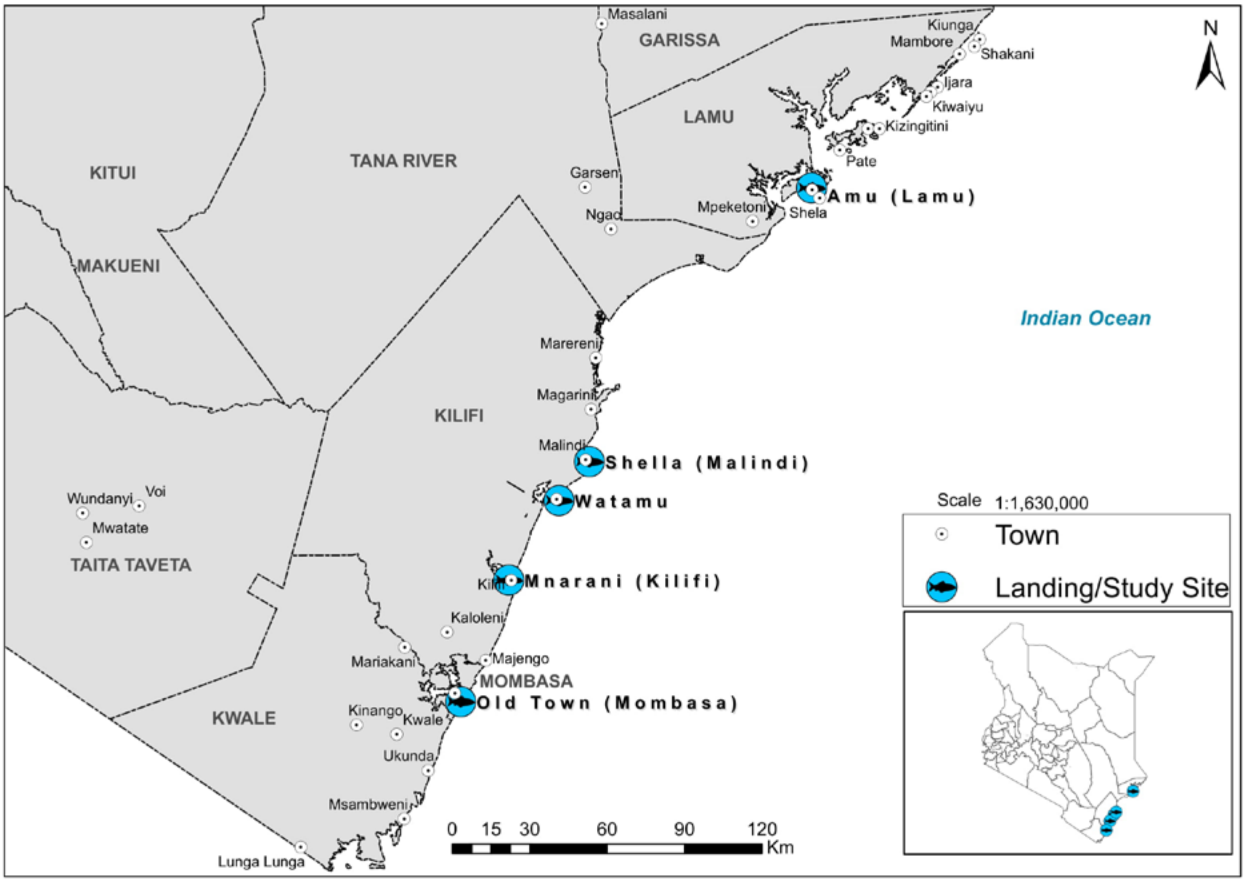

Figure 1. Artisanal yellowfin tuna fishery landing and study sites along the Kenyan Coast.

The species is highly migratory and forms free or associated schools with other tunas (Fishbase, 2018, Majkowski, 2007). Yellowfin is usually caught using various gears including handline, purse seine, gillnet, longline, ringnet, and pole and line (Kaplan *et al.,* 2014; Chassot *et al.,* 2019). Yellowfin tuna is caught in the nearshore waters primarily by artisanal fishers. At least 8,265 Mt, 3,431 Mt and 1, 931 Mt of fishes belonging to the scombrid family were landed by artisanal fishers in Kenyan coastal waters in 2015, 2016 and 2017, respectively (IOTC, 2017; IOTC, 2018). It is estimated that 414 – 800 artisanal fishing vessels target tuna and tuna-like species within 3-5 nautical miles of the coast in Kenyan waters (Ministry of Agriculture, Livestock and Fisheries, 2016; Hoof and Steins, 2017; IOTC, 2018). The fishers employ different gear types to catch yellowfin tuna including longlines, handlines, trolling lines and gillnets (Tuda *et al.,* 2016; Ministry of Agriculture, Livestock and Fisheries, 2016; IOTC, 2017; Alicli *et al.,* 2012; Tuda *et al*., 2016).Generally artisanal fishers lack suitable fishing vessels to venture far offshore (Wakwabi *et al.,* 2003). Fondo (2004) studied catch distribution for different gears in Lamu and Vanga landing sites, although this was for non-tuna species. There is limited information and studies on the biological aspects of tuna and tuna-like species in the coastal waters of Kenya (Fondo, 2004; KMFRI, 2018). Some efforts have been made to collect catch data on the artisanal tuna fishery but this is not disaggregated to species level and is confined only to the quantity of fish catch landed (Ministry of Agriculture, Livestock and Fisheries, 2016; IOTC, 2017). However, some data and information on biological and environmental parameters have been collected though this has not been continuous, consistent and is limited to a few fish landing sites (Wekesa, 2013; Ndegwa and Okemwa, 2017; Fulanda and Wamukota., 2015; KMFRI, 2018). Most studies on yellowfin tuna have been undertaken elsewhere, especially in the Western and Central Pacific Ocean and the East Indian Ocean regions (Mitsunga *et al*., 2013; Abdel-Barr, 2012; Viera, 2005; Kar *et al*., 2012).

In this paper, results are presented on catch rates, growth parameters, mortality rates, gear selectivity and Virtual Population Analysis - VPA (Catch, natural losses, survivors and fishing morality) of yellowfin tuna in Kenyan coastal waters, based on data collected from five fish landing sites along the Kenyan coast over a period of 16 months from August 2015 to December 2016. Sound knowledge and scientific information about the yellowfin tuna fishery in Kenyan waters is necessary to inform policy for effective fisheries management. The findings of this study will contribute to improved scientific knowledge of the yellowfin tuna in Kenyan coastal waters and the entire Indian Ocean region.

Materials and methods

Study sites

The study site was the Kenyan coast and climatic conditions are primarily determined by the reversing Monsoon winds; the dry NEM season occurs between November and March, and the wet SEM season between April and October (McClanahan, 1988; Government of Kenya, 2017; Schott and Mc Creary, 2001). Fish samples were collected from five fish landing sites, namely Lamu (Amu), Mombasa (Old Town), Kilifi (Mnarani), Malindi (Shella) and Watamu (Watamu) (Fig. 1).

Sampling and data collection

Fish samples were collected from the catches of artisanal fishers on a monthly basis over a period of 16 months from August 2015 to December 2016. The Primary Sampling Unit (PSU) was the vessel and the Secondary Sampling Unit (SSU) was the fishing trip while the catch was the tertiary sampling Unit. The fish landing site was the stratification.

Fish samples were identified to the lowest taxon possible using various identification guides/keys (Regional Tuna Tagging Project in the Indian Ocean [RTTP-IO], 2004; Itano, 2005; Anam *et al*., 2012; IOTC, 2013). Each individual yellowfin tuna caught was weighed whole to the nearest kilogram (kg) using an electronic balance. Fork length (FL) of each yellowfin tuna was measured to the nearest centimeter (cm) using a flexible measuring tape. The type of fishing gear, vessel type, the number of fishing crew per boat, date and time of departure and arrival for each fishing trip, name of the landing site and the price per kg of fish were recorded on biological sampling forms for all tuna and tuna-like species, adapted from the IOTC data collection template.

Data analyses

Catch Per Unit Effort (CPUE)

Nominal CPUE was computed by dividing the weight of fish caught (kg) with the number of fishing crew for every fishing trip. This relationship was summarized in the equation below:

$$\text{CPUE} = C_t/E_t \qquad \text{Eqn 1}$$

Where C_t is catch at time *t*, and E is the fishing effort deployed at time *t*.

The CPUE was expressed as kg trip^{-1} and $\text{kg}\cdot\text{Fisher}^{-1}\text{Trip}^{-1}$.

Length-Frequency distribution

The length-frequency data was grouped by month and binned into 10 cm intervals. The Length-Frequency measurements were used to prepare length-frequency distribution graphs and curves. The length and weight measurements were used to determine the length-weight relationship according to the formula:

$$W = aL^b \text{ (Cren, 1951)} \qquad \text{Eqn 2}$$

Where W is total body weight (kg), L is Fork Length (cm), a is a growth coefficient or condition factor (a constant), and b is the relative growth rate (exponent).

The length-weight relationship was calculated by drawing a scatter plot of the data and applying a power regression to determine the *a* and *b* parameters. The degree of association between the length and weight was computed by the determination coefficient R^2.

Estimation of growth parameters

The von Bertalanffy growth function (VBGF) including asymptotic length (L_∞) and the growth coefficient (K) were estimated using the ELEFAN 1 (Pauly, 1987) routine in FISAT II (Gayanilo *et al.*, 1994). The mean annual water surface temperature of 26 °C (for species in the tropics) was used in the model.

The response surface analysis routine with fixed starting points in ELEFAN1 was used to estimate the best fit growth curves to our data (Gayanilo and Pauly, 1997) given a range of the values of L_∞ and K and a fixed starting point (SS), and the starting Length. The following length based VBGF formula (Sparre and Venema, 1998) was fitted to the data:

$$L_t = L_\infty(1-\exp(-K(t-t_0))) \qquad \text{Eqn 3}$$

Where L_∞ is the asymptotic length, K is the von Bertalanffy growth coefficient, t_0 is the theoretical age at length zero, and L_t is the length at age t.

The index of growth performance (ϕ,) (Pauly and Munro, 1984) was estimated using the equation:

$$\phi = \log(K) + 2\log(L_\infty) \qquad \text{Eqn 4}$$

Estimation of mortality rates

The instantaneous rate of total mortality (Z) was calculated using a linearized length converted catch curve (Pauly 1983, 1984) on pooled data used with the estimated growth parameters, Asymptotic Length (L_∞) and the Von Bertalanffy growth constant (K). The following equation was used:

$$\mathrm{Ln}(N_i / Dt_i) = a + b * t_i \qquad \text{Eqn 5}$$

Where N is the number of fish in length class i, Δt is the time required for the fish to grow through length class i, t is the age corresponding to the mid-length of class i, and b is an estimate of the total mortality coefficient (Z).

The natural mortality rate (M) was estimated using indirect methods following the Pauly (1980) empirical relationship expression:

$$\mathrm{Log}\, M = -0.0066 - 0.279 \log L_\infty + 0.6543 \log K + 0.4634 \log T \qquad \text{Eqn 6}$$

Where M is the instantaneous natural mortality rate, L_∞ is the asymptotic length, T is the mean surface temperature at 26 °C and K refers to the growth rate coefficient of theVBGF.

Fishing mortality (F) was calculated using the relationship (Gulland, 1971):

$$F = Z - M \qquad \text{Eqn 7}$$

Where Z is the instantaneous total mortality rate and M is the instantaneous natural mortality rate.

The exploitation rate (E) was calculated as a ratio of the fishing mortality to the total mortality:

$$E = F/Z \qquad \text{Eqn 8}$$

Fishing gear catch selectivity

To estimate the selection parameters at 25 %, 50 % and 75 % of $L_{\max}$ ($Lc_{25}, Lc_{50}, Lc_{75}$), the analysis was based on the logistic curve assuming that selection to be symmetrical, or nearly so, based on the following logistic curve equation:

$$\mathrm{Ln}((1/P_L)-1) = S_1 - S_2 \cdot L \qquad \text{Eqn 9}$$

Where P_L is the probability of capture for length L, and

$L_{25} = (\mathrm{Ln}(3)-S_1)/S_2$

$L_{50} = S_1/S_2 L_{75} = (\mathrm{Ln}(3)+S_1)/S_2$

according to methods described by Pauly (1984a, 1984b, 1990).

Virtual Population Analysis (VPA)

A length based Virtual Population Analysis (VPA) on yellowfin tuna in Kenya coastal waters was conducted in FISAT II (Gayanilo *et al.*, 1994) using methods described by Jones and van Zalinge (1981) adapted for length frequency data to show survivors and losses due to natural and fishing mortalities. VPA required the following inputs that were estimated in this study: the coefficient of growth rate (K); asymptotic length (; natural mortality (M); fishing mortality (F); and the length weight relationship constant (*a*) and exponent (*b*). In this study, the *(a)* and *(b)* values were estimated at 0.0002 and 2.5, respectively. These values were slightly lower than what is reported in Fishbase. This indicated that the growth was negatively allometric since the (*b)* value was less than 3. For the purpose of the VPA, the values a = 0.0224, and b = 2.94 were accessed from Fishbase in October 2018 and used in the analysis.

Results

Yellowfin tuna fish catch rates

A total of 1281 individual yellowfin tuna weighing 12,671 kg were sampled. The minimum and maximum weight of the fish sampled was 1 kg and 97 kg, respectively with a mean weight of 9.9 kg. The size ranged from 11 cm to 205 cm FL, with a mean size of 77.7 cm FL.

Watamu and Shella each accounted for 27 % of the total catch sampled. This was followed closely by Old Town accounting for 21 % of the sampled catch. Amu and Mnarani recorded 15 % and 10 %, respectively. Crew sizes ranged from 3 – 5 fishers per boat with an average of 4 fishers per vessel. A total of 1,808 fisher days were recorded over the sampling period with an average CPUE of 7.2 kg·Fisher^{-1}Trip^{-1}. The highest fishing effort was concentrated at Watamu accounting for 33 % of the fisher days. This was followed closely by Shella with 32 % of the fisher days. Old Town, Mnarani and Amu accounted for 16 %, 13 % and 6 % fisher days, respectively. It is evident that total fish catches correlated with the fishing effort.

Main fishing gears deployed by artisanal fishers targeting yellowfin tuna were handlines, longlines, trolling line and gillnets (Table 1). Trolling accounted for 46 % of the total catch sampled. Handlines, longlines and gillnets accounted for 32 %, 21 % and 1 % of the total catch sampled, respectively. Trolling accounted for 54 % of the fisher days. This was followed closely with handlines which accounted 27 % of the fisher days recorded. Longlines and gillnets accounted for 16 % and 3 % of the fisher days, respectively. The average size of fish harvested using longlines was above 100 cm FL (133.78 cm FL) while from handlines this was 77.2 cm FL. Gillnets recorded the smallest average size of the fish catch sampled. Almost 100 % of the yellowfin tuna in the sample harvested using gillnets were below the size at first maturity (< 100 cm FL), with sizes ranging from 52 cm – 82 cm FL. Trolling, handlines and longlines recorded 99.6 %, 93.7 % and 31.3 % of the catch less than 100 cm FL, repectively.

Temporal variations in yellowfin tuna catches

Monthly variation in yellowfin tuna catch rates was evident (Fig. 2). Highest CPUE was recorded in the month of October 2015 (10.8 kg·Fisher^{-1}Trip^{-1}), with a corresponding catch of 1,430 kg. This was closely followed by November 2015 with a CPUE of 10.7 kg Fisher^{-1}Trip$^{-1.}$ In 2016, high catch rates of 8.6 kg Fisher^{-1}Trip^{-1} and 8.5 kg Fisher^{-1}Trip^{-1} were recorded in February and June, respectively. The lowest CPUE of 3.6 kg·Fisher^{-1}Trip^{-1} was recorded in December 2016. The results indicate monthly variations with better catches reported in the months of May to October (SEM), with an average CPUE of 7.3 kg Fisher^{-1}Trip^{-1} (Table 2). Low catches were reported in the months of November to April (NEM) with an average CPUE of 7.0 kg·Fisher^{-1}Trip^{-1}. High fishing effort occurred during the SEM months, accounting for 62 % of the fisher days compared to 38 % in the NEM season. Nevertheless the Kruskal-Wallis test did not indicate any significant difference between the SEM and NEM catches.

Spatial variations in yellowfin tuna catches

There were variations in fish catch rates between the five fish landing sites (Fig. 3). The results showed that Old Town recorded the highest catch rates with a mean weight of 54.2 kgTrip^{-1} and a mean CPUE of 9.2 Kg·Fisher^{-1}Trip^{-1}. This was closely followed by

Table 1. Comparison of catch rates and under sized individuals (< 100 cm FL) of yellowfin tuna among the main fishing gears in the artisanal fishery in the coastal waters of Kenya.

Gear	Fisher Days	Total catch in the sample (Kg)	Mean Catch/ Day (Kg)	Catch/ Day N	Catch/ Day Std.Err	KgFisher-1Trip^{-1}	Mean Fork Length (cm)	% proportion of under size fish in the sample (Less than 100 cm FL)
Gillnet	60	131	10.08	13	1.99	2.2	65.4	100
Handline	481.75	4038	33.58	119	2.87	9.29	77.2	93.7
Longline	289.00	2717	53.27	51	6.97	9.09	113.78	31.3
Trolling line	977.32	5785	23.61	245	0.96	6.06	73.0	99.6
All Grps	1808.07	12671	29.50	428	1.36	7.21	78.83	

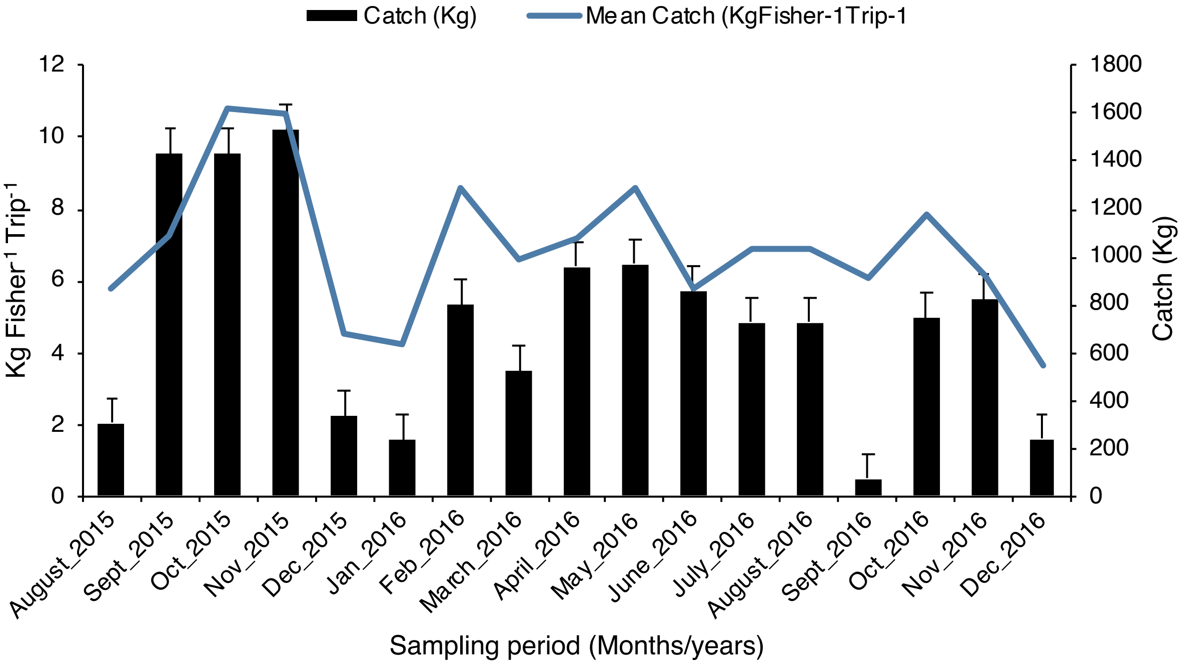

Figure 2. Monthly variation in CPUE of yellowfin tuna (Kg) and KgFisher^{-1}Trip^{-1} from the coastal waters of Kenya for the period August 2015 – December 2016.

Amu with a mean weight of 49.4 kg^{-1}Trip^{-1}and CPUE of 16.7 kg·Fisher^{-1}Trip^{-1}, which was the highest. Shella and Watamu each recorded 24.6 kgTrip^{-1}, with a mean CPUE of 5.8 kg·Fisher^{-1}Trip^{-1} and 5.9 Kg·Fisher^{-1}Trip^{-1}, respectively. Mnarani recorded the lowest mean catch of 19.35 kgTrip^{-1} and CPUE of 5.9 kg·Fisher^{-1}Trip^{-1}. Results of the Kruskal – Wallis test showed a significant difference in yellowfin catches betwee Old Town and other fish landing sitess ($P < 0.05$). The difference in fish catch rates at Mnarani and that of Shella, Watamu and Amu was significant ($P < 0.05$). There was no significant difference in yellowfin tuna catch rates between Watamu and Amu as well as Shella and Amu.

Length-frequency distribution

The length of the 1281 fish measured ranged from 11 cm to 205 cm FL (Fig. 4). Most of the individuals (36 %) were in the length class of 66 cm -76 cm FL. At least 91 % of the individuals sampled were less than 100 cm FL, which is the length at maturity for yellowfin tuna (Viera, 2005; Stequert *et al.*, 1996; Kaplan *et al.*, 2014; Itano, 2000). Three length classes dominated the size structure namely 55-65, 66-76 and 77-87 cm FL. Different gear types were highly selective for size. Mean sizes for yellowfin tuna caught by longline, handline, trolling and gillnet were 113 cm, 77.2 cm, 73.0 cm and 65.4 cm, respectively (Table 1). The results suggest that all (100 %) of the yellowfin tuna harvested by gillnet were undersize (< 100 cm FL). Trolling and handline resulted in 99.6 % and 93.7 % of their catch in the sample being undersize. Longline resulted in some 31.3 % of the undersize catch in the sample. The results indicate that the majority yellowfin tuna caught by the artisanal fishers are juveniles and are more vulnerable to gillnet, handline and trolling gears. However, this study did not look into the specific sizes of the different fishing gears that were deployed by the artisanal fishers at the respective landing sites. It is highly likely that the fishers are using mesh sizes and hooks that are of small sizes. The use these fishing gears (small

Table 2. Comparison of yellowfin tuna catch rates during the SEM and NEM seasons.

Season	KgFisher^{-1}Trip^{-1} Mean	KgFisher^{-1} Trip^{-1} N	KgFisher^{-1} Trip^{-1} Std. Dev	KgFisher^{-1} Trip^{-1} Std. Err
NEM	7.008050	156	8.202849	0.656754
SEM	7.373973	272	6.261162	0.379639
All Grps	7.240599	428	7.023964	0.339516

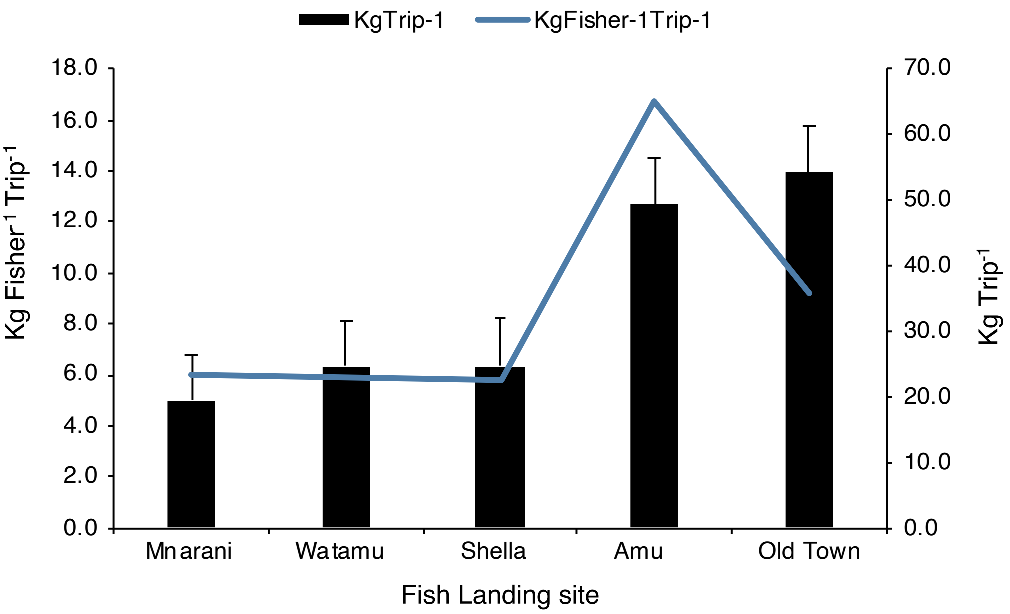

Figure 3. Spatial variation of yellowfin tuna Catch Per Unit Effort (CPUE) from the coastal waters of Kenya for the period August 2015 – December 2016.

mesh sizes and hooks) require close monitoring by the relevant national institutions including KeFS and KMFRI to ensure that the recommended sizes of nets and gears are used by the fishers.

Growth parameters

Life history parameters for yellowfin tuna based on the pooled data set were estimated as follows: Asymptotic Length (L_∞) = 195 cm FL; Von Bertalanffy growth constant (K) = 0.43 year^{-1}; t_0 = 0.82; and the Growth Performance Index (ϕ) = 4.21.

Mortality rates

Total mortality coefficient (Z) was 2.59 year^{-1}. Natural Mortality (M) with a mean annual water surface temperature of 26 °C was estimated at 0.59 year^{-1}. Fishing Mortality (F) was 2.00 year^{-1} and Exploitation Rate (E) 0.77 year^{-1} (Fig. 5).

Fishing gear catch selectivity

Fishing selectivity for yellowfin tuna varied with gear type used (Table 3). The mean length at first capture (L_{50}) for all the gear types combined was 65.4 cm FL.

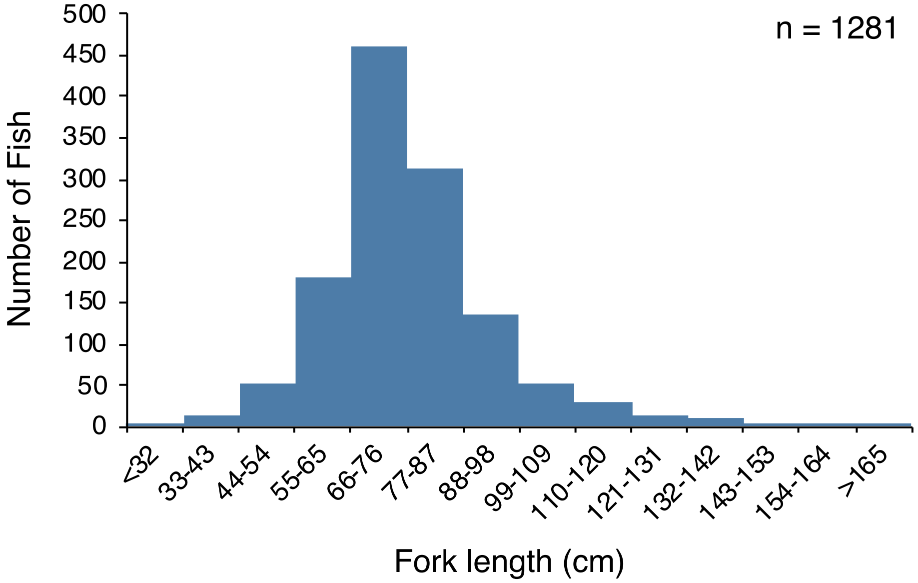

Figure 4. Length frequency distribution of *Thunnus albacares* in the coastal waters of Kenya for the period August 2015 to December 2016.

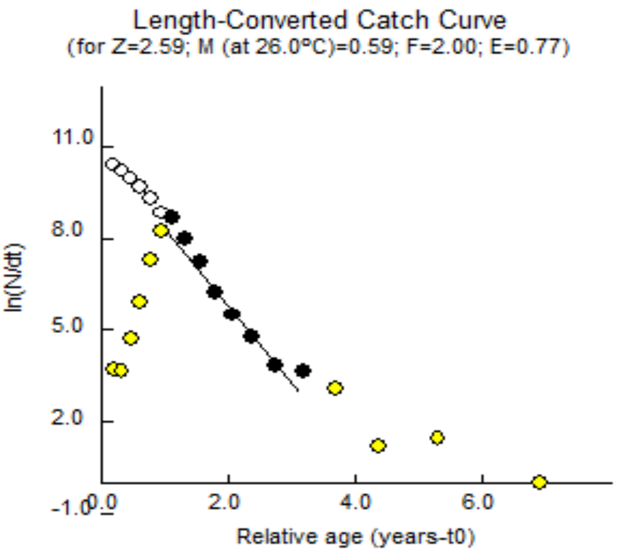

Figure 5. Length-Converted Catch Curve for yellowfin tuna caught in the artisanal fishery in Kenyan waters between August 2015 to December 2016.

However, (L_{50}) in the main gear types used i.e. longline, handline and trolling was 93.3 cm, 75.6 cm and 68.9 cm, respectively. The mean length at which 95 % of the individuals were retained in the fishing gear was 80.66 cm for combined gears, 115.9 cm for longline, 91.4 cm for handline and 83.7 cm for trolling line. These results indicated that specific gears targeted different sizes of yellowfin tuna. Larger individuals were captured by longline as opposed to the other gear types.

Virtual Population Analysis (VPA)

The VPA for yellowfin tuna indicated that individuals were vulnerable to capture from >45 cm FL midlength, with highest catches occurring between 65-85 cm FL midlength (Fig 6). As expected, natural mortality gradually decreased with increasing length (and age). Fishing mortality was highest between the lengths of 65-105 cm FL midlength with a peak at 75 cm FL midlength.

Discussion

There was clear evidence of temporal variation and the results indicated that tuna catches peaked in May-June and October -November during the study period. These findings are comparable to the results of other studies (Kimani and Okemwa, 2019; Kaplan *et al.*, 2014). The temporal variation of yellowfin tuna catch rates observed in this study could be attributed to a number of factors, including ocean dynamics, the quality of the habitat, food availability, type and selectivity of fishing gears, sex and size of individuals, spawning and the migratory nature of the tunas (Tuda *et al.*, 2016; Potier *et al.*, 2007; Abdel-Barr *et al.*, 2012; Kar *et al.*, 2012; Kaplan *et al.*, 2014). The coastal waters of the Somali basin are highly productive in the months of July to October (Kaplan *et al.;* 2014; Chassot *et al.*, 2019) with some upwellings (Chassot *et al.*, 2019; Schott *et al.*, 2009) leading to increased food abundance for tunas, and this would explain why there was an increased abundance of yellowfin tuna during this period.

Yellowfin tuna are highly migratory species and respond to changes in seasons (Boggs, 1994). The monsoon climatic season and weather patterns experienced along the coastline in the Western Indian Ocean region have some influence on the distribution and abundance of marine fishes including the tunas (Koido and Suzuki, 1989; Yamanaka, 1990; Nzioka, 1990; Zudaire *et al.*, 2013). The relatively low catches of yellowfin tuna recorded between December and April (NEM months) demonstrate that some of the individuals could have moved out of the study area for spawning purposes or other natural reasons (Campling,

Table 3. Selectivity parameters of yellowfin tuna from length converted catch curve and the probabilities of capture (Fork Lengths in cm).

Parameter	All gears	Longline	Trolling line	Handline
Total Mortality (Z)	2.59	1.11	3.9	3.9
Fishing Mortality (F)	2.0	0.52	3.3	3.4
Exploitation rate (E)	0.77	0.47	0.85	0.85
Length at capture (Lc 25)	58.9			
Length at capture (Lc 50)	65.4	93.3	68.9	75.6
Length at capture (Lc 75)	71.9	102.4	74.7	81.8
Length at capture (Lc 95)	80.66	115.9	83.7	91.4

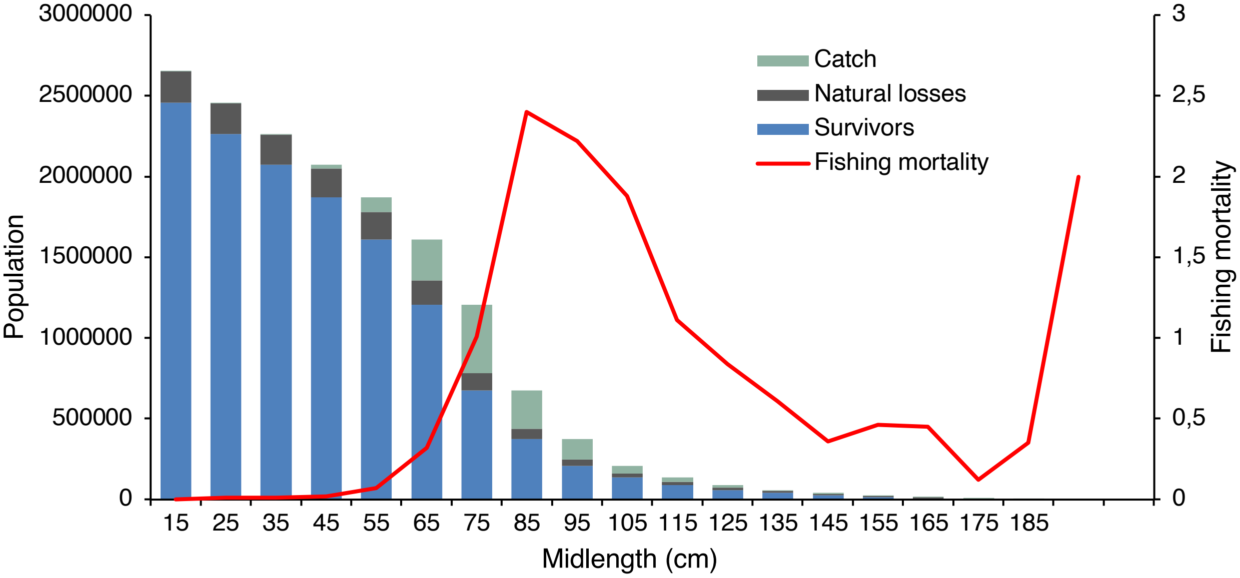

Figure 6. Output of the Virtual Population Analysis showing total population, catches, fishing mortality, natural mortality and survivors (biomass) per length group of yellowfin tuna caught by the artisanal fishers off the coast of Kenya.

2012; Ministry of Agriculture, Livestock and Fisheries, 2013). Peak spawning activity for yellowfin tuna in the Indian Ocean occurs during summer between October and May in the southern hemisphere (Stequert *et al.*,1996; IOTC, 2003; Gouping *et al.*, 2008). Yellowfin tuna catches were recorded throughout the sampling period at all the sites, although with varying high and low peaks in abundance. This suggests that some individuals, particularly juvenile fish, were more resident in the coastal waters of Kenya, however this will require validation using evidence from tagging studies . Similar observations have been reported by other studies which suggest regional residency and limited movements for yellowfin tuna, especially in inshore coastal waters (Chassot *et al.*, 2019; Schaefer *et al.*, 2007; Schaefer *et al.*, 2011; Kaplan *et al.*, 2014).

Analysis of the yellowfin tuna catch sampled revealed variation in fish catch rates across the sites with Shella and Watamu reporting high catches. This observation is partly attributed to the level of fishing effort, the type and size of fishing gears deployed by the fishers as well as the size of fish targeted. The correlation between fishing effort and fish catches had an implication on the CPUE across the fish landing sites. Highest fishing effort was concentrated in Watamu and Shella accounting for at least 33 % and 32 % of the fisher days whereas Amu had the lowest fishing effort (6 %).

Mombasa reported the highest mean catch rate compared to the other fish landing sites. Watamu reported the highest quantities of the total catch sampled (27 %), however , the site also reported the highest fishing effort which resulted to low CPUE for the fisher. The correlation between fishing effort and catches had implication on the CPUE across the fish landing sites. The type of fishing gear also contributed to the difference observed in fish catch rates across the sites. Fishers deployed different gears and gear combinations to maximize the harvest and size of targeted fish. Results show that the highest quantities of fish sampled at Watamu were harvested using trolling lines. On the other hand, longline catches dominated the landings at Old Town. The longline gear on average harvested fairly large sized individuals of yellowfin tuna, ranging from 59 cm FL – 205 cm FL with an average of 113.78 cm FL. Trolling line on the other hand captured individuals within the size range 11 cm FL – 120 cm FL with an average size of 73.0 cm FL. However this study did not investigate the different size ranges of gears including gillnets and hooks deployed by fishers. Further research is required to investigate selectivity of the different sizes of gears on yellowfin tuna fishery in Kenyan coastal waters.

The length -frequency distribution of yellowfin tuna caught suggest that the catch was dominated by juveniles peaking at the 66-76 cm FL size class. Size at first maturity for yellowfin tuna has been estimated at approximately 100 cm FL (Viera, 2005; Stequert *et al.*, 1996; Kaplan *et al.*, 2014; Itano, 2000). The results of this study compare well with the work reported by other authors. For example, the IOTC (2016) reported that most of the yellowfin tuna landed by artisanal fishers in Kenya ranged in size from 70 – 85 cm FL. Further afield, Kaymaram *et al.* (2014) reported a size range of 37 – 172 cm FL from the gillnet fishery in the Oman Sea. Prathibha (2013) estimated a size range of 20 – 185 cm FL for the yellowfin tuna on the east coast of India. Mildenberger *et al.* (2018) reported a size range of 25cm – 199 cm FL for yellowfin tuna in the driftnet fishery in Zanzibar waters.

The growth parameters from this study compare closely with those of Kayamaram *et al.* (2014) who estimated L_∞ = 183.3 cm, K = 0.45 and ϕ = 4.21 for yellowfin tuna in the Oman Sea. Studies conducted by other authors show a range of estimated growth parameters for yellowfin tuna in different parts of the Indian Ocean (Prathibha *et al.,* 2012; Chantawong, 1998; Maldeniya and Joseph 1986; Prathibha, 2013; Nurdin *et al.,* 2016) (Table 4). The growth coefficient (K) in this study was estimated at 0.43 year^{-1} which was found to be higher than 0.30 year^{-1} found in waters on the east coast of India (Prathibha *et al*., 2012; Prathibha, 2013; Nurdin *et al.,* 2016). However, the growth coefficient was lower than that found in a number of other studies from the Indian Ocean (Chantawong, 1998; Maldeniya and Joseph 1986; Nurdin *et al.,* 2016; Kayamaram *et al*., 2014) . This result suggests that yellowfin tuna encountered in the coastal waters of Kenya do not grow at a faster rate than elsewhere. Froese *et al*. (2011) reported that a high proportion of juveniles or adults in the sample may limit the size ranges of the fish, consequently leading to under-estimating or over-estimating the growth parameters. In this study, over 90 % of the fish sampled were below the estimated length at first maturity (100 cm FL). This probably explains why the growth coefficient was lower compared to other studies.

Yellowfin tuna mortality rates estimated in this study were compared with other studies in the Indian Ocean. Kaymaram *et al.* (2014) estimated an exploitation rate (E) of 0.76 year^{-1} and a total mortality rate (Z) of 2.04 year^{-1} which compares favourably to the results of this study (E = 0.77 year^{-1} and Z = 2.59 year^{-1}, respectively). Natural mortality rate (M) estimated in the present study (M = 0.59 year^{-1}) was very similar to a number of other estimates from the same region of between 0.52 year^{-1} and 0.67 year^{-1} (Pillai *et al.,*1993; John, 1995; Kaymaram *et al.,* 2000).

This study revealed that the estimated fishing mortality rate (F = 2.0 year^{-1}) was substantially higher than the natural mortality rate (M) suggesting that fishing pressure is too high and not sustainable. Moreover, the exploitation rate (E) of 0.77 year^{-1} is higher than the recommended optimal rate of 0.5 year^{-1} further emphasizing the high fishing pressure on yellowfin tuna in Kenya's coastal waters. Since most of the individuals being harvested were juvenile fish less than 100 cm FL, this implies that growth overfishing is occurring in this fishery.

The artisanal fishery in Kenya targets multiple species and fishers deploy different gears (Tuda *et.al*., 2016). Major fishing gears deployed by artisanal fishers to catch yellowfin tuna in Kenyan coastal waters are longlines, hand lines and trolling lines. This study reveals that trolling line was the most used gear targeting tuna and tuna-like species accounting for 54 % fisher days. Handline accounted for 27 % of the fisher days. Longline and gillnet accounted for 16 % and 3 %

Table 4. Comparison of growth parameters for yellowfin tuna in the Indian Ocean from different authors.

Study area/region	L_∞	K (Year^{-1})	t_0	Author
Kenya coastal waters	195.0	0.43	- 0.83	This study
India (East Coast)	197.4	0.3	- 0.116	Prathibha *et al.* (2012)
Eastern Indian Ocean	194.0	0.66	- 0.27	Chantawong (1998)
Sea of Oman	183.2	0.45	- 0.184	Kaymaram *et al.* (2014)
Sirilanka (West and South)	178.0	0.47	- 0.28	Maldeniya and Joseph (1986)
East Coast India (Andhra and Pradesh)	197	0.3	- 0. 115	Prathibha (2013)
Eastern Indian Ocean	178	0.47	-0.213	Nurdin *et al.* (2016)
Zanzibar (Tanzania)	165	0.878	-0. 49	Mildenberger *et al.* (2018)

of the fisher days, respectively. The size at first capture (L_{c50}) for yellowfin tuna varied with the different gears used. Longline was highly selective to large sized individuals with length at first capture of 93.3 cm FL and an average size of 113.8 cm FL. There was no significant difference in the average size of yellowfin tuna captured using handlines and trolling line, although the length at first capture (L_{c50}) was slightly different (75.6 cm FL and 68.9 cm FL, respectively.) Similarly, the size of yellowfin tuna captured by different gear combinations varied spatially with landing sites. Old Town recorded the largest mean size of fish landed there (115.04 cm FL) whereas Mnarani reported the lowest mean size (69.82 cm FL). This can be partly attributed to the fact that relatively larger fishing vessels using longlines were predominantly used to catch yellowfin tuna landed at Old Town. These vessels could venture further offshore and remain at sea for longer periods despite the sea conditions being rough. At the other landing sites smaller fishing boats employing handlines and trolling lines were predominantly used by artisanal fishers to target yellowfin tuna. Approximately 78 % of the total fish catch sampled was captured using trolling (46 %) and handlines (32 %).

The results suggest that the selectivity of the gears depends on the size and population dynamics of the fish targeted (Maunder *et. al.*, 2006), the type and size of fishing gear used, and the fishing ground (Tuda *et. al,* 2016). It is evident from this study that fishers employing gillnets, handlines and trolling lines generally target smaller sized yellowfin tuna. Noting that most of the individuals of yellowfin tuna sampled in this study were juveniles and sub-adults, efforts should be directed towards close monitoring of the catch to ensure young fish that are necessary for recruitment are not caught. Development of technical measures and appropriate regulations defining the type of fishing gear, seasons, and fishing grounds to catch larger sizes of tuna is desirable. This would enhance stock sustainability of the target species and minimize the capture of juvenile fish and non-target species in the nearshore waters.

The results of this study showed that the highest fishing mortality (F) of yellowfin tuna was experienced between 65 cm – 105 cm mid-length . These results suggest that the fishing mortality rate was highest at this specific size range due to the selectivity of the fishing gear used (Amponsah *et al.*, 2017; Kar *et al.*, 2012). Furthermore, the high fishing mortality rate over this size range (i.e. mostly juvenile or sub-adult fish) is likely to have a substantial impact on survivorship and the number of yellowfin tuna that reach adulthood and are subsequently able to spawn (i.e. growth overfishing).

Conclusion

Findings from this study show that yellowfin tuna are present in Kenyan coastal waters throughout the year although most of them are small juvenile fish. This indicates the importance of nearshore/coastal waters as a nursery habitat and foraging grounds for juvenile yellowfin tuna; hence the need to put in place appropriate fisheries conservation and management measures to protect these critical ecosystems and fish stocks.

Over 91 % of the yellowfin tuna caught by artisanal fishers in Kenyan coastal waters are juveniles (less than 100 cm FL). Fishing mortality rate (F = 2.0 for combined gears) is substantially higher than the natural mortality rate (M = 0.59). The estimated exploitation rate (E) of 0.77/year is higher than the recommended optimal of 0.5/year. These are clear indications that growth overfishing is occurring in this fishery. These results reinforce the concerns raised by the IOTC that yellowfin tuna stocks within Kenya's inshore waters are being overfished. Unless efforts are made to address this situation, the fishery is most likely to decline. Enforceable measures to reduce fishing effort on yellowfin tuna and putting in place stock recovery plans should be a priority for the relevant national and regional fisheries management authorities including the Kenya Fisheries Service and the IOTC. The IOTC has recommended stock rebuilding for the Indian Ocean yellowfin tuna stocks.

To achieve this the following recommendations are proposed for Kenya's artisanal tuna fishery:

1. A comprehensive study on the biology of yellowfin tuna in the Indian Ocean is necessary to address the complexity of yellowfin tuna growth rates.
2. The artisanal and industrial fishers target the same yellowfin tuna stock. A study on yellowfin tuna resource overlaps and interactions between the artisanal and industrial fishers is recommended. This would yield useful data and information that would assist Kenya and the IOTC to develop effective measures to reduce overall fishing effort on the yellowfin tuna stocks in the Indian Ocean region.
3. It is evident from this study that fishing mortality for yellowfin tuna in the coastal waters of Kenya is

above the optimal levels indicating that the fishery is being overfished. The fishery should be closely monitored with a view to controlling fishing effort, restrictions on gear size and minimum landing size, closed areas and seasons for coastal fisheries.

4. The South West Indian Ocean Fisheries Commission (SWIOFC) should work closely with member states in collaboration with WIOMSA to undertake a regional assessment of yellowfin tuna in coastal waters in the SWIO region. This would inform the development of a regional management strategy for yellowfin tuna that would help drive this fishery to sustainability in order to derive more sustainable socio-economic benefits to the local fishing communities and the national economies of the respective SWIO range states.

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Fibropapillomatosis infection in a population of green turtles at Watamu Bay, Kenya

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Anthropogenic stressors from onshore and offshore activities can act as driving factors of disease for a wide range of marine organisms. Green turtles (*Chelonia mydas*) are prominently afflicted with a tumour-causing disease known as fibropapillomatosis (FP) caused by the chelonid alphaherpesvirus ChHV5. Previous studies indicate that pathways of FP transmission may be genetic (vertical transmission) or linked to causal factors in a turtle's environment (horizontal transmission). In this paper patterns of FP prevalence were examined in 10,896 records of green turtles caught or found stranded around Watamu Bay, Kenya, between 2003 – 2020. Findings were focused on locational and seasonal factors that may potentially influence infection. The findings show that FP prevalence varies significantly on an annual basis. Location significantly influenced infection prevalence, with prevalence higher in open ocean sites than sites located within the creek. Infection prevalence was highest at sites around the creek mouth and north of the creek mouth, with both regions exhibiting disparate annual patterns of infection. This paper is the first to examine long-term trends of FP prevalence in-depth in this region and has implications for the health of turtles and marine biota found along the Kenyan coast, and potentially within the wider Western Indian Ocean region. The findings emphasize the need to distinguish the infection pathways of causative agents via: i) further examination of the links between infection and environmental and/or biont community factors; and ii) the collection of data pertinent to the genetic diversity of green turtles and associated ChHV5 viral strains occurring in the Western Indian Ocean.

Keywords: fibropapillomatosis, Watamu Bay, Kenya

Introduction

The marine biome is one of the largest and most influential on earth; it plays a significant role in global socio-economic health and also provides a range of ecosystem services (Costanza, 1999). However, exposure to anthropogenic stressors has led to the decline of ecosystem services, and caused phenomena such as dead zones and altered food web dynamics (Ravaglioli *et al.*, 2019). Anthropogenic stressors also facilitate the exposure of marine biota to compromised ecosystem function, predation and infections (Diaz and Rosenberg, 2008). Unsustainable practices such as overfishing and habitat destruction can eventually threaten food security and unbalance coastal ecosystems (McClanahan and Muthiga, 1988). Coastal ecosystems especially highlight how food security and people's livelihoods are closely tied to ecological health. In these environments, marine biota have proven to be sound predictors of both ecosystem resilience and human health (Colin *et al.*, 2015).

Sea turtles are long-lived and can cover vast expanses of ocean during different stages of their lives (Schofield *et al.*, 2010; Rees *et al.*, 2012). This exposure positions them as key indicators of ocean health and resilience (Aguirre and Lutz, 2004); however, the alternative consequence is that sea turtles are also exposed to a wide range of anthropogenic stressors. Turtles are often caught as by-catch, injured or killed during the course of fishing activities (Hazel

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and Gyuris, 2006; Wallace *et al.*, 2013). Additionally, various studies show that the ingestion of plastics and other debris discarded in the ocean can significantly impact turtle fecundity (Barnes *et al.*, 2009; Schuyler *et al.*, 2014). Currently all sea turtle species are listed on the red list created by the International Union for the Conservation of Nature (IUCN), in categories ranging from 'vulnerable' to 'critically endangered'. The green turtle (*Chelonia mydas*) is listed as endangered as a result of the continual degradation of their nesting and foraging habitats, as well as incidental mortalities from fishing activities (Seminoff, 2004).

In more recent years, increasing attention has been focused towards the impact of anthropogenic stressors on turtle health (Rees *et al.*, 2016). Fibropapillomatosis (FP), a virulent form of neoplasia, is an additional threat prevailing against the global green turtle population. Although reports on FP extend back to the late 1930s (Smith and Coates, 1938), it remains underreported in various regions of the ocean (Rao *et al.*, 2020). Currently, there are active investigations concerning: (i) the causal pathways leading to an outbreak; (ii) the dominant transmission pathways, i.e. hereditary (vertical) or environmental (horizontal); and (iii) whether pathways vary geographically or in different turtle populations (Greenblatt *et al.*, 2005). Although FP has been recorded in all turtle species (Herbst, 1994; Foley *et al.*, 2005), it is predominantly prevalent in green turtles. The causative agent is thought to be the chelonid alphaherpesvirus 5 (ChHV5) belonging to the family Herpesviridae (Herbst *et al.*, 1995; Jones *et al.*, 2016). A typical FP infection mostly manifests cutaneously in the form of masses or tumours anywhere on a turtle's skin, carapace or plastron. Masses can also occur in the ophthalmic tissue, as well as in the viscera (Schlumberger and Lucké, 1948). Tumour masses can interfere with turtle movements, compromise their feeding ability and increase their vulnerability to hazards, such as bycatch incidents and predators (Flint *et al.*, 2015).

Data from various regions helps to establish an understanding of FP distribution patterns and prevalence globally (Jones *et al.*, 2016). Research on FP has focused on three main priorities: i) spatio-temporal patterns highlighting prevalence and global distribution; ii) the mechanics of vertical transmission (Duffy *et al.* 2018); and to a lesser extent iii) the horizontal transmission (dos Santos *et al.* 2010), although the evidence for this pathway is increasing (Jones *et al.*, 2020). The results of these studies have indicated that juvenile green turtles appear to be most vulnerable to FP, likely as a result of the significant time they spend in neritic environments, which are heavily impacted by anthropogenic activities and degradation (Ene *et al.*, 2005; Foley *et al.*, 2005). Turtles foraging in these habitats are subsequently more vulnerable to pollutants present in the water or incorporated in their algal-based diet (Komoroske *et al.*, 2011; Camacho *et al.*, 2014). Van Houtan *et al.* (2010) found a strong link between nutrient-rich waters and incidences of FP in juvenile turtles in Hawaii. The authors postulated that invasive macroalgae in nutrient-rich waters had higher levels of arginine (processed from anthropogenic nitrogen), which has been implicated in promoting the proliferation of viruses in the Herpesviridae family. Turtles ingesting these algae may be at higher risk of FP; furthermore, the observed elevated FP prevalence is a potential indicator of the habitat quality. Van Houtan *et al.* (2010) also reported great spatial and temporal variability in infection rates, further signalling that infection is triggered by local environmental factors. Similar research (Keller *et al.*, 2014) reported high concentrations of both man-made and organic pollutants in stranded turtles afflicted with FP. Although there was no evidence that these pollutants triggered FP, results indicated that a bio-accumulation of pollutants could be contributing to the progression of the disease. Further evidence for horizontal transmission pathways comes from a study by Greenblatt *et al.* (2004). Their study demonstrated high levels of FPTHV (a suspected causative virus for FP) in leeches and barnacles removed from stranded and free-ranging turtles. This raises the possibility that such organisms may be vector candidates furthering horizontal pathways of viral transmission.

The South Western Indian Ocean basin (which includes the Kenyan coast) is a biodiversity hotspot and is a foraging and nesting ground for a variety of megafauna including sea turtles (Obura *et al.*, 2019). Fishing activities are the predominant income generator for coastal communities in this region, with turtles typically constituting part of the fishing catch or by-catch (Temple *et al.*, 2017). The resilience of marine species in this region is further challenged by the negative impacts stemming from ocean-based industries (Jouffray *et al.*, 2020), onshore activities (the decimation of mangrove stands, influx of untreated effluent from agriculture and tourism) and the impacts of climate change (Obura, 2004; Kirui *et al.*, 2013; Aller *et al.*, 2019). Based on the increasing evidence linking environmental and community dynamics to FP infection, being able to identify the specific dynamics influencing infection patterns will provide a starting point for establishing causative factors and the scale at which they operate.

In this paper, trends of fibropapillomatosis infection observed in a green turtle population occurring in Watamu Bay, Kenya are presented. The focus was specifically on the effects of location and seasonal factors on infection patterns.

and monitored by Kenya Wildlife Services (KWS). The Mida Creek extends inland for approximately 9 km and covers an area of 31.2 km^2. The creek is bordered by mangrove forests on either side covering more than 2000 hectares of land (Kairo *et al.*, 2002). The climate in Watamu Bay adheres to established regional patterns (McClanahan, 1988) influenced by North Eastern monsoon tradewinds (October to March) and South Eastern winds (March to October).

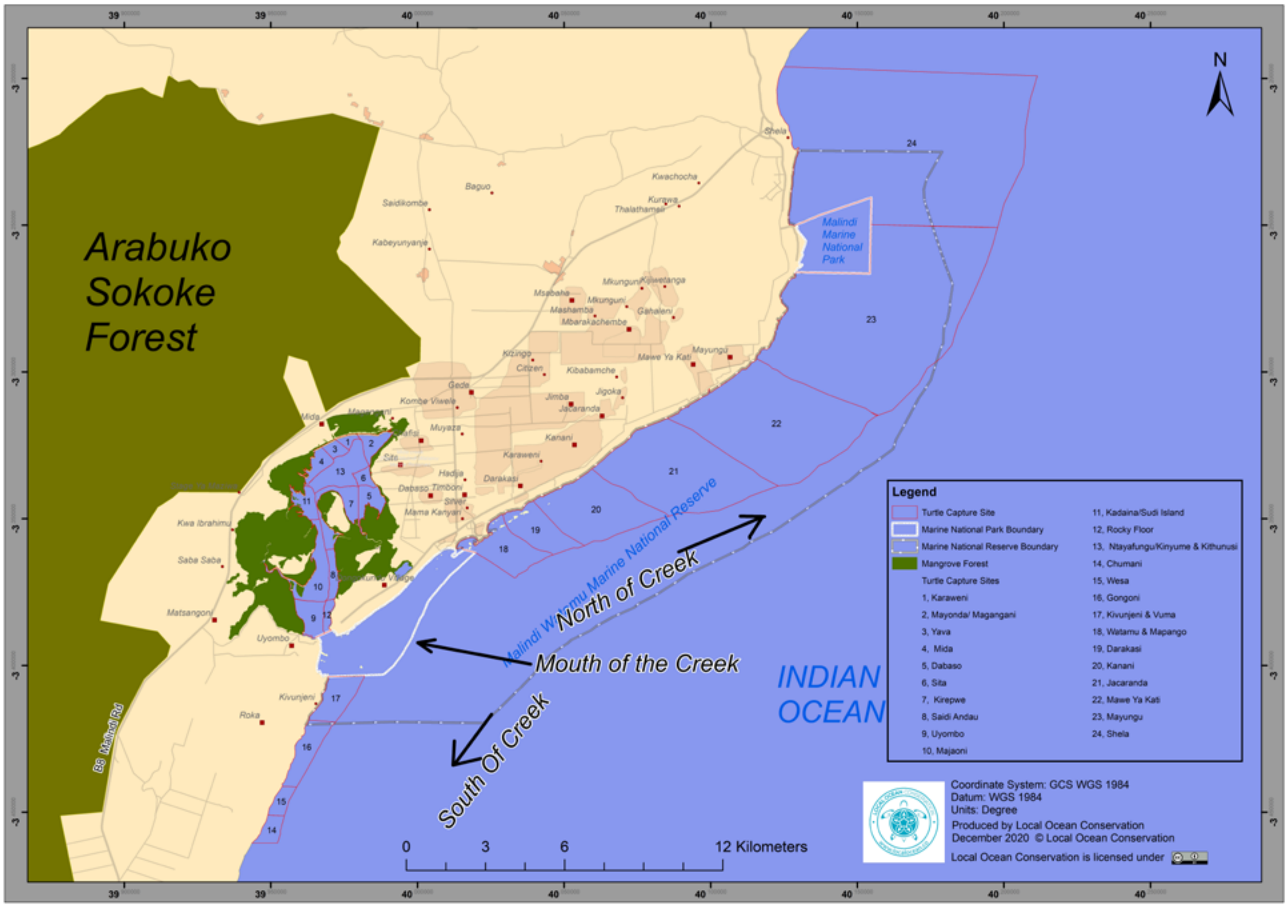

Figure 1. Map of the Watamu area, indicating key marine parks and reserves and capture sites (produced by Local Ocean Conservation).

Methods

Study area

Watamu is a coastal town occurring in the Malindi district of Kenya, located 88 km north of Mombasa and 25 km south of Malindi. Watamu Marine Park and Mida Creek are located in the area, and feature among the marine protected areas of Kenya (Tuda and Omar, 2012; Fig. 1). Both ecosystems are a part of the Malindi/Watamu Marine Reserve. Watamu Marine Park is one of Kenya's oldest marine protected areas (Muthiga, 2009). It forms a conservation area of open ocean approximately 32 km^2 in size that is patrolled and monitored by Kenya Wildlife Services (KWS).

Watamu Bay provides nesting sites for several turtle species including green turtles, and breeding females return to the beaches to lay their eggs.

Data collected by Local Ocean Conservation (LOC), a locally founded marine conservation organization (https://localocean.co/) founded in 1997 was used in this study. LOC operates one of the oldest turtle rehabilitation centres in Africa. It is based in Watamu, Kenya, and runs under the flagship programme of Watamu Turtle Watch. One of the organization's core mandates is to monitor and mitigate activities threatening local turtle nesting sites and populations. LOC routinely receives turtles brought in as by-catch or found stranded in locations around Watamu and other locations along the Kenyan coast.

Data collection

Infection diagnostics

Turtles with visible tumours (on the eyes, body or shell) that were caught in by-catch or found stranded, were brought into the LOC rehabilitation clinic for assessment by a veterinarian. FP tumours typically exhibit a distinct morphology (colour, texture and location) and can manifest on a turtle's skin, carapace, plastron; also on the eye and ocular region (Herbst, 1994). In cases of suspected infection, samples of the tumour(s) were sent to a laboratory for histological analysis. Upon a positive diagnosis of FP, the veterinarian proceeded to cauterize the tumour(s) if possible. Turtles were released after the veterinarian cleared them for release.

Turtle by-catch and stranding data

Standard metric measurements are taken for all captured turtles caught in bycatch and/or found stranded around Watamu Bay. Metric measurements include carapace length and width, weight and turtle ID (tag number). A juvenile turtle was defined as any turtle caught between the curved carapace length (CCL) of 20 - 80 cm, whereas an adult was defined as any turtle with a CCL exceeding 80 cm, as per Kubis *et al.* (2009). New captures are tagged with a titanium metal tag using standard tagging protocols (Limpus, 1992; Heidemeyer *et al.*, 2018). Each tag has a unique identifier number, which can be used to identify turtle individuals upon recapture. For turtles with existing tags, only the tag number and metric measurements were recorded. All tag numbers are recorded, after which turtles are released back into the Watamu Marine National Park. Although LOC occasionally received turtles from other locations along the Kenyan coast, all FP infection records used in this study were restricted to green turtles recorded around Watamu Bay.

Data analysis

Data sorting

Incidents of FP recorded in green turtles from 2003 to 2020 were compiled. This timespan constituted the period of the most reliable data records compiled from the LOC bycatch and rehabilitation programmes. As all FP infections in this time period occurred only in green turtles, other turtle species were excluded from infection analysis. Turtles were counted by cross-checking individual entries in the database using tag numbers (for individual counts), date captured and location (for seasonal and locational counts). In addition to tag number, each turtle was also assigned a unique turtle ID to keep track of their appearance in the database independent of tag replacements. Unique turtle IDs, date and size were used to sort recaptures, whereby turtles with a recurring ID were counted as a recapture whereas turtles that were not tagged or assigned an ID prior were assumed to be unique.

Recaptures were sorted and calculated by year, season and site. Recapture data was taken into account in order to: i) standardize annual and monthly turtle counts (and avoid pseudo replication); and ii) as an indirect indicator of preferred foraging sites around Watamu Bay, to further determine the influence of location (and environmental aspects that turtles are exposed to) on FP prevalence.

Prevalence

Infection prevalence was defined as the sum of turtles infected with FP divided by the total number of turtles captured per unit time or location. Captured refers to turtles recorded as bycatch, stranded, or admitted to the rehabilitation centre. Annual and seasonal infection prevalence were calculated for all capture sites with FP infections. Locational infection prevalence was calculated for capture sites with locational data only.

Location

Location data was obtained from records provided by LOC and organized using allotted capture blocks as shown in Fig. 1. Turtles with no capture site data and capture sites with no cases of infection were omitted from locational analysis.

Sites were allocated into two sets representing broad and finer scale influences: i) broad scale - sites occurring within Mida creek ("creek sites") and sites onshore to the open ocean ("ocean sites"); ii) finer scale – ocean sites north of the creek mouth ("Nocean"; blocks 18 - 22; $n = 11$); ocean sites south of the creek mouth ("Socean"; block 17; $n = 4$); sites around the mouth of Mida Creek ("CMS"; blocks 8 - 10, including the marine park; $n = 6$) and sites within Mida creek ("ICS"; blocks 2 - 7, 11 and 13; $n = 11$).

QGIS software (Hannover version 3.16) was used to map monthly FP prevalence within Watamu Bay sites. Onshore site coordinates were used for turtles found beached or stranded on onshore sites (n = 5), since in these cases, it was difficult to pinpoint the actual foraging location of stranded turtles. GPS coordinates of FP cases were imported and mapped onto a google earth satellite layer (96 DPI resolution) in QGIS at a scale of 1: 100,000 km. A heatmap was generated using a kernel density algorithm (quartic renderer), with a radius of 1 km representing the approximate range of occurrence for each incident, and using FP monthly prevalence as a weighting measure.

Seasonal and epibiont data

Season has been shown to significantly affect marine environmental variables, such as pollutants and nutrient levels and tidal cycles (Espino and Medina, 1993; Li *et al.*, 2016). Seasonal factors were accounted for in this study to determine the influence of associated environmental variables on FP prevalence.

Seasonal data was organized using localized seasons described in Richmond (2011), as Kaskazi (December to March), Kusi (April to mid-September), and Matalai (mid-September to November). The presence of epibionts was accounted for based on evidence indicating that certain species may act as candidate vectors that transmit causative agents for FP (Greenblatt *et al.*, 2004). The presence of epibionts (leeches and barnacles) was accounted for using presence/absence records, both for infected and non-infected green turtles.

Statistical analysis

Statistical analysis was conducted using Python (v3.7) with pingouin (v0.3.8) statistical package and R (v. 6.3.2).

The extent of variation in infection prevalence was determined using Fisher's exact test and ANOVA. Mantel tests (using the vegan 2.5-7 package in R) were used to assess whether there was a spatial correlation between recapture rates and infection prevalence (Oksanen *et al.*, 2020). Matrices for annual infection prevalence and recapture rates were created across all Watamu sites. Matrices were standardized for comparability (by subtracting the mean and dividing by the standard deviation), and an arbitrary constant of 1 added to avoid negative values (for computation using the Bray-Curtis dissimilarity matrix).

Potential effects of seasonality on FP prevalence were tested for using chi-square tests. Logistic regression (using leech and barnacle counts as predictor variables) was used to assess the potential influence of the presence of epibionts (leeches and barnacles) on infection prevalence.

An independent t-test was used to determine the extent of variation in infection prevalence between creek and ocean sites, and Freedman's test was used to determine the extent of variation in infection prevalence between capture sites. A similar approach was applied for seasonal analysis, where an independent t-test was used to determine variation in infection prevalence between monsoon seasons, whereas an ANOVA was used to determine variations between local oceanic seasons.

Results

Annual prevalence

Between 2003 - 2020, 10,869 unique green turtles were brought into the rehabilitation programme or captured as by-catch from 88 sites within Watamu Bay, with a mean of 605 (SD ± 161) turtles captured annually. A further 103 turtles were captured and/or brought in from sites outside of Watamu (n = 10). Juvenile turtles were the most prominently caught age group (n = 10,694), followed by adults (n = 130) and post-hatchlings (n = 45).

A total of 236 turtles (2.4 %) from 40 sites in Watamu Bay exhibited visible FP tumours; of this number, 108 cases (44 %) subsequently died during this time period. Watamu Bay cases showed an annual mean prevalence of 2.9 % during the 2003 - 2020 period. FP prevalence displayed significant annual variation ($F_{1,15} = 8.38$; $p = 0.01$), with the number of cases peaking during 2013 (n = 53) and 2019 (n = 52) respectively (Fig. 2).

Although recapture rates across sites did not vary notably, there was a significant discrepancy between ocean and creek sites ($x^2 = 446.6$; $p < 0.01$) which had recapture rates of 0.6 % and 4 % respectively. High recapture rates were particularly evident at three sites within the creek, which had recapture rates greater than 10 %. FP prevalence and recapture rates displayed a weak negative association ($r = -0.3$; $p > 0.05$), where FP prevalence decreased with higher recapture rates.

Location influence

A total of 194 incidents of FP were recorded around Watamu Bay. Cases of FP were recorded in 19 blocks and at 39 specific capture sites. Overall, FP infection across sites recorded a monthly mean of 24 %.

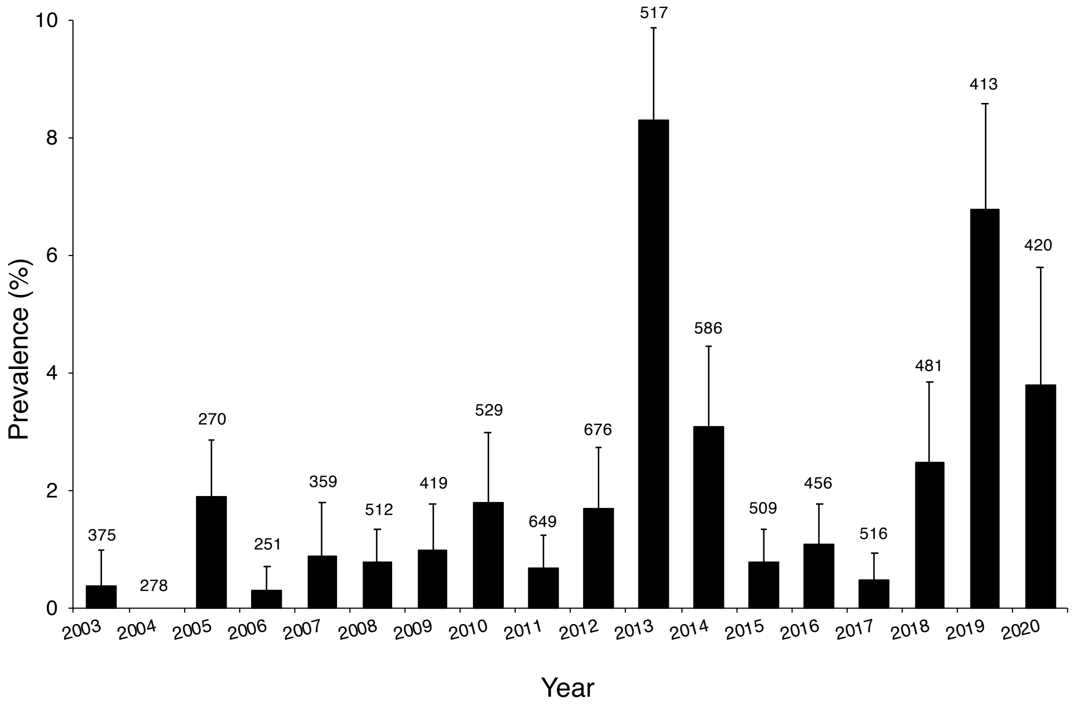

Figure 2. Mean annual prevalence of FP across sites within Watamu Bay (2003 - 2020). Numbers above bars indicate the total number of turtles caught (from by-catch and rehabilitation combined); error bars represent annual (monthly) standard deviation.

Site location significantly affected FP infection prevalence; at a broad scale, ocean sites located offshore or in open ocean displayed a higher infection prevalence per site than sites located within the creek ($F_{1,196} = 12.29$; $p < 0.01$; Table 1). Block size did not affect FP incidents or prevalence.

At a finer local scale, variation in infection prevalence became more significantly pronounced within localised regions of Watamu Bay ($F_{3,\ 194} = 34.76$; $p < 0.01$; Fig. 3b). Sites located around the creek mouth displayed the highest average prevalence, whereas sites located within the creek had the lowest average prevalence (Fig. 4). Sites around the creek mouth and ocean sites south of the creek also exhibited similar fluctuations in annual prevalence, with infections peaking during the same years. Although FP infections were first observed in the creek, with the first case recorded in 2003, sites within the creek recorded the lowest mean annual prevalence throughout the period of study. Annual infection prevalence patterns in ocean sites north of the creek varied in a pattern that was contrasting to those observed in the other regions of Watamu Bay (Fig. 4).

Seasonal and environmental data

FP prevalence showed seasonal variance, with prevalence lowest during the Matalai season, whereas Kaskazi and Kusi seasons showed comparable mean prevalence (Table 2). FP prevalence ($x^2 = 17.7$; $p = 0.47$) and recapture rates ($x^2 = 24$; $p = 0.35$) also displayed similar variance with season, although these were not significant.

Table 1. Turtles infected with FP across sites in Watamu ($N = 131$; SD = standard deviation). Turtles without capture site data are excluded ($n = 8$). Turtles caught are figures adjusted to account for recaptures.

Site Location	Total no. sites	No. of sites with infections	Mean Block Size (km)	Turtles Infected	Turtles Caught	Mean FP Prevalence
Creek	34	19	4.44 (SD ± 2.78)	131 (SD ± 8.5)	8386 (SD ± 690.6)	0.08 (SD ± 0.22)
Ocean	48	22	5.31 (SD ± 2.93)	124 (SD ± 13)	3067 (SD ± 245.6)	0.12 (SD ± 0.15)

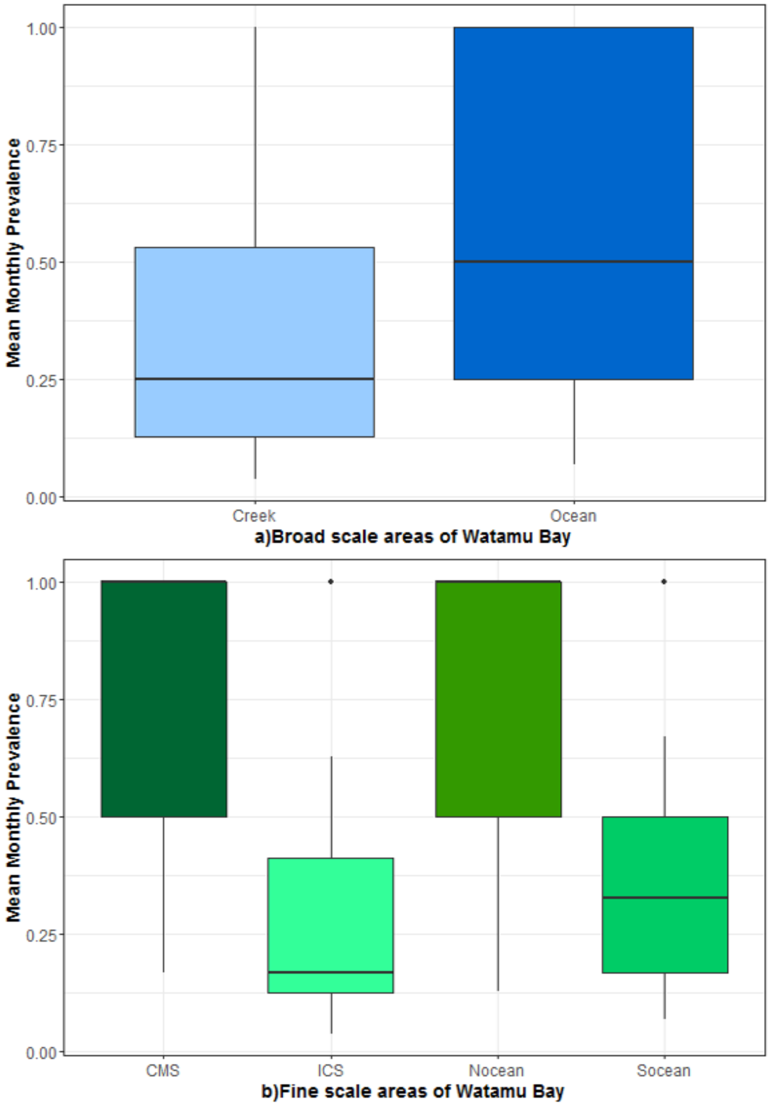

Figure 3. Mean monthly prevalence in Watamu Bay sites at: a) broad scale (creek or ocean); and b) fine scale (CMS = creek mouth; ICS = inner creek; Nocean = northern ocean; Socean = southern ocean).

Logistic regression indicated that barnacle presence increased with a reducing frequency of FP cases while the presence of leeches was correlated with a higher chance of FP (Table 3).

Discussion

Previous studies have shown that FP infections trends can vary temporally (Jones *et al.*, 2016). The finding in the current study are consistent with that variation, as patterns of FP infection prevalence showed prominent annual variation during the period of 2003 - 2020 (Fig. 2). Annual infection patterns indicated the occurrence of two significant outbreaks of FP infection in the Watamu green turtle population, with FP cases peaking during the years of 2013 and 2019 in particular. This suggests that there may have been specific events or significant disruptions within the marine habitat during those years or in the year(s) prior, which triggered an uptick in infection prevalence. Our findings showed that juvenile turtles were the age group most commonly caught around Watamu Bay, and were also the age group most afflicted with FP tumours. As juvenile green turtles remain mostly in neritic habitats during this life stage (Makowski *et al.*, 2006), it is likely that they were the group most exposed to changes in their environment in these years. Annual peaks in infection, especially in sites located around the creek mouth and southern ocean (Fig. 4) may be indicative of a time lag, whereby a period of time lapses between the cause of infection or event triggering infection, and the visible manifestation of infection. An in-depth experimental study by Herbst (1995) demonstrated the presence of a

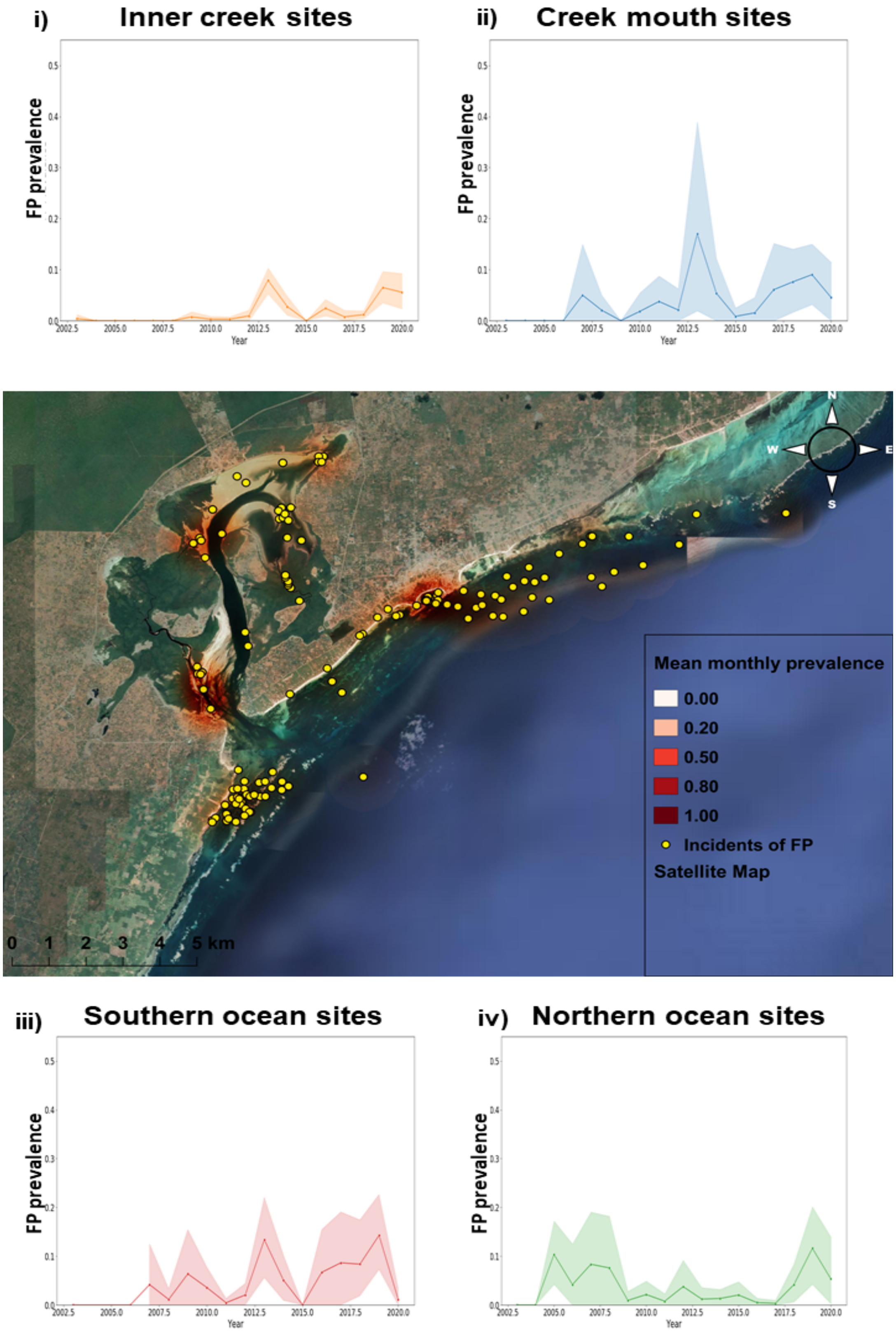

Figure 4. A heatmap depicting monthly incidents of FP infection in sites in and around Watamu Bay (n = 40). Infection intensity is weighted by monthly prevalence; shaded areas depict 95 % confidence intervals derived from annual prevalence in each location. Graphs i - iv show annual variation of infection prevalence in: i) locations around the creek mouth (mean 0.22 ± 0.27); ii) locations within the creek (mean = 0.02 ± 0.016); iii) locations south of the marine park (mean = 0.03 ± 0.02); and iv) locations north of the marine park (mean = 0.12 ± 0.14).

Table 2. Infection prevalence across seasons in positive sites with FP cases in the period between 2003 - 2020 (n = 10,896; SD = standard deviation).

Season	Season Length	Turtles Infected	Turtles Caught	FP Prevalence Range	Mean FP Prevalence
Kaskazi	Nov - Mar	121 (SD ± 8.6)	4400 (SD ± 72.9)	0.00 - 0.10	0.02 (SD ± 0.03)
Kusi	Apr - Aug	99 (SD ± 6.2)	3219 (SD ± 47.2)	0.00 - 0.12	0.03 (SD ± 0.03)
Matalai	Sep - Oct	36 (SD ± 3.1)	1814 (SD ± 35.1)	0.00 - 0.14	0.02 (SD ± 0.04)

lag period between infection and the development of visible tumours ranging between 15 - 43 weeks. However, little remains known about the course of the disease from field studies, such as this one; especially since influential factors are multiple, including the health and age of the turtle, surrounding environmental factors, and the load or type of the infectious agent (Herbst *et al.*, 1994; Greenblatt *et al.*, 2005). The time periods between prominent annual peaks in infection were not consistent, suggesting that the causal agents of infection are diverse and subsequently triggered by various causal factors. Therefore, peaks in infection may also be attributable to various forms or stages of the infection manifesting (Kang *et al.*, 2008).

The other notable outcome of this study was the strong influence of location on FP prevalence in and around Watamu Bay. Additionally, the pattern of annual infection observed across sites in the northern region of Watamu Bay was in contrast to infection patterns observed around the creek mouth and southern ocean regions (Fig. 4). It is possible that infections in the northern ocean may have another underlying factor, such as a different causal agent, or different event. A recent report from Jones *et al.* (2020), demonstrated the diversity of viral strains causing FP occurring at six sites along the Australian coast. Previous studies have found that location plays a significant role on infection - even within the same region, due to the diversity of viral variants that may occur in the same region (Ene *et al.*, 2005). Although the locations of observed FP tumours observed were not a focal point for this study, a closer examination of tumour forms observed on turtles in the region, as well as genetic sampling of the viral strains will help determine whether the peaks in infection are attributable to more than one causative viral strain.

Alternatively, specific onshore events occurring during the course of the years with peak infections may help explain the increases in FP incidents, and also the prominent fluctuations in infection observed at the creek mouth and in surrounding southern ocean locations (Fig. 4). It is likely that the marine habitats at these locations were specifically impacted or exposed to environmental triggers. In Watamu, creek sites are bordered by villages, residential areas, fishing docks and moorings on both sides. Subsequently, onshore discharge from activities such as agriculture and untreated sewage may be washed towards the creek mouth and open ocean and affect neritic habitats in these areas. Mida creek is bordered on either side by extensive mangrove forests, which help with the regulation of ecosystem function and maintenance of water quality (Owuor *et al.,* 2019; Owuor *et al.*, 2017). The dense mangrove habitat bordering the creek may help to explain why FP infection prevalence was consistently lower at sites in the inner creek sites throughout the period of this study.

Although Mida creek (which recorded the lowest overall FP infection prevalence) is affected by tidal ebbs and flows, its ecosystem functions can be viewed

Table 3. Logistic regression model showing the influence of turtle epibionts and algal presence (on shell or body) on FP infection prevalence.

Names	coefficient	Standard error	*p*
Intercept	-3.6	0.07	-
Barnacles	-0.78	0.16	< 0.001
Leech	1.5	0.75	0.046

as distinct to those occurring in the ocean. For example, activities such as the overharvesting of mangroves and pollution have been noted as prominent threats, which potentially also impact the quality of neritic habitats frequented by turtles within the creek (Alamayehu *et al.*, 2014).

It was predicted that a high recapture rate would be an indicator of preferred foraging sites; and that subsequently, habitats with a higher recapture rate would be more commonly frequented by turtles as found by Diez and van Dam (2002). More turtles were caught in capture sites within Watamu creek than in the open ocean (Table 1), with creek sites also reflecting a much higher recapture rate than ocean sites ($p < 0.01$). Foraging trends for green turtles indicate that the species show a preference for seagrass and near-shore habitats (Burgett *et al.*, 2018; Stokes *et al.*, 2019). The findings presented here suggest a higher abundance of preferred foraging habitats in the creek than in the open ocean. However, this finding is confounded by the fact that sites with higher recapture rates in the creek are also frequently visited by fishermen due to their high biodiversity and abundance of marketable species (LOC, personal communication). Recapture is also a measure that carries some potential bias due to realities such as turtles losing their tags over the course of their lives (Heidemeyer *et al.*, 2018) or a lack of available tags. Tag loss is further influenced by tag location, tag type or species tagged (Limpus, 1992; Eckhert *et al.*, 1999). Possible links between FP prevalence and habitat quality indicators (e.g. water quality, nutrient load) is a promising area for deeper consideration, as this study lacked the data to investigate this.

The findings from this study indicated that season was not influential to FP prevalence, with little to no effect on oceanic seasons on FP prevalence (Table 2.) This is similar to the findings of Hirama and Ehrhart (2007), which found that seasonality was weakly associated with FP prevalence. However, as factors pertaining to possible lag periods in infection were not accounted for in this study, there is further opportunity to explore the effects of seasonality in more detail. Overall, findings here indicate that factors more intimately related to green turtle life history traits, such as foraging behaviour, diet and/or turtle community composition, are more likely to act as indirect driving factors for FP.

The incidence of epibionts in this study was varied; the first pattern indicated that barnacle incidence reduces with increased FP prevalence. Barnacle abundance on turtles has been linked with reduced body condition index (Nájera-Hillman *et al.*, 2012), which contrasts with the findings in this study. Turtles not infected with FP were more likely to have barnacles, which indicates a minimal association of barnacle presence with FP prevalence (Table 3). However, this study did find that the presence of leeches increased concurrently with FP prevalence. Prior recent studies have also reported similar findings, where patterns of FP prevalence and the abundance of specific leech species were correlated (Lockley *et al.*, 2020); supporting the potentiality of leeches to act as FP vectors. Observed links between leech infestation and FP prevalence in this study can be further investigated to determine whether: i) leeches, or specific leech species, act as vectors for FP causative pathogens in the Western Indian Ocean region; and ii) whether leech infestations may increase a turtle's susceptibility to FP infection. Further investigations may also determine the range of potential leech vectors, given that the home range of green turtles can vary significantly on an individual scale (Seminoff *et al.*, 2006; Schofield *et al.*, 2015). Therefore, determining the foraging ranges of the Watamu turtle population will be key in helping identify potential environmental factors that turtles are exposed to and which may be driving infection.

Conclusion

The multiple issues facing marine life are widely acknowledged in the sustainable development goals (SDG 14) listed by the United Nations Development Program (UNDP, 2015). There exists an important opportunity to develop knowledge and understanding of turtle populations along the Kenyan coast on multiple levels, with a view to understanding their role as indicators of marine health, and as a priority for their conservation. Besides a lone documented case of FP in the Western Indian Ocean turtle population (Leroux *et al.*, 2010), information pertaining to this disease in this region remains anecdotal. Particularly along the East African coast, information is needed concerning the diversity and type of infectious agents of FP, as well as causal environmental driving factors. This study is the first attempt at an investigation of FP infection patterns in this region. Sites considered in this study were restricted to those within Watamu Bay. However, the emerging associations between location and FP prevalence in this study are highly indicative of the potential influence of environmental factors in the progression of FP, and supports arguments for horizontal transmission of FP. This outcome provides a foundation for further studies examining other sites along the coast to: i) compare whether potential causative conditions are similar; and ii) to determine whether other unique factors are also contributing towards incidences of FP. Subsequently, the findings from this study are also subject to variations in onshore human activities and threats. Watamu is a popular tourist destination along the Kenyan coast, and a majority of the residents derive their income from the tourism industry. It will be important to investigate whether there are any direct or lag impacts from annual population fluxes in residency; factors such as hotel residency rates, new constructions and marine traffic (boat excursions, snorkelling and fishing), can all adversely affect neritic habitats in and around Watamu, especially those located directly offshore. Therefore, factors for further consideration may be related to habitat quality; its influence on turtle ranges and habitat usage; and the level of exposure of various turtle populations to triggers of FP infection. Finally, there is also a need to consider and assess the genetic diversity of turtles along the Kenyan coast as: i) this plays a key role in resilience; and ii) may provide clues to the life-stage at which ChHV5 is transmitted. Such research transcends FP, as characterising the genetic diversity of turtles in Kenya has broader implications for green turtle management as a whole.

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Diversity of mangrove fungal endophytes from selected mangrove species of coastal Kenya

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Endophytes are bacteria or fungi living asymptomatically in the internal tissues of plants. They are symbiotic in nature and can be exploited for novel bioactive metabolites with applications in agriculture, medicine and industry. Mangrove fungal endophytes from the marine environment are abundant and have been recognized as sources of bioactive natural products. The study was designed to isolate, purify and identify mangrove fungal endophytes from selected common mangrove species of Gazi Bay, Tudor and Mida creek on the Kenya coast. The colonization rate and isolation rate of the mangrove fungal endophytes were determined. The studied mangrove species were *Rhizophora mucronata* (red mangrove), *Sonneratia alba* (mangrove apple), *Avicennia marina* (grey or white mangrove), and *Ceriops tagal* (spurred mangrove). Samples from twigs of these mangrove species were collected and analyzed using standard methods. Isolation of pure cultures of the endophytes was performed using Potato Dextrose Agar (PDA) incubated at 28 ± 1°C for 5 days. The fungal isolates were identified under a light microscope based on colony morphology characteristics, type and presentation of conidiophores and conidia. A total of 18 different mangrove fungal endophytes were identified and these belonged to 5 genera. These were *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces*, with *Aspergillus* being the most dominant genus. Tudor Creek recorded the highest fungal community diversity (H' = 1.35) and Gazi Bay had the lowest diversity (H' = 0.45). Fungal community similarity based on the identified genera was highest between Gazi Bay and Mida Creek (0.80) and lowest between Tudor Creek and Mida Creek (0.57). The selected mangrove species recorded a colonization rate of endophytic fungi of between 38.9 – 94.4 % with the highest habitation being associated with *S. alba* and *C. tagal.* There were differences and similarities in the colonization rate within mangrove species across study sites. Findings of this study have confirmed that the selected mangrove species exhibit high diversity of fungal endophytes with host recurrence and spatial heterogeneity.

Keywords: diversity, mangroves, fungal endophytes, colonization, Kenya coast

Introduction

Mangroves differ from other plants in morphology, anatomy and reproduction due to the influence of several factors such as salinity, pH and soil condition (Thorati *et al.*, 2016). Mangrove plants harbor different species of endophytes which are a valuable source of useful metabolites gaining increasing importance in the pharmaceutical industry. The Kenyan mangrove forests are part of the Western Indian Ocean (WIO) region which covers 1 million ha; about 5 % of the global mangrove cover (UNEP-Nairobi Convention/USAID/WIOMSA, 2020). Two of the selected mangrove species in this study, *R. mucronata* (Lamk) and *C. tagal* (Perr.) belong to the largest mangrove family Rhizophoraceae while *A. marina* (Forsk.) is of the family Acanthaceae, and *S. alba* (Sm) from the Lythraceae family (Kairo, 2001). Mangroves have been used in traditional medicines and extracts of some species have been known to exhibit activity against human, animal and plant pathogens (Hamzah *et al.*, 2018).

Endophytic fungi are eukaryotic microorganisms that colonize living internal tissues of plants asymptomatically and are under-explored to meet the various

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needs of mankind, including medicine (Dar *et al*., 2015). These microorganisms represent an important and quantifiable component of fungal biodiversity, affecting plant community biodiversity and structure (Dar *et al*., 2015). Studies have shown that these fungi have been found in every plant examined to date and are a potential source of natural products for exploration in medicine, agriculture and industry (Thorati *et al*., 2016). Recent studies have shown the uniqueness of endophytic fungi with about 1 million species inhabiting plant tissues (Huang *et al*., 2008).

The discovery of penicillin by Alexander Fleming in 1928 from the fungus *Penicillium* sp., coupled with re-isolation and clinical studies by Chain, Florey, and co-workers in the early 1940s, and commercialization of synthetic penicillin, revolutionized drug discovery research to a large extent (Sonia, 2006). This enormous success prompted drug companies and research groups to assemble collections of microorganisms in order to uncover new antibiotics. This led to the discovery of streptomycin, chloramphenicol, chlortetracycline, erythromycin, vancomycin and cephalosporin C. The antibiotic cephalosporin C was extracted from a fungus *Cephalosporium acremonium* and was found to show activity against *Staphylococcus aureus*, *Salmonella typhii* and *Escherichia coli.* All of the compounds stated, or their derivatives, are still in use as drugs to date (Sonia, 2006; Ling *et al*., 2010).

Interest in endophytic fungi has further grown since the discovery of an endophytic fungus, *Taxus brevifolia,* which led to the production of the billion-dollar anti-cancer drug, taxol (Saryono *et al*., 2015). Taxol or paclitaxel was first isolated from an endophytic fungus, *Taxomyces adreanae* by Stierle and colleagues in 1993 (Ling *et al*., 2010). This fungus inhabited the bark of a yew tree (*Taxus brevifolia),* an evergreen shrub. Taxol generated more attention and interest than any other new drug since its discovery, possibly due to its unique mode of action compared to other anticancer agents found to interfere with the multiplication of cancer (Sonia, 2006).

The relationship of endophytic fungi with single or multiple plant hosts can be described in terms of host specificity, host-recurrence, host-selectivity or host-preference (Huang *et al*., 2008). Host-specificity refers to a relationship between a living host and a fungus. This is restricted to a single host or a group of related species but does not occur in other unrelated plants in the same habitat (Nogueira-Melo *et al*., 2017). Frequent or predominant occurrence of an endophytic fungus on a particular host or a group of plant hosts is refered to as host-recurrence while the fungus can still occur in other plant hosts in the habitat (Huang *et al*., 2008). Host-selectivity is a phenomenon whereby an endophytic fungal species forms relationships with two related plant species but demonstrates preference to one of the plant hosts. Endophytic fungi may exhibit host specificity for a particular plant species; the distribution of which may then be influenced by environmental conditions, leading to spatial heterogeneity (Huang *et al*., 2008).

Endophytes in mangrove species along the Kenya coast have scarcely been investigated. The microbial resources of the Kenya coast, particularly the endophytic populations in the mangrove plants, are still under-explored. Jenoh *et al*., (2019) studied the infestation mechanisms of two woodborer species in the mangrove *S. alba* in Kenya and the co-occurrence of endophytic fungi. These authors established the occurrence of secondary infestation by endophytic fungi in the infested branches of *S. alba*. Endophytic fungal isolation was achieved using standard procedures by Barnett (1998) resulting in 15 species distributed in 6 fungal genera namely *Aspergillus*, *Penicillium*, *Trichoderma*, *Giberella*, *Talaromyces*, and *Cladosporium*. Hamzah *et al*., (2018) isolated and identified a total of 78 fungal isolates from the leaves of *R. mucronata* from Matang Mangrove Forest Reserve (MMFR) in Malaysia.

This study therefore aimed at isolating and identifying fungal endophytes from selected mangrove species from samples of twigs collected from Gazi Bay, Tudor Creek and Mida Creek along the Kenya coast. Colonization rate (CR) or the percentage of colonized segments, isolation rate (IR) which is a measure of fungal richness in a sample of plant tissue, relative frequency (RF) (Huang *et al*., 2008), and fungal community diversity were determined. The findings obtained from this study provide preliminary data on the diversity of mangrove endophytic fungi from the Kenya coast for future investigations on marine bioactive molecules.

Materials and methods

Study area

This study was conducted in three selected mangrove ecosystems along the Kenya coast (Fig. 1). These included Gazi Bay on the south coast of Kenya located at 4°25'S, 39°50'E in Kwale County, about 50 km south of Mombasa City. The bay comprises

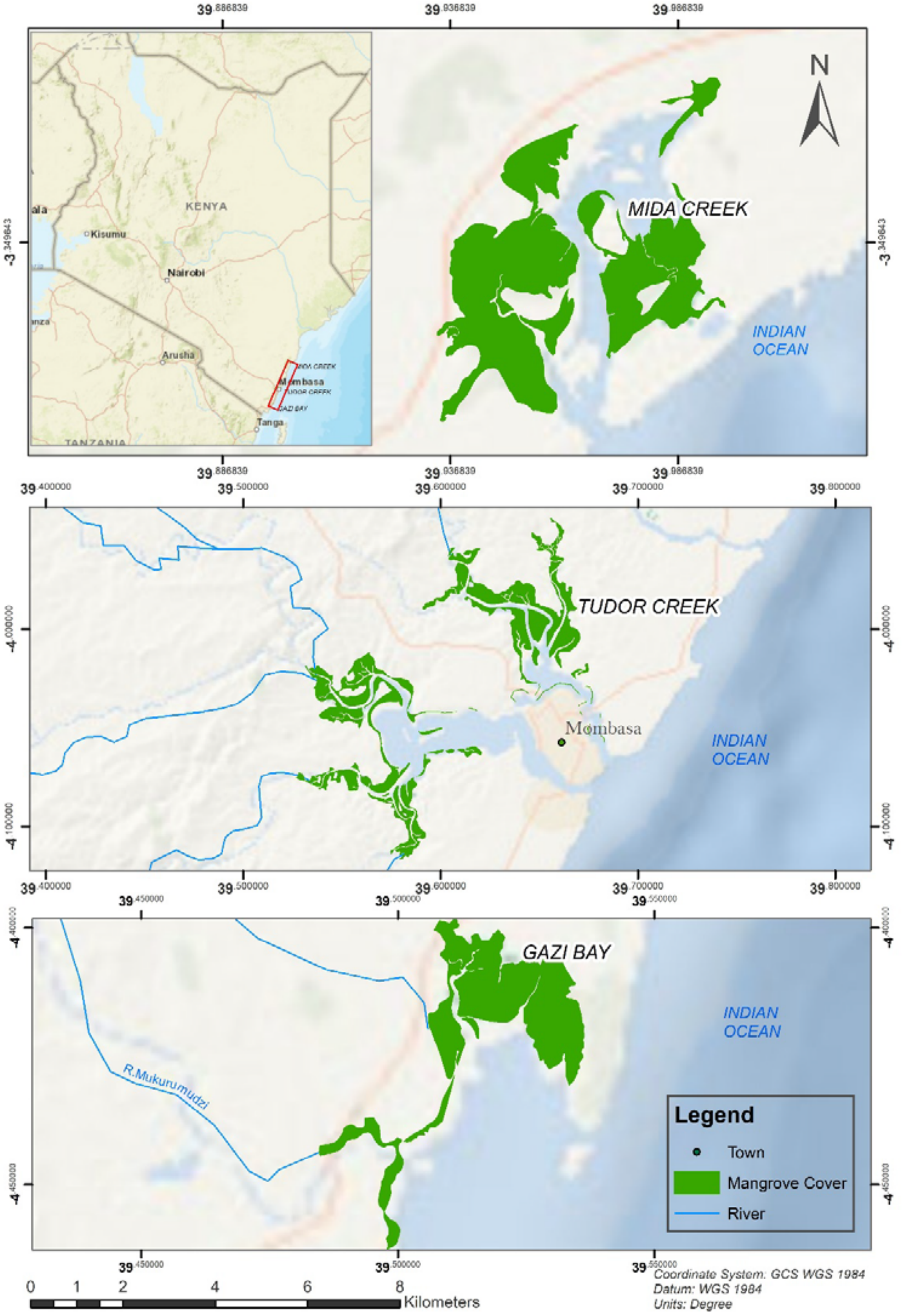

Figure 1. A map of the Kenyan coast showing the mangrove forests of Gazi Bay, Tudor Creek and Mida Creek where samples of mangrove twigs were collected for the study.

a shallow channel approximately 4 km long fringed by mangrove forest dominated by the species *S. alba* and *R. mucronata* (Bosire *et al.*, 2003). Tudor Creek (4°2'S, 39°40'E) which is located at the northwest of Mombasa Island, extends some 10 – 15 km inland with two main seasonal rivers, Kombeni and Tsalu, draining over 45,000 and 10,000 ha, respectively (Bosire *et al.*, 2014). Within the creek is a mangrove forest extending over an area of 1,641 ha, principally composed of *R. mucronata*, *S. alba*, and *A. marina*. The forest does not display distinct mangrove species zonation along the tidal gradient and is covered by sediments that are predominantly made up of mud, and sand in some parts (Bosire *et al.*, 2014). Mida

Creek (03°21'S, 39°59'E) is located in Kilifi County, 88 km north of Mombasa and is approximately 25 km south of Malindi Town (Dahdouh-Guebas *et al.*, 2000). Mangrove forest is the most dominant habitat in Mida Creek and occupies approximately 1,746 ha supporting 7 of the 9 mangrove species found in Kenya (Kairo, 2001; Owuor *et al.*, 2017).

Collection of samples

Identification and sampling of the selected common mangrove species was conducted in each study site. Subjective or non-probability sampling was employed. From the landward side, samples of twigs of *A. marina* were collected. Between the landward and seaward side, samples of twigs of *C. tagal* and *R. mucronata* were collected, and to the seaward side, samples of twigs of *S. alba* were collected. A total of 3 twig cuttings from 2 mangrove trees of each species in the 3 study sites were randomly sampled using a sharp laboratory knife; [3 x 2 x 4] x 3. This gave an overall total of 72 twig cuttings comprising of 18 segments from each mangrove species.

Isolation and purification of fungal endophytes

All 72 twig cuttings were taken to the laboratory where tissue segments were washed in running water to remove soil and debris. Mangrove fungal endophytes were isolated according to Arnold (2001) with a few modifications. The twig cuttings were surface sterilized with 1 % sodium hypochlorite for 30 seconds instead of 0.525 % for 2 minutes. Traces of sodium hypochlorite were removed and then rinsed in sterile distilled water before treatment with 90 % for 30 seconds instead of 70 % ethanol for 2 minutes according to Arnold (2001). The outer tissues from the twigs were removed after drying the plant tissues under sterile laminar air flow and passing through a flame for 30 seconds. The internal tissues were then cut into smaller pieces of between 0.5 and 1 cm and inoculated on Potato Dextrose Agar (PDA) plates to capture a broad spectrum of the fungal community. The plates were later incubated at 28 ± 1°C for 5 days (Saunders, 2010; Bijaya, 2015; Prabukumar, 2015). Hyphal tips of fungi, emerging out of the mangrove fungal endophytes were then isolated and sub-cultured on PDA at an optimum temperature of 28 ± 1°C and a pure culture of each isolate obtained (Xing *et al.*, 2011).

Morphological and microscopic identification of the fungal isolates

Identification of the mangrove fungal endophytes was based on macroscopic morphological features such as fungal colony characteristics, fungal growth, colony color (front and reverse), characteristics of the spores and discernible vegetative features on the PDA plate. Microscopic slides of each endophyte were prepared by placing a drop of lacto phenol- cotton-blue on a clean slide. A small tuft with spores and spore bearing structures of the fungus was picked using a sterilized inoculation needle and placed onto the drop. The stain was mixed with the mold structures and a cover glass was placed over the preparation. The slides were observed under a light microscope (Primostar) at x40 and x100 with oil emulsion. Microscopic features of the endophytes were photographed and processed by an Axiocam ER 5s camera linked to a Zen blue 71 software. Features included conidial development, shape of conidia and conidial head, size of and attachment of conidia (Barnett and Hunter, 1998; Huang *et al.*, 2008; Xing *et al.*, 2011).

Determination of colonization rate, isolation rate and relative frequency

Colonization rate (CR) was calculated as the total number of segments colonized by endophytic fungi divided by the total number of segments incubated for a given plant sample and expressed in percentage. This compares the degree of infection by endophytic fungi between plant tissues. Isolation rate (IR) is a measure of fungal richness in a sample of plant tissue and the incidence of plant infections per segment. It was calculated as the total number of segments incubated from the twig cuttings of a given mangrove species divided by the endophytic fungi isolated from the segments. This gave an indication of the fungal richness per sample of plant material. Fungal density or relative frequency (RF) was calculated as the number of isolates of one fungal genus divided by the total number of isolates and expressed in percentage (Huang *et al.*, 2008; Liu *et al.*, 2019).

Data analysis

Determination of fungal isolate diversity

Diversity of fungal isolates (taxa richness) contained in each selected mangrove species and the relative abundance of the fungal isolates were determined. The Shannon Wiener diversity index (*H'*) was used as a measure reflecting the number of different isolates and how evenly the individuals are distributed among the species of the selected mangroves (Pielou, 1977). The degree of community similarity of endophytic fungi between the individual selected mangrove species was determined by employing Sorenson's Coefficient (CC) (Magurran, 2004).

Results

Isolation of fungal endophytes

Cultural identification of endophytic fungi isolates resulted in a total of 50 mangrove endophytic fungal isolates from the 72 twig cuttings of *R. mucronata*, *S. alba, A. marina*, and *C. tagal*. Due to the large number of the isolated fungi, selection was made based on good growth characteristics, color and margin characteristics. The resulting fungal endophytes were classified into 4 groups according to their host mangrove species (Fig. 2). The isolated fungal endophytes demonstrated a brown color with a white margin and different shades of brown, green and white.

Morphological characterization of mangrove fungal endophytes

A total of 19 endophytic fungal isolates were selected for further morphological characterization based on their colony morphology, microscopic and macroscopic features (Table 1) (Fig. 6-10). Sporulated cultures were examined for colony color, conidial size, shape and development. A total of 18 isolates were successfully identified morphologically into 5 mangrove endophytic fungal genera namely; *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces* (Fig. 4). One of the isolates was not fully characterized because its conidia did not grow in PDA media. The dominant fungal genus identified in this study was *Aspergillus* (55 %). This was followed by *Penicillium* (22 %) and *Fusarium* at 11 %. The least dominant were *Cephalosporium* and *Blastomyces* each recording an abundance of 5.5 %. *Aspergillus* was found in all the 3 study sites of Gazi Bay, Tudor and Mida creek and in all the selected mangrove species of *A. marina, C. tagal, R. mucronata* and *S. alba.* The genus *Penicillium*, the second most abundant, was distributed in *A. marina* from the 3 study sites and in *S. alba* from Tudor Creek. The 2 isolates of *Fusarium* were both found in Tudor Creek in *S. alba* and *C. tagal,* respectively. *Cephalosporium* isolate 1 and *Blastomyces* isolate 1 were found in *S. alba* as well as in *R. mucronata* of Tudor Creek. A total of 4 out of 5 fungal genera were found in Tudor Creek alone, namely *Aspergillus*, *Penicillium*, *Fusarium* and *Cephalosporium*. Gazi recorded 2 fungal genera, *Aspergillus* and

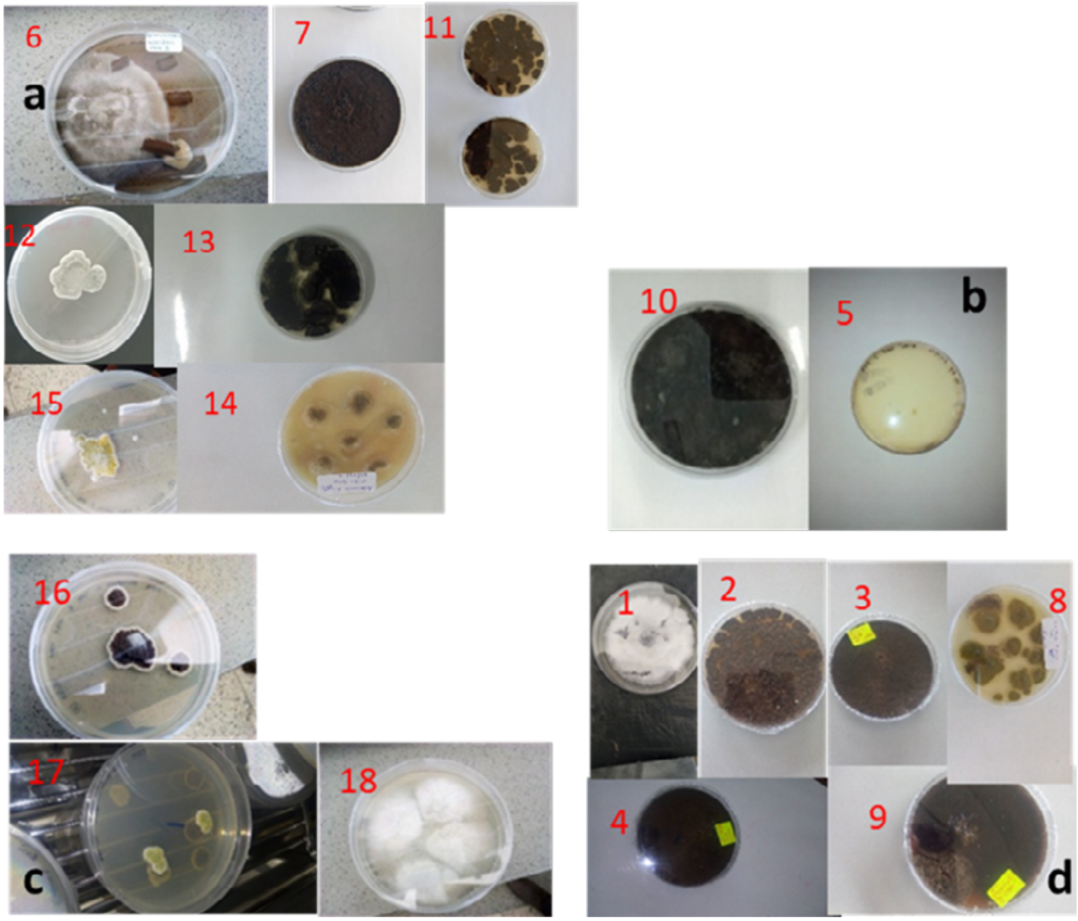

Figure 2. A section of the culturally identified mangrove fungal endophytes from the twig cuttings of selected mangrove species of coastal Kenya. **(a)** *Avicenia marina*, **(b)** *Ceriops tagal*, **(c)** *Rhizophora mucronata*, **(d)** *Sonneratia alba*.

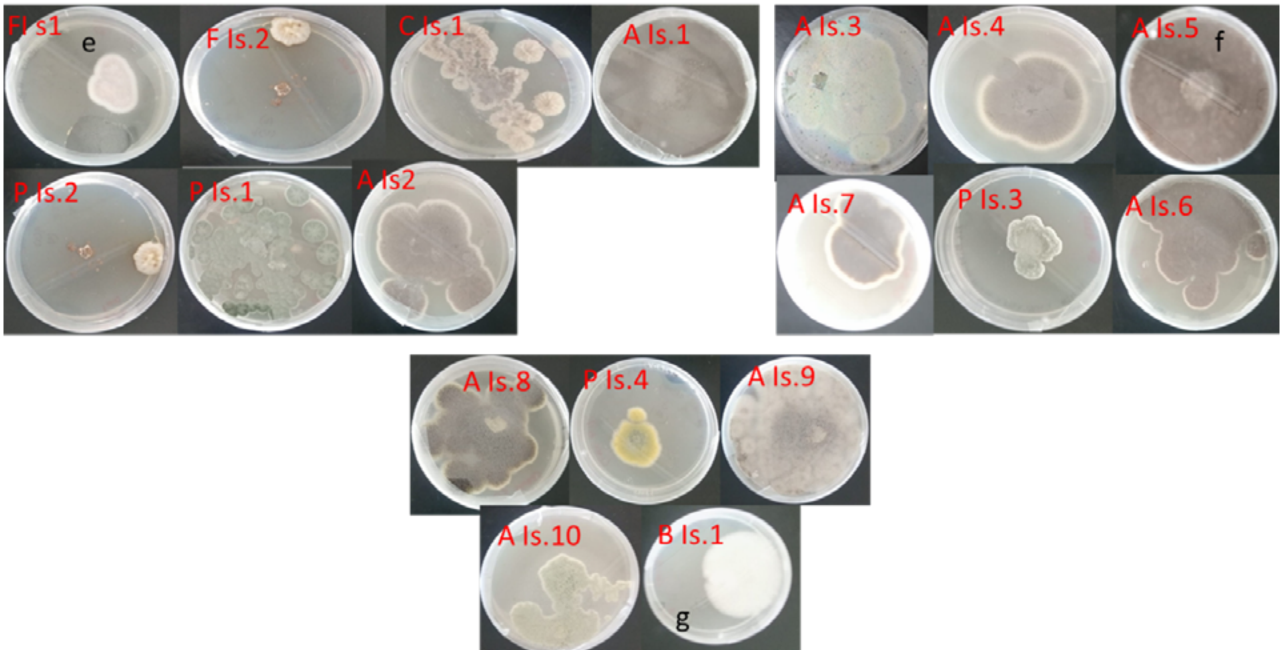

Figure 3. Spatial distribution of mangrove endophytic fungal genera in **(e)** Tudor Creek, **(f)** Gazi Bay, and **(g)** Mida Creek.

Table 1. Morphological characteristics of fungal mangrove endophytes isolated from selected study sites in coastal Kenya.

S/No	Fungal genera	Macroscopic features	Microscopic features	Reverse side
1	*Aspergillus*	Fast growing brown colony with a white margin a powdery texture	Have regular mycelium and hyphae that are septate, Conidiophores are erect and terminate in a vesicle. The vesicle and conidia form the conidial head	Cream
2	*Penicillium*	Grow moderately fast, colony green and velvet like	Hyphae are septate, hyaline simple conidiophores. Phialides are branched in brush like clusters at the tips of conidiophores. Conidia are round and unicellular	Yellow
3	*Fusarium*	White colony filled the plate with aerial mycelia	Hyaline, septate hyphae leading to branched conidiaphore with both macro and microconidia. Microconidia are sickle-shaped and produced from phialides of branched conidiophores	White
4	*Cephalosporium*	Dark grey in color and cream in reverse. Initially glabrous and shortly became felt like	Well-developed hyaline slender hyphae with unbranched conidiophores. Conidia hyaline and unicellular. Phialides are erect, unbranched, tapering and are form directly on narrow hyphae	Cream
5	*Blastomyces*	The texture is membranous and downy to woolly. Produced aerial mycelium	They have septate hyaline hyphae and unbranched short conidiophores. Conidia are hyaline and unicellular. Conidia are round and are produced perpendicular to the hyphal axis on short, thin conidiophores.	White

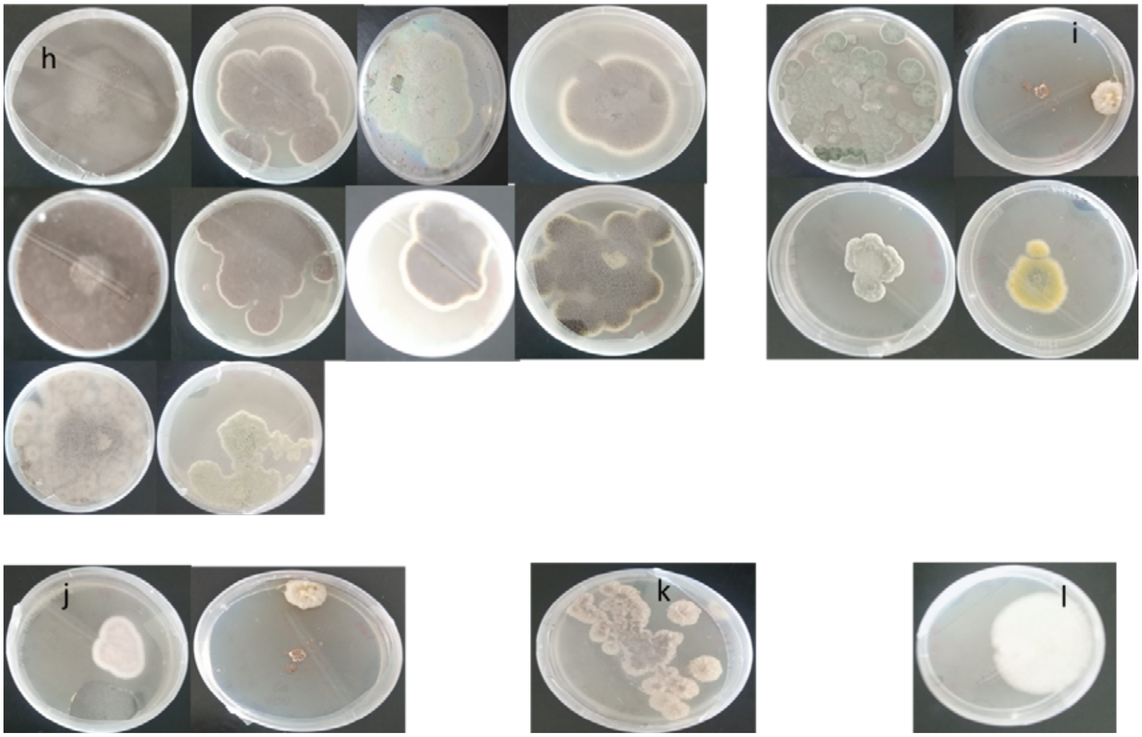

Figure 4. Morphologically identified mangrove endophytic fungal genera from selected mangrove species of coastal Kenya. (**h**) *Aspergillus*, (**i**) *Penicillium*, (**j**) *Fusarium*, (**k**) *Cephalosporium*, (**l**) *Blastomyces*.

Penicillium while Mida Creek recorded 3 fungal genera, *Aspergillus*, *Penicillium* and *Blastomyces*. The mangrove species *A. marina* hosted the largest number (7) of mangrove endophytic fungal genera while *S. alba* hosted 6 isolates, *R. mucronata* 3 isolates, and 2 isolates were found in the mangrove species *C. tagal* (Fig. 4).

Colonization and isolation rates

Endophytic CR ranged between 38.9 – 98 % for the mangrove species in this study. The highest CR was 94.4 % associated with the mangrove species *S. alba* and *C. tagal* in Tudor Creek. This was followed by *R. mucronata* (88.9 %) in Tudor Creek, *R. mucronata* (83.3 %) in Mida Creek, and *A. marina* with 72.2 % in Tudor Creek, *C. tagal* and *A. marina*, 66.7 % in Mida Creek and Gazi Bay, respectively (Fig. 5). Endophytic IR was highest in *A. marina* at 0.30 followed by *C. tagal* and *R. mucronata* each at 0.24, and lowest for *S. alba* at 0.14. Fungal diversity varied across the study sites. The highest fungal community diversity (H' = 1.35) was found in Tudor Creek followed by Mida Creek (H' = 0.95) and Gazi Bay was the least diverse (H' = 0.45).

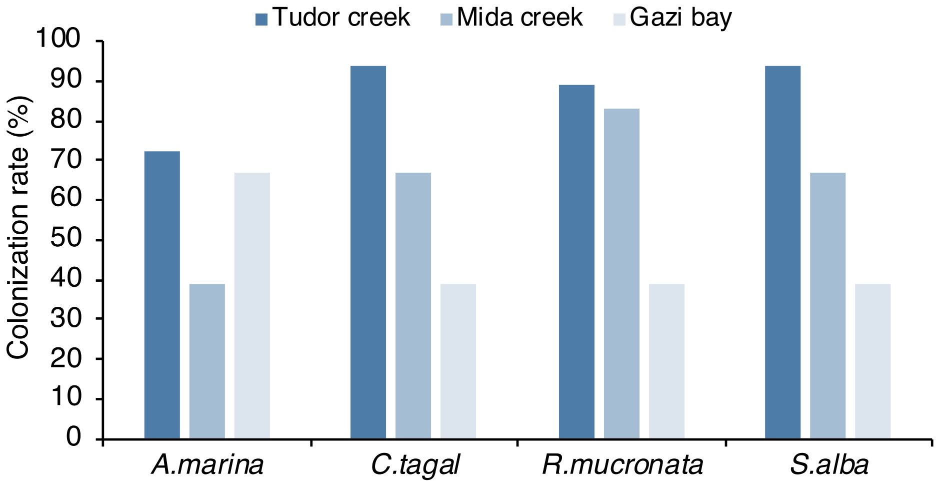

Figure 5. The colonization rates of mangrove fungal endophytes across the study sites in coastal Kenya.

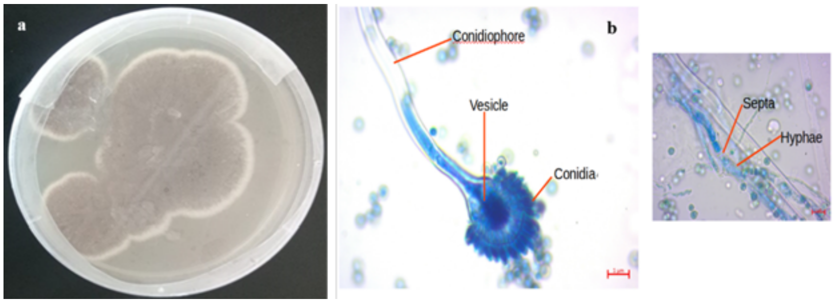

Figure 6. Colony morphology of *Aspergillus*: a) front view; and b) microscopic features of *Aspergillus* (x 100).

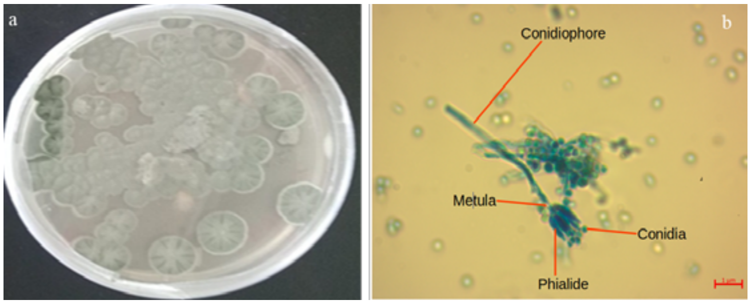

Figure 7. a) Colony morphology of *Penicillium*; b) microscopic features of *Penicillium* (x 100).

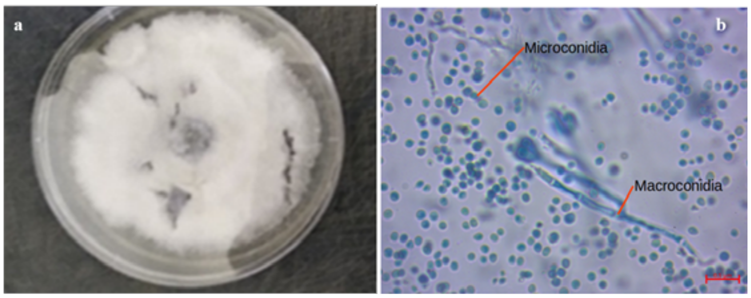

Figure 8. Colony morphology of *Fusarium*: a) front view; b) microscopic features of *Fusarium* (x 100).

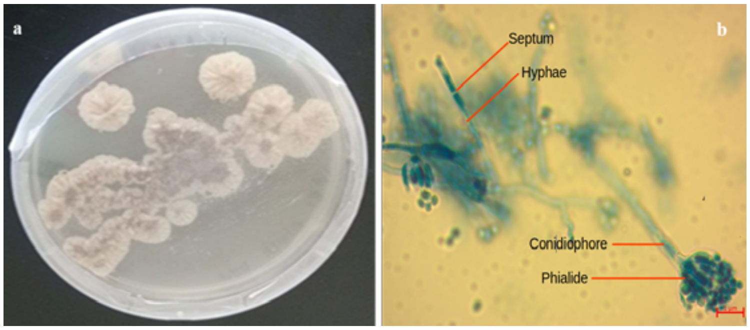

Figure 9. Colony morphology of *Cephalosporium*: a) front view; and b) microscopic features of *Cephalosporium* (x 100).

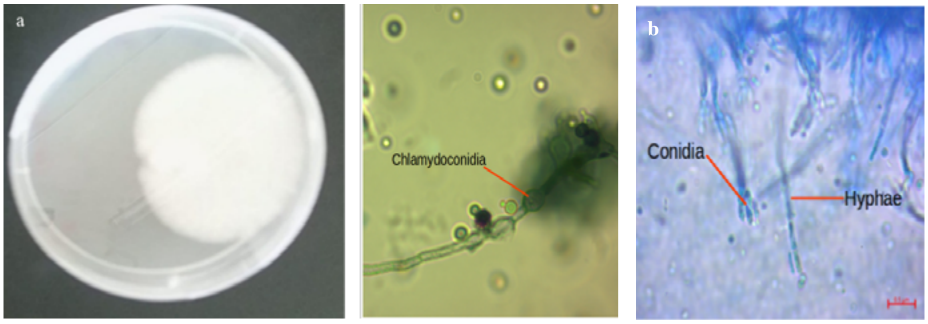

Figure 10. Colony morphology of *Blastomyces*: a) front view; and b) microscopic features of *Blastomyces* (x 100).

Fungal community similarity based on the identified genera was highest between Gazi Bay and Mida Creek (CC = 0.80) and lowest between Tudor Creek and Mida Creek (CC = 0.57).

Mangrove endophytic fungal genera found in Tudor Creek were *Aspergillus* isolate 1 and 2, *Penicillium* isolate 1 and 2, *Fusarium* isolate 1 and 2 and *Cephalosporium* isolate 1. Gazi recorded a total of 6 mangrove endophytic fungal isolates; *Aspergillus* isolate 3, 4, 5, 6 and 7 and *Penicillium* isolate 4. A total of three out of ten isolates of *Aspergillus* (8, 9 and 10), and *Blastomyces* isolate 1 were recorded in Mida Creek (Fig. 3). The isolates were clustered in 5 groups (h-l) according to color of their colonies and other morphological characteristics. Group h represents the genus *Aspergillus* sp. ranging from different forms of green, whitish brown, dark and various forms of brown with different colony morphology. Group i represents *Penicillium* genera, j *Fusarium* genera, k *Cephalosporium* genera and l *Blastomyces* genera (Fig. 4).

A summary of morpho taxonomic characteristics of fungal endophytes by study site and mangrove species showed that the brown fungal endophyte recorded the highest relative frequency (RF) of 16 % followed by green (12 %), and white and black with 10 % each. The brown fungal endophyte is found in *R. mucronata* and *S. Alba* whereas the green fungal endophyte appears more in *A. marina* of Gazi Bay and Mida Creek. A total of 2 isolates (greenish white and the yellowish green) of the green fungal endophyte was observed in *R. mucronata* of Mida Creek only. The black and the grey fungal endophytes occurred in *C. tagal* more than in any other mangrove species. Generally endophytic fungi were recorded in more than one location except the stranded brown and the yellowish green fungal endophyte that was recorded only in Tudor and Mida Creeks, respectively (Fig. 3).

Discussion

Diversity of mangrove fungal endophytes

Morphological characterization resulted in a total of 5 fungal genera, namely *Aspergillus*, *Penicillium*, *Fusarium*, *Cephalosporium* and *Blastomyces* (Fig. 4). The most abundant endophytic fungal genus was *Aspergillus*; an observation that has been commonly encountered in other endophytic studies (Prihanto *et al.*, 2011; Rossiana, *et al.*, 2016; Jenoh *et al.*, 2019). Thorati *et al.* (2016), recorded the prominence of *Aspergillus* in a study of isolation and identification of endophytic fungi from mangrove roots of *R. apiculata, R. mucronata* and *Bruguiera gymnorrhiza* along the coast of South Andaman sea, Andaman and Nicobar Islands, India. The genus *Penicillium* (Fig. 6) was moderately abundant and had been identified previously along the Kenyan coast in the infested *S. alba* of Gazi Bay and Mida Creek (Jenoh *et al.*, 2019). *Fusarium* (Fig. 7) was the third most abundant mangrove fungal endophyte genus in this study and was also observed in other endophytic fungal studies (Prihanto *et al.*, 2011; Hamzah *et al.*, 2018). Hamzah *et al.*, (2018) identified the genus *Fusarium* in the leaves of *R. mucronata* growing in the Malaysian mangrove forest. The 2 isolates of *Fusarium* in this study were isolated from the twigs of *S. alba* and *R. mucronata* in Tudor Creek. The least encountered endophytic fungal genera were *Cephalosporium* isolate 1 and *Blastomyces* isolate 1. *Cephalosporium*, only found in *R. mucronata* of Mida Creek, is listed among fungal endophytes with the potential for producing bioactive compounds (Kumar, 2020).

Tudor Creek recorded the highest diversity of endophytic fungi and these were mostly present in *A. marina* located on the landward side of the creek. On the other hand, Mida Creek recorded the least diversity and the associated fungi came mostly from the mangrove species *C. tagal* that was located between the landward side and the seaward zone of the mangrove forest (Fig. 3). The highest number of fungal isolates from Tudor Creek was attributed to differences in environmental conditions where samples of twigs were collected towards the landward side of the creek that was more exposed to air, land and water pollution from land-based sources. Such differences in environmental conditions may include elevated levels of pollution along Tudor Creek arising from both domestic and industrial sources (Mohamed *et al.*, 2008). Endophytic fungi in plant tissues are known to help the host plant adapt to both biotic and abiotic stress factors (Wang *et al.*, 2014). Therefore, Tudor Creek being a highly polluted environment is the likely cause of the presence of the highest number of endophytic fungal genera. This has been cited as an adaptation strategy for marine plants in surviving stressful environments as documented by Thatoi *et al.* (2013).

Most of the mangrove fungal isolates showed colony pigmentation attributed to the production of pigments such as carotenoids, melanins, flavins, phenazins and quinones which are associated with crucial antifungal, antibacterial and herbicidal activities. Previous studies on endophytic fungi have also shown that fungal isolates produce pigmentation in response to adverse conditions such as low moisture, pH and UV light (Hamzah *et al.*, 2018). A summary of morpho-taxonomic characteristics revealed that the most frequently encountered fungal endophyte was the brown isolate identified as *Aspergillus* (Fig. 6) (Barnett and Hunter, 1998). The least common fungal endophytes were stranded brown and the yellowish green fungal isolate from Mida Creek, each with a relative frequency of 2 %. These results corroborate with findings from a study conducted in Hong Kong Island by Huang *et al.* (2008). Tudor Creek recorded the highest fungal community diversity whereas the lowest diversity was encountered at Gazi Bay. This is indicative of variations in environmental conditions in the respective study sites. Similarity in fungal community based on the identified genera was highest between Gazi Bay and Mida Creek and lowest between Tudor Creek and Mida Creek. This may be attributed to the fact that Mida Creek is located in a less polluted environment and has similarities to Gazi Bay which is a natural harbor pushing deep into the mainland coast.

Host recurrence of mangrove endophytic fungi

A phenomenon whereby an endophytic fungus frequently occurs or dominates a particular plant host or a group of plant hosts is termed 'host-recurrence'. However, the endophytic fungi can still colonize other plant hosts in the same habitat. Host selectivity occurs when endophytic fungi form relationships with two related plant species but demonstrates preference to one plant host (Huang *et al.*, 2008; Nogueira-Melo *et al.*, 2017). All the selected mangrove species under study were found to harbor different types of endophytic fungi, an observation that was also made on endophytic fungi from 29 traditional Chinese medicinal plants (Huang *et al.*, 2008). There were variations in the kind of endophytic fungi isolated from different mangrove species. Endophytic fungi colonized the same mangrove species from different locations. The brown fungal endophyte for instance occurs in many forms and was isolated mainly from *R. mucronata* and *S. alba* and was identified as belonging to the genus *Aspergillus* (Barnett and Hunter, 1998). *Aspergillus* is a common genus and has been reported in many endophytic fungal studies (Barathidasan and Panneerselvam, 2015). The black and grey fungal endophytes identified as *Aspergillus* isolate 1, 5, 6 and 7 and isolated from *C. tagal* exhibit host-recurrence as described by Nogueira-Melo *et al.*, (2017) since they are rarely observed in other mangrove species. These 4 dominant fungal isolates (brown, white, black and green) showed preference for specific mangrove species. The brown fungal isolate was mainly isolated from *A. marina* of Gazi Bay and Mida Creek whereas the white fungal endophytes were consistently common to *R. mucronata.* The white mangrove fungal isolates identified as *Blastomyces* (Figure 11 a and b) was found in *R. mucronata* of Mida Creek while 2 other white endophytic fungal isolates identified as *Fusarium* isolate 1 and 2 were isolated from *S. alba* and *C. tagal* of Tudor Creek, but are also more rarely observed in *R. mucronata* and *A. marina,* thereby exhibiting host-specificity and host-recurrence according to Huang *et al.*, (2008). The other 3 endophytic fungi (stranded brown, pinkish fluffy and yellowish green) were only isolated from *S. Alba, C. tagal* and *R. mucronata* of Tudor and Mida Creeks respectively, thus exhibiting host selectivity (Nogueira-Melo *et al.*, 2017).

Spatial heterogeneity of mangrove endophytic fungi

Mangrove twig cuttings were obtained from different sites, however the overall CR and IR differed slightly such that they ranged from between 38.9 % to 94.4 % and 0.14 to 0.30, respectively. This observation corroborates the study by Huang *et al.* (2008) where CR values of endophytic fungi of medicinal plants ranged from 58.3 % to 83.3 %. Differences in CR may be due to differences in structure and nutrient content of the mangrove species (Liu *et al.*, 2019). Different genera of mangrove fungal endophytes showed different relative frequencies in different plant species (Huang, 2008). The fact that *S. alba* was most colonized is significant in that endophytic fungi are known to be associated with plant hosts for shelter and in turn protect the host plant against invasion by pathogens. Protection against invasion by pathogens is enhanced through the synthesis of biologically active secondary metabolites. Huang *et al.* (2008) suggested that differences in endophytic assemblages from different hosts may be related to chemical differences of the plant. The fact that *S. alba* is the most colonized may be due to its location on the seaward side where it is submerged in water during high tides. However, though the CR of *S. alba* was high, its IR was much lower because it was mainly colonized by *Aspergillus*. According to Kairo (2001) *S. alba* is found on the seaward side because it cannot tolerate wide salinity fluctuations. *A. marina* which enjoys double zonation and tolerates high salinity levels on the landward side of the intertidal zones (Kairo, 2001) had the highest number of fungal genera. A relationship exists between extreme environments and physiological adaptations of mangroves; some of which may be the colonization of fungal endophytes which in turn produces secondary metabolites that aid host plants fight against invasion by pathogens (Jose and Christy, 2013; Ling *et al.*, 2016).

Conclusion

Mangrove fungal endophytes of the Kenyan coast are diverse, depicting host recurrence and spatial heterogeneity. The most abundant and widely distributed fungal genus was *Aspergillus* with 10 isolates representing 55 % of the total fungal genera identified morphologically. Most of the fungal genera were found to be host specific despite differences in location and hence exhibiting host-specificity. The dominance of endophytic fungal genera, diversity and similarity coefficients of mangrove fungal endophytes differed across the study sites. The colonization of Kenyan mangrove species by fungal endophytes coupled with the high diversity indicate that these endophytes can further be explored as sources of novel bioactive molecules contributing to the blue economy. This further emphasizes the need for continued conservation of mangroves along the Kenya coast.

Most fungal endophytes identified in this study grew well in PDA and poorly in PDB, therefore posing a challenge in the examination of their crude extracts since the study was method specific. It is recommended that other types of media and broths be explored to overcome this challenge leading to further investigation of a wider range of mangrove fungal endophytes in coastal Kenya which are largely underexplored. It is proposed that confirmation of the 18 fungal isolates be subjected subjected to DNA extraction, amplification, sequencing and analysis for complete identification of the fungal isolates to species level. Antimicrobial properties of the fungal isolates should be determined and bioactive compounds responsible for the different activities of isolated compounds purified, and structures elucidated by appropriate techniques.

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Presence of microplastics in jellyfish (*Crambionella orsini*) along the Kenyan coast

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Abstract

Microplastics are plastic particles less than 5 mm in diameter. These plastics mostly result from degradation of larger plastics. Due to their small size, they are often accidentally ingested by sea faunas, particularly the deposit and filter feeders. However, information on the ingestion of microplastics by sea fauna such as jellyfish is rare. This paper provides evidence of ingestion of microplastics by jelly fishes (*Crambionella orsini*) along the Kenyan Coast. Samples were taken from three stations (Mikindani and Makupa in Mombasa, and Dabaso in Mida Creek) between 31st January 2018 and 3rd February 2018 using tow nets. Samples were digested using 10 % KOH at 60 °C for 24 hrs and sieved through a 38 µm sieve. Products below 38 µm were filtered using a 0.8 µm Whatman filters, then dried in an oven and viewed under a dissecting microscope for microplastics. Suspected microplastics were confirmed using a hot needle test. Microplastics obtained were mainly fibres of different colours: black, blue, green, colourless, purple, red and yellow. Colourless fibres were the majority accounting for 53 % of the total number of fibres while purple fibres were the least at only 1 %. Mean concentration of microplastics was highest in Dabaso (0.05 mp/g of tissue), whereas in Mikindani and Makupa were almost equal (i.e., 0.03 ± 0.003 mp/g in Mikindani, and 0.03 ± 0.01 mp/g in Makupa). Statistically, the means were not significantly different between the stations ($F_{1, 2} = 1.34$; $P = 0.43$). This study presents evidence of contamination of the Kenyan coastal waters by microplastics and their ingestion by sea fauna such as jellyfish. Results of this study will help reinforce the plastic ban in the country to prevent further accumulation in the environment.

Keywords: microplastics, jelly fish, Kenyan coast, dissecting microscope, whatman filters

Introduction

Study background

This study formed part of a broader study whose aim was to investigate the presence and concentration of microplastics in marine waters along the Kenyan coast. During the sampling for microplastics from the water column, a few jellyfishes were caught by chance in tow nets at the three stations (Mikindani and Makupa in Mombasa, and Dabaso in Mida Creek). The jellyfishes were thus investigated for microplastics. Data obtained from this study will help to increase understanding of the interaction of microplastics with the sea fauna along the Kenyan coast, and especially the zooplankton feeders such as jellyfishes that accidentally ingest the microplastics in the water column. Currently, only a few studies have demonstrated the presence of microplastics in jellyfishes globally.

Introduction

Since the discovery of plastics in the 1950s (GESAMP, 2015), an increase in their production has been witnessed (Dehaut *et al.*, 2016). Plastics are used for a variety of purposes including for: packaging, construction of houses, agriculture, clothing, footwear, personal cleaning products and electronics (Boucher and Friot, 2017). This wide application is due to their durability, excellent thermal and electrical insulation as well as their ability to be moulded into various shapes (Dris *et al.*, 2015). The most widely used plastics include Polyethylene (PE), Polypropylene (PP), Polyvinyl Chloride (PVC), Polystyrene (PS) and Polyethylene Terephthalate (PET), representing about 90 % of the world's total production, thus making them the major pollutants in the environment (Ivar do Sul and Costa, 2014).

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Plastics are ubiquitous in both the marine and coastal ecosystems (Dris *et al.*, 2015). Of particular concern are the microplastics (<5 mm in diameter), which are classified either as primary or secondary microplastics (EFSA, 2016: Smith *et al.*, 2018; Wright *et al.*, 2013). Primary microplastics are plastics that are designed to be microscopic, and include materials such as beads, fibres, pellets and resins (EFSA, 2016). Secondary microplastics normally result from fragmentation of larger plastic materials (Milisenda *et al.*, 2014).

The small size of microplastics makes them invisible, especially to suspension, deposit and detritic feeders such as oysters and crabs which mistake them for prey (Lusher *et al.*, 2017). Ingestion of microplastics has been observed in a number of marine fauna including fishes, echinoderms, crustaceans, cetaceans and bivalves (Jamieson *et al.*, 2019). Microplastics taken by organisms at the lower trophic levels, that is, zoo- or phytoplankton, are likely to be incorporated into the food chain (Katija *et al.*, 2017). According to Robinson *et al.* (2014), jellyfish inhabit the pelagic environment, hence their diet tends to overlap with those of the forage fish. Mesozooplankton for instance, contribute greatly to the diet of *Aurelia* spp. Other jellyfish species such as *Rhizostoma octopus* are predators, feeding mainly on fish eggs and larvae. Morais *et al.* (2015) observed that jellyfish diet is not only restricted to zooplankton with some species such as *Blackfordia virginica* feeding also on phytoplankton, detritus and ciliates. Such jellyfish, therefore, are likely to ingest microplastics by mistaking them for prey leading to serious effects. On the other hand, jellyfishes act as food for various sea organisms including seabirds, sea turtles, sunfish and juvenile fish (Robinson *et al.*, 2014). Ingestion of microplastics by jellyfish, therefore, has implications on the marine food web as well humans as some of the jellyfish predators such as fish are highly valued human food.

The objective of this study was to establish the presence and concentration of microplastics in jellyfish from three sites: Makupa, Dabaso and Mikindani, located along the Kenyan coast. In addition, the shape, length and colours of the plastics were determined.

Materials and methods

Field methods

Sampling was carried out during the spring low tide between 31st of January and 3rd of February 2018. Jellyfish were encountered at all the three stations and were caught by towing 500 µm mesh size nets for approximately 10 minutes. Samples were stored in cooler boxes to be transported to the laboratory for further analysis.

Laboratory methods

Jellyfish were weighed using a weighing balance and the weights recorded. Samples were then rinsed in distilled water to remove any microplastics attached on the surface. Replicates of each sample were put in separate beakers in which 10 % KOH was added until the sample was completely submerged, and then incubated at 60 °C for 24 hrs. After digestion, samples were sieved using a 38 µm sieve and filtered through filter membranes (0.8 µ Whatman filters). The membranes were dried in an oven for 12 hrs and viewed under a dissecting microscope. Possible microplastics were isolated into a glass petri dish and confirmed using a hot needle. Materials that were plastics melted at the point of contact with the hot needle. The shape, colour, and length of the plastics were determined. There was, however, no attempt to identify the types of plastics owing to their microscopic size.

Quality control

Contamination of the samples was minimized by working in a laboratory with minimum movement, wearing a cotton lab coat, using glass equipment,

Table 1. Mean (± SE) lengths (mm) of microplastics of different colours in jellyfish at different stations along the Kenyan coast.

	Black	**Blue**	**colourless**	**Green**	**Red**	**Purple**	**Yellow**	***F***	***p***
Dabaso	1.31	3.00	1.50	0.00	2.50	0.00	0.00		
Makupa	0.79 ± 0.79^{aA}	2.34 ± 2.34^{Aa}	3.21 ± 0.71^{aA}	0.94 ± 0.93^{aA}	2.34 ± 2.34^{aA}	0.75 ± 0.74^{Aa}	-	0.83	0.59
Mikindani	3.24 ± 3.24^{aA}	1.5 ± 1.49^{Aa}	1.62 ± 0.03^{aA}	0.37 ± 0.37^{aA}	0.94 ± 0.94^{aA}	-	1.5 ± 1.49^{aA}	0.58	0.76
$F_{2,2}$	0.29	0.11	3.17	0.33	0.2	0.6	0.6		
P-Value	0.78	0.9	0.24	0.76	0.83	0.63	0.63		

Means ($\bar{x} \pm SE$) within columns followed by the same lowercase letters are not significantly different; means ($\bar{x} \pm SE$) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$).

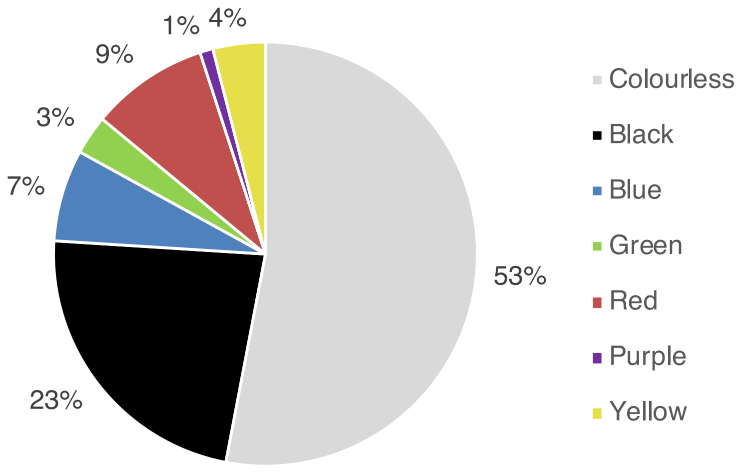

Figure 1. Proportion of microplastics of different colours ingested by jellyfish

using distilled water, and rinsing all the equipment with distilled water before use. A control was set up on the working table using a membrane filter. The filter was observed under a dissecting microscope and no microplastics were found, therefore it was assumed that the study was at minimum risk of plastic contamination.

Data analysis methods

Data analysis was performed using the Rcmdr package in R-console. One-Way ANOVA was used to compare the mean concentration, length and colours of microplastics in jellyfish sampled from different stations. Where means were significantly different, the Tukey's post-hoc test was used to check the differences ($p < 0.05$).

Results

Jellyfish distribution

A total of 9 jellyfish were obtained for the study, with Makupa having the highest number (n = 5). Dabaso and Mikindani had a total of 2 jellyfish each. The jellyfish belonged to the genus *Crambionella* sp. Weights of individual jellyfishes ranged between 200 g and 1000 g. Jellyfish from Mikindani were heavier (890 g – 1000 g) than those from Dabaso and Makupa, and were therefore considered as separate samples, whereas the small-sized jellyfish were grouped together to form a sample.

Microplastic occurrence in jellyfish samples

Microplastics obtained from the jellyfishes were mainly fibres of seven different colours (black, blue, green, colourless, purple, red and yellow (Fig 1). Colourless fibres were the most dominant fibres accounting for 53 % of the total number of fibres, whereas purple fibres were the least at 1 % (Fig. 1). The length of the fibres ranged between 0.3 mm – 3 mm (Table 1). Colourless fibres had a relatively longer mean (±SE) length (2.23 ± 0.46 mm) than the other fibres, whereas purple fibres were the shortest with a mean of 0.30 ± 0.30 mm. Variations in these lengths were however

Table 2. Mean weights of jellyfish and their corresponding microplastic (mp) concentration (mp/g tissue).

Station/replicate	Mean Weight (g)	Mp Conc (mp/g tissue)
Mikindani A	890	0.028
Mikindani B	1000	0.022
Makupa A	897	0.041
Makupa B	831	0.012
Dabaso A	298	0.05

not significant ($F_{6, 28} = 1.3$; $p = 0.29$). Mean concentration of microplastics in jellyfish was determined in terms of the number of microplastics per gram of their tissue (mp/g tissue). Mean concentrations were 0.05 mp/g tissue in Dabaso, 0.03 ± 0.01 mp/g tissue in Makupa, and 0.03 ± 0.003 mp/g tissue in Mikindani (Table 2). The mean concentrations of microplastics between the sites were not statistically significant ($F_{1, 2} = 1.34$; $p = 0.4$).

Discussion

This study has established the presence of microplastics in jellyfish on the Kenyan coast, particularly along the creeks where most subsistence fisheries occur. Results of this study represent the second evidence of microplastic ingestion by jellyfish, with the first evidence being reported by Macali *et al.* (2018) on *Pelagia noctiluca*. Jellyfish play an integral role in the marine food web as either predators or prey. They trap their prey by the use of tentacles, and are therefore likely to ingest plastic particles in the process (Mandal and Gosh, 2010). Ingested microplastics may be passed on to their predatory fish including bogue (*Boops boops*), chum salmon (*Oncorhynchus keta*), and filefish (*Stephanolepis cirrhifer*), which are commercially valuable (Milisenda *et al.*, 2014). Eventually, microplastics in the fish tissues may end up in human diets and lead to health complications.

Sites for this study were chosen based on their susceptibility to plastic waste pollution. For instance, Makupa creek is located next to the Kibarani dumpsite, and hence there is potential leakage of nutrients from the dumpsite into the creek which favours phytoplankton growth and abundance of zooplankton that are eaten by jellyfish (Purcell *et al.*, 2007). This explains the high number of jellyfish in Makupa compared to Dabaso and Mikindani.

Microplastics obtained from the jellyfish samples were classified according to their shape, colour and length. These microplastics were mainly fibres and as reported in other studies, ingested fibres may have come from urban surface runoff, fisheries, wastewater treatment plants, shipyards, rivers, synthetic textiles, and personal care products (Graca *et al.*, 2017). The microplastics were of different lengths and colours. Variation in the colour of microplastics is an indication that they were from multiple sources. Of all the colours, colourless fibres were dominant suggesting that the sea was highly contaminated by these types of plastics.

It was further noted that the concentration of microplastics among the stations was not statistically different. In fact, the concentration in Dabaso, which is a nature reserve, was higher than Makupa and Mikindani. This reveals the trans-boundary nature of plastic pollution to the extent that even protected areas are not exempted. This study reveals the contamination of Kenyan coastal waters by microplastics and their ingestion by sea fauna such as jellyfish which mistake them for food. Results of this study will help policy makers to make informed decisions regarding plastic waste pollution so as to prevent their future accumulation in the environment.

Conclusion

This study has established ingestion of microplastics by jellyfish along the Kenyan coast and especially within the creeks where most subsistence fisheries occur. This suggests the contamination of these environments by microplastics. Microplastics obtained from the study were mainly fibres that were of different colours suggesting that the plastics came from multiple sources. Colourless fibres were the dominant fibres indicating high contamination of the ocean with these types of fibres. This study reinforces the need for the plastic bag ban policy in the country and recommends proper plastic waste management strategies to reduce their accumulation in the environment.

Recommendation

This study recommends further investigations to establish which body parts of the jellyfish accumulated these microplastics and what polymers constituted the microplastics to enable prediction of the possible sources of microplastics entering the ocean. Further research should also be conducted to establish the concentration of microplastics in the water column that these organisms inhabit.

Acknowledgements

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