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Table of Contents

Assessment of reef fish and benthic cover of the North and South Dar es Salaam Marine Reserves system before the 2016 El Niño

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Abstract

The status of reef fish density, diversity, species richness, biomass and coral cover was evaluated by comparing the conditions in two Dar es Salaam Marine Reserves (DMRs); the North Dar es Salaam Marine Reserve (NDMR; gazetted in 1975), and the South Dar es Salaam Marine Reserves (SDMRs; gazetted in 2007), before the 2016 El Niño. A 10 m line-intercept transect was used to characterize benthic cover and a 50 m belt transect was used to assess reef fish population status. Sampling occurred between August 2014 and April 2015. The results showed that fish biomass in the (NDMRs) was 2.7 times higher than that in the (SDMRs) and live hard coral cover was 3 times higher. Higher reef fish density, biomass, diversity, species richness and live hard coral cover were revealed before 2016 El Niño in NDMRs as compared to the SDMRs. Differences in status are linked to differences in time of gazetting and level of effective management in the marine protected areas (MPAs), where NDMRs has a General Management Plan (GMP) while SDMRs does not, and the differences in management are likely to have contributed to the differences in fish biomass and coral cover.

Keywords: reef fish, coral reef, marine reserves, conservation, El Niño

Introduction

The DMRs comprising of the NDMRs and SDMRs were gazetted in 1975 and 2007 respectively. The NDMRs has a GMP that was developed in 2005 while the SDMRs lacks a GMP. The GMP serves as guidance to ensure that resource protection and recreational activities and developments remain balanced and compatible with one another. It also sets out an active process which guides subsequent planning and implementation on how to effectively conserve and manage the resources (URT, 2005). Before being gazetted, the DMRs were characterized by unregulated fishing, including widespread use of beach seines and spear fishing.

Informed management intervention in MPAs includes understanding the impacts of El Niño in order to institute adaptive management as part of a disaster response mechanism. The impact could easily be detected if data were collected before the event. El Niño is often termed the "Southern Oscillation", or ENSO, where the atmosphere and ocean collaborate together (Trenberth, 1997). However, some scientists confine the term to the coastal phenomenon, while others use it to refer to the basin wide phenomenon (Trenberth, 1997; Aceituno 1992; Glantz, 1996).

Surface temperatures in the Eastern and Central tropical Pacific Ocean during the ENSO in late 2015 exceeded 2 degrees Celsius above average (Glantz, 1996), providing evidence that the 2015-16 El Niño was one of the strongest on record, comparable with the 1997-98 and 1982-83 events. Subsequently, it led

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to unusually high levels of warming and changes in the local and regional coral reef ecology, including coral bleaching and mortality. It was reported that the threshold on the Sea Surface Temperature (SST) for three consecutive months was only 0.4 °C (Glantz, 1996). Throughout 2014, the inter-tropical Pacific SST rose steadily from the below average values observed in 2013. They remained near borderline values for some time (October to February) before finally breaking the El Niño threshold (+0.5°C) in March 2015 (FAO, 2014).

High SST can lead to coral bleaching, which refers to the loss of the zooxanthellae by the host (i.e. the coral), or the loss of photosynthetic pigments within the coral structure itself, and can cause coral mortality (Muhando, 1999; Wagner, 2004).

Consequently, El Niño events are a serious public concern, and forecasting is critical to highlight the need for society to get ready for the potential impacts of the event. Additionally, El Niño is also responsible for larger magnitude weather anomalies such as floods, drought, heat waves, hurricanes, and tsunamis resulting in disease outbreaks and water shortages, among other challenges. Knowledge on El Niño can provide usable information for decision makers to choose whether to pursue strategic or tactical disaster risk reduction policies (Glantz *et al.*, 2018). El Niño intensities can easily be quantified ranging from weak to very strong (Glantz *et al.*, 2018). If severe, El Niño can result in coral bleaching and subsequent mass coral mortality. Baseline data from before the event is therefore critical for tracing the impact on the ecosystem.

Studies on coral reef systems have been carried out along the Tanzanian coast to describe the coral fauna of the East African Coast (Hamilton, 1975), to assist with the establishment of MPAs in southern Tanzania (Muhando *et al*, 1998), and assess the status of coral reefs in the DMRs and other MPAs in Tanzania (Muhando and Francis, 2000). These studies have also served to assess coral reef degradation in Tanzania (Mohammed *et al.,* 2000), assist with coral reef management (Wagner, 2004), and to determine the role of improved fisheries management in increasing the biomass of fish and benthic communities in Tanzania (McClanahan *et al,* 2009). Prior to the establishment of the DMRs their natural systems had been degraded due to the widespread use of dynamite and other destructive fishing techniques (Benno, 1992).

Friedlander (2007) reported high species richness, biomass, density, habitat complexity and good habitat quality in protected areas as compared to areas open to fishing in Hawaii. FAO (2011) suggested that the most common types of indicators of biological response within protected areas include increased density, biomass and size of animals. Syms and Jones (2000) observed that disturbance plays a substantial role in structuring communities of coral-reef fishes by modifying both spatial and temporal heterogeneity.

There is substantial scientific evidence that areas with increased management (when designed appropriately) have more and bigger fish and a higher biomass than those without management (Côte *et al.*, 2001; Friedlander and De Martini, 2002; Friedlander *et al.*, 2003a; Friedlander *et al.*, 2003b; Dulvy *et al.*, 2004; Kamukuru *et al.,* 2004; McClanahan *et al.,* 1999).

However, only a few studies have been undertaken in the DMRs. Hamilton (1975) and Wagner (2004) indicated that some parts of the DMRs had significant live coral cover which was valuable as a tourist attraction while some areas were already degraded as a result of dynamite fishing. Kamukuru (1997, 2009) assessed the biological status of the coral reefs, the trap fishery and reproductive biology of the white spotted rabbit fish *Siganus sutor* (Siganidae), respectively.

Understanding the relationship between reef habitat and fish population structure is becoming increasingly recognised as important for the sustainable management of fisheries and MPA resources (see for e.g., Anderson and Millar 2004; García-Charton *et al.*, 2004). The physical structure of the reef has been observed to play a key role in the organization of fish assemblages, protection of reef fish from predators and providing access to food (Tuya *et al,* 2011). Thus, this study was aimed at investigating and establishing the status of reef fish density, biomass, richness and live hard coral cover in the DMRs before the 2016 El Niño event. El Niño has been reported to cause tremendous impacts including the collapse of coral reef ecosystem. Taking the 1998 El Niño as an example, coral cover was 81.2 % before bleaching, and dropped to 37 % after bleaching at Bongoyo West (Wagner, 2004). Around Mbudya Island, coral mortality was 40-60 % (Wagner *et al.*, 2001), while at Pangavini, 77.5 % of the coral reef died (Mrema, 2001). At Fungu Mkadya, 60 % died (Bipa, 2000) while at Fungu Yasini southwest, almost 100 % died (Peter, 2002). Similarly in Mnazi Bay in the Ruvuma Estuary Marine Park only 50 % of the coral reef survived after bleaching (Wilkinson, 1998). Reef fish density has been reported to be correlated with live coral in terms of both density and biomass which implies that when coral reefs are affected, the whole ecosystem is jeopardized (Kamukuru, 1997; Julius *et al.*, 2016). Taking the above into account, coral cover and the associated reef fish were assessed before the predicted 2016 El Niño event.

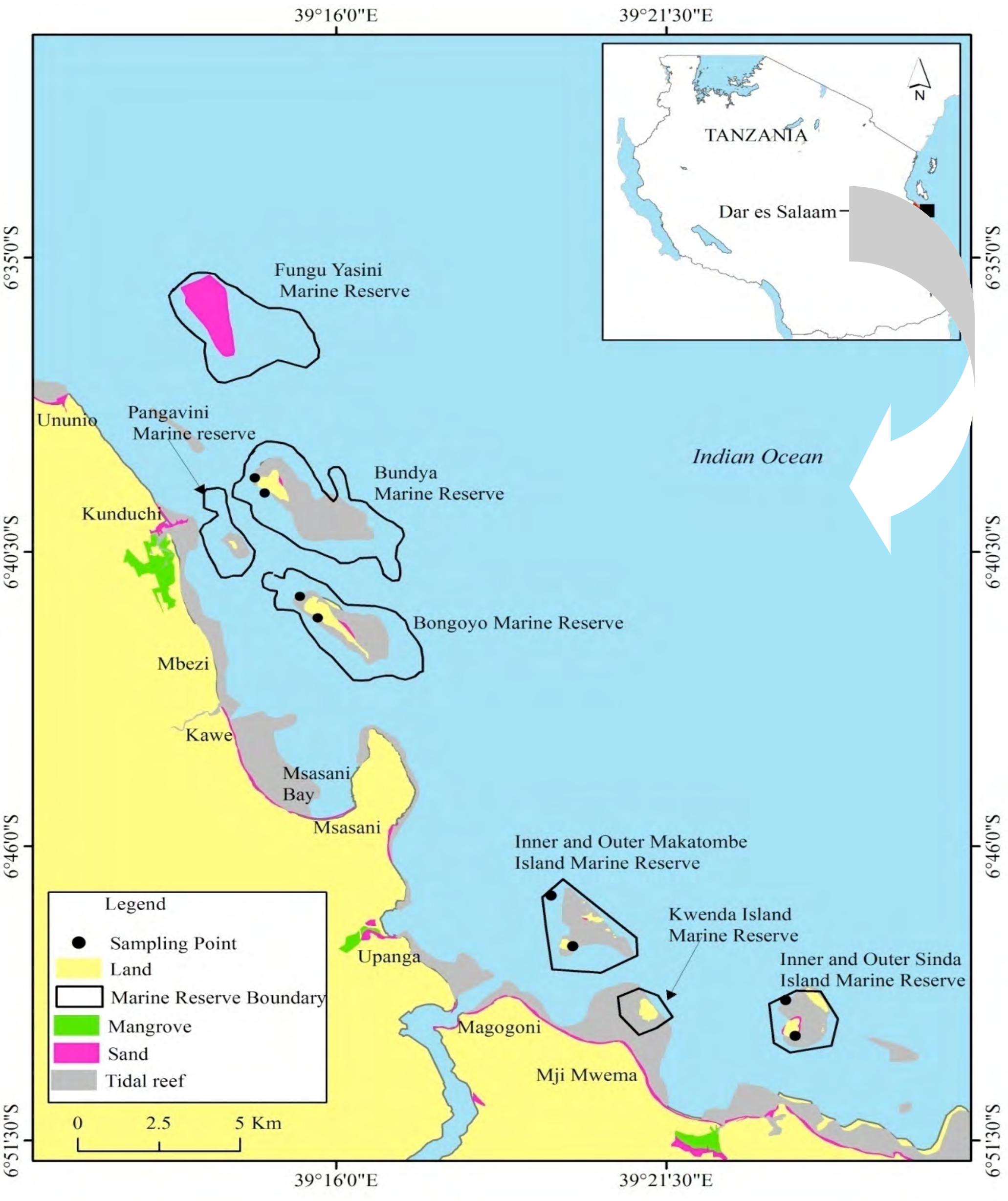

Figure 1. Map of Dar es Salaam coast showing the DMRs study sites.

Study site and methodology

Study site

The study was conducted within the DMRs which extends from north to south of Dar es Salaam City between 06°35' - 06°45' S and 39°13' - 39°17'E. The North Marine Reserve includes Mbudya, Bongoyo and Pangavini Islands, while the Southern Marine Reserve is comprised of Inner and Outer Sinda and Inner and Outer Makatumbe islands (Fig. 1). The respective locations of the islands are given in Table 1. The islands are surrounded by diverse and unique habitats including coral reefs, sea grass beds, sandy beaches and rocky shores and lie on a shallow continental shelf with waters of less than 20 metres deep (URT, 2005). Fishing and collection of shellfish in the reserves (no take), recreational and tourist activities are common on and around the islands.

Methodology

Data was collected during low tide. Three methods were used to assess the habitats and resources of the DMRSs. Firstly, a rapid assessment using a Manta tow survey was conducted around all islands (Mbudya, Bongoyo, Makatumbe and Sinda) to select the sampling site. Coordinates of the selected sites were recorded using a GPS. Sampling was done on the southern and western sides of the islands because they were sheltered and easily accessible, and the corals were well-established.

Secondly, a visual census survey was used to assess the habitats and quantify fishes. All fish species observed along each transect were identified with the aid of a field fish identification guide (Richmond, 2002). Fish size was classified based on their total length. Specimens from 1-10 cm were considered as juvenile, from 11 to 20 cm as recruits, and 21cm and above as adult. Slates were used to record the data, which included fish description, size and number of individuals falling of a particular species and size. The fish were counted by tallying the information from the slates and where larger numbers of reef fish were encountered, a rough estimated was done. An underwater camera was used for taking photos of fish species which were not easily identified on the spot. Further detailed identification in the laboratory was carried out using the field guide by Fischer and Bianchi (1984), the Coral Reef Fishes Pocket Guide (Lieske and Myers, 2001), and Bianchi (1985).

Fish counting was undertaken by adopting the method of Samoilys and Carlos (2000) by swimming at a slow, constant speed along the transect at 3-4 metre min^{-1}, depending on fish abundance and complexity of the habitat or rugosity of coral reef, covering 33 m^{2} min^{-1}. A break period of 20 minutes between transects was allowed for fish to return to the area.

Coral reef fish diversity was determined by the Shannon index (H'):

$$H' = -\sum_{i=1}^{s} p_i \ln(p_i)$$

Where p_i is the proportion of all observations in the i^{th} species category, and S is the total number of species. The Shannon index measures both richness (the number of species) and evenness, or how evenly individuals are distributed among species. High values of H' denote high biodiversity.

The third method used was a Line Intercept Transect (LIT). A 10 m LIT was used to characterize benthic cover along a 50 m wide belt to assess reef fish density, biomass, species richness and diversity based on Underwater Visual Census (UVC) techniques (English, 1994). Eight belt transects running from immediately above the reef crest to the reef slope were conducted in the study. Two divers recorded data on either side of the transects. Three surveys were conducted from August to September, 2014, January to February, 2015, and April, 2015. A total of 24 swim tracks were performed for each sampling phase per site.

Data analysis

Fish densities obtained from direct field counts (UVC) were organized using Microsoft Excel 2013 before analysis. Fish biomass values were computed from length estimates using the conversion equations (W = a * Lb) of published length-weight relationships from FishBase (www.fishbase.org), where L is fish length in centimetres estimated from the field during data collection, W is fish weight in grams computed from the equation, a is the y-intercept and b is the slope of the equation when the natural logarithm is applied. The values obtained for an individual fish was multiplied by the number of fish of each species counted and sizes observed, providing an estimate of total biomass (g.500m-2) per transect. The mean fish biomass (± standard error) and mean density (± standard error) was also calculated. Live coral benthic cover was also organized in Microsoft Excel in a different file before analysis. Statistical analysis was carried out using Graph Pad Instant Statistical software, version 3.06.

Data were tested for normality before reef fish density, biomass and live coral cover were evaluated for homogeneity and heterogeneity. The Mann-Whitney, Signed-ranks and Kruskal-Wallis tests were used for testing the data.

Reef density and biomass were tested for normality before analysis using Graph Pad Instant Statistical software version 3.06 for statistical analysis. The Mann-Whitney test was used to evaluate differences in reef fish biomass and density between the North and South DMRs, and the Wilcoxon matched-pairs signed-ranks test was used to evaluate coral reef fish population structure between the marine reserves. Live coral cover was tested by the Wilcoxon matched-pairs signed-ranks test.

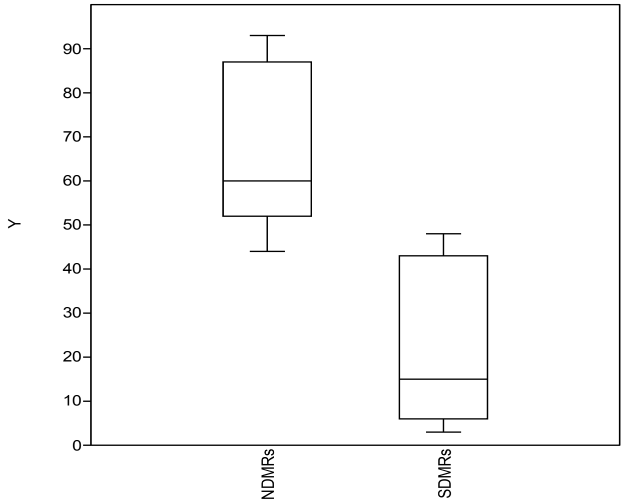

Figure 2. Live coral cover (%) in the DMRs.

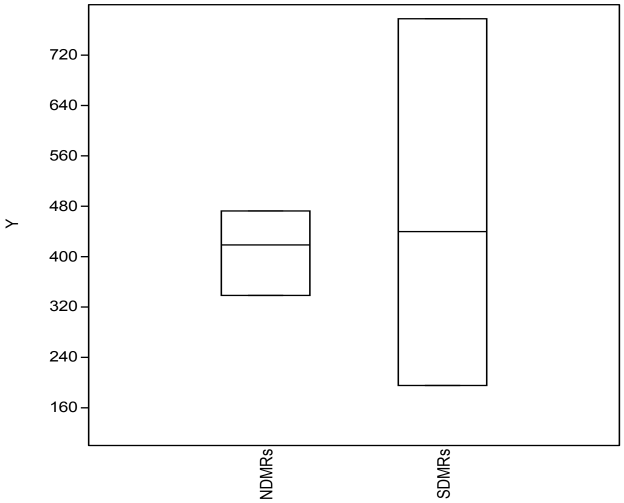

Figure 3. Reef fish density (#/ha) in the DSMs.

Results

There was significant a difference in reef fish density and biomass (Mann-Whitney Test (U); P = 0.0004 for both abundance and biomass). Additionally, hard coral cover was significantly different between sites; higher in NDMRs than in SDMRs (Wilcoxon matched-pairs signed-ranks (T); p<0.0001). The test also revealed that similarity existed among the northern islands in the NDMRs (Mbudya and Bongoyo) and in those in the SDMRs (Sinda and Makatube) for both fish biomass and abundance (Mann-Whitney Test (U); p<0.05).

Coral cover, fish density and biomass

The mean live coral in % ± SE was significantly higher (Wilcoxon matched-pairs signed-ranks test, $T_{0.05,\ 32}$ = 528, p<0.0001) in the NDMRs (69.688 + 3.249) than in the SDMRs (22.969 ± 2.966) (Fig. 2). Likewise, the mean fish density was significantly higher ($U'_{0.05}$ *188. 69 = 8372*; $p < 0.05$) in the NDMRs (442.6 ± 69.4 individuals per 500 m^2) than in the SDMRS (408.4 ± 104.2 individuals per 500 m^2) (Fig. 3). The NDMRs had significantly higher reef fish biomass (27.7 ± 5.4 kg/500 m^2) than the SDMRs (10.1 ± 2.6 kg/500 m^2); $U'_{0.05}$ *188.69 = 8944*; $p <$ 0.05 (Fig 4). A similar situation was observed for the juvenile fish at P < 0.0001 (Fig. 5). A very strong positive correlation (r^2=0.955) was revealed between live coral cover and fish abundance in both the NDMRs and SDMRs (Figs. 6 and 7).

Reef fish diversity and species richness

The study revealed that there were 59 species within 26 families in NDMRs (Fig. 8) and 40 species within 22 families in SDMRs (Fig. 9). The NDMR was dominated by the butterfly fishes (Chaetodontidae) which contributed 17 %, followed by Pomacentridae at 15 %, and Pomacanthidae at 8 % of fish families. The fish family

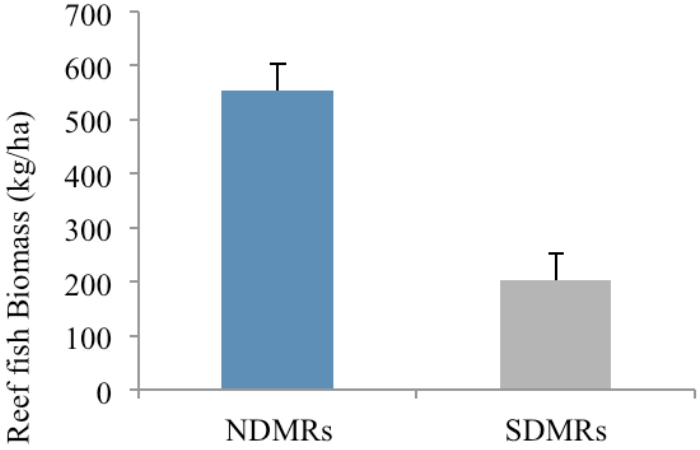

Figure 4. Reef fish biomass in the DMRs.

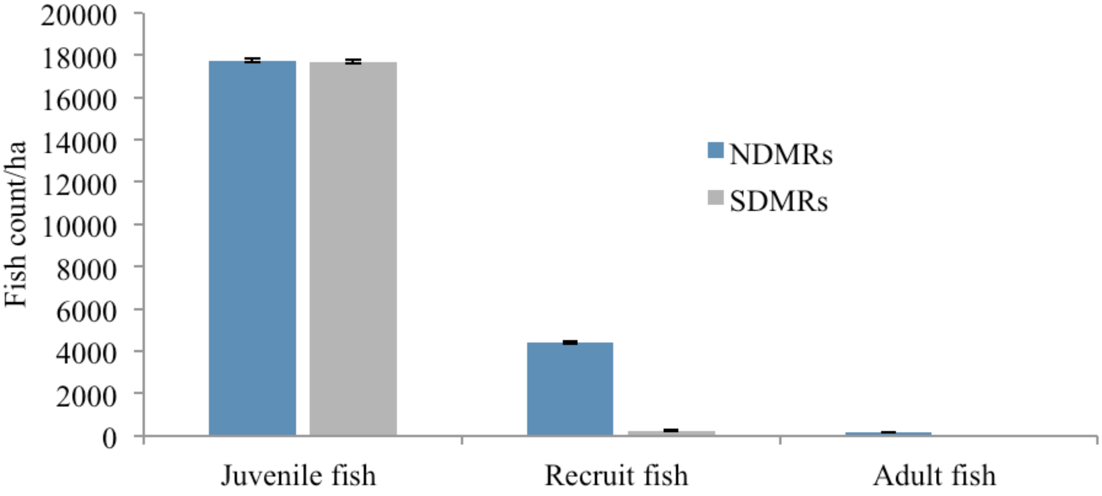

Figure 5. Reef fish population structure in the DMRs.

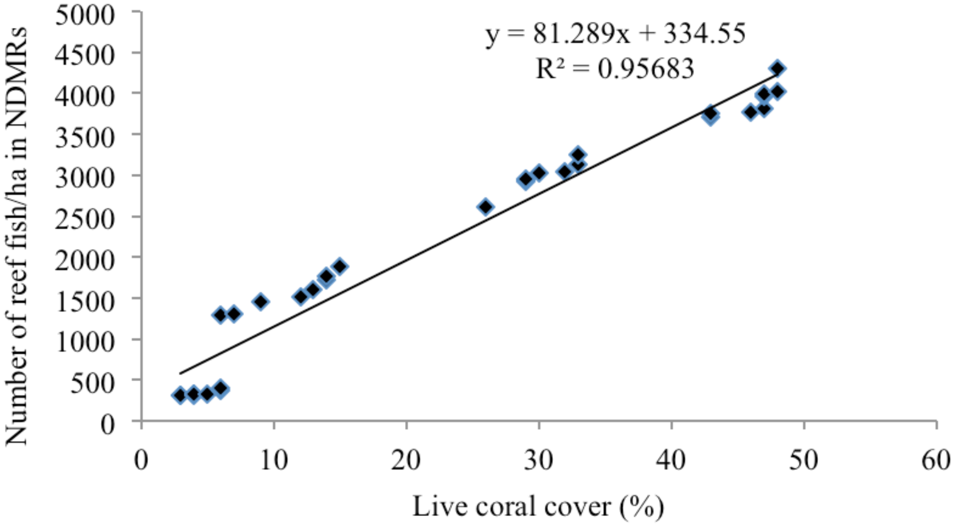

Figure 6. Correlation of reef fish abundance with live coral cover in NDMRs.

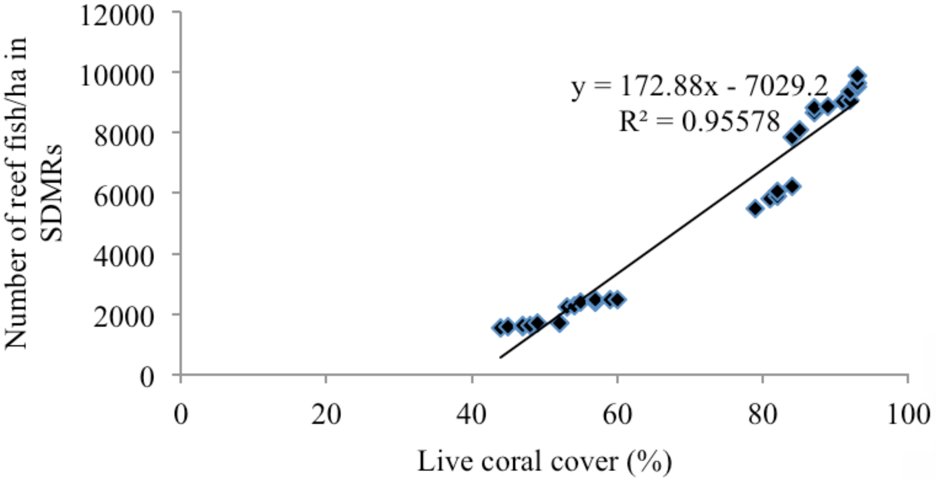

Figure 7. Correlation of reef fish abundance with live coral cover in SDMRs.

Table 1. Number of reef fish families and species recorded in DMRs.

Study site	Shannon index (H´)	No. of Reef fish family	No. of Reef fish species
NDMRs (with GMP)	4.3	26	59
SDMRs (without GMP)	3.2	22	40

composition in SDMRs was dominated by small bodied individuals, namely Pomacentridae (damselfish and clown fishes) at 18 %, followed by Pomacanthidae at 13 %. Mullidae and Labridae both contributed 8 % and the remaining proportion was shared by other groups. The fish diversity was higher in the NDMRs than in the SDMRs with Shannon Wiener diversity indices (H´) of 4.323 and 3.22692, respectively (Table 1, Figs. 7 and 8).

Discussion

The status on reef fish and benthic cover of the North and South DMRs before the 2016 El Niño is now established. The higher reef fish density, biomass, species diversity and live hard coral cover observed in the NDMRs compared to the SDMRs indicates the impact of differences in management effectiveness and the implication of differences in the time since the reserves were gazetted, as well as the level of management between the marine reserves though the guidance of a GMP.

Reef fish were dominated by the families Chaetodontidae, Pomacentridae and Pomacanthidae in the NDMRs; probably because butterflyfishes (Chaetodontidae) have been observed globally to constitute almost half of the coralivorous fish families, followed by other families including the Pomacentridae (Cole *et al.*, 2008). It has been observed by Garpe and Öhman (2003) and Halford *et al.* (2004) that the loss of structural reef complexity often affects the health of fish communities. Sano *et al.* (1987) also observed that the abundance and diversity of the coral reef community was observed to have declined by approximately two-thirds after the reef collapsed into a formless rubble state. The low fish diversity in SDMRS could be a response to loss of coral cover (Cole *et al.*, 2008). Both dominant family groups indicate a disturbed habitat which is attributed to destructive fishing practices impacting coral growth as well as causing physical damage.

Prevalence of juvenile fishes in both sites emphasises the role of coral reefs as nursery grounds (Fig. 5). Higher abundance of both recruits and adult fishes in NDMRs indicates the value of high coral cover. The low number of recruits and adult fish observed in the SDMRs suggests their excessive removal by unregulated fishing activities in the area as a contributing factor. The better biological status in the NDMRs was possibly due to highly regulated fishing activities as well as the older age of the reserve compared to the

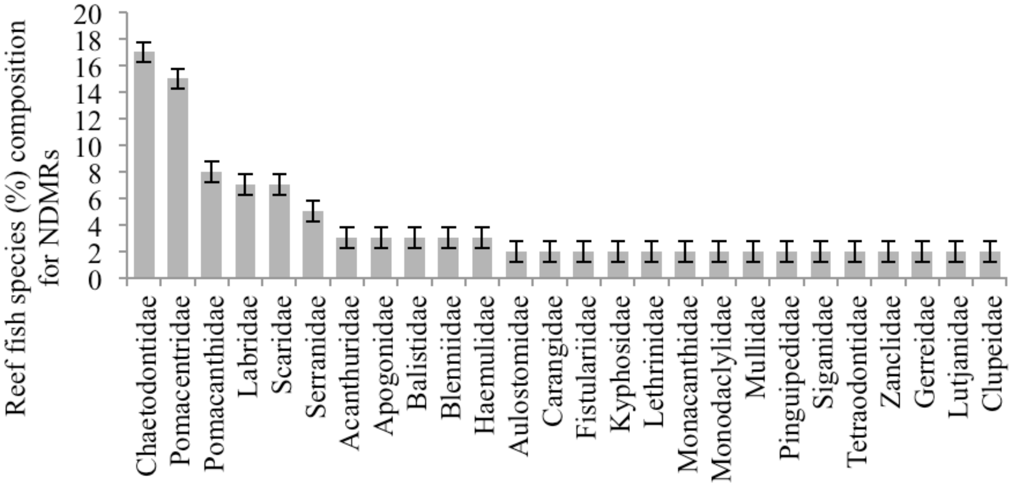

Figure 8. Reef fish families in NDMRs.

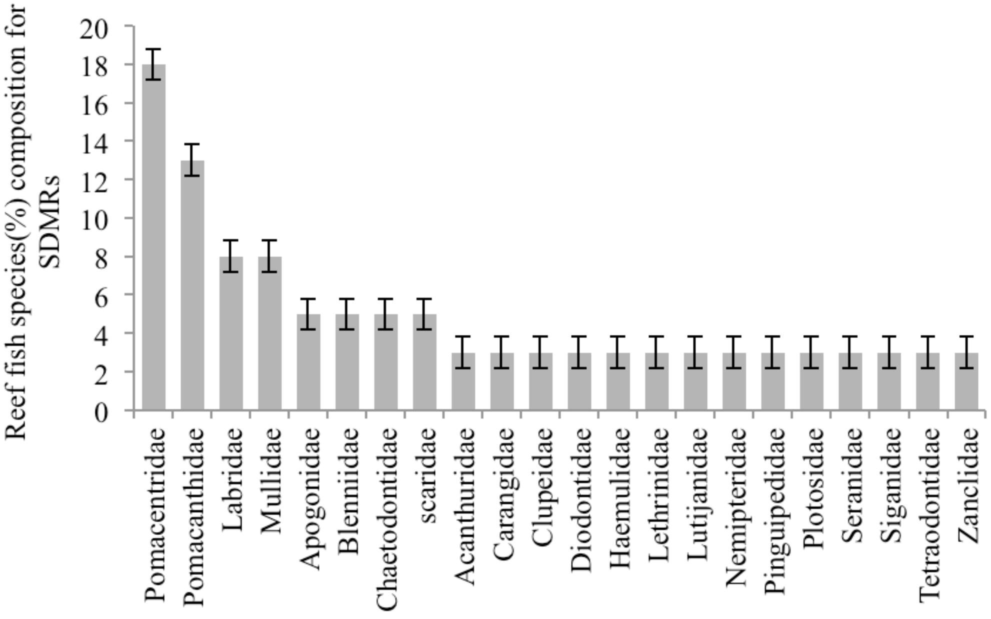

Figure 9. Reef fish families in SDMRs.

SDMRs. In contrast to the SDMRs, the existence of a GMP for the NDMRs provides guidance and attributes accountability to all key stakeholders in the management and conservation of resources in this area.

With respect to fish biomass status in other areas in the WIO, Kamukuru *et al.* (2004) reported the existence of over six times the biomass of *Lutjanus fulviflamma* in Mafia Island Marine Park compared to unprotected areas. McClanahan *et al.* (2009, 2015) reported that the biomass of fish rose continuously from 260 to 770 kg/ha from 1994 to 2007 on Tanga reefs because of stability of coral cover due to increased management, and that the reefs exhibited more resilience due to management.

Reef fishes have been reported to exhibit a strong positive correlation with live hard coral cover substratum, with this being considered critical for the provision of food, shelter and living space for fishes (Beukers and Jones, 1997). Also, Garpe and Öhman (2003) observed that sites with the highest proportion of dead coral exhibited the highest degree of dispersion of fish assemblages. Habitat characteristics play a dominant role in determining fish assemblage composition on coral reefs (Garpe and Öhman, 2003). The high percentage coral reef cover in the NDMRs is associated with the presence of both high reef fish abundance and biomass in NDMRs. This has management implications, as reef fish are automatically conserved if the coral reef is maintained in good condition.

Conclusion

This study revealed that the NDMRs has higher reef fish density, biomass, diversity, species richness and live hard coral cover compared with the SDMRs. This study recommends another survey using similar methods after the 2016 El Niño to assess the impact of the event on the ecology in the DMRs. This will assist in improved management and sustainability of the Marine Reserves through regular documentation on their biological status.

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Carbon dynamics and sequestration by the urban mangrove forests of Dar es Salaam, Tanzania

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Abstract

This study intended to 1) determine spatial and temporal changes of mangrove forests, 2) identify drivers of mangrove deforestation and forest degradation, 3) determine historical carbon storage, sequestration and deforestation emissions by mangrove forests, and 4) determine whether mangrove forests are a source or sink of CO_2 in Dar es Salaam, Tanzania. Mangrove forests have decreased from 4,813 hectares in 1986 to 1961 hectares in 2016. The following were prominent drivers of deforestation in descending order: clearing mangrove forests for salt pans; hotel construction; settlement; and charcoal making. Tree removals for firewood and building poles were also prominent drivers of mangrove forest degradation. Similarly, carbon stored in mangrove forests has decreased from 1,131,055 tonnes CO_2e in 1986 to 460,835 tonnes CO_2e in 2016. Sequestration of CO_2 by mangrove forests is estimated at 133,516 (1986-1995); 106,110 (1995-2006) and 69,616 (2006-2016) tonnes CO_2e year^{-1}. Conversely, mangrove deforestation has resulted in emissions of about 27,400, 16,500 and 24,000 tonnes CO_2e year^{-1} in 1986-1995, 1995-2006 and 2006-2016, respectively. Urban mangrove forests play an important environmental role in mitigating climate change and amelioration of local weather through the large carbon stocks they store and sequester. Mangrove forests in the study area remain a net carbon sink, however, the sink role played by mangrove forests in the study area is decreasing rapidly. The declining spatial and temporal trends of urban mangrove forest cover has resulted in a systematic decrease in the total carbon stored and sequestered by mangrove forests. In the absence of timely measures of preserving and rehabilitating degraded mangrove areas, the mangrove forests of Dar es Salaam may become the source of CO_2. The study recommends effective urban land use planning and effective law enforcement to ensure a win-win situation through sustained ecosystem services offered by urban mangrove forests to support economic growth.

Keywords: carbon storage, carbon emission, carbon sink, drivers of carbon stock change, mangrove forest

Introduction

Global carbon emissions are increasing rapidly. Recently, IPCC (2014) reported that CO_2 emissions have increased by about 90 % since 1970. Emissions from fossil fuel combustion and industrial processes contribute about 78 % to the increase of the total greenhouse gas emissions from 1970 to 2011 (IPCC, 2014; Boden *et al.*, 2017). Urban areas and their development are characterised by high concentrations of transport and industries which yield high emissions of CO_2 and other greenhouse gases (Parrish and Zhu, 2009; Bettencourt and West, 2010; Hillman and Ramaswami, 2010; Seto and Satterthwaite, 2010; URT, 2014). Consequently, emissions cause climate change, air pollution and affect human health (Epstein, 2001; Hunter, 2003; Svirejeva-Hopkins *et al.*, 2004; Parrish and Zhu, 2009; IPCC, 2014; URT, 2014).

There is a strong interest in stabilizing the atmospheric abundance of CO_2 and other greenhouse gases to mitigate the risks of global warming (Kerr, 2007; IPCC, 2014). In order to mitigate climate change, UN member parties have set a target, in the Paris Agreement, of limiting average warming to 2 °C above pre-industrial

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temperatures. Three strategies aimed at lowering CO_2 emissions for mitigating climate change have been proposed (IPCC, 2014); these include (i) reduction of global energy use, (ii) development of low or no-carbon fuel, and (iii) sequestration of CO_2 from point sources or the atmosphere.

Urban areas are characterised by high concentrations of transport and industries and are thus a source of high emissions. Despite being sources of high emissions, mangrove forests, other forest types and trees outside forests exist in many urban areas. The forests and trees serve as carbon sinks. A carbon sink is any process, activity or mechanism that removes a greenhouse gas from the atmosphere.

Destroying forests and trees release CO_2 into the atmosphere. For this reason, forests and trees such as mangroves are vital in fighting global warming because they counteract carbon emissions from such sources as industries and transportation (Saenger, 2002; Donato *et al.*, 2011; Njana *et al.*, 2018; Mauya *et al.*, 2019).

Apart from carbon storage and sequestration, mangrove forests attenuate storm surges (Zhang *et al.*, 2012) and offer other environmental and livelihood benefits (Saenger, 2002; FAO, 2007). Therefore, when mangrove forests are disturbed (e.g., land use/land cover conversion) they release large amounts of CO_2 and result in loss of many other environmental and livelihood benefits. Effective management of urban mangrove forests is thus important.

Mangroves are known for the immeasurable ecological and economic ecosystem services they provide. However, the literature shows that mangroves are among the most vulnerable ecosystems worldwide and are declining rapidly (Kuenzer *et al.*, 2011). According to FAO (2007) approximately 3.6 million ha of global mangroves have been lost between 1980 and 2005 due to conversion of mangrove forests to other land uses. Both natural and anthropogenic factors are responsible for the loss of mangroves (Ilman *et al.*, 2016; Gevaña *et al.*, 2018).

The impact of different factors causing losses of mangroves and the response of the mangroves varies between developed and developing countries and may also vary between urban and rural settings. For example, industrial pollution could be one of the most important factors in urban settings (Tam and Wong, 2000) while conversion of mangrove areas to agriculture could be a factor in rural settings (Mwansasu, 2016).

In Mainland Tanzania, mangroves are distributed along the coastline from the north (Tanga) to the south (Mtwara) with Dar es Salaam (which is the former capital of Tanzania) having about 2,500 hectares (Semesi, 1992; Wang *et al.*, 2003) of mangrove forest. Various studies have been conducted in an attempt to generate information to support effective management of mangroves in the country. A study by Semesi (1992) provided countrywide baseline information on the distribution, status, coverage and uses of mangroves in the country. Other studies (Alavaisha and Mangora, 2016; Njana *et al.*, 2018) have reported on biomass or carbon densities. These studies are either generic in nature or provide information at national scale and are not entirely focused on urban mangroves.

A few site-specific studies, for example Mwansasu (2016), have looked at causes and perception of environmental changes in mangroves of the Rufiji delta in rural areas of south-eastern Tanzania, while Katundu (2006) carried out a similar study in urban areas of Kunduchi and Mbweni in Dar es Salaam. The former study focused on mangroves in rural areas while the later study addressed social and ecological resilience of mangrove ecosystems in urban areas.

The current study 1) determined spatial and temporal changes of mangrove forests, 2) identified drivers of mangrove deforestation and forest degradation, 3) determined historical carbon storage, sequestration and deforestation emissions by mangrove forests, and 4) determined whether the mangrove forests of Dar es Salaam were sources or sinks of CO_2.

Methods

Study area

This study was conducted in the Dar es Salaam Region of Tanzania. Dar es Salaam is the former capital and remains the main commercial centre and most industrialized and urbanized city in Tanzania, with a population of more than 5 million people. Dar es Salaam Region is located along the Indian Ocean on the east coast, situated between latitudes 6° 36' and 7° 0' S and longitudes 39° 0' and 33° 33' E. The region is estimated to cover 1 800 km^2 and comprises five Districts (Ilala, Kigamboni, Kinondoni, Temeke, and Ubungo). Only Ubungo District is not located on the coast and has no mangrove forests.

Dar es Salaam is divided into three ecological zones; lowlands, middle plateau and the hilly areas in northern and western parts of the region. Dar es Salaam is characterized by a modified equatorial climate with a mean temperature of 29°C. The average rainfall is 1000 mm ranging from 800 to 1300 mm. Humidity is around 67 % and 96 % in the morning and afternoon, respectively. Prevailing winds are south-westerly from April to October and north-westerly monsoon winds from November and March (URT, 2004). Mangrove forests in Mainland Tanzania cover about 110,000 hectares, 2,500 hectares of which are found is Dar es Salaam. Mangrove forests were declared as forest reserves in 1947 (MNRT, 1991).

Research methods

Both quantitative and qualitative research methods were used in this study. Spatial and temporal changes of mangrove forests, carbon storage and sequestration, and carbon emissions due to mangrove deforestation were determined using quantitative research methods. Quantitative methods were also used to determine whether the urban mangrove forests of Dar es Salaam are net carbon sinks or otherwise. Conversely, qualitative methods were applied to assess the drivers of mangrove deforestation and forest degradation. In this study, deforestation refers to the conversion of mangrove forest cover to non-mangrove forest cover while mangrove forest degradation refers to selective tree removal resulting into a decrease of forest quality and productivity.

Quantitative research methods

Spatial and temporal changes of mangrove forests were determined through mapping and change detection of mangrove forest cover and productivity (or forest condition) in the study area. Temporal change of mangrove forest cover was carried out for 20 years, from 1986 to 2016, divided into four data points of 1986, 1995, 2006 and 2016. The study period of 20 years was considered suitable for determining historical carbon storage, sequestration and emissions in the study area and is in line with the time series studies in the literature (Wang *et al.*, 2003; Long *et al.*, 2013; Njana *et al.*, 2021). Accordingly, the study utilised Landsat images, which were previously successfully used in spatial and temporal analysis of mangrove cover (e.g. Wang *et al.*, 2003; Hong *et al.*, 2020). Landsat images provided time series data at a resolution of 30 m since 1980s.

Freely available Landsat images were downloaded from the US Geological Survey National Centre for Earth Resources Observations and stacked using ERDAS Imagine software to obtain colour composite images. Dry season images which ensure relatively higher classification accuracy (e.g., Liu *et al.*, 2015; Kenduiywo *et al.*, 2020) were selected where possible from Landsat archives. Although coastal areas in the study area are often cloudy, only images with less than 10 % cloud were selected. Image enhancement was employed to improve the interpretability, while the histogram equalization method was used for contrast enhancement of the image so that all the information from the input image was represented. After the enhancement of colour composites, supervised classification was used for image classification. The Spatial Analyst Tool in ArcGIS 10.3 was used for image classification. Sample points collected in the field were used for validation of the results. The interpretation was validated with information collected from local and especially older people who had good knowledge of changes in the mangrove cover over time, including the areas covered by forest, buildings, and water.

The classified images for different years were then vectorised and clipped to obtain the area in hectares (ha) of mangroves forest at each location. Productivity or condition of mangrove forests was assessed using the Normalised Difference Vegetation Index (NDVI). NDVI values were derived using Eq. (1).

$$NDVI = \frac{NIR - RED}{NIR + RED} \quad \text{............................ Eq. 1}$$

Where NIR = Near infra-red band and RED = Red band.

NDVI for each location was calculated for each year using ERDAS Imagine software. The maximum, mean and minimum NDVI values were recorded for each study site. NDVI has been applied successfully in many studies on mangrove forests (e.g. Zhu *et al.*, 2015; Gupta *et al.*, 2018; Hong *et al.*, 2020).

Total carbon stored and sequestered by mangrove forests may be estimated using two datasets: (i) mangrove forest cover/change (ha); and (ii) CO_2 equivalent density (tonnes CO_2e ha^{-1})/CO_2 equivalent sequestration density (tonnes CO_2e ha^{-1} $year^{-1}$). The later dataset is commonly derived using forest inventory data. However, forest inventory was beyond the scope of the current study, and secondary information was applied for this purpose. Recently, Njana *et al.* (2018) reported aboveground and belowground CO_2 equivalent densities for mangrove forests using national forest inventory data. Similarly, the study reported aboveground CO_2 equivalent and belowground CO_2 equivalent sequestration densities for mangrove forests using national level permanent sample plot data.

This study considered both above- and belowground carbon pools. Therefore, the total CO_2 equivalent density (TCD, tonnes CO_2e ha^{-1}; i.e. sum of aboveground CO_2 equivalent and belowground CO_2 equivalent densities) and the total CO_2 equivalent sequestration density (TCSD, tonnes CO_2e ha^{-1} $year^{-1}$; i.e. sum of aboveground CO_2 equivalent and belowground CO_2 equivalent sequestration densities) from Njana *et al.* (2018) were applied in the estimation of total carbon stored and sequestered by the mangrove forests of Dar es Salaam. TCD was also applied in the estimation of carbon emissions due to mangrove deforestation. The total annual carbon stored (TC), sequestered (TACS, tonnes CO_2e $year^{-1}$) and emitted (TACE, tonnes CO_2e $year^{-1}$) due to deforestation of mangrove forests in Dar es Salaam were derived using Eq. 2, Eq. 3 and Eq. 4 respectively. Similar procedures were applied by Njana *et al.* (2021) in the estimation of carbon storage, sequestration and emissions from forests in relation to land use and land cover change.

$$TC = A * TCD \quad \text{.......................... Eq. 2}$$

$$TACS = A_{FF} * TCSD. \quad \text{........................ Eq. 3}$$

$$TACE = A_{FN} * TCD.. \quad \text{........................ Eq. 2}$$

Where A = Area of mangrove forest cover (ha), A_{FF} = Area of mangrove forest cover remaining (ha), A_{FN} = Area of mangrove forest cover converted to non-mangrove forest cover divided by time; i.e. annual mangrove deforestation (ha $year^{-1}$).

Net carbon sink/net carbon source was determined by subtracting TACE from TACS. A positive resultant value implied a net carbon sink and a negative resultant value implied a net carbon source.

Qualitative research methods

Qualitative data for identifying drivers of mangrove deforestation and forest degradation was derived from Participatory Rural Appraisal (PRA) methods (Sandham *et al.*, 2019), and field observations. PRA methods employed in this study included focus group discussion (FGD) and indepth interviews with key informants. Four Districts with patches of mangrove forests were purposively selected for FGD. These include Kinondoni, Temeke, Ilala, and Kigamboni. The results from the analysis of mangrove forest cover and change were the basis for defining the mangrove forest patches (i.e. 11 patches) in the study area, and FGDs were conducted at each of these sites. FGD involved people living adjacent to mangrove forests and who were asked to identify driving factors of mangrove forest cover change in the study sites.

A neighbourhood Chairperson or the Neighbourhood Executive Officer (NEO) or their representative assisted in mobilizing participants for FGD and prepared the venue for the exercise. FGD comprised of 5 to 8 people including both males and females living closest to the individual patches of mangrove forests. The discussion was guided by open-ended questions such as *why, how, what, when*, and by the extent of change and productivity (quality) of mangrove forest cover in the study area from 1986 to 2016. The historical timeline was also conducted with at least two people in every site in order to 1) understand the history of the neighborhood/street and the people who are living in the study site, and 2) identify key events and how they related to the trends in the mangrove forests. The results from FGD were triangulated during in-depth interviews with key informants, who included District Forest Managers from Tanzania Forest Service (TFS) and ward and sub ward leaders in all the four districts endowed with mangrove forest patches.

Results from both FGD and in-depth interviews were subjected to content analysis according to Cavanagh (1997). Detailed analysis of research and other official reports such as the Mangrove Forest Management Plan for Dar es Salaam was also carried out to obtain further historic information. Finally, the results on drivers of mangrove deforestation and forest degradation were summarised in tabular form.

Results

Spatial and temporal changes of mangrove forests in Dar es Salaam

Land cover (LC) analysis revealed that there are 11 patches of mangrove forests in Dar es Salaam (Fig. 1). The patches were of variable size. The largest and smallest patches of mangroves were at Mtandika (1986-2006) and Ras Kilomoni (1986-2016). From 2016 the largest patch of mangrove forest changed to Mbezi river. Overall, Dar es Salaam had 4,813 ha of mangrove forests in 1986 which declined to 1,961 ha in 2016 (a decrease of 59 %). All sites exception Kunduchi creek (1995-2006) experienced a decline in mangrove forest cover (Fig. 2). Sites with larger patches recorded relatively higher loss of cover than did the smaller patches.

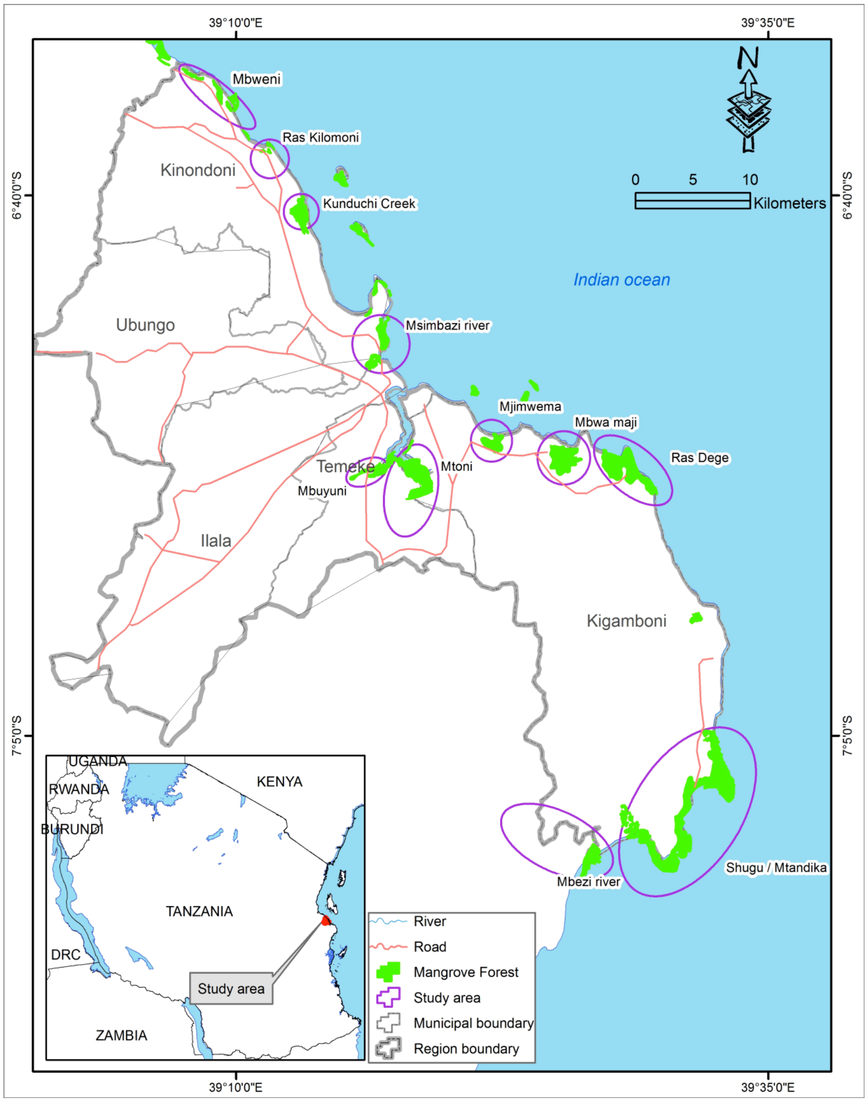

Figure 1. Patches of mangrove forests in Dar es Salaam.

Generally, the trend of NDVI values shows a decreasing trend from 1986 to 2006, yet for the period of 2006 to 2016 there was a significant increase in NDVI values for all sites (Fig. 3).

Drivers of deforestation and degradation of mangrove forests

Drivers of deforestation and degradation of mangrove forests in the study area are presented in Table 1. In descending order, clearing mangrove forests for salt pans, hotel construction, settlements, and charcoal making were prominent drivers of mangrove deforestation across sites. Similarly, tree removal for firewood and building poles were prominent drivers of mangrove forest degradation across sites.

Trends of carbon stored in the mangrove forests of Dar es Salaam

The total carbon stored in mangrove forests of Dar es Salaam has been declining over time from 1,131,055 tonnes CO_2e in 1986, 883,835 tonnes CO_2e in 1995, 702,415 tonnes CO_2e in 2006, and 460,835 tonnes CO_2e in 2016. Site-specific statistics also show a similar declining trend over time. However, between 1995 and 2006 there was an increase of CO_2e of about 1,200 tonnes CO_2e (Fig. 4).

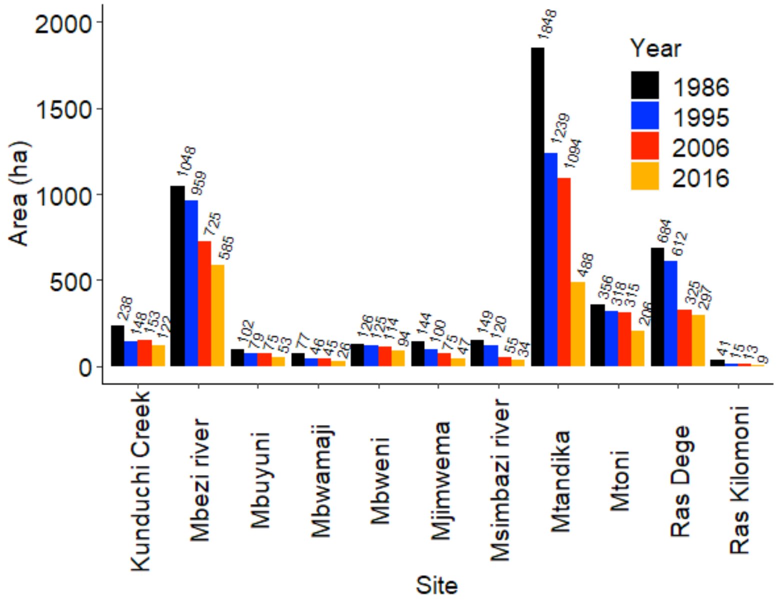

Figure 2. Mangrove forest cover in Dar es Salaam over time.

Trends of total annual carbon sequestration by mangrove forests of Dar es Salaam

Sequestration of CO_2 by mangrove forests of Dar es Salaam is estimated at 133,516 (1986-1995), 106,110 (1995-2006), and 69,616 (2006-2016) tonnes CO_2e year^{-1}. Overall statistics showed a decreasing trend of CO_2 sequestration with a decrease of more than 50 % between 2006 and 2016, relative to CO_2 sequestration observed between 1995 and 2006. Although, the site-specific distribution of CO_2 sequestration showed mixed results, the proportions are in general the same across time (Fig. 5).

Trends of total annual carbon emissions due to mangrove deforestation in Dar es Salaam

Mangrove deforestation resulted in emissions of about 27,400, 16,500 and 24,000 tonnes CO_2e year^{-1} in 1986-1995, 1995-2006 and 2006-2016, respectively. Site-specific results in Fig. 6 further show that loss of mangrove forest cover in Mtandika between 1986 and

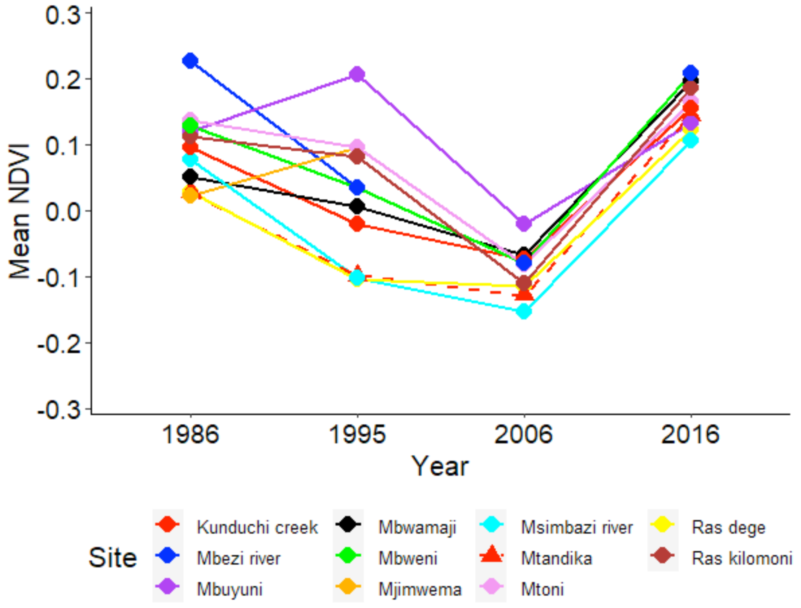

Figure 3. Trends of NDVI at the study sites.

Table 1. Drivers of deforestation and forest degradation.

SN	**Drivers**	**Study sites**											
		Kunduchi	**Mbezi river**	**Mbuyuni**	**Mtoni**	**Mbwa maji**	**Mbweni**	**Mjimwema**	**Msimbazi river**	**Ras Dege**	**Ras Kilomoni**	**Mtandika**	**%**
Drivers of deforestation													
1.	Charcoal making		√					√		√		√	40
2.	Hotel construction	√				√		√		√	√		50
3.	Industrial waste			√	√				√				20
4.	Infrastructure construction								√	√	√		30
5.	Rice farming			√	√	√				√			30
6.	Salt pans	√		√	√		√	√		√	√		60
7.	Settlement	√				√		√	√				40
Total cases		3	1	3	3	3	1	4	3	5	3	1	
Drivers of mangrove forest degradation													
1.	Boat making										√		10
2.	Firewood	√		√	√	√	√	√		√			60
3.	Medicines	√											10
4.	Building Poles	√				√		√			√	√	50
Total cases		3	0	1	1	2	1	2		1	2	1	

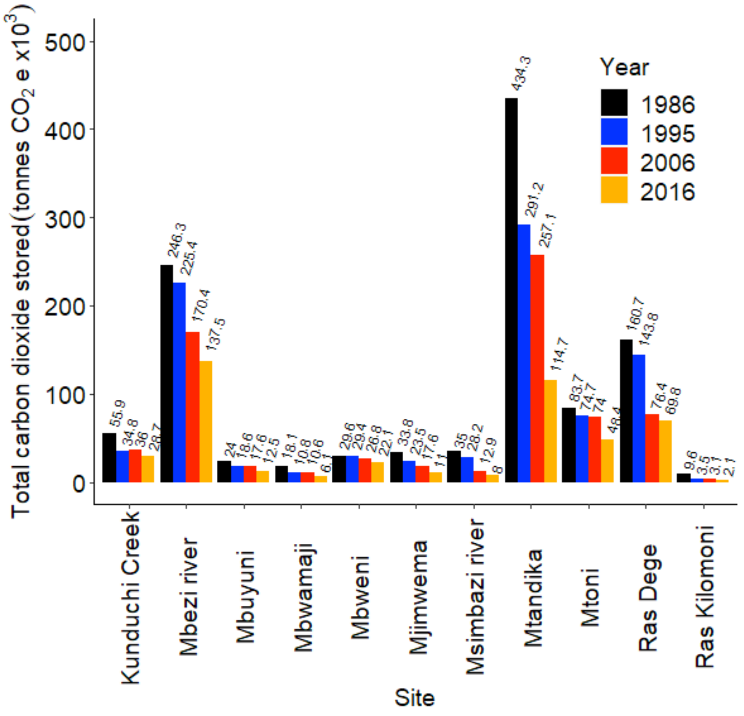

Figure 4. Carbon stored in the mangrove forests of Dar es Salaam over time.

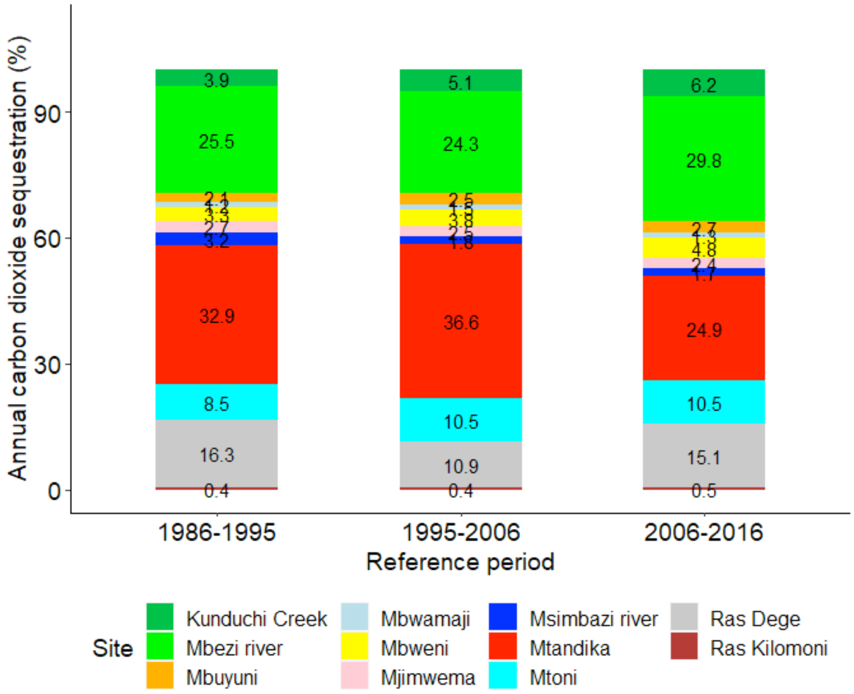

Figure 5. Total annual carbon sequestration by mangrove forests across sites.

1995 as well as from 2006 to 2016 contributed more than 50 % of total emissions in the study area. Similarly, loss of mangrove forest cover between 1995 and 2006 in Ras Dege and Mbezi river was responsible for more than 30 % of the total emissions from Dar es Salaam mangroves.

Mangrove forests of Dar es Salaam: Are they a net carbon sink or a net carbon source?

Whether mangrove forests of Dar es Salaam are a net sink or a net source of CO_2 was determined by comparing TACS and TACE for different periods under consideration. The results in Fig. 7 show that TACS was

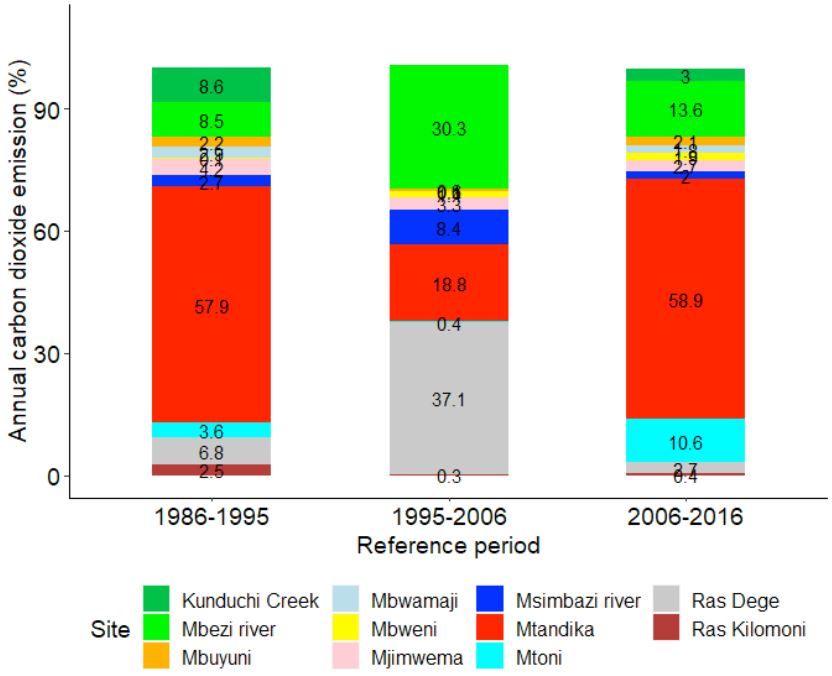

Figure 6. Total annual carbon emission from mangrove forests across sites.

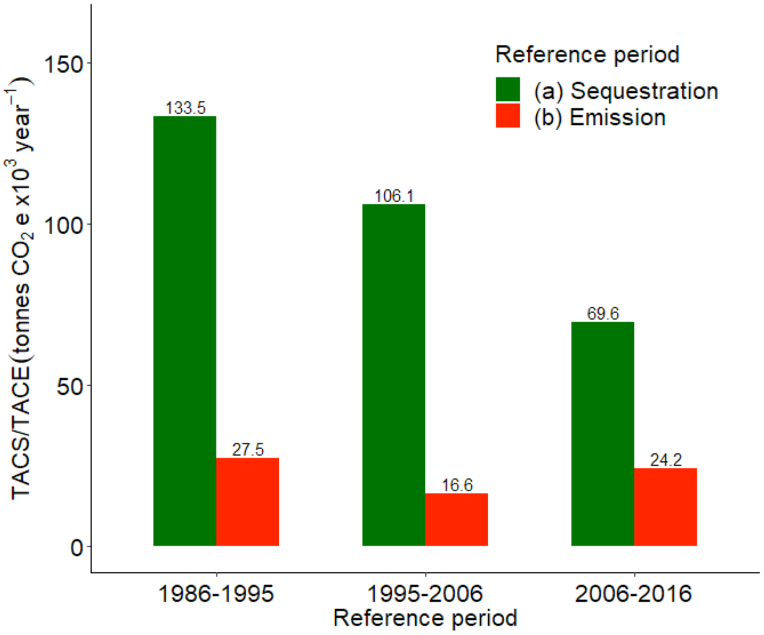

Figure 7. Annual carbon sequestration and emissions over time.

systematically higher than TACE from 1986 to 2016 implying that the mangrove forests of Dar es Salaam are a net sink of CO_2. The net carbon sink was 106, 89.5 and 45.4 tonnes CO_2e year^{-1} in 1986-1995, 1995-2006 and 2006-2016, respectively.

Discussion

Urban ecosystems such as the mangrove forests of Dar es Salaam regulate climate and local weather by removing greenhouse gases (GHG) such as CO_2 from the atmosphere, reducing heat strains and filtering air pollutants (Njana *et al.*, 2018). Such ecosystems may on the other hand propagate climate change when they are destroyed through their conversion into other land uses/land covers or through unsustainable exploitation. Given that mangroves store large quantities of carbon per unit area (Njana *et al.*, 2018; Mauya *et al.*, 2019), their conversion to other land uses/covers or unsustainable use may cause large emissions per unit area. Emissions cause climate change and resultant extreme weather conditions, air pollution affecting human health, and impair sustainable development in general (Epstein, 2001; Hunter, 2003; Svirejeva-Hopkins *et al.*, 2004; Parrish and Zhu, 2009; IPCC, 2014; URT, 2014). Therefore, global efforts aim at, among other things, stabilizing the atmospheric abundance of CO_2 and other greenhouse gases through sequestering CO_2 from point sources or from the atmosphere.

The analysis of land use and land cover changes are central in supporting global efforts of stabilizing the atmospheric abundance of CO_2 and other greenhouse gases. More importantly, the results from land use and land cover and their changes are pre-requisites for effective urban land use planning. The findings from this study show a decreasing trend of mangrove forests from 1986 to 2016 in Dar es Salaam. Such results are consistent with the national, regional and global trends of mangrove forests both in urban and rural areas (Wang *et al.*, 2003; FAO, 2007; Hong *et al.*, 2020). However, there is a discrepancy in the estimated acreage of mangrove forests lost in Dar es Salaam compared to the findings by Wang *et al.*, (2003). Such discrepancy is attributable to differences in methodological approaches, analysis techniques, cloud cover in the images used, the data used and the reference period. For example, differences in the estimates of mangrove cover between the Wang *et al.* (2003) study and the present study could be due to misclassification of mangrove forests (e.g. Long *et al.*, 2013).

The quality of mangrove forests was estimated using NDVI which declined from 1986 to 2006. The trend of mangrove NDVI during this period was similar to the trend of mangrove forest cover for the same period. However, contrary to the further declining trends of mangrove forest cover between 2006 and 2016, NDVI results showed that the quality of mangrove forests improved from 2006 to 2016. In the early 1990s, the Forest and Beekeeping Division developed mangrove management plans for all mangroves in mainland

Tanzania including Dar es Salaam (MNRT, 1991). The management plans proposed a number of activities such as replanting in degraded mangrove stands, environmental conservation education to the local communities and enhancement of law enforcement. Effective implementation of such activities in Dar es Salaam started around 2000s through the Mangrove Management Project funded by NORAD under the Forest and Beekeeping Division. Replanting of mangroves in degraded mangrove stands and natural regeneration of mangroves as a result of effective law enforcement and compliance resulted into improved productivity of mangroves in Dar es Salaam; hence the observed rise of NDVI values from 2006 to 2016. This finding is consistent with the findings of Feller *et al.* (2017) who revealed that conservation and rehabilitation efforts coupled with natural regeneration may promote gains in specific mangrove areas.

The study also identified anthropogenic drivers of mangrove deforestation and degradation. Similar drivers are reported in the existing literature (e.g. Wang *et al.*, 2003; Mohamed *et al.*, 2009; Kithiia and Lyth, 2011; Troung and Do, 2018; Hong *et al.*, 2020). Drivers of deforestation and degradation of urban mangrove forests identified in the present and many other studies are very much associated with population growth. High population in urban areas such as Dar es Salaam is characterised by high demands for natural resources (e.g. charcoal for energy) and land uses other than forestry. Studies show that growth of cities affects the environment directly and indirectly and has been a source of much pollution due to large consumption of fuel for transportation, for example (Bettencourt and West, 2010; Hillman and Ramaswami, 2010). The literature indicates that, since 1950, the proportion of the world's population living in urban areas has increased from 13 % to more than 50 % (UNPD, 2011) suggesting that urbanization is the main obstacle to sustainable development (McDonald, 2008). Although the total urban area worldwide remains a relatively small fraction of the earth's terrestrial surface, urbanized areas account for roughly 75 % of the global consumption of resources (Angel *et al.*, 2005; Pacione, 2009). The drivers of mangrove deforestation and degradation identified in this study are useful in designing and developing strategies for REDD+. Direct drivers and underlying causes of deforestation and forest degradation such as those reported in this study are the basis for development and designing of REDD+ strategies that are aimed at the reduction of emissions from deforestation and forest degradation, improvement of management of forests, as well as conservation and enhancement of forest carbon stocks.

The findings of this study demonstrate that the urban mangrove forests of Dar es Salaam store and sequester large quantities of atmospheric CO_2 and thereby contribute to climate regulation. In addition, studies show that urban mangroves ameliorate local weather and filter air pollutants due to economic development activities in the cities (Alavipanah *et al.*, 2015; Fan *et al.*, 2015; Rotem-Mindali *et al.*, 2015; Kayet *et al.*, 2016; Rousta *et al.*, 2018). Both total stored and sequestered carbon by the urban mangrove forests of Dar es Salaam had been declining from 1986 to 2016. A decreasing trend of forest cover due to urbanization was also previously reported by Delphin *et al.* (2015). The current study shows that trends of carbon stocks, sequestration and emissions are linked to spatial and temporal dynamics of mangrove forest cover in the city. Accordingly, emissions due to mangrove deforestation are high. Although it was revealed that mangrove forests in the study area are a net carbon sink, the sink role played by mangrove forests is decreasing rapidly. It is expected that these findings will stimulate dialogues and pave the way for making appropriate decisions on the management of urban mangrove forests.

The loss of urban mangrove forests does not only threaten regulatory ecosystem services of storing and sequestering greenhouse gases, which poses environmental challenges to the present and future generations, but also has other severe negative impacts. Such impacts include loss of other ecosystem services including coastal protection against storm surge and loss of breeding sites for fish and habitat for several marine species. Therefore, management of urban mangrove forests needs to be enhanced.

Success of conservation efforts of urban mangrove forests relies on strategies and interventions aimed at addressing drivers of mangrove deforestation and degradation. Priorities should be focused on that include addressing the drivers causing substantial impacts on carbon storage and sequestration (e.g. charcoal making, salt pans etc.) since such ecosystem services are fundamental for the mitigation of climate change that affects many sectors of the economy. Therefore, effective urban land use planning, law enforcement, as well as awareness creation and education are recommended.

Conclusion

Urban mangrove forests play an important environmental role in mitigating climate change and ameliorating the severity of local weather conditions through the large carbon stocks they store and sequester. However, the declining spatial and temporal trends of urban mangrove forest cover have resulted in a systematic decrease of the total carbon stored and sequestered by mangrove forests in Dar es Salaam. Such a trend is largely and indirectly caused by high urbanization. Consequently, emissions due to mangrove deforestation are high. In addition, mangrove forests in the study area are still a net carbon sink. However, the sink role played by mangrove forests in the study area is decreasing rapidly. In the absence of timely measures for preserving and rehabilitating degraded mangrove areas, mangrove forests of Dar Salaam may be transformed into a source of CO_2. The findings from this study are expected to stimulate dialogues and pave the way for making appropriate decisions and taking appropriate measures on the conservation of urban mangrove forests. This study therefore recommends effective urban land use planning and law enforcement, awareness raising and education aimed at achieving both sustained ecosystem services offered by urban mangrove forests and economic growth.

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Composition and structure of the mangrove fish and crustacean communities of Vanga Bay, Kenya

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Abstract

Mangroves support coastal fisheries, particularly by providing nurseries for juvenile fish. However, much remains unknown about the fish and crustacean communities in mangroves and about the lifecycles and behaviours of individual species. This study is the first to describe the fish and crustacean communities in the Vanga mangrove ecosystem, the largest mangrove forest and the most important marine fisheries landing site in southern Kenya. Using fyke nets, 14 mangrove creek sites were sampled once every 3 months during spring tides, between September 2015 and September 2017. The sampling period covered the north east (NEM) and south east (SEM) monsoon seasons. A total of 1,879 fishes and 1,132 crustaceans were caught, represented by 59 and 16 species, respectively. Over 95 % of the fishes caught were juveniles with 50 % of both fish and crustacean species being of commercial importance. About 70 % of the catch was dominated by only 6 fish species with *Yarica hyalosoma* and *Acropoma japonicum* contributing 44 % of the total abundance. *Penaeus semisulcatus* and *Penaeus indicus* dominated the crustaceans. Fish abundances differed significantly between seasons, with the SEM catches almost three times higher than those in the NEM, while crustaceans displayed no clear seasonality. The study documents for the first time the fish and crustacean communities in Vanga mangroves and confirms their importance as juvenile habitat for commercial species. The importance of mangrove and near mangrove habitat as nursery habitat for both fish and crustaceans emphasizes the need for conservation particularly of seaward edges

Keywords: nursery habitat, juvenile, size distribution, intertidal, seasons

Introduction

Estuarine habitats are often important nursery areas for juvenile fishes and crustaceans (Laegdsgaard and Johnson, 1995; Rozas and Minello, 1997; Sheridan and Hays, 2003; Barbier, 2011; Lefcheck *et al*., 2019). Nursery habitats are defined as areas that contribute disproportionately to adult populations of fish, with juveniles occurring in high densities, or achieving better growth or survival, or all of these combined (Beck *et al*., 2001). The nursery function of mangroves has been studied in most mangrove regions of the world, including South America and the Caribbean, South East Asia, Australia and East Africa (Primavera, 1997; Laegdsgaard and Johnson, 2001; Lugendo *et al*., 2007; Nagelkerken *et al*., 2008; Abrantes and Sheaves, 2009; Gajdzik *et al*., 2014). These studies and others give evidence that mangroves can provide shelter and food for juvenile fish, but it is still challenging to prove that these juveniles successfully move from mangrove nurseries to adult habitats (Beck *et al*., 2001; Gillanders *et al*., 2003). The scarcity of permanent residents in intertidal mangrove habitats means that fish within commonly found families such as Lethrinidae, Lutjanidae and Monodactylidae (Kimani *et al*., 1996; Wainaina *et al*., 2013), which may be heavily reliant on mangroves at a given site, must utilise other habitats as well.

Penaied shrimp contribute to artisanal and commercial fisheries in the Western Indian Ocean (WIO) which includes mainland continental states of Kenya,

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Tanzania, Mozambique and the small island states of Madagascar, Mauritius, Seychelles, Comoros and Reunion. The commercially important penaeid shrimp species in WIO nearshore habitats include: *Penaeus indicus, Penaeus. semisulcatus, Penaeus monodon* and *Metapenaeus monoceros* (Wakwabi and Mees, 1999; Macia, 2004) and have been shown to use mangrove habitats at various life stages (Rönnbäck *et al.*, 1999; Crona and Rönnbäck, 2005). *P. indicus* has been found to prefer vegetated, muddy bottoms, and other species found in mangrove areas such as *Penaeus japonicus* prefer bare areas (due to its ability to burrow and hide from predators) while *P. semisulcatus* prefers deep, less clear waters (Macia, 2004; Crona and Rönnbäck, 2005; Munga *et al.*, 2013). In Kenya, penaeid shrimp generally exhibit no seasonality (Ndoro *et al.*, 2014).

Given the broad association between healthy mangrove ecosystems and fisheries production (e.g. Barbier, 2000), it is unfortunate that mangroves in the WIO region continue to face a wide range of anthropogenic pressures, such as overharvesting and conversion to other uses, which have resulted in long-term declines in extent and quality (Kairo *et al.*, 2002; Ochiewo, 2004; Fulanda *et al.*, 2009; Huxham *et al.*, 2015). Securing a reliable and sustainable occurrence of fishes and crustaceans, for food and as well as for biodiversity, will require sound mangrove and coastal management, which ideally should be informed by a better understanding of how fishes and other fauna are using the habitat. However, conducting the research that would allow this is difficult; sampling mobile fishes and crustaceans within mangrove forests is challenging. The complex root network in mangroves makes it difficult to use most conventional fish sampling gear to carry out effective and quantitative sampling. Faunce and Serafy (2005) highlighted that most mangrove fish sampling surveys conducted between 1955 - 2005 had failed to sample inside the mangrove forest due to such limitations. Instead, many studies on mangrove fishes restricted sampling to adjacent bays or large, permanently inundated creeks. Whilst this literature provides important insights into the range of species present, doubts remain over whether, and to what extent, individual fish captured adjacent to mangroves venture into the forest at high tide. It is possible that some or many of these species that use permanent open water habitats never enter the inter-tidal areas. To overcome this problem, barrier enclosure samplers (stake nets) and visual methods (whenever water is clear enough) have been used to collect data on fish species within forests (Vance *et al.*, 1996; Rönnbäck *et al.*, 1999; Huxham *et al.*, 2004; Crona and Rönnbäck, 2005; 2007). Although such approaches provide thorough quantitative data, they are very labour intensive, cumbersome, and restricted to sampling small (and possibly unrepresentative) areas and therefore limited to mangrove areas that are easily accessible or (in the case of visual surveys) low in turbidity.

Work on mangrove fish communities typically features large spatial and temporal variability, which may arise through these methodological limitations; fine-grained approaches at small sites within a forest may sample statistical noise that would disappear in larger data sets. However, this variability may be more than noise; it could imply major differences in the value of sampled areas within mangrove sites for individual fish species or communities as a whole. Explaining this variability remains a major research challenge with important management implications, since identifying forest characteristics that are of importance for the utilization of mangroves by fishes, crustaceans and other fauna would allow managers to target conservation efforts at such areas and features.

The Vanga mangrove ecosystem is one of the largest contiguous mangrove blocks in Kenya and supports rich fishing grounds (Obura, 2001; GoK, 2017). The fishery resource is transboundary and attracts a substantial number of migrant fishers from Tanzania over the fishing seasons. There is evidence that the fishery is under pressure and declining; a recent analysis suggests total officially recorded catches have declined by ~40% over the past ten years (Fortnam *et al.*, 2020). The causes of this decline are unknown; whilst they may be related to changes in mangrove extent and quality (in common with other Kenyan mangroves, the forest here has suffered declines in total area and increasing evidence of human impact such as cutting over the past four decades), there is a major gap in knowledge with respect to the Vanga mangroves; the only peer-reviewed study published on mangrove ecology from the site is Gress *et al.* (2016), which documents carbon storage. The present study therefore is a first step in filling this gap by assessing the community structure of fishes and crustaceans (based on species composition, abundance and size) in the Vanga mangrove ecosystem. In addition, seasonal and spatial variations of these variables for the dominant species were assessed. This will help to understand ontogenetic changes in habitat use in fish, and seasonality effects on fish and crustacean species.

Study sites

The study site is Vanga on the south coast of Kenya (4° 39' 38.42"S, 39° 13' 9.71"E; Fig. 1). The climate of Vanga is typical for east African coastal areas where the Inter Tropical Convergence Zone (ITCZ) partitions the year into two distinct seasons i.e. the South East Monsoon (SEM) and North East Monsoon (NEM). The rainy SEM season is from March to October and the dry NEM season from September to March (McClanahan, 1988). Fish catch and reproduction typically peak during NEM at the east African coast when the waters are more stable and nutrition available (McClanahan, 1988). The tidal regime is semi diurnal and ranges between amplitudes of 1.5m at neap tide and up to 4 m at spring tides (Obura, 2001). The Vanga mangrove complex covers a total area of > 4000 ha, and comprises 7 species of mangrove trees, although forests are dominated by 5 common species: *Avicennia marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Rhizophora mucronata*, and *Sonneratia alba* (GoK, 2017). The rivers Umba and Mwena drain fresh water into the Vanga mangrove ecosystem, with the River Umba, (source in Tanzania), discharging circa 16 million m^3 of fresh water into the sea annually (UNEP, 1998; GoK, 2017).

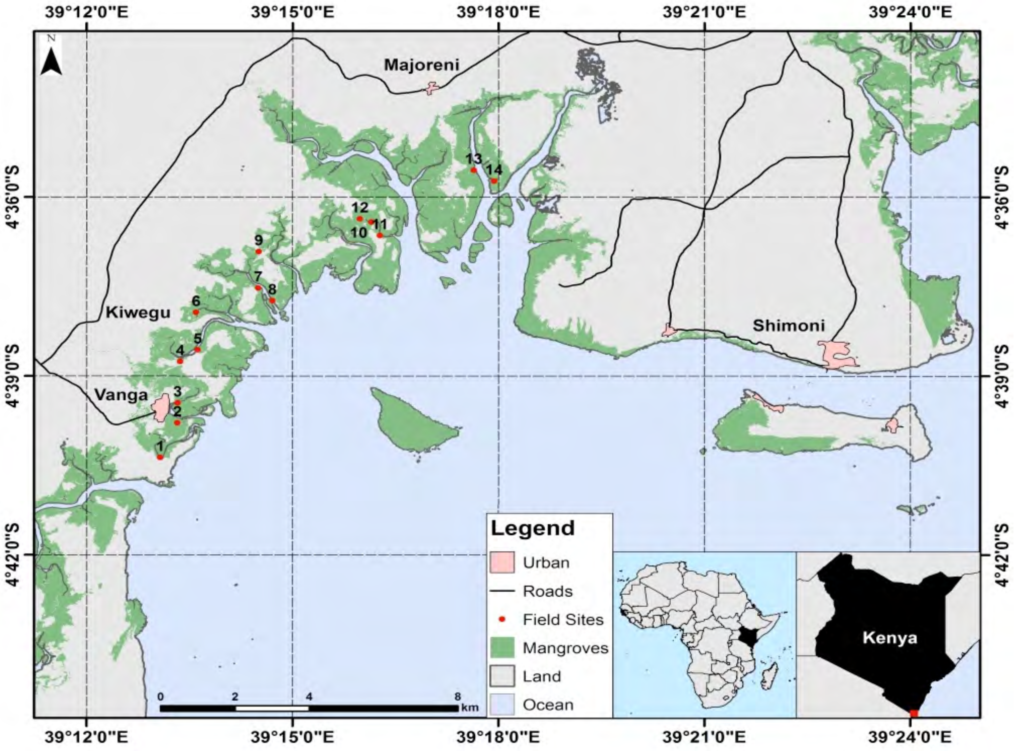

Figure 1: Location of Kenya (inset) and the sampling sites across the Vanga mangrove ecosystem (sampling sites shown as red dots and site numbers in black).

Sampling design and methodology

Fishes and crustaceans were sampled at 14 mangrove sites, situated between Jimbo and Majoreni villages in the Vanga Bay along around 11 km of coastline. Sites were chosen to cover a wide geographical area of the Vanga mangrove forest, whilst still being accessible enough to allow regular sampling. Fyke nets were deployed and retrieved after 24 hours within small creeks that drained the 14 mangrove sites, in order to sample fish communities leaving these areas during the ebb tide. The nets had two wings each of 9.5 m length and a height of 1m. The length of the body frame was 3.6 m. The main frame was made of metal measuring 1 m x 1 m with three rings (0.9 m, 0.7 m, and 0.6 m in diameter) distributed along the body frame. The net had a mesh size of 1.9 cm when stretched. Sites were sampled once every three months between September 2015 and September 2017. Each sampling campaign was spread over a six-day period during new moon spring tides. Hence replicate samples were taken eight times from each of the 14 sites giving a total of 112 samples. March 2017 was not sampled due to logistical reasons. Fish and crustacean samples collected in the field were placed in a cool box and later sorted and identified in the laboratory to the lowest possible taxonomic level using Anam and Mostarda (2012) and Richmond (2011). Fish standard length and total length were measured (to the nearest 0.1 cm) and individual mass recorded (to the nearest 1 g). Crustaceans were counted and weighed to the nearest 0.1 g. To classify the fishes into size classes, the maximum length of each species was sourced from FishBase (Froese and Pauly, 2017). Using guidelines from Nagelkerken and Velde (2002), fishes with total length ≤1/3 maximum length were classified as small juveniles, between >1/3 to ≤ 2/3 maximum length classified as large juveniles/ sub adults and those >2/3 maximum length were classified as adults. Fishes were also classified into their trophic groups and importance to fisheries as guided by information on FishBase (Froese and Pauly, 2017).

Statistical analysis

Fish and crustacean data were analysed separately. 'Fish' in this case stands for all the teleost fish species caught while crustaceans included shrimp and crabs. Data were analysed using R Core Team (2013) and Plymouth Routines In Multivariate Ecological Research (PRIMER) version 6.0 Clarke and Warwick (2001). Shannon's diversity index (H'), Margalef's (D) and Pielou's evenness index (J') were used to compare fish and crustacean community structure between seasons. Statistical analysis used all 112 samples as raw data in this analysis.

To test for differences between seasons, September and June abundance data were pooled under the season 'South East Monsoon (SEM)' and March and December data under 'North East Monsoon (NEM)'; separate analyses were performed for fishes and crustaceans.

Non-metric multidimensional scaling (nMDS) ordination plots based on the Bray Curtis similarity index were then developed to visualize seasonal differences (Clarke and Warwick, 2001). One-way Analysis of Similarities (ANOSIM) was used to test for significant differences among fish and crustacean community structure between seasons, after which similarity of percentages (SIMPER) was used to establish the fish and crustacean taxa that mainly contributed to the differences found.

ANOVA was used to test for differences between mean fish abundance in NEM and SEM seasons and a significance level of $p < 0.05$ was used. A Kruskal -Wallis H test was used to test the differences between mean crustacean abundance in both seasons since the datasets could not meet the assumptions of normality. A significance level of ($p<0.05$) was used. *Yarica hyalosoma, Acropoma japonicum* and *Gerres oyena* were sufficiently abundant (both overall and between dates) to allow comparisons of size-frequency distributions among some of the sampling dates, in order to explore growth patterns. Chi – square tests of association ($p<0.05$) were performed to test for differences between size classes distributions at different sampling times for *Y. hyalosoma, A. japonicum* and *G. oyena*.

Results

Fish and crustacean community structure

A total of 1,879 fishes were caught, represented by 28 families and 59 species. Six species dominated the catch and contributed about 70 % of the total abundance: *Y. hyalosoma* (25 %), *A. japonicum* (18.9 %), *Ambassis natalensis* (11.2 %), *Ambassis ambassis* (7.2 %), *Leiognathus equulus* (7.2 %) and *G. oyena* (5.7 %) (Table 1). The 5 most common families were Apogonidae, Ambassidae, Acropomatidae, Gerreidae and Leiognathidae. Serranidae was the most diverse family (5 species) followed by Lutjanidae and Haemulidae, which had 4 species each. *Gerres filamentosus* and *G. oyena* were the most widely spatially distributed with each being caught at 10 different sites. The species *Lutjanus fulvus, L. bohar*, *Pterois volitans* and *Tylosurus crocodilus* (single individuals each) were considered rare. Circa 50 % of the total species caught were of commercial importance, based on information from FishBase (Froese and Pauly, 2017) (Table 1).

A total of 1,161 crustaceans were recorded, distributed between 16 species. Most of them belonged to infra-order Brachyura for crabs and family Penaeoidea for the shrimp. The most abundant crustacean species were *P. semisulcatus* (46.5 %) and *P. indicus* (36.2 %) (Table 2). *P. indicus*, *P. semisulcatus* and *Thalamita crenata* were encountered in all 14 sites sampled. The penaeid species *Macrobrachium rude*, *Scylla serrata* and *T. crenata* are of high commercial value.

Seasonal variations in fish assemblages (SEM – NEM)

The mean catches of fish (pooled across all sites) during the SEM months were 330 and 294 in September and June, respectively. By comparison, the NEM months of December and March had much lower means of 80 and 139, respectively (Fig. 2), a difference which was highly statistically significant (one-way ANOVA $F_{(1,110)} = 8.58$, $p=0.004$). September and June (SEM) also

Table 1. Summary information on fish communities including fish abundance (catch per net), respective families, associated fish species in the families (fish that were not identified to species level are shown as unidentified), total abundance and total species per site. The commercial importance of the fish abbreviated: HC=highly commercial, C=commercial, MC=minor commercial, AQ=Aquarium, NCI = no commercial importance; based on Froese and Pauly (2017) at the 14 sampling sites in Vanga, Kenya. The symbol (*) was used to indicate fish whose importance has not been documented.

Family	**Species**	**Importance**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Acropomatidae	*Acropoma japonicum*	C	29	6	96	49	29	23	4	14	7	5	0	0	0	0
Ambassidae	*Ambassis ambassis*	Non	0	0	70	25	14	26	0	0	0	1	0	0	0	0
	Ambassis gymnocephalus	MC	0	0	0	0	8	0	0	0	0	0	0	0	0	0
	Ambassis natalensis	C	0	2	107	13	28	53	0	0	6	0	0	0	1	0
Antennariidae	Frog fish (unidentified)	*	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Apogonidae	*Yarica hyalosoma*	Non	178	45	99	1	7	0	27	40	16	22	9	4	9	21
Atherinidae	*Atherinomorus lacunosus*	C	2	0	0	0	0	0	20	44	0	11	3	6	0	0
Belonidae	*Tylosurus acus melanotus*	C	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Tylosurus crocodilus	C	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Carangidae	*Carangoides ferdau*	C	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	Caranx ignobilis	C	0	0	0	0	0	0	1	0	0	1	0	1	0	0
Chanidae	*Chanos chanos*	HC	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Clupeidae	*Sardinella gibbosa*	HC	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Engraulidae	*Spratelloides gracilis*	MC	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Stolephorus commersonnii	C	0	0	0	0	1	0	0	0	0	0	0	1	0	0
	Thrysa setirostris	MC	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Gerreidae	*Platax orbicularis*	MC	0	0	0	0	0	0	1	0	0	0	0	0	1	0
	Platax pinnatus	MC	0	0	0	1	0	0	1	0	0	0	0	0	0	1
	Gerres longirostris	C	1	0	0	0	0	2	0	0	0	0	0	0	1	1
	Gerres filamentosus	MC	20	25	12	1	10	0	1	0	1	0	4	1	13	0
	Gerres oyena	C	28	0	1	4	4	1	17	0	0	15	1	8	22	7
Gobiidae	*Acentrogobius nebulosus*	*	0	0	1	0	1	0	0	0	1	0	0	0	0	0
	Goby	AQ	0	1	0	0	3	0	0	1	1	0	0	0	0	0
	Periopthalamus spp.	*	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Gynglymostomatidae	Blue spotted goby	*	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Haemulidae	*Plectorhinchus plagiodesmus*	C	0	1	1	0	0	0	0	1	0	0	0	0	0	0
	Pomadasys argenteus	C	0	0	1	1	0	1	2	0	0	3	0	0	0	0
	Pomadasys multimaculatus	C	0	1	0	1	1	3	0	3	0	2	0	2	0	0
	Pomodasys kaakan	C	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Family	Species	Importance	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hemiramphidae	*Hyporhamphus gamberur*	Non	1	0	2	1	0	0	1	0	0	0	0	0	0	0
	Hyporhamphus affinis	Non	13	0	10	6	2	0	2	4	0	3	1	0	0	0
	Zenarchopterus dispar	C	1	0	2	5	0	0	0	0	0	0	1	0	0	0
Leiognathidae	*Gazza minuta*	C	0	0	0	7	0	1	0	0	40	0	0	0	4	0
	Leiognathus equulus	MC	2	0	0	1	0	3	0	0	125	3	0	2	0	0
Lethrinidae	*Lethrinus harak*	C	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Lutjanidae	*Lutjanus argentimaculatus*	C	1	2	0	1	3	0	0	0	0	3	0	0	0	0
Lutjanidae	*Lutjanus bohar*	C	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Lutjanus fulviflamma	C	3	0	1	0	0	0	1	0	0	4	2	1	0	0
	Lutjanus fulvus	C	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Monodactylidae	*Monodactylus argenteus*	MC	7	0	0	0	4	4	7	0	0	0	0	0	15	0
Mugilidae	*Crenimugil crenilabis*	C	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Moolgarda seheli	C	0	0	15	4	3	0	0	0	1	0	0	0	0	0
	Mugil cephalus	HC	0	10	0	0	5	0	0	0	0	0	0	0	0	0
Percophidae	*Bembrops platyrhynchus*	Non	1	0	0	0	0	0	1	0	0	0	0	0	0	0
	Bembrops caudimacula	*	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Platycephalidae	*Platycephalus indicus*	C	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pomacentridae	*Dascyllus* spp.	*	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Scorpaenidae	*Pterois volitans*	C	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Serranidae	*Epinephelus coeruleopunctatus*	MC	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Epinephelus coioides	C	0	1	0	2	1	0	1	0	0	0	0	0	0	0
	Epinephelus lanceolatus	C	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Epinephelus malabaricus	HC	0	1	0	2	2	0	0	0	0	1	0	0	1	0
	Epinephelus spilotoceps	C	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Sillaginidae	*Sillago sihama*	C	0	0	0	5	0	7	0	0	13	2	0	9	0	0
Sphyraenidae	*Sphyraena barracuda*	MC	2	0	1	0	0	0	0	0	0	1	1	1	1	0
	Sphyraena jello	C	2	2	2	0	0	0	3	0	1	3	1	0	1	0
	Sphyraena putnamae	C	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Terapontidae	*Terapon jarbua*	MC	0	0	5	0	0	0	0	0	3	0	0	1	0	0
Tetraodontidae	*Arothron immaculatus*	MC	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Total individuals (N)			293	100	427	135	128	125	102	108	217	81	23	37	69	34
Total species (S)			18	15	18	22	20	12	24	8	13	17	9	12	11	7

Table 2. Summary of crustacean community structure at the 14 sampling sites, including crustacean abundance (catch per net), respective families, number of species and the total abundance and species per sites. The importance of species is abbreviated: NAI=No available information, C=commercial, HC=highly commercial, NCI= no commercial importance.

Family/ infaorder	Species	Percentage (%) of total N	Importance	Sampling sites													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
Brachyura	Crab 1	0.2	NAI	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Brachyura	Crab 2	0.2	NAI	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Brachyura	Crab 3	0.2	NAI	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Caridea	Caridean shrimp	1.5	C	1	0	0	2	6	0	0	1	1	2	1	3	0	0
Matutidae	*Ashtoret lunaris*	0.2		1	0	0	0	0	1	0	0	0	0	0	0	0	0
Ocypodidae	*Uca* spp.	0.2	NIC	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Palaemonidae	*Macrobrachium rude*	2.7	HC	1	0	0	16	5	0	0	3	0	3	0	3	0	0
	Metapenaeus stebbingi	0.2	HC	1	0	0	0	0	0	0	0	0	1	0	0	0	0
	Metapeneaus monoceros	1.7	HC	1	0	0	1	0	1	0	5	0	5	0	7	0	0
	Penaeus indicus	36.2	HC	1	42	19	48	77	82	28	24	13	42	2	18	19	5
Penaeidae	*Penaeus monodon*	5.9	HC	1	1	1	2	4	6	4	5	0	12	5	7	8	12
	Penaeus semisulcatus	46.5	HC	1	11	4	4	4	15	13	22	15	177	10	75	13	176
Portunidae	*Scylla serrata*	1.7	HC	1	4	0	2	1	4	0	1	1	1	0	0	3	2
Portunidae	*Thalamita crenata*	2.5	HC	1	3	1	1	1	3	0	3	1	2	2	1	7	3
	Shrimp 1 (unidentified)	0.2	NAI	1	0	0	0	0	0	0	0	0	0	0	0	1	0
	Shrimp 2 (unidentified)	0.2	NAI	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Total individuals N				16	61	26	77	98	112	45	64	33	245	21	114	51	198
Total species				16	5	5	9	7	7	3	8	7	9	6	7	6	5

Table 3. Margalef's species richness (D), Pielou's evenness index (J') and Shannon diversity index (H') (±sd) for fish species in the different sampling months/seasons.

Sample	Sep-15ᵃ	Dec-15ᵇ	Mar-16ᵇ	Jun-16ᵃ	Sep-16ᵃ	Dec-16ᵇ	Jun-17ᵃ	Sep-17ᵃ
No. of species	26	15	15	27	18	15	17	17
No. of individuals	578	48	139	385	183	112	202	230
Margalef's species richness (D)	3.9±0.5	3.6±0.7	2.8±0.6	4.4±0.8	3.3±0.7	3.0±0.5	3.01±0.6	2.9±0.7
Pielou's evenness index (J')	0.6±0.2	0.7±0.1	0.6±0.2	0.7±0.2	0.7±0.2	0.7±0.2	0.6±0.2	0.7±0.2
Shannon Weiner index (H')	1.9±0.5	2.0±0.5	1.7±0.5	2±0.6	1.9±0.5	1.8±0.4	1.6±0.5	2.0±0.5

ᵃ=SEM, ᵇ=NEM seasons

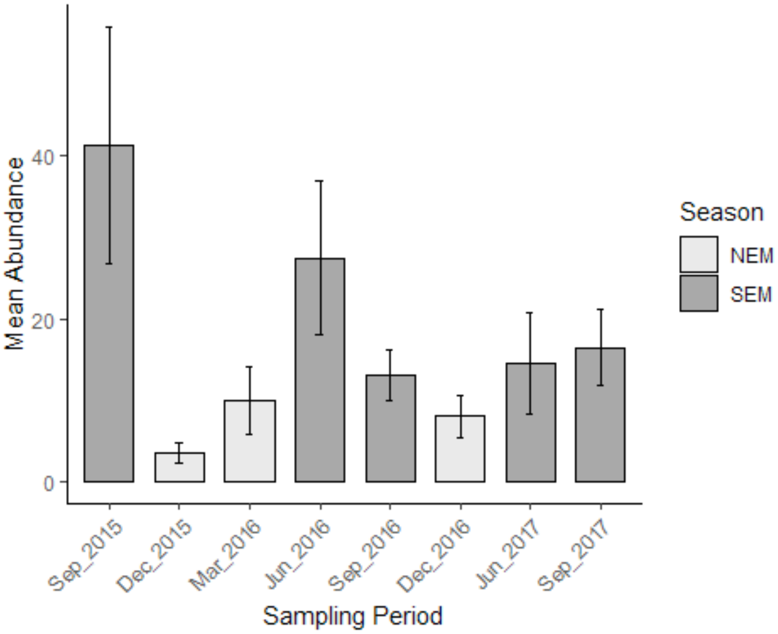

Figure 2. Mean abundance (± 95 % CI) of individuals caught at each of the sampling dates. Data are means for all 14 nets at each sampling date.

showed higher diversity compared to December and March (NEM) (Fig. 3). The most abundant species in SEM were *Y. hyalosoma*, *A. ambassis*, *A. natalensis* and *L. equulus*. In NEM the most abundant species were *A. japonicum*, *Y. hyalosoma*, *L. equulus* and *S. sihama.* The family Ambassidae, that comprised of *A. ambassis, A. natalensis* and *A. gymnocephalus,* showed strong seasonality as it only occurred in SEM. *Acropoma japonicum* had a higher mean abundance in NEM than in SEM i.e. 40 and 28 individuals respectively. Other species that showed marked seasonality included *G. oyena* and *A. lacunosus*. The highest species diversity H' was in June 2016 (H'=2.15±0.6) and the lowest was in June 2017 (H'=1.64±0.5). The species evenness index (J) was more stable, varying between 0.58±0.2 (June 2017) and 0.72±0.2 (Dec 2015) (Table 3). ANOVA revealed no significant differences in Margalef (D) and Pielou's evenness (J') indices of fishes for the 8 sampling dates, p=0.111 and p=0.170 respectively. However, there was a significant difference in Shannon's diversity index (H') p= 0.036. A non-metric multi-dimensional (nMDS) ordination plot of square root transformed abundance data of all fish species in the sites during NEM and SEM seasons showed separation between SEM and NEM seasons (Fig. 4). ANOSIM revealed significant differences of fish assemblages between NEM and SEM

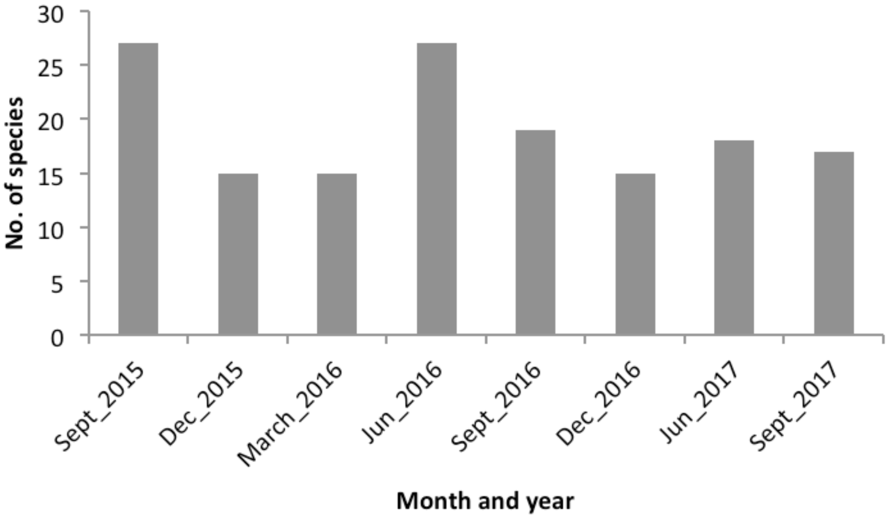

Figure 3. Total number of fish species caught in each of the 8 sampling periods.

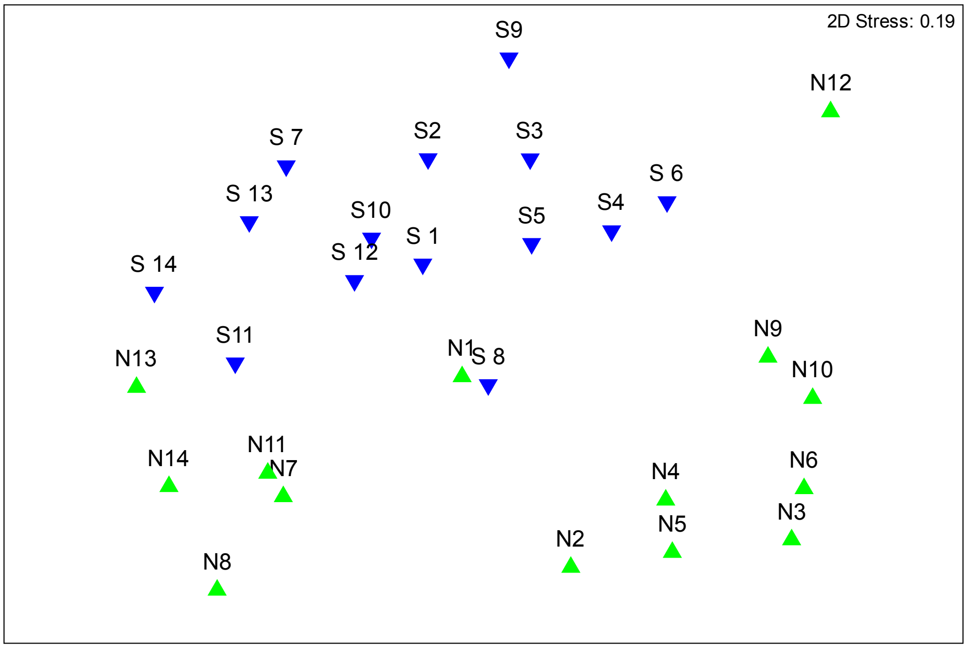

Figure 4. Nonmetric multi-dimensional scaling (nMDS) of seasonal fish abundance (pooled number of individuals per season) based on Bray–Curtis similarity using square root transformed data of crustaceans in all sampling locations for both NEM and SEM seasons. Blue triangles and letter S stand for SEM and green triangles and letter N for NEM. Numbers refer to separate sampling locations.

seasons on square root transformed abundance data, with R=0.263 and p=0.004. SIMPER analysis showed an average dissimilarity of 82.62 % between the NEM and SEM seasons and the species *A. hyalosoma, A. japonicum, G. oyena, A. natalensis, G. filamentosus* and *A. lacunosus* contributing to over 40 % of this dissimilarity.

Seasonal variations in crustacean assemblages (SEM – NEM)

Penaeus indicus did not show any marked seasonality while *T. crenata* and *M. rude* showed tendencies to seasonality as they mostly occurred in SEM (Fig. 5). Generally, the highest crustacean abundance was recorded in the NEM as opposed to fish where the highest abundances were in the SEM. The highest species diversity H' was found in March 2016 (H'=0.9±0.3) and the lowest in December 2015 (H'=0.5±0.3). The species evenness index (J) varied between 0.6±0.3 (December 2015) and 0.9±0.1 (September 2016) (Table 4). ANOVA revealed no significant differences in Margalef's species richness (D), Pielou's evenness index (J') and Shannon diversity index (H') in crustaceans in the 8 sampling seasons with p=0.634; p=0.149 and p=0.419 for D, J' and H' respectively. A Kruskal Wallis

Table 4. Margalef's species richness (D), Pielou's evenness index (J') and Shannon diversity index (H') (±sd) for crustacean species in the different sampling months/seasons.

Sample	Sep-15^{a}	Dec-15^{b}	Mar-16^{b}	Jun-16^{a}	Sep-16^{a}	Dec-16^{b}	Jun-17^{a}	Sep-17^{a}
No. of species	6	5	7	11	7	9	7	54
No. of individuals	45	341	151	125	49	164	79	5
Margalef's species richness (D)	0.9±0.4	0.5±0.3	1.0±0.3	0.9±0.5	0.9±0.4	0.9±0.5	1.0±0.5	0.9±0.4
Pielou's evenness index (J')	0.8±0.2	0.6±0.3	0.8±0.2	0.7±0.2	0.9±0.1	0.6±0.7	0.8±0.2	0.8±0.2
Shannon Weiner index (H')	0.8±0.2	0.5±0.3	0.9±0.3	0.8±0.3	0.7±0.2	0.7±0.4	0.7±0.4	0.7±0.2

^{a}=SEM, ^{b}=NEM seasons

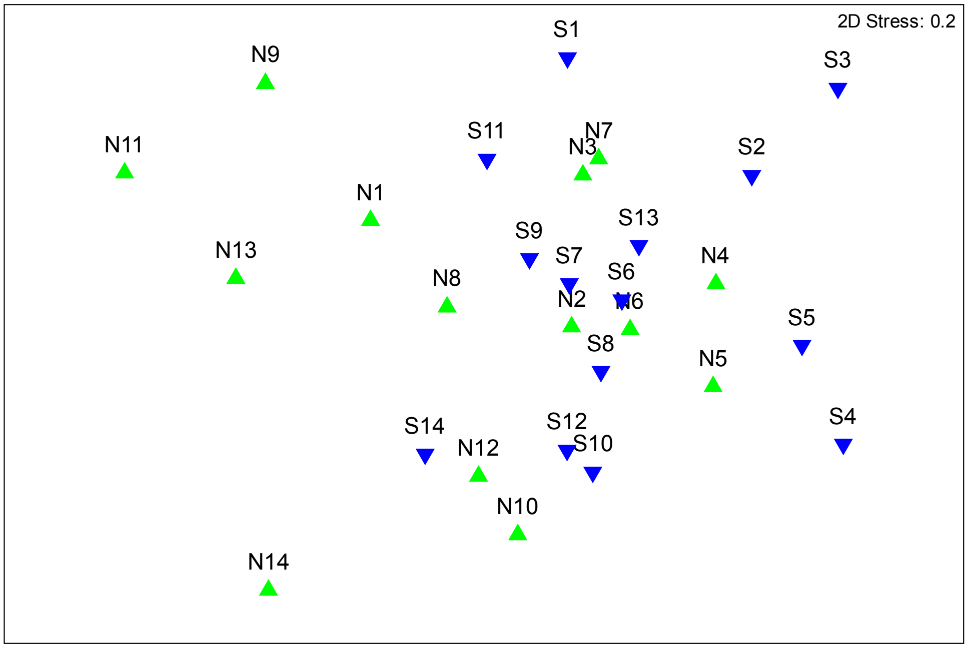

Figure 5. Nonmetric multi-dimensional scaling (nMDS) of seasonal fish abundance (pooled number of individuals per season) based on Bray–Curtis similarity using square root transformed data of fish in all sampling locations for both NEM and SEM seasons. Blue triangles and letter S stand for SEM and green triangles and letter N for NEM. Numbers refer to separate sampling locations.

test was used to test for differences in mean crustacean abundance between NEM and SEM season and no significant differences were detected (p =0.843). The pattern of crustacean seasonality was visualised in an nMDS ordination plot (Fig. 5)., The nMDS plots (Fig. 5) revealed differences in the crustacean assemblages between the seasons. One-way ANOSIM showed significant differences in crustacean assemblages on square root transformed abundance data between NEM and SEM seasons (R=0.158, p=0.003). SIMPER analysis revealed an average dissimilarity of 52.9 % in the assemblages between the two seasons, with *P. semisulcatus* (26.16 %), P. *indicus* (22.92%), *P. monodon* (11.32%) and *T. crenata* (9.97 %) contributing to 70.37 % of the difference between the seasons.

Size classes of the most abundant fishes

Circa 60 % of all individuals caught were classified as small juveniles while 37 % appeared as large juveniles/sub adults. A total of 32 species appeared as small juveniles only while 8 species appeared as 100 % large juveniles/sub adults. Of the 6ix most abundant species, *L. equulus* and *A. natalensis* appeared as 100 % small juveniles and 100 % large juveniles/sub adults, respectively. Ninety seven per cent (97 %) of *G. oyena* were small juveniles (Table 5).

Size classes for 3 of the most abundant species *A. japonicum, Y. hyalosoma,* and *G. oyena,* as shown in Figure 6 – 8, were used to conduct cohort analyses to investigate evidence for spawning periods and growth rates. For *A. japonicum* there were significant differences between size frequency distributions (X^2=9.98, df=1, p=0.041).

A fairly stable mode was demonstrated by *Y. hyalosoma* over most sampling months, although the large dispersion was reflected in the significant difference among frequency distributions (X^2=52.85, df=4, p<0.001) (Fig. 7). In contrast with *A. japonicum* the largest individuals were found in December with the smallest in June and September, suggesting a later spawning date during the NEM. For *G. oyena*, the greatest variation was found between size frequency distributions (X^2=19.288, df=1 and p<0.001) with most smaller fish found in September and most larger ones in June, suggesting a late NEM or early SEM spawning time.

Discussion

Fish communities in intertidal areas have been studied using varying sampling techniques and gears. Here, an approach intermediate to sampling large areas adjacent to the forest (such as seine netting in bays) and sampling very small, possibly unrepresentative plots (such as stake netting quadrats) was taken; in order to capture forest variability, fishes were sampled at multiple discrete sites within a forest canopy using repeat samples. Using passive gear (fyke nets) situated in small mangrove creeks, the present study documented 59 fish

Table 5. Maturity stages of fish species caught in Vanga from September 2015 to June 2017. The maturity stage was determined according to Nagelkerken and Velde (2002). Individuals with a third or less of the max length were classified as small juveniles, between one third and two thirds of maximum length as big juveniles to sub adults and above two thirds maximum length as adults. Max length, standard length, preferred habitats and environment were sourced from Froese and Pauly (2017). TL = total length, and SL = standard length.

Fish species	Min TL (cm)	Max TL (cm)	N	% Juveniles (Juv)	% Sub – adults (Sa)	% Adults (Ad)	Max length (cm)	Preferred habitat and environment
*Acentrogobius nebulosus**	6.1	9.7	3	0	100		18.0 SL	sandy shorelines, reefs
Acropoma japonicum	1.7	9.3	252	86.9	13.1	0.0	20.0 TL	sand, sandy mud bottoms
*Ambassis ambassis**	3.2	5.7	136	2.2	97.8		15.0 TL	no information
Ambassis gymnocephalus	5.2	5.9	5	20.0	80.0		16.0 TL	no information
Ambassis natalensis	4.6	7.5	178	0.0	100	0.0	9.0 SL	no information
Yarica hyalosoma	1.0	9.3	470	41.9	58.1	0.0	17.0 TL	mangrove estuaries, tidal creeks (Adults)
Arothron immaculatus	5.3	5.4	2	100			30.0 TL	weedy areas, estuaries, seagrass
Atherinomorus lacunosus	3.5	9.2	84	90.5	9.5	0.0	25.0 TL	sandy shorelines, reef margins
Bembrops caudimacula	8.0	9.0	3		100		24.1 TL	no information
Bembrops platyrhynchus	6.5	6.5	1	100	0.0	0.0	25.0 TL	inhabits offshore trawling grounds
Carangoides ferdau	6.1	6.1	1	100			70.0 TL	sandy beaches; near reefs
Caranx ignobilis	10.6	11.8	3	100			170 TL	Clear lagoons, seaward reefs (Adults)
*Chanos chanos**	14.7	16.5	2	100			180 SL	offshore marine waters, shallow coastal embayments
Crenimugil crenilabis			1	100			60.0 TL	sandy/muddy lagoons, reef flats
Epinephelus caeruleopunctatus	41.8	41.8	1	100	100		76.0 TL	coral-rich areas, deep lagoons,
Epinephelus coioides	15.0	27.8	5	100	0.0	0.0	120 TL	brackish water, mangroves (Juveniles)
Epinephelus lanceolatus	45.0	45.0	1	100			270 TL	caves, estuaries
Epinephelus malabaricus	12.5	35.0	7	100			234 TL	coral reefs, estuaries, mangroves
Epinephelus spilotoceps	22.3	22.3	1		100		35.0 TL	lagoon, reefs,
Gazza minuta	1.2	7.0	49	100	0.0	0.0	21 TL	young ones enter mangrove estuaries/ silty reef areas
Gerres filamentosus	4.7	15.2	88	65.9	34.1		35.0 TL	mangrove (Juveniles)

Fish species	**Min TL (cm)**	**Max TL (cm)**	**N**	**% Juveniles (Juv)**	**% Sub – adults (Sa)**	**% Adults (Ad)**	**Max length (cm)**	**Preferred habitat and environment**
Gerres longirostris	5.1	7.0	5	100	0.0	0.0	44.5 TL	adults, coastal waters (Adults), estuaries (Juveniles)
Gerres oyena	4.9	11.0	106	97.2	2.8	0.0	30.0 TL	saltwater lagoons, estuaries
*Hyporhamphus affinis**	5.0	13.4	40	90.0	10.0		38.0 SL	coral reefs
Hyporhamphus gamberur	12.0	13.7	3	33.3	66.7	0.0	37.0TL	common around reefs
Leiognathus equulus	2.3	6.4	136	100			28.0 TL	muddy inshore areas, mangroves
Lethrinus harak	6.3	6.3	1	100			50.0 TL	shallow sandy, mangroves, seagrass
Lutjanus argentimaculatus	11.4	19.6	10	100			150 TL	Mangrove (Juveniles and young adults)
Lutjanus bohar	15.5	15.5	1	100			90.0 TL	coral reefs
Lutjanus fulviflamma	6.5	15.5	36	100	0.0	0.0	35.0 TL	coral reefs (Adults), mangrove (Juveniles)
Lutjanus fulvus	5.8	5.8	1	100			40.0 TL	lagoons (Adults), mangroves (Juveniles)
*Monodactylus argenteus**	3.9	8.2	36	100			27.0 SL	bays, mangroves
Moolgarda seheli	6.7	10.8	23	100			60.0 TL	coastal waters, estuaries
*Mugil cephalus**	5.3	12.9	15	100			100.0 SL	coastal waters
Platax orbicularis	5.5	7.3	2	100			60.0 TL	sandy areas (Adults), mangroves (Juveniles)
Platax pinnatus	3.1	5.2	3	100			45.0 TL	reef slopes (Adults), mangroves (Juveniles)
Platycephalus indicus	9.8	9.8	1	100			100.0 TL	sandy and muddy bottoms
Plectorhinchus plagiodesmus	7.1	19.4	3	100			90.0 TL	coastal, coral crevices
Pomadasys argenteus	5.6	10.4	8	100			70.0 TL	coastal waters
Pomadasys kaakan	9.4	9.4	1	100	0.0	0.0	80 TL	inshore waters (sandy to muddy bottoms), estuaries
Pomadasys multimaculatus	4.9	15.6	13	100	0.0	0.0	76.0 TL	coastal waters, tidal estuaries
Pterois volitans	20.0	20.0	1		100		38.0 TL	lagoons, reefs, turbid inshore
*Sardinella gibbosa**	6.1	6.4	2		100		17.0 SL	no information

Fish species	**Min TL (cm)**	**Max TL (cm)**	**N**	**% Juveniles (Juv)**	**% Sub – adults (Sa)**	**% Adults (Ad)**	**Max length (cm)**	**Preferred habitat and environment**
*Sillago sihama **	9.0	14.7	33	87.9	12.1	0.0	31.0 SL	beaches, sandbars, mangrove
Sphyraena barracuda	11.3	28.7	7	100			200 TL	murky harbours (Adults), mangroves (Juveniles)
Sphyraena jello	4.0	21.4	15	100	0.0	0.0	150 TL	Reefs, bays, estuaries
Sphyraena putnamae	14.6	17.5	2	100			90.0 TL	lagoons, seaward reefs, bays, turbid lagoons
*Spratelloides gracilis**	3.5	3.5	1		100.0		10.5 SL	coastal, reefs
*Stolephorus commersonnii**	10.0	10.2	2	0.0	0.0	100	10.0 SL	coastal waters, brackish water
Terapon jarbua	4.0	11.3	9	100			36.0 TL	shallow sandy bottoms (Adults) sandy intertidal areas (Juveniles)
Thrysa setirostris*	7.5	7.8	2		100.0		18.0 SL	no information
Tylosurus acus melanotus	29.8	29.8	1	100			100.0 TL	offshore, coastal waters
Tylosurus crocodilus	35.8	35.8	1	100			150 TL	lagoons, seaward reefs
Zenarchopterus dispar	8.6	14.5	9		88.9	11.1	19.0 TL	shallow water, mangroves

*indicates standard and not total length was used

species in the Vanga mangrove system. This is higher than the species numbers recorded from nearby Gazi forest in studies using block nets (another passive gear), which found 30 (Huxham *et al.*, 2004) and 49 species (Crona and Rönnbäck, 2007). Studies in Kenyan mangroves that use active gears, such as seine nets and beam trawls, report higher numbers, for example 128 fish species were recorded in Gazi (Kimani *et al.*,1996) while at Tudor Creek, 83, 84 and 75 species were recorded by Little *et al.* (1988), Wainaina *et al.* (2013) and Wakwabi and Mees, (1999) respectively. Hence the choice of gear clearly has a large influence on the numbers and diversity of fish caught. Netting in bays and large permanent creeks produces bigger samples (and is logistically more straightforward) than placing passive gear under the canopy (Franco *et al.*, 2012; Tietze *et al.*, 2011). However such sampling is less able to discriminate between forest areas of different quality and may include fauna that are not using mangrove habitats at all.

It is generally true that fish communities, like almost all biological communities from inshore habitats, tend to have highly right skewed and steeply sloped rank abundance curves, with only a few species, typically 3 - 7, contributing over 70 % of the total abundance (Bell *et al.*, 1984; Giarrizzo and Krumme, 2007; Shervette *et al.*, 2007). Such a pattern was observed in the present study in Vanga, where 6 species contributed around 70 % of the total abundance. A similar pattern was found in the bay habitats of neighbouring Tanzania, where 9 species contributed 70 % of the total individuals (Lugendo *et al.*, 2007). In Thailand, Tongnunui *et al.* (2002) found that 20 fish species contributed 88.5 % of the total abundance in the Sikao creek mangrove estuary.

Studies of mangrove fish communities consistently show that juveniles dominate. Ninety nine per cent of the fish reported by Tongnunui *et al.* (2002) were not adults; 71 % were small juveniles and 28 % were large juveniles/sub adults. Out of the 54 encountered species in this study 34 occurred only as small juveniles, while 9 species occurred only as large juveniles. These results conform to research findings in other mangrove systems. In Tudor creek Kenya, 90 % of the

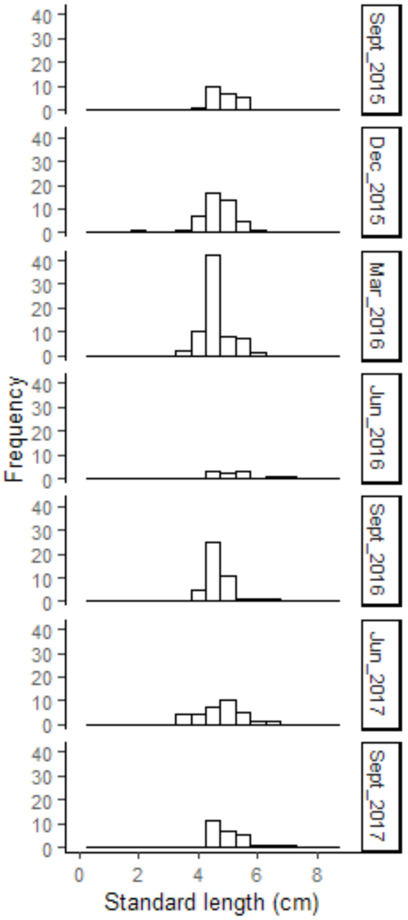

Figure 6. Size classes of *Acropoma japonicum* (max. length= 20 cm) over the study period (Individuals with a third or less of the max length were classified as small juveniles, between one third and two thirds of maximum length as big juveniles to sub adults and above two thirds maximum length as adults).

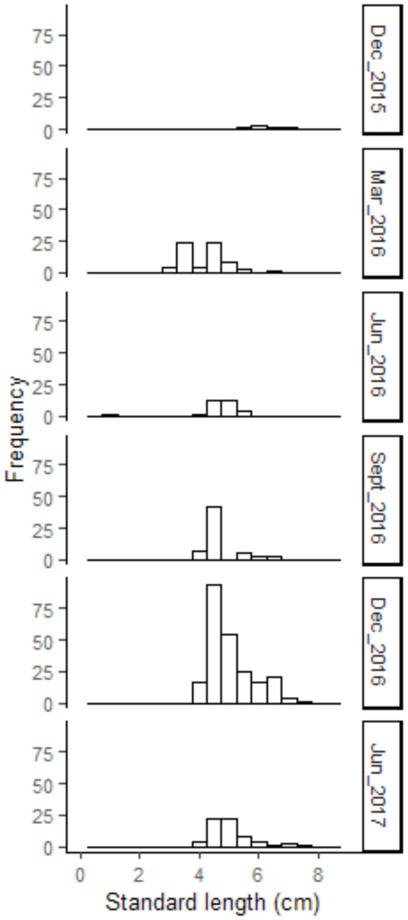

Figure 7. Size classes of *Yarica hyalosoma* (max. length=17 cm) over the study period (Individuals with a third or less of the max length were classified as small juveniles, between one third and two thirds of maximum length as big juveniles to sub adults and above two thirds maximum length as adults).

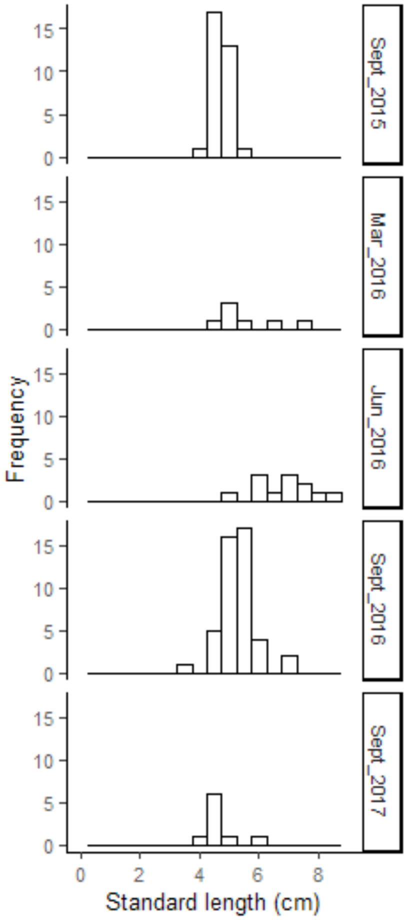

Figure 8. Size classes of *Gerres oyena* (max. length=30 cm) over the study period (Individuals with a third or less of the max length were classified as small juveniles, between one third and two thirds of maximum length as big juveniles to sub adults and above two thirds maximum length as adults).

fishes caught were juveniles (Little *et al.*, 1988) while in Gazi bay, 63 % of the species caught included juveniles with 29 % caught as juveniles only (Kimani *et al.*, 1996). Crona and Rönnbäck (2007) recorded juveniles in almost all fish taxa encountered in Gazi bay. Over 70 % of the fishes caught in bay habitats of Tanzania were juveniles (Lugendo *et al.*, 2007), whilst in Thailand 57 % of the species were caught as juveniles only (Ikejima *et al.*, 2003). In the current work, only 2 % of individuals caught were classified as adults. Hence the findings of this and other studies are consistent with the nursery hypothesis for mangroves (Beck *et al.*, 2001), although a rigorous test of this would require comparisons of fish densities with other habitats and tracing of fish migration and survival to adult habitats.

The most abundant species in this study – *Y. hyalosoma*, (previously known as *Apogon hyalosoma*), *A. japonicum*, *A. natalensis*, *A. ambassis*, *L. equulus* and *G. oyena* – are reported in most relevant studies in the WIO and also in the Mediterranean (Kimani *et al.*, 1996; Huxham *et al.*, 2004; Crona and Rönnbäck, 2007; Wainaina *et al.*, 2013; Lugendo *et al.*, 2007; Mwandya *et al.*, 2010; El-Regal and Ibrahim, 2014). They have been reported in other regions such as in the intertidal mangrove areas of Thailand where *Y. hyalosoma* was found to contribute up to 7 % of the total catch (Krumme *et al.*, 2015). In Gazi bay, *A. japonicum* was strongly associated with mangrove plantations (Crona and Rönnbäck, 2005). *Leiognathus equulus* is a widely distributed species in inshore areas of the Indo – Pacific region (Blaber and Milton, 1990). Though present in Tudor and Mida creeks in Kenya, it contributed <2% of the total catch in these mangrove systems (Wakwabi and Mees, 1999, Wainaina *et al.*, 2013; Gajdzik *et al.*, 2014). The current study recorded a higher percentage (7.4

%), close to what was recorded (5.4 %) in mangrove habitats of Tanzania (Lugendo *et al.*, 2007). In subtidal seagrass areas of Peninsular Malaysia, *L. equulus* accounted for circa 19 % of fishes sampled in the seagrass beds (Aziz *et al.*, 2005). High percentages (92 %) of *L. equulus* were also encountered in sub tidal areas compared to intertidal areas in Thailand (Ikejima *et al.*, 2003). It could therefore be concluded that this species is found in both intertidal and subtidal inshore areas but prefers the latter over the former. Fishes of the Ambassidae family have been found to contribute up to 10 % of the total catch on the north coast (Little *et al.*, 1988; Gajdzik *et al.*, 2014) but are scarcely reported on the south coast of Kenya (Kimani *et al.*,1996; Crona and Rönnbäck, 2005). In the current study at Vanga (south coast) *Ambassidae*, which contributed to 18.4 % of total fish abundance, were found in the NEM season only with over 80 % of the individuals being subadults. This concurs with the findings from Mida creek (Gajdzik *et al.*, 2014) and Tanzania (Mwandya *et al.*, 2009) where subadults and adults of this species dominated the catch (Mwandya *et al.*, 2010). In Tanzania Ambassidae were among the dominant families in mangrove areas (Lugendo *et al.*, 2007). *Gerres oyena*, the sixth most abundant species in this study (contributing 5.7 % of total abundance), is considered a mangrove resident and that could explain its abundance in these habitats. In Tanzania, *G. oyena* was the most abundant species in forested mangrove sites (Mwandya *et al.*, 2009) while Huxham *et al.* (2004) recorded the species as the most abundant in the small cleared sites in Gazi, Kenya. Further, *G. oyena* had the greatest abundance in both seaward and landward sites in the Gazi bay mangroves (Huxham *et al.*, 2008). The variation in the other dominant species caught in mangrove habitats along the coast could be due to site specific differences, seasonality or their nature of migrating into and out of mangrove and other habitats.

Serranidae was the most diverse family, with 5 species found, all from the genus *Epinephelus*. The Vanga reefs have 8 species of *Epinephelus,* which is lower than other Kenyan reefs such as Msambweni (14 species) and Shimoni (36 species) (Agembe *et al.*, 2010). *Epinephelus* sp. have suffered sharp declines at the Kenya coast over the last three decades (Kaunda-Arara, 1996; Kaunda-Arara *et al.*, 2003). The high commercial value and sedentary nature of the species in this genus have exposed them to threats leading to high mortality (Kaunda-Arara *et al.*, 2003; Sadovy, 2005). Dynamite fishing in reef areas of Vanga by fishermen from neighbouring Tanzania has also been suggested as the cause of these low numbers (Ochiewo, 2004; Samoilys and Kanyange, 2008). The low abundance (<1 % of total abundance) and relatively low diversity recorded in this study are consistent with other findings, including that of declines in most *Epinephelus* species at Vanga (Fortnam *et al.*, 2020) indicating that this diverse and commercially important genus could be threatened, creating concern for its future survival.

Size classes

The maximum length of *G. oyena*, a common fish species caught in Vanga mangroves, was 11 cm TL compared to large sized individuals (29.2 cm TL) of the same species caught in Gazi bay (Kimani *et al.*, 1996). However, *G. oyena* caught using passive gear inside Gazi mangrove forests were comparable in size to those caught in the current study i.e. 10.8cm TL for Gazi (Crona and Rönnbäck, 2007). From this comparison, it could be suggested that *G. oyena* juveniles are likely to be encountered inside the forests and adults in the creeks and nearshore waters. It could also be speculated that size classes are likely to differ between habitat types within similar ecosystems such as mangrove forests and mangrove creeks. It was not possible to compare the sizes of *Y. hyalosoma* and *A. japonicum* with other sites due to a lack of relevant literature; it seems that very little is known about the growth and possible ontogenetic shifts in these species.

The size frequency distributions compared over time for 3 species i.e. *G. oyena, Y. hyalosoma* and *A. japonicum* gave an indication of decreases in numbers as the standard length of the fish increased. It was difficult to conclusively point to particular times of spawning for these species due to a lack of supporting literature and insufficient detail in the current cohort analyses. For *A. japonicum* for instance, the significant differences between size frequency distributions, did not provide an unambiguous indication of likely spawning times and growth rates since the mode remained fairly constant; the smallest individuals were found during December and the largest in September, consistent with spawning in the early NEM season. Increases in size between sampling dates could indicate a single cohort that grows whilst in the mangroves and then leaves to be recruited into the coastal fishery later, however much more information would be needed to confirm such a pattern.

Seasonality

Environmental variables such as salinity and temperature are influenced by seasonality and in turn these may influence fish community structures by affecting feed availability, reproduction and ontogenic migrations (McClanahan, 1988). In Vanga, like the rest of the Kenyan coast, sea surface temperatures may range between 24 °C and 29 °C during the year with salinity levels ranging between 34.5 ‰ and 23.8 ‰. (McClanahan, 1988; Mwashote, 2003).

The overall mean catch per net (abundance) of juvenile fishes and the number of fish species in Vanga was significantly higher during the SEM (rainy) than NEM (dry) seasons. Fish community structures (abundance and diversity) in the WIO region have been found to vary with monsoon seasons (Lugendo *et al.*, 2005; Crona and Rönnbäck, 2007). While juvenile fishes occupying mangrove habitats have been found to be more abundant in SEM than in NEM, in the current and previous work (Crona and Rönnbäck, 2007), densities of planktonic fish larval assemblages in marine parks in Kenya were higher in NEM when waters were calm compared to the rougher SEM season (Mwaluma *et al.*, 2011). McClanahan (1988) also recorded fish spawning in the East African region to peak during the NEM season (McClanahan, 1988). It is possible that fishes spawned offshore in the NEM season move into the mangrove nursery habitats during the rougher SEM season. Fishes in the Ambassidae family, which constituted a large proportion of the catch in the current study, were only found during the NEM season. Most individuals caught were relatively large sub-adults, and hence may not be showing this pattern since they were not using the Vanga mangroves as habitat during the small juvenile stages. Further studies into the life cycles of these species may help shed more light on their ecology.

Crustacean community structure

Penaeid shrimps comprised over 80% of the crustaceans caught with *P. semisulcatus* and *P. indicus* dominating the catch. These two species have previously been recorded as the most abundant penaeid shrimp in Malindi – Ungwana bay in Kenya (Munga *et al.*, 2013). Similarly, in the same area, *P. indicus* was among the dominant penaeid species (Munga *et al.*, 2013; Ndoro *et al.*, 2014). Munga *et al.* (2013) found significantly higher prawn densities in SEM compared to NEM while Ndoro *et al.* (2014) found the biomass of penaeid prawns to be high in NEM with no seasonality in abundance. Generally, crustaceans did not show any clear seasonality in the present study. This contrasts strongly with the fish catches reported here and with commercial and artisanal catches of fish in Vanga waters. In Malindi-Ungwana bay, Kenya (a commercial prawn trawling area), gravid prawns of all penaeid species were recorded all year round with their abundance peaking in December, February and March (Mwatha, 2002). The apparent lack of seasonality for penaeid shrimp means that exploitation can continue throughout the year. There is a real risk of overfishing, especially for juveniles that use intertidal areas as nurseries. In order to protect the stocks which use mangroves as nurseries from overfishing, Mwatha (2002) suggested that the fishery should be closed in February, December and March, the peak months for gravid females. Uncontrolled fishing especially of juveniles is likely to lead to low returns. This work supports the idea of mangrove and near mangrove habitat as nursery habitat for both fish and crustaceans so emphasising the need for conservation particularly of seaward edges. Future studies should focus on size classes of penaied shrimp species in nearshore habitats in both the NEM and SEM seasons and this could guide management options such as closed seasons.

Conclusion

Most of the fish families and species encountered in Vanga are common on the East African coast. The dominance of a few species is commensurate with most findings from tropical mangrove ecosystems where a few species occur in high densities. *Gerres oyena* has been reported as a common species in several other mangrove sites on the Kenya coast; it is strongly associated with and possibly dependent upon mangroves. Close to 50 % of the species caught at mangrove sites in Vanga are exploited for commercial fisheries while 32 of the total 59 species were reef associated. Most of these species however occurred in very low numbers. This could mean that they prefer other nursery habitats such as seagrass. If that is not the case, then it can be speculated that their recruitment into the fishery could also be low.

Almost all the individuals encountered in this study were juveniles. This is expected in mangrove habitats. Thus, this study further affirmed the hypothesis that mangroves provide habitats for juvenile fauna, which later migrate offshore.

The strong seasonality in fish catches, with much greater abundance and diversity being found during the SEM season, supports the hypothesis that many species are spawning offshore during the NEM season and then moving into mangroves as juveniles, before migrating offshore again as sub-adults. In contrast there was no clear seasonality for penaeid shrimp suggesting they show a different lifecycle with more rapid reproduction and more consistent affinity with inshore habitats.

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Modelling spillover effects of a marine protected area in the Western Indian Ocean

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Abstract

This study estimated the abundance gradient of fishes in the waters surrounding a marine protected area (MPA) and used this information as evidence of spillover from the MPA. Fish landing data were collected from trap fishermen over a 12-month period from the Blue Bay Marine Park on the west coast of Mauritius in the Western Indian Ocean. Two indicators of abundance were used: catch per trap, and number of fish per trap. A Generalised Linear Model was used to standardise the catch data by removing the effects of individual fishermen's productivity from the abundance measurements while the negative-binomial distribution was used for the number of fish. The study found a slight declining gradient of catch beyond the MPA up to a distance of 4 km. The study also established that the individuals belonging to key species caught near the MPA were larger than those of the same species caught more than 4 km away. The negative gradient reinforces the evidence of spillover occurring from the MPA.

Keywords: marine reserve, spillovers, gradient assessment, standardisation, Indian Ocean

Introduction

In the context of this paper a marine protected area (MPA), or marine reserve, is a portion of ocean where fishing and other human activities are prohibited (Hannesson, 1998; Crowder *et al*., 2000; Sladek-Nowlis and Roberts, 1999; Lorenzo *et al*., 2016). When an overfished area is closed to harvesting and exploitation, its ecosystem and its resident fish populations recover, leading to so-called "reserve effects" in terms of an increase in biomass, fecundity as well as the proportions of older and larger fish (Bohnsack, 1996; Sladek-Nowlis and Roberts, 1999; Hallwood, 2005; Horta e Costa *et al*. 2013). Over time, the undisturbed area, if it is large enough, returns to a naturally bio-diverse equilibrium (Sladek-Nowlis and Roberts, 1999) and depending on density-dependent mechanisms, the carrying capacity of the protected and adjacent areas, and connectivity of suitable habitats, this translates into an export of post-settlers to the adjacent areas, commonly referred to as "spillover effects" (Chapman and Kramer, 1999; Gell and Roberts, 2003; Forcada *et al*., 2009; Bellier *et al*., 2013, Lorenzo *et al*., 2016). Evidence that MPAs can lead to spillover effects provides opportunities for them to be used as fisheries management tools to sustain fishers in the adjacent areas.

This paper investigates the evidence of spillover effects of a small MPA, the Blue Bay Marine Park (BBMP), located in a heavily fished area in the southeast of Mauritius. As an attempt to conserve the marine ecosystem as a main tourist asset and to reduce fishing pressure in key sites, Mauritius began to establish MPAs around its coasts in 1983. Two marine parks and six fishing reserves have been established, while the process of establishing new protected areas and expanding existing ones is ongoing, especially as part of the marine spatial planning process (Smith, 2017).

The BBMP was declared an MPA and designated a Marine Park in June 2000 under the Fisheries and Marine Resources Act 1998 (Convention on Biological Diversity [CBD], 2013). The total area of the Marine Park is currently 353 hectares. Since the last inventory of the park carried out in 2012, a marked improvement in the fish population (biodiversity and density) has been noted (CBD, 2013). Since the proclamation

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of Blue Bay as an MPA, no fishing activities have been allowed in the conservation area (Fig. 1) while pole and line fishing for leisure is allowed from the shoreline only. No commercial fishing activities are presently being carried out in the park. Conand *et al.* (2016) concluded that biodiversity inventories show some improvements over time. Improvement of the habitats in the BBMP, despite its relatively small size, explains the rising diversity of holothurians (sea cucumbers). Without a comprehensive assessment of the reserve effect, these findings are assumed to be as a result of this phenomenon and are used as the basis to examine the spillover effects.

Fishery scientists have employed various tools to analyse the spillover effects of MPAs (Russ *et al.,* 2004). One of these is to compare variables such as fish density, biomass, size of organisms, and species diversity before and after the establishment of MPAs (Halpern, 2003). However, in many cases, these biological data are not available, and such before-and-after analyses cannot be made (Chapman and Kramer, 1999). A common alternative is therefore to assess the differences in fish population density (and other variables of interest) between sites in a reserve, and sites which have the same ecological features but are located in adjacent areas outside of the MPA. If emigration determines the distribution of fishes, fish density should be higher in the centre of the reserve and decrease gradually toward and beyond the boundaries (Rakitin and Kramer, 1996; Abesamis *et al.,* 2006). Spillover is typically observed through patterns of abundance or catch that decline with distance from reserve boundaries (Halpern *et al.*, 2009).

This, and other gradients of biological features, can be obtained by visual census and tagging of fish inside and outside the MPA (Chapman and Kramer, 1999; Abesamis *et al.,* 2006). However, this method may be costly and time consuming. Moreover, according to Chapman and Kramer (1999), the quantification of the spillover effects should take into account both the spatial and temporal variation in fish distribution. Such gradients can more feasibly be estimated using the catches made by fishers in adjacent areas (Vandeperre *et al.*, 2011). Whilst catch per unit of effort (CPUE) is a poor indicator of abundance for some species, for others it is taken as evidence of spillover when CPUE higher nearer the MPA (Chapman and Kramer, 1999; Murawski *et al.*, 2005; Goñi *et al.*, 2006; Stelzenmuller *et al.*, 2007; Forcada *et al.*, 2009; Bellier *et al.*, 2013). Such fish landing data are commonly used to measure fish abundance (Beverton and Holt, 1957; Kimura, 1981; Harley *et al.*, 2001; Pascoe and Herrero, 2004; Bordalo-Machado, 2006; Stobart *et al.*, 2009), and their use to test for a decreasing abundance gradient with distance from the MPA is commonly justified on both technical and practical grounds. Such fishery-dependent data not only offers greater coverage in space and time but are economically cheaper to collect (Ye and Dennis, 2009).

Translating data on catch rates into an abundance gradient can be an issue in that the coefficient of catchability is stable. The latter is the parameter which relates catch rates as an index of relative abundance to the stock of fish (Squires and Vestergaard, 2015). It is well established that this varies across species. However, even within a species it may not be stable. Only if catchability is constant does catch data reflect abundance. Noting the range of factors that can affect catchability, fishery scientists have adopted a statistical approach to 'standardisation' – the process through which these factors influencing catchability are 'controlled' so that the catch rate data is a truer reflection of abundance. Such standardisation typically uses the Generalised Linear Models (GLMs) or Generalised Additive Models (GAMs).

The main objective of this paper is to provide evidence on the spillover effects of the BBMP by using fish landing data which was collected from a sample of trap fishermen over a 12-month period on the east coast of Mauritius in the Indian Ocean. Two indicators were used from the data: (i) catch per trap (ii) and number of fish per trap. The data was collected through post-trip inquiries with the assistance of professional fish landing officers, and fishermen were required to indicate the location they fished for that trip on a map. Consequently, the statistical analysis had to consider the many factors which may influence catch, including fishers' characteristics, seasonality and habitat characteristics. This is captured through applying the GLM to standardise the catch by assuming a particular distribution for the indicators. Once the extent of a declining gradient was obtained, the study sought to identify the main associated fish species which could be driving the results. It was assumed that the results may be influenced by habitat, and given the limited information on this aspect, the study collected data on the depth of adjacent waters and some characteristics of the main fish species which could be the drivers of the spillover effects. The mean size of the main identified fish species in and the adjacent to the Marine

Park was also determined. No studies on potential spillover from the Marine Park had been undertaken previously, and it is anticipated that this study using spatially-collected catch data will open avenues for further research to further confirm the fisheries benefits of MPAs.

Materials and methods

Study site and data

Mauritius is located in the Indian Ocean approximately 800 km east of Madagascar. The BBMP is located on the southeast coast of Mauritius and was proclaimed a National Park in October 1997 (Fig. 1) and declared a Marine Protected Area and designated a Marine Park in June 2000 under the Fisheries and Marine Resources Act 1998 (Convention on Biological Diversity, 2016). The total area of the Marine Park is currently 353 hectares. The water depth in the park varies from 1 to 150 m (Albion Fisheries Research Centre [AFRC], 2008). Fishing activities with pole and line and basket traps are allowed in the multiple use zone lagoon). According to the CBD (2013), the level of human-induced disturbance or degradation is low, and the area harbours high coral biodiversity. Surveys carried out have revealed the presence of 72 fish species representing 41 genera and 31 families (CBD, 2013). Commercial species and many reef fish, including those that display schooling behaviour, are present in the park. The main fish families found in surveys in the Park include Acanthuridae, Labridae, Scaridae and Serranidae (AFRC, 2008).

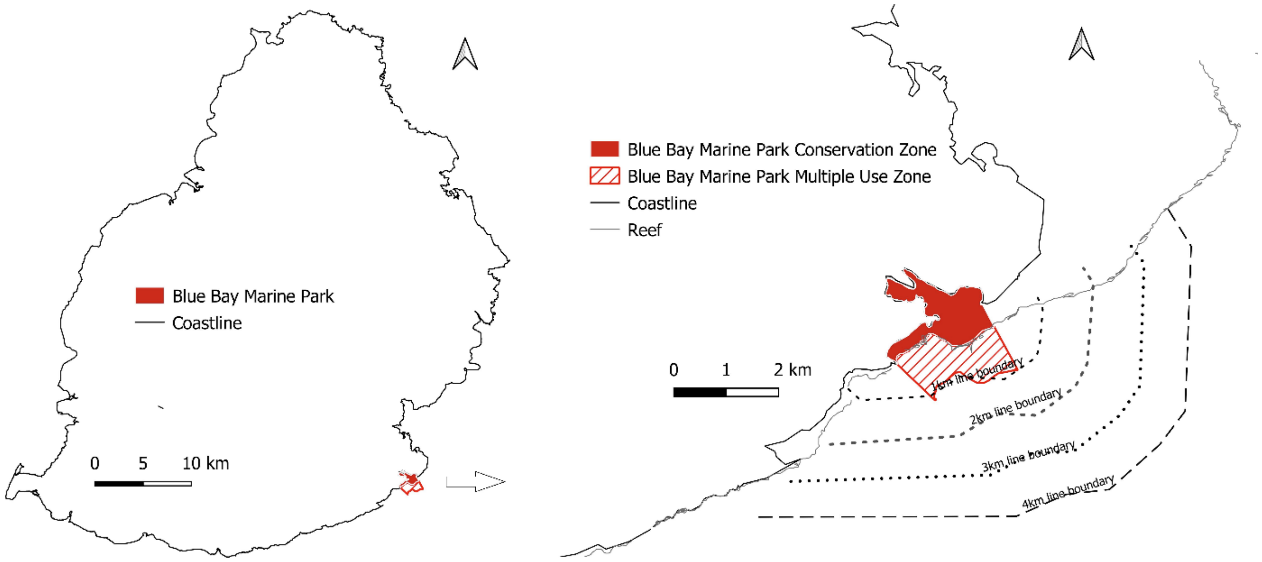

Figure 1. The study area: Blue Bay Marine Park, located on the southeast coast of Mauritius.

There are approximately 350 trap fishers in the area covered by this study. No official list of fishermen was available, however, with the help from fish landing officers, a list of regular fishermen was prepared. This was supplemented by an on-site survey of regular fishermen over approximately one month (December 2014). A total of 179 regular full-time fishermen were noted, from whom 100 were randomly selected. The study attempted to record the fishing locations of this sub-sample of fishermen, as well as details of fish catch for 10 trips spread evenly over the year. The random selection was limited in that it was observed during the interviews that around 15 % of the fishermen were either unable or reluctant to provide the information needed. They were eventually replaced. The survey was conducted from January 2015 to December 2015 and 10 trips were recorded for each fisherman, creating a panel of 100 by 10 observations. For each trip the interviewer recorded the 'total catch of the fisher for the trip in kg'. To ensure that the data was collected properly, assistance was sought from experienced fish landing officers who were fully acquainted with the study sites and were known to the fishermen. The questionnaire was used to record the number of fish of each fish species as well as the weight of the fish from each trip. Fishers were given a map as shown in Figure 1 on which the reef and the waters surrounding the reserve were shown. The map was divided into grid blocks which were numbered and positioned spatially on the map using the QGIS software. Fishers indicated on the map where their traps had been located and the route taken to and from those traps. Data collected also included the characteristics of fishermen and of their fishing technology, including the trap sizes and the numbers of traps used.

Conceptual framework and estimation methods

The conceptual framework assumes that a fisherman's catch is proportional to the abundance of fish. While this is a common assumption, some basic information is provided as shown by equation (1) (Maunder and Punt, 2004):

$$C_{ji} = q_{ji} E_{ji} X_i \tag{1}$$

Where C_{ji} = catch for fisher j in area i; q_{ji} = catchability coefficient for fisher j in area i; E_{ji} = effort; and X_i = population density in area i. It follows that catch per unit of effort (*CPUE*) is:

$$CPUE_{ji} = \frac{C_{ji}}{E_{ji}} = q_{ji} X_i \tag{2}$$

Changes in $CPUE_{ji}$ can therefore be due to either changes in the stock density, X_i, or changes in the catchability coefficient (q_{ji}). *Ceteris Paribus* (i.e. with q constant) spatial changes in *CPUE* may reflect other factors, such as habitat differences, rather than overall physical abundance.

In order to estimate stock abundance, statisticians standardise the *CPUE* by adding additional structure through the catchability coefficient (Maunder, 2001). The variables forming the additional structure can be continuous (e.g. sea-surface temperature, price of fish, vessel size). Once the additional structures for modelling the catchability coefficient have been incorporated, the remaining variation in *CPUE* is linked to distance from the MPA to analyse the declining gradient hypothesis. Catch per trip as well as catch per trap were both used as a measure of abundance. This conceptualisation is similar to that used by Goni *et al.* (2006) and Stelzenmuller *et al.* (2007). Following Halpern *et al.* (2009) an exponential decay relationship is given by:

$$X_i = \exp\left(-\beta_{DIS}\, DIS_{im}\right) \tag{3}$$

Where DIS_{im} is the distance from location i to the location of the marine reserve m.

Since the focus was on the artisanal fishers using traps, the number of basket traps (*NBAS*) , and the size of basket trap (*SBAS*) were used as additional structures. Seasonal effect on catches may be significant and, hence, quarterly effects were included through a categorical variable representing the four quarters of the year.

Habitat differences could lead to differences in abundance and therefore play a key role in the findings. There is currently a lack of information on the geographical characteristics of the habitats. The only accurate indicator is the depth of the water which was included as a continuous variable to capture potential habitat differences. In order to probe this issue further, the analysis was supplemented by examining the characteristics of the fish species and their associated habitats.

The predictive response indicator is specified as follows:

$$\eta = q' + quarter + NBAS + SBAS + DEPTH + (quarter \times NBAS) + (quarter \times SBAS) + (quarter \times DEPTH) + (NBAS \times SBAS) + (NBAS \times DEPTH) + (SBAS \times DEPTH) + DIS_m + DIS_m^2 + error \tag{4}$$

The square of the distance from the MPA is added to estimate the strength of the relationship. In particular, if there is an L-shape, the term will be redundant while a U-shape will provide a cut-off point.

To further provide insights on the spillover effects, the total individual fish per trip and per trap were also used as the response indicators. Since these data are discrete and positively skewed the response variable was modelled using a negative binomial (Bellier *et al.*, 2013). The log-linear specification is commonly used in count data models to ensure that the conditional expectation is positive (Hausman *et al.*, 1994; Delgado and Kniesner, 1997).

Results

A summary definition of the covariates used in the analysis is provided in Table 1. Distance from the MPA was measured as a linear transect from the border of the MPA to the middle of the 1×1 km grid where the fishing had taken place during the trip.

The goodness of fit was evaluated using the model's scaled deviance and two other criteria; the Akaike Information Criterion (AIC) (Akaike, 1973), and the Schwarz Bayesian Information Criterion (BIC) (Schwarz, 1978). If the selected model fits the data reasonably well, the AIC and the BIC should be low (Su *et al.* 2008; Ye and Dennis 2009).

Table 1. Summary definition of variables.

Variable	Definition	n	Mean	Standard Deviation	Minimum	Maximum
$CPBT_{ji}$	Catch per basket trap for fisher *j* in location *i*	1000	1.57	1.20	0.00	25.00
$NBAS_{ji}$	Number of baskets used in the trip for fisher *j* in location *i*	1000	8.19	1.70	1.00	13.00
$SBAS_{ji}$	Size of basket (volume) in meter cube for fisher *j* in location *i* (feet^3)	1000	21.88	28.58	1.50	216.00
DIS_{im}	Distance from location *i* to marine reserve *m*	1000	6.72	2.44	1.00	12.00
$DEPTH_i$	Depth measured in meters in location *i*	1000	7.06	10.12	1.00	85.00
QU_n for $n = 1, 2, 3, 4$	Categorical variable representing quarter: Quarter 1: January, February, March Quarter 2: April, May, June Quarter 3: July, August, September Quarter 4: October, November, December					

The analysis started with the null hypothesis that none of the covariates have any influence on the stochastic response variable (catch per fishermen per trip and catch per trap per trip). Table 2 shows the relative performances of the model.

The residual deviance, AIC and BIC decreases as covariates are added, confirming their explanatory power. For instance, adding *NBAS* and *SBAS* reduce the residual deviance (to 220.3) as expected. Correcting for seasonal factors by adding categorical variables representing quarters again reduces the residual variation substantially (and the interaction variables add further explanatory power. Adding distance from the MPA improved the model fit as can be seen from Table 2. So too did adding the square of distance. Table 3 shows a similar analysis when using catch per trap.

Using the outcomes shown in Table 2 and 3, the effect of distance from the MPA on standardised catch per trap was simulated. Figure 2 and 3 show these results. A first observation shows that there is a slight U-shaped relationship between standardised catch and distance, but the rising segment takes place beyond 4 km. Standardised catch per trip declines non-linearly consistently for 4 kms from the MPA. This finding indicates a declining gradient from the spillovers.

It is important to highlight that the analysis took catch from the boundary of the reserve which is the conservation zone (Fig. 4). The multiple use zone where pole

Table 2. Analysis GLM fitted to catch per trip.

	GLM-Normal			
	DF	Residual deviance	AIC	BIC
Null hypothesis	999	392.95	1.91	-6507.90
$+ NBAS_{ji}$	998	267.36	1.53	-6626.57
$+ SBAS_{ji}$	997	220.08	1.33	-6666.95
$+ NBAS_{ji} \times SBAS_{ji}$	996	211.22	1.29	-6668.90
$+ QU_n + QU_n \times NBAS_{ji} + QU_n \times SBAS_{ji}$	987	168.91	1.08	-6649.35
$+ DEPTH_i + DEPTH_i \times NBAS_{ji} + DEPTH_i \times SBAS_{ji} + DEPTH_i \times QU_n$	977	159.68	1.05	-6584.28
$+ DIS_{im} + DIS_{im}{}^2$	975	156.54	1.03	-6574.62

Table 3. GLM analysis fitted to catch per trap.

	GLM-Normal			
	DF	**Residual deviance**	**AIC**	**BIC**
Null hypothesis	999	302.45	1.64	-6598.40
$+ NBAS_{ji}$	998	291.03	1.61	-6602.82
$+ SBAS_{ji}$	997	237.99	1.41	-6649.03
$+ NBAS_{ji} \text{ x } SBAS_{ji}$	996	231.85	1.38	-6648.28
$+ QU_n + QU_n \text{ x } NBAS_{ji} + QU_n \text{ x } SBAS_{ji}$	987	180.87	1.15	-6637.08
$+ DEPTH_i + DEPTH_i \text{ x } NBAS_{ji} + DEPTH_i \text{ x } SBAS_{ji} + DEPTH_i \text{ x } QU_n$	977	168.11	1.10	-6576.85
$+ DIS_{im} + DIS_{im}^{\ 2}$	975	164.15	1.08	-6567

and line, and basket trap fishing are allowed occurred one km from this zone.

The second model relates the number of individual fish per basket trap to the distance from the MPA. Table 4 shows the performance of this model.

Adding each subsequent variable reduces the residual variance significantly as well as the AIC and BIC. The simulation exercise is shown in Figure 4.

Figure 4 presents a very different picture. While the mass of fish per trap decreases with distance, the number of fish caught increases continuously with distance from the MPA to 8 km, then stabilises and falls slightly. The suggests that the reserve is contributing large fish to the catch in the adjacent waters.

Discussion

The relationship between catch per trip (in kg) and distance from the MPA observed in this study accords with the negative exponential slope typical of such studies (e.g., Bellier *et al.,* 2013). The effects of fishing characteristics and the seasonal effects in explaining variations in the catch data were expected from the literature (Stelzenmuller *et al.*, 2007). The study infers a declining fish abundance for 4 km from the MPA when using the standardised catch in kg per trip and per trap, as a measure of abundance. This may support the evidence of spillover effects observed in marine reserves as in similar studies (e.g. Roberts *et al.*, 2001; Rakitin and Kramer, 1996; Goni *et al.*, 2006). An important policy issue is the strength of the effect. In other words, when the direct spillover of adult fish is effectively at its minimum extent, by how much has

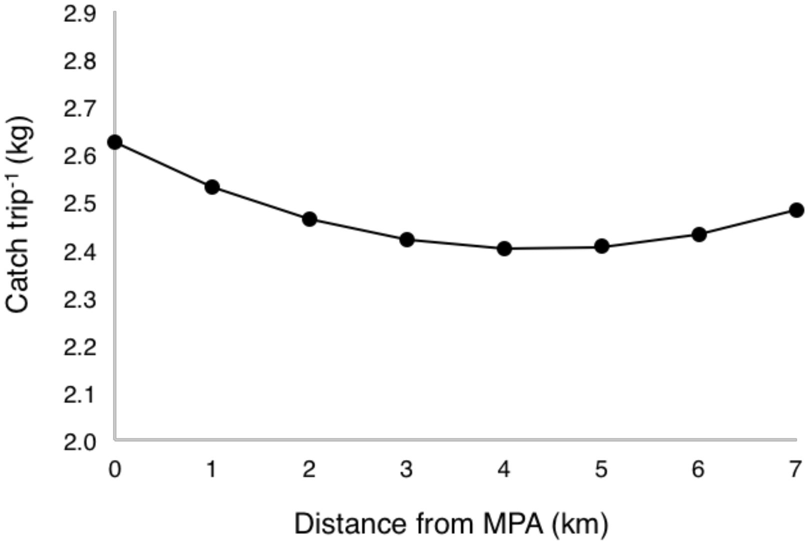

Figure 2. Standardised catch per trip and distance from MPA based on GLM.

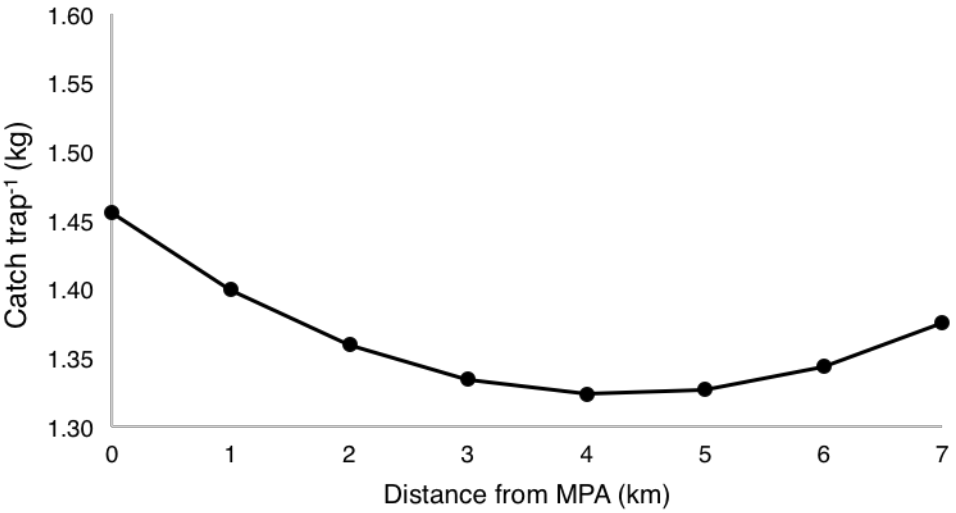

Figure 3. Standardised catch per basket trap and distance from MPA based on GLM.

the catch fallen? Catch per trap from the boundary to 4 km of the MPA shows a decline of 18.5 %.

A fundamental question is why the negative gradient prevails even after controlling for the many fishermen specific effects? If commercial species are mobile and fishermen have free access, fish yield is expected to stabilise, unless the spillovers from the MPA are continuous and systematic. These results may also reflect changes in habitat. It is important to establish that the observed gradient was caused by distance from the reserve and not an additional factor related with habitat characteristics that can change the fish community structure. Unfortunately, this is not easy as there was limited information on geographical characteristics. Some insights may be obtained from an analysis of the depth and specific fish species which exist in the adjacent areas. Figure 4 shows the depth of the waters beyond the boundary of the MPA. At 3 km, the water reaches a depth of around 325 m. Do these habitats host the fish species which may drive the results? An identification of the fish species in those waters may assist in answering this question.

Table 5 shows that there were four main fish species which were present on most of the fishing trips. The percentage of total fishing trips within 4 km of the MPA that caught Bluespine unicornfish *(Naso unicornis,* Forsskål, 1775) was 60.77 %, Spangled emperor (*Lethrinus nebulous* Forsskål, 1775) 34.4 %, Shoemaker spinefoot

Table 4. GLM analysis fitted to the number of fish per basket trap.

	Individual fish per trip				Individual fish per trap			
	DF	Residual deviance	AIC	BIC	DF	Residual deviance	AIC	BIC
Null hypothesis	999	428.48	8.40	-6472.37	999	353.56	4.56	-6547.29
$+ NBAS_{ji}$	998	423.96	8.40	-6469.99	998	315.91	4.52	-6578.03
$+ SBAS_{ji}$	997	401.16	8.37	-6485.88	997	303.23	4.51	-6583.80
$+ NBAS_{ji}$ x $SBAS_{ji}$	996	400.59	8.38	-6479.53	996	302.83	4.51	-6577.30
$+ QU_n$	993	3.96.68	8.38	-6462.73	993	299.26	4.51	-6560.15
$+ QU_n$ x $NBAS_{ji}$, QU_n x $SBAS_{ji}$	987	374.79	8.37	-6443.16	987	275.79	4.51	-6542.16
DIS_{im}	986	367.10	8.37	-6443.95	986	268.28	4.50	-6542.77
$DIS_{im}{}^2$	985	362.92	8.36	-6441.22	985	265.52	4.50	-6538.62

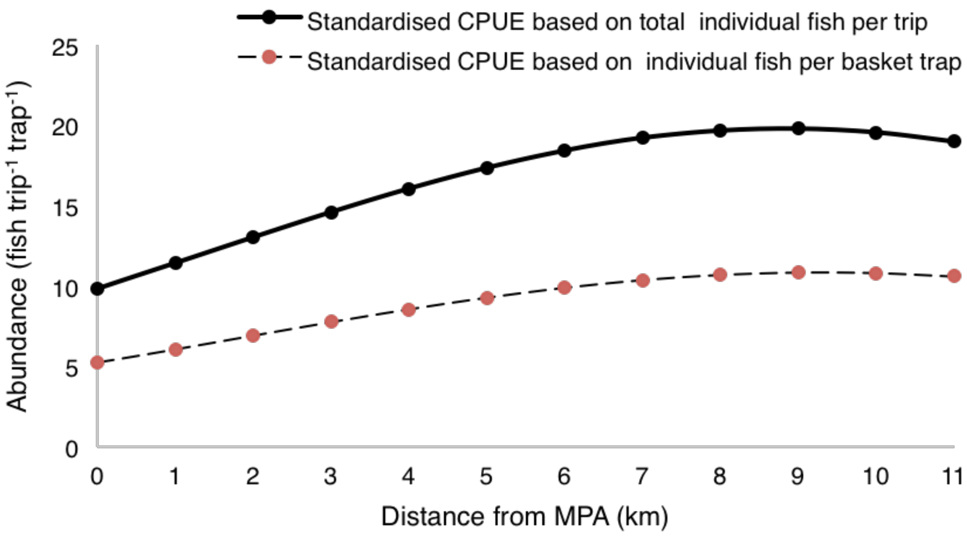

Figure 4. Number of individual fish per trip and per basket trap and distance from MPA.

(*Siganus sutor*, Valenciennes, 1835) 18.1 %, and Blue-barred parrotfish (*Scarus ghobun*, Forsskål, 1775) 13.9 %.

N. unicornis has a home range which extends a linear distance of 0.3 km to 1 km (Hardman *et al*., 2010; Marshell *et al*., 2011; Green *et al*. 2015). It is reef-associated and has been found within a depth range 1 – 180 m (Froese and Pauly, 2021). *L. nebulosus* inhabits nearshore and offshore coral reefs, coralline lagoons, seagrass beds, mangrove swamps, coastal sand and rock areas, to depths of 75 m (Froese and Pauly 2021). According to Pillans *et al*. (2014), the average home range for resident individuals is about 8 km compared to average sanctuary zone size of 30 km^2. *S. ghobun* is found in a depth range of 1 – 90 m while *S. sutor* inhabits seagrass beds and rocky/coral reefs with a depth of 1-50 m, but typically 1-12 m.

These variations across fish species provide relevant information on the extent of selective fishing effort targeting high value species and on the behavioural characteristics of each species. According to the literature, relatively mobile fish should exhibit a shallower gradient of abundance across the reserve boundaries in a hyperbolic shape, whereas sedentary fish should exhibit a steep linear gradient and highly mobile fish a flat gradient. Species that spend part of their life in the reserve, but then move three or four kms away include species such as *S. sutor* and *L. nebulous. N. unicornis* is also a highly mobile fish.

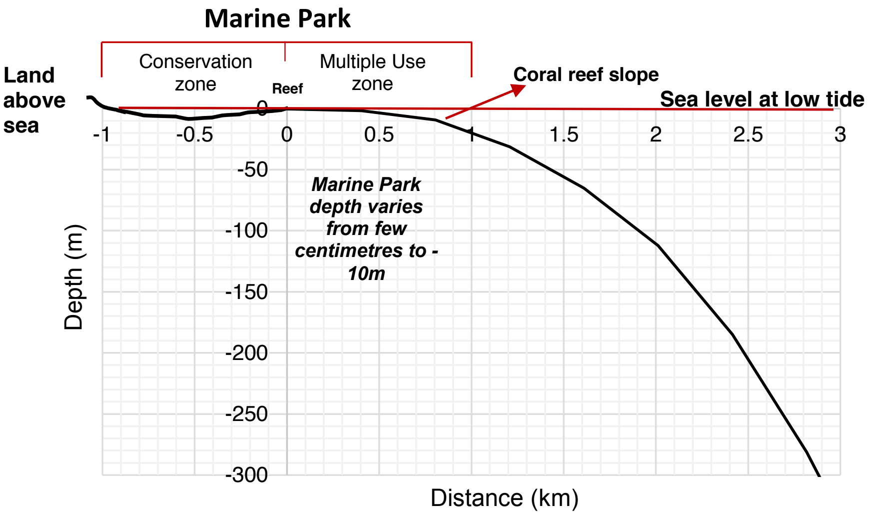

Figure 5. Depth of the Blue Bay Marine Park.

Table 5. Main fish species near the MPA.

Common names	Fish species	% of trips with the fish species	
		Within 4km from MPA	Beyond 4km from MPA
Shoemaker spinefoot	*Siganus sutor*	18.03	48.29
Bluespine unicornfish	*Naso unicornis*	**60.66**	30.87
Black grouper	*Epinephelus fasciatus*	7.38	22.32
Spangled emperor	*Lethrinus nebulous*	**34.43**	11.62
Rock flagtail	*Kuhlia rupestris*	6.56	3.53
Sky emperor	*Lethrinus mahsena*	6.56	8.66
Blue-barred parrotfish	*Scarrus ghobun*	**13.93**	26.42
Goatfishes	*Parupeneus* sp.	5.74	16.51
Doctorfish	*Acanthurus chirurgus*	3.28	6.61
Kingfishes	*Caranx* sp.	0.82	5.35

Further insights were obtained from the analysis of the weight of these fish species.

When individual number of fish per trap is considered a rising gradient of abundance is apparent; i.e., there seems to be more fish caught in the traps the further one moves from the reserve. A naïve interpretation of this is that this is inconsistent with spillover effects from an MPA. However, in waters close to the MPA the mass of fish per trap is higher even though the number of fish per trap is less; i.e., the fish caught near the MPA are larger, while the abundant juveniles are found further away. This is in fact consistent with the observed effect of MPAs; mean size should be smaller in non-reserve than in reserve areas because fishing mortality will reduce the proportion of older (hence larger) fish in the non-reserve (Rakitin and Kramer, 1996). Gell and Roberts (2003) point out that inside reserves, when the individuals of which those populations are comprised grow larger, they also develop increased reproductive potential. Reserves should serve to increase the mean sizes of sexually mature fish of each species in the community. Fish whose home range is fully located in the reserve should be bigger than those whose home range is only partly in the reserve, which in turn will be bigger than those whose home range is entirely outside the reserve. Moreover, in consequence of such growth in populations and amongst individuals, density-dependent emigration is expected to increase. This is a consequence of rising frequency of aggressive interactions between conspecifics as density and average size of targeted fish increase (Abesamis and Russ, 2005). These higher rates of aggressive interactions induce subordinate fish to relocate to home ranges outside the reserve (Kramer and Chapman, 1999). If such density-dependent aggressive interactions occur, with larger fish dominating smaller fish, a consequence is a gradient of mean sizes declining with distance from the reserve (Abesamis and Russ, 2005).

In order to examine whether mean size was higher near the MPA, the weights of the main fish species which are recorded near the MPA were collected, and the difference in their sample means was tested. These results are shown in Table 6. A clear observation from these results is that 9 out of the 11 fish species showed a higher weight within a 4 km radius of the MPA than in a zone more than 4 km from the MPA, with 6 of them having differences in means which are statistically significant. The main differences in mean weight was displayed in *N. unicornis, L. mahsena,* and *S. ghobun* and to a lesser extent *S. sutor* and *L. nebulous.* However, *L. mahsena* did not make up much of the catch. Consequently, the four other identified fish species could explain the declining gradient of individual weights with distance from the Park.

Table 6 also shows the fish species which were caught beyond the 4 km boundary. The finding that there was greater abundance of different types of fish species in those waters explains the rising segment of the standardised catches. Moving further away from the marine reserve, the fishing area is located outside the reef where

Table 6. Average weight of individual fish near the MPA.

Common names	Fish species	Average weight of individual fish (g)		Differences in mean test t-statistics (p-value)	Types of fish S=sedentary, V=vagile fish, HV=highly vagile
		Within 4km from MPA	Beyond 4km from MPA		
Shoemaker spinefoot	*Siganus sutor*	582.72	522.24	-2.03**	V
Bluespine unicornfish	*Naso unicornis*	1959.46	1484.21	-4.92***	HV
Black grouper	*Epinephelus fasciatus*	193.75	166.75	-0.766	S
Spangled emperor	*Lethrinus nebulous*	1102.33	922.88	2.44 **	V
Rock flagtail	*Kuhlia rupestris*	1116	828	-3.32 ***	HV
Sky emperor	*Lethrinus mahsena*	725	569.38	-1.77*	V
Blue-barred parrotfish	*Scarrus ghobun*	1085.29	680.03	3.75***	V
Goatfishes	*Parupeneus* sp.	285.71	292.25	0.11	V
Doctorfish	*Acanthurus* sp.	150	139.66	0.37	V
Kingfishes	*Caranx* sp.	NA	666.67	NA	HV

***=significant at 1%, **=significant at 5% and *=significant at 10%
NA: Not available

both the stock of fish and the number of fish species are relatively higher, given the depth of the waters (Fig. 4). The inclusion of the fish catch from these waters in the analysis was deemed important since the data was collected at fish landing sites and the estimation requires sufficient observations to produce an appropriate fit of the data to the degrees of freedom.

Conclusion

The results of this study show that catches were slightly higher in waters adjacent to of the Marine Park and the size of the fish near the reserve was relatively larger. These findings reinforce the available evidence of the spillover effects of marine reserves and consequent changes in fish age distribution, with a greater number of older fish within and close to the reserve. The negative gradient was most likely driven by the four fish species *N. unicornis*, *L. nebulous*, *S. sutor*, and *Scarrus ghobun*. Spatial catch data could be an effective instrument to assess the impact of MPAs on adjacent waters on a regular basis, as compared to comprehensive oceanographic assessments and visual census techniques which require greater financial and logistic resources. There is a major caveat, however. While the catch data was collected with a degree of accuracy, the findings depended solely on fishermen's responses regarding their fishing locations. The use of GPS would greatly enhance the precision of these locations. Moreover, the study only measured changes in fish abundance and size from the edge of the MPA where fishing is allowed. Theory suggests that there should be a decline from a point inside the MPA boundary. It is plausible that these results considerably understate the impacts of the reserve. Lastly, the study did not consider the likely impacts of fishing intensity on the fish population which may also influence the results. These issues open avenues for further research.

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Distribution of organochlorine pesticides and polychlorinated biphenyls present in surface sediments of the Sabaki and Tana estuaries, Kenya

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Abstract

Polychlorinated biphenyls (PCBs) and Organochlorine pesticides (OCPs) in the surface sediments of the Sabaki and Tana estuaries were studied to determine distribution as well as their ecological risk. The concentration range of PCBs and OCPs were 0.65-9.29 ng/g dw and 0.47-9.84 ng/g dw, respectively. Sabaki Bridge in the Sabaki River and Tana 4 in the Tana River recorded high levels of both PCBs and OCPs. The calculated values of DDD+DDE/DDT showed that the presence of DDT from both the rivers is a result of historical input. The ecotoxicological impact of sediment pollution by PCBs and OCPs was assessed using sediment quality guidelines specified by the Canadian Council of Ministers of the Environment. The concentration values for PCB, HCB, Heptachlor epoxide, and Chlordane in this study indicate low ecological risk to sediment dwelling organisms. DDT showed an adverse biological effect to sediment dwelling organisms. As a result, there is a need to establish a programme for monitoring persistent organic pollutants in Kenya, so that any elevation in concentration above the environmental quality standards can be detected and appropriate actions taken to minimize potential adverse effects to the environment and public health.

Keywords: estuarine, ecotoxicological risk, surface sediments, polychlorinated biphenyls, organochlorine pesticides

Introduction

OCPs and PCBs are among the group of POPs which have been banned or restricted globally under the Stockholm Convention (UNDP, 2001). These environmental contaminants are of major concern on regional and global scales due to their long-range transportation, high lipophilicity, environmental persistence, and potential toxic biological effects to both humans and wildlife (Dierking *et al.* 2009; Barakat *et al.* 2013).

OCPs were extensively used in agriculture and for mosquito control for several decades (1940s through to 1960s). Even though many of these compounds were banned globally under the Stockholm Convention on POPs (UNDP, 2001), some developing countries continue to use them. Most of the pesticides are developed with the ability to have a wide spectrum of applications, but some are developed with the concept of target organism toxicity, even though quite often non-target species are also adversely affected under the latter conditions. Due to their high toxicity, slow degradation, and bioaccumulation, the use of OCPs can adversely affect environmental health and ecosystem services. Pesticides are reported to cause extinction, behavioral changes, loss of safe habitat, and population decline in several bird species. Their prolonged use has been shown to cause a drastic decrease in birds like the peregrine falcon, sparrow hawk, and bald eagle (Mitra *et al.*, 2011). They are also reported to affect many aquatic and terrestrial species such as microorganisms, invertebrates, plants, and fish (Jayaraj *et al.,* 2016).

PCBs are organochlorine compounds that have been widely deployed industrially in the past few decades in many different products. They have been used for instance as dielectric, coolant, and heat exchange

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fluids in electrical equipment such as transformers and capacitors. They have also been used as additives in pesticides, cutting oils, surface coatings, heat transfer fluids, hydraulic lubricants, sealants, carbonless copy paper, flame retardants, adhesives, and paints (Mohammed *et al.,* 2011; Kassegne *et al.,* 2020). PCBs are introduced into the environment through human activities. In particular, they are derived from the discharged waste of some chemical and electrical industries, mostly through leaks from industrial facilities, evaporation, and inappropriate disposal practices (Pazi *et al.,* 2011; Gakuba *et al.,* 2015; Kassegne *et al.,* 2020).

PCBs are known to possess varying toxicological and physicochemical properties related to their chemical structures, and as such, they elicit adverse effects on humans and ecosystems (UNEP, 2009). They have been classified as probable human carcinogens and associated with cancers of the liver, gall bladder, gastrointestinal tract, breast, brain, and disruption of hormone function (Montuori *et al.,* 2015; Montuori *et al.,* 2020).

Most of the studies on POPs in Kenya have been focused on OCPs and targeted freshwater and river environments. Lalah *et al.* (2003) determined the concentrations of OCPs (e.g., DDT, DDE, DDD, endrin, lindane, endosulfan) residues in fish, water, and sediment samples from the Tana and Sabaki rivers, while Everaarts *et al.* (1996) reported the presence of low concentrations of OCPs and PCBs residues in sediments and macroinvertebrates from some sites along the Indian Ocean coast of Kenya. This study aimed to determine the distribution of persistent OCPs and PCBs in sediments from the Sabaki and Tana estuarine areas and identify their possible sources and probable environmental risks.

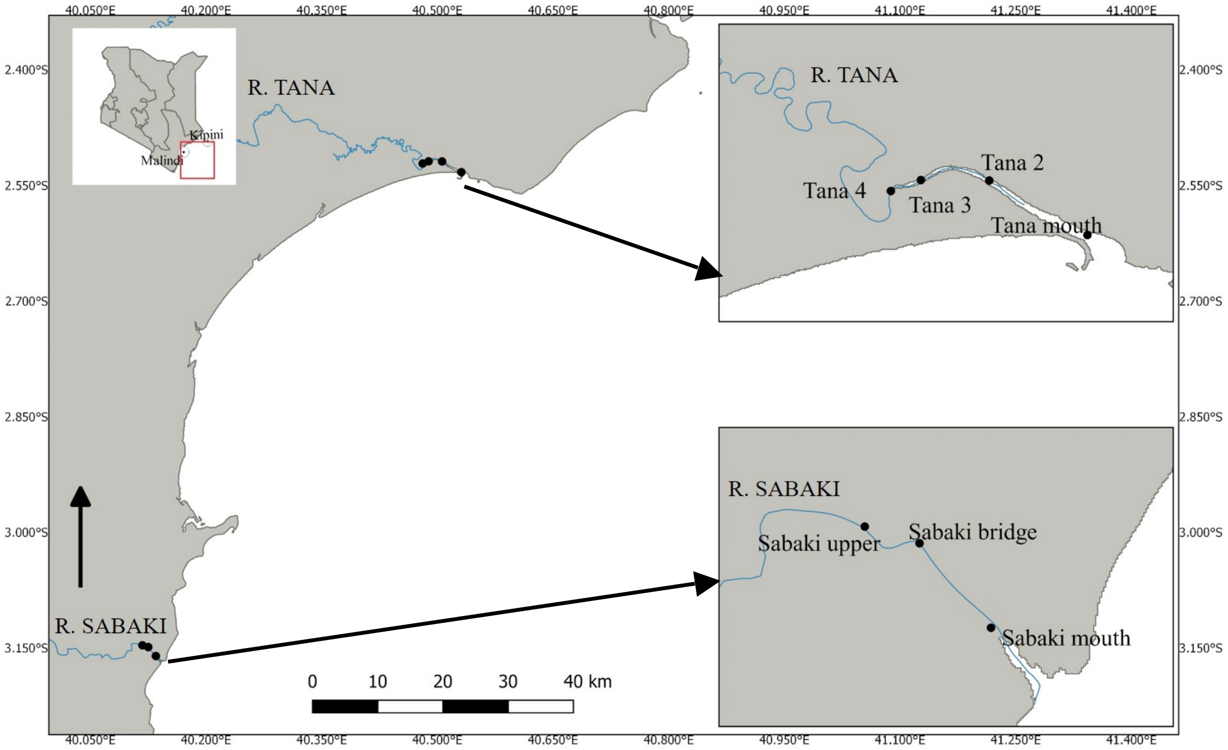

Figure 1. Map showing the selected sampling locations in the Tana and Sabaki estuarine systems.

Materials and methods

Study area

The study was carried out in th3 Tana and Sabaki estuarine systems of Kenya (Fig. 1). Tana River is the largest river in Kenya rising from the Aberdares and Mount Kenya ranges of central Kenya. It runs through the arid and semi-arid lands in the eastern part of the country to enter the Indian Ocean through a roughly triangular-shaped delta, with its apex at Lake Bilisa in the north of Garsen (Okuku *et al.,* 2013). The Athi-Galana-Sabaki River is the second largest river system in Kenya. It originates from the Ngong hills in central Kenya (as River Athi). It flows mainly through sand and rocks and mining industries for cement and other inorganic products as the Athi River. It further flows through areas with major industries and coffee and tea farms where agrochemicals and fertilizers are used on a large scale before discharging into the Indian Ocean (Okeyo 1998; Lalah *et al.,* 2003).

Sample collection and storage

Sediment samples were collected from selected locations in the Tana and Sabaki estuarine systems (Fig. 1). Surface sediment samples of about 100-200 g were collected using a grab sampler, put in aluminum containers previously cleaned with hexane HPLC grade (sigma Aldrich), and stored in a cool box at a sufficiently low temperature (~4 °C) to limit biological and chemical activity. The samples were transported to the Kenya Marine and Fisheries Research Institute laboratory and kept frozen at -20 °C before transportation to the Laboratory of Physics and Toxico-Chemistry (LPTC) at the University of Bordeaux 1, France, for analysis.

Analytical procedures

Grain size and carbon analysis

Sediment samples were dried in an oven at 105 °C to constant weight. Grain size analysis of samples was performed through sieving (Folk, 1980), while total organic matter was analyzed using the ashing method. Total organic carbon (TOC) content was determined by oxidation with 1 N $K_2Cr_2O_7$ acidified with concentrated H_2SO_4 and titration with 0.5 N $[Fe(NH_4)_2(SO_4)_2]$ (Loring and Rantala, 1992).

Extraction and clean-up

Sediment samples were processed and analyzed in the laboratory as described by Thompson *et al.* (1999). Wet sediment samples of about 100 g were weighed, put in clean pre-weighed aluminum containers, covered with clean aluminum sheets with holes, and freeze-dried for 48 hours using a Heto power dry LL 3000 freeze dryer (Thermo Scientific). After freeze-drying, the samples were weighed to obtain the dry weight, and sieved through a 2 mm mesh-sized metallic sieve to remove any coarse materials. The samples were then stored in clean, well-labeled glass amber bottles awaiting analysis. Prior to extraction, about 3 g of each sample was put in the sample extraction vial and spiked with 30 µL of an internal standard containing a mixture of PCB 30, PCB 103, PCB 155, PCB 198 (Promochem, Dr Ehrenstorfer GmbH, France) and d8 4,4' DDT (Cambridge Isotope Laboratory, France). The added internal standard was used to quantify the recovery of the total extraction procedure. The samples were then extracted for 20 minutes with 30 mL dichloromethane (Pro-labo, Fontenay-sous-Bois, France) using the START E microwave-assisted extraction system (Milestone, Italy). The extracts were mechanically filtered into 25 mL amber glass vials through clean glass funnels stuffed with pre-cleaned glass wool. The filtered extracts were concentrated to about 1 mL using Rapidvap LABCONCO (Serlabo Technologies, France). The concentrated samples were subjected to a clean-up process during which the lipids and sulfur were removed from the extracts using a micro-column containing acidified silica (Silica gel, 0.063 ± 0.2 mm, Merck, Darmstadt, Germany) and activated copper (40 mesh, 99.5 % purity, Aldrich, Saint Quentin Fallavier, France). The PCBs were then purified on the micro-column by eluting 3 x 5 mL with a mixture of n-pentane and dichloromethane (90/10 v/v). The extracts were finally concentrated under nitrogen and transferred to 100 µL isooctane (99 % extra pure, Scharlau, ICS, St Medard en Jalles, France). The solution was further re-concentrated to 100 µL in an injection vial, and 1 µL of the sample was injected for analysis using Gas Chromatography-Mass Spectrometry.

Gas Chromatography-Mass Spectrometry (GC-MS) analyses

Analysis was performed using a Hewlett-Packard 5980 Series II gas chromatograph equipped with a ^{63}Ni electron-capture detector using a HP5 capillary column (60 m × 0.25 mm internal diameter × 0.25 µm film thicknesses). The GC conditions were set by injecting 1 µL through a split-less liner at a temperature of 280 °C; detector temperature: 290 °C; initial oven temperature: 60 °C held for 2 min, heated to 120 °C at 6 °C/min and held for 5 min, then heated to 280 °C at 2 °C/min and held for 20 min. Helium gas was used as the carrier gas. The PCB congeners were analyzed individually, whenever possible, though in some cases the concentrations were reported as the sum of overlapping congeners due to co-elution on the GC column. The relative response factors of the different compounds were determined by injecting a standard solution SRM 2262 for PCB (NIST, MD, USA) spiked with the same solution of internal standards (PCB 30, PCB 103, PCB 155, PCB 198, and OCN) and SRM 2261 for OCP spiked with (d8 4,4' DDT and OCN) as that used for spiking the sediments. The response factors were determined after every four samples. Blank injections of isooctane were performed between each injection of a sample to prevent cross-contamination.

Quality control and quality assurance

Laboratory glassware and reagent containers were thoroughly cleaned and dried in an oven for 4 hours at 450 ˚C. All reagents, solvents, and standards were of HPLC quality and tested for suitability before sample analysis. Milli-Q water (organic grade quality) was used for all cleaning throughout the analytical process. A solvent blank and reagent blank were run with each batch under the exact condition of the sample to trace any potential contamination during sample preparation. The average recovery of the internal standards ranged from 82-99 % for OCP and 89-104 % for PCB. The reported values in this study were corrected according to the values found in the blank, Instrumental detection limits (IDLs), and method detection limits (MDLs).

Results and discussion

Sediment physicochemical characterization

The results of surface sediment TOC, organic matter (OM), and particle grain size are presented in Table 1. High levels of TOC (16.3 mg/g), OM (1.45 %) and silt particle size (78.70 %) were reported in samples from Sabaki Bridge. Tana River mouth samples had high levels of TOC (14.2 mg/g) and sand particle size, > 63 µm (95.91%). Tana 2 samples had a high level of organic matter (8.02 %) compared to samples from the other stations. The surface sediments from the Sabaki and Tana rivers had sandy particle size, an indication that the grain size of the sediments with a high percentage of sand tends to have high porosity and high permeability (Kilunga *et al.,* 2017).

Distribution of POPs in surface sediments

The concentration of POPs, namely PCBs and OCPs, in surface sediments of the Sabaki and Tana estuarine systems are illustrated in Figure 2. The POPs concentrations in Sabaki estuary were in the range of 1.37-7.93 ng/g dw, whereas in the Tana estuary, the concentrations were in the range of 1.13-16.99 ng/g, dw. High concentrations of POPs as well as TOC, organic matter and % silt content was observed at Sabaki Bridge. This shows a positive relationship between POPs and TOC confirming that POPs tend to have a high affinity for organic matter (Wu *et al.,* 2015; Wu *et al.,* 2016). Both the Sabaki and Tana river mouths recorded the lowest concentrations of POPs due to continuous flushing of sediment by the sea resulting in sediment with a high sand proportion (94.5 % and 95.9 %, respectively).

PCBs in surface sediment

The total concentrations of PCB (sum of 12 congeners) in the surface sediment samples (Table 2) in Sabaki estuary was low (0.68-3.87, mean: 2.01 ng/g) compared to Tana estuary (0.65-9.29 ng/g, mean 5.59 ng/g). The concentration of PCBs reported in this study was comparable to levels reported by Okuku *et al.* (2019) for Sabaki estuary (0.58-2.40 ng/g) and Tana estuary (4.94-7.99 ng/g). PCB concentrations in the Tana estuary were also similar to those of the Yellow River: 1.4–5.2 ng/g (He *et al.,* 2006), Tonghui River: 0.78-8.47 ng/g (Zhang *et al*., 2004), Jiulong River: 1.0-8.1 ng/g (Wu *et al*., 2016) and Makelele river: 0.9-10.9 ng/g (Kilunga *et al.,* 2017). High concentrations of PCBs have been found in the Awash River Basin, Ethiopia: 0.85-26.56 ng/g (Dirbaba *et al.,* 2018), Umgeni River, South Africa: 102.60-427.80 ng/g (Gakuba *et al.,* 2015), Msunduzi River, South Africa: 214.21-610.45 ng/g (Adeyinka *et al.,* 2018), Haihe River, China: nd–253 ng/g (Zhao *et al.,* 2010), Damietta estuary, Egypt: 0.29 to 377 ng/g (Barakat *et al.,* 2013) and Soan River, Pakistan: 37.4-187 ng/g (Malik *et al.,* 2014).

PCBs in the Sabaki estuary may have resulted from several activities in the catchment area including industrial installations and companies around Athi River

Table 1. TOC concentration (mg/g), organic matter (%) and < 63 µm particles fraction (%) in sediment from the Sabaki and Tana estuaries.

STN	TOC (mg/g)	% Organic matter	% Silt (< 63 µm)	% Sand (> 63 µm)
Sabaki upper	6.64	0.72	35.21	64.79
Sabaki bridge	16.30	1.45	78.65	21.35
Sabaki Mouth	3.45	0.14	5.54	94.46
Tana Mouth 1	14.91	1.02	4.09	95.91
Tana 2	11.92	2.92	41.12	58.88
Tana 3	6.18	8.02	35.93	64.07
Tana 4	13.45	4.67	37.26	62.74

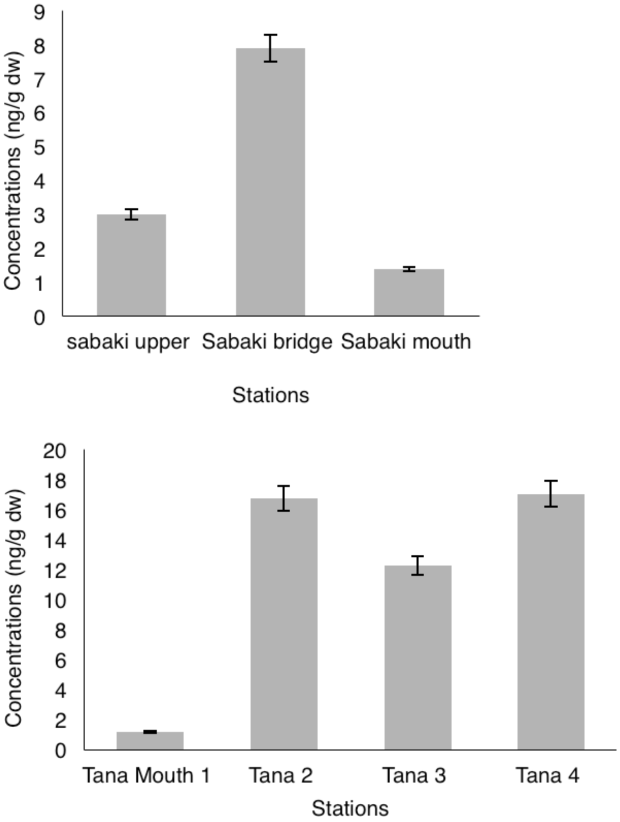

Figure 2. POPs concentration in the Sabaki and Tana estuarine areas.

and Nairobi that discharge their effluent into the river system. Sabaki Bridge had the highest concentration of PCBs in the Sabaki estuarine system which may have been the result of road traffic at the bridge.

PCB concentration in Tana 2 (9.29 ng/g dw) was high compared to the other stations in the estuary, probably due to oil leakage from the boats used by the fishermen given that this is a landing site where most of the boats are anchored. However, the low PCB concentrations at the Tana Mouth station (0.65 ng/g dw) and Sabaki mouth (0.68 ng/g dw) were due to tidal dilutions. High levels of PCBs in the Tana River system could be attributable to the possible leakage or inadvertent disposal of materials used in transformers and capacitors in the Seven Folks electricity-generating dam. Leakage from broken transformers and used electrical equipment has been reported elsewhere to be a major anthropogenic source of PCBs transferred to the environment (Fu *et al.*, 2003; Kassegne *et al.*, 2020). The Tana River holds the Seven Folks Scheme, a serially cascaded dam used for hydroelectricity generation (Masinga, Kamburu, Gitaru, Kiambere, and Kindaruma Dams). Such installations use large transformer and capacitor systems that would apply Aroclor formulations as dielectric and heat retardants in the past, before the ban on PCB production and use by various PCB producing countries following the Stockholm Convention (UNEP, 2009). Even though

Table 2. Descriptive statistics (Sum, mean, and standard deviation values) of PCB concentrations (in ng/g dw) on surface sediment samples from the Sabaki and Tana estuarine systems.

Compounds	Sabaki River			Tana River			
	Sabaki upper	Sabaki bridge	Sabaki mouth	Tana Mouth 1	Tana 2	Tana 3	Tana 4
PCB (ng/g)							
28+50	0.23	0.51	0.10	0.07	0.80	0.47	1.16
44	0.14	0.25	0.05	0.05	0.37	0.17	0.31
101	0.10	0.22	0.05	0.08	0.54	0.21	0.00
87	0.10	0.18	0.05	0.06	1.47	0.71	0.74
118	0.40	1.14	0.17	0.15	2.89	1.80	3.03
153	0.32	1.10	0.13	0.13	1.84	1.18	1.48
138	0.04	0.20	0.04	0.04	0.51	0.23	0.10
128	BDL	BDL	0.02	0.01	BDL	BDL	BDL
187	0.05	0.09	0.03	0.02	0.17	0.11	0.07
180	0.04	0.08	0.02	0.02	0.44	0.20	0.07
170	0.05	0.09	0.03	0.02	0.26	0.17	0.17
206	BDL	BDL	BDL	BDL	BDL	BDL	BDL
∑ PCB congeners	**1.48**	**3.87**	**0.68**	**0.65**	**9.29**	**5.26**	7.15
Mean	0.15	0.39	0.06	0.06	0.93	0.53	0.71
STDEV	0.13	0.41	0.05	0.05	0.87	0.56	0.96

BDL-Below detection limit

Kenya ratified and implemented the Convention, due to the recalcitrance of PCBs, it is expected that residues of these contaminants still exist in various environments, including aquatic systems.

The PCBs with congeners of Penta-Hexa and hepta chlorobiphenyls were the most prominent homologous groups. Heavier PCB congeners dominated due to their adsorption to particulate material resulting in their accumulation and deposition in the sediments (Malik *et al.,* 2014). In the Sabaki and Tana estuarine systems, the most abundant PCB congeners reported were PCB 118 (Sabaki 1.71 ng/g; Tana 7.87 ng/g) followed by PCB 153 (Sabaki 1.55; Tana 4.63 ng/g) (Fig. 3). These are among the most persistent PCB congeners and have a long half-life (Kilunga *et al.,* 2017). As much as commercial use of PCBs has been banned, the presence of the PCBs congeners indicated historical pollution as also reported elsewhere by Kilunga *et al.* (2017). The persistence of PCBs in aquatic sediments is due to their low rate of degradation and vaporization, low water solubility, and partitioning to particles and organic carbon (Aly Salem *et al.,* 2013; Montuori *et al.,* 2020).

OCPs in the surface sediment

The levels of total OCPs, including HCHs, DDT, and its metabolites DDXs, heptachlor, heptachlor *epoxide* chlordane, Nanochlor, and mirex, were detected in the Sabaki and Tana estuarine systems (Table 3). The total OCP concentrations in surface sediments in the Sabaki estuary were in the range of 0.69-4.01 ng/g dw with a mean of 0.12 ng/g dw, while in the Tana estuary, the concentrations were in the range of 0.47-9.84 ng/g dw with a mean of 0.56 ng/g dw. The highest concentrations of OCPs were reported at Sabaki Bridge and Tana 4 with values of 4.01 ng/g dw and 9.84 ng/g dw, respectively. Lalah *et al.* (2003) reported a concentration range of < 0.003 to 108.51 ng/g in the Sabaki estuary (Lalah *et al.*, 2003). This suggests that although OCPs use has been banned in Kenya, there might be an illegal or minor application of OCPs for malaria vector and tsetse fly control (Wandiga *et al.,* 2002). OCPs in the Sabaki and Tana River estuaries showed similar concentrations with Bizerte lagoon, Tunisia, at 1.1-14.0 ng/g (Barhoumi *et al.,* 2014), and the Volturno River, Italy, at 0.52- 9.89 ng/g (Montuori *et al.,* 2020). Higher concentrations of OCPs have been reported by Unyimadu *et al.* (2019) in the Niger River, Nigeria (4672-7009 ng/g), Dirbaba *et al.* (2018) in the Awash River Basin, Ethiopia (6.63–206.13 ng/g) and Kilunga *et al.* (2017) in tropical urban rivers of the Congo (21.6 - 146.8 ng/g).

The concentration of Heptachlors (including heptachlor and its metabolite, heptachlor epoxide) was 0.51 ng/g dw, and Cis chlordane at 0.09 ng/g dw in the Sabaki estuary. In the Tana estuary, Heptachlor was only detected at Tana Mouth due to its use in termite control by the community in the area (Lalah *et al.,* 2003; Zhao *et al.,* 2009), while its metabolite, heptachlor epoxide was present in all the other sites except in Tana Mouth with a concentration of 0.11 ng/g dw.

HCN has been reported to have a total of eight HCN isomers, among which only α, β, ɣ, ε and δ isomers are stable and commonly identified in the environment (Wang *et al.,* 2009). Although HCN is no longer in use, HCN concentrations in Sabaki and Tana estuarine systems in this study were 0.07 ng/g dw and 0.75 ng/g dw, respectively. At the same time, the presence of ɣ- HCN in Sabaki (0.02 ng/g dw) and Tana (0.12 ng/g dw) systems may be as a result of the application of

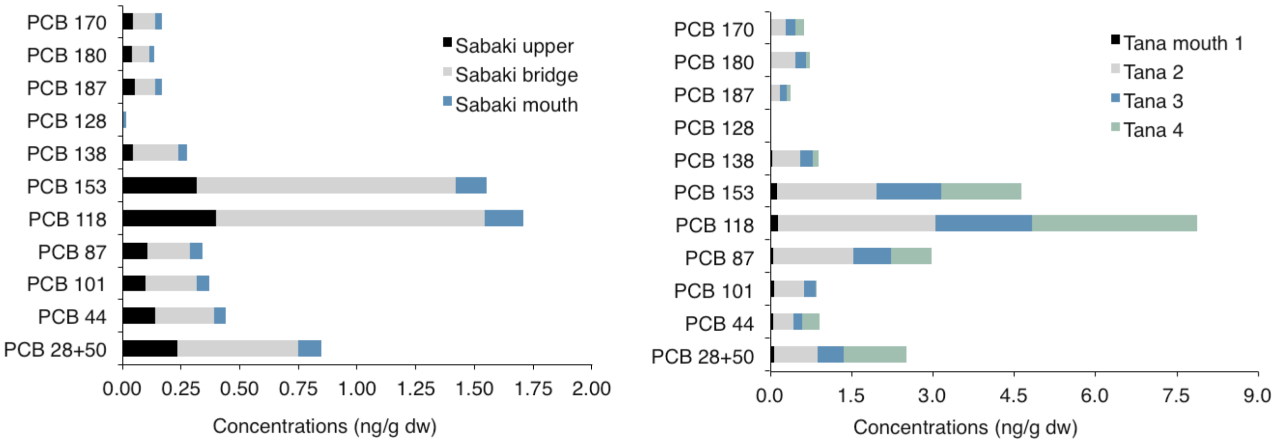

Figure 3. PCB congeners in Sabaki and Tana Rivers estuarine systems.

Table 3. Descriptive statistics (Sum, mean, and standard deviation values) of OCP concentrations (in ng/g dw) on surface sediment samples from the Sabaki and Tana estuarine systems.

Compounds	**Sabaki River**			**Tana River**			
	Sabaki upper	**Sabaki bridge**	**Sabaki mouth**	**Tana Mouth 1**	**Tana 2**	**Tana 3**	**Tana 4**
OCP (ng/g)							
HCB	0.04	0.10	0.02	0.03	1.16	0.78	0.54
gamma-HCH	0.00	0.05	0.01	0.00	0.25	BDL	0.10
Heptachlor	0.16	0.11	0.19	0.04	BDL	BDL	BDL
Hep.Hepoxide	0.04	BDL	BDL	BDL	0.18	0.05	0.11
Cis chlordane	0.04	0.08	0.03	BDL	0.30	0.09	0.19
Trans Nonachlor	0.20	0.07	0.02	0.02	0.18	0.09	0.16
o,p' DDE	0.03	0.20	0.02	0.03	0.43	0.33	0.46
p,p'DDE	0.10	0.22	0.03	0.03	0.57	0.31	0.41
o,p' DDD	0.56	1.51	0.21	0.22	BDL	2.38	3.65
p,p' DDD	0.10	0.70	0.05	0.04	1.80	1.29	1.82
o,p' DDT	0.18	0.67	0.07	0.06	1.68	1.14	1.67
p,p' DDT	0.05	0.31	0.01	0.01	0.64	0.45	0.70
Mirex	BDL	BDL	0.02	BDL	0.21	0.01	0.04
∑ OCP	**1.51**	**4.01**	**0.69**	**0.47**	7.40	6.93	9.84
∑ DDT	1.03	3.49	0.41	0.36	4.99	5.67	8.43
Mean	0.13	0.36	0.06	0.05	0.67	0.63	0.82
STDEV	0.15	0.44	0.07	0.06	0.60	0.73	1.07

BDL-Below detection limit

Lindane (ɣ- HCN being an active ingredient) which has been used in Kenya since 1949 for both agriculture and pest vector control purposes (Lalah *et al.*, 2003; Wang *et al.*, 2009; Dirbaba *et al.*, 2018) and is still used under restriction for termite control. Kassegne *et al.* (2020) also detected Lindane (ɣ- HCN) in Akaki River (mean 371.78 ng/g) despite the ban.

DDT and it's metabolites like p,p'DDE, o,p'DDE, o,p'DDD, p,p' DDD, o,p'DDT and p,p'DDT were detected in the surface sediment in both the Sabaki and Tana estuaries (Table 2). The highest concentrations of ∑6 DDTs were recorded at Sabaki Bridge (3.49 ng/g) and Tana 4 (8.43 ng/g) (Fig. 4). Tana estuary samples showed a decrease in the total concentration of DDT from the upper parts towards the Indian Ocean because it passes through areas with a history of organochloride pesticide use (Lalah *et al.*, 2003). The concentration of total DDT in the Tana estuary was comparable to the Volturno River, Italy, at 0.10 to

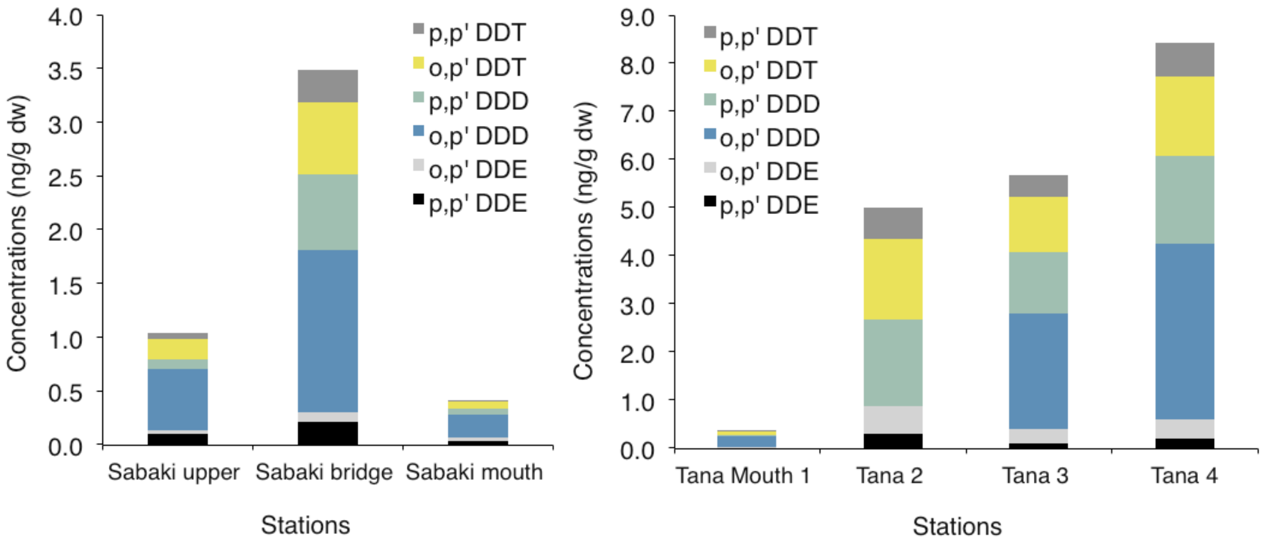

Figure 4. Concentration of DDTs metabolites in the Sabaki and Tana estuarine systems.

5.22 ng/g (Montuori *et al.,* 2020), Bizerte lagoon, Tunisia, at 0.3–11.5 ng/g (Barhoumi *et al.,* 2014) and Jiulong River, Taiwan at 2.3-11.8 ng/g (Wu *et al.,* 2016). A higher concentration of DDT was reported in the Awash River Basin, Ethiopia by Dirbaba *et al.* (2018) ranging from 1.99–139.68 ng/g, in the Niger River, Nigeria by Unyimadu *et al.* (2019) ranging between BDL and 107.6 ng/g, and Barakat *et al.* (2013), in the Damietta estuary, Egypt ,ranging between 0.07 and 81.5 ng/g.

The concentration of DDT isomers were found to be p,p'DDE >o,p'DDD > o,p'DDE > p,p'DDD > o,p'DDT > p,p'DDT in the Sabaki estuariy while in the Tana estuary they were as follows: p,p' DDE >o,p'DDE> o,p'DDD > p,p'DDD > p,p'DDT > o,p'DDT. This was also observed by Zhao *et al. (*2009) when they conducted a study in Taihu Lake, China, as well as by Kilunga *et al*. (2017) on tropical urban rivers in Kinshasa, Democratic Republic of the Congo. However, this was contrary to the findings of Malik *et al.* (2014) on the Soan River, Pakistan.

It has been reported that DDT can be biodegraded under aerobic and anaerobic conditions to DDE and DDD, respectively (Malik *et al.,* 2014; Nguyen *et al.,* 2019). Thus, the ratio of DDE/DDD has been used to denote the DDT degradation status. If the ratio of DDE/DDD is greater than one (> 1), it indicates the anaerobic degradation of DDT into DDD while if it is less than one (< 1), it shows the DDT conversion into DDE via aerobic degradation (Ali *et al.,* 2016). In this study, the Tana estuarine system reported a DDE/DDD ratio < 1, suggesting an aerobic degradation of DDT to DDD via dehydrochlorination and oxidation processes, and further degradation into DDD by reductive dechlorination in the environment (Cheng *et al.,* 2014; Kilunga *et al.,* 2017). Whereas in the Sabaki estuarine system the DDE/DDD ratio was > 1, indicative of anaerobic degradation of DDT into DDD. A similar study on the difference in DDE/DDD ratios in sediment was also reported on the Egyptian Mediterranean coast (Aly Salem *et al.,* 2013). The source of DDT in the environment either as a historic or of recent origin has been determined by applying the DDD+DDE/DDT ratio. If the ratio DDD+DDE/DDT is > 0.5, this indicates historical input, and if <0.5, this indicates recent input (Kilunga *et al.,* 2017; Montuori *et al.,* 2020). In this study, the values of DDD+DDE/DDT in the Sabaki and Tana estuarine systems were 0.57 and 2.73, respectively, suggesting historical input of DDT in both the estuaries due to their capability to remain in the agricultural soil for a long time and re-mobilize through runoffs. A similar observation was reported by Aly Salem *et al*. (2013), and Montuori *et al.* (2020). However, this was contrary to the findings of Zhao *et al.* (2009) which indicated recent DDT input due to illegal input of DDT or usage of other pesticides containing DDT, such as dicofol.

Table 4. Comparison of OCP and PCB concentrations (ng/g dw) in surface sediments in this study with those from various locations in the world.

Location	PCB	OCP	DDT	References
Sabaki estuary, Kenya	0.68-3.87	0.69-4.04	1.03-3.49	This study
Tana estuary, Kenya	0.65-9.29	0.47-9.84	0.36-8.43	This study
Tana estuary, Kenya	4.94–7.99	-	-	(Okuku *et al.,* 2019)
Sabaki estuary, Kenya	0.58–2.40	-	-	(Okuku *et al.,* 2019)
Ramisi Estuary	ND-0.15	-	-	(Okuku *et al.,* 2019)
Awash River Basin, Ethiopia	0.85–26.56	6.63–206.13	1.99–139.68	(Dirbaba *et al.,* 2018)
Bizerte lagoon, Tunisia	0.8–14.6	1.1-14.0	0.3–11.5	(Barhoumi *et al.,* 2014)
Niger River, Nigeria	-	4672-7009	Bdl-107.6	(Unyimadu *et al.,* 2019)
Umgeni River, South Africa	102.60–427.80	-	-	(Gakuba *et al.,* 2015)
Msunduzi River, South Africa	214.21–610.45			(Adeyinka *et al.,* 2018)
Damietta estuary, Egypt	0.29 to 377	0.27–288	0.07-81.5	(Barakat *et al.,* 2013)
Tropical urban rivers, Congo	0.9 - 10.9	21.6 - 146.8	1.23-270.6	(Kilunga *et al.,* 2017)
Taihu Lake, China	-	4.22 - 461	0.25 - 375	(Zhao *et al.,* 2009)
Haihe River, China	nd–253	0.997– 2447	n.d. – 155	(Zhao *et al.,* 2010)
Volturno River,Italy	4.3 to 64.3	0.52 to 9.89	0.10 to 5.22	(Montuori *et al.,* 2020)
Jiulong River, Taiwan	1.0-8.1	5.2 - 551.7	2.3-11.8	(Wu *et al.,* 2016)
Soan River, Pakistan	37.4- 187	NA	6.98–30.1	(Malik *et al.,* 2014)

NA-data is not available, nd-not detected

Table 5. Comparison of OCP and PCB concentrations in sediments of the study area with TEC and PEC values in ng/g dw.

	TEC		PEC		Sabaki estuary (Range ng/g)	Tana estuary (Range ng/g)
	TEL	ER-L	PEL	ER-M		
DDD	3.54	2	8.51	20	0.27-2.21	0.26-5.47
DDE	1.42	2	6.75	15	0.06-0.30	0.03-0.87
DDT	NG	1	NG	7	0.09-0.98	0.07-2.36
Total DDT	7	3	4.45	350	0.41-3.49	0.36-8.43
Heptachlor epoxide	0.6	NG	2.74	NG	BDL-0.04	BDL-0.18
HCB	0.3	NG	1.0	NG	0.02-0.10	0.03-1.16
Chlordane	4.5	0.5	8.9	6	0.02-0.20	0.02-0.18
PCB	34.1	50	277	400	0.68-3.87	0.65-9.29

NG- No guideline

Ecotoxicological assessment

Contaminated sediments can represent potential risks to sediment-dwelling organisms such as benthic and epibenthic species. To evaluate the ecotoxicological significance of sediment pollution by PCBs and OCPs in the Sabaki and Tana estuarine systems, sediment quality guidelines (SQGs) specified by the Canadian Council of Ministers of the Environment (CCME) were applied (CCME 2002). They include threshold effect concentrations (TEC) and probable effect concentrations (PEC). TEC was intended to identify contaminant concentrations below which harmful effects on sediment-dwelling organisms were not expected. PEC was used to identify contaminant concentrations above which harmful effects on sediment-dwelling organisms was expected to frequently occur (MacDonald *et al.,* 2000; Gómez-Gutiérrez *et al.,* 2007). Effects range-low (ER-L) and effects range-median (ER-M) values are used to predict the potential impacts of contaminants in sediments. In addition, the threshold effect level (TEL) and the probable effect level (PEL) was used as the criterion for the prediction of toxicity (Long and Morgan, 1990; MacDonald *et al.,* 2000; CCME, 2002; Gómez-Gutiérrez *et al.,* 2007).

Sediment quality criteria and concentration ranges of major organochlorine contaminants are summarized in Table 4. PCB, HCB, Heptachlor epoxide, and Chlordane concentrations in sediments from the Sabaki and Tana estuarine systems were below ER-M, ER-L, PEL, and TEL values at all the stations, suggesting rather low toxicity to benthic organisms. However, total DDTs values were higher than the threshold effect concentrations (TECs), including TEL and ER-L in both estuarine systems, suggesting that DDT could potentially pose adverse toxic effects to benthic organisms.

Conclusion

This study determined the concentration of various POPs in the sediments from selected locations in Kenya and predicted any potential risk to environmental health. The concentrations of POPs were relatively low suggesting low ecological risk. DDT metabolites were relatively high in the Sabaki and Tana estuaries, indicating potential toxic effects on benthic organisms. This study recommends a need to establish a POPs monitoring programme for continual assessment of potential ecotoxicological risks in Kenya estuaries.

Acknowledgments

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Seagrass restoration trials in tropical seagrass meadows of Kenya

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Abstract

The degradation of seagrasses is becoming prevalent in the Western Indian Ocean (WIO) region due to anchor damage, sea urchin herbivory, extreme events such as cyclones and floods and anthropogenic factors such as pollution and sediment inflows. Consequently, there have been numerous efforts to advance the restoration of degraded seagrass beds in several countries in the region. In Kenya, experimental restoration efforts were started in 2007 in response to seagrass habitat degradation due to sea urchin herbivory. Although the initial efforts experienced challenges, there were lessons learned which provided insights into subsequent restoration work using different techniques. In this paper, insights are provided into three types of restoration techniques; the sod technique, the seagrass mimic technique, and the Hessian bag technique. In the case of the sod technique, *Thalassodendron ciliatum* showed a decline from 20 ± 1.7 shoots sod^{-1} in the first three weeks to 7 ± 4.4 shoots sod^{-1} at the end of the experimental period of the study, while *Thalassia hemprichii* sods showed an increase from 28 ± 3.4 shoots sod^{-1} to 32 ± 2.7 shoots sod^{-1} over the same period. For the Hessian bag method, the expectation was that the pilot site would be filled with the transplanted seagrass species, *Thalassia hemprichii*, but the findings showed that different species including *Halodule uninervis*, *Syringodium isoetifolium*, *Halophila stipulacea*, *Cymodocea rotundata*, and *Cymodocea serrulata* colonized the area. This indicated that it was not possible to restore the area to its original status, but that the area could be rehabilitated. The costs of restoration have also been assessed as well as community participation in such initiatives. These findings provide insights for restoration efforts in Kenya and provide a baseline for future work.

Keywords: *Thalassodendron cililatum*, *Thalassia hemprichii*, rehabilitation, sods, mimics, Hessian bag

Introduction

Seagrasses are widely distributed in shallow coastal areas throughout the world with over 60 described species, 12 genera and four families (Short *et al.*, 2007; Orth *et al.*, 2006). Seagrasses have been traditionally used for roof covering, as medicine, fertilisers, and their seeds as a food source (de la Torre-Castro and Rönnbäck, 2004). They provide shelter for breeding, nursery and feeding grounds for herbivorous fish, dugongs and turtles (Björk *et al.*, 2008). They also support a diverse assemblage of plant and animal species which include macroalgae that grow as epiphytes on the stems and leaves of the seagrasses (Uku, 2005), and invertebrates that include sea cucumbers, sea urchins, shrimps and lobsters (Ochieng and Erftemeijer, 2003). Due to these attributes they function as an important food source and support the livelihoods of local communities. Seagrass beds are important in sediment stabilization, nutrient cycling, shoreline protection, enhancement of water transparency, biological system support and carbon sequestration because of their slow decomposition rate (Muthama and Uku, 2003; Orth *et al.*, 2006; Juma *et al.*, 2020). They therefore have a high economic value (Costanza *et al.*, 1997; Björk *et al.*, 2008) which is further elaborated by Mtwana Norlund *et al.* (2016) who highlighted ecosystem services and values associated with water purification, provision of cultural artefacts, coastal protection, fertilizer and pharmaceutical uses.

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In recent years, seagrass beds have been altered due to frequent anthropogenic and natural disturbances (Waycott *et al.*, 2009; Paulo *et al.* 2019). Anthropogenic threats that have led to the decline of seagrasses globally include pollution, dredging, destructive fishing activities, aquaculture, invasive species introduction and overfishing of predators, among others (Waycott *et al.*, 2009). Climate change effects include rising sea levels, increase in sea temperature and flooding, leading to a rate of seagrass loss of approximately 7 % per year and this places seagrasses at the same level as mangroves and corals as the most threatened ecosystems on earth (Waycott *et al.,* 2009). In Kenya, the loss of seagrasses over time has been documented to be from 0.29 % yr^{-1} in 2000 to 1.59 % yr^{-1} in 2016, which is lower than the global rate of loss (Harcourt *et al.*, 2018), but has the potential to increase due to the increasing threats in these systems. These threats affect seagrass beds and their ability to provide ecosystem services including their ability to sequester carbon.

Seagrass beds have been degraded in Kenya due to sea urchin herbivory (Eklof *et al.*, 2008) and this has implications on the functionality of these important critical habitats. Further to this, some of the seagrass species within the coastline of Kenya fall among those that are considered threatened on a global scale, such as *Zostera capensis* (Short *et al.*, 2011). The loss in these critical habitats threaten critical ecosystem functions such as nutrient cycling, photosynthesis, carbon sequestration, sediment stabilization and key fisheries and biodiversity that are supported by these ecosystems (Irving *et al.*, 2014). In Kenya and Mozambique, loss of seagrass cover has been documented to lead to a loss of fauna, and decreased sediment stabilization leading to erosion exposure in degraded areas (Eklof *et al.*, 2008; Amone-Mabuto *et al.*, 2017).

Seagrass meadows take several years for natural recovery from disturbances, with some species like *Posidonia spp.* taking up to 100 years to cover a cleared substratum (Kirkman and Kuo, 1990). Therefore, in an attempt to enhance recovery times, various restoration methods have been developed (Irving *et al.*, 2014, Paulo *et al.*, 2019). Two approaches can be undertaken to restore ecosystem services. This can be done through (i) the reduction of the threats facing these ecosystems, or (ii) by physically restoring the ecosystems through harvesting seagrasses from a donor site and transplanting these plants in the degraded area. Physical restoration efforts have been undertaken worldwide with some successes and failures reported (Paling *et al.*, 2009; Bayraktarov *et al.*, 2016).

Conservation and restoration of seagrass beds can be enhanced by transplantation of plants from natural seagrass beds (Paling *et al.,* 2001). Their restoration, usually conducted in areas affected by eutrophication, coastal construction and mechanical damage from boat propellers and fishing nets (van Katwijk *et al.*, 2016), is expected to lead to recolonization and creation of new meadows (Paling *et al.*, 2007; van Katwijk *et al.*, 2016). Restoration has been undertaken in several parts of the world with the most successful work being undertaken in Australia by Murdock University (van Keulen *et al.,* 2003; Paling *et al.,* 2003,) using the species *Posidonia spp.* and *Amphibolis griffithii* (Black, den Hartog) (Paling *et al.,* 2001). Seagrass transplantation has also been conducted on a large scale using mechanically transplanted sods with the help of specially designed underwater seagrass harvesting and planting machines with a capacity of planting of up to 18 sods day^{-1} (Paling *et al.*, 2001). Such restoration efforts have been performed world-wide to compensate or mitigate seagrass losses and have been shown to enhance the associated ecosystem services (Orth *et al.*, 2020; Tan *et al.*, 2020; UNEP, 2020).

Several lagoons in Kenya including the Diani – Chale lagoon have been affected by seagrass degradation. The area is dominated by tourist activities and the seagrass beds form artisanal fishing grounds. Available information shows extensive seagrass decline especially for the dominant species, *Thalasodendron ciliatum* Forskal den Hartog (Uku *et al.*, 2005). This decline was attributed to the proliferation of the sea urchin, *Tripneustes gratilla* (L.) (Eklof *et al.*, 2008). The rapidly increasing rate of seagrass degradation compared to the low rate of natural recovery has increased the demand for seagrass restoration in Kenya. This widespread seagrass loss led to the experimental trials of restoration using different methods with varying measures of success.

In this paper the prospects of seagrass restoration efforts in Kenya are described through the evaluation of trials of seagrass restoration in the country, and recommendations are made on the methods that are suitable for restoration. Experiences are shared in seagarass restoration experiments conducted on the south coast of Kenya, in Diani and Wasini, from 2007 to 2015. It was attempted to determine the following aspects of restoration in these experiments: (i) which species of the 11 seagrasses found in the degraded areas could be used for restoration; (ii) which time of the year is best suited for seagrass restoration; and (iii) which restoration method yields the best outcome. Over this period, areas were worked in that were impacted by natural degradation caused by sea urchin herbivory and an area that was impacted by anthropogenic degradation caused by boat anchorage and trampling.

This paper is aimed at sharing some of the lessons learnt in the seagrass restoration pilot studies carried out in Kenya whose methodologies could potentially be used in other areas of the WIO Region.

Methods

Data has been compiled in this paper from three restoration trials conducted in Kenya between 2007 and 2015. All the trials occurred on the south coast of Kenya, which had been greatly impacted by sea urchin herbivory. By the time the trials were being undertaken the seagrass herbivory had halted and the sea urchin numbers had declined. The trials consisted of different planting techniques, in shallow subtidal areas, and different monitoring frequencies. The frequency of monitoring for the different techniques was varied and in some cases the only data available was for the initial planting effort versus the final monitoring of the transplanted seagrasses.

To test for success of the rehabilitation technique, the survival rate was determined, as explained in the different transplantation techniques, and also documented colonization by other seagrass species. In most of the restoration work, regular monitoring was not possible due to financing gaps and the measure of restoration success was based on the site assessments at the start and end of the experimental period that varied with the different methods used.

Seagrass transplantation using the sod technique

The experimental work using the sod technique was conducted in 2007 in Diani. Three experimental seagrass plots of 10 m^2 within the Diani lagoon were established. The plots were separated by 20 meters from each another. The three plots of 10 m^2 were planted with seagrass making a total area of 300 m^2 (0.03 ha). The sod/plug technique was used following a protocol developed by van Keulen *et al.* (2003). Although the initial intention was to establish equal replicates of *Thalassodendron ciliatum* and *Thalassia hemprichii* plots, the final work yielded two plots of planted *Thalassodendron ciliatum* sods and one plot of *Thalassia hemprichii* sods, therefore covering a final area of 0.03 ha.

The two species were selected due to their previous occurrence in the study site. Each sod/plug consisted of bundles of viable shoots, with attached rhizomes, which were collected from healthy beds and transported to the recipient sites to be transplanted within 2 hours. The sods were collected using a corer of 18 cm diameter and planted by carefully fitting them into prepared holes. The sods were planted 1m apart and there were approximately 20 shoots per sod. The planted sods were initially monitored weekly for shoot density, canopy height and the number of sods remaining during each visit (Fig. 1). Recovery success was measured in terms of percentage survival of

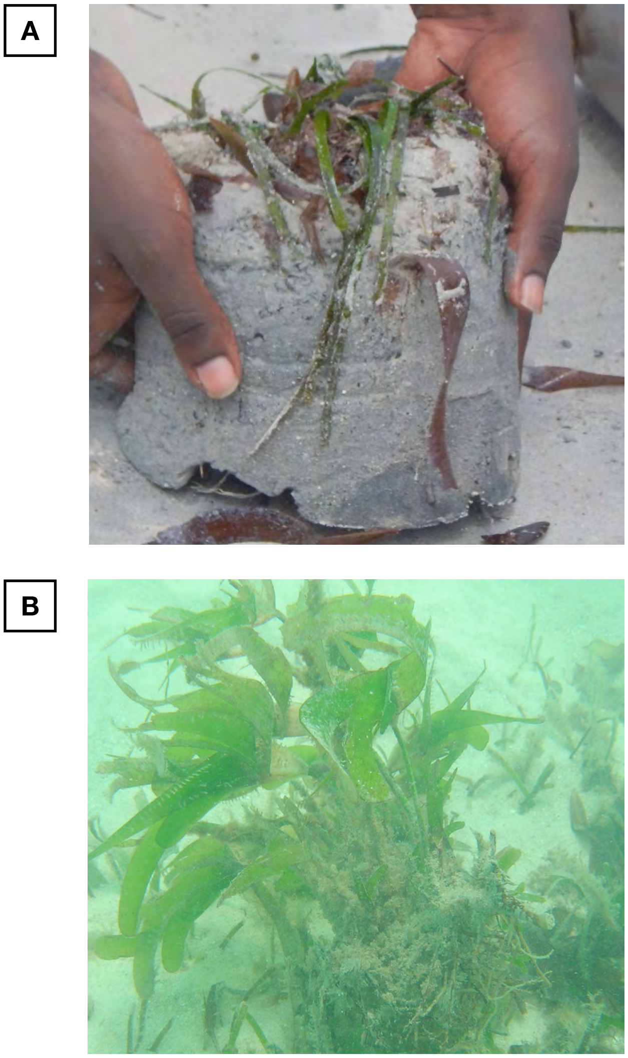

Fig. 1. (a) A sod retrieved from a healthy area and (b) sods planted within the degraded area.

transplanted sods by counting the number of remaining sods during every field visit. The initial monitoring was conducted over three weeks during the North East Monsoon (NEM) which is represented by calmer sea conditions, while the rest of the monitoring was conducted within the South East Monsoon (SEM) season which is represented by rough sea conditions.

Restoration using plastic seagrass mimics

Plastic seagrass mimics resembling *Thalassia hemprichii* were set up in the Diani lagoon in 2008 to undertake a three-week experiment on colonization of degraded areas by epiphytic meiofauna (Daudi *et al*, 2013). The mimics had an average plant surface area of 146.8 cm^2 (SE ± 17.3). The mimics were anchored into the sediment using stay rods with an oval eye and cable ties were used to secure them to the rods (Fig. 2). A total of six mimics were set in each plot providing a total 18 mimics in the experimental restoration site of 0.03 ha. Although the seagrass mimics were set up for the assessment of colonization by epiphytic meiofauna, they yielded results that enhanced understanding of the potential for the use of artificial seagrasses in the rehabilitation process (Tuya *et al*., 2017). The mimics were harvested after 21 days to collect associated epiphytic meiofauna and the findings of this work have been published in Daudi *et al.* (2013).

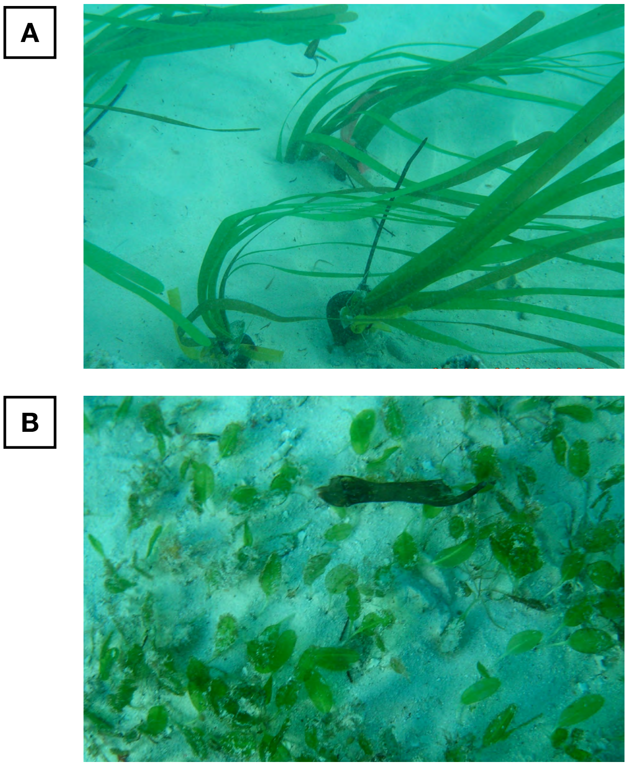

Fig. 2. (a) Seagrass mimics anchored with an oval eyed metal rod and (b) *Halophila ovalis* observed around the seagrass mimics.

Seagrass transplantation of seedlings using the modified Hessian bag method

The use of the Hessian bag technique was undertaken on the south coast at Wasini Island in 2015. The seagrass seedlings were harvested using a PVC corer from healthy *T. hemprichii* meadows in Mkwiro (*Nyuma ya maji*), approximately 8 km from the restoration site. The harvested sods were carefully transported to the restoration site within two hours of harvest to avoid desiccation. The sods were further processed by separating them into individual *T. hemprichii* seedlings.

The method used was a modification to the biodegradable Hessian bag protocol by Irving *et al.* (2014). In the original method, the Hessian bags were filled with sand and placed on the substrate to provide an anchorage for recruits of *Amphibolis antarctica* (Labill.) Asch. In the present study, the Hessian bags (jute bags), which were 1 m wide and 1.2 m long, were cut open lengthwise on one side and the bottom and flattened out before the planting process. They were punched with small holes using dive knives and the seedlings were planted in these holes. The bags were anchored in the restoration site using mangrove poles and 50 bags were joined to make an area of approximately 0.012 ha. A spacing interval of 20 cm for the seedlings was used. Each plant unit consisted of approximately three shoots with attached rhizomes grouped together as a cluster. Fifty jute sack bags were used with each sack having approximately 40 planted seedlings (Fig. 3). This resulted in approximately 2000 seedlings over a surface area of 0.012 ha.

Community engagement in the seagrass restoration work

Members of the fishing community from the nearby Beach Management Units (BMU) were included in the survey and establishment of the transplantation plots in all the seagrass restoration work. In Wasini, as the restoration effort was within their co-management area (CMA), more effort was made to involve the BMU members fully. The project design was disclosed to the community members at the onset of the work. The members selected the restoration site. As a follow up, 30 community members from the Beach Management Unit were trained in the establishment and monitoring of the experimental site. They also had the responsibility of safeguarding the site from other threats. As the technical team was unable to visit the site as envisioned, the community members from the BMU were expected to provide an in-kind contribution by monitoring the site and providing feedback on what they saw.

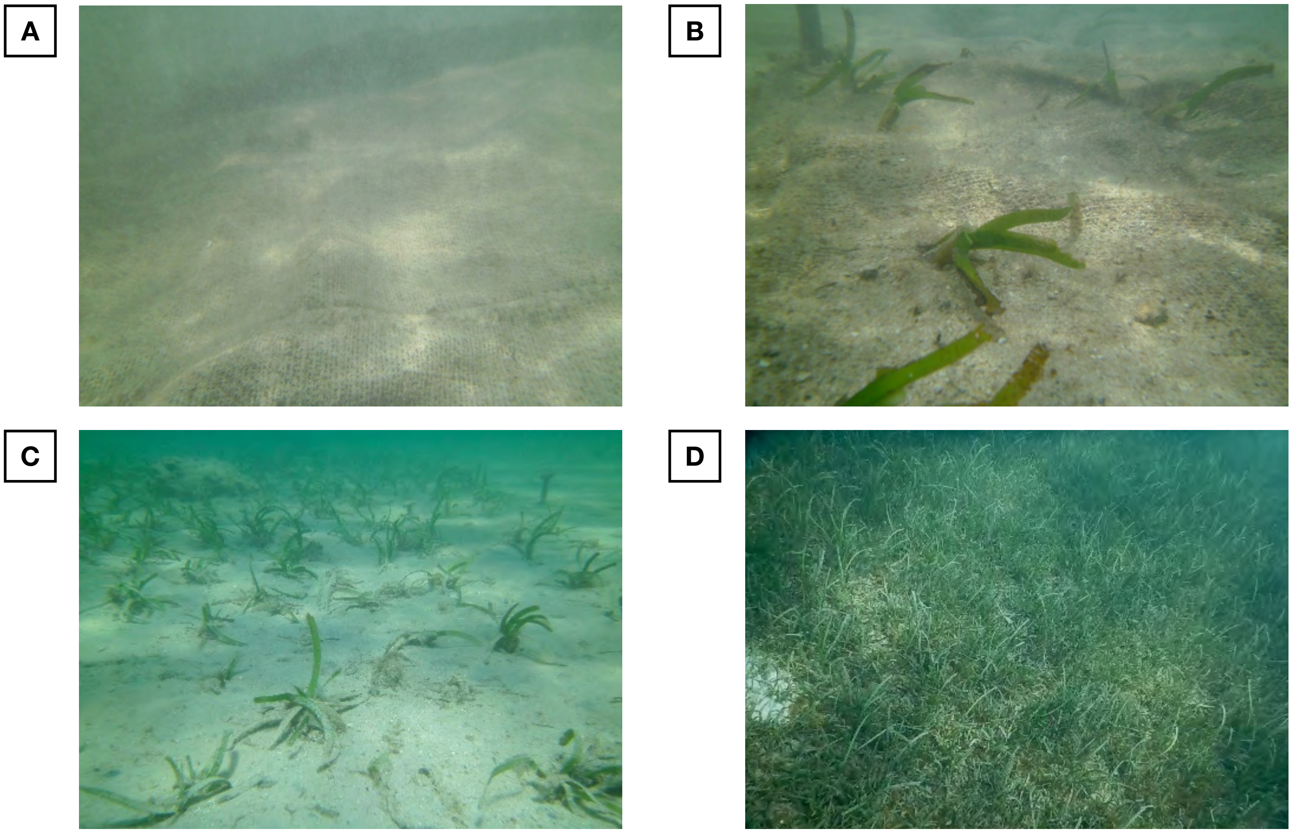

Fig. 3. (a) Hessian/Jute bags anchored for planting seedlings, (b) freshly planted *T. hemprichii* seedlings, (c) healthy growing *T. hemprichii* 6 months after establishment of the site, and (d) rehabilitated area colonized by other seagrass species in the restoration site in Wasini.

Results

The survival of seagrasses using the sod technique in Diani

The survival of the entire sod was estimated in Diani. The sod survival of *T. ciliatum* ranged between 26 ± 0.9 sods plot^{-1} to 5 ± 2.9 sods plot^{-1} while that of *T. hemprichii* ranged from 22 sods plot^{-1} to 15 sods plot^{-1} during the monitoring period which was in the first three weeks after transplantation, and later in the 32nd week after transplantation, Sod survival in the first three weeks was significantly higher than the last six weeks of monitoring for both species (Mann Whitney U=0.00; $p<0.05$ (Fig. 4). *T. ciliatum* experienced a high rate of loss of sods at 81 % whereas for T. *hemprichii* the loss was much lower from the first week to the 37th week of the experiment. Significant differences were observed between shoots per sod for the two species with a decrease in the number of shoots per sod for *T. ciliatum,* from 20 ± 1.7 shoots sod^{-1} in the first three weeks to 7 ± 4.4 shoots sod^{-1} at the end of the experimental period (Fig. 5). Monitoring from week 32 however showed a general switch between the two species with an increase in the number of shoots per sod for *T. hemprichii* and a decline of the same for *T. ciliatum* (Fig. 5). *T. hemprichii* increased from 28 ± 3.4 shoots sod^{-1}at the start of the experiment to 32 ± 2.7 shoots sod^{-1}.

The canopy heights were highest for *T. ciliatum* (17 cm) at the beginning of the experiment but reduced continuously over time up to approximately 7 cm at the end of the monitoring period, showing a reduction of about 60 % (Fig. 6). Contrary to this, *T. hemprichii*, which recorded initial canopy of approximately 7 cm, showed an increase in canopy height of 8 cm at the end of the experiment. Significant differences were observed for canopy height between both species as well as between the different monitoring periods (Mann-Whitney U=5050 for differences between species and U=1791.5, U=433.5 for *T. ciliatum* and *T. hemprichii*, respectively).

Seagrass restoration using mimics in Diani

Follow up observations of the seagrass mimics showed that none of the seagrass mimics were uprooted in Diani. Observations, though not quantitative, indicated that the mimics were surrounded by the pioneer seagrass species *Halophila stipulacea* (Forsskål)

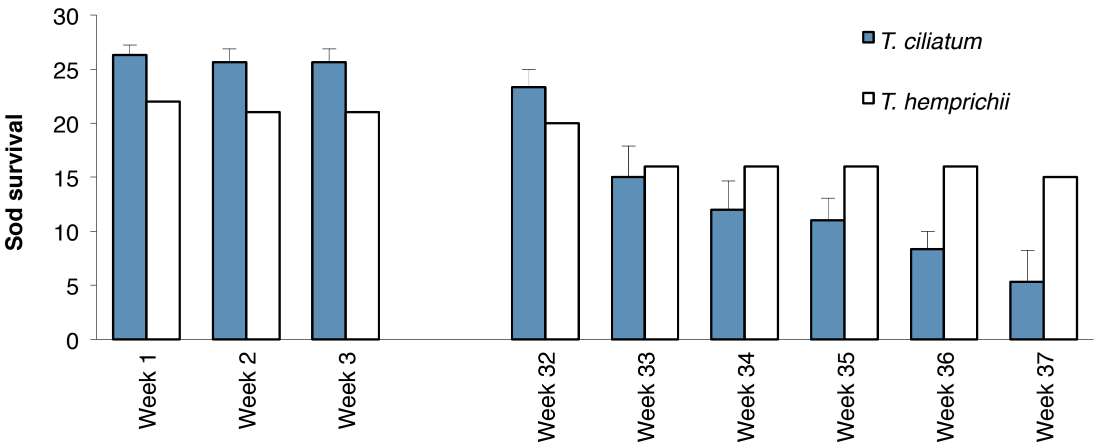

Figure 4. Variation in sod survival of *T. ciliatum* and *T. hemprichii* for the monitoring period (Note that as there was only one plot for *T. hemprichii* there is no standard error for this species).

Ascherson towards the end of the three weeks of deployment. These observations indicated that the mimics were important in stabilizing sediments around them thus allowing colonization by the pioneer species.

Seagrass restoration using seedlings on Hessian bags in Wasini

Approximately 2000 seedlings of *T. hemprichii* were planted in the study area at Wasini. Due to the disruption in funding, the site was not monitored as it should have been by the technical team. The community members reported the recolonization by associated epiphytes and seagrasses six months after replantation. They also reported instances of herbivory of the shoots but did not estimate the quantity of the loss of shoots. The community monitoring was irregular and not as rigorous as was anticipated.

An assessment conducted three years later in 2018, revealed colonization by other seagrass species other than the replanted species, *T. hemprichii*, and seagrass cover was documented to be approximately 75 % in the area that was previously bare of seagrasses. The new colonizing species included *Halodule uninervis* (Forskål) Ascherson (29 % cover), *Syringodium isoetifolium* (Ascherson Dandy) (39 % cover) and *Halophila stipulacea* (Forskål) Ascherson (7 % cover). *Cymodocea rotundata* Ehrenberg & Hempr. ex Ascherson and *Cymodocea serrulata* (R. Br.) Ascherson & Magnus, were also noted at the site. The spread of the species around the restored area showed a wider expanse of spread of these species, though the coverage was not estimated.

The costs of restoration using the different methods

Table 1 shows the associated costs of restoration which need to be considered when adopting such activities.

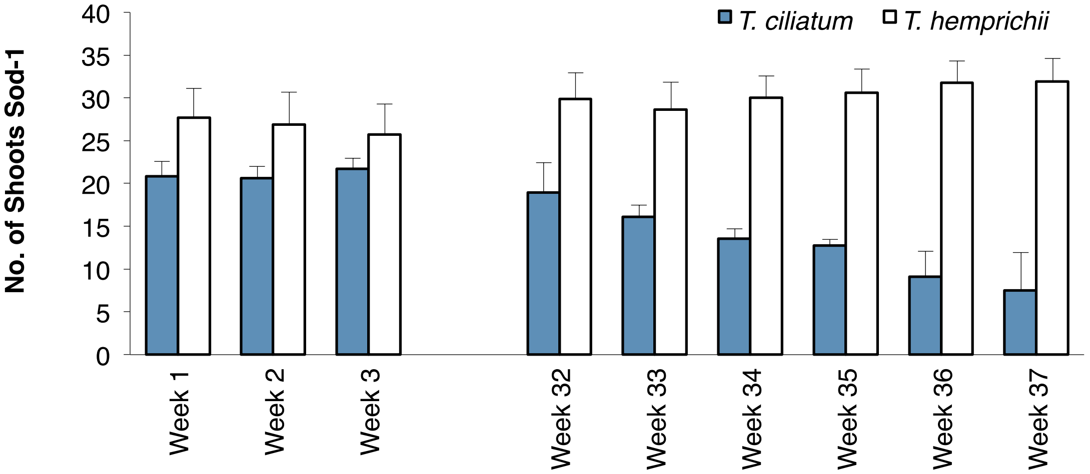

Figure 5. Variation in the number of shoots per sod of *T. ciliatum* and *T. hemprichii* for the monitoring period.

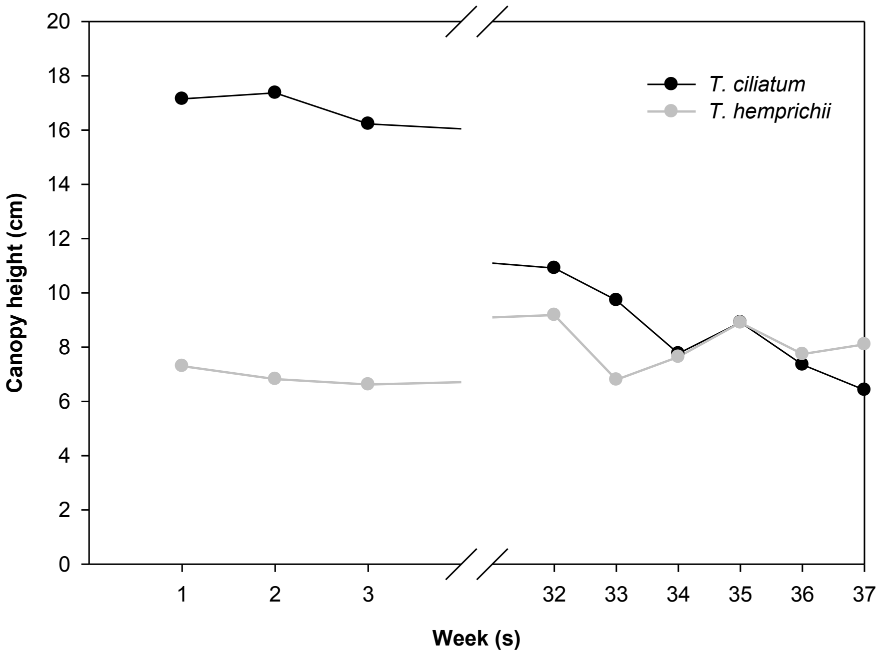

Figure 6. Canopy height for *T. ciliatum* and *T. hemprichii* during the monitoring period.

The costs include the costs of manpower through technical expertise from researchers, community participation from local fishermen, materials/equipment, and the costs of monitoring and evaluation. The costing does not consider the time spent doing the work for the fishers who monitored the sites as part of their fishing effort. The highest cost incurred was for the Hessian bag method (Table 1).

Discussion

Seagrass restoration using the sod method

Restoration using the sod technique has been documented to yield higher chances of transplant success (van Katwijk *et al.*, 2009) and this was expected from this work for the two species used. However, the sods were impacted by the effect of seasonality. Planting of the sods was carried out during the NEM season and this represented the period of stable sod survival for both *T. ciliatum* and *T. hemprichii*. This is a result of the calm weather conditions that are normally associated with this season. On the other hand, the SEM season is characterized by rough sea conditions that destabilized the planted sods and this was evident in this study with lower survival of sods in July/August 2008. Van Katwijk *et al.* (2009) indicate that the outcome of transplantation of seagrasses is dependant on hydrodynamic stressors and disturbances while Diego *et al.* (2019) indicate that transplants

Table 1. Cost estimates for the seagrass restoration work.

Site	Year	Planted area	Plant material used	Cause of degradation	Approximate costs of rehabilitation (costs in USD for the restored area)
Diani	2007 to 2008	0.03 ha	Sods/Plugs (*Thalassodendron ciliatum, Thalassia hemprichii*)	**Urchin herbivory (natural causes)**	Technical support = 2,609 Community costs = 862 Equipment costs = 2,529 Costs per ha = 200,000
Diani	2008	0.03 ha	Seagrass mimics	**Urchin herbivory (natural causes)**	Technical support = 500 Equipment costs = 360 Costs per ha = 28,667
Wasini	2015	0.012 ha	Seedlings using Hessian/ Jute bags (*Thalassia hemprichii*)	**Boat propeller damage and trampling (Anthropogenic causes)**	Technical support = 6,373 Community costs = 760 Equipment costs = 2,667 Costs per ha = 817,000

did not survive winter conditions and strong storms in Marine Park Professor Luiz Saldanha in Portugal. The timing of planting is critical and the NEM is most appropriate for planting seagrass rehabilitation meadows in the WIO region which is influenced by the monsoonal seasonal variations. Sods must be planted at the onset of the low energy NE monsoon season when there are no strong winds and waves, as the seagrass roots and rhizomes need to have time to stabilize in order to ensure success.

The sod technique in this study showed greater success for *T. hemprichii* suggesting that this species was more successful for transplantation using the sod method and was more resilient to seasonal environmental changes. The increasing shoots observed for *T. hemprichii* also suggests a more successful vegetative reproduction mode for this species than for *T. ciliatum.* In this assessment, the sod method provided a loose anchorage for *T. ciliatum* and the shoots were carried off easily by wave action. It can only be postulated in this paper that the success of *T. hemprichii* could be due to their growth form and adherence to sandy substrates on shallow intertidal flats, while *T. ciliatum* favors growth on mixed sandy and rocky or hard substrates in deeper infra-littoral zones (Aleem, 1984; Gullstrom *et al*., 2002). *T. cilatum* sods may have also required additional anchorage to enable them to settle on the substrate as demonstrated by van Katwijk (2016). Further to this, the stocking density may also have had an influence as more shoots were contained in the *T. hemprichii* sods compared to the *T. ciliatum* sods. *T. ciliatum* was originally the dominant species at the restoration site, and its loss from the areas may have led to seabed erosion (Ekloff *et al.,* 2008) and altered the sediment to the extent that anchorage by this species, which has shallow rhizomes (Ekloff *et al*., 2008), was not possible.

Seagrass restoration using mimics

Although the experiment using the seagrass mimics was set up to address a different set of questions, it provided insights on the opportunity that the mimics can provide for the settlement of sediments in bare sand areas. The mimics promoted the settlement of pioneer species such as *Halophila stipulacea* (Forsskål) Ascherson. This demonstrates an opportunity for use as technique in areas that may be too rough for the sod technique and Hessian bag method and yet require rehabilitation. It is further recommended that instead of metal anchors, biodegradable material such as bamboo can be considered as this can be bent to provide strong anchors on the substrate (Calumpong and Fonseca, 2001). Tuya *et al*. (2017) also demonstrated the usefulness of artificial seagrasses in increasing the survival of transplanted seagrass as they functioned as a shield against herbivory. In some instances, such seagrass mimics can be used to stabilize sediments and allow for the establishment of seagrasses through natural recovery (UNEP, 2020).

Seagrass restoration using the Hessian bag technique

Given the success of *T. hemprichii* restoration using the sod technique in 2007, it was decided to use this species in the work undertaken using the Hessian bag method in Wasini in 2015. Irving *et al*. (2014) developed the Hessian bag method for facilitating recruitment of *Amphibolis antarctica* seedlings *in situ*, where bags were filled with sediment from donor beds and deployed in restoration sites. This method was modified in the current study to use vegetative shoots rather than filling the bags with sand. Since the donor bed was further away from the transplantation site, this also ensured that it was not necessary to depend on donor meadows as a source of seed as was the case in Irving *et al*. (2014). The Hessian bag served as anchoring material for the planted seedlings to prevent them from being uprooted due to waves and currents. The bag also facilitated the trapping of sediments which served as a substrate for root and rhizome development. From this study it is recommended that this modified method is adopted as it is convenient for transplantation of *T. hemprichii* seedlings in shallow subtidal sites which have a sandy substrate and could also be applied to other species with similar morphological characteristics. Further, the Hessian bag method is environmentally friendly as the sisal material disintegrates in the water and the mangrove poles are eventually uprooted and disintegrate. As the bags and mangrove poles can easily be found locally and since they are biodegradable they do not harm the environment. Further success in this area was achieved through the removal of the threat from boat anchor damage and trampling by the introduction of mooring buoys in the area outside of the seagrass beds.

The Hessian bags provided settling substrate for colonization by pioneer species and other intermediate species such as *H. uninervis*, *S. isoetifolium, H. stipulacea*, *C. rotundata* and *C. serrulata* apart from *T. hemprichii* which was the transplantation species.

The costs of restoration

The overall cost of transplantation using the different methods in these experiments was within the reported costs of seagrass restoration projects of USD 630,000 per hectare reported by Calumpong and Fonseca (2001), although the Hessian bag technique was slightly higher. The Hessian bag method required a large amount of manpower to establish a plot and more work needs to be done to reduce the costs of using this method. Further investigations using the mimic method, for a longer duration of time, may yield a much cheaper and cost-effective restoration approach.

Conclusions

The experimental restoration work showed that there are key aspects that need to be considered to ensure the success of seagrass restoration projects. These include the following:

1. Seasonal stressors are important as demonstrated by the success achieved in the sod establishment method during the calmer NEM period;
2. More work is needed to determine a better approach to the restoration of climax species such as *T. ciliatum* in shallow subtidal areas;
3. Site selection is also critical as it is important that sites are sheltered from high wave action to provide the transplanted seagrass with an opportunity for settlement and spread;
4. Identification and removal of the stressors is important and crucial in providing for re-establishment of seagrass areas (Katwijk, *et al.* 2016) that have been stabilized, as demonstrated through the Hessian bag method where anchoring buoys were introduced, and in the sod method where the work was undertaken when the sea urchin numbers had declined;
5. Preservation of seagrass beds is critical to ensure that there are donor communities from which to obtain seed materials;
6. There is need to monitor the restored areas over long time periods and to share the costs with communities to ensure lower costs of the work. In this study the science support covered the technical aspects of restoration while the community support entailed monitoring of the sites. However, this work revealed the need for repeated training and greater technical oversight in order to ensure that monitoring data is correctly documented by community members;
7. Seagrass restoration success can be hindered by costly and time consuming methods of restoration, absence of a scientific method (such as the use of controls) which may affect evaluation of success, site selection, bioturbation caused by marine organisms, the size of the area targeted, and elevated tidal and wind driven current energy, among other factors (Bell, *et al.*, 2008). Controls should be set in sites that are similar in nature to the restoration site and should be monitored alongside the restoration site; and
8. In this study, the expectation was that the pilot site using the Hessian bag technique would be filled with the transplanted seagrass species, *Thalassia hemprichii*, but the findings showed that different species colonized the area thus indicating that it was not possible to restore the area to its original status. It was however, still rehabilitated with seagrasses. Long term monitoring (beyond 5 years) is required to monitor the colonization process and determine whether the original state is ever achieved.

In summary, if scaled up with consideration of the lessons learnt, this seagrasss restoration trial using successfully tested restoration techniques would contribute to Kenya's efforts towards the sustainable development goal of supporting ecosystem rehabilitation efforts where feasible. Globally such efforts contribute towards the achievement of SDG 14.2 which indicates that by 2020, marine and coastal ecosystems should be sustainably managed and protected to avoid significant adverse impacts, including by strengthening their resilience, and taking action for their restoration in order to achieve healthy and productive oceans. In light of this global target, the findings of the seagrass restoration work in Kenya provide several insights into the processes that can be adopted for the future in this field.

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Improvement of live coral shipping conditions using an illuminated box

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Abstract

The transportation of live coral nubbins is a major constraint of the coral trade. Corals must be transported within a timeframe of <20 hours using concentrated oxygen to avoid high post-transportation mortality. To understand the effects of transportation water quality on coral nubbin growth and post-transportation mortality, a series of transportation simulations were performed on *Seriatopora hystrix.* Different water volumes (dry method, 125 ml, 190 ml, and 325 ml) and oxygen concentrations in the gas phase (21 %, 40 %, and 85 %) were tested, and a 24 LED Handy Lamp was added to provide light irradiance of >100 µmol m^{-2} s^{-1} in the transportation box. During transportation nubbins extracted calcium carbonate for growth reducing water alkalinity and consequently pH to 6.67. Dissolved oxygen concentration also rapidly decreased to 0.19 mg L^{-1}. The concentration of inorganic nitrogen, particularly ammonium ions, increased after 24 hours and reached very high concentrations after 48 hours. Before 48 hours, the larger the water volume and oxygen concentration, the faster the nubbin growth after transportation. Beyond 48 hours, in all cases, reduction in water quality became critical for nubbins and resulted in partial mortality. Illuminating the transportation box improved water quality for 72 hours using normal air.

Keywords: coral transportation, *Seriatopora hystrix*, light irradiance, resistance

Introduction

Over the last few decades, research on coral reefs has mostly focused on coral biology, and the impact of global climate change and ocean acidification (e.g., Salvat and Allemand, 2009; Leblud *et al.*, 2014). This was due to the unprecedented decline of coral reef health status in the majority of coral ecoregions (Wilkinson, 1998, 2008). Coral reefs continue to be mainly exploited for fishery resources and tourism, but also for commercially valuable coral species.

Despite the significant volume of the coral trade, the live coral market remains poorly understood. Aquarium shops or public aquariums constitute the main market with an increasing demand from several countries, especially the USA and Europe (Smit, 1986; Green and Shirley, 1999). Since the 1990s, the harvesting and trade of wild live corals became regulated by the Convention on International Trade of Endangered Species of wild fauna and flora (CITES). All scleractinian corals are registered in Appendix II of CITES (CITES, 2016), and CITES certificates are required for their trade.

Coral aquaculture has been developing worldwide (Ellis and Sharron, 1999; Department of Fisheries of Western Australia, 2009; Albert *et al.,* 2012, Ng *et al.*, 2012; Leal *et al.*, 2013; Todinanahary *et al.,* 2017). According to data from CITES (2016), from 1990 to 2014, total traded live scleractinians reached 156,252 pieces of live aquacultured corals. This is relatively low compared to wild harvesting that represents more

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than 80 % of the total trade, compared to only 1 % for aquaculture (the source of ~19 % being unknown). These results were calculated from importer-reported quantities (data from the CITES database). The principal importer countries are the USA, followed by Japan and France. Corals are mainly imported from Fiji, the Marshall Islands, and Indonesia. Considering the large distances between the importers and exporters, shipping is one of the main constraints of the live coral trade.

With the advent of modern air travel and an increase in flight connections to remote tropical locations, the transportation of live coral across the world has become common and rarely exceeds 30 hours travel time (Delbeek, 2008). However, the COVID19 pandemic has complicated the shipping process. The transportation techniques used are determined on a case-by-case basis, and depend on coral size, stage (larva, recruit, juvenile, or adult colony), and species. A short transportation duration can result in approximately >90 % post-transport survival, using mainly semi-dry (corals are packed in wet tissue or similar) and wet (corals are submerged in seawater) transportation techniques (Carlson, 1999; Petersen *et al.,* 2005). Corals can tolerate a wide range of environmental conditions, but they usually do not tolerate a sudden change in parameters such as temperature, salinity, pH, dissolved oxygen concentration, inorganic nitrogen, and phosphorus concentrations (Osinga *et al.*, 2011; Wijgerde *et al.*, 2014). Light irradiance is also an important factor; its intensity influences coral symbiotic algae photosynthesis and skeletal growth rate (Marubini *et al.*, 2001; Schutter *et al.*, 2008; Juillet-Leclerc and Reynaud, 2010).

Maintaining these parameters within the tolerated range for corals requires large financial investment and production costs in coral aquaculture, especially in *ex-situ* aquariums (Osinga *et al.*, 2011). Moreover, the coral trade involves relocation into a new environment (either directly or after transportation) and requires adaptation to that new environment. The adaptation process generally requires acclimation either upstream (before transportation) or downstream (after transportation), and appropriate conditions must match those in the aquariums (Delbeek, 2008). The range of physicochemical characteristics of water allowing optimal coral growth within these aquariums has been widely studied (Osinga *et al.* 2011). However, both water conditions and coral ecophysiological processes during transportation remain unclear and poorly studied. To date, most studies have focussed on coral larvae (Petersen *et al.*, 2005) or large colony transportation (Petersen *et al.*, 2004). In the context of the aquarium coral market, it is important to understand the variations in the physical and chemical parameters (conditions) of the transportation water to identify the main limiting factors of successful coral nubbin transportation and ensuing husbandry.

The main aim of the present study was to improve coral shipping conditions in order to increase the resistance of small size coral nubbins and decrease post-shipping mortality. The objective was to identify the main water parameters affecting the post-transportation survival and growth of coral nubbins. Experiments were performed to simulate transportation and create well-defined conditions involving parameters such as water volume, dissolved oxygen concentration, and light irradiance.

Materials and methods

Studied species

The species *Seriatopora hystrix* (Dana, 1846) was used for all experiments. This species was chosen due to its availability at the laboratory facility, and because it is one of the most common species globally, and has been one of the most studied over the last few years (Leblud *et al.*, 2014).

Experimental design

All simulation experiments were performed in a laboratory using 1-L borosilicate glass bottles with a wide mouth (GL80) as nubbin transportation containers. The bottles differ from soft plastic bags used for real transportations (lighter, cheaper, and more resistant to breakage than glass), but have the advantage of a perfectly constant shape and volume. The nubbin containers, the number of which depended on the replicates and the experiment, were packaged in cardboard boxes. To better standardize the simulated transportation conditions, the boxes were kept in the dark at ambient temperature in a thermoregulated room of the laboratory (25±1°C). The ratio of water/air (air was enriched in dissolved oxygen in Test 3) in each transportation container was maintained at 1/3, except for in the dry transportation method test.

At least 10 days prior to each transportation simulation, the required number of coral nubbins +30 % were fragmented from mother colonies derived from the ECONUM artificial reef mesocosm (Leblud *et al.*, 2014). Each fragmented nubbin of ~2 g buoyant weight was attached with a nylon line and suspended in the mesocosm for acclimation. The buoyant weight of each nubbin was measured regularly before and after the transportation, and at each sampling period. In addition, each nubbin was photographed after each buoyant weighting for colour aspect observation, using a Canon EOS50D camera (Canon Inc., Ōta, Tokyo, Japan) with a Canon Macro Lens EF 100 mm (Canon Inc.).

The nubbin used for each transportation container was chosen randomly among the acclimated ones (function sample () in the R statistical software).

The first experiment (Test 1) was performed to determine whether the wet or dry transportation method improved the health status of the coral nubbins after transportation. The wet method consisted of transporting a nubbin immersed in a ~415 mL of water (V4 hereafter), while the dry method involved an imbibed strip of a plastic bag, which wrapped the nubbin. For the dry method, the total volume of water used in each transportation container was ~100 mL. The simulation lasted 36 hours and sampling was performed every 12 hours. Triplicate transportation containers were tested for both methods, at each sampling time. The containers of the same sampling time (for both V4 and the dry method) were packaged in the same box. Control containers (without coral nubbins) were also sampled after 36 hours.

In the second experiment (Test 2), the wet method was repeated to test the influence of water volume with an extended sampling time. This experiment aimed to extend the monitoring of the influence on coral nubbin physiology. Three volumes were tested: V1 = 125 mL; V2 = 190 mL; and V3 = 325 mL. 250 mL, 500 mL, and 1000 mL borosilicate glass laboratory bottles with a wide mouth (GL80) respectively were used to maintain the ratio of water/air of 1/3. The simulation lasted 72 hours and sampling was performed every 24 hours. Triplicate transportation containers were tested for both volumes, at each sampling time. Triplicates of control containers of V3 (without coral nubbins) were sampled after 72 hours.

In the third experiment (Test 3), a water volume of 325 mL in 1-L borosilicate containers was used. Three oxygen concentrations (OC) were added to the gas phase of the containers: OC1 = 21 %; OC2 = 40 %; and OC3 = 85 %. OC1 corresponded to the natural concentration of oxygen in the air. OC2 was obtained using oxygen enriched compressed gas mix (Nitrox 40) and OC3 was produced with a mobile oxygen concentrator (Weinmann OXYMAT 3, Hamburg, Germany). The simulation lasted 72 hours and sampling was performed every 24 hours. Five replicates of the transportation container were used for each OC at each sampling time. Five replicates of control containers for each OC were sampled after 72 hours.

The fourth experiment (Test 4) investigated how the addition of a light source in the transportation container affected the nubbins, and then consequently influenced water physicochemical parameters and

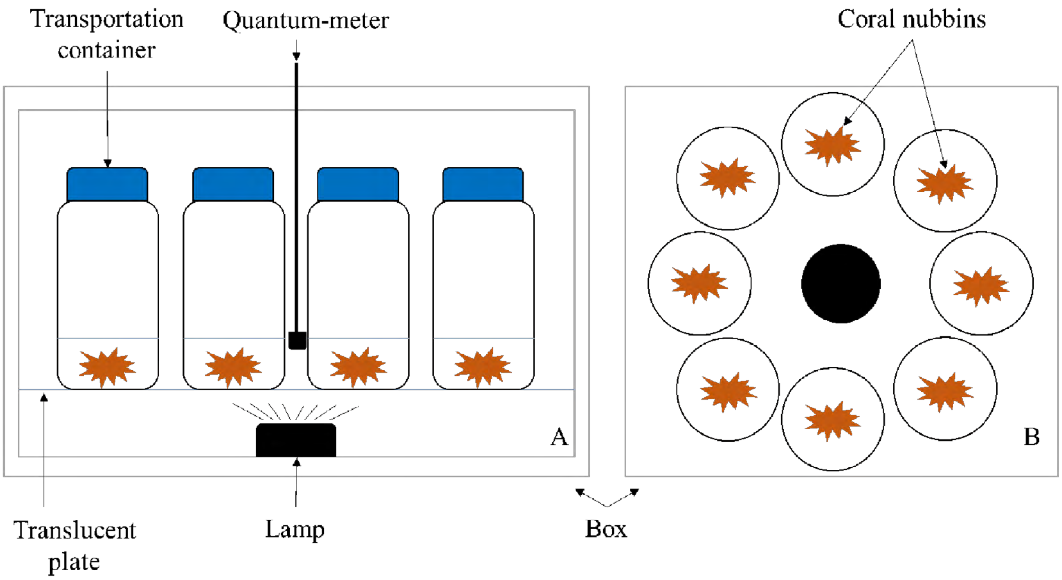

Figure 1. Schematic representation of Test 4. A. Profile view of the box; B. Location of the containers and the light, seen from the top.

post-transportation coral nubbin growth. A water volume of 325 mL and a duration of 72 hours was used. Five replicate transportation containers were established for both light (LB) and dark (DB) boxes, at each sampling time. Frigolite boxes were used to enhance the light irradiance (reflecting white walls). Five replicate control containers for both LB and DB were sampled after 72 hours. Each LB was illuminated with a 24 LED Handy Lamp (Hama GmbH, Monheim, Germany), which was powered by 3 Duracell Ultra alkaline LR20 batteries of 1.5 Volts. This light source was chosen as a representative, realistic, cheap, and single-use system that could be deployed in practice. The lamp was placed below the containers to better disperse the light in each container (Fig. 1). Light irradiance was measured from inside the boxes using a quantum meter (Apogee Quantum MQ-200, Apogee Instruments, Logan, Utah, USA). During the experiment, the intensity of the light irradiance varied from 380 at the beginning to 11 µmol m^{-2} s^{-1} after 72 hours due to battery drain. The irradiance decreased to almost 100 µmol m^{-2} s^{-1} after the first 12 hours before decreasing gradually until the end of the experiment (Fig. 2).

Water physico-chemical parameter monitoring

Salinity was measured with a Cond 340i WTW (Weilheim, Germany, 2002) salinometer. This meter was also used to measure temperature. Dissolved oxygen was measured with a pH/Oxi 340i WTW (Weilheim, Germany). Before each measurement, the CellOx 325 probe was calibrated in water-vapor-saturated air, supplied by the OxiCal®-SL air calibration vessel (WTW, Weilheim, Germany, 2002).

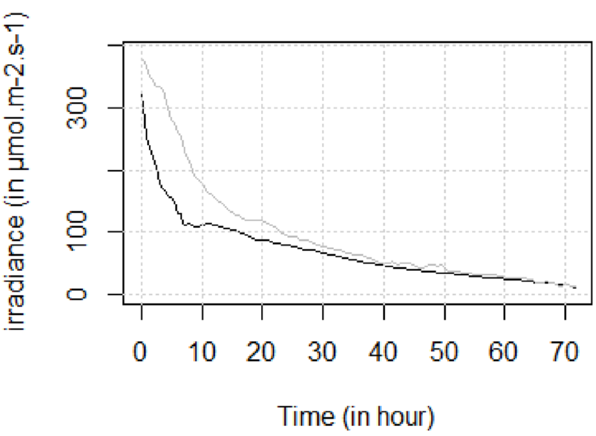

Figure 2. Irradiance variation during Test 4. Each line represents the variation from one box.

The pH_T (pH, total scale) was measured using a m-cresol pH-indicator, following a method adapted from SOP 6b (Dickson *et al.*, 2007). M-cresol colours the sample from yellow (acid) to purple (basic). pH_T is calculated from the absorbance values of the sample at three wavelengths: 548 nm; 434 nm; and 730 nm using the Spectroquant® Pharo100 spectrophotometer (Merck, Darmstadt, Germany; Merck, 2012). The wavelength 548 nm indicates the maximum absorbance for the basic form, while 434 nm applies to the acid form. The 730 nm wavelength allows for water-colour error correction. Absorbance was measured three times at both wavelengths: without m-cresol; with 50 µL; and with 100 µL m-cresol added to 5 mL of the sample. This enabled for the adjusting of the effect of the indicator addition to the real pH of the sample. Calculations were performed with R statistical software (R Core Team, 2015).

Total alkalinity was measured at each sampling time by potentiometric titration. The apparatus, measurement, and calculation methods were adapted from Dickson *et al.* (2007), and are described in Leblud *et al.* (2014).

The concentrations of nitrite (hereafter NO_2^-), nitrate ions (hereafter NO_3^+), ammonium ions (hereafter NH_4^+), and orthophosphates (hereafter PO_4^{3-}) were also determined using an automated colorimetric analysis: a Seal AA3 nutrient analyzer coupled to an XY-2 autosampler (Seal Analytical, Mequon, Wisconsin, USA). Calibrations were performed using standard solutions according to standard Seal procedures. During Test 1 and Test 2, NH_4^+ was dosed by fluorimetry adapted from Holmes *et al.* (1999), modified by Li *et al.* (2005), and refined by Stumpp *et al.* (2012). This method uses a reagent o-phtaldialdéhyde with a reducing agent (sodium sulphite) and a fluorimeter AquaFluor 8000-010 (Turner Designs, San Jose, CA, USA).

Coral nubbin growth monitoring

Weight measurements were performed by suspending the nubbins, which were immersed in seawater, to a hook connected to an electronic balance (Sartorius LE623P, precision = 1 mg; Sartorius Corporate, Goettingen, Germany). The salinity and temperature of the seawater the nubbins were immersed in during the measurements were quantified to calculate the density of the medium. The buoyant weight was converted into skeletal weight using the following equations (Jokiel *et al.*, 1978) that consider the density of the seawater and the skeleton (Davies, 1989):

$$Wstd = \frac{\text{WB}(\rho\text{skeletal} - \rho\text{std})}{\rho\text{skeletal} - \rho\text{sw}}$$

$$WS = \frac{\text{Wstd}}{1 - \frac{\rho\text{sw}}{\rho\text{skeletal}}}$$

where, WB is the measured buoyant weight, WS is the skeletal weight, Wstd is the standard buoyant weight, ρskeletal is the skeletal density (the value used here is the density of aragonite [ρskeletal = 2930]), ρstd is the density of seawater at a temperature of 25°C and salinity of 35 psu, and ρsw is the density of seawater during the measurement.

The growth rate was calculated using the following equation:

$$k = \frac{100\,\frac{\text{WSt}-\text{WSt0}}{\text{WSt0}}}{t}$$

where, k is the growth rate (% day^{-1}), WSt is the skeletal weight at time t (g), WSt0 is the skeletal weight at time t = 0 (g), and t is the time (day). Measurements were performed at the same time on each measurement day.

In addition to the growth rate measurements, the nubbins were photographed at each sampling time to evaluate their health status by comparing their colour and visual aspect. The following three main nubbin health statuses were recorded: healthy; bleached; and (partly) dead (Fig. 3).

Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2015). Descriptive statistics were calculated first. The normality of the data was determined using a Shapiro-Wilk test, and the homogeneity of the variance was calculated using Levene's test. The significance of difference in means was determined using one-way ANOVA or t-test according to the number of the compared variables, with a level of 5 %. The Kruskal-Wallis test was used for non-normal data and/or data with non-homogeneity of the variances. For multiple comparison analysis, Tukey's test was used for pairwise comparisons between stations where the null hypothesis of equal means was rejected by ANOVA. The Pearson correlation was also calculated.

Results

Influence of volume, oxygen concentration, and light on water parameters

In the dry and wet method (V4), the concentration of dissolved oxygen, total alkalinity, and pH_T rapidly and significantly decreased compared to the initial values (Fig. 4A, B, C). Dissolved oxygen and pH_T decreased significantly more in the wet method within 24 hours ($p < 0.01$), while no difference was observed after 36 hours. There was a significant decrease in total alkalinity after 36 hours ($p < 0.05$), but no difference was observed between V4, the dry method, and the control containers.

The nutrient concentration results revealed a significant increase in total inorganic nitrogen (hereafter N_{ti}) in the dry method, compared to V4 and the control containers ($p = 0.0006$). This was strongly influenced by the high concentration of NH_4^+ and NO_2^- in the transportation water, which increased respectively from an initial concentration of 0.491 µmol L^{-1} (NH_4^+) and 0.160 µmol L^{-1} (NO_2^-) to 9.835 µmol L^{-1} (NH_4^+) and 0.355 µmol L^{-1} (NO_2^-) after 24 hours (Fig. 5A).

During Test 2, there was a significantly larger decrease in dissolved oxygen concentration and pH_T in V1, V2, and V3 containers compared to the initial

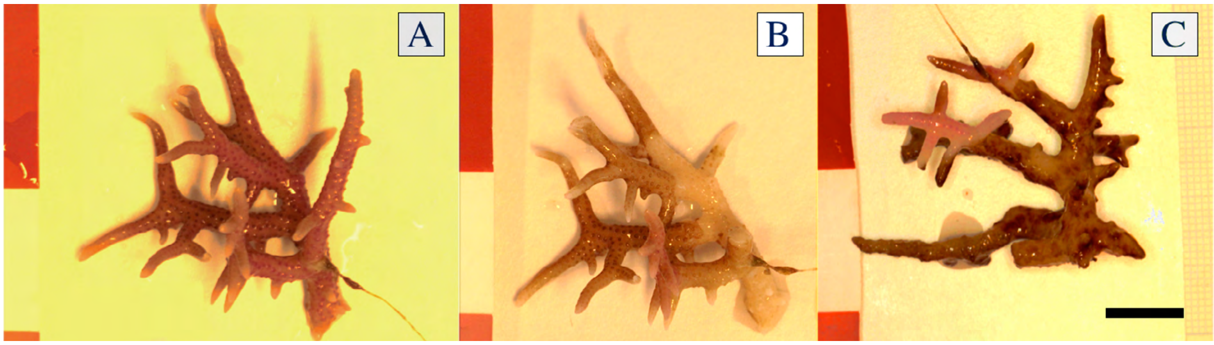

Figure 3. Colour and visual aspect of a nubbin before (A), at unpacking (B), and 2 weeks after transportation (C). A represents a healthy nubbin; B represents a bleaching/partly died nubbin; and C is a dead nubbin. Scale bar = 1 cm

concentration after 24 hours (respectively $p = 0.002$ and $p < 0.0001$) (Fig. 4D). These values remained stable for 72 hours, except in V3, in which the concentration of dissolved oxygen significantly decreased to 0.02 mg L^{-1} compared to V1, V2, and the control containers ($p = 0.0003$), with 3.34 ± 0.21 mg L^{-1}, 2.4 ± 0.57 mg L^{-1}, and 4.55 ± 0.62 mg L^{-1}, respectively. pH$_T$ significantly ($p = 0.0001$) decreased to 7.03 ± 0.06, compared to 7.77 ± 0.01, 7.51 ± 0.03, and 7.92 ± 0.03 in V1, V2, and control containers, respectively (Fig. 4F).

In contrast to the dry method, total alkalinity gradually increased in V1 and V2 over 72 hours, while in V3, it decreased over 48 hours comparable to the dry method and in V4; however, it promptly increased after 72 hours (significant difference between 48 hours and 72 hours, with 2.24 ± 0 mmol kg-$^{\text{soln-1}}$, and 3.03 ± 0.17 mmol kg-$^{\text{soln-1}}$, respectively [$p = 0.0008$]).

In V1 and V2, N_{ti} significantly decreased over 72 hours ($p = 0.0008$), while an increasing concentration was observed in V3 from 24 hours (Fig. 5C). The overall concentration of PO4^{3-} remained below 0.1 µmol L^{-1} before gradually increasing to 1.72 ± 0.16 µmol L^{-1} in V1 and V2 after 72 hours. It was sharply increased to 26.09 µmol L^{-1} (maximum) in V3 after 72 hours (Fig. 5D). This concentration significantly differed from the rest of the tested volumes and dry method ($p < 0.0001$).

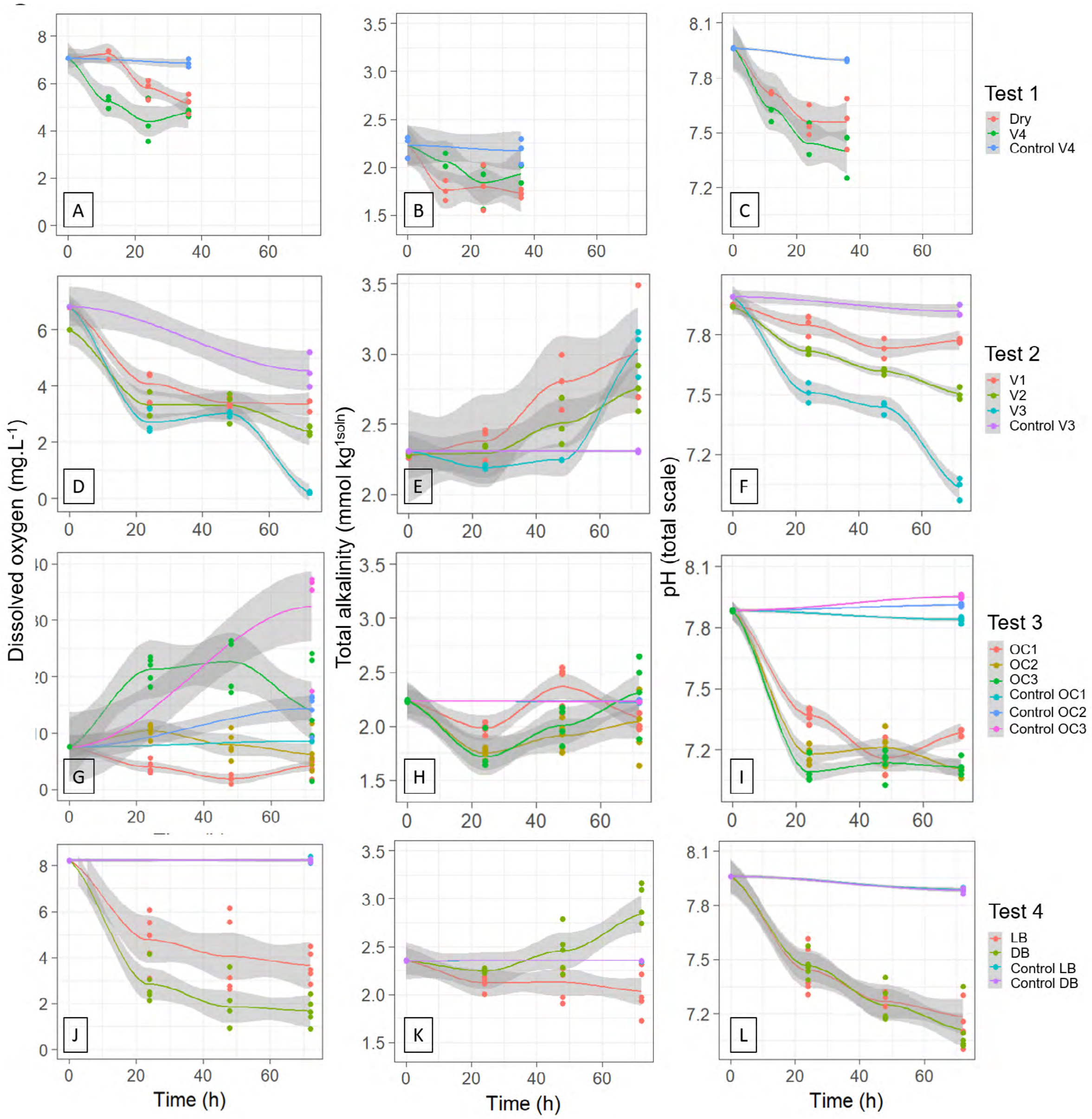

Figure 4. Changes in the dissolved oxygen concentration, total alkalinity, and pH of the transportation water. Grey shape: confidence interval; n=3 for Test 1 and Test 2; n=5 for Test 3 and Test 4. Note the different scale of the Y-axis on sub-figure G.

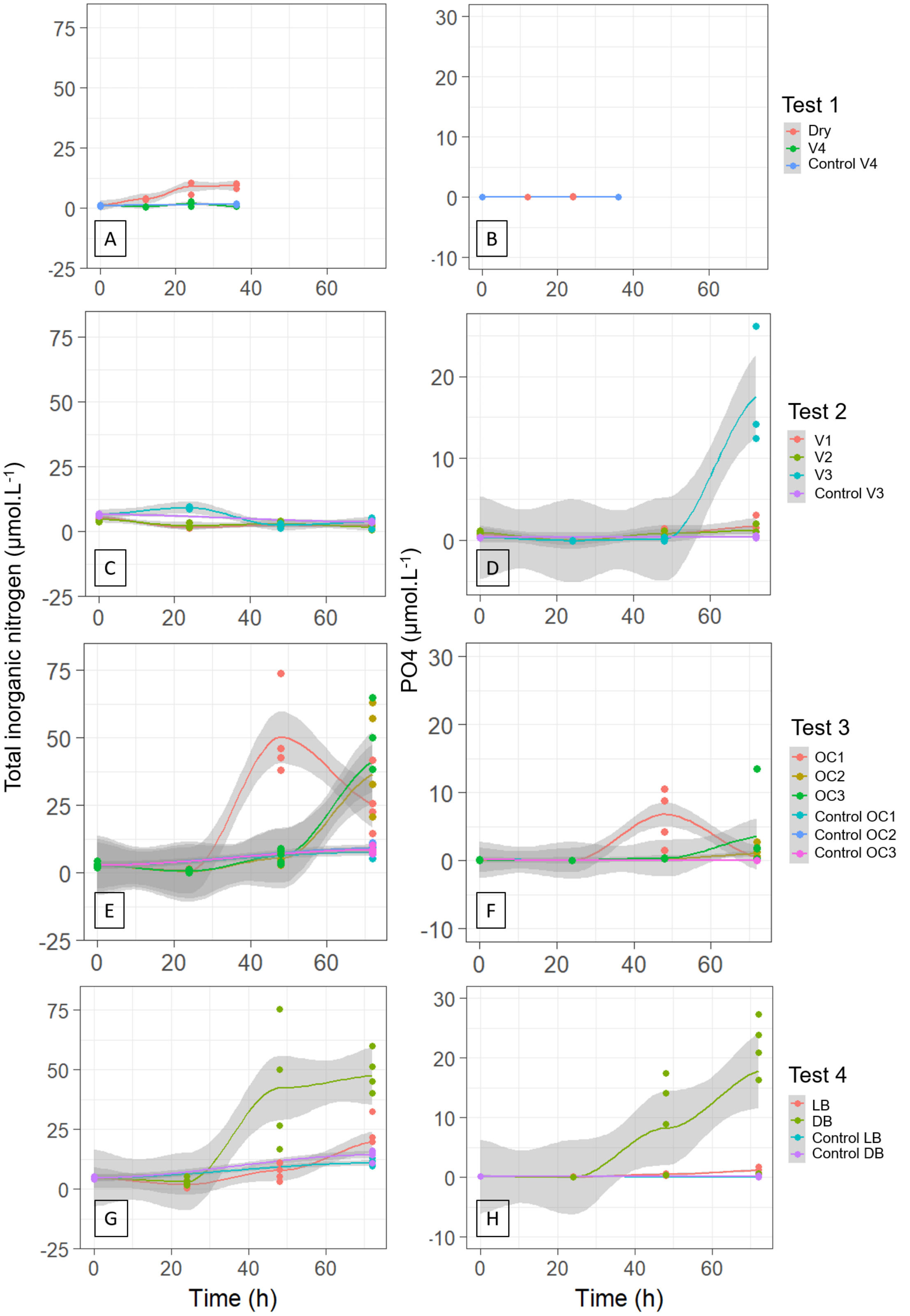

Figure 5. Variation in total inorganic nitrogen and PO_4^{3-} concentrations in the transportation water. Grey shape: confidence interval (method: loess, 95 %); n=3 for Test 1 and Test 2; n=5 for Test 3 and Test 4.

Adding a high concentration of oxygen in the gas phase to the transportation container resulted in an increase in the dissolved oxygen concentration of the water. In OC1, dissolved oxygen decreased to 1.85 ± 0.63 mg L^{-1} during transportation (Fig. 4G). A significant difference from the initial concentration was observed ($p = 0.0012$). In OC2 and OC3, a rapid and significantly increasing concentration was observed, reaching 10.4 ± 1.15 mg L^{-1} ($p = 0.0418$) and 21.29 ± 2.25 mg L^{-1}, ($p = 0.0109$), respectively after 24 hours compared to an initial concentration of 7.53 ± 0.03 mg L^{-1} in the transportation water. These concentrations stabilized until 72 hours.

Compared to an initial concentration of 2.23 ± 0.01 mmol $kg^{-soln-1}$, total alkalinity significantly decreased in OC1, OC2, and OC3, to 1.98 ± 0.05 mmol $kg^{-soln-1}$, 1.75 ± 0.05 mmol $kg^{-soln-1}$, and 1.71 ± 0.15 mmol $kg^{-soln-1}$, respectively, during the first 24 hours, before slightly increasing and matching the control values (Fig. 5H). There was no significant difference observed in the control containers at 48 and 72 hours. In OC1, OC2, and OC3, pH_T sharply decreased significantly after only 24 hours ($p < 0.0001$), but remained stable at 7.3 ± 0.19 until 72 hours (Fig. 4I).

N_{ti} also decreased during the first 24 hours, but increased to the level of the initial concentration at 48 hours, except in OC1, in which it increased to almost 20 times the initial values (from 2.85 ± 1.10 µmol L^{-1} to 56.97 ± 20.56 µmol L^{-1} ($p = 0.0002$) (Fig. 5E). A sharp increase was only observed in OC2 and OC3 at 72 hours, with 36.61 ± 23.20 µmol L^{-1} ($p = 0.0006$) and 50.40 ± 28.50 µmol L^{-1}, respectively ($p = 0.0002$).

N_{ti} were significantly influenced by NH_4^+; the Pearson correlation was $r = 0.9998$. In all containers, NO_3^+ decreased from the initial concentration while NO_2^- was stable below 0.02 µmol L^{-1}. PO_4^{3-} remained below 0.1 µmol L^{-1} before gradually increasing to 0.25 ± 0.01 µmol L^{-1} and to 0.33 ± 0.07 µmol L^{-1}, respectively in OC2 and OC3 after 48 hours (Fig. 5F). At 72 hours, the concentrations sharply increased to 13.52 µmol L^{-1} in some containers. PO_4^{3-} in OC1 showed a significant increase to 6.79 ± 3.74 µmol L^{-1} at 48 hours ($p = 0.001$), but at 72 hours it decreased to the same level as PO_4^{3-} in OC2 and OC3.

In both LB and DB, dissolved oxygen concentration rapidly and significantly decreased from the initial concentration of 8.22 ± 0.02 mg L^{-1} to 4.77 ± 1.17 mg L^{-1} ($p = 0.0424$) and 2.84 ± 0.80 mg L^{-1} ($p = 0.0144$), respectively, after 24 hours (Fig. 4J). It continued to decrease to 3.65 ± 0.66 mg L^{-1} and 1.67 ± 0.58 mg L^{-1}, respectively at 72 hours. There was no significant difference observed between the concentrations at 24 and 72 hours. There was a significant difference in total alkalinity between LB and DB ($p = 0.0148$). Both decreased during the first 24 hours ($p < 0.0001$), but then increased to 2.84 ± 0.33 mmol $kg^{-soln-1}$ after 72 hours in DB ($p = 0.0072$), and slightly decreased to 2.03 ± 0.23 mmol $kg^{-soln-1}$ ($p = 0.0259$) in LB (Fig. 4K). pH_T significantly decreased from 7.96 ± 0.0 ($p = 0.0001$) to 7.18 ± 0.14 and 7.10 ± 0.14 ($p = 0.0004$) in LB and DB, respectively (Fig. 4L).

N_{ti} was also highly influenced by NH_4^+; the Pearson correlation was $r = 0.993$. A slight decrease in NH_4^+ at 24 hours was observed in LB and DB, followed by an increase up to 19.77 ± 8.39 µmol L^{-1} and 47.46 ± 8.41 µmol L^{-1}, respectively at 72 hours. Compared to the control containers, there was only a significant increase in concentration in DB ($p = 0.0039$) (Fig. 5G). In addition, N_{ti} in DB started to significantly increase from 48 hours to 51.13 ± 29.97 µmol L^{-1} ($p = 0.0006$). There was a significant increase in PO_4^{3-} in DB containers, reaching 17.81 ± 10.44 µmol L^{-1} after 72 hours compared to an initial concentration of 0.15 ± 0.02 µmol L^{-1} ($p = 0.007$) (Fig. 5H).

Nubbin growth and survival

On the one hand, the nubbins transported during 36 hours with the wet method (V4) showed slow growth during the transportation and continued to grow thereafter, while those transported with the dry method did not completely recover to the initial growth rate (Fig. 6). Two weeks after transportation, the skeletal growth rate of the nubbins from dry transportation remained significantly lower than those transported with the wet method, with 0.42 ± 0.20 % day^{-1} and 0.62 ± 0.11% day^{-1}, respectively ($p = 0.0216$). On the other hand, during Test 2, within 48 hours of transportation, results showed that compared to the very small volume (V1), the larger water volumes (V2 and V3) allowed the nubbins to better tolerate the transportation, according to visual observation of their colour (Fig. 3B), even if their growth rate was not significantly different (Fig. 6). However, after 72 hours, nubbins from V3 were severely impacted and only a small portion of the colony survived (Fig. 3C). After transportation, the mean growth rate of the nubbins from V3 (0.27 ± 0.05 % day^{-1}) was significantly lower than those from V1 and V2 (0.50 ± 0.10 % day^{-1}) ($p = 0.017$) (Fig. 6).

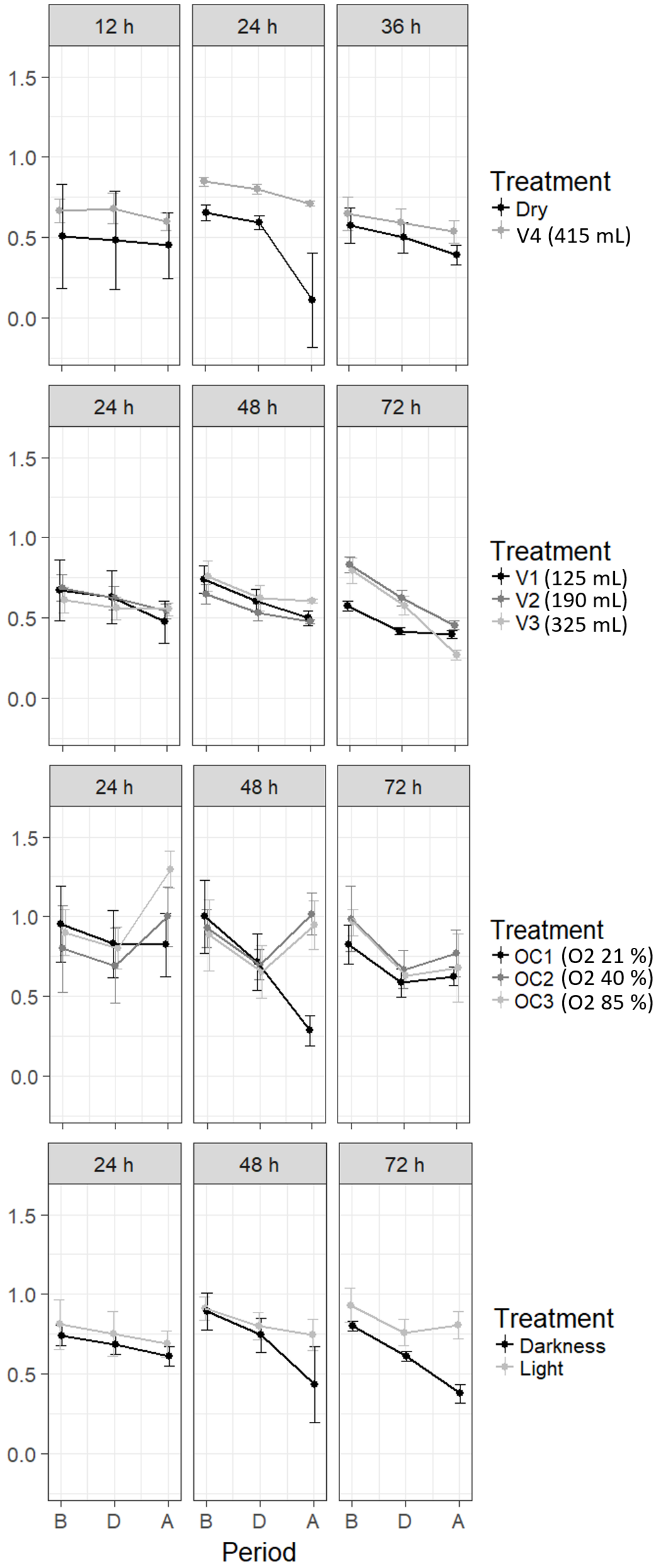

Figure 6. Skeletal growth rate (% day^{-1}, mean ± standard error, n=3 for Test 1 and 2, n=5 for Test 3 and 4) of the nubbins before (B), during (D), and after (A) transportation. Each subfigure corresponds to the nubbin transportation duration (12, 24, and 36 hours for Test 1; 24, 48, and 72 hours for Tests 2, 3, and 4).

Two weeks after transportation, nubbins transported in OC1 showed a significantly lower growth rate (0.58 ± 0.31 %day^{-1}, p = 0.0028) compared to those in OC2 (0.93 ± 0.28 % day^{-1}) and OC3 (0.97 ± 0.37 % day^{-1}) (Fig. 6). This low rate was highly influenced by the near-mortality of >50 % of the nubbins transported during 48 hour or longer. Sixty percent of the nubbins from both OC2 and OC3 presented some bleaching but recovered a few days after transportation and their growth rate remained higher than those from OC1.

Two weeks after transportation, the growth rate of the nubbins transported in LB (0.75 ± 0.15 % day^{-1}) was significantly higher than those transported in DB (0.47 ± 0.25 % day^{-1}) (p = 0.0013) (Fig. 6). This difference was particularly obvious when the duration of the transportation exceeded 48 hours. In LB, the longer the transportation duration, the higher the growth rate, while in DB the opposite effect occurred. Sixty percent of the nubbins transported during 48 hours in DB bleached, but had completely recovered 2 weeks later; however, 80 % of those transported for 72 hours showed part mortality (Fig. 3B, C; Fig. 6).

Discussion

The present results show that water volume and oxygen concentration, and the addition of a light source in the shipping box, differentially influence the health status and post-transportation growth of coral nubbins. They improve the understanding of how the coral uses the chemical and organic elements presents in the transportation water, and how these elements vary during transportation and effect coral holobiont physiology, as reported in previous studies (e.g., Osinga *et al.*, 2011; Wijgerde *et al.*, 2014). When using normal air, dissolved oxygen and pH rapidly decreased during the first 24 hours. The addition of a high oxygen concentration addressed this problem, but it did not prevent a decrease in pH in all tested conditions. These results support the findings of Wijgerde *et al.* (2014) highlighting the important role of oxygen in coral calcification. The authors suggested that within the current (natural seawater) oxygen and pH range, oxygen exerts a substantial control on coral growth, whereas the role of pH is limited (Wijgerde *et al.*, 2014). Indeed, the results from the present study showed that, even without the light source, the transported corals continued to grow slowly during the first 24 hours, and steadily consumed the dissolved oxygen. This also led to a decrease in water alkalinity due to skeletogenesis. Without the possibility of zooxanthellae performing photosynthesis, the dissolved oxygen concentration continued to decrease, and the resulting modified carbonate system lowered the water pH (Kleypas *et al.,* 1999).

Coral colonies can survive and grow in a well-conditioned small volume of water such as a chemostat, but only if these conditions are maintained for a given period (Leblud, 2015). In the present study, except in the presence of light, the concentration of inorganic nitrogen, especially ammonium ions, increased dramatically beyond 24 hours. With the critically decreased pH, the level of inorganic nitrogen continued to increase due to degradation of parts of the coral tissue in some cases. The part mortality of the coral also implies skeleton dissolution and a subsequent increase in alkalinity. This mainly occurred after 48 hours of transportation, in dark containers; which confirms the conclusion by Gattuso *et al.* (1999), stating that coral calcification is, on average, three times higher in the light than in the dark.

The lower post-transportation growth rate of the coral nubbins with the dry method suggests that it was not appropriate for small nubbins compared to the wet method. Indeed, several real transportation experiences propose the use of the dry method only for large colonies (~18.3 cm), with respect to a particular preparation and packing system (Petersen *et al.*, 2004). However, in contrast to the results of these experiences, in the present study, all small nubbins survived the transportation and changes in coral health status were only noticed when the duration exceeded 24 hours. This can be explained by the appropriate post-transportation conditions in the mesocosm that provided sufficient light irradiance to allow rapid recovery (Leblud *et al.,* 2014). It is important to note that, in these transportation experiments, the pre- and post-transportation conditions were identical (same as the mesocom). Thus, coral nubbins did not have to deal with, for example, different seawater chemistry or different pathogen strains when acclimatizing to the post-transportation conditions. While this increases post-transportation success, it also ensures that the changes observed in coral nubbin growth are due to the transportation itself, rather than other factors, which is what the present study aimed to investigate.

With the wet method, the use of a larger volume and normal air is suggested when the transportation duration does not exceed 48 hours, otherwise the sudden changes in parameters result in a markedly higher nubbin mortality (Osinga *et al.*, 2011). Nubbins transported in smaller volumes during 72 hours presented a lower post-transportation growth rate, but better health status compared to those in larger volumes, suggesting that using a small volume of water is much more profitable. This result is counterintuitive, and it is not easy to elaborate upon a hypothesis to explain such observations. Since both oxygen and pH decreased at a slower rate in the smaller volume, it seems that nubbin metabolism is rapidly limited, resulting in better oxygenation and pH values over a longer term in the transportation water, which would be overall less stressful to the coral. Further investigations are required to confirm these results and to determine the optimal water volume/nubbin size ratio. A previous study proposed to increase the volume of water to 70 % when packing corals for more than 20 hours and to reduce the amount of oxygen to decrease the risk of oxygen toxicity (Delbeek, 2008). The present results clearly do not support this hypothesis, except when the transportation time exceeds 48 hours. For shorter transportation, there was a marginal effect of water volume only. In addition, the results from this study suggest that the use of a high oxygen concentration with larger water volumes is only suitable for a transportation duration under 48 hours. The use of an oxygen concentration greater than 40 % may induce high mortality or reduce nubbin recovery capability post-transportation. However, the use of 100 % oxygen represents a huge financial investment in coral shipping (Osinga *et al.,* 2011).

This is thought to be the first study to propose the addition of a light source. Gattuso *et al.* (1999) reported a very strong correlation between photosynthesis and calcification at the organism level for corals. The results from the present study seem to support the hypothesis that calcification is dark-repressed rather than light-enhanced (Gattuso *et al.*, 1999). However, the addition of a light source may be logistically problematic in airplanes, where electronic devices cannot be transported switched on, although it should be possible to design certified systems that are safe for airplanes. The concept of adding a light source to the container is to trigger coral zooxanthellate photosynthesis during transportation to (partly) recycle oxygen, nitrogen, and phosphorus, and to attempt to stabilize the pH. This effect relates only to photoautotrophic organisms that can utilize light (Shi *et al.,* 2012).

Adding a light source in the transportation box greatly influenced the survival and growth of the nubbins. These results suggest that adding sufficient light in the transportation box prevents the need to use pure oxygen and instead permits the filling of the containers with much cheaper normal air. The cost of a single-use LED light in addition to a couple of cheap batteries is not much higher than the price of using pure oxygen in the bags. This technique also potentially allows an increase in the transportation duration for up to 72 hours, and may be useful for transportation to remote locations, or as a safety margin in more traditional transportations (for instance, to mitigate against flight delay, which can be a common issue). Indeed, it has been shown that with a relatively higher water volume and use of normal air, the post-transportation status of coral was better and the growth rates were higher compared to the other conditions. In this study, 24-LED Handy Lamps were used, one per box, which are readily available and cost less than 20 Euros (including batteries). However, the use of a supplementary accessory and a greater water volume will result in extra weight in the transport box. Several coral farming projects, especially in Madagascar, have stopped their production due to, among other reasons, frequent major loss occurring because of missed flight connections. This illuminated box method may represent a solution for such cases.

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Morphometric study of humpback whale mother-calf pairs in the Sainte Marie channel, Madagascar, using a simple drone-based photogrammetric method

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Abstract

Morphometric studies of humpback whales (*Megaptera novaeangliae*) occurring in the Indian Ocean area have been limited by the technology currently available. In the Sainte Marie channel, Madagascar, straightforward aerial single-camera photogrammetry was tested on mother-calf pairs that combines standard Unoccupied Aerial Vehicle (UAV) with free, easy-to-access, and user-friendly software. The goals of the study were to estimate mother and calf body measurements and to investigate the effect of maternal parity (primiparous versus multiparous, based on length) on calf size. A mean length of 12.4±1.2 m for mothers (*N* = 16) and 5±0.9 m for calves (*N* = 16) was estimated. The size of calves did not depend significantly on maternal parity. The photogrammetry method used was simple and cost-effective, yet produced convincing morphometric measurements with acceptable precision and accuracy. The coefficients of variation (CVs) of repeated estimates and the level of error were relatively low (CV = 2.31 % for a known-sized object and average CV = 2.52 % for individual whales; average error = 1.8 % for a known-sized object). These results will encourage more teams to study the morphometry of large marine mammals despite limitations in terms of resources.

Keywords: aerial photogrammetry, breeding grounds, parity, Unoccupied Aerial Vehicle (UAV)

Introduction

Morphometric data is the numerical expression of an animal's morphological characteristics that can be used to address various biological questions (Schmidt-Nielsen and Knut, 1984). Previous studies have demonstrated the efficiency of using body length for determining growth rate, age-structures, and population demographics in cetaceans (Chittleborough, 1965; Perryman and Lynn, 1993). At the individual level, morphometric data can be examined to assess body condition and reproductive capacity (Perryman and Lynn, 2002; Miller *et al.*, 2012; Christiansen *et al.*, 2016; Fearnbach *et al.*, 2018). In addition, a time series of morphometric data can be used to assess population responses to environmental and anthropogenic changes (Hanks, 1981). For example, long-term changes in size distribution can provide a signal of overexploitation of a population (Stevens *et al.*, 2000).

Live capture-release schemes are generally not applicable to large whales due to their size. Hence in the past, morphometric studies of large whales relied mainly on direct measurements on stranded or commercially harvested specimens (e.g., Chittleborough, 1955, 1958, 1965; Omura, 1955; Nishiwaki, 1959, 1962). Later, the development of photogrammetry for

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studying whales allowed for the extension of morphometric studies to live animals. Known as the science of measuring objects using photographs, photogrammetry is better than direct measurements as it does not require physical capture of animals and is thus non-invasive and less opportunistic. Photogrammetry used in whale studies can be divided into two main approaches; either stereo-photogrammetry or single-camera photogrammetry. Stereo-photogrammetry uses overlapping photographs to estimate length (Cubbage and Calambokidis, 1987; Dawson *et al.*, 1995). It allows for accurate measurements of whales since the measurements are done in 3-dimensions. However, it requires a complex pre-configuration such as a precise and controlled stereo-camera mounting and synchronization. On the other hand, single-camera photogrammetry requires only a single photograph and uses either a known-size object in the frame for scale (Christiansen *et al.*, 2016) or a measurement of the range to the individual (Best and Rüther, 1992; Perryman and Lynn, 2002; Jaquet, 2006; Fearnbach *et al.*, 2011; Miller *et al.*, 2012; Durban *et al.*, 2015; Dawson *et al.*, 2017; Christiansen *et al.*, 2018; Burnett *et al.*, 2019). To achieve accurate measurements from single photographs while optimizing the cost, a variety of combinations of tools, detailed in Table 1, has been used in the field. One of the most notable advances is the use of Unoccupied Aerial Vehicles (UAVs) or small aerial drones to perform aerial photogrammetry.

UAVs have facilitated an array of methods for monitoring wildlife and studying spatial ecology. They provide an ideal solution if the studied animals are scared by the presence of humans in the area or if the animals are dangerous for human observers (Linchant *et al.*, 2015). UAVs allow researchers to observe animals in their environment from above, are less invasive than Occupied Aircrafts (OA), and are significantly much more cost-effective. UAVs have therefore been used for a number of marine mammal research applications (Goebel *et al.*, 2015; Fiori *et al.*, 2017), including measuring individual animals (Christiansen *et al.*, 2016, 2018; Durban *et al.*, 2016; Dawson *et al.*, 2017; Burnett *et al.*, 2019).

The models of UAVs commonly used in aerial photogrammetry of whales vary from standard UAVs, such as DJI Phantom 3, to the more expensive UAVs specifically designed for scientific research and/or above-water operations. To obtain an approximation of the range between the camera on the UAV and the whale, researchers generally rely on an altimeter, i.e., an altitude measurements tool (as mentioned in Table 1). The altimeter can be a barometric one (Durban *et al.*, 2015, 2016; Burnett *et al.*, 2019) or an external customized tool such as Light Detection and Ranging technology or LIDAR (Dawson *et al.*, 2017; Christiansen *et al.*, 2018). LIDAR can provide very accurate and precise altitude measurements (Dawson *et al.*, 2017). However, the deployment of LIDAR on an UAV can incur additional cost. Therefore, although barometric altimeters are less accurate and less precise than LIDAR, they are still suitably reliable for photogrammetric purposes and are commonly integrated into most UAVs (Durban *et al.*, 2015, 2016; Burnett *et al.*, 2019). It should be noted that since the lens of the UAVs camera often distorts the images, some photogrammetric studies incorporate prior image correction to improve photo accuracy during the image processing step (Dawson *et al.*, 2017; Burnett *et al.*, 2019). Alternatively, other studies follow a set of specific framing rules in order to minimize the distortion effects on the image (Durban *et al.*, 2015, 2016; Christiansen *et al.*, 2016, 2018).

The logistical challenges in utilizing advanced research tools for studying whales may offer an explanation as to why comprehensive surveys on humpback whale morphometry in some regions is lacking. Adding elements to an existing UAV or designing a new UAV can be for example a complex task for teams lacking a Research & Development or electronics department. In this paper, the performance of a simple and cost-efficient single-camera photogrammetry approach is established and validated that combines a standard UAV (here a DJI Phantom 4) with open-source software to target mother-calf pairs from the Sainte Marie channel, Madagascar.

Humpback whales are a highly migratory species. They spend the majority of the summer in their mid- or high-latitude feeding grounds. In winter, they breed and give birth in warm tropical waters (Clapham, 2018). The Sainte Marie channel, located on the eastern coast of Madagascar, is part of the humpback whale's breeding grounds in the South Western Indian Ocean. It is an important breeding ground in terms of the presence of mother-calf pairs as the channel is relatively calm and shallow (Trudelle *et al.*, 2018). Humpback whales arrive here between June and September.

Female humpback whales start to calve between 5 to 9-years old (Clapham, 1992; Gabriele *et al.*, 2007) where they give birth to a single calf, approximately

Table 1. Non-exhaustive list of photogrammetric approaches used for measuring whales at sea. For Aerial vehicle: (***) expensive, time and resource consuming aerial vehicle. (**) efficient/specialized but expensive aerial vehicle. (*) Low price/standard vehicle. (.) Aerial vehicle not required. For Range measurement tool: (***) Efficient/specialized but expensive tool. (**) Moderate price/standard tool or pre-integrated in the vehicle. For Image measurement tool: (***) highly specialized scientific tool. (**) Paid software/Script available for free but requiring paid software. (*) Free software/Script and software available for free. (a) Based on linear measurements of a known-sized object. (b) Based on linear measurements of individual adult whales. (c) Based on measurements of the surface area of individual whales.

Reference	Aerial vehicle	Type	Range measurement tool	Scale basis	Lens distortion correction	Image measurement tool	CV (%)	Error (%)
Cubbage & Calambokidis (1987)	Airplane (OA) ***	Stereo-photogrammetry	Radar altimeter **	Independent reference object	No	Stereoplotter ***	1.7 (a)	0.5 (a)
Best and Rüther (1992)	Helicopter (OA) ***	Single-camera-photogrammetry	Radar altimeter **	Independent reference object	No	Stereoplotter ***	1.3 (b)	-
Dawson *et al.* (1995)	(Not required) .	Stereo-photogrammetry	(Not required)	Camera characteristics (obtained from calibration)	No	Stereoplotter ***	4.4 (b)	< 2.5 (a)
Spitz *et al.* (2000)	(Not required) .	Single-camera-photogrammetry	Sonar **	Camera characteristics (obtained from calibration)	Yes	Adobe Photoshop software (Adobe) **	4.3 (a)	4.6 (a)
Perryman & Lynn (2002)	Airplane (OA) ***	Single-camera-photogrammetry	Radar altimeter **	Camera characteristics (factory specifications)	No	Image Image Pro Plus (Media Cybernetics) **	2 (b)	1 (a)
Jaquet (2006)	(Not required)	Single-camera-photogrammetry	Laser range finder **	Independent reference object	No	Adobe Photoshop software (Adobe) **	1.3 (b)	0.27 (a)
Fearnbach *et al.* (2011)	Helicopter (OA) ***	Single-camera-photogrammetry	GPS ***	Camera characteristics (factory specifications)	No	ImageJ software (NIH) *	-	< 3.2 (a)
Miller *et al.*(2012)	Airplane (OA) ***	Single-camera-photogrammetry	Radar altimeter **	Independent reference object	No	Image Pro Plus (Media Cybernetics) **	1.7 (b)	-
Durban *et al.* (2015, 2016)	APH-22 hexacopter (UAV) **	Single-camera-photogrammetry	High precision barometer **	Camera characteristics (factory specifications)	No	-	-	< 1 (a)
Christiansen *et al.* (2016)	Splashdrone quadcopter (UAV) **	Single-camera-photogrammetry	(Not required)	Reference object in the same frame as the target animal	No	Custom-written script in R *	< 12 (c)	-
Dawson *et al.* (2017)	DJI Inspire Pro (UAV) **	Single-camera-photogrammetry	LIDAR ***	Camera characteristics, (obtained from calibration)	Yes	Custom-written script in MATLAB **	1.2 (b)	1 (a)
Christiansen *et al.* (2018)	DJI Inspire Pro (UAV) **	Single-camera-photogrammetry	LIDAR ***	Camera characteristics (factory specifications)	No	Custom-written script in R *	0.3 (b)	4.75 (b)
Burnett et *al.* (2019)	DJI Phantom 3 Pro/4/4 Pro (UAV) *	Single-camera-photogrammetry	Barometer **	Independent reference object	Yes	Custom-written script in MATLAB **	< 5 (b)	-

every 2 years (Clapham, 2018). Humpback whale mothers are left with the responsibility of the survival of their young until the calf reach the age of approximately 1-year old (Clapham, 2018). The size of sexually mature females ranges between 11 m to 15 m (Omura, 1955; Nishiwaki, 1959, 1962). Christiansen *et al.* (2016) reported a mean length of 12 m for females accompanied by a calf off Australia. A similar measurement has been reported by Spitz *et al.* (2000) in Hawaii. The mean length of calves is about 4.2 m at birth (Chittleborough, 1965) and they have a growth rate of approximately 3 cm per day (Christiansen *et al.*, 2016).

In pinnipeds (Bowen, 2009) and some large whale species (Laws, 1961; Gambell, 1972; Best and Rüther, 1992), it has been found that primiparous mothers (females having their first young and thus inexperienced mothers) tend to produce smaller offspring compared to multiparous mothers (mothers that have previously calved and thus more experienced). This trend may be related to numerous factors, such as a physiological change in the mother following her first parturition that favours the development of future foetuses, a more favourable external environment for the mother, or a behavioural change in the mother gained through previous experiences (Ellis *et al.*, 2000). Female investment in offspring size can be considered as a form of maternal contribution to the survival of the offspring since larger offspring are known to have higher chances of survival in mammals (Ronget *et al.*, 2018). In humpback whales, the effect of the maternal parity on offspring size has not yet been investigated. Therefore, the goals of this study were to: 1) estimate mother and calf body measurements (standard length and maximum width), and 2) test whether the calf's size is related to the parity of the mother (primiparous or multiparous).

Materials and methods

Study site

Field studies were conducted in the Sainte Marie channel, in Madagascar (Indian Ocean). About 60 km long and 7 to 30 km wide (Trudelle *et al.*, 2018), the channel is located between Sainte Marie Island (between latitudes 17° 19' and 16° 42' South, and longitudes 49° 48' and 50° 01' East) and the East coast of Madagascar's mainland. The data collection, conducted in conjunction with an ongoing study on humpback whale mother-calf interactions, were completed between August and September 2018 under the national research permits #28/18 MRHP/SG/DGRHP.

UAV platform

The DJI Phantom 4 UAV is a quadcopter weighting 1380 g, with a diameter of 350 mm. It is equipped with a built-in barometer that provides real-time altitude measurements (in m), and a gimballed camera with a 3.61-mm focal length, infinite focus and 0.0015-mm pixel size. Within a centred radius equivalent to 60 % of the video frame height, the distortion-related displacement on an image from the camera is less than five pixels (Burnett *et al.*, 2019), which is low. In this study, the video resolution was set at 4096×2160-pixels (4K) with a framerate of 24 frames per seconds.

Whale visual searches protocol

All methods and approaches were carried out in accordance with relevant guidelines and regulations in force in Madagascar. Dedicated visual searches for humpback whale mother-calf pairs were conducted from a 6.40 m rigid motor boat during days with moderate weather conditions (Beaufort scale ≤ 3, corresponding to gentle breeze, wind speed less than 12 km h^{-1}, and wave height not exceeding 0.5 m) between 0630 and 1730 hours. The crew consisted of at least 3 trained observers (one on an elevated platform at the back of the boat and two covering the lateral view) and 1 or 2 experienced drone pilots. When a mother-calf pair was spotted, it was approached at idle speed to a distance between 100 and 200 m. All mother-calf pairs were photo-identified to ensure that there was no double-sampling during the study period. The ventral face of the tail fluke (visible when the whale is about to dive) and/or the dorsal fin of each individual was/were photographed using a Nikon digital camera (model D5600) fitted with a 50-300 mm lens. The photographs obtained within the season were then manually compared in order to check that indeed no double-sampling of mother-calf pairs occurred. Depending on the degree of dorsal furl, the relative age of each calf was also estimated (neonate versus non-neonate, Cartwright and Sullivan, 2009; Faria *et al.*, 2013, Saloma, 2018). All sighting data were integrated into the local dataset CETANET (www.cetanet.org, managed by Cétamada Association) that gathers all marine mammal sighting information recorded in Madagascar since 2009.

UAV flight protocol for whale images acquisition

The UAV was deployed from the boat to video-record the spotted mother-calf pair vertically overhead from an altitude around 15 m to 60 m, at a vertical speed of approximately 0.5 m s^{-1}. Sometimes the mother and the calf were not close to each other or were not

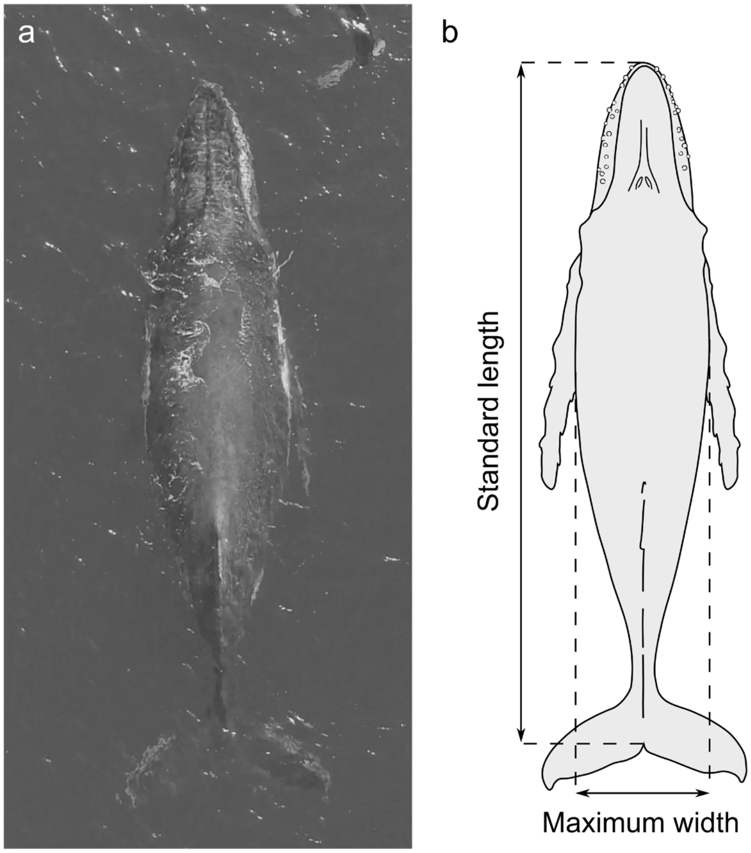

Figure 1. (a) Example of acceptable aerial photograph of humpback whale extracted from the video recording and used for photogrammetry. The original image was of 4096×2160-pixel but was cropped in this figure to highlight the fixed criteria. The whale must be flat at the surface, dorsal side facing up, emerged as much as possible, static or travelling at relatively slow speed, with a contour not masked by chops and with a non-arching body axis and peduncle. (b) Measurements in pixel recorded for each photograph.

at the surface at the same time. In these cases, they were filmed separately. The drone initialization (altitude zeroing) was performed before each flight on an on-boat platform 0.6 m above the sea level (the variation of the on-boat load between outings was fairly low and assumed to not have a very significant effect on this zeroing height). A maximum of two flight sessions was conducted for each pair (10-15 min duration per flight). Photographs were extracted afterward from the video recordings (see Images extraction and digitization).

Calibration images acquisition

To estimate the whales' dimension from photographs in the absence of known size scale markers in the frames and to account for systematic error in ranging, the camera needed to be calibrated. This can be done using images of an object with a known length taken at various distances between the object and the camera, i.e., ranges (Jaquet, 2006; Burnett *et al.*, 2019). To perform calibration in this study, a static floating kayak of 2.75 m in length was video-recorded vertically overhead at altitudes between 5 m to 45 m at the end of the study period. In addition, for testing, a supplementary filming flight was performed to acquire video material that was independent to the one dedicated to the calibration. The equipment, settings used, and weather conditions present during calibration were consistent with the whale survey flights.

Image extraction and digitization

The photogrammetric method was based on 4096×2160-pixel photographs (no cropping) extracted from the collected nadir pointing video using the frame capture function in GOM Player v2.3.32.5292 (GOM & Company, www.gomlab.com). Video recordings were viewed frame by frame for the process. For each filmed individual, one photograph which was of the highest quality was extracted. A high quality photograph is one which includes the whale lying flat at the surface, dorsal side facing up, emerged as much as possible, static or travelling at relatively slow speed, with contour not masked by chops, and with a non-arching body axis and peduncle (Fig. 1a). As the unadjusted lens is likely to distort images around the outside of the frame, a suitable high quality photograph was selected if the subject was positioned within the 60 % radius in the middle of the frame (see lens description in UAV platform). For each photograph, the standard length and the maximum width of the whale were measured in pixels using the software Inkscape v0.92 (www.inkscape.org) (Fig. 1b).

With respect to the kayak video dedicated to calibration, eight photographs were extracted at approximately 5 m altitude intervals (reference photographs). Only photos with clear, centred frames containing the entire kayak were selected. Additionally, from the supplementary independent kayak video, several photographs (tests hereafter) were taken at an altitude ranging from 5 to 45 m. The length of the kayak in all photographs was then measured in pixels.

Calibration process

For each of the reference photographs, the measured length from the photographs (in pixels) and the real length of the kayak (in m) were used to calculate the corresponding scale (in m pixel^{-1}):

$$\text{Scale} = \text{real length} / \text{length in pixels} \qquad (1)$$

Then, the scale was regressed against the altitude at which the reference photographs were taken (Fig. 2):

$$\text{Scale} = 0.0004197 \times \text{altitude} + 0.0001814 \qquad (2)$$

From (2), a formula was then derived with which the metric length could be estimated from vertical images taken at known altitudes:

$$\text{Estimated length} = \text{scale} \times \text{number of pixels} \qquad (3)$$

Barometric altimeter accuracy evaluation

The UAV model used did not have a suitable alternate altitude measurement for comparison. Therefore, the accuracy of the barometric altimeter was assessed directly using the optical properties of the camera. All of the test photographs were used to back-calculate the expected distance between the camera and the object (in m) as done by Krause *et al.* (2017):

$$\text{Expected camera-object distance} = (\text{Real length} \times \text{Focal length}) / (\text{Number of pixels} \times \text{Pixel size}) \qquad (4)$$

This was then compared with the barometric altitude reading while accounting for the zeroing height. To assess if the accuracy of the barometric altimeter varies with the expected altitude (expected camera-object distance minus zeroing height), a Spearman's correlation test was performed using the R statistical software v4.0.3 (R core team, www.R-project.org).

Measurement accuracy evaluation

It was not possible to directly address the accuracy of the measurements as it was impractical to include a scale object with a known size in each photograph of whales. However, as a proxy, the measurement error was evaluated based on the test photographs. From each photograph, the length of the kayak was estimated and the measurement error was then calculated as the percent difference as follows (Krause *et al.*, 2017):

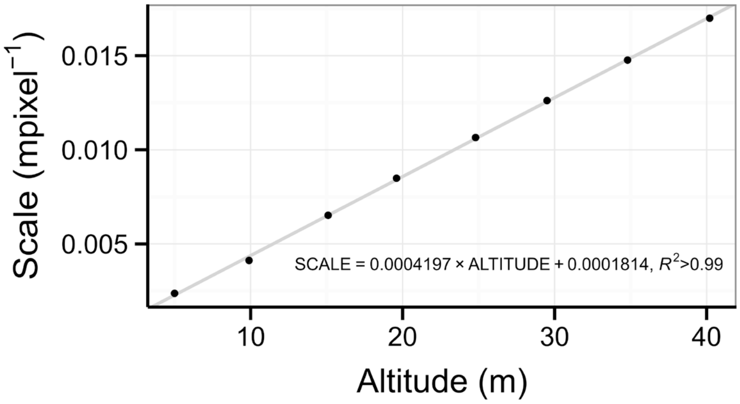

Figure 2. Relationship between photographic scale and height at which the photos were taken. Multiplied by the number of pixels, the regression equation gives length in meters.

% Error = | 1 - (real length / estimated length) × 100 | (5)

To investigate whether the error varies with the altitude at which the photographs were taken, a Spearman's correlation test and a Kruskal-Wallis test was used (using three altitude classes: 5 to 15 m, 15 to 25 m and > 25 m) in R.

Measurement precision evaluation

To indirectly assess the precision of the approach used, the coefficient of variation (CV) was calculated of the length estimations of the test object. Additionally, to address whether different observers consistently derive similar measurements, four independent observers estimated the length of the test object. The results were compared among observers using Kruskal-Wallis test in R.

Whale data analysis

The numbers of pixels of the whales were related to the estimated dimension in meters using the formula (2) and (3). Based on the estimated length and considering the life history of the humpback whale (Omura, 1955; Nishiwaki, 1959, 1962; Chittleborough, 1965; Clapham, 1992; Gabriele *et al.*, 2007; Clapham, 2018), the mothers were sorted into two categories: primiparous, i.e. likely accompanied by their first calf, for mothers < 13 m, and multiparous, likely already had previously one or more calves, for mothers ≥ 13 m. To assess if the body lengths of calves differ between the two parity categories of mothers, a Wilcoxon test was performed in R.

Age has previously been used to categorize females as primiparous or multiparous when complete birth records for each individual were not available (Ellis *et al.*, 2000). Such an approach is possible when the life history of the species is known. As mentioned previously, the age at which female humpback whales have their first calf is between 5-9-years old (Clapham, 1992; Gabriele *et al.*, 2007) and the birth interval is about 2 years (Clapham, 2018). Based on these data, it can be assumed that females < 9-years old whom are accompanied by a calf are likely to be primiparous mothers, while those that are ≥ 9 years old can be assumed to be multiparous. At age 9, a female humpback whale should reach 13 m (Chittleborough, 1965) and incidentally, this value is consistent with data obtained by whaling operations (i.e., data from direct measurements); the average size of sexually mature female humpback whales is 13 m (Omura, 1955; Nishiwaki, 1959, 1962). Therefore, 13 m was set as the threshold to define parity.

In some instances, it was possible to extract more than one suitable, high quality photograph of individual whales. Therefore, for these whales, it was possible to make additional length estimations. The CVs on individual whales was thus calculated as well to obtain a more direct precision assessment.

Results

Using a DJI Phantom 4, a total of 16 mother-calf pairs were photographed between August and September 2018. All calves had an unfurled dorsal fin, indicating that they were not neonates (yet aged less than 3 months). All photographs were obtained at an altitude ranging between 17 and 60 m (mean = 27±11 m); the majority (29 out of 32) being obtained at an altitude < 45 m. The empirical calibration formulas (2) and (3) allowed estimation of the standard length and the maximum width of mothers and calves from these photographs.

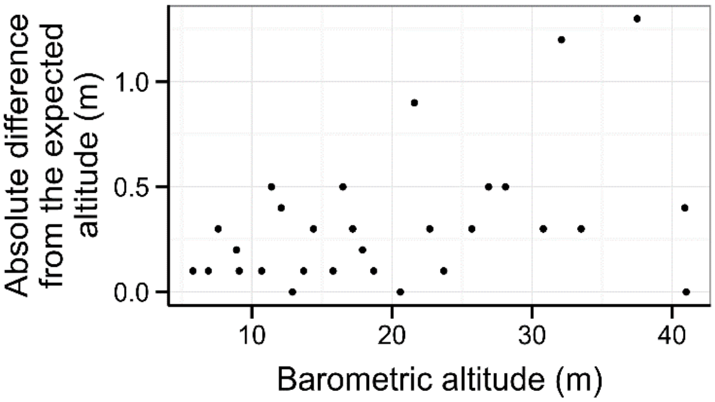

Figure 3. Barometric altitude reading versus its absolute difference from the expected altitude. The correlation was low and not statistically significant (Spearman's correlation test; rho = 0.354, S = 2624 and p = 0.06).

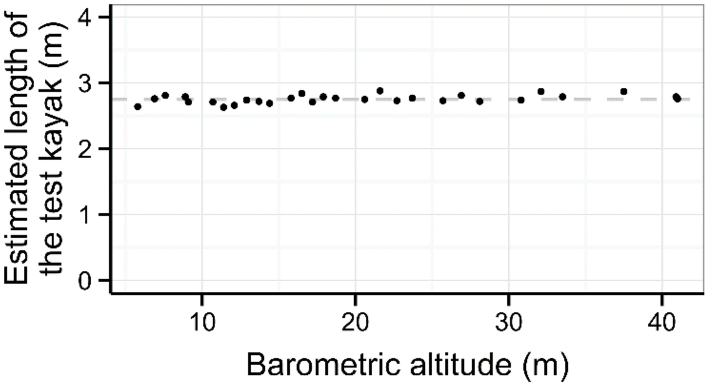

Figure 4. Repetitive estimation (N = 29) of the length of the test kayak at an altitude from 5.8 m to 40.9 m. The grey horizontal dashed line indicates the real length of the kayak (2.75 m). The coefficient of variation (CV) of the estimations was of 2.31 %.

Accuracy of the barometric altimeter

The absolute difference between the altitude reading provided by the barometric altimeter of the UAV and the expected altitude (expected camera-object distance minus zeroing height) was low (mean = 0.3±03 m, min = 0 m, max = 1.3 m, N = 29; Fig. 3). Furthermore, the correlation between this difference and the barometric altitude was low and not statistically significant (Spearman's correlation test; rho = 0.354, S = 2624 and p = 0.06).

Accuracy of the method using an object with a known size

The results of repetitive estimations of the length of an object with a known size, a 2.75-m test kayak, at an altitude between 5.8 and 40.9 m showed an average error of 1.8±1.41 % with respect to the real length (min = 0 %, max = 4.56 %, N = 29). The errors showed no statistically significant variation with respect to altitude (Spearman's correlation test; rho = -0.16, S = 4711 and p = 0.406; Kruskal-Wallis test; χ^2 = 1.664, df = 2 and p = 0.435; Fig. 4).

Precision of the method using an object with a known size

The repeated estimations of the length of an object with a known size showed a CV of 2.31 %. No statistically significant differences were detected for the estimated length of the object among four independent observers (Kruskal-Wallis test; χ^2 = 0.511, df = 3 and p = 0.916; Fig. 5).

Morphometric measurements of whales

All estimations are presented in Table 2. The mothers showed a mean body length of 12.4±1.2 m (min = 10.2 m, max = 14.7 m, N = 16). Their average maximum width was 2.8±0.4 m (min = 2.1 m, max = 3.6 m, N = 16). The calves presented a mean body length of 5±0.9 m (min = 3.6 m, max = 7.2 m, N = 16) and a mean maximum width of 1±0.2 m (min = 0.7 m, max = 1.5 m, N = 16).

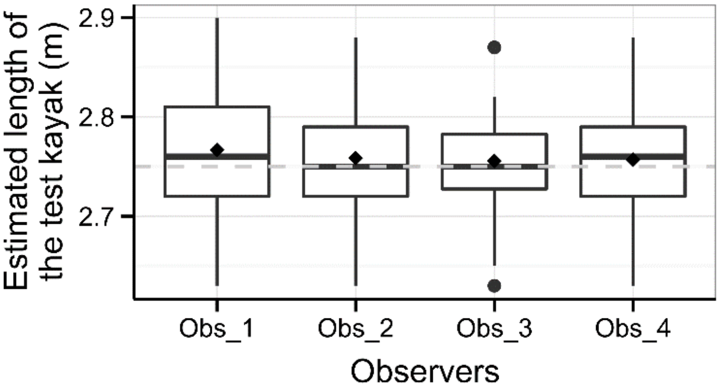

Figure 5. Repetitive estimation (N = 29) of the length of the test kayak compared between four independent observers. The grey horizontal dashed line indicates the real length of the kayak (2.75 m). The difference found was not statistically significant (Kruskal-Wallis test; χ^2 = 0.511, df = 3 and p = 0.916).

Table 2. Morphometric measurements of mothers and calves from photogrammetry and reproductive category of the mothers based on their estimated length. Length corresponds to standard length, i.e., length from the tip of the snout to the notch of the tail fluke. Width represents the maximum body width.

	Mother			Calf	
Pair ID	**Length (m)**	**Width (m)**	**Category**	**Length (m)**	**Width (m)**
1	13.3	2.9	Multiparous	4.9	0.9
2	14	3.6	Multiparous	4.8	1.1
3	13.1	2.9	Multiparous	4.9	0.9
4	14.7	3.2	Multiparous	7.2	1.5
5	12.7	3.1	Primiparous	4.6	1
6	10.2	2.3	Primiparous	3.6	0.7
7	12.1	3	Primiparous	5	1.1
8	12.1	2.6	Primiparous	4.9	1
9	12.4	2.7	Primiparous	4.6	0.9
10	13.7	3	Multiparous	6.6	1.4
11	12.1	2.8	Primiparous	4.3	1
12	10.7	2.2	Primiparous	4.4	1
13	12.4	2.6	Primiparous	4.9	1
14	11.2	2.7	Primiparous	4.9	1
15	12.6	3	Primiparous	4.6	1
16	11	2.1	Primiparous	5.3	1

Relation between calf size and parity

The majority of the mothers (11 out of 16) were < 13 m in length and were classified into the primiparous category. The remaining 5 individuals were classified as multiparous mothers (≥ 13 m in length). The length of calves from primiparous mothers was smaller than those from multiparous ones on average (Fig. 6). However, there was no statistically significant difference (Wilcoxon test; W = 12 and p = 0.084).

Precision of the whale measurements

For most individual whales (N = 24), it was possible to extract supplementary photographs at different altitudes. Including the initial whale photographs, two length estimations for 23 individuals and three for one individual were obtained. With these estimations, an average CV of 2.52±1.65 % (min = 0.02 %, max = 5 %) was found.

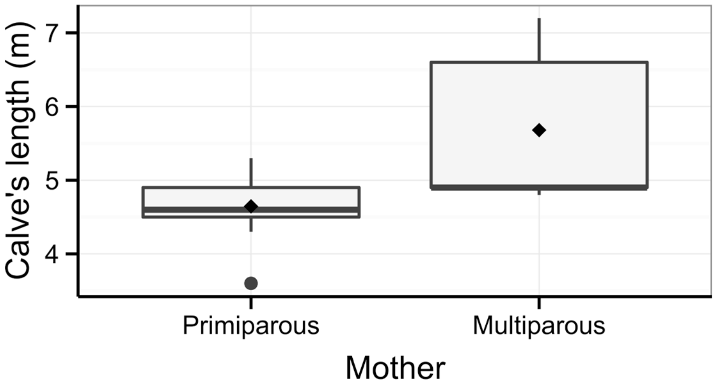

Figure 6. Calves' estimated standard body length according to the parity of their mother. The bold black lines represent the median and the diamonds represent the mean. The difference found was not statistically significant (Wilcoxon test; W = 12 and p = 0.084).

Discussion

The photogrammetric approach used relied on a formula which was based on several vertical-aerial photographs of a reference object with a known size (a 2.75-m kayak) placed on the sea surface. This empirical formula allowed assessment of morphometric data of both humpback whale mothers and their calves using nadir pointing aerial photographs taken at a known altitude with a DJI Phantom 4 UAV. In this method, errors are likely due to 1) the distortion of the lens, 2) the human error in the digitization process, 3) the accuracy of the UAV's barometric altimeter, and 4) the whales' body flex and varying submersion level. To minimise lens distortion, all the images were centred, with the targeted object avoiding the outer frames. With framing, the pixel displacement normally does not exceed 5 pixels (Burnett *et al.*, 2019). Thus, the associated error is likely to be relatively small. Also, the contribution of human error is likely to be negligible, as it was found that independent observers systematically derived similar estimations.

With the assumption that local environmental barometric pressure is relatively uniform within and between individual flights in a given site (Burnett *et al.*, 2019), the tests suggested that the measurements obtained with this method are relatively precise and accurate. Although higher than those reported by Dawson *et al.* (2017) and by Durban *et al.* (2015, 2016), the CVs of repeated estimates (2.31 % for an object with a known size and 2.52 % on average for individual whales) and the level of error (1.8 % on average, as calculated using an object with a known size) did not differ greatly from those reported in Christiansen *et al.* (2016) and in Burnett *et al.* (2019).

Regarding the barometric altimeter of the drone, the altitude it provided, used as a proxy for range, was accurate to within 1.3 m. Compared to the LIDAR altimeter like the one used by Dawson *et al.* (2017) which is accurate to < 0.06 m, the barometric altimeter of the drone was less accurate, which in turn, likely contributed greatly to the estimated errors and variation over repeated measurements.

With respect to the contribution of the body flex and varying body submersion of free-ranging whales, precise quantification is difficult as the true size of each individual is not known in advance for comparison. The dorso-ventral flexing of the body may result in underestimation of length if the method is based on nadir-pointing images (Cubbage and Calambokidis, 1987; Dawson *et al.*, 1995). Body submersion may also contribute to additional underestimation and variation because the altitude was used as a proxy for range (camera-object distance). As whales are always partially submerged (at least), the range is always slightly greater when photographing a whale than when photographing a kayak from the same altitude. Also, because the degree of submersion may vary slightly amongst photographs and individuals, there could be additional ranging imprecision which was not taken into account during this study. Incidentally, this may partially explain why the CV of repeated estimates is greater for whales than for the test kayak. Further studies involving this method and other alternatives at the same time that can take into account the whales' body flex and body submersion (e.g., stereo-photogrammetry, Cubbage and Calambokidis, 1987; Dawson *et al.*, 1995) would allow the quantification of these errors and are thus encouraged. In all cases, the method used in the present study ensured that only photographs of the whale lying flat at the surface, emerged as much as possible from the water, and with straight body axis and caudal peduncle were used. The authors are thus confident that these whale related issues were minimized.

The estimated standard lengths of the mothers were consistent with those obtained from underwater images by Spitz *et al.* (2000) in Hawaii and from aerial vertical photographs by Christiansen *et al.* (2016) off Australia. Using a threshold that was defined according to the known life history of humpback whales, the mothers were categorized as primiparous (< 13-m mothers) or multiparous (≥ 13-m mothers). The calves produced by mothers categorized as multiparous seemed larger than those produced by mothers categorized as primiparous. It has been reported that novice (primiparous) females generally produce smaller offspring (Clutton-Brock, 1991), while multiparous females are capable of producing larger offspring, as they are physically and physiologically more mature than primiparous females (Ellis *et al.*, 2000). For both fin whales (*Balaenoptera physalus*) and sperm whales (*Physeter macrocephalus*), it has been documented that the offspring of primiparous females are smaller than those of multiparous females examined at the same time (Laws, 1961; Gambell, 1972). For right whales (*Eubalaena australis*), calves from primiparous females have a smaller mean length than calves from older females (Best and Rüther, 1992). In this study, the difference in size between calves from multiparous mothers and primiparous mothers was however not statistically significant. It should be considered that this study was conducted at the end of the breeding season, and all calves observed had an unfurled dorsal fin and were longer on average compared to the mean length at birth (4.3 m; Chittleborough, 1965). This means that the calves have already grown significantly since birth. The difference in size, while likely evident at birth, is likely less evident with time as calves may grow throughout the season at different rates depending on various external factors, as seen in pinnipeds (Bowen, 2009). Milk intake by humpback whale calves and the milk production from each mother may for example differ amongst mother-offspring pairs, meaning the postnatal growth rate may vary. Although the sample size was relatively small, the results provide a first assessment of the morphometry of the South Western Indian Ocean's female humpback whales and their calves.

In conclusion, this study allowed the estimation of the body measurements of humpback whale mother-calf pairs and the investigation of the effect of maternal parity on calf size using a relatively straightforward photogrammetric method that combined a standard UAV and free, easy-to-access, and user-friendly software. This method was not as precise and as accurate as methods involving more advanced equipment and tools such as drones equipped with an acute altimeter (Durban *et al.*, 2015, 2016; Dawson *et al.*, 2017) and paid software (Dawson *et al.*, 2017; Burnett *et al.*, 2019). However, it was demonstrated that the method presented in the present study produce convincing morphometric measurements with both satisfactory precision and accuracy. While the level of error limits the suitability of the method presented in the present study for comparing individuals, the method still has potential applications to study the global morphometric trend of a population. The method presented in the present study can be further adapted for studying population structures or for investigating population responses to a changing environment as has been done previously (Chittleborough, 1958; Hanks, 1981; Stevens *et al.*, 2000; Perryman and Lynn, 2002). It is hoped that this study will encourage more teams to study the morphometry of large marine mammals despite limitations in terms of resources. Such studies would especially help in conservation decision-making as it may help identifying environmental issues.

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Integrated approach to analyse benthic images from an autonomous underwater vehicle deployed at Pemba Island, Tanzania

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Abstract

Manual analysis of large amounts of benthic images is time consuming and costly. This challenge has led to the development of automated image analysis techniques such as CoralNet. The technique combines an online repository and machine learning to completely or partially automate classification of benthic images. Here, the integration of Coral Point Count with Excel Extensions (CPCe) and CoralNet is showcased. CPCe was applied to manually annotate images captured by an autonomous underwater vehicle (AUV) deployed at Pemba Island, Tanzania and then to train and build confidence in CoralNet to automatically annotate more images. Further, possible outputs that can be derived from assessing the relationships between the cover of benthic variables and depth are demonstrated.

Keywords: photo quadrat, benthic communities, annotation, depth effects

Introduction

Coral reefs are undergoing dramatic changes and shifts in their community structure, indicating the necessity to monitor these changes rapidly and on a large scale (Bryant *et al.*, 2017). Until now, human observers have been the major source of information on coral reefs. This is limited to time and resources available for monitoring the reefs (Obura *et al.*, 2019). The commonly used monitoring techniques for coral reefs include Length Intercept Transect (LIT), Point Intercept Transect (PIT), photo quadrat and video transect (Hill and Wilkinson, 2004). LIT and PIT both involve counting the number of times lifeforms are found along a transect. The photo quadrat captures digital images of substrates along the transect, whereas the video transect survey captures benthic communities as movies, allowing surveyors to collect sufficient data with minimal sampling effort and time (Leujak and Ormond, 2007). Random Point Count is applied on these photos and videos to estimate the percentage cover of benthic communities (Kohler and Gill, 2006). The photographic surveys are rapid to conduct, and are now widely adopted by coral reef monitoring programmes to determine benthic status and trends while also creating a permanent archive suitable for subsequent analysis (Williams *et al.*, 2019). Collection of photos has also been improved by underwater vehicles (González-Rivero *et al.*, 2016). However, it takes more time to annotate the photo or video quadrats (Molloy *et al.*, 2013) because manual analysis of benthic images is time consuming and costly (Williams *et al.*, 2019).

Coral Point Count with Excel Extensions (CPCe) is a software designed to improve the efficiency and convenience of performing the numerous image annotations. The tool works by randomly spreading a defined number of points over an underwater photo and then allowing the viewer to visually identify the benthos that lies underneath each point (Kohler and Gill, 2006). The annotation output may then be obtained from the programme, which includes both the point count and the benthic percentage cover from each individual photo quadrat, as well as the aggregated mean cover for all annotated photo quadrats. Although photos can be annotated with relative ease with CPCe, picture processing still requires manual

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processing by specialists who are able to identify the image features (Stokes and Deane, 2009). Additionally, varying human analytical accuracy may lead to bias (González-Rivero *et al.*, 2016). Currently, benthic photo analysis has been simplified mainly to a collaboration between marine and computer scientists (Wilson *et al.*, 2017).

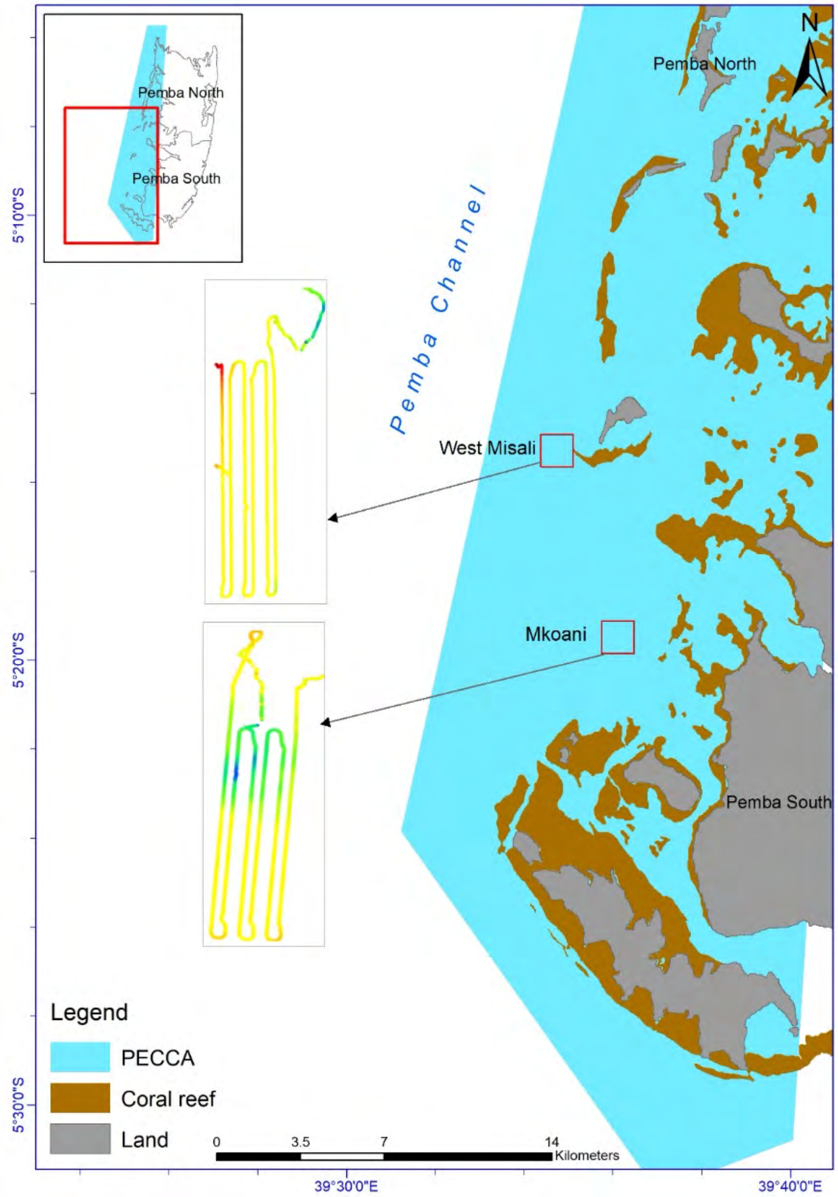


Figure 1. Map showing the sites of West Misali and Mkoani within the Pemba Channel Conservation Area (PECCA) in Tanzania and an inset of the track lines of autonomous underwater vehicle (AUV) deployed at each site. (Adapted from Osuka *et al.*, 2021).

The challenge of accurately and rapidly analyzing large amounts of photos has led to the development of automated image analysis techniques (González-Rivero *et al.*, 2016). CoralNet (https://coralnet.ucsd.edu/) is widely used, particularly for benthic image analysis. It combines an online repository, a tool for manually annotating photographs, and machine learning techniques to completely or partially automate classification of benthic photos (Beijbom *et al.*, 2015; Williams *et al.*, 2019). CoralNet's beta version has an accuracy comparable to human analysts of 80 % for corals and 48-66 % for algal groups such as macroalgae, turf algae, and coralline algae (Williams *et al.*, 2019). On average, González-Rivero *et al.* (2016) discovered that machine annotation and approximation of benthic cover differed by 2.5 % from human annotators' estimates. In the other study by Williams *et al.*

(2019), they demonstrated how spatial and temporal changes in coral reef benthic cover may be accurately assessed using CoralNet through a completely automated image processing algorithm.

Oceanographic variables, either separately or in combination, can predict marine benthic composition with a high level of accuracy (Belanger *et al*., 2012). In benthic studies, depth has consistently been a powerful explanatory variable (Gray, 2001). It is the most important factor influencing marine benthic communities (Bergent *et al*., 2001). It is not a direct driver structuring benthic variables, but it frequently corresponds with predictions and gradients of a variety of dispersal variables particularly temperature, day length, and light penetration (McArthur *et al*., 2010). Olabarria (2006) revealed a correlation between depth and water quality parameters, as well as seafloor characteristics, which affect the settlement, recruiting, primary production and survival of benthic communities. This culminates in diverse benthic habitats and communities at various depths.

Habitat with high supply of resources and good conditions for survival are highly favoured by benthic communities. In particular, environments with a variety of resources, minimal tidal change, inaccessible or least interference by humans, are the most desirable habitats for the benthic organisms (Thrush *et al*., 1998). Since most photosynthesising biota are depth-limited, vertical water depth regulates the spatial distribution of benthic habitats (Wicaksono *et al*., 2019). Besides that, the type of substrate that supports the growth of the benthic community changes with depth. In the shallow and upper mesophotic depths, hard substrata are common, while sand predominates in the lower mesophotic depths (Osuka *et al*., 2021). In terms of benthic cover, hard coral cover has been found to peak at a depth of 10 m, while soft coral and turf algae peak at 20 m whereas the cover of crustose coraline algae (CCA) decreases with depth (Williams *et al*., 2013).

Here, CPCe is applied to manually annotate survey images captured by autonomous underwater vehicle (AUV) deployed at Pemba Island, Tanzania (Osuka *et al*., 2021), and then train and build confidence in CoralNet to automatically annotate more images. Further, the cover of benthic categories is derived from the annotation process and the relationships between the cover of benthic variables with depth is assessed.

Materials and methods

Study area

The shallow reefs of Pemba Island show a broad range of reef conditions, with some reefs in healthy states and dominated by hard coral cover, while others are in a degraded state with low coral cover (Grimsditch *et al*., 2009). An AUV was deployed in the Greater Pemba Channel at Misali, Mkoani and Tumbatu Shoals (see Osuka *et al*., 2021). This study utilised images collected by a low-flying AUV deployed at West Misali and Mkoani (Fig. 1).

Methods

Data were collected by the Teledyne Gavia Offshore Surveyor AUV in depths ranging from 10 and 45 m as described in Osuka *et al*. (2021). The AUV surveyed seafloor and water column properties and took seabed images using a Grasshopper, Sony ICX285 CCD sensor camera. The AUV was programmed to survey from 2 m above the seafloor for approximately 1-hour to capture detailed seabed photography. The control and command centre module of the AUV ensured that the vehicle followed a pre-designed track, collected high-resolution data including photos of the benthic community, and stored the data for later retrieval. The surveys were conducted from the RV *Angra Pequena*. Images of the 2 m-AUV mission had a length and width of ~1.7 m X ~1.3 m giving a footprint of 2.21 m^2. A total of 22 and 19 transects were created from AUV mission pathways in West Misali and Mkoani respectively. Each transect was 100 m in length within which 24 images were randomly selected and 25 points randomly assigned on each image (Table 1). A total of 984

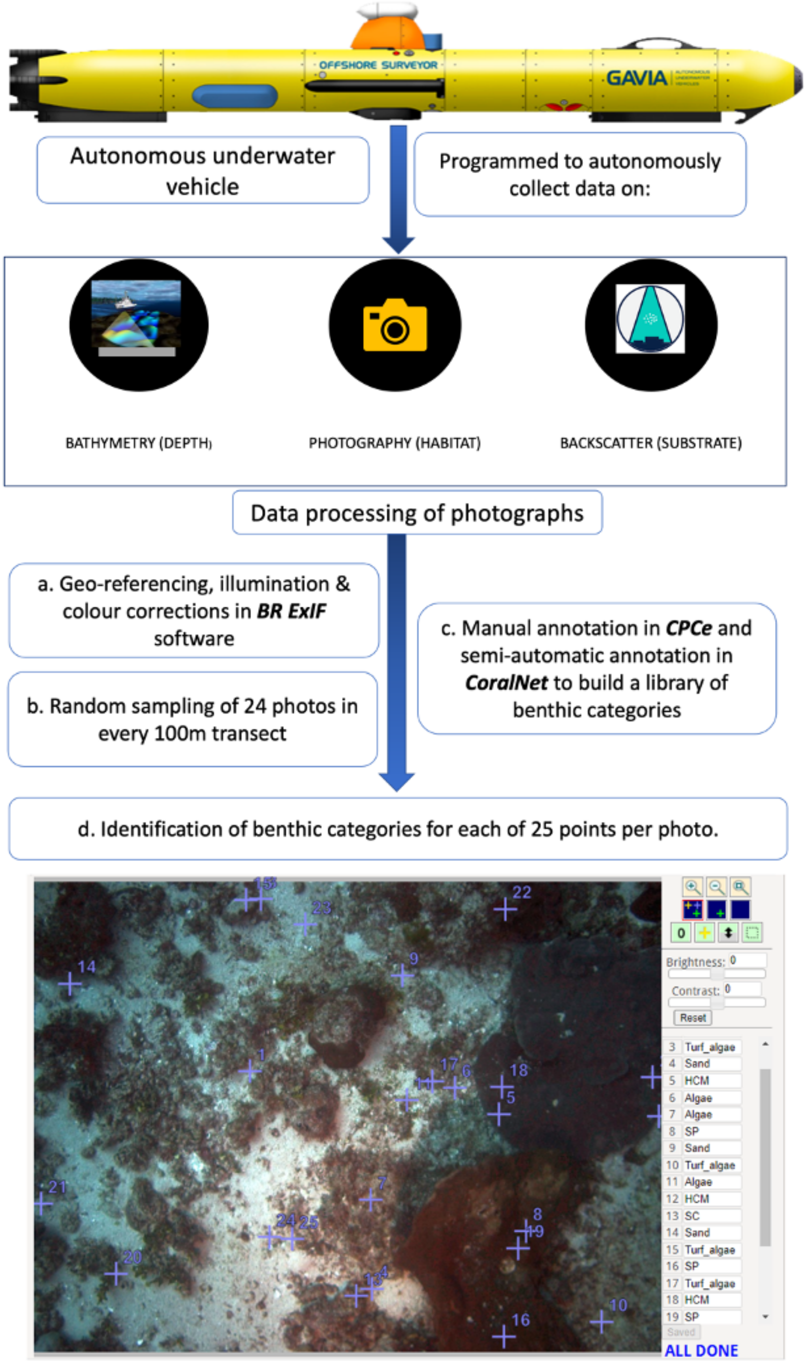
Table 1. Sampling design for two sites showing the hierarchy from transect per site, number of images captured per transect and points selected per image.

Site	Transect (100 m)	Photos per transect (random)	Points per image (random)	Total points per site
West Misali	22	24	25	13,200
Mkoani	19	24	25	11,400

images (528 in West Misali and 456 in Mkoani) were selected for annotation.

Annotation process

The images were manually annotated with CPCe before being semi-automatically annotated in CoralNet (Fig. 2). The goal was to increase CoralNet's confidence in automating annotation of more images collected by the AUV. The annotation process was initially slow while using CPCe because an annotator had to identify the category of each of the 25 points overlaid on the image in the same way for each other image. Later, CPCe images and other raw photos were uploaded to CoralNet, increasing its confidence to around 60 %. The remaining photos were easily annotated using CoralNet, which provides autosuggestions of annotation points. Following the completion of the annotation process on CoralNet, the confidence



3	Turf_algae
4	Sand
5	HCM
6	Algae
7	Algae
8	SP
9	Sand
10	Turf_algae
11	Algae
12	HCM
13	SC
14	Sand
15	Turf_algae
16	SP
17	Turf_algae
18	HCM
19	SP

Figure 2. A graphic showing the autonomous underwater vehicle, data collected, data processing steps, and a sample of the photo with the benthic categories. HCM = hard coral massive, SC = soft coral, and SP = sponges.

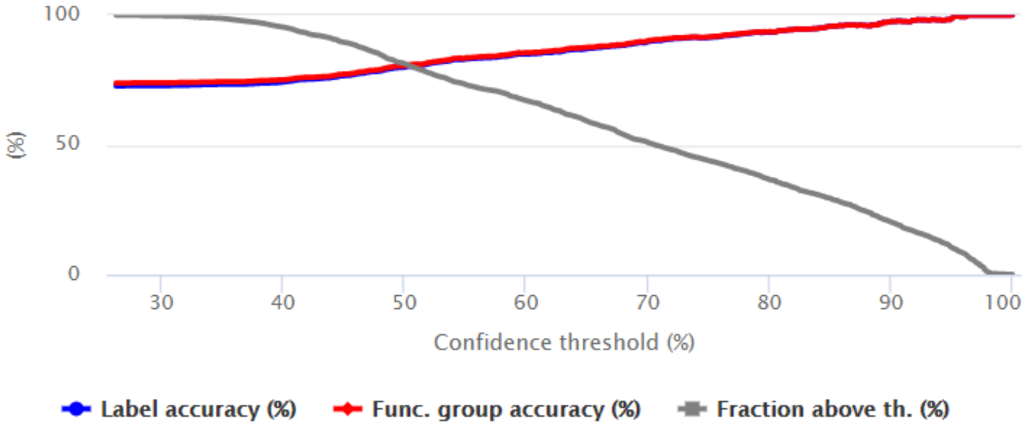


Figure 3. CoralNet computer vision backend showing sweeps of confidence thresholds (th.) for label and functional (func.) group accuracy and fraction above threshold.

threshold raised to 72.7 % (Fig. 3), with the goal of reaching at least 80 – 90 % for future automatic annotation and at least 50 % for within group accuracy. The final output from the CoralNet was uploaded and used for analysis in this study.

Analysis

The cover of benthic variables: hard coral, soft corals, crustose coralline algae (CCA), halimeda, sponge, turf algae, fleshy algae, invertebrates, rubble and sand determined from each photo, were summarised using

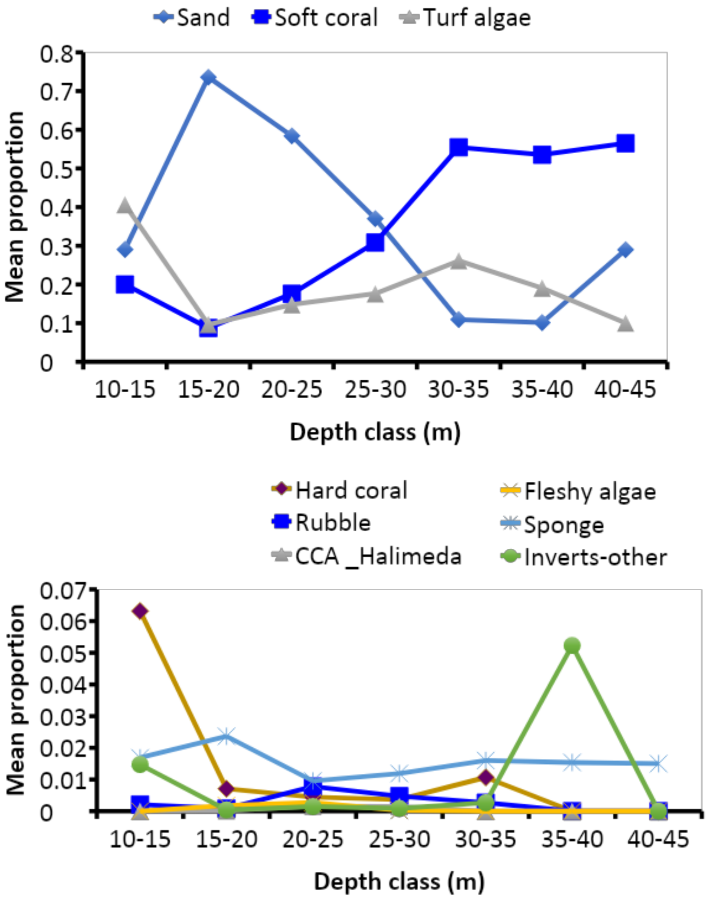


Figure 4. Mean proportion of nine benthic variables sampled from images captured by autonomous underwater vehicle.

means. Relationships between depth and cover of benthic variables were assessed using ordinary least squares regression.

Results

Sand, soft coral and turf algae were the most common benthic variables found in all depths with a mean cover of more than 1 % (Fig. 4). The cover of sand showed a peak of 75 % at 15-20 m depth class but decreased in the subsequent depth classes plateauing at 30-40 m before increasing again in 40-45 m water depth. Soft corals showed an upward trend reaching a maximum cover of about 55 % at 30-35 m and maintaining the cover all through to 45 m. The cover of turf algae was highest at 10-15 m but reduced to around 10 % at 15-20 m before increasing gradually in the subsequent depths up to 30-35 m. The cover then showed a downward trend reaching about 10 % at 40-45 m. Other variables notably hard coral, rubble, CCA-halimeda, fleshy algae, sponge and inverts-other showed low mean cover of <10 % in all depth classes (Fig. 4). Hard corals decreased in cover from about 6 % in 10-15 m to <1 % in depths >15 m. The cover of sponges oscillated between 1-2 % across all depth classes while invertebrates showed two peaks at 10-15 m and 35-40 m registering a cover of 1.5 % and 5 % respectively (Fig. 4).

Cover of certain benthic reef variables showed a significant relationship with depth (Table 2). Negative relationships with depth were evident in hard corals, sponges and sand while positive associations existed for soft corals and turf algae (Fig. 5). Every 10 m increase in the depth was associated with a 1.0 % and 33 % reduction in the cover of hard coral and sand respectively. On the contrary, soft corals and turf algae showed an increase of 24 % and 4.0 % for every 10 m increase in depth (Table 2). Other variables like sponges, rubble, fleshy algae and CCA did not show significant effects.

Discussion

The value of integrating tools to analyse images captured by AUVs is demonstrated. Despite several benefits of using CPCe to analyze photo-quadrats, it is a cumbersome, labour intensive process. However, integrating it with CoralNet helped improve the automation process. Indeed, several efforts globally have been undertaken to automate the benthic image processing and CoralNet is proving to be one possible option. Further research into its use and feasibility for the western Indian Ocean is still needed.

Hard coral and sand showed a significant decline with increasing depth, while soft coral and turf algae showed a different trend. High cover of benthic communities is expected in shallower than in deeper waters (Olabarria, 2006; Stefanoudis *et al.*, 2019). Studies have shown benthic communities at 15–30 m are dominated by reef-building corals (Stefanoudis *et al.*, 2019). Presence of sandy substrates is low in shallow depths where there is a high frequency of coral-algal interactions (Johns *et al.*, 2018) but are expected to dominate in greater depths of >40 m (Osuka *et al.* 2021). While most benthic community classes decrease with depth, others such as turf algae can increase in coverage with depth (Stefanoudis *et al.*, 2019). Dominance of turf algae in shallow reefs constitute an unstable phase that is moving towards a coral or macroalgae attractor (Mumby *et al.*, 2007), with evidence suggesting a shift towards macroalgae dominance if herbivore density is low (Diaz-pulido and Mccook, 2002). Indeed, algae can survive in depths greater than 45 m (Nelson *et al.*,

Table 2. Regression coefficients of the relationship between depth and proportion of benthic variables from Pemba Island, Tanzania. Bolded p-values indicate significant relationships.

Variable	Slope	Error	Intercept	Error	r	p
Hard coral	-0.001	0.000	0.022	0.006	-0.091	**0.005**
Soft coral	0.024	0.002	-0.342	0.035	0.456	**0.001**
Sponge	-0.001	0.000	0.026	0.007	-0.061	0.059
Turf algae	0.004	0.002	0.052	0.037	0.088	**0.006**
Sand	-0.033	0.002	1.299	0.053	-0.421	**0.001**
Rubble	0.000	0.000	0.001	0.006	0.027	0.399
CCA _Halimeda	0.000	0.000	0.000	0.002	0.030	0.360
Fleshy algae	0.000	0.000	0.003	0.005	-0.010	0.748
Inverts-other	0.001	0.000	-0.009	0.003	0.139	**0.001**

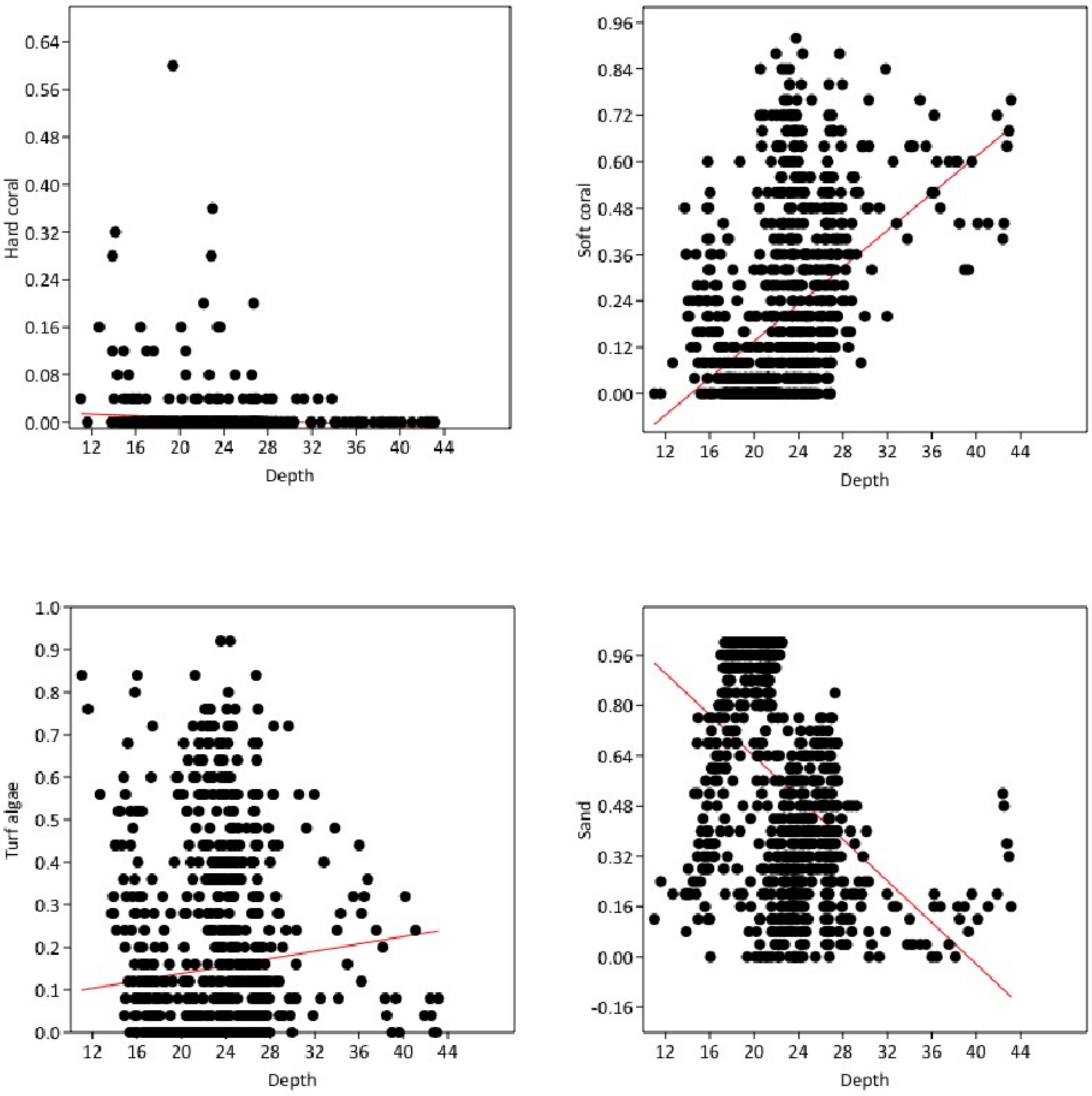


Figure 5. Linear relationships between depth and cover of benthic variables.

2015) and even in depths with 1 % of the surface irradiance, in contrast to seagrass and the majority of hard corals, which require more than 10 % of the surface irradiance to undergo photosynthesis (Wicaksono *et al.*, 2019). On the other hand, soft coral supplement themselves by heterotrophy, which allows them to thrive in deeper waters (Williams *et al.*, 2013). Because of variations in light attenuation underwater, benthic communities become restricted to particular depths (Duarte, 1991; Olabarria, 2006).

Image annotation is a time-consuming process, therefore finding a tool that will make the process easier becomes important, especially where a high number of images are collected by autonomous vehicles like the AUVs. CoralNet, Collaborative and Automated Tools for Analysis of Marine Imagery (CATAMI), SQUIDLE (https://squidle.org/about/) and BIIGLE (https://biigle.de/) are all web-based annotation tools that require consistent online connections to operate, but CPCe is a computer window-based tool that can be used offline but more importantly integrated with CoralNet to help improve the automation process, thereby reducing the amount of time used in analysing images. An alternative improvement would be the development of a computer-based annotation tool that could fully or semi automate the annotation process without requiring access to the internet.

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Erratum

The following article published on the WIOJMS Vol. 20 No.1 (2021) had the legends of the Figures 3 and 4 swapped:

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

Authors:
Mathew O. Silas, Mary A. Kishe, Johnson G. Mshana, Masumbuko L. Semba, Said S. Mgeleka, Bigeyo N. Kuboja, Benjamin P. Ngatunga, Muhaji A. Chande, Patroba Matiku

The correct legends are:
Figure 3. Recruitment pattern of Octopus cyanea of Mafia Archipelago. Jibondo Lα = 23.1, K = 1.5, WP = 0, t0 = 0 (a) and Bwejuu (b) Lα = 23.1, K = 1.2, WP = 0, t0 = 0.

Figure 4. 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 = E0.1., Red = E0.5 and Yellow = Emax).

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