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Original Article

Seagrass cover reduction in Zanzibar from 2006 to 2019

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Abstract

Seagrass meadows are abundant in Zanzibar, Tanzania and provide essential ecosystem services, yet they have been less researched or protected than other marine ecosystems. Evidence suggests that anthropogenic impacts, such as seaweed farming, small-scale fishing, and tourism, are their greatest threats. Using geographic information systems (GIS) to estimate seagrass cover, this study conducted Spearman's rank correlation analyses to estimate correlations between the scale of seaweed farming, fishing, and tourism or the implementation of marine protected area (MPA) management plans, and the change in percent of seagrass cover from 2006 to 2019. On average, seagrass cover decreased by 10.98 % over this period. The scale of tourism was negatively correlated with seagrass cover ($r_s(9) = -0.64$, $p = 0.044$). No other variables were correlated with declines, though seaweed farming had a weak protective effect on seagrass cover ($r_s(9) = 0.28$, $p = 0.426$). To improve the management of seagrass meadows, plans must identify seagrasses as critical ecosystems, expand seagrass restoration projects, and address harmful practices in the tourism industry.

Keywords: seagrass, change detection, anthropogenic impact, MPA, coastal management

Introduction

Seagrasses have been less commonly researched or protected than other flora and fauna in tropical coastal marine environments in Africa. However, there is increasing evidence that seagrasses are essential to humans and coastal environments and must be considered in coastal marine management (Githaiga *et al.*, 2016; Staehr *et al.*, 2018). Due to a lack of data on density and areal extent of seagrass meadows and their threats in Zanzibar, Tanzania (Aller *et al.*, 2019; Staehr *et al.*, 2018), coastal marine management plans cannot adequately target specific threats and address their underlying factors.

Seagrass meadows are located in the subtidal and intertidal zones around the islands of Zanzibar (Aller *et al.*, 2019; Khamis *et al.*, 2017); there have been 12 documented species in East Africa dominated by the climax species *Thalassodendron ciliatum* and *Thalassia hemprichii* (Lyimo *et al.*, 2008; Ochieng and Erftemeijer, 2003). They provide extensive ecosystem services in tropical and temperate regions around the world (Nordlund *et al.*, 2016; Staehr *et al.*, 2018). Seagrasses accumulate and stabilize sediment in the intertidal and subtidal zones, which facilitates coastal geomorphology and provides coastal protection by dampening waves (Belshe *et al.*, 2018; Nordlund *et al.*, 2016; Staehr *et al.*, 2018). They also fertilize sediment with nitrogen and other nutrients, creating ideal environments for feeding grounds, nurseries, and habitats for aquatic and nonaquatic species (Belshe *et al.*, 2018; Nordlund *et al.*, 2016; Staehr *et al.*, 2018). Globally, seagrasses also sequester an estimated 50-64 % of global organic carbon and a substantial proportion of blue carbon (Nchimbi and Lyimo, 2019; Nordlund *et al.*, 2016). One study concluded that the East African coast had the greatest total seagrass biomass in Africa, followed by the Southern Mediterranean coast (Githaiga *et al.*, 2016). Seagrasses are essential to coastal ecosystems and for mitigating climate change.

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Previous research has focused primarily on the impacts of seaweed farming, small-scale fishing, and coastal tourism activities as drivers of seagrass degradation in Zanzibar. Commercial seaweed farming was introduced to Zanzibar in 1989 in Jambiani and Paje and expanded to Chwaka Bay in 1990 (Eklöf *et al.*, 2012; Msuya, 2013). Commercial seaweed is the largest marine export from Zanzibar, with production reaching >16,500 metric tons in 2016 (Kamer, 2022), and seaweed farming remains a high-volume economic venture for several coastal villages (Moreira-Saporiti *et al.*, 2021; United Republic of Tanzania, 2008). The historical practice of uprooting seagrass meadows to remove sea urchins that destroy seaweed has mostly ceased in favor of situating farms in nutrient-rich seagrass meadows that maintain low surface temperatures (Hedberg *et al.*, 2018; Lyimo *et al.*, 2006). The majority (92 %) of seaweed farms in Zanzibar are located at least partially on seagrass meadows, demonstrating their interlinkages (Hedberg *et al.*, 2018). Physical disturbances like trampling or removing seagrasses and sediment disturbance can cause degradation, including a loss of seagrass biomass, shoot length, and cover (Lyimo *et al.*, 2006; Moreira-Saporiti *et al.*, 2021). Situating seaweed farms above seagrass meadows shades seagrasses and hinders their ability to photosynthesize (Lyimo *et al.*, 2006). Among two rural villages where 50-60 % of villagers engaged in seaweed farming, Nchimbi and Lyimo (2019) found that seagrass meadows were visibly degraded and had lower shoot heights, biomass, and density.

The preferred fishing grounds for small-scale fisheries in Zanzibar are seagrass-dominated areas (de la Torre-Castro *et al.*, 2014; Hedberg *et al.*, 2018), yet due to the limited monitoring of seagrass, it is unclear how small-scale fishing practices have impacted seagrass meadows (Staehr *et al.*, 2018). Seagrass meadows and proximate coral reefs support 70 % of small-scale fishing by providing habitats, nurseries, and feeding grounds for finfish, prawns, and bivalves (Khamis *et al.*, 2017; Staehr *et al.*, 2018; UNEP-Nairobi Convention and WIOMSA, 2021). The fishing industry, which provides livelihoods for about one-fifth of the Zanzibar population, primarily uses traditional low-technology gear, such as seine nets and wooden basket traps, and vessels, such as sail-powered dhows and canoes (de la Torre-Castro *et al.*, 2014; Jiddawi and Öhman, 2002; Khamis *et al.*, 2017; Staehr *et al.*, 2018). Annual fish catches have increased from 4,100 tons in 1980 to 34,100 tons in 2015 (Staehr *et al.*, 2018), yet the fish catch per fisherman has decreased, suggesting overfishing is occurring (Khamis *et al.*, 2017). Destructive fishing techniques, such as the illegal use of seine nets, spear-guns, and dynamite, degrade and destroy seagrass meadows and coral reefs (Jiddawi and Öhman, 2002; Khamis *et al.*, 2017). Overfishing and destructive fishing methods are increasingly used to satisfy the swelling demands of a rapidly growing population of inhabitants and tourists (Staehr *et al.*, 2018). However, most Zanzibari fishermen make an income of less than 6 USD per day and cannot afford to alter their practices (de la Torre-Castro *et al.*, 2014), and there is limited capacity to enforce laws that protect the coastal environment (Jiddawi and Öhman, 2002; Khamis *et al.*, 2017).

Since 1985, tourism has grown more than sixteen-fold (Staehr *et al.*, 2018), comprising 27 % of Zanzibar's gross domestic product in the mid-2010's (Khamis *et al.*, 2017). Hoteliers build lodgings along the most attractive sections of the coast, clearing the coastline of seagrass meadows and dredging the seafloor of muddy silt, which is pushing the ecological capacity of coastal marine ecosystems to a state of degradation or destruction (Khamis *et al.*, 2017). These harmful practices compromise the seafloor integrity, the health of seagrasses, coral reefs, and mangrove forests, and the welfare of vertebrates and invertebrates that depend on them (Khamis *et al.*, 2017; Staehr *et al.*, 2018). Other impacts of the booming tourism industry include the runoff of pollution and waste into the ocean and increased oil spills from maritime transport (Khamis *et al.*, 2017; Staehr *et al.*, 2018). Few hotels have onsite water treatment facilities, and many hotels discard waste directly into the ocean (Khamis *et al.*, 2017; Staehr *et al.*, 2018). Zanzibar does not have systematic waste management on the islands to manage the large increase of solid and sewage waste, which increasingly contain plastic products (Staehr *et al.*, 2018). There is an inverse relationship between biomass of seagrass meadows and coastal development, which means that as the Zanzibar population, mariculture, and tourism increase, seagrass meadow densities decrease (Khamis *et al.*, 2017; Staehr *et al.*, 2018).

Although seagrasses are vulnerable to environmental changes, evidence suggests that they typically recover quickly to changing environmental conditions such as changing water temperature (Aller *et al.*, 2019). For example, Aller *et al.* (2019) measured how changes in cloud cover, sunspot activity temperature, tidal amplitude and height, and storm occurrence affected seagrass cover and composition of six species in three transects of the Chumbe Island protected area over a 10-year period. Each transect gradually declined and then increased in composition and cover within the end of the 10-year period, demonstrating the resilience of the seagrass meadows to environmental variables. Although researchers speculate that increasing sea temperatures, sea levels, and storm occurrences due to climate change may be long-term environmental threats to seagrass viability, there is currently no evidence to suggest that environmental changes have affected long-term seagrass viability (Aller *et al.*, 2019; Khamis *et al.*, 2017; Lyimo *et al.*, 2008; Staehr *et al.*, 2018).

Zanzibar has made strides to protect coastal marine environments. Multiple public and private marine protected areas (MPAs) cover the majority of the Zanzibar's 370 km of coastline, protecting approximately 1,300 km^2 of Unguja, the main island commonly referred to as Zanzibar (see Table 1; IUCN, 2020). Private MPAs strictly regulate no-take zones in which mariculture is prohibited to enhance conservation efforts, whereas public MPA zones are regulated to ensure sustainable use of marine ecosystems, such as preventing overfishing (IUCN, 2020). Notably, seagrasses have not been identified as important or vulnerable ecosystems in coastal marine management plans, yet they may benefit from the protections implemented to preserve biodiversity and protect adjacent ecosystems, such as mangroves and coral reefs (Cullen-Unsworth *et al.*, 2014; de la Torre-Castro *et al.*, 2014; Unsworth and Cullen, 2010; Unsworth *et al.*, 2018).

To date, previous studies on seagrasses in Zanzibar have focused on the overall health of seagrasses within particular regions of Zanzibar (e.g., Chwaka Bay) or specific anthropogenic impacts (e.g., seaweed farming) on seagrasses. Yet absent from the discussion is whether particular anthropogenic activities have caused greater harm to seagrasses over time and whether implementing MPA management plans has had a protective effect. Using geographic information systems (GIS) satellite imagery and field data, the current study assessed the percent of seagrass cover in five sites located within MPAs around Unguja Island, Zanzibar in 2006 and 2019 to evaluate whether 1) coastal activities (seaweed farming, small-scale fishing, and tourism) at varying scales, and 2) the implementation of MPA management plans are correlated with long-term changes in seagrass cover. It was hypothesized that high-intensity tourism will have the largest negative correlation with seagrass cover, and the implementation of MPA management plans will have the largest positive correlation.

Materials and methods

Study area

The study area included seagrasses on the coast of Unguja Island, which is located approximately 30 km off the coast of eastern Africa in the Western Indian Ocean. Zanzibar's climate is tropical and defined by two rainy seasons from March to May ("Masika" or the long rains) and October to December ("Vuli" or the short rains), and the Monsoon wind system influences the local currents with slightly stronger winds during the March-May rainy season (Khamis *et al.*, 2017; Staehr *et al.*, 2018). The coastlines alternate between rocky terrain, sandy beaches, and mangrove forests, and the coastal marine environments often include dense seagrass meadows, algae, and fringing coral reefs.

Table 1. Established marine protected areas (MPAs) on Unguja Island, Zanzibar, modified from IUCN (2020).

MPA	Declaration date	Status	Management plan implementation date	MPA area (km^2)
Chumbe Island Coral Park (CHICOP)	1994	Private MPA on western side of island	1994	0.55
Mnemba Island-Chwaka Marine Conservation Area (MIMCA)	2002	Public MPA, no-take zone around Mnemba Atoll	2010	337.3
Menai Bay Conservation Area (MBCA)	1997	Public MPA	2010	717.5
Tumbatu Marine Conservation Area (TUMCA)	2015	Public MPA	Not implemented	162.9
Changuu-Bawe Islands Marine Conservation Area (CHABAMCA)	2015	Public MPA	Not implemented	118.2

Most seagrass beds and meadows are located in shallow water depths of less than 5 m, and meadows are typically comprised of two or more seagrass species (Aller *et al.*, 2017; Belshe *et al.*, 2018; Ochieng and Erftemeijer, 2003). The dominant seagrass species in Zanzibar include *T. ciliatum*, *T. hemprichi*, *Cymodocea rotundata*, *Cymodocea serrulata*, and *Enhalus acoroides* (Lyimo *et al.*, 2008).

There have been two major El Niño Southern Oscillation (ENSO) events in the Western Indian Ocean in the last 30 years, the first lasting from 1997-1998 and the second lasting from 2014-2016 (Lin *et al.*, 2018; Nowicki *et al.*, 2017). Heatwaves resulting from ENSO events have caused episodic declines in seagrasses, and there is currently little research on the recovery time for seagrasses following ENSO events, especially for seagrass meadows on the East African coast (Nowicki *et al.*, 2017; UNdata, 2021).

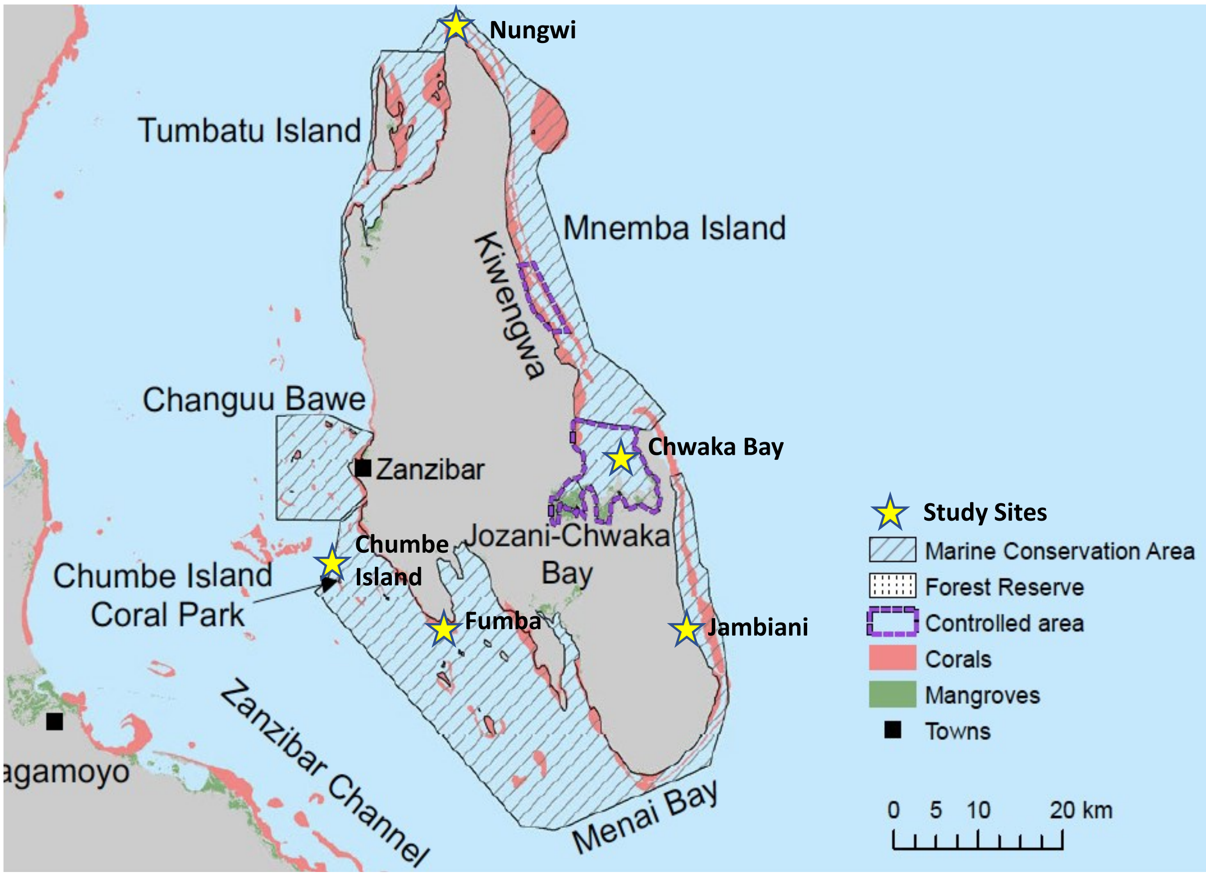

Figure 1. Study site map with MPA boundaries, modified from WIOMSA (UNEP-Nairobi Convention and WIOMSA, 2021).

Site selection

Seagrasses were mapped in five sites within MPAs on and off the coast of Unguja: Chumbe Island, Chwaka Bay, Fumba, Jambiani, and Nungwi (see Fig. 1). Sites were selected that represented a diversity of MPA protections and coastal activities. Chumbe Island, located 6 km off the southwest coast of Unguja, is a small private island surrounded by coral reefs and seagrasses with one small ecolodge. The western side of the island is a no-catch zone established and managed by the Chumbe Island Coral Park (CHICOP) MPA. Chwaka Bay is a large economically important bay on the eastern central coast that supports mariculture activities, including small-scale fishing and seaweed farming. Nungwi is the northernmost village on the island and economically relies on large-scale tourism and small-scale fishing. The Mnemba Island-Chwaka Marine Conservation Area (MIMCA) includes the coasts of Chwaka Bay and Nungwi. Jambiani is located on the southeastern coast of Unguja and supports small-scale fishing, seaweed farming, and large-scale tourism. Fumba is located on the southwest coast and relies primarily on small-scale fishing immediately off the coast. The Menai Bay Conservation Area (MBCA) includes the coasts of Jambiani and Fumba.

Data sources

The study used Google Earth satellite images taken between 2005-2007 and 2019-2021, existing field data from a data repository, regional statistics, and evidence from peer-reviewed articles to map and analyze seagrass and habitat characteristics as well as MPA management plans and coastal activities. To compare the distribution and characteristics of seagrasses over time, site-specific satellite images and field data were included. The field data used for validation are part of SeagrassNet (Global Monitoring Network), were collected by CHICOP, Ltd., and were made available through the database at www.seagrassnet.org. Field data included the percentage of seagrass cover per 0.25 m^2 quadrat collected by CHICOP, Ltd. in October 2006 and September 2019, which aligned with the time periods of the Google Earth images to ensure field samples and satellite images reflected the same ecological conditions. Regional statistics, peer-reviewed articles, and Google Earth images provided information about the implementation and scale of MPA management plans and coastal activities, including seaweed farming, small-scale fishing, and tourism, occurring in coastal villages around Unguja. Due to the limited availability of data on the scale of coastal activities, all available relevant data was included.

Time period selection

The study compared the change in the percentage of seagrass coverage at each site from 2006 to 2019. Appropriate baselines were determined for seagrass cover based on timelines of the growth of Zanzibar's overall economy, mariculture, and tourism sectors and the availability of high-quality satellite images. Zanzibar's per capita GDP has steadily increased from 445 USD in 2006 to 1,111 USD in 2019 (UNdata, 2021), indicating a nearly threefold increase in capital in the measurement period. Tanzania's overall seaweed production increased from approximately 7,000 tons in 2004 to 10,000 tons in 2019 (Kamer, 2022; Msuya, 2020). Following a decline in fish catches in the 1980's, annual artisanal fish catches in Zanzibar have steadily increased from >20,000 tons in 2006 to >30,000 tons in 2016 (Rehren *et al.*, 2020). The number of tourists visiting Zanzibar has increased fivefold from >100,000 tourists in 2005 to >500,000 in 2018 (World Bank, 2019).

In addition to considering the increasing intensity of coastal activities, the limitations in available historical satellite imagery and field data had to be factored in. The earliest available high-quality satellite images on Google Earth Engine were between 2005 to 2007 for all sites, and CHICOP, Ltd. collected its first set of field data in 2006.

Study variables

Each site was assigned codes in 2006 and 2019 based on the relative scale of the following variables: MPA management plans, seaweed farming, small-scale fishing, and tourism. For MPA management plans, each site received a "1" or "2" if the site was contained within a partial or full MPA, respectively, that implemented a management plan for at least two years. A two-year minimum implementation period was included to account for the time it takes to implement new policies that lead to changes in practices in the use of coastal marine ecosystems. Sites contained outside of MPA boundaries or in MPA boundaries without management plans implemented for > two years were assigned a "0".

To assign the scale of seaweed farming at each site, the number of seaweed farmers were used as a proxy for assessing the impact of trampling, shading, and removal of seagrasses. The Joint Frame Survey scale of the number of seaweed farmers in each region of Unguja was used to assign codes (United Republic of Tanzania, 2008). Since the quantity of seaweed farmers is aggregated and reported at the regional level, sites that produced commercial seaweed within regions with >1,000 seaweed farmers were coded with "2"; commercial seaweed producing sites within regions with <1,000 seaweed farmers were coded with "1". Sites that did not harvest commercial seaweed were coded with "0".

Due to a lack of comprehensive fisheries data available for each site, a proxy of the number of fishermen was used to determine the scale of small-scale fishing at each site. As the number of fishermen increases, the number of destructive fishing practices and harmful equipment (e.g., use of seine nets and fishing boat engines) that can damage seagrasses are likely to increase as well. The Joint Frame Survey scale for the number of fishermen in each region was used to assign codes; sites within regions with >4,000 fishermen were coded with "3", sites within regions with 2,501-4,000 fishermen were coded with "2", and sites within regions with ≤2,500 fishermen were coded with "1" (United Republic of Tanzania, 2008). Sites within no-take zones were coded with "0". It should be noted that the mapping boundaries for Chumbe Island were limited to the no-take zone on the western side of the island.

The number of hotels situated on the coastline within each site mapping boundary were used as a proxy for the tourism variable, the same method employed by Khamis *et al.* (2017). This indicator is a suitable measure of the impact of tourism because the construction and operation of hotels on the coast can directly contribute to removal of seagrass meadows and other vegetation, coastal erosion, sewage and solid waste runoff, and increased degradation of coastal ecosystems due to higher volumes of tourists swimming in the intertidal and subtidal zones. Google Earth was used to identify and calculate the percent of hotels on the coastline in 2019. Sites with <10 %, 10-50 %, or >50 % of hotels located on the coastline were assigned a "1", "2", or "3", respectively. Due to a lack of available data on the number of hotels on the coast in 2006, it was estimated that the scale of tourism in 2006 was one-third of the volume in 2019 since the number of tourists had increased more than threefold from 2006 to 2019 (World Bank, 2019). Each site was, therefore, assigned a "1" for low-scale tourism in 2006.

A variable for the combined score of coastal activities was also created to assess whether coastal activities had a collective impact on seagrass health. For example, Chwaka Bay's combined score for coastal activities in 2006 was calculated as follows: 3 (high-scale seaweed farming) + 3 (high-scale fishing) + 1 (low-scale tourism) = 7. The MPA management plan status and scale of coastal activities for each site in 2006 and 2019 are displayed in Table 2.

Developing coastal maps

Remote sensing methods have been utilized globally to map the distribution of seagrass meadows and measure habitat characteristics, such as seagrass biomass, water depth, and water quality (Amran, 2017; Hossain *et al.*, 2016; Knudby and Nordlund, 2011). The distribution of seagrass beds and meadows at both time points using Google Earth Engine images was mapped first at a resolution of approximately 15 m. Only seagrass beds and meadows located in relatively shallow areas (<5 m) were included to ensure data quality. Seagrass beds and meadows were mapped in heterogeneous polygons to measure the area of each patch.

For sites that did not have clear satellite images in 2006 or 2019 or during the same corresponding seasons, the primary image between the range of 2005-2007 and 2020-2021 was selected and the mappings compared to 2006 and 2019 images for accuracy. Images of final site mappings are displayed in Figures 2a-j, and the seasons during which each image was taken are listed in the figure caption. One challenge to mapping was distinguishing algae-covered coral reefs from seagrasses in sites with high coral mortality, such as at Nungwi, so potential seagrasses located in coral reefs were not included unless seagrass meadows within coral reefs were visibly dense (for example, see Fig. 3).

Once the mappings were completed, the percentage of seagrass cover for each site was calculated by

Table 2. Site characteristics of MPA management plan status and scale of coastal activities in 2006 and 2019.

Site	MPA zone	Year	Management plan status	Scale of Coastal Activities		
				Seaweed farming	Small-scale fishing	Tourism
Chumbe Island	CHICOP	2006	Implemented – Full MPA	None	None	Low
		2019	Implemented – Full MPA	None	None	Low
Chwaka Bay	MIMCA	2006	None	High	High	Low
		2019	Implemented – Partial MPA	High	High	Medium
Fumba	MBCA	2006	None	None	Medium	Low
		2019	Implemented – Partial MPA	None	Medium	Low
Jambiani	MIMCA	2006	None	High	High	Low
		2019	Implemented – Partial MPA	High	Low	High
Nungwi	MBCA	2006	None	None	High	Low
		2019	Implemented – Partial MPA	None	High	High

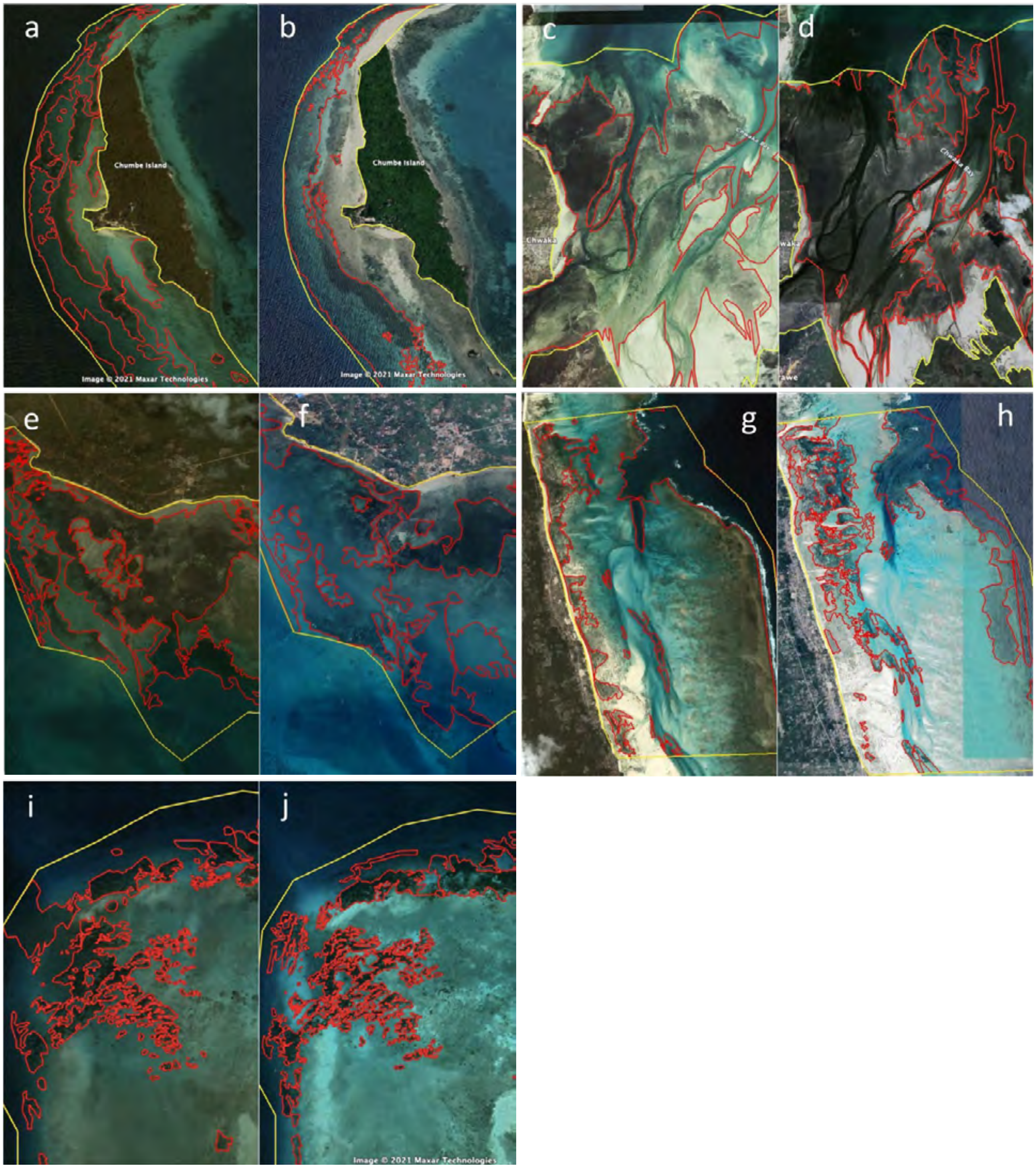

Figure 2a-j. Google Earth images of sites with site boundaries outlined in yellow and seagrass mappings outlined in red: (**a**) Chumbe Island, October 2005; (**b**) Chumbe Island, February 2021; (**c**) Chwaka Bay, February 2006; (**d**) Chwaka Bay, January 2019; (**e**) Fumba, January 2006; (**f**) Fumba, July 2019; (**g**) Jambiani, September 2005; (**h**) Jambiani, August 2020; (**i**) Nungwi, November 2005; and (**j**) Nungwi, February 2019.

summing the area of each polygon to calculate the total area (km^2) of seagrasses at each site and then dividing the total area of seagrass cover by the total mapping area for each site. The percent change in seagrass cover from 2006 to 2019 was then calculated per site.

Statistical analyses

All analyses were conducted with StatPlus:mac v5.0 statistical analysis software (AnalystSoft Inc., 2021). First, the mappings were validated by conducting a two-samples paired t-test to compare the percent of seagrass coverage in 2006 and 2019 in the validation field dataset

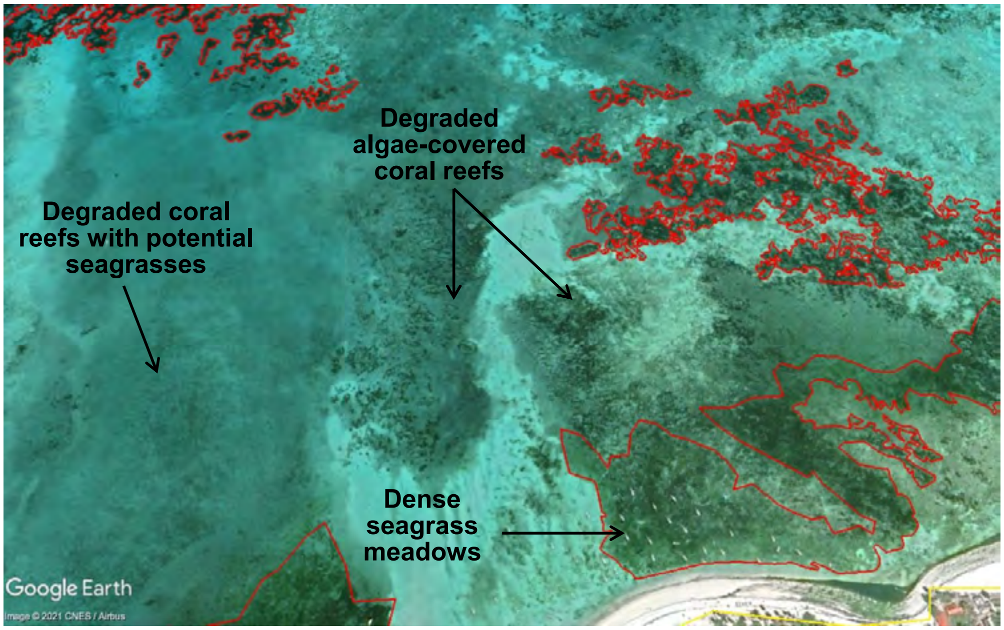

Figure 3. Examples of degraded coral reefs with potential seagrasses, degraded algae-covered coral reefs, and dense seagrass meadows located in Nungwi, February 2019.

and the study GIS dataset. To determine whether the implementation of MPA management plans, coastal activities (seaweed farming, small-scale fishing, and tourism), or the combination of coastal activities were correlated to changes in seagrass coverage from 2006 to 2019 at selected sites, Spearman's rank correlations tests were conducted and scatterplots generated.

Results

Total mapping areas, percent of seagrass cover in 2006 and 2019, and total percent change for each site are listed in Table 3. Chwaka Bay had the largest mapping area of 59.12 km², and Chumbe Island had the smallest area of 0.57 km², defined by the boundaries of the no-take zone. Chwaka Bay also had the highest percentage of seagrass cover in both measurement

Table 3. Total area and percentage of seagrass cover per site.

Site	Area included in mapping (km²)	Total area of seagrass cover (km²), 2006	Percentage of seagrass cover, 2006	Total area of seagrass cover (km²), 2019	Percentage of seagrass cover, 2019	Percent change from 2006 to 2019
Chumbe Island	0.57	0.27	48.11%	0.22	39.08%	-18.76%
Chwaka Bay	59.12	37.58	63.57%	32.68	55.28%	-13.04%
Fumba	6.72	3.29	49.00%	3.09	45.91%	-6.30%
Jambiani	20.49	5.83	28.47%	6.01	29.34%	+3.07%
Nungwi	6.47	1.25	19.30%	1.00	15.47%	-19.86%

Table 4. Correlation coefficients and p-values for correlations between seagrass coverage and the implementation of marine protected area (MPA) management plan and the scale of coastal activities between 2006 and 2019.

MPA or coastal activity	Correlation coefficient (rs)	p-value
MPA management plan	-0.01	0.972
Seaweed farming	0.28	0.426
Small-scale fishing	-0.12	0.734
Tourism	-0.64	0.044
Combined coastal activities	-0.08	0.825

periods (63.57 % and 55.28 %), followed by Fumba (49 % and 45.91 %). Nungwi had the lowest percent of seagrass cover (19.3 % and 15.47 %) and had large areas of degraded or dead algae-covered coral reefs. On average, seagrass cover decreased by 10.98 % from 2006 to 2019. Nungwi had the highest decrease in seagrass cover (19.86 %), followed by Chumbe Island (18.76 %). Only Jambiani had an increase in seagrass cover from 2006 to 2019 (3.07 %).

The percent change of seagrass cover from 2006 to 2019 was -32.61 % for the validation field dataset compared to -18.76 % for the study GIS dataset. In the paired two-samples t-test, no significant difference was found in the percent change in seagrass cover between the validation and study datasets ($t(3) = 2.65$, $p = 0.230$). Therefore, it was concluded that the Chumbe Island GIS data adequately aligned with the field data and that the mappings of satellite images were valid.

Table 4 lists the correlation coefficients and p-values for MPA management plans and coastal activities. Spearman's rank correlation coefficients in the range of 0.60-0.69 are considered moderate to strong correlations (Akoglu, 2018). A strong negative correlation was found between changes in seagrass coverage from 2006 to 2019 and the scale of tourism ($r_s(9) = -0.64$, $p = 0.044$). In other words, seagrass coverage is more likely to be lower in sites with higher-scale tourism. Figure 4 displays a scatterplot of the relationship between tourism and the percent of seagrass cover. The linear slope of the line of best fit from the top left to the bottom right indicates a strong negative relationship between the variables.

There were no significant correlations between changes in seagrass coverage and MPA management plan implementation ($r_s(9) = -0.01$, $p = 0.972$), seaweed farming ($r_s(9) = 0.28$, $p = 0.426$), small-scale fishing ($r_s(9) = -0.12$, $p = 0.734$), or the combined score for coastal activities ($r_s(9) = -0.08$, $p = 0.825$).

Discussion

This study is the first to identify correlations between different coastal activities and decreases in seagrass cover over time. As expected, tourism had the greatest significant negative correlation with seagrass cover from 2006 to 2019 ($r_s(9) = -0.64$, $p = 0.044$), whereas the use of seaweed farming, small-scale fishing, or the combination of activities were not significantly correlated with changes in seagrass cover. Several studies evaluating nutrient concentrations in coastal waters near cities around Unguja have found higher nutrient concentrations in population and tourism centers and closer to the shore, which they attribute to untreated sewage and pollution (Limbu and Kyewalyanga, 2015; Moto and Kyewalyanga, 2017; Moynihan *et al*., 2012; Ngusaru, 2000). Staehr *et al*. (2018) suggested that eutrophication resulting from increased levels of dissolved organic matter and nutrients from untreated sewage in intertidal and subtidal zones were related to population and tourism increases in Zanzibar. Algal overgrowth due to eutrophication can harm seagrasses through light reduction and ammonium toxicity (Burkholder *et al*., 2007). Although the links between tourism and deteriorating marine environmental conditions are well established, the current study is the first to the authors' knowledge to demonstrate correlations between growing tourism and long-term seagrass declines in Zanzibar.

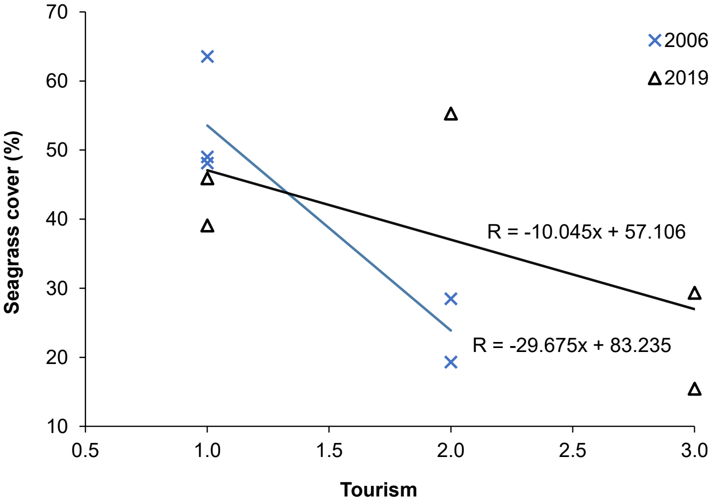

Figure 4. Scatter plot of the linear relationship between the scale of tourism and the percent of seagrass cover in 2006 compared to 2019.

Small-scale fishing had a weak negative correlation with the percent of seagrass cover ($r_s(9) = -0.12$, $p = 0.734$), suggesting that small-scale fishing still has a negative impact on seagrasses that has been documented in other research. For example, overfishing of finfish residing in seagrass meadows has caused multiple crown of thorn starfish (*Acanthaster planci*) outbreaks in Zanzibar, leading to significant coral reef damage (Staehr *et al.*, 2018). Additional research on the long-term impact of small-scale fishing practices on seagrass health is needed to establish whether small-scale fishing poses a substantial threat to seagrasses over time and should be identified as a threat in coastal marine management plans.

Contrary to hypothesis used in this study, seaweed farming had a weak positive correlation with seagrass cover ($r_s(9) = 0.28$, $p = 0.426$), suggesting that areas with higher scales of seaweed farming were more likely to have greater seagrass cover. Jambiani was the only site to show an increase in seagrass coverage from 2006 to 2019, which was likely reflective of the high baseline degradation of seagrass meadows and historical practices of uprooting seagrasses in seaweed farms (Gullström *et al.*, 2006; Lyimo *et al.*, 2006). For example, Figure 5 is a satellite image from 2006 of rows of seaweed farms in Jambiani that were built over bare sand that had been cleared of seagrasses. Seaweed farmers have since developed an understanding that the sediment nutrient enrichment of seagrasses supports better growth of seaweed, and the practice of uprooting seagrasses is less common (Lyimo *et al.*, 2008).

Contrary to the study hypothesis, the implementation of MPA management plans had no effect on seagrass cover ($r_s(9) = -0.01$, $p = 0.972$). However, this is not entirely surprising given the mixed evidence for the efficacy of MPAs to conserve seagrasses and other marine ecosystems (de la Torre-Castro *et al.*, 2014) and the complexity of developing MPA management plans that adequately account for the unique ecological, sociopolitical, and economic contexts both within and outside the boundaries of the MPA. Several studies have demonstrated the influence of coastal land-use on seagrass conservation within MPAs in Zanzibar and in other tropical environments. Aller (2018) compared seagrass cover and species composition in public MPAs, private MPAs, and unprotected sites in Zanzibar. Although the study's results suggested that MPAs increased the temporal stability of seagrass habitat-dependent fish, management of MPAs did not effectively protect seagrasses from negative land-use effects. Eklöf *et al.* (2009) compared the effectiveness of protecting seagrasses from sea urchin predation in two large Kenyan MPAs and ultimately found that the targeted approaches of MPAs were ineffective at preventing sea urchin overgrazing. They suggested that both ecological factors and impacts from coastal land use contributed to the unsuccessful management of sea urchin overgrazing. Additional research is needed to evaluate

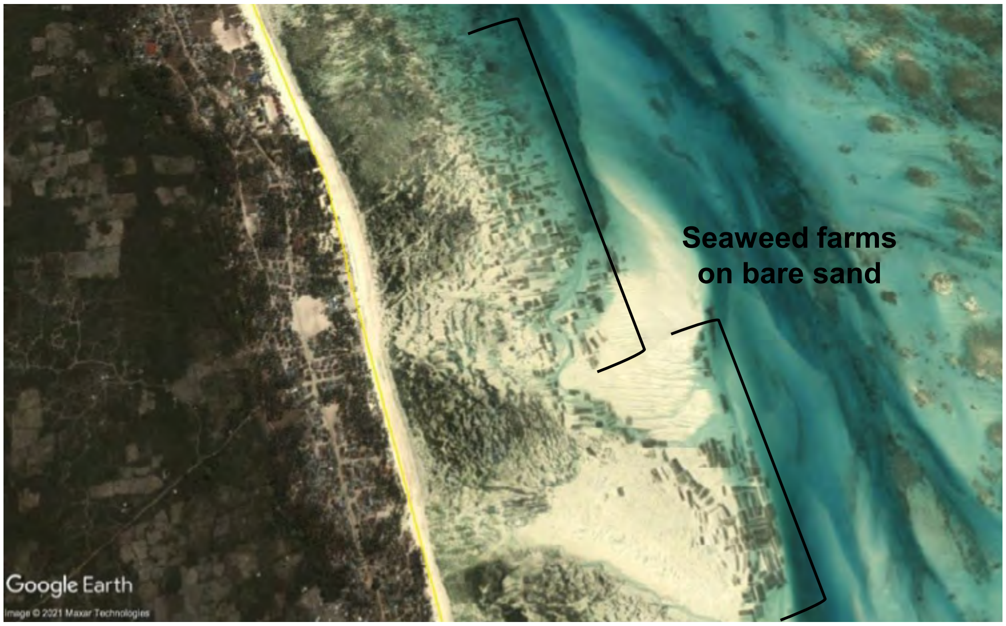

Figure 5. Rows of seaweed farms located on bare sand in the intertidal and subtidal zones of Jambiani, September 2005.

specific characteristics of Zanzibar MPAs, effects of coastal land use, water pollution, and associated seagrass conservation outcomes. In addition, a global framework for determining a site-specific hierarchy of variables (i.e., ecological factors within MPAs and outside of MPAs, sociopolitical factors, land-use conditions, among others) that influence the effectiveness of MPAs could be a useful tool for integrated coastal marine planning and management.

It remains a possibility that environmental factors are also driving seagrass declines, though the available literature does not support this conclusion. Aller *et al*. (2019) found that changes in cloud cover, sunspot activity temperature, tidal amplitude and height, and storm occurrence did not impede seagrass recovery in Chumbe Island within a 10-year period. The timing of seagrass declines and recovery varied by transect, rather than by the quarter of year, so they concluded that seasonality may not be the main driver of seagrass cover variation. In addition, two studies evaluating the seasonal variation of seagrass cover using satellite images and field data in Chwaka Bay and Chumbe Island found no seasonality in the percent of seagrass cover (Gullström *et al*., 2006; Knudby *et al*., 2010, respectively). More generally, Kamermans *et al*. (2002) found very little variability of porewater salinity in the intertidal zone across seasons, which may indicate that bi-annual monsoons do not substantially affect coastal water composition. More research is needed to evaluate the long-term impacts of environmental factors and climate change on seagrasses in Zanzibar.

There are several limitations to the current study. First, it was difficult to distinguish algae-covered coral reefs from seagrasses in several sites, so the percent of seagrass cover was likely underestimated in sites with high coral reef mortality, such as at Chumbe Island. More extensive and comprehensive field data could improve the accuracy of seagrass mappings. In addition, the percent of seagrass cover provides limited information on the overall health of seagrass meadows, therefore collecting in-situ data at multiple sites and utilizing more indicators in addition to seagrass cover can strengthen the assessment of overall seagrass health. Finally, results comparing the relative impact of each coastal activity should be interpreted with caution as detailed data on the number of seaweed farmers and small-scale fishers at each site and water quality indicators were not available.

Recommendations

First, it is recommended that Zanzibar's coastal marine management plans identify seagrass meadows as critical ecosystems; implement regional efforts to measure and track changes to seagrass cover, volume, and species composition; and develop evidence-based plans to restore and conserve seagrass meadows. Future research should evaluate the long-term return on investment for seagrass restoration projects to ensure they are cost-effective and beneficial to communities in Zanzibar.

Second, there is an opportunity for Zanzibar to promote sustainable practices and accountability in the tourism industry by creating an ecotourism network that uses a transparent rating system to evaluate participating hotels and lodges on their environmental practices. Although many tourists already participate in ecotourism in Zanzibar, there is currently no mechanism for tourists to determine the environmental and socioeconomic practices of hotels and lodges.

Finally, addressing harmful environmental practices of the tourism industry, such as polluting coastal waters and uprooting seagrass meadows on hotel coastlines, should become a high long-term priority for integrated coastal marine management. There are numerous models with demonstrable success from which Zanzibar could draw, such as levying fines on noncompliant hotels and lodges or instituting tourism taxes that fund waste management systems and conservation projects. Policymakers in Zanzibar should consult the appropriate stakeholders and conduct market-based analyses on each approach to determine the best approach to address harmful environmental practices in the tourism industry.

Conclusions

The current study found support for the hypothesis that tourism, compared to seaweed farming or small-scale fishing, was significantly correlated with a decline in seagrass cover in Zanzibar from 2006 to 2019. A weak insignificant correlation between small-scale fishing and seagrass cover decline was also found, as expected. In contrast, the results showed that seaweed farming had a weak, though insignificant, positive correlation with seagrass cover. Surprisingly, support was not found for the hypothesis that the implementation of MPA management plans would have a protective effect on seagrass cover. This may be due to community reliance on coastal resources and the ineffective enforcement of MPA regulations, which may limit the effectiveness of MPAs throughout Zanzibar (UNEP-Nairobi Convention and WIOMSA, 2021).

This research contributes to a growing body of evidence that tourism, urbanization, and other anthropogenic impacts are driving declines in seagrass ecosystems in Zanzibar, and implementing MPAs alone is not enough to protect seagrasses and the rest of the coastal marine environment. Yet this study is the first of its kind to compare the long-term impacts of coastal activities and the implementation of MPA management plans on seagrasses in Zanzibar.

Acknowledgments

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Original Article

The first attempts made towards the domestication of sea cucumbers in Kenya

Western Indian Ocean
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Abstract

The potential for culture of sandfish Holothuria scabra in Kenya was investigated based on a sample of 1000 wild individuals with an average weight of 40-80 g. This experiment was conducted to identify suitable methods of collection, transportation, acclimatization, and growth at different stocking densities. Packing methods tested during collection were oxygen filled plastic bags and open basins. For method 1, Sample 1 sandfishes were carried individually in oxygen filled plastic bags with sea water. Sample 2 included a total of five sandfishes per bag, and Sample 3 had a maximum of ten sandfishes. Under method 2 specimens were transported using open basins containing sand and aerated sea water in densities of 10, 20 and 30 individuals per basin. The mean (±SD) percentage evisceration rate during transportation in method 1 was 0 for a density of 1, 3.33 ± 5.77 % for a density of 5, and 20 ± 10 % for a density of 10. In method 2 evisceration occurred at all the three densities; 23.3 ± 15.28 % for the low density, 23.33 ± 2.8 % for the middle density and 36.6 ± 3.33 % for the higher density. The findings of this study provide information to contribute to the development of marine aquaculture of sea cucumber in Kenya.

Keywords: sea cucumber, *Holothuria scabra*, collection, transportation, acclimatization

Introduction

Sandfish *Holothuria scabra*, Jaeger 1883, has been classified as the most commercially valuable species of sea cucumber commonly found in tropical and sub-tropical countries (Conand and Muthiga, 2007). The species *H. scabra* has high grade of beche-de-mer (dried body wall) that over the years has commanded the highest prices in the international market (Purcell *et al.*, 2018). The main uses of the processed products from sea cucumbers include food applications, medicinal purposes, and as an aphrodisiac that are highly prized in Asian countries like China and Malaysia (Rahman, 2014). It has been sold in Hong Kong at prices ranging from USD 115-640 kg^{-1} dried (Purcell *et al.*, 2012). Due to these traits the organism has been vulnerable to exploitation resulting in a decline in populations in the wild that has rendered the species endangered. With the market being so lucrative worldwide, fishermen are searching for and expanding to new fishing grounds (Rahman, 2014).

Community-based sea cucumber fisheries have been on the increase in the Western Indian Ocean region in countries like Madagascar and Tanzania (Beltran-Gutierrez *et al.*, 2014; Robinson and Pascal, 2012). The main attributes of the fishery include the sedentary behavior of sea cucumbers, high product value, low processing cost and huge international market which is mostly in Asian countries (Lovatelli *et al.*, 2004; Bruckner, 2006; Conand and Muthiga, 2007). On the Kenyan coast, the sea cucumber fishery is mostly artisanal, where it has been in existence since the early 1900's but there has been no tradition of consuming sea cucumber in Kenya. The fishery involves collecting sea cucumbers either as bycatch or targeting them through hand picking and fishing by using snorkel or

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SCUBA equipment (Conand and Muthiga, 2007). The processed sea cucumbers are purchased by dealers at the landing beaches who in turn sell the products to exporters based in the county capital of Mombasa who then export to Hong Kong. The major landing beaches for *H. scabra* are on the southern coast of Kenya in Vanga, Shimoni and Majoreni.

Studies have shown that over 50 species of tropical sea cucumbers have been commercially exploited and traded including *H. scabra,* the most valuable sea cucumber species exploited in tropical areas (Purcell, 2010; Purcell *et al*., 2012). Other holothurians facing high exploitation are *H. fuscogilva* and *H. nobilis,* which are also found in Kenyan waters and are highly valued (Conand and Muthiga, 2007). Overexploitation in the sea cucumber fishery has created a worldwide management concern with the main reasons being worldwide demand, high value, serial local depletions, and fishermen migration to new fishing areas (Lovatelli *et al.,* 2004). A study by Uthicke (2004) states that the slow nature of sea cucumber population recovery after depletion has led to a situation that has compromised sustainability. Further research by Hasan (2005) and Friedman *et al.* (2011) found that a heavily exploited sea cucumber population could take more than 50 years to recover. In addition, low *H. scabra* densities may decrease chances of successful spawning thus impeding population recovery and increasing the risk of an Allee effect (Bell *et al*., 2008).

From the aforementioned discussion it is clear that sea cucumber have been under intense fishing pressure, warranting need for effective conservation measures. The organisms provide an important contribution to livelihoods of coastal communities which has fostered the need for domestication to ascertain viability of culture and farming options. Further, cultivation of *H. scabra* has increasingly become necessary to support stock enhancement programmes (Giraspy and Ivy, 2005) and to meet the export market demand (Purcell *et al*., 2012). Domestication will protect the organisms from identified threats such as habitat destruction and unsustainable fishing until the sea cucumbers reach marketable size or a minimum size for restocking. Hatchery production of *H. scabra* has been carried out in different countries across the globe (Kumara and Dissanayake, 2017). Other countries have initiated restocking and sea ranching (Purcell *et al.,* 2012; Eriksson *et al*., 2014; Watanabe *et al*., 2014). In China, *H. scabra* aquaculture has achieved milestones where 5 g juveniles have been produced and cultured to commercial harvestable adults of 300 to 500 g weight (Purcell and Wu, 2017). Studies have shown that success of *H. scabra* aquaculture is limited to hatchery production with reliance on viable broodstock collected from wild populations (James, 2012).

Hatchery production of *H. scabra* juveniles relies on control of broodstock collection, maintenance, spawning, fertilization, larval rearing, and post larvae settlement rearing (Hamel *et al.*, 2022). For domestication purposes, transportation of wild collected individuals is essential and it follows different procedures which vary from one hatchery to another (Hamel *et al*., 2022). Studies by Battaglene (1999) mentioned that sea cucumber collection needs to be done under minimal salinity and temperature variations to avoid evisceration during transport. In other studies, individuals have been placed individually in oxygen filled bags with seawater (Ito, 2014; Abidin *et al*., 2016; Kumara and Dissanayake, 2017). Studies by Tuwo *et al* (2019) assessed the evisceration rate of *H. scabra* using closed and open transportation modes and their findings showed that evisceration was triggered by the presence of decaying individuals in the transportation bags that had eviscerated before transport. Acclimatization in pre-prepared holding tanks aids in the quick recovery of *H. scabra* individuals that survive during transportation (Hamel *et al*., 2022).

In Kenya, there has been no culture trials of *H. scabra* so far. The aim of this study was therefore twofold; to identify a suitable method of sea cucumber transportation, and to understand and establish optimal acclimatization conditions at different densities. This article documents the first attempts made towards domestication of sea cucumber in Kenya as initial steps towards *H. scabra* culture for natural stock enhancement and as an opportunity for an alternative livelihood for the fisher communities.

Materials and methods

Experimental design

The study tested conditions for sea cucumber transportation and acclimatization. Two transportation modes were investigated including varying packing methods and sandfish density. Plastic aerated bags were used for Method 1 (closed model) and aerated open basins for Method 2 (open model). The treatment had three replicates for both methods. Potential evisceration using both methods was monitored while transporting the sea cucumbers. Water quality parameters including salinity, temperature, dissolved oxygen, pH, total dissolved solids (TDS) and conductivity were monitored before transport, during transport and also within the culture facility by using a multi-parameter meter kit (Hanna Instruments). After acclimatization, a three-month study was designed using a portion of the sea cucumbers that survived during transportation to determine growth performance at three different stocking densities (5, 10, and 15 individuals) with replicates in flow through culture tanks.

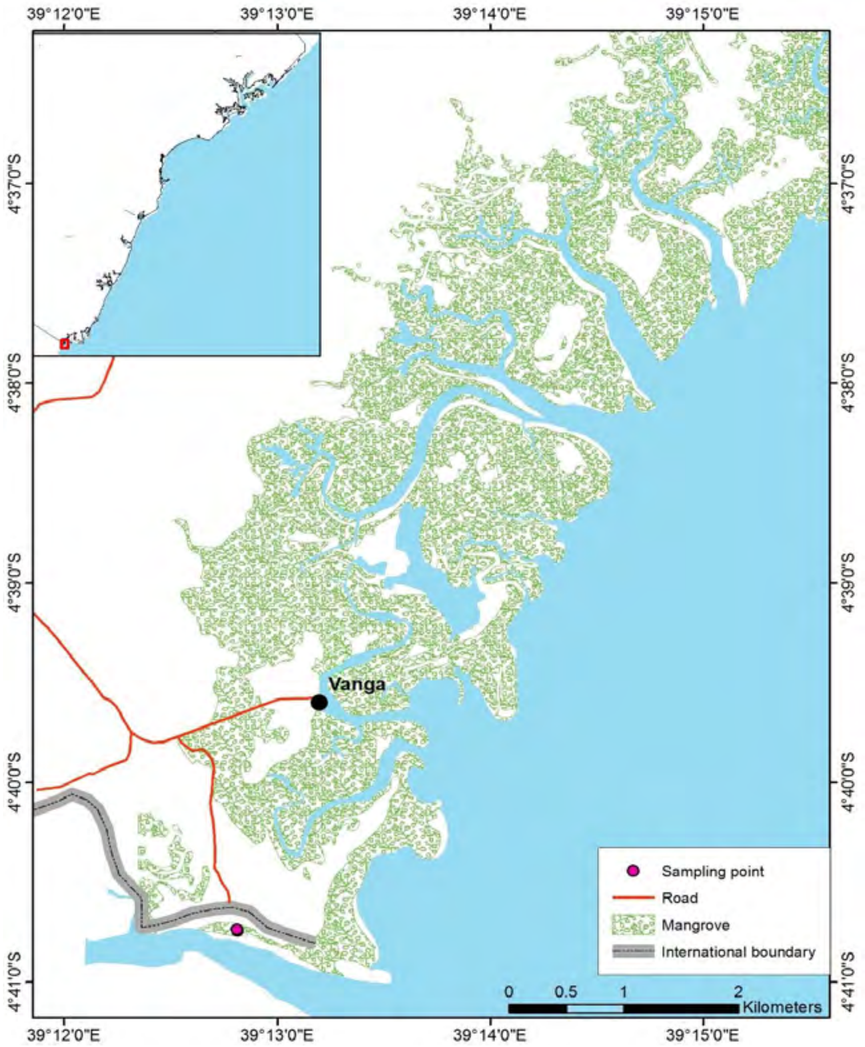

Figure 1. Collection site of the experimental organisms.

Culture site and systems

The marine hatchery at Kenya Marine and Fisheries Research Institute (KMFRI) located 5 km from Mombasa town (GPS 4.0552°S and 39.6821°E) was used as the experimental site to conduct the *H. scabra* hatchery trials. A concrete outdoor tank measuring 5m x 3m x 1m was used as a holding facility for the *H. scabra* specimens collected from the wild. The bottom of the tank was covered with 15 cm of sand to provide the sandfishes with an environment that simulated their natural habitat. This provided a burrowing medium to allow for their usual behavioral patterns as well as to provide a source of food and shelter from adverse environmental conditions and sometimes predation (Wiedemeyer, 1992; Mercier *et al*., 2000). The holding tank was continuously supplied with seawater extracted from a borehole with a salinity of 27 ppt and a temperature of 25 °C. An air blower maintained continuous aeration to the water as the sandfish acclimatized for a period of two weeks before subjecting them to different experimental treatments.

Collection of specimens

During this study a total of 1000 individuals with an average weight of 40 g to 80 g were collected in four trips from the Jimbo area of Vanga ward based at Kwale County, Kenya (Fig. 1), with 250 individuals collected per trip,. Transportation was carried out by road for four hours to the hatchery at KMFRI. Local sea cucumber fishermen with indigenous knowledge about the fishery were engaged to do the collection through hand picking at low spring tide during the dark moon phase after obtaining a permit from the Kenya Wildlife Service (KWS).

Handling of collected organisms

The sampling points were chosen based on ease of accessibility and availability of the organisms within the sandy seagrass beds. Upon arrival and after retrieving the sandfishes, the water quality parameters of the sea water that was used to hold the organisms were measured including salinity, temperature, dissolved oxygen, pH, TDS and conductivity. These were taken at 1:00 am using a multi-parameter kit followed up by counting and packing the organisms for transportation. The animals were carefully handled to avoid evisceration, then they were cleaned by washing their body with seawater in preparation for transportation using the two test methods.

Transportation

The first experiment (Method 1) was carried out by packaging the sea cucumbers in 10 l plastic bags at densities of 1 individual per bag, 5 individuals per bag, and a maximum of 10 individuals per bag. The plastic bags used were transparent and filled in with 4 l of seawater. Each treatment had three replicates. Pure oxygen was used to inflate the packaging bags after adding water and sandfishes. The ratio of oxygen to water was 40 % to 60 %. The bags were sealed with a rubber band and placed in a 1 m^3 plastic tank quarter-filled with seawater to maintain a temperature within the range of 24 to 26 °C in the bags during the four-hour trip to the KMFRI hatchery. The collection was carried out on four different occasions. The second transportation mode (Method 2) entailed the use of 20 l open basins filled with a layer of sand and half-filled with seawater. Individuals of average size were cleaned with sea water and placed in the transportation basins at a density of 10, 20 and 30 individuals per basin. A battery pump (E-jet BP-3) was used to aerate the water in the basins during transportation. Table 1 shows the treatment detail of sandfish during transportation.

Acclimatization

Upon arrival at the KMFRI hatchery, the test organisms were checked for mortality and evisceration that might have occurred during transport. The test organisms were acclimatized while still in the transport bags with ambient culture water for 30 minutes and later transferred to the holding tank with running oxygenated sea water from the KMFRI borehole. Water quality parameters of the holding tank were monitored using a multi-parameter meter kit before releasing the organisms from the transport bags.

Acclimatization was carried out by carefully placing the basin inside the holding tank and slowly introducing the water and releasing the sea cucumbers. Water quality was monitored on a daily basis in the morning and afternoon while allowing water to flow into the holding tank. The organisms were left to rest and recuperate for a day after which feeding was introduced gradually from day two while still monitoring the water quality parameters at 9 am and 4 pm daily. Feeding of all sea cucumbers was carried out once a day by applying portions of seaweeds mixed with broken pellets and also by using muddy sand collected from mangroves, mixed with the sand substrate in the holding tank. This was done in order to test feed acceptability for one week, after which a daily feed ration of 3 % body weight per day (Broom *et al.*, 2021) was introduced.

During the acclimatization period, the water exchange rate in the holding tank was maintained at 50 % per day to remove waste and supply oxygen. Periodic cleaning of the tank was carried out fortnightly with renewal of the sand substrate according to Purcell *et al.* (2012) and

Table 1. Treatment detail of sandfish during transportation.

Transport model	Treatment	Density	Replicates
Open model	Low density	5	3
Open model	Medium density	10	3
Open model	High density	15	3
Closed model	Low density	10	3
Closed model	Medium density	20	3
Closed model	High density	30	3

Table 2. Evisceration rate of broodstock during transport using the two trial methods.

Sandfish density	Number of sandfishes that evicerated		
(Individuals/Bag or basin)	Replicate 1	Replicate 2	Replicate 3
Method 01			
1.1 (1)	0	0	0
1.2 (5) 0 0 1	0	0	1
1.3 (10) 2 3 1	2	3	1
Method 02			
1.1 (10) 2 4 1	2	4	1
1.2 (20) 5 5 4	5	5	4
1.3 (30) 12 11 10	12	11	10

Duy (2011). Mortality in the acclimatization tank was recorded every day.

Monitoring of sea cucumber growth at three different stocking densities

After acclimatization, the growth rate of test organisms was observed for three months in different stocking densities of 5, 10 and 15. Total Length (TL) and total weight measurements were recorded after every two weeks using a string and meter ruler and an analytical weighing balance with the precision of 0.01 g to obtain length and weight gain in grams. The Specific Growth Rate (SGR) of the three stocking densities was calculated as described by (Novoa *et al.*, 1990) as follows:

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e\,\text{(final weight)-log}_e\,\text{(initial weight)} \times 100\%}{\text{Culture Days}}$$

Data analysis

Comparisons of the different treatments for transportation Methods 1 and 2 and among the sandfish density treatments were analyzed by calculating the mean values of different variables and expressing the results in table and graphical formats. Statistical analyses such as the T test were conducted using Excel version 2013 to test the equality of means. Data are presented as mean (±SD). Prior to the analysis, data sets were examined for normality and homogeneity of variances with an F test. Results were considered significant at $p < 0.05$.

Results

Evisceration rate of the sandfish for the different methods and different densities is shown in Table 2. The mean (±SD) percentage evisceration rate of the collected stocks of *H. scabra* in Method 1 was 3.33 ±5.77 % for the density of 5, and 20 ± 10 % for the density of 10 as shown in Table 3. Density 1 had no evisceration. In Method 2 evisceration occurred at all the three densities; 23.3 ±15.28 % for the low density of 10 individuals, 23.33± 2.8 % for the middle density of 20 individuals, and 36.6 ± 3.33 % for the higher density of

Table 3. Evisceration of *H. scabra* transported by Method 1 showing the mean and standard deviation (SD).

NO	Bag No	Density in bag	Evisceration in bag	
			Σ	%
1	5	1	0	0
2	4	1	0	0
3	10	1	0	0
	Average		0	0
	SD		0	0
1	8	5	0	0
2	6	5	0	0
3	3	5	1	10
	Average		0.33	3.33
	SD		0.58	5.77
1	9	10	2	20
2	7	10	3	30
3	1	10	1	10
	Average		2	20
	SD		1	10

Table 4. Evisceration of H. scabra transported by Method 2 showing the mean and standard deviation (SD).

NO	Basin no	Density	Evisceration in basin	
			Σ	%
1	3	10	2	20
2	5	10	4	40
3	4	10	1	10
	Average		2.33	23.33
	SD		1.53	15.28
1	2	20	5	25
2	6	20	5	25
3	1	20	4	20
	Average		4.67	23.33
	SD		0.58	2.89
1	9	30	12	40
2	7	30	11	36.67
3	8	30	10	33.33
	Average		11	36.67
	SD		1	3.33

30 individuals as shown in Table 4. The sea cucumbers that had been packed in plastic bags were assessed and found to be in good state except for the eviscerations in the replicate bags carrying 10 individuals. While assessing the second method of transportation using the open basin it was found that eviscerations occurred in every basin. The individuals in the Sample 3 basin that carried 30 individuals appeared weak on arrival, and experienced more evisceration. However, they became more active after putting them into the acclimatization tank. Sample 1 which had 10 individuals had the least number of eviscerations among the replicate basins. Statistical analysis proved that a significantly higher rate of evisceration occurred when *H. scabra* specimens were transported in aerated open basins (t-test, $p<0.05$, n=6).

Twenty sea cucumbers died on the fourth and sixth day during the first three weeks of acclimatization. Water quality parameters collected before transport, during transport and in the culture facility are presented in Table 5. Temperature, pH, salinity, transparency, and dissolved oxygen did not show marked variations between the treatments during transportation and acclimatization.

The stocking density treatments showed a 100 % survival of the test organisms. The final mean weight of sea cucumbers stocked at a density of 5 in plastic tanks was the highest at 88.96 ± 4.09 g compared to the stocking density of 10 and 15 which had a final mean weight of 60.93 ± 21.11 g and 46.91± 1.11 g respectively. The weight gain of sea cucumbers stocked in different stocking densities of 5, 10, and 15 decreased with an increase in stocking density (Table 6; Fig. 2). The specific growth rate of sea cucumbers stocked at a density of 5 was 1.03± 0.56 g followed by 0.22± 0.41 g for the stocking density of 10 and -0.37 ± 0.02 g for the stocking density

Table 5. Water quality parameters taken during the three-week acclimatization period showing the mean and standard deviation in the morning and evening.

Water quality parameters	Morning	Evening
Temperature (°C)	25.62 ± 0.25	25.86 ± 0.38
Salinity (ppt)	27.27 ± 0.45	27.17 ± 0.25
TDS (mg/l)	27624.13 ± 0.32	27517.93 ± 0.55
DO (mg/l)	4.52 ± 0.42	4.63 ± 0.38
Conductivity (mmho/cm)	42795.93 ± 0.28	42156.23 ±0.3
pH	8.49 ± 0.22	7.77 ± 0.24

Table 6. Results of analysis for the initial weight (IW), final weight (FW) and weight gain (WG) in grams, and specific growth rate (SGR) for three stocking densities (ind./m3) in replicate tanks. Data are represented as mean ± standard deviation.

Stocking Density	IW	FW	%WG	SGR
5	39.07 ± 16.76	88.96 ± 4.09	184.56 ± 1.33	1.03 ± 0.56
10	42.13 ± 0.2	60.93 ± 21.11	29.47 ± 45.34	0.22 ± 0.41
15	55.21 ± 0.31	46.91 ± 1.11	-28.06 ± 0.7	-0.37 ± 0.02

of 15 sea cucumbers (Fig. 3). The test organisms showed a high acceptance of the mangrove mud as a feed as compared to the seaweed and pellet mixture.

Discussion

During this study transportation took place at night to avoid high temperature fluctuations. The sandfish that were transported in open basins were higher in density and most likely the shock and friction from the walls of the transporting basins could have exposed the sandfishes to greater stress during the four-hour transport time as compared to those that were packaged in lower densities in oxygenated polythene bags. The study indicated that with the 40-60 % ratio of oxygen to water used, 0 evisceration, 3 % and 20 % evisceration occurred at the low, middle, and high sandfish densities in Method 1. In Method 2, an evisceration rate of 23 %, 23 %, and 36 % was experienced with the low, middle, and high densities. From this analysis the middle density seems to be most appropriate for transportation from a cost effort benefit perspective. Oxygenation is therefore essential for long distance transportation as results show low evisceration rates in samples that had oxygen provision (Ito, 2014; Tuwo *et al.*, 2019).

Of the total transported individuals, 2 % mortality (20 individuals) occurred in acclimatization tanks. Upon investigation these individuals seemed to have been injured during collection and transportation and while adapting to the new tank culture conditions. The findings of the study indicated that the open basin model did not work well as evidenced by the high evisceration during transport which was attributed to shock and friction of basin walls as compared to the aerated plastic bags. This is in accordance with the findings of Tuwo *et al.* (2019).

The physico-chemical parameters monitored during the acclimatization period (Table 5) were within the optimum range required for sea cucumber rearing (Agudo, 2006). Wild collected holothurian species have been observed to eviscerate as a reflex response to danger, adverse environmental conditions and handling stress that makes them release their internal organs to the surrounding environment (Battaglene *et al.*, 2002). In this study, most organisms were found to be in good condition since most did not eviscerate. However, viscera were always removed from the water once noticed during transport in order to maintain good water quality and the well-being of the sea cucumbers. Studies have shown that *H. scabra* are relatively hardy organisms that can tolerate low dissolved oxygen levels (Agudo, 2006).

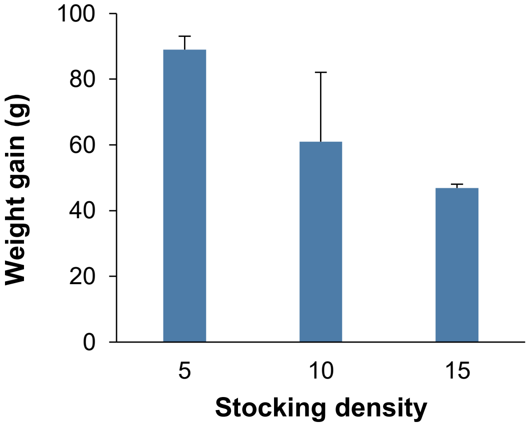

Figure 2. Weight gain in grams of sea cucumber in different stocking densities with standard deviation indicated for each density.

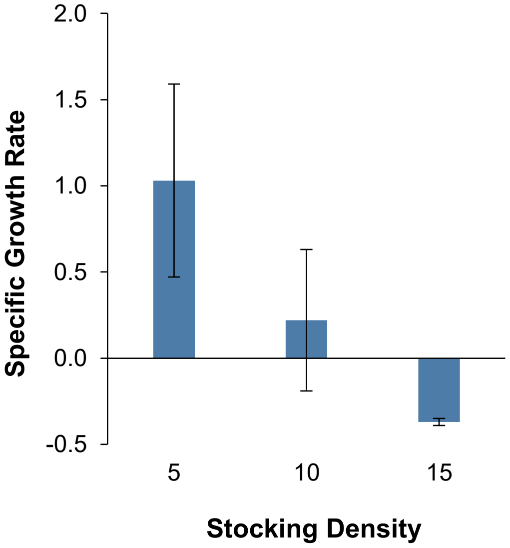

Figure 3. Specific growth rate (SGR) in different stocking densities with standard deviation indicated for each density.

However, provision of aeration is essential during long distance transportation in order to overcome the stress encountered during fishing, manipulation at packaging and while transporting.

Holothuroids including *H. scabra* are deposit feeders and studies have shown that they normally take the available food from their surrounding by ingesting deposited materials on the surface of the substrate (Roberts *et al.,* 2000). In this study the feeds were introduced to the tank in areas where the sandfishes were observed to aggregate. Active feeding was not observed during the day. The organisms consumed the feeds on the sand substrate mostly at night when they are believed to be most active (Hamel *et al*., 2022). The feeds given were observed to reduce in quantity and topping up was done in small portions while taking care of the water quality in the culture facilities.

The current study revealed that sea cucumber survival was not dependent on stocking density after all treatments recorded 100 % survival. Stocking density was seen to be a factor in sea cucumber growth rate (Battaglene *et al.*, 1999, Pitt and Duy, 2004, Lavitra *et al.*, 2010). High growth rate was observed in treatments with low stocking density and the lowest in the high stocking density treatments. Competition for resources and space could have attributed to the varied growth rates observed (Davies *et al.*, 2011, Slater and Carton, 2009). Improved growth rates of sea cucumber in low stocking density as seen in the present study were also observed in other studies on *H. scabra* (Battaglene *et al.*, 1999; Beltran-Gutierrez *et al.*, 2014).

The average daily growth rate of the 5 stocking density treatment in the current study was seen to range from 0.47-1.59 g which appeared to align with the results of Davies *et al.* (2011) who reported growth rate of 0.06-1.39 g in Zanzibar, and Beltran-Gutierrez *et al.* (2014) who reported rates of 1.6 and 0.9 g. The slight growth rate difference could have been attributed to the initial stocking weight of the organisms used (96 ± 31 g) for Beltran-Gutierrez and (39.07 ± 16.76 g) for the current study. Lower growth rates of sea cucumbers were observed in the higher stocking densities as observed by Pitt *et al*. (2004) who recorded a growth rate of 0.24 g at a stocking density of 10 ind./m-^{2}, while Battaglene *et al.* (1999) and Asha and Diwakar (2013) reported an average growth rate of 0.2 g at a stocking density of 10 ind./m2. In this study, the weight gain of the sandfish decreased with an increase in stocking density.

The findings of this study show that transport method and sandfish density affect evisceration rate. However, appropriate handling before and during transportation can increase survival even at high densities. The study further gives important information for future mariculture development in Kenya, Africa and the world. Stocking density could be of importance for initial domestication. However, gaps still remain in assessing growth of sea cucumber under different culture conditions.

Conclusions

Aquaculture of sandfishes remains the most viable option to ensure the sustainability of these species and this formed the key motivation for the current study. It is concluded that proper handling during broodstock collection, packaging, and transportation using oxygenated packaging bags reduced stress levels of the organisms. Low stocking densities contributed to high growth rates of sea cucumbers. Diversification into the culture of marine organisms like sea cucumber can be a potential livelihood for coastal communities in Kenya; especially women in self-help groups. Therefore, embracing sea cucumber culture as an alternative livelihood should be a priority on the Kenyan coast. From the findings of this study, it is recommended that further research be carried out on optimizing transport practices and stocking densities, assessing the efficacy of oxygenated closed bags as opposed to non-oxygenated closed bags, and optimizing cost-effort-efficiency transportation methods.

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Short note

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New record of the Bluntnose scorpionfish, *Neomerinthe erostris* (Alcock 1896) (Teleostei: Scorpaenidae) from Mayotte, southwestern Indian Ocean

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Abstract

The Bluntnose scorpionfish *Neomerinthe erostris* is recorded for the first time from Mayotte, southwestern Indian Ocean, based on a specimen photographed in November 2022, at a depth of 115 m. The live colour pattern of this species is described for the first time.

Keywords: biodiversity, scorpionfishes, new record, Mayotte, distribution

Introduction

The scorpionfishes of the genus *Neomerinthe* Fowler 1935 are distributed in tropical and temperate seas in the Atlantic Ocean, Red Sea and Indo-West Pacific (east to Hawaiian Islands and French Polynesia). They inhabit benthic habitats, mostly dwelling on rocky or coral reefs. The genus includes a total of 14 valid species (Fricke *et al.*, 2022) (Tab. 1). It is characterised within the family Scorpaenidae by the dorsal rays XII, 9-11, the last split from the base; dorsal-fin spines short, usually less than 1/2 body depth; dorsal-fin membranes not incised nearly entire length of all spines; anal-fin rays normally III, 5; at least some pectoral-fin rays branched; pectoral fins rounded or elongate, but not strongly notched or bilobate; vertebrae 24 (rarely 23); scales on flank ctenoid, particularly above lateral line; scales on breast ctenoid or cycloid, small, but never absent; bones of cranium strongly or moderately ossified; occiput inclined, but flat or slightly convex, never concave; palatine teeth present on roof of mouth; lacrimal and suborbital bones (infraorbital bones 1 to 3) relatively narrow and usually somewhat convex laterally and usually strongly ossified; lacrimal bone with strong spines along ventral margin; ventral margin of lacrimal bone usually with numerous spines; lacrimal bone relatively immobile, strongly bound to the lateral-ethmoid; posterior lacrimal spine relatively strong, pointing postero-ventrally (not hooked forward); third infraorbital bone (suborbital 2) extends nearly straight posteriorly and relatively broadly connected to preopercle; skin at gill openings connected to each other or connected to isthmus only narrowly anteriorly, not broadly connected to isthmus; opercle with 2 distinct ridges that diverge at an acute angle, both usually strongly developed and bearing spines; lateral line normal, continuing onto or near base of caudal fin; lateral-line scales forming relatively complete tubes, that are sometimes buried; peritoneum pale; caudal peduncle at base of caudal fin without scattered large melanophore (Eschmeyer, 1969; Poss, 1999; Poss and Motomura, 2022; modified).

Species of the genus Neomerinthe were first discovered by Alcock (1896), who described two species,

Table 1. Species of the genus *Neomerinthe* Fowler 1935 and their distribution.

Species	Primary types	Synonyms	Distribution
N. amplisquamiceps (Fowler 1938)	Holotype: USNM 98883		Eastern Indian Ocean, western Pacific
N. bauchotae Poss & Duhamel 1991	Holotype: MNHN 1989-0273		Southern Indian Ocean: Saint-Paul Island
N. beanorum (Evermann & Marsh 1900)	Holotype: USNM 49534		Western Atlantic
N. bucephalus (Alcock 1896)	Syntypes: ZSI F13737 (1), F14084 (1)		Indo-West Pacific
N. erostris (Alcock 1896)	Lectotype: ZSI F 12977 [selected by Motomura *et al.* (2015: 530)]	*Scorpaena gibbifrons* Fowler 1938; *Neomerinthe rotunda* Chen 1981; *N. bathyperimensis* Zajonz & Klausewitz 2002	Red Sea, Indo-West Pacific
N. folgori (Postel & Roux 1964)	Holotype: MNHN 1963-0600		Eastern Atlantic
N. hemingwayi Fowler 1935	Holotype: ANSP 63482	*Neomerinthe tortugae* Hildebrand 1940	Western Atlantic
N. kaufmani (Herre 1952)	Holotype: USNM 202511		Western Pacific
N. megalepis (Fowler 1938)	Holotype: USNM 98897		Southwestern Pacific
N. naevosa Motomura, Béarez & Causse 2011	Holotype: MNHN 2010-0952		South Pacific: Marquesas Islands
N. nielseni (Smith 1964)	Holotype: ZMUC P79192		Western Indian Ocean
N. pallidimacula (Fowler 1938)	Holotype: USNM 98889		Western Pacific
N. procurva Chen 1981	Holotype: CAS 47306		Eastern Indian Ocean, western Pacific
N. rufescens (Gilbert 1905)	Holotype: USNM 51628		Central Pacific: Hawaiian Islands and Johnston Atoll

Scorpaena bucephalus from India, and *S. erostris* from Sri Lanka. The species description of *N. erostris* by Alcock in 1896 (as *Scorpaena erostris*) was based on two specimens collected off southern Sri Lanka, at 62 or 77 metres depth. The species was redescribed by Motomura *et al.* (2015), recently revised by Poss and Motomura (2022), and recorded from the southern Red Sea and the Indo-West Pacific, at depths of 52-505 m.

A specimen of *N. erostris* was photographed in the mesophotic zone of Mayotte (France), Western Indian Ocean. This new record is reported and discussed in the present paper.

Materials and methods

On 11 Nov. 2022, a *ca.* 100 mm SL specimen of *N. erostris* was photographed by Gaby Barathieu in the mesophotic zone east of Mayotte at 12°52.964'S, 45°16.557'E, at a depth of 115 m. The specimen was photographed while diving, using a rebreather with trimix gas. The size of the specimen was estimated by the diver, according to his experience.

Counts and measurements followed Hubbs and Lagler (1947); the standard length is abbreviated SL. The genus and species classification follows Fricke *et al.* (2022), the family classification follows van der Laan *et al.* (2014), the head spine terminology follows Eschmeyer (1969), fin-ray counts follow Fricke (1983). Collections are abbreviated according to Fricke and Eschmeyer (2022).

Specimens of *Neomerinthe erostris* used as comparative material included: MNHN 1988-1528 (1), Madagascar; MNHN 1991-0752 (2), La Réunion; MNHN 2004-0061 (Madagascar); MNHN 2004-0056 (2), Madagascar.

Results

Neomerinthe erostris (Alcock 1896)

Description

Proportions shown in Table 2 are part of this description.

Dorsal-fin spines XII, third spine longest, dorsal-fin soft rays 9 (last divided at base); pectoral-fin rays ca. 17 (left side), with 3rd to 5th rays branched, membranes strongly incised. Lateral lacrimal spine absent; anterior lacrimal spine simple, directed ventrally; posterior lacrimal spine simple, directed postero-ventrally;

Figure 1. *Neomerinthe erostris* (Alcock, 1896). Specimen photographed in the mesophotic zone at Mayotte at 115 m depth on 11 November 2022. Photograph: Gaby Barathieu.

suborbital ridges with 3 spines. Preopercle with 5 spines. Pored lateral-line scales ca. 25 (left side); vertical scale rows in longitudinal series ca. 39; predorsal scale rows ca. 8; scales on sides of body ctenoid; scale rows above lateral line 6, below lateral line 12. Occiput flat. Supraorbital tentacle large, posteriorly with 7 branches. Head with numerous additional tentacles; also numerous dermal tentacles on back and sides of body. Standard length *ca*. 100 mm.

Live colouration (Fig. 1): Head and body pale orange, snout pale, eyes with 7 orange bars; back with white saddles below 4th dorsal-fin spine, end of spinous and beginning of soft dorsal fin, and end of soft dorsal-fin to caudal peduncle. Fins translucent, dorsal fin with basal and distal scattered white spots, pectoral fin with two indistinct distal white bars; caudal fin orange, with indistinct, narrow, vertical white bars.

Table 2. Proportions of Mayotte specimen of *Neomerinthe erostris* (Alcock, 1896) (ca. 100 mm SL), compared with values given by Motomura *et al.* (2015).

	Mayotte specimen [% of SL]	Motomura *et al.* (2015) [% of SL]
Head length	46.2	46.3-53.9
Orbit diameter	13.1	13.0-19.5
Preorbital length	13.4	10.1-13.8
Orbital tentacle length	9.7	
Maxillary length	23.0	21.8-26.6
Predorsal length	41.6	39.1-45.9
Prepectoral length	42.5	
Length of 1st dorsal-fin spine	6.6	6.9-12.9
Length of 2nd dorsal-fin spine	12.8	12.5-21.6
Length of 3rd dorsal-fin spine	18.9	16.8-25.5
Length of 4th dorsal-fin spine	18.5	17.3-23.7
Length of 5th dorsal-fin spine	15.4	16.6-21.6
Longest dorsal-fin ray	15.6	18.1-21.0
Length of 5th dorsal-fin ray	11.5	
Length of last dorsal-fin ray	6.6	
Pectoral-fin length	27.1	27.1-38.1

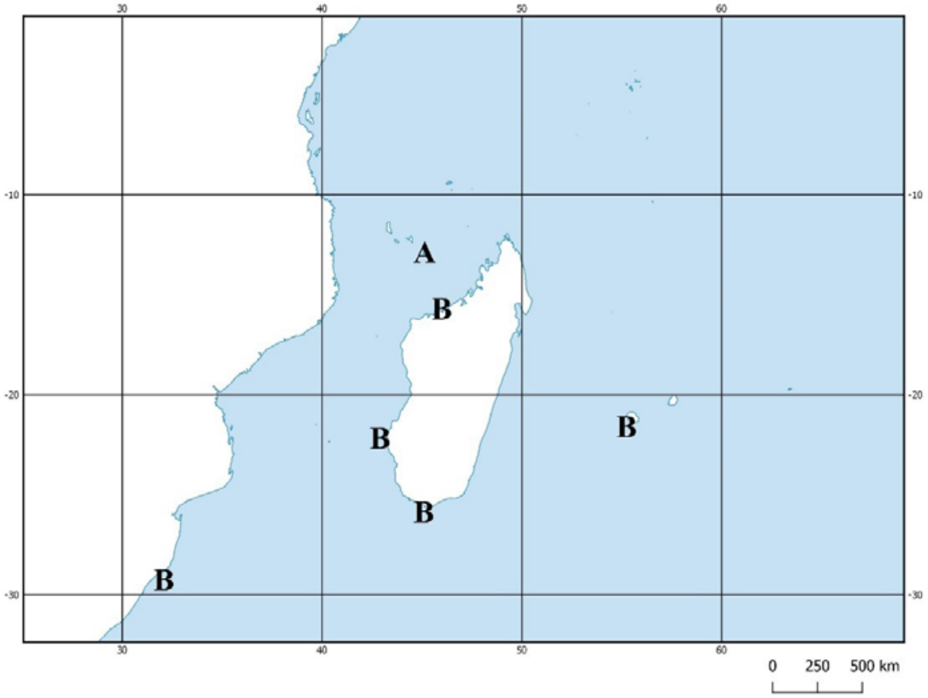

Figure 2. Geographical distribution of *Neomerinthe erostris* (Alcock, 1896) in the southwestern Indian Ocean. A - New record from Mayotte; B - Other records based on examined specimens and literature.

Discussion

Neomerinthe erostris was revised by Motomura *et al.* (2015), who treated *Scorpaena gibbifrons* Fowler 1938, *Neomerinthe rotunda* Chen 1981, and *N. bathyperimensis* Zajonz and Klausewitz 2002 as junior synonyms, and reported the species based on specimens from Madagascar, La Réunion, Yemen, Sri Lanka, Taiwan, Philippines, Indonesia, Solomon Islands, New Caledonia, Vanuatu, and Wallis and Futuna Islands. They also listed a literature reference from Hong Kong (China). The known distribution of *N. erostris* in the southwestern Indian Ocean is illustrated in Figure 2.

During the CORCOMA project, a specimen of *N. erostris* was photographed in the mesophotic zone of Mayotte. This represents a range extension from the closest known locality (northwestern Madagascar) of ca. 300 km to the northwest, and the first record of this species from the Comoros Archipelago. Although the lower parts of the specimen are not visible on the photographs, it is clearly identifiable as a species of *Neomerinthe*, and distinguished from *N. nielseni* by its different head shape, higher dorsal fin, more numerous scales, and absence of a black spot on the spinous dorsal fin. The characters of the specimen on the photograph agree well with those of *N. erostris*; the proportions visible on the photograph are compared with those provided by Motomura *et al.* (2015) in Table 2.

Motomura *et al.* (2015: Fig. 1F) illustrated a freshly dead specimen of *N. erostris* from Taiwan, which was overall mostly red, dorsally speckled with orange. The present photograph (Fig. 1) is the first documentation of the live colouration of this species. Here, the fish is rather pale orange, dorsally variegated with white, and with white saddles.

The present specimen of *N. erostris* was photographed at a depth of 115 m, on a steep, sloping rock surface covered with yellow sponges, directly above a drop-off (Fig. 3). At this depth, the habitat is characterized by hard substrate colonized by Porifera, Hydrozoa, Antipatharia and Octocorallia. These are the dominant hard substrate benthic communities in the lower mesophotic zone of mesophotic coral ecosystems in Mayotte and along coral reef drop-offs (Mulochau *et al.*, 2021). That depth record is not unusual for the species, which was previously reported from 52-505 m (Motomura *et al.*, 2015). The preferred depth may be related to the water temperature; the depth of the Mayotte specimen is similar to that of the northwestern Madagascar and La Réunion specimens deposited at MNHN, which were collected at depths of 90-130 m.

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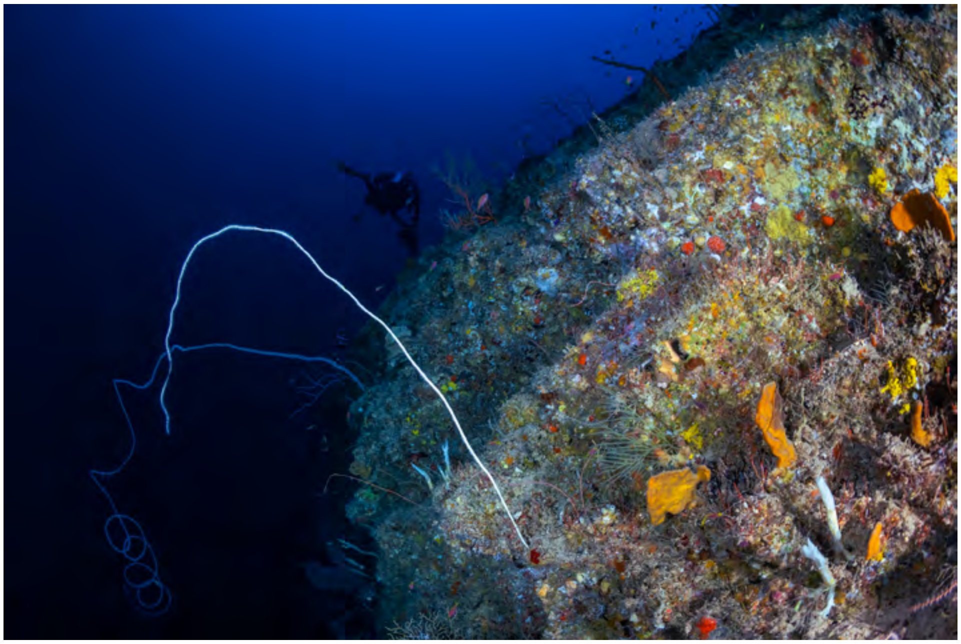

Fig. 3. Habitat of *Neomerinthe erostris* (Alcock 1896), off Mayotte, southwestern Indian Ocean. Photograph: Gaby Barathieu.

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Original Article

Willingness and drivers of community participation in mangrove conservation in the Rufiji Delta, Tanzania

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Abstract

Promoting local community participation in recognition of their indigenous knowledge is important for effective resource conservation. The aims of this study were to evaluate local community willingness and drivers of participation in mangrove conservation activities in the Rufiji Delta. Data were collected through focus group discussions, key informant interviews, semi-structured household interviews and participant and non-participant observations, across three designated management blocks - Northern delta (ND), Central delta (CD) and Southern delta (SD). Qualitative data were analysed using content analysis while quantitative data were analysed for descriptive and inferential statistics. Overall, the majority of respondents (88 %) agreed that it was important for the community to participate in mangrove conservation activities and 50 % of them expressed immediate readiness to participate if called upon. Zone wise, 50 % of respondents in ND and CD, and 42 % in SD indicated a moderate level of community willingness to participate in conservation. Age of household head, education level, source of income and awareness of mangrove governing laws were the most significant drivers positively associated with community willingness to participate in conservation activities. These findings suggest that for long-term management of mangroves, local communities should be adequately recognized as partners rather than as foes.

Keywords: willingness, mangroves, conservation, local communities, Rufiji Delta

Introduction

Mangrove forests are intertidal ecosystems made of trees that have evolved to survive in wet, salty and often unstable environments (Spalding and Leal, 2021). They are special, unique and vulnerable ecosystems found globally in tropical and sub-tropical coasts (Astuti *et al.,* 2017). There are about 8,349,500 ha of mangroves globally (Romanach *et al.,* 2018) with Southeast Asia harbouring almost a third of the global total (Spalding and Leal, 2021). The Western Indian Ocean Region consists of 745, 518 ha of mangroves which represents 25 % of Africa's mangroves or 5 % of all the mangroves in the world. Mangroves in Tanzania cover an area of 110,787 ha with 41 % (45, 583 ha) located in the Rufiji Delta (Erftemeijer *et al.,* 2022).

There is a growing recognition of the role of mangroves in providing services that enhance livelihoods of millions of people, often the rural poor, by providing wood resources and support to fisheries. In addition, the role of mangroves in protecting coastlines and properties and ameliorating climate change-related impacts through their high capacity for carbon sequestration is increasingly appreciated (UNEP, 2014; Biswas and Biswas, 2019; Aye *et al.,* 2019; Gallup *et al,*. 2020; Spalding and Leal, 2021). Despite their recognized roles, mangroves are among the most threatened global ecosystems (Gallup *et al.,* 2020). Direct and indirect human pressures including conversion of mangrove areas to accommodate infrastructure, urbanization, aquaculture, agriculture and coastal tourism are some of the drivers for mangrove loss globally (Romanach *et al.,* 2018; Goldberg *et al.,* 2020; Spalding and Leal, 2021). To address this situation, a number of countries have placed mangrove protection firmly into policy guidelines or framework legislation (Gallup *et al.,* 2020), which varies greatly across nations. Repeated calls to action, including reforms in policy and management strategies have been made (UNEP, 2014; Spalding and Leal, 2021) to support mangrove rehabilitation programmes in many countries throughout the world (Gallup *et al.,* 2020).

Like in other major mangrove countries of the WIO region including Kenya, Mozambique and Madagascar (Erftemeijer *et al.,* 2022), Tanzania has not escaped mangrove degradation and loss, which is reported from various parts of the country including the Rufiji Delta which is the largest contiguous mangrove forest in the country (Monga *et al.,* 2018). Due to the high dependence of local livelihoods on mangrove areas and resources in the Rufiji Delta, the area has become a victim of a tacit common pool for firewood, charcoal-making, building poles, boat making and conversion to other land uses (Mangora *et al.,* 2016; Mwansasu, 2016; Mshale *et al.,* 2017; Monga *et al.,* 2018; Japhet *et al.,* 2019). These pressures have led to substantial degradation and loss of mangrove cover, where Monga *et al.* (2018) reported that between 1991 and 2015, 9,089 ha of mangrove forests in the delta were lost, translating to a net loss of 12.4 % in the period of 24 years, largely from conversion to rice farms.

To respond to repeated calls for protection and restoration of mangroves at different times, a number of government bodies and international conservation Non-Government Organizations (NGOs) such as the World Wide Fund for Nature (WWF) (Longopa, 2018), International Union for Conservation of Nature (IUCN) (Richmond *et al.,* 2002) and Wetlands International (WI) (Japhet, 2018) have funded conservation initiatives with components of protection and restoration of mangroves and promoting community participation in the Rufiji Delta. Reportedly, most of such initiatives are concentrated in the north delta block which is characterized by greater mangrove losses due to conversion into rice farming than the south and central delta blocks (Monga *et al.,* 2018). Local NGOs like Pakaya Culture and Environmental Group (PCEG) have also been conducting community sensitization and awareness raising in the delta to ensure sustainable conservation of mangroves. At the village level, Village Natural Resource Committees (VNRCs) and Beach Management Units (BMUs) manage mangroves in the delta (Mshale *et al.,* 2017; Japhet, 2018; Nyangoko *et al.,* 2021).

Local community participation in natural resource conservation is gaining interest and encouraged in various countries and by various practitioners as an effective approach to ensure sustainability (Astuti *et al.,* 2017; Garekae *et al.,* 2017; Thuy *et al.,* 2019). This is due to the growing recognition that indigenous knowledge should be blended with contemporary conservation techniques to enhance the capacity to carry out long-term natural resource conservation plans (Ellison, 2012). However, it is evident in the literature that local communities in different areas have not actively participated in conservation programme planning and decision-making for various reasons (Kariuki, 2014; Gumede and Nzama, 2021). Participation is promoted as a voluntary process (Biswal, 2006), local community participation in natural resource conservation is not guaranteed because communities argue that their willingness is compromised by the absence of incentives (Zahabu *et al.,* 2010; Hassan, 2015). The Tanzanian Forestry Policy of 1998 and Forest Act of 2002 emphasize participatory management to achieve sustainable forest management through the Participatory Forest Management (PFM) framework (Zahabu *et al.,* 2010). The PFM has two strategies; Community-Based Forest Management (CBFM) and Joint Forest Management (JFM). In CBFM, a community becomes the forest owner and duty bearers of forest management, while JFM requires a community to sign an agreement with the government and other forest owners regarding forest management (MNRT, 2008). However, both CBFM and JFM strategies in Tanzania have not been effective and successful, not only in mangrove forests, but in some other terrestrial forest reserves where attempts have been made (Kajembe *et al.,* 2006; Mpokigwa *et al.,* 2011; Kilemo *et al.,* 2014). This is because there is no clear or binding agreements on how forest benefits and revenues are to be shared between the government and local communities (MNRT, 2008). An impact evaluation report by Persha and Meshack (2016) stated that JFM in Tanzania has enabled higher levels of local level governance, particularly the functions of VNRCs, but lacked in improving livelihoods of local communities participating in JFM, which calls into question the long-term sustainability of the strategy. These examples highlight why an adequate understanding of drivers influencing community willingness to participate in conservation in the delta is important for designing and implementation of effective community-based mangrove conservation. In the present study, the willingness of local communities to participate in mangrove conservation in the Rufiji Delta was explored. The study specifically set out to answer the following questions: (1) What is the importance of community participation in conservation?; (2) How do the local community perceive their role in participation in conservation initiatives?; (3) What is the level of community willingness to participate in conservation?; and (4) What are the drivers of community willingness to participate in conservation?

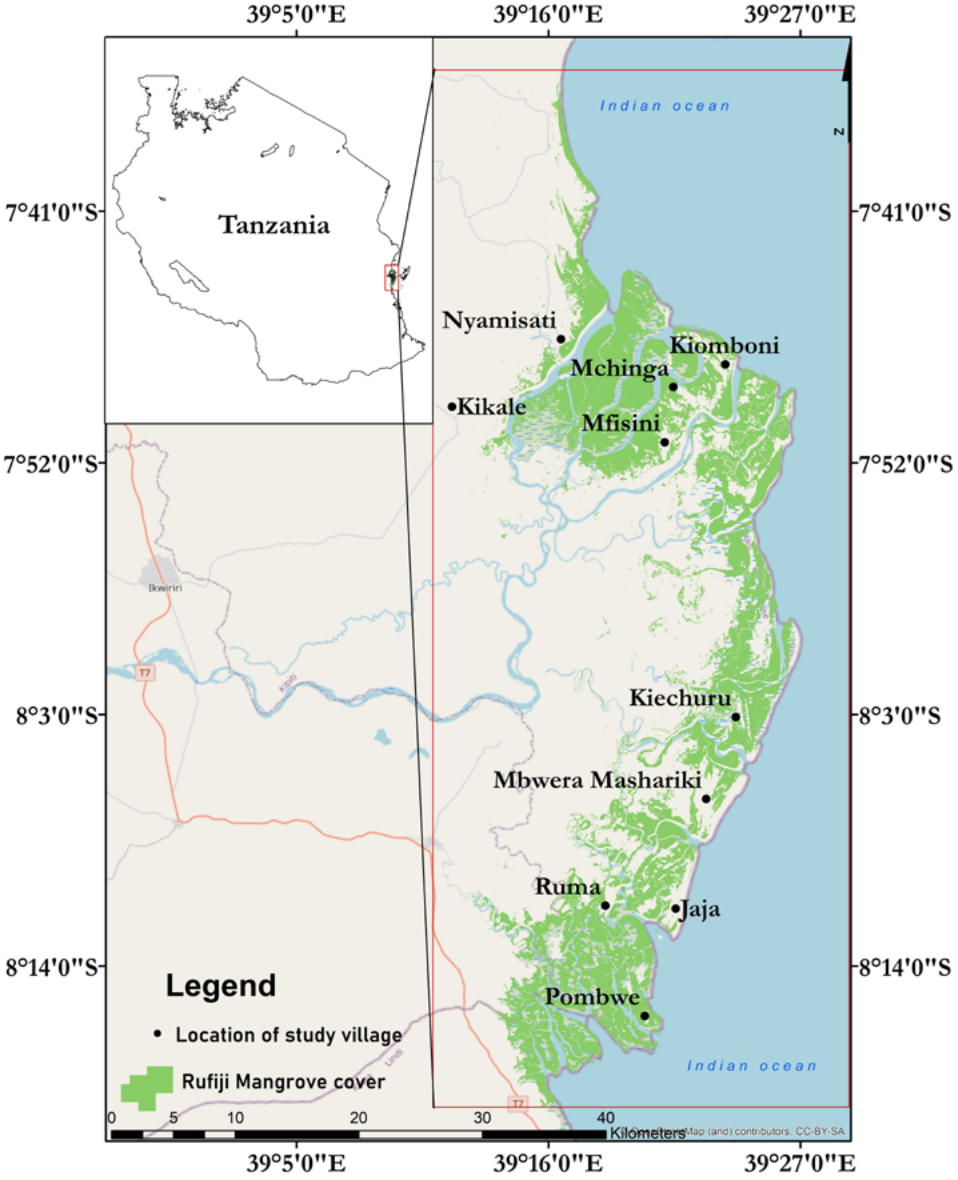

Figure 1. Map of Rufiji Delta showing location of study villages and mangrove cover.

Materials and methods

Study area

The Rufiji Delta lies between latitudes 8°20’00” and 7°35’00”S and longitudes 39°10’00” and 39°20’00”E. The delta is home to the largest mangrove ecosystem in Tanzania, with about 48,030 ha of mangrove forests (Monga *et al.*, 2018). For management purposes, the delta is divided into three blocks; namely, Northern delta (ND), Central delta (CD) and Southern delta (SD) (Mangora *et al.*, 2016; URT, 2020). On the basis of proportional coverage of the forest and utilization and dependence of local communities on mangrove forest, ten (10) villages were selected for the study.

These are Mfisini, Kiomboni, Mchinga, Nyamisati and Kikale villages (North delta, which makes up 46 % of the mangrove area); Mbwera Mashariki and Kiechuru villages (Central delta, which comprises 28.5 % of the mangrove area); Ruma, Jaja and Pombwe villages (South delta, which contain 25.4 % of mangroves) (Semesi, 1992) (Fig. 1). Major livelihood activities in the delta include rice farming, fishing and trade of mangrove products (Kangalawe and Masao, 2018). Multi-ethnic groups live in the study villages, with the majority being "Ndengereko" and "Nyagatwa". Other minor tribes who are mainly immigrants that reside in and around the Rufiji Delta include Zigua, Kurya, Matumbi, Makonde, Mwera, Zaramo, Ngoni, Ngindo, Shirazi and Hehe.

The Rufiji Delta experiences an average temperature of 24 °C and 28 °C during cold and hot months throughout the year, respectively (Ndesanjo *et al.,* 2012), with an annual rainfall of 750 mm to 1,250 mm (Mwansasu, 2016). The area experiences two rainy seasons; the short rains usually start in October and end in December, and the long rains are from February to May.

Research design and data collection

Before the actual data was collected, a preliminary survey was carried out to introduce the objectives of the study and seek the consent of communities to participate. The study applied a triangulation approach by using both qualitative and quantitative social science research approaches (Schoonenboom and Johnson, 2017) to gather primary data with increased credibility and validity at household and community level (Table 1). Qualitative research methods involved Focus Group Discussions (FGDs) and Key Informants Interviews (KIIs), while quantitative method involved Household Semi-Structured Interviews (HSSI). With the help of village leaders, simple random sampling was applied to select respondents who were knowledgeable about mangrove issues and activities, and of different ages and gender. All interviews, recordings, and photographs were conducted after receiving the verbal consent of the respondents, which is generally considered acceptable. Relevant and retrievable literature was collected and reviewed to complement information collected from primary data sources.

Focus group discussions

In each village, one FGD was conducted at community level with six mixed sex participants (4 males, 2 females) of various age groups following a suggestion by Krueger and Casey (2018). Although coastal traditions make it difficult for women to speak in public, the FGD facilitator made sure that women participants were given adequate room to speak out. This helped to resolve the risk of bias in the results. The

Table 1. Research design including data collection and analysis.

Data collection	Method Description	Data collected	Data analysis
Community level	1 Focus Group Discussion per each village with 6 participants of different sex, age groups and livelihood occupation 32 KIIs (i.e., 10 village leaders, 10 Village Natural Resource Committee (VNRC) leaders, 10 village elders, 1 TFS DFC and 1 local NGO Pakaya Culture and Environmental Group leader	Community livelihood system, mangrove conservation issues (status, importance, threats, resource conflicts, conservation activities) and community perceptions on their willingness to participate in conservation (need, importance, level, drivers)	Content analysis
Household level	300 Households for semi structured interviews, 30 per each village	Socio-demographic characteristics, socio-economic conditions, community uses of mangrove areas and resources, mangrove conservation initiatives and community willingness to participate in conservation.	Descriptive and Statistical analysis
Participant and Non-participant observation	Engaging either directly or indirectly with local communities	Observed day-to-day activities and understand of real-world phenomenon of local community livelihoods and mangrove conservation	Content analysis
Literature review	Comprehensive review of related literature	Community willingness and participation in mangrove conservation, and the influencing factors	Content analysis

selected participants were residents who depended on mangrove resources for livelihoods, well-versed in mangrove issues and different mangrove-related livelihood occupations such as fishers, crop farmers, salt chefs, beekeepers, livestock keepers, fish traders, food and vegetable vendors, mangrove cutters, handcrafters, traditional healers and boat captains. Discussions were guided by a prepared checklist of questions, which were posed by the facilitator to allow participants to contribute their own ideas which were recorded on a flipchart. The checklist questions aimed at capturing information on community livelihood systems, mangrove conservation issues (status, importance, threats, resource conflicts, conservation activities) and community perceptions on their willingness to participate in conservation (need, importance, level, drivers).

Key informant interviews

A total of 32 key informants including village leaders, VNRC leaders, village elders, a Tanzania Forest Service (TFS) District Forest Conservator (DFC) and a local NGO Pakaya Culture and Environmental Group leader were interviewed using another prepared checklist of questions. The questions and interviews aimed to complement on issues raised in the FGDs and HSSIs in order to gain more understanding on village livelihood history, mangrove conservation initiatives and community willingness to participate in mangrove conservation.

Household semi-structured interviews

Prior to actual data collection, a household questionnaire was pre-tested to determine the reliability and applicability of the questions and revised accordingly. Two enumerators from each study village were selected and trained on administering household questionnaires using the Open Data Kit (ODK) on mobile devices. Following recommendation by Angelsen *et al.* (2011), a total of 300 households, 30 from each village were interviewed. The interviews were conducted with the heads of households, but in their absence, a household representative regarded to have sufficient knowledge and information about the household was interviewed. The questionnaire and interviews aimed to collect information on socio-demographic characteristics, socio-economic conditions, community uses of mangrove areas and resources, mangrove conservation initiatives and community willingness to participate in conservation. Questions on community perceptions of their willingness to participate in different conservation activities (restoration, dissemination programmes, giving information on violation of laws, cooperating with government authorities in law enforcement, implementing alternative income activities, compliance to rules governing mangroves and formation of special groups for monitoring mangroves) involved statements in the form of a three-point Likert-scale 1= Disagree, 2 = Neutral, 3 = Agree (Hassan, 2015). The five-point Likert scale (1 = Very Low, 2 = Low, 3 =Medium/moderate, 4 = High, 5 =Very High) was used to record responses on the level of community willingness to participate in any conservation initiative. To minimize bias and ensure that respondents were not duplicated, the Geographical Positioning System (GPS) location of the respondent's home or workplace was registered.

Participant and non-participant observations

These observations involved directly or indirectly participating in community activities in order to physically identify socio-economic activities, mangrove forest resources and conservation activities. It was carried out to supplement the information from FGDs, KIIs, and HSSI and to better comprehend the real-world phenomena.

Data analysis

Based on Shannon and Hsieh (2014), qualitative data from FGDs, KIIs and observations was subjected to content analysis, where detected themes were highlighted and coded to assess and give meaning to the data as reported by respondents. Quantitative information collected from household surveys were analysed for descriptive and inferential statistics using SPSS Statistical software version 20 and Microsoft Excel 2010 for Windows. The binary logistic regression model was used to deduce the relationship between 10 independent variables (drivers) and one dependent variable (community willingness). Variable description is shown in Table 2. The model was chosen based on Sperandei (2014) because it can simultaneously analyse impacts of both continuous and categorical explanatory variables. The following logistic regression model was used:

$$\ln(\pi/1-\pi) = \alpha + \beta i\, Xi + \beta k\, Xk + e \text{ ....(i)}$$

Where:

$(\pi/1-\pi)$ = Y is dependent variable, α is constant at y-axis (intercept)
β_i to β_k are coefficients of regression
X_i to X_k are independent variables
e is the distribution error term of independent variables

Table 2. Description of variables used in logistic regression (Drivers of community willingness to participate in conservation).

Variables	Description	Modules of quantities
Dependent variable		
Willingness to participate in mangrove conservation	Whether community is ready to participate	Categorical, if yes=1, no=0
Independent variables		
Gender	Gender of household head	Categorical, 1=Male, 0= Female
Age	Age of household head	Continuous, Number of years
Education level	Education level of household head	Continuous number of years spent in school
Household size	Total number of members in household	Continuous, Number of members
Residence time	Length of time household settled in an area	Continuous, Number of years
Source of income	Main livelihood activity of household head	Categorical, 1= Mangrove based activities, 0= non-mangrove-based activities
Participation in mangrove conservation training	Whether household head has participated in mangrove training	Categorical, if yes=1, no=0
Knowledge on mangrove laws	Whether household head know any laws governing mangroves	Categorical, if yes=1, no=0
Participation in communal activities	Whether household head has participated in any communal activities	Categorical, if yes=1, no=0

Results

Socio-demographic characteristics

The socio-demographic characteristics of the study respondents are summarized in Table 3, indicating that more than 68 % of the interviewed respondents were male, and 56 % were in the age group of 18-45 years. Nearly 81 % of the respondents were married. The major ethnic groups were Ndengereko (71.8 %) and Nyagatwa (19.9 %). Nearly 60 % of respondents in ND reported a household size of 5-8 people, while less than 50 % reported this household size in CD and SD. In terms of literacy, over 63 % of respondents had primary education and 24 % had no formal education. Generally, about 45 % of the respondents had lived in the delta for 21-40 years. Mangrove-based rice farming was the major livelihood occupation of most respondents in ND (75 %) while about 43 % and 42 % reported to depend on non-mangrove-based crops farming in CD and SD, respectively.

Participation in mangrove conservation

Responses to community perceptions on the importance of participating in mangrove conservation activities are summarized in Figure 2, indicating a significant difference (χ^2 = 9.846, *p-value* = 0.007) across study zones. Overall, more than 80 % of the respondents across the delta agreed that it is essential for communities to participate in mangrove conservation activities because they are natives with local knowledge of delta areas, and the primary implementers of the initiated conservation activities. For example, one FGD respondent from ND disclosed that:

It is crucial that the community get involved in mangrove conservation efforts since doing so will help us learn about conservation, provide us with income to meet our needs, and serve as the first custodians of these resources for the benefit of future generations.

Willingness to participate in mangrove conservation

The three study zones of the Rufiji Delta had different perspectives on their willingness to participate in conservation activities. Figure 3 presents community perceptions on seven conservation activities and the corresponding willingness to participate in such activities. Overall, over 70 % of respondents agreed that it was important to participate in all conservation activities. Mangrove restoration was in particular highly regarded as important by the majority (over 85 %) of respondents.

Level of willingness to participate in mangrove conservation

Respondents were required to rank the level of community willingness to participate in conservation activities. Figure 4 presents the level of community willingness to participate in conservation, indicating a significant difference (χ^2 = 29.204, *p-value* = 0.000) on the level of community willingness between the three zones, where 60 % of respondents in ND and 50 % in

Table 3. Socio-demographic characteristics of respondents in the study sites (n=300).

Variable	Category	Percentage of responses			
		ND	CD	SD	Overall
Gender	Male	66.7	66.7	71.1	68.2
	Female	33.3	33.3	28.9	31.8
Age	18-35	23.3	43.3	25.6	30.7
	36-45	28.7	21.7	24.4	24.9
	46-59	25.3	20	21.1	22.1
	≥60	22.7	15	28.9	22.2
Marital status	Married	85.3	80	76.7	80.7
	Single	2	10	5.6	5.9
	Divorced	6	5	10	7
	Widower/widow	6.7	5	7.8	6.5
Tribe	Ndengereko	33.3	98.7	83.3	71.8
	Nyagatwa	58.7	0	1.1	19.9
	Hehe	1.3	0	3.3	1.5
	Konde	0.7	0	1.1	0.6
	Matumbi	2	0	6.7	2.9
	Zigua	0.7	0	0	0.2
	Ngindo	1.3	1.7	1.1	1.4
	Others	2.7	0	3.4	1.9
Household size	1-4	27.3	50	47.8	41.7
	5-8	59.3	45	48.9	51.1
	9-11	11.3	3.3	2.2	5.6
	12-15	2	1.7	1.1	1.6
Education level	No formal education	21.3	30	20	23.8
	Primary level	69.3	55	65.6	63.3
	Didn't finish primary	2	10	2.2	4.7
	Secondary level	4	3.3	6.7	4.7
	Didn't finish secondary	0.7	1.7	0	0.8
	Didn't finish advanced	0	0	1.1	0.4
	Adult education	2.7	0	4.4	2.4
Residence time	1-20	15.3	18.3	23.3	18.9
	21-40	40	56.7	36.7	44.5
	41-60	30.7	20	26.7	25.8
	61-80	13.3	5	8.9	9.1
	81-100	0.7	0	4.4	1.7
Source of income	Mangrove based rice farming	74.7	10	0	28.2
	Fishing	6.7	33.3	34.4	24.8
	Non-mangrove-based crops farming	4	43.3	42.2	29.8
	Mangrove harvesting	2.7	0	0	0.9
	Food vendors	0.7	3.3	6.7	3.4
	Livestock keeping	2.7	0	1.1	1.3
	Casual labour	2	0	0	0.6
	Formal employment	2	0	0	0.6
	Fish trading	0.7	0	2.2	0.9
	Driving	0	0	1.1	0.4
	Small business	0	3.3	7.8	3.7
	Boat captains	0	1.7	1.1	0.9
	Beekeeping	0	3.3	0	1.1
	Vocational works	3.3	1.7	3.3	2.8

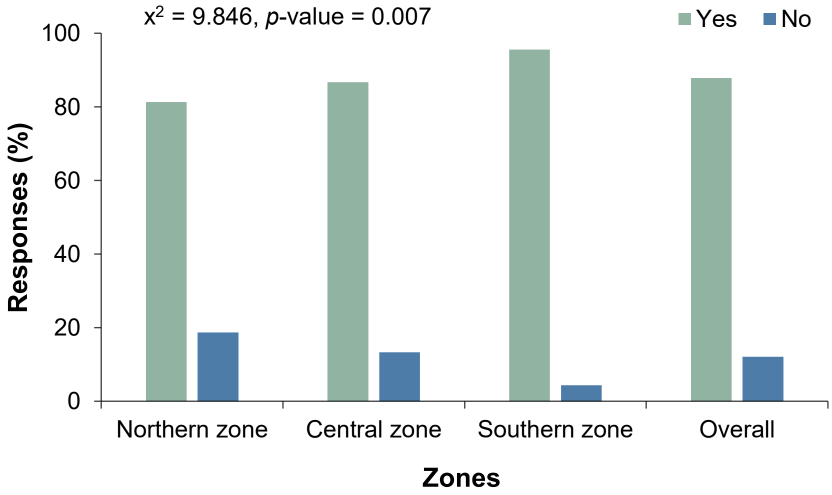

Figure 2. Percentage responses of importance of community participation in mangrove conservation activities in the study area.

CD indicated moderate levels of community willingness to participate in conservation, compared to 42 % of their counterparts in SD. Overall, only 6 % and 4.7 % of respondents agreed that the community could participate in conservation at very high and very low levels, respectively.

Drivers of willingness to participate in mangrove conservation

Logistic regression analysis indicated four drivers (independent variables) were strongly associated with community willingness to participate in mangrove conservation in the study area, including age of household

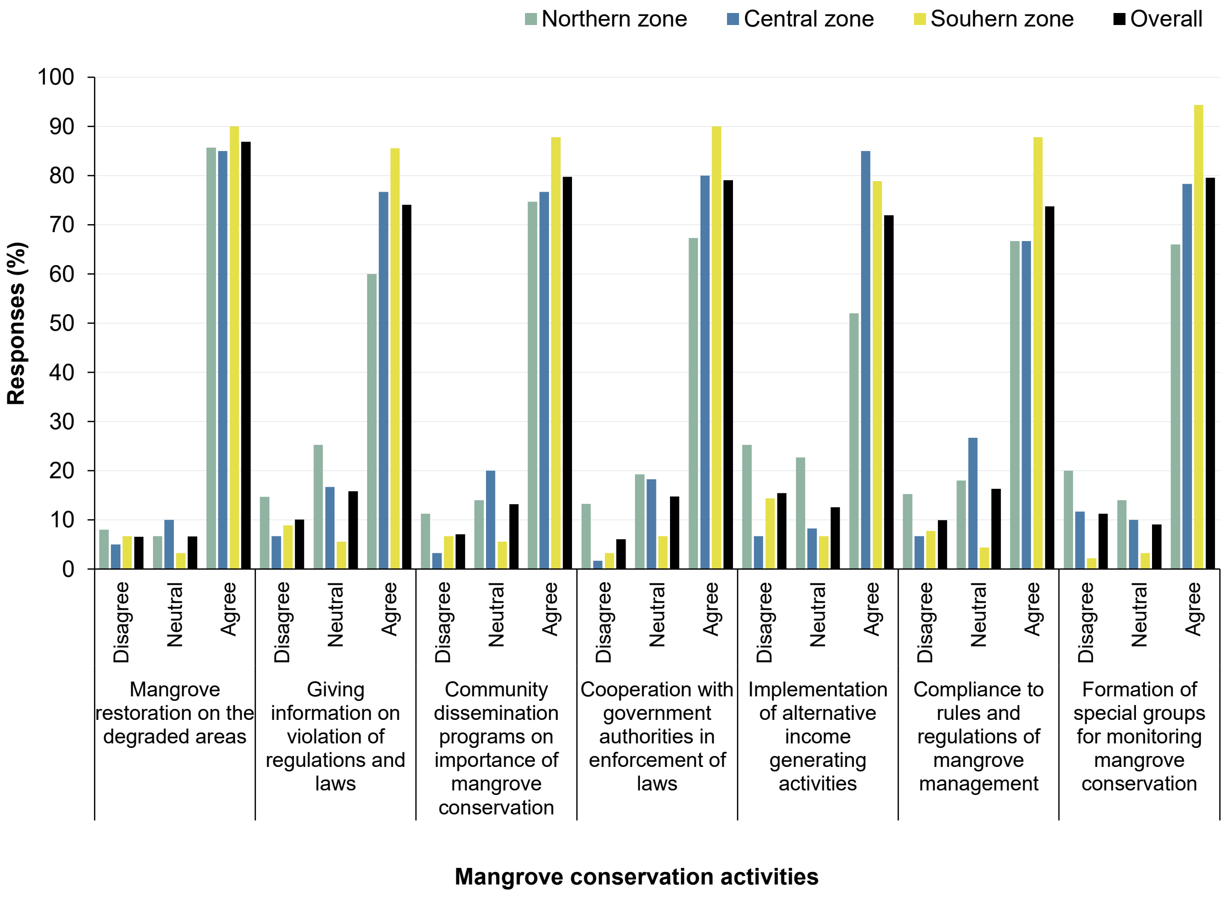

Figure 3. Responses on the list of conservation activities in which communities would be willing to participate in the study area.

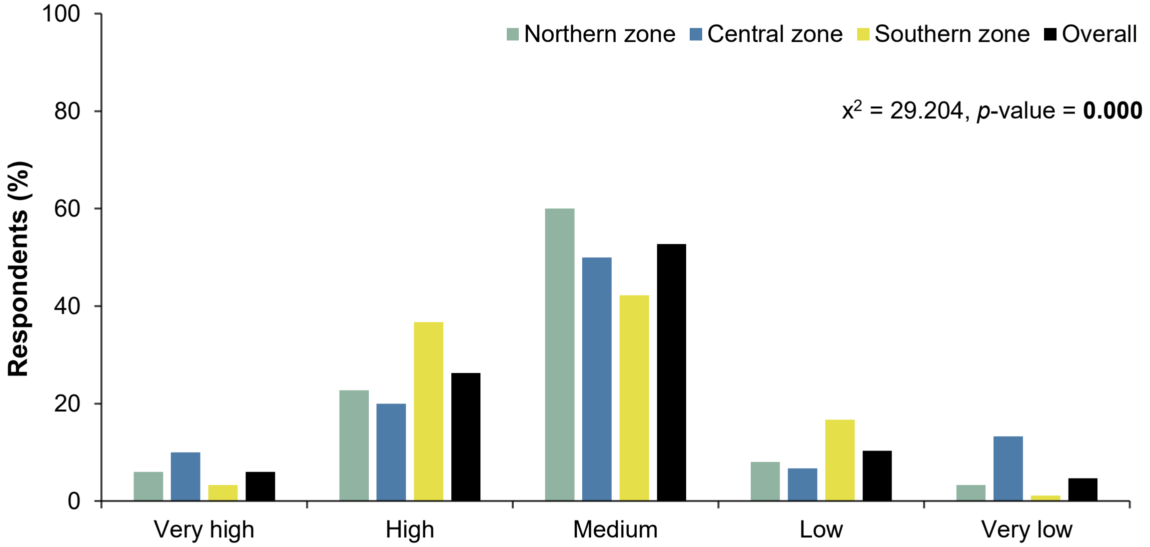

Figure 4. Level of community willingness to participate in mangrove conservation activities in the study area.

head, education level, source of income and awareness of mangrove governing laws. The coefficients of these variables were positive, and so a unit increase translated to an increase in number of people who were willing to participate in mangrove conservation by a factor of Exp (β). The findings are summarized in Table 4.

Discussion

Various government and non-governmental organizations such as TFS, WI, IUCN (Ntibona *et al.*, 2022), and environmental groups such as PCEG, VNRCs and BMUs (Mshale *et al.*, 2017; Japhet, 2018) working in the conservation field have extensively acknowledged the significance of community participation in conservation and management practices. In this study, 52 % of the respondents agreed to participate in various mangrove conservation activities in the study area. This implies that the local community in the study area is ready to take part as partners in conservation programmes. The key benefit of local community participation in conservation is that it effectively reduces protection costs in terms of time and money input by eliminating the need for outside technical expertise and human resources (Xu *et al.*, 2022). It was seen during interviews and discussion that the community is eager to be considered and included in conservation. Respondents articulated that it is essential for them to participate because they are natives of the delta, have enough local traditional knowledge to manage mangroves, use mangrove resources in their daily livelihoods and are able to implement conservation programmes for a longer period. Furthermore, their participation would increase public awareness on the benefits of conservation. The benefits are categorized into ecological (good air quality, increased mangrove quality, coastal area protection, increased fisheries), economic (increased income, creation of job opportunities, addressing social needs), cultural (culture preservation, increased social values), and legal (provision of licenses and proper harvesting plans). Xu *et al.* (2022) reported that local communities in Southeast China were willing to take part in ecosystem conservation due to increased awareness on forest protection and the myriad of ecosystem services provided by forests which support their wellbeing. Sesabo *et al.* (2006) found that coastal villages in Tanzania were willing to support establishment of Marine Protected Areas (MPAs) as a conservation initiative due to economical, ecological and social benefits that will be accrued from the MPAs. In this study, about 53 % of respondents indicated a medium level of willingness to participate in conservation, indicating that there is increased awareness on mangrove conservation as a result of several awareness training programs established by conservation NGOs (WWF and WI) (Nyangoko *et al.*, 2021, Ntibona *et al.*, 2022). People who are aware of the environmental costs of their actions are more likely to support the relevant environmental management programmes (Kangalawe, 2012). Astuti *et al.* (2017) who carried out research in Demak, Indonesia reported that local communities in some villages indicated mixed high and low levels of willingness. The low level of willingness was due to recurring shoreline advances and periodic floods caused by industrial activities that damaged mangroves, causing communities to be less concerned about the importance of

Table 4. Results of logistic regression on determinants of community willingness to participate in conservation in the study area.

Driver variables	β	SE	Wald	*p* value	Exp (β)
Gender of household head	0.607	0.428	2.010	0.156 ns	1.835
Age of household head	0.914	0.273	11.173	**0.001***	2.493
Education level	0.729	0.345	4.459	**0.035***	2.073
Household size	0.013	0.353	0.001	0.971 ns	1.013
Residence time	0.023	0.338	0.004	0.947 ns	1.023
Source of income	0.194	0.091	4.572	**0.033***	1.214
Participation in mangrove conservation training	18.954	5235.9	0	0.997 ns	170385159
Awareness on mangrove governing laws	1.497	0.425	12.414	**0***	4.468
Participation in communal activities	0.120	0.430	0.078	0.780 ns	1.128
Land ownership	-0.488	0.542	0.810	0.368 ns	0.614
Constant (intercept)	-3.098	1.418	4.773	**0.029***	0.045

Chi-square = 59.819 df = 10 Nagelkerke R square = 0.344

-log likelihood = 164.289 Overall percentage correctness = 89 Valid n = 300

β = Coefficient of regression, + and - are positive and negative relations respectively, p value = Significance level, SE is the standard error, * represents statistically significant at $p<0.05$, ns = indicates statistically non-significant at $p<0.05$. Wald value determines statistical significance of the independent variables.

mangroves. The high level of participation was credited to the potential for mangrove ecotourism as well as the support of government and private stakeholders to enable community-based management.

Local community participation is however contingent on incentives and benefits they will receive. Lack of benefits discourages local communities from participating in conservation initiatives (Paudyal *et al.,* 2018; Valenzuela *et al.,* 2020). Conservation activities should ensure socio-economic and socio-cultural wellbeing of local communities which are essential for maintaining conservation development (Gumede and Nzama, 2021). However, Kangalawe and Masao (2018) reported a number of challenges facing effective community engagement in conservation initiatives in the delta including little trust between community and government officers, and poor knowledge on the importance of conservation among communities. In other areas, similar and additional challenges have also been reported including political influence, inadequate capacity, time and desire to maintain natural resource management (Kangalawe, 2012; Abdullah *et al.,* 2014; Gumede and Nzama, 2021). The National Forest Policy of 1998, National Forest Policy Implementation Strategy of 2018-2028 and the Forest Act of 2002 emphasize community participation in mangrove conservation through implementation of PFM approaches (JFM and CBFM) (Zahabu *et al.,* 2009; Mshale *et al.,* 2017). In 2013, The Ministry of Natural Resources and Tourism through the forest sector developed specific guidelines for benefit sharing mechanisms under JFM which offer a framework for outlining roles and the associated community benefits (Mshale *et al.,* 2017). However, the problem has been on implementation of the guidelines. JFM, not only in mangroves, but also in other productive state forest reserves has remained relatively ineffective and limited to written guidelines. This is because there is no clear binding agreements on how forest benefits and revenues are shared equally between government and local communities (Nurse and Kabamba, 2001; MNRT, 2008). Substantial evidence from wildlife management programmes and other participatory forest management cases in Tanzania indicate that revenue sharing at the community level is still uneven (Melita and Mendlinger, 2013; Carius and Job, 2019; Harris *et al.,* 2020). An impact evaluation report by Persha and Meshack (2016) stated that JFM in Tanzania has enabled raising the level of local level governance, particularly through the function of VNRCs, but lacked in improving livelihoods of local communities participating in JFM which necessitates questioning the long-term sustainability of the strategy at the local level.

Understanding the need and importance of conservation triggers local communities to take part as partners in conservation programmes. Findings of this study are in line with observations that willingness of the local community to participate in conservation of natural resources is shaped by a mixture of drivers that are both demographic and behavioural (Ward *et al.,* 2017; Htay *et* *al.,* 2022), including age of household head, education level, source of income and personal knowledge on mangrove governing laws. In this study, age of household head was statistically significant and positively correlated with community willingness to participate in conservation, implying that old people were more willing to participate in conservation than young ones. This might be due to the fact that young individuals do not value conservation activities and see this as a waste of time. Overall, the majority of respondents were in the age group 18-45 which is the most productive age to engage in livelihood activities for household income generation. Also, young people are high mobile and frequently move to towns in quest of employment and other opportunities (Garekae *et al.,* 2017). A feeling of respect, absence of jobs and an expectation to get even small monetary benefits from conservation activities might influence old people to participate in conservation. However, old people have long-term experience of the negative consequences of mangrove degradation and understand their surrounding environment well, therefore being willingly to protect their environment. The findings contradict articulations of Mogomotsi *et al.* (2020) who reported that older respondents were less likely to participate in wildlife conservation in the Okavango Delta in Botswana and recommended the provision of conservation education especially to young people who can influence the elderly. Kirupakaran and Thiruchelvam (2010) reported that old people were unwilling to participate in conservation of freshwater turtles in Sri Lanka because they will not live long enough to enjoy the benefits of preserving resources in later years. Furthermore, observations by Mpokigwa *et al.* (2011) and Hassan (2015) indicated that older people are unwilling to participate in environmental natural resource conservation because they are less sensitive and concerned about environmental conditions compared to young people.

Level of education was statistically significant with a positive regression coefficient, implying higher educated respondents were more willing to participate in conservation than lower educated individuals. Educated people understood the importance of mangrove conservation to their livelihoods and environment and were therefore able to positively participate in conservation. Ponrahono and Sunoto (2019) reported a similar observation that education level positively influences community willingness to participate in mangrove conservation in Malaysia. In other cases of natural resource conservation (Kirupakaran and Thiruchelvam, 2010) higher levels of education is a determinant of coastal community willingness to participate in conservation of freshwater turtles in Sri Lanka. People with higher education would likely have better awareness of environmental issues which may result in a higher willingness to participate (Grazhdani, 2014; Xue *et al.,* 2022). Likewise, source of income was statistically significant with a positive regression coefficient indicating that people whose major sources of income are mangrove-based were more likely to participate in conservation to enable them to air their views and attitudes to conservationists regarding different laws and regulations that affect their access to utilize mangrove areas or related resources. This finding corroborates observations by Sesabo *et al.* (2006) who claimed that poor households who are dependent on common and open resources are highly motivated to support establishment and conservation of marine protected areas in Tanzania. Astuti *et al.* (2017) reported farmers are more participative in mangrove conservation due to their day-to-day interactions in the field as compared to non-farmers. Conversely, Mwanyoka (2006) claimed that people whose income depended on environmental resources are less likely to support conservation initiatives with the feeling that conservation initiatives might hinder their income-generating activities. Similarly, awareness of mangrove governing laws was statistically significant and positively influenced community willingness. The majority of respondents indicated that they know and understand the laws governing mangroves in their areas following awareness raising programmes initiated by respective authorities (TFS, VNRCs). Several studies by Sesabo *et al.* (2006), Mpokigwa *et al.* (2011) and Macharia (2015) have reported that an increase in public awareness on the existence of rules governing the use of coastal resources increases community willingness in forestry conservation interventions.

Other variables did not statistically influence community willingness to participate. Positive regression coefficient on gender implied more men are willing to participate than women. Sterian and Soutsas (2005) and Garekae *et al.* (2017) reported that men have more positive attitudes and are ready to participate in forestry conservation activities than women. According to coastal traditions, men are heads of household and play the major role in household decision making (Mshale *et al.,* 2017; Nyangoko *et al.,* 2021). Women are obliged to take care of house chores while men go out to search for income. This tradition has supported views that women are inferior and the exclusion of women in important village matters including participation in conservation initiatives. Several studies (Mpokigwa *et al.* 2011; Hassan, 2015; Garekae *et al.*, 2017; Ponrahono and Sunoto, 2019) have indicated that an increase in the residence time in the area influences community willingness to participate in conservation, in agreement with the findings of this study. The majority of respondents indicated that they had been living in the delta for 21-40 years. Longer staying residents are more familiar with their environment and keen to engage in conservation efforts (Nyangoko *et al.*, 2022). Household size was also positively related with community willingness to participate in conservation. This implies that larger households are more willing to participate than small households. In large households, there is the possibility of division of labour among household members. This creates opportunity for some members to attend conservation activities while others participate in production activities. Larger households have increased demands for basic necessities which increases their mangrove dependency (Nyangoko *et al.*, 2022), hence triggering their participation. Suggestions by Kirupakaran and Thiruchelvam (2010) and Garekae *et al.* (2017) that larger household are more likely to participate and support conservation programmes, are in agreement with findings form this study.

Participation in mangrove conservation training and communal activities were positively related to community willingness to participate in mangrove conservation activities. Respondents who regularly participate in trainings and communal activities agreed to willingly participate in conservation activities. The findings indicated that over 60 % have voluntarily participated in communal activities including construction of government buildings (dispensaries, schools) and village infrastructure for their village development. Responsible government authorities and conservation stakeholders should support more trainings programmes to raise community awareness. Macharia (2015) who researched in Meru County in Kenya found that the majority of respondents who have attended various trainings and workshops on mangrove conservation have awareness that contributed to effective participation in implementing forest conservation projects. In this study, land ownership did not positively relate to community willingness to participate in conservation activities, implying that regardless of the amount of farmland owned, many households were willing to participate in conservation. The findings indicated that the majority of respondents in the delta owned 1-3 acres of farmland within the mangrove area outside their villages. The negative regression coefficient implies that as the number of people who own farmland in mangroves increases, their willingness to participate decreases. Since 2010 the government has actively restricted rice farm expansion in the delta through conservation laws and regulations (Mshale *et al.*, 2017). Respondents might have been reluctant to support mangrove conservation activities thinking that this might increase their chances of being evicted from mangrove areas. Mogomotsi *et al.* (2020) found that people owning ploughing fields within wildlife conservation areas were less likely to participate in conservation in the Okavango Delta in Botswana. In contrast, Mndolwa *et al.* (2009) who carried out research in terrestrial community-based forests in Tanzania found that high participation of people in forest management was enhanced by the fact that many owned forest land.

Conclusions

This study set out to evaluate local community willingness to participate in mangrove conservation activities in the Rufiji Delta. It was found that in general local communities have knowledge of the importance of their participation in conservation activities towards sustainable use of natural resources. The majority disclosed moderate levels of willingness. Mangrove restoration activities were rated as highly important by the majority (over 85 %) of respondents. Age of household head, education level, source of income and awareness on mangrove governing laws are significant factors and were positively associated with community willingness to participate. Altogether, these findings suggest that for effective conservation, it is important for responsible conservation authorities to recognize and include locals in conservation activities. The government should strengthen implementation of participatory forest management approaches as emphasized by the National Forest Policy of 1998, National Forest Policy Implementation strategy of 2018-2028 and Forest Act of 2002. This can be supported by reviewing how benefits for communities can be generated and shared equally, capacity building of local level institutions such as VNRCs, and improving TFS capacity with human, technical and financial resources to enhance community forest management mechanisms. Communities would be willing to engage in conservation if their position is adequately recognized and they are given the opportunity to exercise their mandate. This can be achieved by engaging locals in planning and decision-making and implementation of laws and regulations governing their own areas. Local communities should be provided with incentives to encourage them to develop the capacity and desire to sustainably manage conservation programmes such as payment to mangrove planters, donation of livestock to poor households, giving rewards to illegal use reporters, revenue sharing, establishment of local mangrove protection teams and supporting trainings.

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Original Article

Comparison of growth and survival rates of big blue octopus (*Octopus cyanea*, 1849) fed on natural and formulated diets in captivity

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Abstract

Comparative studies on growth and survival rates of *Octopus cyanea* fed on natural and formulated diets in captivity have never been conducted in Tanzania. This study aimed to investigate the growth and survival rates of *O. cyanea* using natural and formulated diets. The three formulated diets were made up of a mixture of sardines, fish waste, and alternating ratios of crab paste content across the different diet treatments. Treatment B had 75 % crab paste content, Treatment C had 50 % crab content and Treatment D had 0 % crab content while Treatment A was based on a natural diet of frozen crabs (*Scylla serrata*) and was used as a control. After five weeks of feeding, the effect of each diet was analyzed on growth performance and survival rates for the octopus. Results showed that there was a significant difference in growth rate in all the diet treatments (H=13.243, p=0.004, DF=3). Specific growth rates (SGR) were higher in octopuses fed in feed treatment A and lowest in feed treatment D. The survival rates were 100 % for treatment A and feed treatment B and 75 % for treatments C and D respectively. More research is needed to develop optimal nutritional diets for faster growth rates of *O. cyanea* in captivity.

Keywords: octopus, nutrition, formulated diets, captivity

Introduction

Octopus cyanea, also known as the big blue octopus, is one of the most common exploited species in the Western Indian Ocean regions (Guard and Mgaya, 2002; Roper and Hochberg, 1988a). *O. cyanea* is found within phylum Mollusca, class Cephalopoda, and family Octopodidae and is distributed throughout the Indo-Pacific Ocean from Hawaii to the eastern coast of Africa. Their preferred habitat is in either the lower reaches of the intertidal reef flat or along the reef edge where they live in small holes (dens) and crevices often hidden by small stones, rubble and pieces of shell (Norman, 2000; Guard and Mgaya, 2002). Octopus play an essential ecological role in the marine ecosystem where they act as predators and potential prey to larger fishes such as sharks and some neritic tuna (Forsythe and Hanlon, 1997; Guard, 2009). In Tanzania, octopus fishing is practiced by small-scale fishers and occurs extensively along the coast providing a source of protein and improving the livelihoods of the fishermen (Jiddawi and Ohman, 2002). Despite of the high economic value of the octopus fishery, global octopus catch declined to 335,865 tonnes in 2012 from 380,000 tonnes in 2007. In Zanzibar and Tanzania Mainland, octopus catch peaked at around 1700 tonnes in 2003 before dropping sharply to 703 tonnes in 2006 (FAO, 2014). The reported declines in octopus catches have been linked to several factors, including

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over-exploitation for export markets, increasing number of octopus fishers and tourists, poor management practices, seasonal change in sea temperatures, habitat degradation, disease outbreaks, pollution and predation (Katsanevakis and Verriopoulos, 2006; Van Heukelem, 1973; Sparre, 1998; Rocliffe and Harris, 2016). In 2020, the global export of cephalopods amounted to USD 10.2 billion which is equivalent to 6.8 % of the total value of exports of aquatic products; this trend resulted in the share of cephalopods in global trade increasing over time and put supplies at risk due to poor management (FAO, 2022). Efforts have been focused on octopus fishery closures to assist in fishery sustainability, and little attention has been paid to exploring the viability of successfully culturing octopus species for commercial industrial production. The first feeding and behaviour experiments on the rearing of octopus in captivity were performed by the Instituto de Ciencias Marinas de Vigo and the Instituto Español de Oceanografía in Spain (Guerra, 1978; Nixon and Mangold, 1998). Octopus juveniles were grown in tanks and floating cages obtaining promising results and since then, many Spanish research centers have shown interest in the development of production techniques for octopus, such as Ciencias Marinas del Mar of CSIC, Barcelona (Villanueva, 1995). Such studies have not been explored across the Western Indian Ocean (WIO) region although octopus species present a series of favorable characteristics for rearing in captivity and commercial farming. These include: a great tolerance to captive conditions (Iglesias *et al.*, 2000), very fast growth rate (Mangold, 1983; Van Heukelem, 1973; Guard, 2009, Roper and Hochberg, 1998b)

1988b), high feed conversion efficiencies (Mangold and Von Boletzky, 1973), high reproductive rate of up to 400,000 paralarvae (Boyle and Rodhouse, 2005) and a high market price (Vaz-Pires *et al.*, 2004). Several cephalopod species have been studied worldwide, and various species of octopus have been reared in captivity to sexual maturity in the 1960s to 1970s, including *Octopus joubini*, *O. briareus* and *O. vulgaris*. In the early 2000s, the first successful commercial-scale octopus farming operation was established in the state of Yucatan for *O. maya* (Lopez *et al.,* 2015).

Nevertheless, making formulated feeds of appropriate nutritional composition is the main challenge to the development of aquaculture of octopus (Vaz-Pires *et al.*, 2004; Cerezo and Garcia, 2016). A series of experiments have shown that formulated feed is the determinant of successful growth and intensification of octopus aquaculture production. So far, considerable effort has been made to develop artificial diets (Aguila *et al.*, 2007; Domingues *et al.,* 2007; Cerezo *et al.*, 2008). Future development of octopus aquaculture depends on the development of nutritionally sound and cost-effective feeds that can support production levels (Kirimi *et al.*, 2016). Despite octopus being recognized as an ideal candidate for aquaculture, studies on octopus aquaculture in relation to formulated diets remain limited. This study aimed at investigating the performance of formulated diets on growth and survival rates of *O. cyanea* in comparison with a natural diet based on frozen crab. The selection of the ingredients to formulate experimental diets for the octopus was based on past successes reported in the literature from similar studies with different octopus species, and the availability of these ingredients locally in large quantities.

Material and methods

Description of the sampling site

The experiment was carried out for 35 days at the Institute of Marine Sciences, Buyu campus, Zanzibar. *O. cyanea* used in the study were obtained from local fishermen in Kizimkazi Mkunguni (Fig. 1) who used free diving to collect the specimens. Kizimkazi Mkunguni is located on the southern coast of Unguja Island, Zanzibar with geographical coordinates 6°26'60.0"S, 39°27'60.0"E and is characterized by sandy beaches and extensive nearshore coral reefs inhabited by abundant numbers of *O. cyanea*. Fishing is the main economic activity supporting livelihoods.

Experimental design and data collection techniques

Collection of juvenile Octopus cyanea

A total of 32 live juveniles of *O. cyanea* with an average weight of 20 to 250 grams were collected at a depth of 1 to 3 meters during low spring tide at Kizimkazi Mkunguni by free diving, the 32 octopuses were graded according to their weights and kept in 10L buckets separately in pairs. The buckets had openings in the lid to allow flow of air, while small pebbles were placed on the bottom to mimic the wild environment. No aerators were used during the approximately 2-hour transportation to the Institute of Marine Sciences, Buyu campus where the experiments took place. The octopuses were randomly distributed in sixteen tanks of 1000 L (two octopus per tank) in a flow-through saltwater system at the experimental site.

Water quality parameters (temperature, salinity, dissolved oxygen, and pH) were measured twice a day (morning and afternoon after feeding). Growth in weight was evaluated after every seven days. Coral fragments were kept inside the tanks to allow hiding and attachment as in the natural wild habitat. The octopuses were acclimatized for two days before the onset of the experiment and during this period they were not fed to allow them to get used to the captive conditions (after Sen, 2019).

The crab paste content in Treatment B was 75 %, 50 % in Treatment C, and 0% in Treatment D. Treatment A used a natural diet of frozen crabs (*Scylla serrata*).

Each tank contained a PVC tube of 10.16 cm diameter that was placed at the bottom of the tanks for the octopus to use as a refuge. Other habitat substrates such as pieces of dead hard corals and pebbles were also kept in the tanks. Seawater was exchanged regularly to maintain a conducive condition for the survival of the *O. cyanea*. To prevent escape, each tank was covered with a plastic mesh lid designed with two removable hatches. Seawater inlet was through a pipe that was connected to the tank reservoir containing water from the sea. The seawater outlet was located at the end

Figure 1. Map of Unguja Island showing location of sampling area at Kizimkazi Mkunguni.

Experimental design

The experimental tanks were distributed randomly in the experimental site and labeled based on the feed treatment provided. There were three feed treatments; each with four replicates. The feeds used in this study were composed of sardines, fish waste, and varying amounts of crab paste in the different treatments.

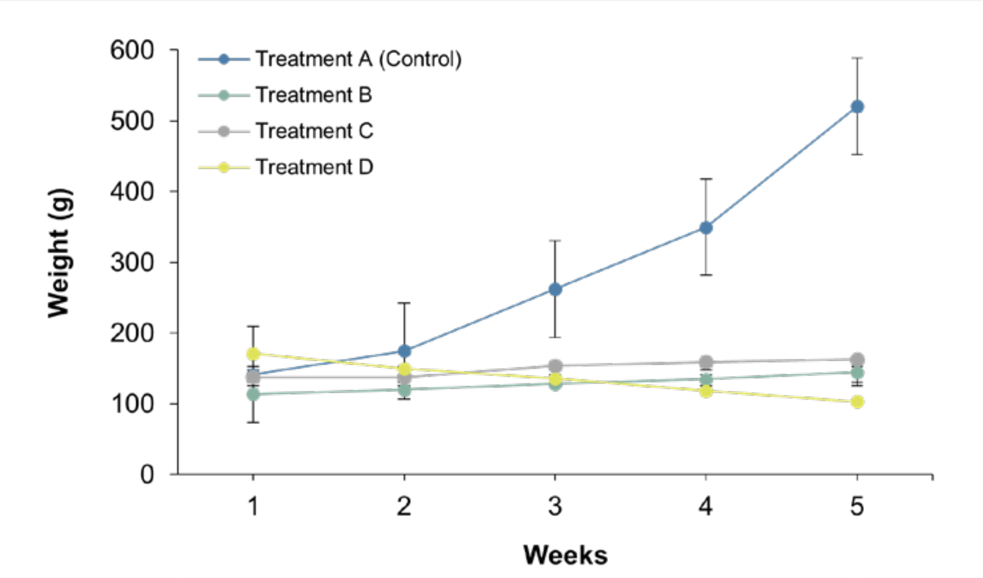

Figure 2. Mean weight of *Octopus cyanea* fed on formulated diets and frozen crab (Treatment A) for 5 weeks.

of each tank and sealed with a cork. The outlet was less than 2 cm diameter to prevent the octopus from escaping during opening and closing. Temperature, salinity, dissolved oxygen (DO), and pH were checked twice daily (morning and afternoon after feeding). The three formulated diet treatments and the control were supplied to the octopus once a day at (0900 hrs) at 5 % body weight for all regimens (after Farìas *et al*., 2010). Unconsumed feed was siphoned every day (at 1700hrs). Overnight faeces was removed from the tanks before the octopus were given the initial feeding. The experiment was carried out for a period of 35 days.

Preparation of the formulated feeds for the experimental diets

Three formulated diets and a natural diet consisting of a crab diet (*Scylla Serrata*) were used in the experiment. The formulated diets (Fig. 3) were a mixture of sardines (*Sardinella longicep*s), fish waste that consisted of fish stomach contents, intestines, heads and fins that are often discarded at fish markets, and crab paste (soft parts of the crab meat after the skeleton has been removed). Cassava flour was used as a binder. Fifty kg of each ingredient was purchased, dried for 24 hours under the sun and ground using a grinding machine, then sieved using 0.5 mm mesh to remove indigestible parts and to obtain a fine powder for all ingredients. This was followed by mixing thoroughly by hand to form a uniform single mixture; however, for the case of crab, the hard parts including the carapace and hard shells which are less nutritious and less digestible were first removed before other processes. Formulated diets of different crab paste content (75 %, 50 % and 0 %) for the diet treatments were obtained. Hot boiled water was added to cassava flour as a binder

Table 1. Feed composition in % for *Octopus cyanea* formulated diets: crab paste, sardines and fish wastes.

Ingredients	Feed Treatment B	Feed Treatment C	Feed Treatment D
Crab paste	75.0	50.0	0.0
Sardines	12.5	25.0	50.0
Fish waste	12.5	25.0	50.0

Table 2. Proximate analysis for *Octopus cyanea* formulated diets: crab paste, sardines and fish waste.

Ingredient	Dry Matter	Ash	Crude protein	Crude Fiber	Ether Extraction
Crab paste	95.0±0.30	41.5±0.50	31.1±0.40	7.6±1.30	0.0±0.10
Sardines	94.4±4.90	38.3±0.50	47.2±0.20	0.6±0.30	4.9±0.20
Fish wastes	95.0±2.20	37.9±1.60	8.6±1.20	0.2±0.10	2.0±0.10

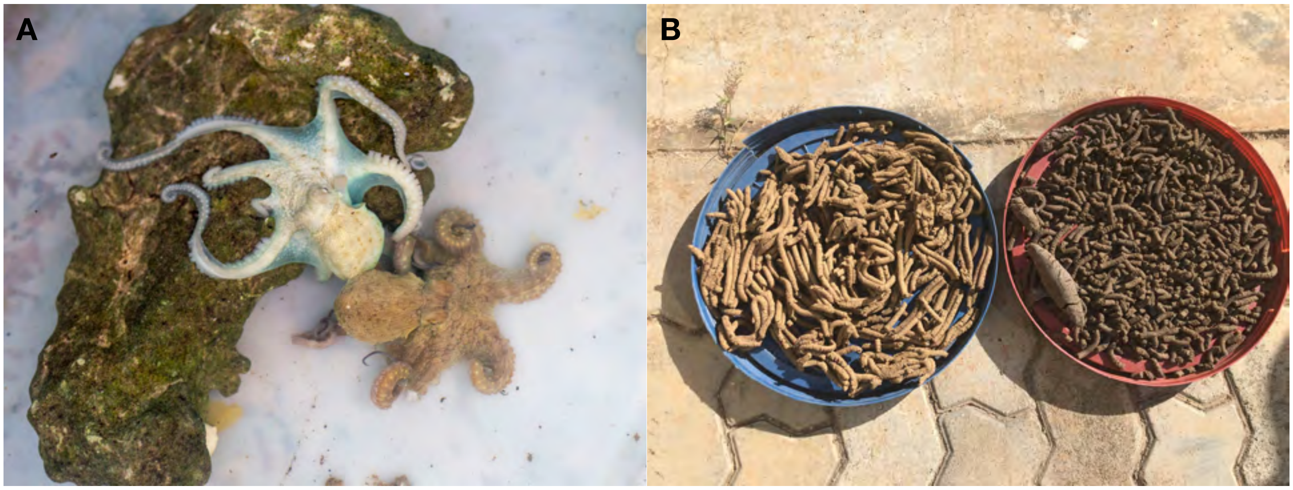

Figure 3. (A) Photo showing *O. cyaena* in a tank. (B) Photo showing formulated feed with crab paste content (left) and with no crab paste content (right).

to obtain separate doughs containing the various dietary ingredients. The dough was then passed through a pelleting machine to obtain 1.5mm diameter pellets. Immediately after pelleting the feeds, they were dried under the sun and then stored in an air-conditioned room at a temperature below 18 °C. The crabs were purchased from Darajani fish market in Zanzibar and then stored in the freezer at the experimental site at Buyu Campus. Proximate analysis for each feed ingredient was conducted at Sokoine University of Agriculture to assess their nutritional content (Table 2).

Data analysis

The data obtained are presented as mean ± SE (standard error). Calculations of growth performance parameters for the different feed treatments were conducted for Specific Growth Rates (SGR), Weight Gain (WG), Average Daily Gain (ADG) and Survival Rates (SR) by using the formulae below as described by Abarike *et al.* (2012). To determine the growth rates of *O. cyanea* fed on the different formulated diets, growth data was tested for normality and homogeneity by Shapiro's test of normality and Levene's test for homogeneity respectively, using R programme 4.6. The data were found not to be normally distributed therefore the non-parametric test of Kruskal Wallis was conducted followed by a post-hoc pairwise Wilcoxon test to depict where the significant difference in growth rates lies within the different diets.

Specific Growth Rate (% per day) = [(Ln final body weight – Ln initial body weight)] × 100/Experimental period.
ADG (g) = Weight gain/culturing days
Weight Gain (g) = Final weight (g) - Initial weight (g)
Survival Rate (%) = (Initial number of octopus stocked – mortality)/initial number of octopus stocked × 100

Results

Growth rates of *O. cyanea* fed on different formulated diets

Growth rates for *O. cyanea* were calculated and are presented in (Table 3) below, together with initial mean body weight (g), final mean body weight (g), weight gain (g), SGR (%/day) and ADG (g) with mean ± standard error. The highest SGR was displayed by

Table 3. The Growth performance of *Octopus cyanea* with different formulated feeds. Values show mean ± standard error.

Parameters	Feed Treatments			
	Feed Treatment A	Feed Treatment B	Feed Treatment C	Feed Treatment D
Initial mean body weight (g)	141.50±40.30	113.80±29.30	137.50±25.90	171.00±40.50
Final mean body weight	520.50±131.50	144.75±30.70	162.75±18.30	103.00±32.80
Weight gain (g)	379.00±106.80	25.00±7.70	31.25±17.30	-68.00 ± 15.20
SGR (%/day)	3.98±1.20	0.97±0.90	0.58±0.90	-1.58±0.50
ADG (g)	54.00±15.30	4.40±1.10	3.61±2.50	-9.70±2.20
Survival (%)	100	100	75	75

Table 4. Temperature, salinity, water pH, and DO of the experiment at different feed treatments.

Parameters	Feed Treatment A	Feed Treatment B	Feed Treatment C	Feed Treatment D
Temperature (°C)	25±0.20	26±0.20	24±0.20	26±0.00
DO (Mg/l)	9.0±0.10	7.0±0.20	8.5±0.60	7.4±0.00
pH	8.7±0.00	8.7±0.00	8.8±0.00	8.7±0.00
Salinity	38.9±0.20	38.5±0.00	39.0±0.20	40.0±0.10

a natural diet based on frozen crab followed by feed treatment B and C, with the lowest SGR displayed by feed treatment C (Fig. 1).

Survival rates

The survival rate was 100 % in treatment A and B, and 75 % in feed treatment C and D.

Water quality parameters

In all treatments the temperature ranged from 24 °C to 26.4 °C DO varied from 6.8 to 9.5 mg/l, pH varied from 8.6 to 8.8, and salinity from 38.5 to 40. (Table 4).

Discussion

Growth and survival rates of *O. cyanea* in captivity

Octopus showed a slow positive growth rate for feed treatments B and C while a negative growth rate was recorded in feed treatment D. There was no significant difference in growth rate shown in all the three formulated feeds (B, C and D). These results can be compared to a study by Martínez *et al.* (2014) in which best growth rates in juvenile stages of *O. maya* were obtained by using a moist crustacean-based diet and this was attributed to high protein assimilation and digestibility levels of this diet. Furthermore, Aguado and García (2002) reported that growth and food intake were higher with a crab diet when rearing *O. maya* in captivity. Other studies agree that the natural diet is still the most reliable, especially when based on crustaceans (Sánchez *et al.*, 2014; Gutiérrez *et al.*, 2015). The success of these studies are attributed to the longer period of the experiment. Replacement with formulated diets must be carried out using crustaceans-based diets, squid or fish, using freeze-dried meals (Estefanell *et al.*, 2013; Rosas *et al.*, 2013; Rodríguez *et al.*, 2015). However, studies that have used a fish diet for octopus have exhibited lower growth rates and survival compared to those using a crustacean-based diet (Cagnetta and Sublimi, 2000; Domain, 2000; Aguado and García, 2002). The lowest growth rates have been attributed to low acceptability (López-Uriarte and Rios-Jara, 2009; Farías *et al.*, 2010; Estefanall *et al.*, 2013;), and low digestibility (Martínez *et al.*, 2014). To date, species such as *O. maya* and *O. vulgaris* have shown acceptable developmental rates based on formulated moist feeds allowing them to replace fresh natural diets at the experimental level (Cerezo *et al.*, 2008). This is evident after a series of feed experiments from the 1960s to date, exploring different nutritional requirements aiding fast growth. The present study provided initial experimental information on *O. cyanea* using diets that included locally formulated feeds in Tanzania. The results show a slow positive response and this provides room for more experiments with various feeds to determine the best nutritional requirements for octopus aquaculture. There was a significant difference in survival rates observed amongst feeds in this study; those fed with less crab had lower survival rates compared to those with more crab in their diet (100% survival).

In term of water quality, the salinity range for octopus is between 35 to 39.5 psu (Mangold, 1983). In this study, they survived well between 38 up to 40 psu. There were occurrences of temperature fluctuations recorded within the experiment, but they fell within *O. cyanea's* survival and growth range (24 - 26 °C). The present study did not estimate what temperature ranges supported the most efficient metabolic activities of the animals. For example, within the survival temperature range, *O. vulgaris* responded to temperature rises by increasing food intake and growth (Mangold, 1983); this can be an area of interest for future studies to compare different temperature ranges for *O. cyanea* growth and survival. The tanks were well aerated with aerators providing sufficient DO throughout the experiment, and there was regular water exchange twice a day, maintaining the DO at > 6 mg/l which proved to be within the survival range for *O. cyanea.* However, the species showed the ability to survive in low oxygen supply conditions for short periods. For instance, they endured 3 hours of transportation from the wild to the experimental site, with no aerators. Nevertheless, the release of ink into the water as a mechanism to defend themselves from predators (usually occurring during weighing operation) clouded the water, and the accumulation of this ink lowered the DO.

Formulated feeds and proximate analysis

O'Dor *et al.* (1984) reported a 96 % protein and 46 % lipid digestibility for *O. vulgaris,* indicating clearly that this species has the capacity to digest and assimilate protein rapidly. Crude protein (CP) is the major constituent and most costly component of fish feed (Kaushalendra *et al.*, 2016). CP in feeds provides essential amino acids; it was the most significant nutritional composition amongst the three feeds in this study for promoting growth in the animals. The highest CP values were associated with sardines followed by crab paste while the lowest was found in fish waste. Studies by Mangold and Boletzky (1973), Aguado and Garcia (2002), and Villanueva *et al.* (2002) have shown that crustacean-based diets have high protein, assimilation and digestibility levels that favour growth and survival of octopus. Furthermore, lipid content is known as a limiting factor to growth, due to the low digestibility and assimilation levels of lipids by octopus. This suggests that for fast growth of octopus there should be appropriate matching of levels of crude protein and lipids content in the diet. In this study fish waste had the highest lipid levels (4.85 ±0.20), followed by sardines (1.99±0.06) and crab paste (0.04±0.13). Thus, despite the sardine having high crude protein content, crab paste has the right proportions of protein and lipid that favors positive growth of octopus. This is due to lower lipid content that enhances better digestibility and assimilation of the ingredients in octopus compared to sardines and fish waste. This is supported by Cerezo *et al.,* (2008) who reported that matching nutritional proportions of protein and lipids contributes greatly to high growth and survival rates of the octopus species.

Water quality parameters

Temperature, salinity and DO were observed to be the most important parameters to the growth and survival rates *of O. cyanea* in tanks. In this study the parameters were measured in the morning before feeding (0900hrs) and in the evening (1700hrs). The highest salinity recorded was 40 psu and the minimum was 38 psu. To maintain acceptable levels of DO water was exchanged in the morning before feeding and in the evening after feeding. Disturbances can cause octopus to release ink, thus clouding the water and lowering the DO (pers. obs.). The optimum range was observed to be within 6.68 - 9.05 mg/l; this was well maintained throughout the experiment through aeration. The pH was observed not to fluctuate as often as other water parameters, and throughout the experiment it remained between the safe range for *O. cyanea* of 8 - 8.5.

Conclusions

This study observed *O. cyanea* to have high selectivity and preference to the crustacean-based diets compared to the mixed non-crustacean diets (inclusion of fish waste and sardines). This selectivity and preference explains the prolonged time it took for octopus to adapt to the mixed ingredients, and explains the slow growth on the mixed diets with feed treatment B and C of 75 % and 50 % of the crab paste content respectively, and the negative growth for the feed treatment D of 0 % crab content. Treatment A showed fast growth because this diet contained the same content (crabs) as in the wild and did not require time for the octopus to adjust to it. However, the length of the experiment was only 5 weeks, a factor that may have affected the ability of octopus to adapt to the mixed ingredients, hence causing slow growth. Further research should consider the time factor of the experiments to determine appropriate feed combinations that work best for *O. cyanea* in captivity. Determination of the nutritional requirements for rearing *O. cyanea* is still at an infantile stage in Tanzania compared to other countries such as Spain. Therefore, this area remains largely unexplored and the suitable feed combinations that support highest growth levels are yet to be determined. Different countries have had trials with different feed mixtures in rearing different species of octopus which have provided good results over time, therefore future collaborations with countries that have pioneered successful trials should be established for more intensive research and experimental modifications that can contribute to octopus farming in the future.

Recommendations

The current study suggests the following recommendations in order to increase knowledge relevant to establishing octopus aquaculture with suitable feed formulation in Tanzania:

The current study assessed the effect of formulated feeds in *O. cyanea* in terms of growth and survival rates in captivity for only 35 days. Further studies should extend the culture period until the marketable size is attained to confirm any other effects associated with the formulated feeds.

Future research should focus on studying the matching nutritional requirements of *O. cyanea* for attaining higher growth rates in captivity; This aspect is still a challenge for octopus aquaculture research studies. Once the best feed combination with the correct nutritional quantities is determined octopus aquaculture can be successful. This requires resources in terms of funds, time and human capacity.

Other studies should focus on further research on replacing the "crustacean-based diet", which has proven crucial to the growth of *O.cyanea* and other octopus species worldwide, with an alternative, cheaper source which is nutritionally sufficient and acceptable to the animal. This is because crabs are expensive to purchase, and identifying an alternative best feed option will make this farming possible and affordable.

Different countries have had trials with different feed mixtures in rearing different species of octopus which have provided excellent results over time, therefore future collaborations with countries that have pioneered successful trials of octopus rearing should be established for more intensive research, knowledge exchange, resource mobilization and modifications.

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Original Article

DNA barcoding reveals endangered and protected elasmobranchs in Tanzanian fish markets

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Abstract

Western Indian Ocean countries have banned elasmobranch finning and enacted legislation to protect endangered elasmobranchs, however finned and morphologically deformed specimens are still landed and traded on Tanzanian fish markets. Such specimens are difficult to identify morphologically, and it is possible that protected elasmobranchs are among these. This study used DNA barcoding to uncover protected elasmobranchs in 102 specimens traded on Tanzanian fish markets. The sampled specimens revealed 23 elasmobranch species, 12 of which (52.1 %) were classified as endangered (EN) or critically endangered (CR) on the IUCN Red List. Three of the identified species (great hammerhead *Sphyrna mokarran*, oceanic whitetip shark *Carcharhinus longimanus*, and pelagic thresher *Alopias pelagicus*) are protected by Tanzanian laws. Hence, it is advised that steps be taken to strengthen law enforcement at landing sites and fish markets in the country. Furthermore, the Third Schedule of Fisheries (Amendment) Regulations of 2009 should be updated to include 11 EN and CR elasmobranchs that are not on the list. Additionally, national and regional elasmobranch conservation plans should be developed to prevent the exploitation of endangered elasmobranchs.

Keywords: DNA barcoding, cytochrome c oxidase subunit I (COI) gene, illegal trade, sharks and rays, endangered elasmobranch, East Africa

Introduction

The elasmobranch fishery has long been an important source of income and employment for coastal communities in the Western Indian Ocean (WIO) (Jiddawi and Ohman, 2002). In 2020, it accounted for 4.05 % of the total catch from Tanzanian marine waters, which is equal to 2 581.58 metric tons (URT, 2020). Total annual earnings from the fishery in 2020 were 12.9 billion TZS (approximately 5.5 million USD) (URT, 2020). The main fishing grounds for elasmobranchs in Tanzania are in areas associated with coral reefs, mangrove creeks, seagrasses, and sandbanks (Jiddawi and Ohman, 2002). The fishery has historically been exploited by traditional fishers using longline, gillnets, demersal nets, and drift gillnets (Schaeffer, 2004). In the 1990s, about 26 different species of elasmobranch were harvested in Tanzania, with the silky shark (*Carcharhinus falciformis)* dominating catch at many landing sites (Shehe and Jiddawi, 2002). Most of the harvested elasmobranchs in the country are traded in either processed or unprocessed form in local fish markets in Tanga, Dar es Salaam, Nungwi, and Mtwara. However, because elasmobranch oil and other products are used for painting fishing boats, traditional medicine, and a variety of other purposes, the species have been severely overfished (Muhando and Rumisha, 2008). The high demand for elasmobranch fins in Asian markets has also resulted in overfishing and increased destructive fishing practices in the country (Muhando and Rumisha, 2008; Hobbs *et al.*, 2019; Sachithanandam and Mohan, 2020). Hence, the elasmobranch fishery has declined by over 80 %, and over 30 % elasmobranch species have been fished to the brink of extinction (Dulvy *et al.*, 2017; Simwanza and Rumisha, 2023).

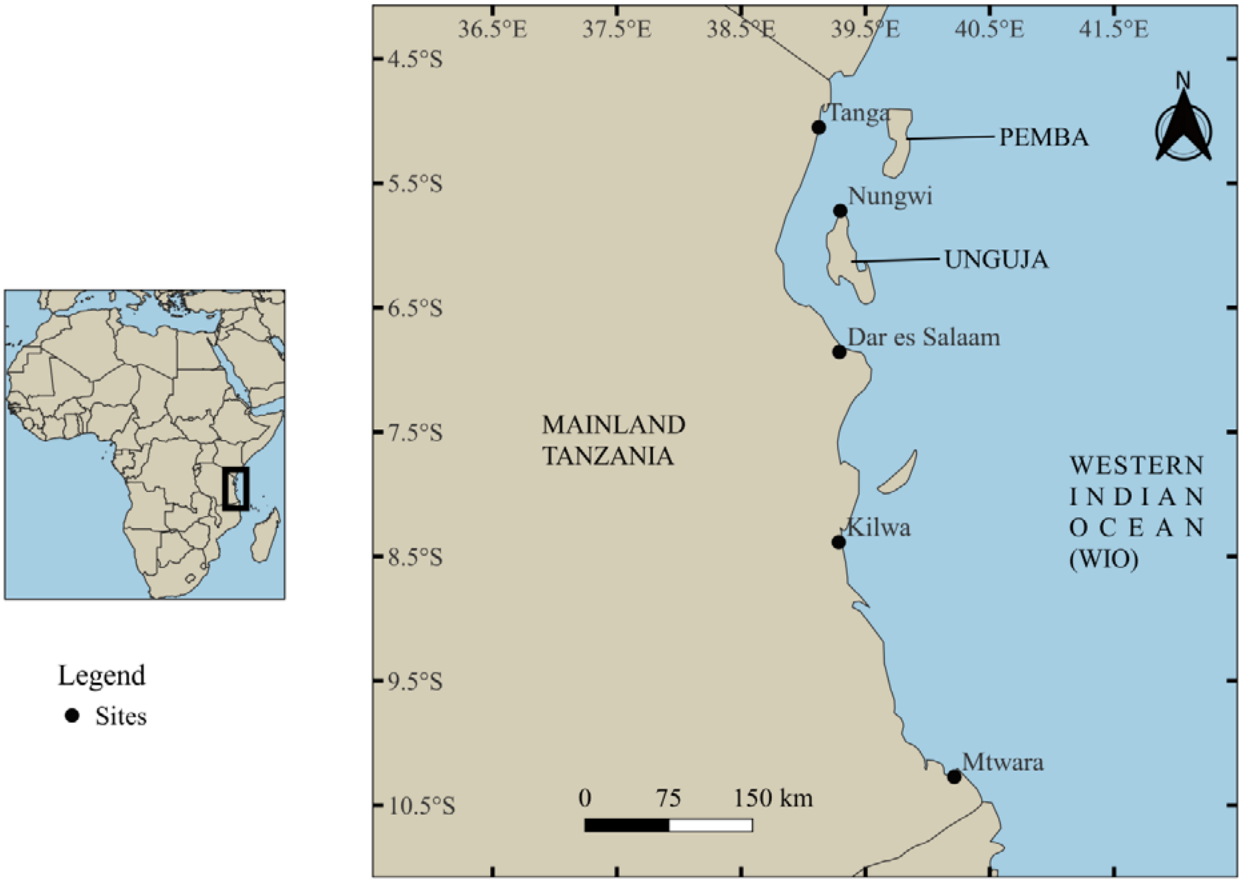

Figure 1. Map showing the sites where elasmobranch samples were collected between 2020 and 2022.

In response, international treaties such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have acted to regulate international trade of threatened elasmobranchs (Cardeñosa *et al.,* 2018). To date, 54 species of elasmobranch are listed in CITES Appendix II, including: the great white shark (*Carcharodon carcharias*), basking shark (*Cetorhinus maximus*), whale shark (*Rhincodon typus*), silky shark (*Carcharhinus falciformis*), oceanic whitetip shark *(Carcharhinus longimanus*), porbeagle shark (*Lamna nasus*), hammerhead sharks (*Sphyrna lewini, Sphyrna mokarran, Sphyrna zygaena*), thresher sharks (*Alopias pelagicus, Alopias superciliosus, Alopias vulpinus*), short fin mako shark (*Isurus oxyrinchus),* long fin mako shark (*Isurus paucus*), *Manta* spp., *Mobula* spp., and *Rhinidae* spp. Furthermore, Tanzania banned elasmobranch finning in its waters (Regulation 6 (b) of the Deep Sea Fisheries Management and Development Regulations, 2021) and enacted measures to prohibit the purchase, offer for sale and sale of shark fins which have been removed on-board, retained on-board, trans-shipped or landed in contravention to the IOTC resolution 17/05. Additionally, the country prohibited fishing, processing, trade and export of parts, products or derivatives of any elasmobranch species listed as endangered in any International Convention to which the United Republic of Tanzania is a party (Regulation 13 (11) of the Fisheries

(Amendment) Regulations, 2009). Also, the country enacted Regulation 67 of the Fisheries (Amendment) Regulations of 2009, which prohibited fishing and trade in any part or product derived from any of the ten elasmobranch species listed in the third schedule (whale shark *Rhincodon typus*, basking shark *Cetorhinus maximus*, longheaded eagle ray *Aetobatus flagellum*, reticulate eagle ray *Aetomylaeus vespertilio*, knifetooth sawfish *Anoxypristis cuspidate*, largetooth sawfish *Pristis microdon*, wide sawfish *Pristis pectinata*, narrowsnout sawfish *Pristis zijsron*, bottlenose skate *Rostroraja alba* and great hammerhead *Sphyrna mokarran*. Furthermore, the country outlawed fishing of Oceanic white tip sharks (*Carcharhinus longimanus*) and all thresher sharks (*Alopias* spp.) in accordance with Regulation 8 (1) (a) of the Deep Sea Fisheries Management and Development Regulations, 2021. Despite the existence of such regulations, finned and morphologically deformed specimens are still landed and traded in the country's fish markets. Because such specimens are difficult to identify morphologically, it is possible that protected elasmobranchs are among those traded specimens. Thus, this study was conducted to reveal the composition and the conservation status of landed and traded elasmobranchs in Tanzanian fish markets through morphological identification and DNA barcoding. Similar approaches have been used around the world to reveal the composition of protected species of fauna in traded specimens (Haque *et al.*, 2019; da Silva Ferrette *et al.*, 2019; Villate-Moreno *et al.*, 2021).

Material and methods

Study area

This study was conducted along the Tanzanian coast, specifically in Tanga, Mtwara, Kilwa, Dar es Salaam, and Nungwi (Fig. 1), where the main landing sites and fish markets are located (Muhando and Rumisha, 2008). These areas have a variety of marine ecosystems, such as coral reefs, mangroves, seagrass beds, and sandbanks, which support a diverse range of elasmobranchs and other marine fauna (Richmond, 2002; Rumisha *et al.*, 2015). The coastal waters in these areas are characterized by seasonal variations in

Table 1. Summary of the BLAST results obtained after comparing the obtained COI sequences of elasmobranchs from Tanzanian fish markets with those in the NCBI database.

Order	Family	Species	Sequence name	Base pair size	Accession number	Maximum score	Query cover (%)	E-value	% identity
Carcharhiniformes	Carcharhinidae	*Carcharhinus altimus*	RSS3.42	557	OQ359493	1029	100.0	0.0	100.0
		Carcharhinus falciformis	RSS3.4	567	OQ361640	1048	100.0	0.0	100.0
		Carcharhinus longimanus	RSS5.51	404	OQ361641	747	100.0	0.0	100.0
		Carcharhinus melanopterus	RSS1.19	612	OQ361642	1098	100.0	0.0	99.0
		Carcharhinus plumbeus	RSS3.11	613	OQ361643	1131	100.0	0.0	100.0
		Carcharhinus sorrah	RSS4.29	454	OQ361644	830	100.0	0.0	99.6
		Galeocerdo cuvier	RSS3.27	598	OQ361645	1127	100.0	0.0	100.0
		Loxodon macrorhinus	RSS4.28	582	OQ361648	1048	97.0	0.0	99.8
		Rhizoprionodon acutus	R11	605	OQ361660	1118	100.0	0.0	100.0
		Triaenodon obesus	RSS1.35	599	OQ361666	1107	100	0	100
	Hemigaleidae	*Hemipristis elongata*	RSS4.10	515	OQ361646	941	100.0	0.0	99.6
	Sphyrnidae	*Sphyrna lewini*	RSS4.11	566	OQ361662	1046	100.0	0.0	100.0
		Sphyrna mokarran	R4	613	OQ361663	1112	100.0	0.0	99.7
		Sphyrna zygaena	R58	623	OQ361664	1151	100.0	0.0	100.0
	Triakidae	*Mustelus asterias*	RSS5.14	576	OQ361657	1064	100.0	0.0	100.0
Lamniformes	Alopiidae	*Alopias pelagicus*	RSS3.3	635	OQ359492	1162	100	0	99.69
	Lamnidae	*Isurus oxyrinchus*	RSS3.19	584	OQ361647	1079	100.0	0.0	100.0
Myliobatiformes	Dasyatidae	*Maculabatis gerrardi*	RSS3.15	566	OQ361656	985	100.0	0.0	98.1
Orectolobiformes	Stegostomatidae	*Stegostoma tigrinum*	RSS1.39	566	OQ361665	1046	100.0	0.0	100.0
Rhinopristiformes	Rhinobatidae	*Acroteriobatus variegatus*	RSS1.29	404	OQ359491	697	100.0	0.0	97.8
		Rhina ancylostomus	RSS1.1	369	OQ361658	665	100.0	0.0	99.2
		Rhinobatos annandalei	RSS1.94	601	OQ361659	1033	100.0	0.0	97.7
		Rhynchobatus australiae	RSS5.71	609	OQ361661	1114	100.0	0.0	99.7

water circulation associated with the periods of northeast monsoon (NEM) and southeast monsoon (SEM). The NEM occurs from November to March and the SEM from April to October (Mahongo and Shaghude, 2014). Generally, there is more fishing activities during the NEM due to higher air temperature and weaker winds (Jiddawi and Ohman, 2002). The average temperatures range between 25.0 and 30.2 °C and water surface salinity between 34.5 and 35 parts per thousand (Mahongo and Shaghude, 2014).

Sampling and DNA extraction

Sampling was conducted between May 2020 and February 2022. A total of 102 elasmobranchs were sampled from landing sites, fish markets, and artisanal processors in the study area (Fig. 1). Each individual elasmobranch was first morphologically identified to species level using the available keys (Richmond, 2002; Kiszka *et al*., 2016). About 3 g of the fin tissue was dissected from each fish and stored in microcentrifuge tubes containing 99.9 % ethanol for further analysis. Genomic DNA was extracted from the sampled fin tissues by using the Quick-DNA™ Miniprep Plus Kit (Zymo Research Inc., CA, USA) according to the instructions of the manufacturer. The quality of the DNA extracts was checked on a 1 % agarose gel (Rumisha and Kochzius, 2023).

Identification of landed and traded species

Fragments of the cytochrome oxidase subunit I gene (COI) with ca. 650 base pairs were amplified from the DNA extracts of each sample in a T100™ Thermal cycler machine (Bio-Lab Inc, GA, USA) using the forward primer FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and the reverse primer FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward *et al*., 2005). Amplification reactions were done in a total volume of 25 µl consisting of 1 x OneTaq 2X Master Mix with Standard Buffer (New England BioLabs Inc., MA, USA), 0.25 µM of each primer, and 0.5 mg bovine

Table 2. Percentage composition of elasmobranch species at landing sites and fish markets in Tanzania between 2020 and 2022.

Species	Common name	Percentage composition (%)				
		Tanga	Dar	Nungwi	Kilwa	Mtwara
Carcharhiniformes		**76.0**	**83.2**	**63.2**	**75.4**	**55.5**
Carcharhinus altimus	Bignose shark			5.3		
Carcharhinus falciformis	Silky shark			15.8		
Carcharhinus longimanus	Oceanic whitetip shark					11.1
Carcharhinus melanopterus	Blacktip reef shark	3.5				
Carcharhinus plumbeus	Sandbar shark			5.3		
Carcharhinus sorrah	Spottail shark	3.5	8.3	10.5	30.0	
Galeocerdo cuvier	Tiger shark			10.5	6.0	
Loxodon macrorhinus	Sliteye shark				6.1	
Rhizoprionodon acutus	Milk shark		16.6			
Triaenodon obesus	Whitetip reef shark	65.5			3.0	
Hemipristis elongata	Snaggletooth sharks				3.0	
Sphyrna lewini	Scalloped hammerhead		16.7	10.5	27.3	11.1
Sphyrna mokarran	Great hammerhead		25.0			
Sphyrna zygaena	Smooth hammerhead	3.5	8.3	5.3		
Mustelus asterias	Starry smooth-hound		8.3			33.3
Lamniformes		**0.0**	**0.0**	**31.6**	**0.0**	**33.3**
Alopias pelagicus	Pelagic thresher			5.3		
Isurus oxyrinchus	Shortfin mako shark			26.3		33.3
Myliobatiformes		**0.0**	**0.0**	**5.3**	**0.0**	**0.0**
Maculabatis gerrardi	Whitespotted whipray			5.3		
Orectolobiformes		**3.5**	**0.0**	**0.0**	**0.0**	**0.0**
Stegostoma tigrinum	Zebra shark	3.5				
Rhinopristiformes		**20.7**	**16.7**	**0.0**	**24.2**	**11.1**
Acroteriobatus variegatus	Stripenose guitarfish	6.9				
Rhina ancylostomus	Bowmouth guitarfish	3.5				
Rhinobatos annandalei	Bengal guitarfish	3.5				
Rhynchobatus australiae	Bottlenose wedgefish	6.9	16.7		24.2	11.1

serum albumin. Each reaction was initially denatured at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min. The final extension of 72 °C for 10 min was added to ensure complete elongation. The quality of each PCR product was checked on a 1 % agarose gel. The successful PCR amplicons were sanger sequenced by the Macrogen Europe Lab in the ABI 3730XL automated sequencer (Applied Bio systems, Foster City, USA) using the primer FishF1. The obtained sequence for each sample was trimmed and translated into amino acid sequences using the vertebrate mitochondrial genetic code routine in the software MEGA ver. 11 (Tamura *et al.*, 2021), in order to identify and remove nuclear pseudogenes and sequencing artifacts from the dataset (Rumisha *et al.*, 2018; 2023). The taxonomic identity of each elasmobranch was revealed by comparing each edited COI sequence with the COI barcode records published in the NCBI GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST).

Results

Composition of landed and traded species

A total of 23 different shark and ray species, representing five orders and nine families, were identified from the sampled tissues, with percentage identities ranging from 97.7 to 100 % (Table 1). Similarly, the expected value for all sequences analysed was 0, while the bit score and query coverage ranged from 665 to 1162 and 97 to 100 %, respectively. Identifications derived from DNA barcoding were concordant with those resulting from morphological analysis, whenever the latter was possible.

The Carcharhiniformes were the most common, accounting for 15 (65 %) of the identified species, while

Table 3. Conservation status of elasmobranch species traded in Tanzanian fish markets between 2020 and 2022. Threat categories for IUCN: VU – Vulnerable, NT – Near Threatened, EN – Endangered, CR – Critically Endangered. CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora, CMS - Convention on the Conservation of Migratory Species of Wild Animals.

Species	Common name	Proportion in the traded samples (%)	IUCN Red List	CITES listed	CMS listed
Carcharhiniformes		72.5			
Carcharhinus altimus	Bignose shark	1	NT	Not listed	
Carcharhinus falciformis	Silky shark	2.9	VU	Appendix II	Appendix II
Carcharhinus longimanus	Oceanic whitetip shark	1	CR	Appendix II	Appendix I
Carcharhinus melanopterus	Blacktip reef shark	1	VU	Not listed	
Carcharhinus plumbeus	Sandbar shark	1	EN	Not listed	
Carcharhinus sorrah	Spottail shark	13.7	NT	Not listed	
Galeocerdo cuvier	Tiger shark	3.9	NT	Not listed	
Loxodon macrorhinus	Sliteye shark	2	NT	Not listed	
Rhizoprionodon acutus	Milk shark	2	VU	Not listed	
Triaenodon obesus	Whitetip reef shark	19.6	VU	Not listed	
Hemipristis elongata	Snaggletooth sharks	1	VU	Not listed	
Sphyrna lewini	Scalloped hammerhead	13.7	CR	Appendix II	Appendix II
Sphyrna mokarran	Great hammerhead	2.9	CR	Appendix II	Appendix II
Sphyrna zygaena	Smooth hammerhead	2.9	VU	Appendix II	Appendix II
Mustelus asterias	Starry smoothhound	3.9	NT	Not listed	
Lamniformes		8.8			
Alopias pelagicus	Pelagic thresher	1	EN	Appendix II	Appendix II
Isurus oxyrinchus	Shortfin mako shark	7.8	EN	Appendix II	Appendix II
Myliobatiformes		1			
Maculabatis gerrardi	Whitespotted whipray	1	EN	Not listed	
Orectolobiformes		1			
Stegostoma tigrinum	Zebra shark	1	EN	Not listed	
Rhinopristiformes		16.7			
Acroteriobatus variegatus	Stripenose guitarfish	2	CR	Appendix II	
Rhina ancylostomus	Bowmouth guitarfish	1	CR	Appendix II	
Rhinobatos annandalei	Bengal guitarfish	1	CR	Appendix II	
Rhynchobatus australiae	Bottlenose wedgefish	12.7	CR	Appendix II	Appendix II

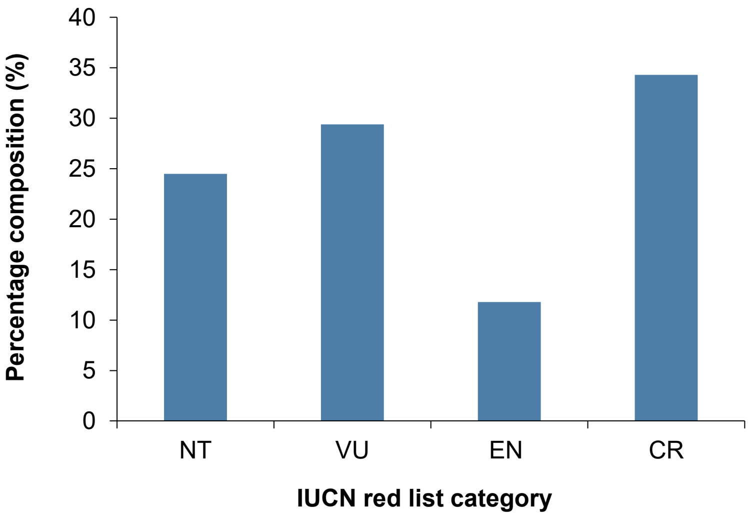

Figure 2. Percentage of threatened species in elasmobranchs traded in Tanzanian fish markets between 2020 and 2022. NT, Near Threatened; VU, Vulnerable; EN, Endangered; CR, Critically Endangered.

the Rhinopristiformes and Lamniformes were represented by four and two species, respectively (Table 1). Eight of the identified species are being reported for the study area for the first time. These include the bignose shark *Carcharhinus altimus,* the oceanic whitetip shark *Carcharhinus longimanus,* the smooth hammerhead *Sphyrna zygaena,* the starry smooth-hound *Mustelus asterias,* the pelagic thresher *Alopias pelagicus,* the whitespotted whipray *Maculabatis gerrardi,* the stripenose guitarfish *Acroteriobatus variegatus,* and the Bengal guitarfish *Rhinobatos annandalei*. Despite differences among landing sites, the spottail shark (*Carcharhinus sorrah*), the bottlenose wedge fish (*Rhynchobatus australiae*), the scalloped hammerhead (*Sphyrna lewini*) and the whitetip reef shark (*Triaenodon obesus)* were the most commonly landed and traded species in the study area (Tables 1 and 2). The whitetip reef shark (*Triaenodon obesus)* dominated the catch in Tanga, accounting for about 65.5 % of all the landed elasmobranch at the site. On the other hand, the great hammerhead shark (*Sphyrna mokarran)* dominated the catch in Dar es Salaam, accounting for 25 % of the total samples collected from the site. Conversely, the short fin mako shark (*Isurus oxyrinchus*) dominated the catch in Nungwi, while the spottail shark (*Carcharhinus sorrah)* dominated the catch in Kilwa. The catch in Mtwara was dominated by the shortfin mako shark (*Isurus oxyrinchus*) and starry smooth-hound (*Mustelus asterias)*.

Conservation status

It was observed that 78 % of the elasmobranch species identified at landing sites and fish markets in Tanzania are threatened with extinction, while about 22 % of them are near threatened (Table 3). Of those, 52.1 % are either endangered (EN) or critically endangered (CR), and accounted for approximately 46.1 % of the total catch (Fig. 2 and Table 3). Furthermore, it was revealed that about 47.8 % of the landed and traded elasmobranch species in the country are listed in CITES Appendix II, implying that they should not be exported outside the country without a CITES permit. Thresher sharks, which are protected under IOTC Resolution 12/09, great hammerhead sharks, and other elasmobranchs, which are protected under the Tanzania Fisheries Regulation 13 (11) and 67 (2) of 2009, were detected in specimens collected from fish markets.

Discussion

This study revealed 23 different elasmobranch species among the fish landed and traded in Tanzanian fish markets (Table 1). The observed number of species is lower than the number previously reported in South Africa (Fennessy, 1994) and Zanzibar (Shehe and Jiddawi, 2002) but it is higher than the number previously reported in Mozambique (O'Connor and Cullain, 2021). Because all of the aforementioned studies were conducted in the WIO, differences in the reported number of species may be attributed to the region's diverse marine ecosystems and the fact that management approaches in each country differ. However, because all of the aforementioned studies relied solely on morphological identification, which can occasionally fail to disentangle cryptic species and species with similar morphologies, the variations in the reported number of species may also be the result of different identification techniques used. Of the 23 identified species, the Carcharhiniformes, particularly the spottail shark (*Carcharhinus sorrah*), the scalloped hammerhead (*Sphyrna lewini*) and the whitetip reef shark (*Triaenodon obesus*) were the most commonly landed and traded. High abundance of the Carcharhiniformes was also previously reported in Zanzibar (Shehe and Jiddawi, 2002; Schaeffer, 2004) and in other parts of the WIO (Fennessy, 1994). Furthermore, Rhinopristiformes, particularly the bottlenose wedgefishes (*Rhynchobatus australiae*), which are common in the WIO (Temple, 2018), were also among the most commonly landed and traded elasmobranch species in the country. The results obtained per site showed that Tanga is dominated by the whitetip reef shark (*Triaenodon obesus),* Dar es Salaam by the great hammerhead shark (*Sphyrna mokkaran),* Nungwi by the short fin mako shark (*Isurus oxyrinchus*), Kilwa by the spottail shark (*Carcharhinus sorrah)* and Mtwara by the short fin mako shark (*Isurus oxyrinchus*) and the starry smooth-hound (*Mustelus asterias*). This shows that the distribution of elasmobranch species in Tanzanian waters is not uniform and that some species may be better adapted to specific coastal habitats.

The findings of this study revealed that 18 of the 23 identified elasmobranch species (78 %) from landing sites and fish markets in Tanzania are classified as threatened by the IUCN (VU, EN or CR, Table 3). The fact that EN and CR species accounted for 46.1 % of the total elasmobranch catch shows that despite varying degrees of protection, elasmobranchs that are in danger of going extinct are still caught in large numbers and supplied to fish markets. These results are comparable to previous studies which reported that 58 % and 93 % of the traded elasmobranch products in Bangladesh and Southeast Asia are categorized as threatened by the IUCN (Sembiring *et al.*, 2015; Haque *et al.*, 2019). Three of the 12 EN and CR elasmobranchs caught are legally protected and specifically mentioned in Tanzanian laws: the great hammerhead (*Sphyrna mokarran*), which is protected by Tanzania Fisheries (Amendment) Regulation 67 (2) of 2009, and the oceanic whitetip shark (*Carcharhinus longimanus*) and pelagic thresher (*Alopias pelagicus*), which are both protected by Tanzania Deep Sea Fisheries Management and Development regulation 8 of 2021. The fact that the great hammerhead was previously reported in the catch (Shehe and Jiddawi, 2002) and that it accounted for approximately 2.9 % of the total elasmobranchs traded in Tanzanian fish markets between 2020 and 2022 (Table 3), shows that fisheries regulations are not strictly enforced. The presence of the oceanic whitetip shark (*Carcharhinus longimanus*) and pelagic thresher (*Alopias pelagicus*) in the catch, despite the fact that this is the first time they have been identified in the catch, provides additional evidence for a lack of law enforcement. Limited enforcement has been identified as one of the major reasons why illegal trade in protected elasmobranchs continues to thrive in many parts of the world (Haque *et al.*, 2019). However, because Fisheries Officers were present at every sampled fish market and were regularly inspecting the landed and traded elasmobranchs, it is unclear why they are not enforcing the existing regulations. Therefore, if the shark fishery is to thrive, the main reasons why fisheries officers do not enforce the existing regulations should be identified and addressed.

Results of the present study also suggest that Tanzania's fisheries regulations should be revised. This is crucial because nine of the 12 EN and CR elasmobranchs are neither listed in the Third Schedule of the Tanzania Fisheries Regulations of 2009, nor are they mentioned in the Deep Sea Fisheries Management and Development Regulations of 2021, implying that they are not protected by these regulations. The fact that CR species such as the scalloped hammerhead shark (*Sphyrna lewini)* and the bottlenose wedgefish (*Rhynchobatus australiae)* were among the most traded species is alarming and calls for immediate measures to protect threatened elasmobranch in Tanzanian waters. The present study is concordant with that of Van Beuningen (2020), which showed that 50 % of traded elasmobranchs on the Tanzanian island of Pemba are threatened with extinction, implying that either existing regulations are not enforced or the regulations themselves do not adequately protect threatened elasmobranchs. Because many of the EN and CR elasmobranch species identified from specimens collected in Tanzanian fish markets are not listed in the Third Schedule of the Tanzania Fisheries regulations of 2009, it is advised that the Third Schedule be updated to include these species.

This study also revealed that among the 23 identified elasmobranch species in Tanzanian fish markets, 11 species (47.8 %) are listed in CITES appendix II. These findings are comparable to those of a previous study, which reported 14 CITES protected elasmobranch species in fish markets and landing sites in the Tanzanian island of Pemba (Van Beuningen, 2020). Because most of the fish exports come from the sampled fish markets, there is a chance that these CITES-listed elasmobranchs are exported outside the country in contravention to CITES regulations. Therefore, there is a need to strengthen enforcement of current fisheries regulations to ensure that protected elasmobranch species do not enter fish markets and are not exported outside the country without permits.

Conclusions

Many WIO countries including Tanzania have established legislation to protect threatened elasmobranch species (Tanzania Fisheries (Amendment) Regulation, 2009; Kenya Fisheries Management and Development Act No. 35, 2016; Mozambique Marine Fisheries' Regulation (REPMAR), 2020). However, the level of protection varies across the region, with countries such as Mozambique prohibiting threatened mobulids, thresher sharks, whale sharks, basking sharks, great white sharks, and oceanic whitetip sharks, while others have prohibited only a few of the aforementioned species and other species that may not be protected in Mozambique. Because species protected by Tanzanian laws, such as great hammerhead (*Sphyrna mokarran*), oceanic whitetip shark (*Carcharhinus longimanus*), and pelagic thresher (*Alopias pelagicus*) were detected in fish markets inspected on a regular basis by fisheries officers, the main reason why fisheries officers do not enforce existing regulations should be identified and addressed. Additionally, because nine of the EN and CR species traded in Tanzanian fish markets (Table 3) are neither listed in the Third Schedule of the Tanzania Fisheries Regulations of 2009 nor mentioned in the Deep Sea Fisheries Regulations of 2021, it is advised that the regulations be urgently updated to include these species and other EN and CR elasmobranchs on the IUCN Red List. The fact that the CR scalloped hammerhead shark (*Sphyrna lewini*) and the CR bottlenose wedgefish (*Rhynchobatus australiae*) were among the most traded elasmobranchs in Tanzanian fish markets, indicates that they are in grave danger of extinction and should be protected immediately. Furthermore, because all identified elasmobranch species are either threatened or near threatened, it is recommended that steps be taken to promote sustainable fishing in Tanzanian waters and that a national plan of action for elasmobranch conservation be developed to halt exploitation of threatened elasmobranchs. Additionally, the country should implement multilateral agreements to which it is a party, particularly trade controls in CITES listed elasmobranchs and an embargo on shark finning (IOTC resolution 17/05). Lastly, a regional plan of action for elasmobranch conservation should be developed to ensure that WIO countries with similar elasmobranch stocks collaborate on a shared management strategy.

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Original Article

Billfish in coastal visual art in Kenya: a resource for ocean science communication

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Abstract

Approaches to marine conservation and management issues has often been limited to using scientific research to propose technical and policy interventions. However, communicating outputs as scientific publications and policy briefs has limited reach and impact for non-academic audiences. Art markets in Mombasa and Kilifi Counties in Kenya were sampled to determine how the creative arts can be used to communicate ocean science. Artwork with terrestrial themes was popular and increasingly available to buyers compared to ocean art. Billfish as a representative species was popular in ocean art culture, underscoring its potential in a niche market for ocean art. Our study highlights opportunities for active collaboration between scientists and artists in communicating messages from scientific work to non-academic audiences. Engagement of Western Indian Ocean countries in the UN Decade of Ocean Science for Sustainable Development will benefit from the use of ocean art in science communication and enhance ocean literacy in the region.

Keywords: ocean art, billfish, ocean literacy, science communication

Introduction

The advent of the United Nations Decade of Ocean Science (2021-2030) has sparked global interest and an increased focus on the development of innovative solutions to pressing ocean challenges. Actions within the Ocean Decade aim to address human-induced impacts affecting the world's oceans such as climate change, ocean acidification, coral bleaching, and overfishing (Claudet *et al.*, 2020). The successful implementation of these anticipated actions requires collective action and uptake across a diverse non-scientific stakeholder spectrum which ranges from local coastal communities to policy makers (Jefferson *et al.*, 2021). Incorporating these multiple stakeholders to promote Ocean Decade priorities and targets requires added emphasis on fostering effective communication to ensure increased knowledge sharing for decision making, behaviour change, and sustainable ocean resource use (Paterson *et al.*, 2020).

In Kenya, the focus on SDG 14 has been amplified by the increasing attention on achieving a sustainable blue economy. Several towns and counties along Kenya's coastline are heavily reliant on the productivity and health of the ocean and are significant contributors to the nation's economy through revenue generating industries such as fisheries, maritime shipping, and tourism (Rasowo *et al.*, 2020; Muigua, 2018). Sustainably leveraging these marine-based economic sectors to advance Kenya's Blue Economy requires integrating science-based measures that utilise inclusive societal approaches to

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ocean conservation (Okafor-Yarwood, *et al.*, 2020). The authors recognize that effective uptake of science-driven information for sustainable development agendas by the public and private sector calls for effective knowledge sharing and dissemination of scientific findings to the users of marine and coastal resources.

Current forms of communicating science using scientific publications, policy briefs and contemporary media have a limited reach and low impact on the behaviours of non-academic and non-scientific audiences (Guest *et al.*, 2015; Wilson *et al.*, 2016). This gap in communicating science to non-scientific communities can create obstructions in collaborative decision-making processes closely associated with ocean governance (Kopke *et al.*, 2019). Science communication approaches can be innovatively and contextually curated to address specific outcomes such as enhancing stakeholder engagement in decision making for coastal resource management (Cooke *et al.*, 2017; Garcia and Cater, 2022). Strengthening stakeholder engagement in marine conservation efforts has been linked to communication strategies which emphasise cultural connections and human values (Cvitanovic *et al.*, 2015; Chambers *et al.*, 2019). One such avenue that is intrinsically interconnected with these ideals is the creative arts sector. Art can uniquely and expansively communicate and connect scientific findings to human values and emotions therefore motivating behavioural change, supporting evidence-based science and enhancing capacities for tackling challenges associated with coastal and marine resource use (Worm *et al.*, 2021; Santoro *et al.*, 2017; Kendall-Bar *et al.*, 2021).

Successful efforts in utilising visual art for various environmental awareness campaigns over the years reveal the capacity the arts hold as a tool for enhancing public involvement with conservation actions (Hicks and King, 2007). This success is attributed to the nature of art itself; its ability to trigger emotive and intuitive responses in the human mind (Hicks and King, 2007; Lesen *et al.*, 2016; Savoie, 2022). Ocean literacy, which emphasises the necessity for generating awareness and understanding of oceanic ecosystems,

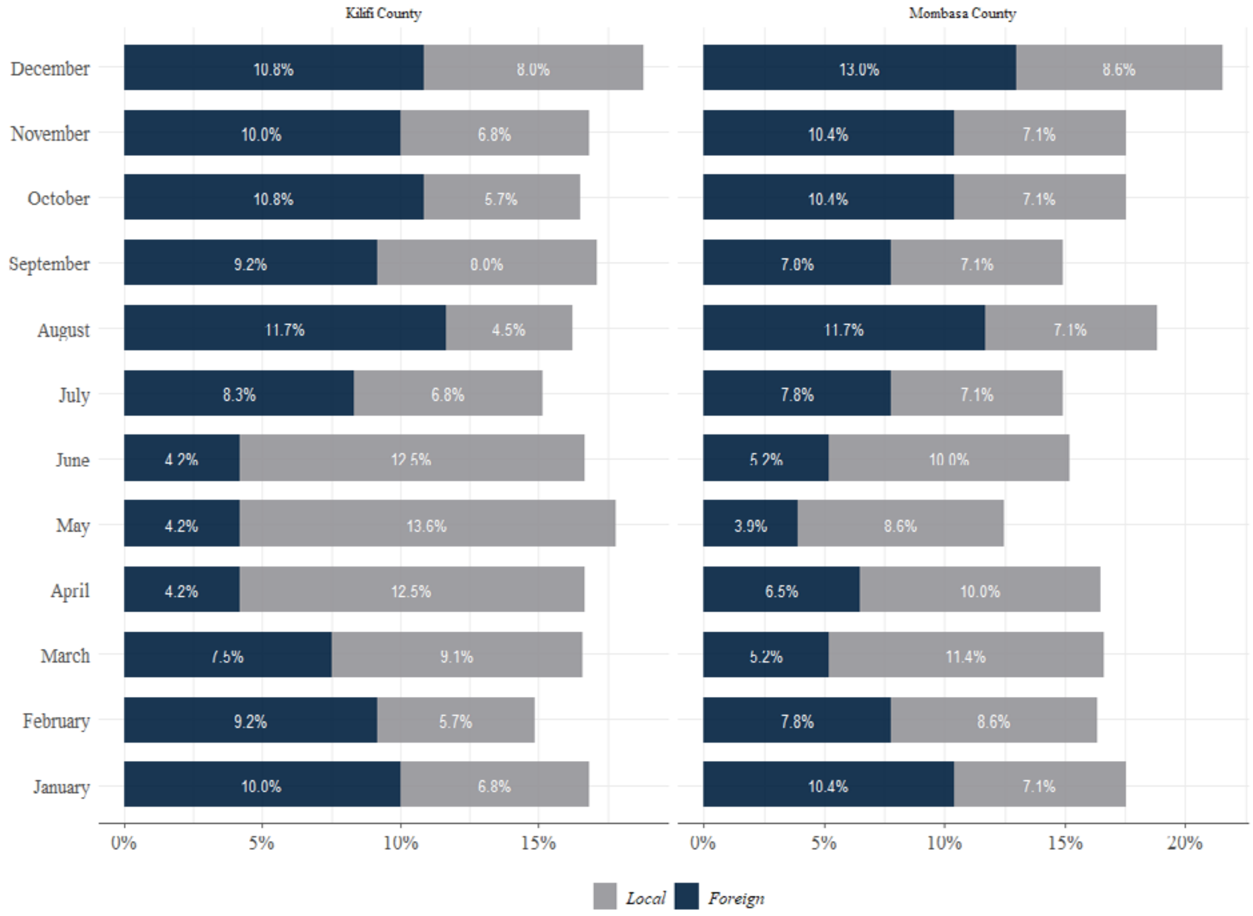

Figure 1. Artist description of local and foreign buyers of artwork per month (pre-COVID-19).

has also been coupled with arts-based approaches in advancing the accessibility of ocean knowledge to the public (Worm *et al.,* 2021).

In Kenya, the coastal visual art sector has operated since the colonial era and is an industry that is still largely informal with historical linkages to the tourism industry (Kasfir, 2004; Mahoney, 2012). Kenyan coastal visual artists are based in towns and cities adjacent to the ocean and are well situated in easy-to-access locations that can be used to convey ocean-based messaging. Nonetheless, the scope of Kenya's coastal visual artists and the avenues for their inclusion into ocean science communication remains unexplored.

This pilot study was developed to examine the role coastal visual artists in Kenya play in fostering the understanding of the ocean and marine life through their art. The study sought to enhance the understanding of (1) the demographics of the coastal artist population, (2) their level of awareness of ecological and ocean matters, and (3) possibilities of the integration of their art into ocean science communication.

The study also attempted to further understand how locally landed fish species like billfish are understood and represented by coastal visual artists. Specifically, the drivers were explored behind art production with a focus on billfish, a renowned migratory fish species especially in sport and recreational fisheries that is landed along Kenya's coast (Kadagi *et al.*, 2011). Due to their legendary charismatic nature, agility and speed, billfish have a history of strongly influencing ocean cultures across the globe (Ulanski, 2013). The marlin, a billfish species, features prominently in Ernest Hemingway's famous allegory of the *Old Man and the Sea,* which revolves around strong connections between a fisherman, a billfish, and relationships with nature through the sea (Alexis *et al.,* 2014). Previous studies linked to assessing pro-environmental behaviour have utilised a flagship species that fits in local contexts as avenues to engage with surrounding communities for conservation efforts (Bowen-Jones and Entwistle, 2002; Senzaki *et al.,* 2017). Thus, this study used familiarity with billfish as a topical entry point to investigate the perceptions of environmental and ocean stewardship for visual artists in Kenya. The choice of billfish reflects the use of a charismatic marine fisheries species in ocean art which has the potential to transform ocean literacy, a critical component for the Ocean Decade and promoting sustainable blue economy both in Kenya and the Western Indian Ocean (WIO).

Materials and methods

Site selection

This study focused on Mombasa and Kilifi Counties which are situated along the Kenyan coast (Fig. 2). Within the two counties, the specific study sites were public locations where there were popular art markets and artists present. In Mombasa County, the artist survey covered the Old Town area, Akamba handicraft market, Bombolulu workshop, art vendors outside Fort Jesus, Jomo Kenyatta (Pirates) and Voyager public beaches, Swahili Pot, Swahili Cultural Centre and Kibarani. In Kilifi County, artists were surveyed along Watamu Beach, the Gede-Watamu roadside, in Malindi art market and Kilifi town.

Methods

The artist survey was undertaken from 24th May to 16th June 2021 in the counties of Mombasa and Kilifi, Kenya. The specific study sites were locations where there were artists and art markets. A mixed methods approach was used and obtained (1) quantitative data from questionnaires, and (2) qualitative data and insights from focus group discussions.

To gain an introductory understanding of the dynamics of the Kenyan coastal visual artist population and sector, a questionnaire was used as the primary survey tool to guide inquiry into the area of interest (Milena *et al.*, 2008; Showkat and Parveen, 2017; Kabir, 2016). The surveys were carried out with the artists at their respective point of sale and the questionnaire was sequenced to understand the artists demographics; the content of art that artists had on display; the artists' familiarity with billfish and their ocean awareness; and the drivers of their art production (Glithero and Zandvliet, 2021). The coastal visual artist population in Kenya has not been the primary focus of prior research and as such, a defined sample population is not yet known. For this reason, this study employed the purposive sampling method whereby respondents were selected based on the artist information available (Neetij and Thapa, 2015).

The study was complemented by a series of focus group discussions (FGDs) which were held with the same artists whom were engaged in the questionnaire data collection. The guiding questions in the FGDs were thematically categorised as: interactions with the ocean, awareness of human impact on the ocean, current ocean knowledge, and ocean art market information. The purpose of the FGDs was to interrogate social and cultural issues that may not have been captured in the questionnaire.

Data collection and analysis

Data from administering the questionnaire was collected digitally via Google Forms on the researchers' phones or on the artists' personal phones. For areas where internet connectivity was limited, printed copies of the questionnaire were filled by the artists and later keyed into Google Forms by the researchers. Data from the questionnaires was analysed using R software (version 4.1.3). Responses to the themed questions in the FGDs were sequentially recorded with respondent's consent, noted down according to main statements made, and keyed into an Excel spreadsheet. These data were then coded to extract categories for each specific question. This was followed by refining these categories into overarching themes that highlighted additional information.

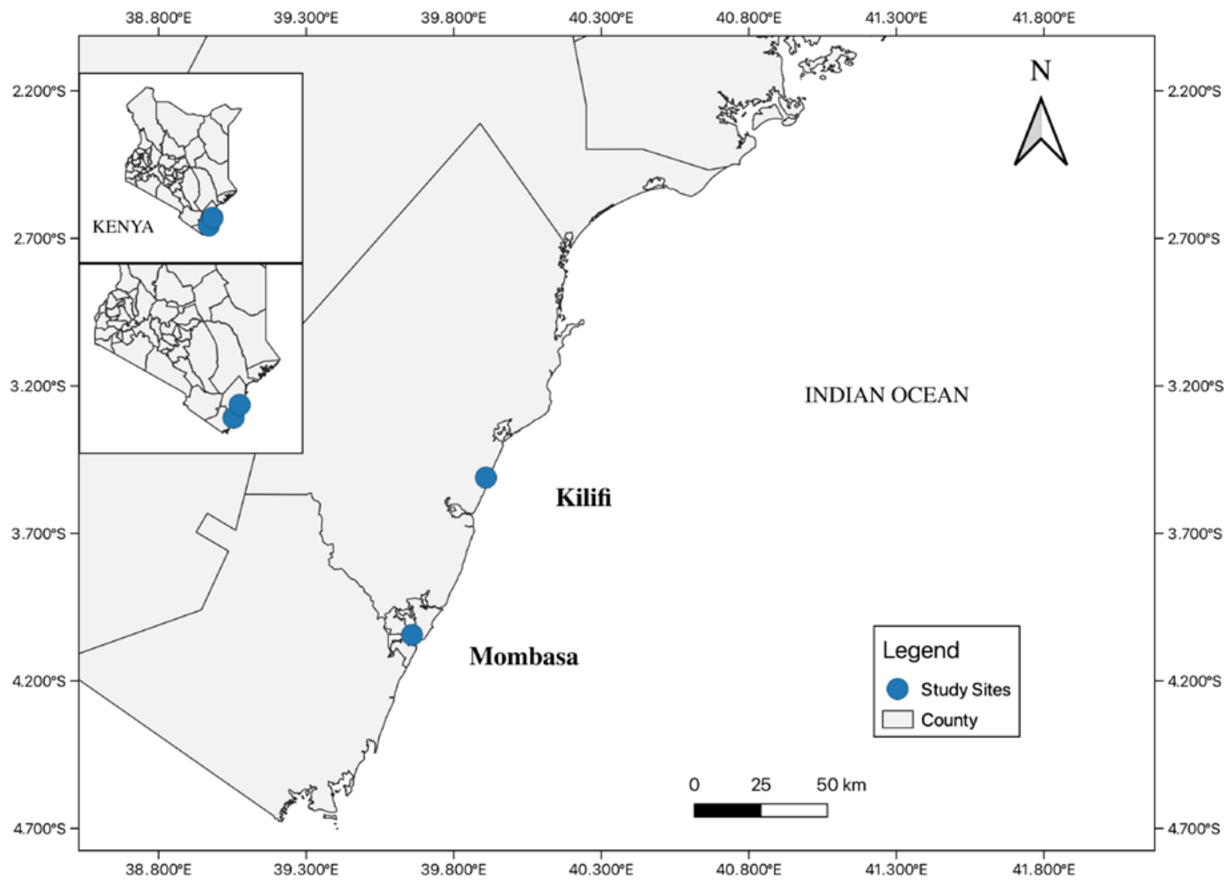

Figure 2. Map of Kenya alongside a close-up of the study sites in Kilifi and Mombasa Counties.

Results

Demographics of the art community

A total of 29 artists were surveyed in the two counties of Mombasa (14) and Kilifi (15). The study was conducted during the COVID-19 pandemic period which limited the number of artists that could be accessed. A total of five FGDs were held.

In Mombasa County, a majority of the artists were male and with an average age of 45 years old. In Kilifi, male artists were a majority and were younger than their Mombasa counterparts with an average age of 34 years. In both counties, the female artists were notably fewer and made up approximately 10 % of the artists identified and interviewed in the study.

Less than 25 % of the respondents in both Mombasa and Kilifi Counties had received formal art education. One female participant in Mombasa County had prior art training accounting for 8.3 % of the total number of participants while the majority of the artists (83.3 %) who were male had no formal art training. Two male artists in Kilifi counties had received art education accounting to 14.3 % whereas the majority (78.6 %) of the artists who were male, and one female artist (7.1 %) did not have any formal art training. The artists who did not receive formal training explained that they had developed their artistic skills through observation, apprenticeship, and individual creative expression.

Twenty eight out of 29 of the artists encountered highlighted their dependency on the art industry for their livelihood. A majority of them in both Kilifi (86.7 %) and Mombasa (92.3 %) noted that the art sector was their primary occupation. More than half of the respondents completely depended on art as their source of livelihood (60.7 %) while 21.4 % were very reliant on art income, whereas only 17.9 % moderately relied on art as their source of income.

Content of art on display

Twenty-eight of the artists interviewed provided an affirmative response to the production of nature-based art. When specifically asked about the visual content of the art they made, more than half of the artists revealed that they produced both land and ocean themed artwork. In Mombasa, eight out of 13 (61.5 %) artists indicated that they produce art with both land and ocean themes. Only four (30.8 %) of the Mombasa artists noted that they produced purely land based art and only one (7.7 %) of them solely focused on oceanic features. In Kilifi, most (93.3 %) of the artists reported to depict both land and ocean features in their art while only one artist (6.7 %) primarily portrayed only oceanic themes in his artwork.

Familiarity with billfish and ocean awareness of artists

In Mombasa County, 14.3% of the artists were extremely familiar with billfish and 28.6 % were slightly familiar. In Kilifi County, 41.7% of the artists were extremely familiar with billfish and 33.3 % were slightly familiar with this group of fish.

When asked about their awareness of the ocean and its systems, 53.8 % of the Mombasa artists and 20 % of the Kilifi artists reported that they were very aware. Most of the Kilifi artists (40 %) cited that they were slightly aware. In terms of awareness of their counterparts (other artists), the respondents in both counties indicated a slight awareness. In Mombasa County, the artists believe that 38.5 % of the total artist population was slightly aware of the ecosystems while in Kilifi County, the level of awareness of other artists was cited to be 47 %.

Drivers of art production

The majority of the artists from both Kilifi and Mombasa counties reiterated that their production of art is primarily driven by market demands and the opportunity to make sales. 86.7 % of the artists in Kilifi County and 64.7 % of the Mombasa County based artists reported that their main buyers were foreigners/international tourists. Local buyers were fewer; 35.3 % in Mombasa and 13.3 % in Kilifi. All the artists interviewed noted that their sales were affected by the COVID-19 pandemic.

Additionally, more than 60 % of the artists interviewed in both Kilifi and Mombasa counties reported that their nature-based artwork portraying terrestrial features were more popular with buyers compared to ocean-based art. Kilifi County recorded the highest foreign demand for artwork in the month of August, followed by the months of December and October respectively, while the lowest demand was in the months of April, May, and June. The highest local demand in Kilifi was in the month of May followed by the months of April and June respectively, with the lowest demand in the months of August, October, and February. The highest local demand for artwork in Mombasa County was in the month of March, followed by the months of April and June respectively, with the lowest demand recorded in January and July through November. The highest foreign demand for arts was in the month of December followed by August with the lowest demand recorded in the month of May (Fig.1).

Focus Group Discussions

The FGDs were focused on thematic aspects which included the interactions of the artists with the ocean, the artists' awareness of human impact on the ocean, the artists' current ocean knowledge, and additional information the artists considered significant about the ocean art market (Table S1).

The artists indicated that they interacted relatively often with the ocean through various activities such as fishing, selling art on the beach, and collection of material such as driftwood for art pieces from the beach. They also demonstrated an awareness of anthropogenic activities on the ocean by highlighting challenges such as plastic and chemical pollution, illegal fishing, and climate change, among others. Regarding the depiction of these trends of human activity in their artwork, some noted that the use of recycled material and subtle colour schemes captured some of these themes.

The artists also shared that the sizes of fish that are part of their art pieces are determined by the actual sizes of the fish they encounter. Billfish was the focal species to determine ocean knowledge for this study, and when asked about billfish, some artists noted that billfish are iconic for their large sizes, have been consumed for food, and that they have interacted with sport fishers who commissioned them to produce billfish sculptures.

Further information from the FGDs highlighted that ocean art does not sell as fast as other themes, as evidenced by one artist who had a marine piece in Malindi on display for over a year. Aside from market demand, the artists in both counties during the FGDs indicated that watching oceanic films, admiring aquatic life, having experiences around the marine realm, and wanting to create awareness were other motivations for creating ocean art.

Most of the artists in the FGDs in Mombasa were aware of challenges affecting the ocean such as pollution, climate change, and oil spills and felt that this may be linked to the urban nature and higher population of Mombasa County.

The artists explained that they would be motivated to create more ocean-based art if they had more knowledge and understanding of it as well as having buyers for the ocean art. They expressed interest in ocean art but noted that one of the main challenges in its production is fewer sales of ocean art in comparison to terrestrial-themed art.

The few female respondents highlighted that they face challenges as artists which include the outdoor nature of the art production process in locations without sanitary amenities and responsibilities that come with gender roles in the family context.

The artists explained that the majority of them began seeking other livelihood generating business streams during the COVID – 19 pandemic due to the drop in foreign buyers who they attributed to be the main driver of the art industry. In the FGDs, the artists reiterated that low tourism numbers during the pandemic greatly affected their sales. They further explained that the increase in domestic tourism during the pandemic resulted in a notable increase of local art buyers.

During the FGD with the artists in Watamu, the artists expressed that they would like to find out how to unite as artists and work as a group to access better opportunities and have better resource management. The artists in Kilifi town were organised into a Community Based Organisation (CBO). During one of the FGDs in Mombasa, a group of artists explained that although they had run a vibrant group, membership and activities significantly declined during the COVID-19 pandemic.

Discussion

This first-time pilot study aimed at exploring the potential roles that coastal visual artists could play in ocean science communication. This was done through gaining a baseline understanding of the coastal visual art industry characteristics and drivers in two coastal counties in Kenya. The findings from this inquiry provide a starting point to map out possible collaborative pathways in the creation of science communication outputs between ocean scientists and coastal artists. Owing to the charismatic nature of billfish species and their significance as a food source, this study used familiarity with the species as a topical focal point to investigate the perceptions of environmental and ocean stewardship for visual artists in Kenya.

These findings based on the artists encountered convey that the visual art industry is dominated by males and that apprenticeship is the main method of growth in the industry particularly in the recruitment of youth into the sector. It is envisioned that the use of artwork for science communication would present an opportunity for the inclusion of more youth across genders through formal training workshops which can be matched with apprenticeship from older artists. It is also noted that the inclusion of more female artists in this sector may be made possible through the creation of more conducive operating environments that include proper sanitary amenities to support women working in beach-based art studios.

The majority of the artists encountered were engaged in art production as their primary source of livelihood. The artists' awareness of the ocean and marine ecosystems was present, however art is a business, and like any other business, the artists produce the type of art that sells. They placed emphasis on market demands as the determinant for the type and content of art they produce, and they therefore focussed on the production of terrestrial art since it sells. Responses given by some of the artists revealed that the active creation of demand for ocean art would contribute to the promotion of the production of ocean-centric art. This is considered to be an opportunity for the creation of market demand by the marine scientific community through the incorporation of the creative arts in campaigns and workshops to disseminate scientific findings to a non-scientific and non-academic audience. This would be an effective method in promoting effective ocean science communication whilst improving the livelihoods of coastal artists (Lesen *et al.*, 2016).

The increase in domestic tourism during the COVID-19 pandemic resulted in a notable increase of local art buyers suggesting that there is a growing local art market within the country that is purchasing nature-based artwork. This market can be aligned to the purchase of ocean art through appropriate messaging and visibility of this type of artwork.

In terrestrial art, elephants and other animals are seen as representatives of the living organisms of the terrestrial environment. For the ocean, billfish were selected as a representative of an organism living in the ocean and it was found that the artists encountered in this study had a clear awareness of billfish, ocean ecosystems, and the environmental challenges affecting ocean health. Several artists were also fishers, and their knowledge came from individual experiences with the ocean and observations they had made. Herein lies an opportunity to tap into the observations made by artists who are fishers to show changes in the ocean and creatures that they encounter while at sea. This awareness and knowledge sharing of ocean resources may also be used to propel the FAO -Blue Growth Initiative (BGI) for sustainably developing fisheries and aquaculture.

In Kenya, Kilifi County is one of the major billfish fishing zones contributing to the country's prominence in big game fishing dating back to the 1950s (Kadagi *et al.*, 2020). The localization of sport fishing in Kilifi contributes to the higher familiarity with billfish among the artists in Kilifi compared to Mombasa. The artists in Kilifi County have captured the unique opportunity of creating fibreglass sculpture models from the catches made by sport fishers thus enhancing their livelihoods.

Kelly *et al.* (2018) rightly observed that marine scientists should not assume that the non-academic community share their concerns about marine environments and threats that they face. They further indicate that the scientific method of research communication through scientific papers is ineffective as the readability of papers has declined (Kelly *et al.*, 2018). Dupont *et al.* (2017) indicate that the scientific process does not end until the science is communicated and that scientists need to become effective communicators by using simple messages, and narrative stories that can motivate environmental actions. The appreciation of nature has been long acknowledged to be understood by children, as highlighted in Woodsworth's Ode to Immortality (1820), and by artists; thus art is a medium in which science may be perfectly explained and understood (van Boeckel, 2013).

The findings of the current study provide opportunities for art to be used as a creative outlet for science communication. The ability of the arts to evoke emotions and propel self-reflection as well as the will to take actions (Dupont *et al.*, 2017) forms a key consideration for this linkage of creative arts to science. This was noted in a survey conducted for public schools in Canada where the knowledge and value of the ocean was predetermined by the exposure to ocean related activities and hence the interest in the ocean resources (Guest *et al.*, 2015).

As advocated by Chambers *et al.* (2019), the creation of a community of practice in Kenya and across the Western Indian Ocean region through the creation of a network of scientists and artists that can work together across disciplines, is here again recommended. They would need to share knowledge and expertise in a collaborative manner to create innovative messaging that can drive the creative sharing of scientific information (Garcia and Cater, 2022). As this recommendation is made, the challenges in evaluating the impact of such trans-disciplinary collaborations is recognised (Paterson *et al.*, 2020). It is also important to consider that there is a need for the use of a variety of media platforms which bring inclusivity as they allow more stakeholders to be part of the conversation (Paterson *et al.*, 2020).

Furthermore, consideration has to be given to the different expectations of the artists and scientists due to differences of mind set, training, communication styles, values and societal status (Paterson *et al.*, 2020). Despite these challenges, marine scientists in the region are urged to consider actively engaging and collaborating with coastal artists in the artistic visualisation and co-production of key messaging they would like to convey to specific audiences such as policy makers, the youth, and coastal communities (Lesen *et al.*, 2016).

Artist and ocean scientist workshop residencies are recommended approaches worth exploring for the co-designing and development of art-centred outputs for ocean science communication that connect ocean ecosystem concepts to emotive-linked art (Jung *et al.*, 2022; van Boeckel, 2013). Such collaborative engagements can further consider making the outputs accessible to local markets through avenues such as affordable pricing, and sculptures and murals in public spaces. Different age sets ranging from children to the old can also be engaged in the artistic methodologies for qualitative oceanic research. Further possibilities for multidisciplinary and collaborative engagements between cross-disciplinary artists at all levels and ocean scientists include holding ocean art workshops and competitions for students such as the World Oceans Day Art Contest in Chumbe Island, Tanzania and the development of projects such as the South African based lalelauLwandle project that created community ocean-focused art using theatre (van Bressem *et al.*, 2006).

Ongoing ocean literacy platforms within the Kenyan context surround marine thematic campaigns for learners. In addition, recent innovative approaches such as Science Cafes have been facilitated in Mombasa, Kenya, which are conversations between ocean scientists, artists and the public (Uku pers. comm.; IOC-UNESCO, 2022). It is further recommended that attention be given to the creation and adoption of programmes and curricula aimed at inculcating art appreciation and marine species science for the young through exciting ocean-themed activities such as excursions, song, dance, and storytelling. A growing focus on such engagements as a pathway to fostering artist inclusion in the science communication of the region is envisioned.

Conclusions

To propel the marine community of practice, it is important to define a shared vision, develop a clear strategy of engagement between artists and scientists as well as diverse stakeholders- which demonstrates the benefits of the collaboration and provides tools to evaluate progress in the uptake of co-designed messages (Heritage and Golfomitsou, 2016; Santoro *et al.*, 2018; Pocock *et al.*, 2019).

The findings from this study underscore the use of various forms of artistic expression to promote citizen engagement with environmental issues. The significance of marine art which could provide alternative sources of income for communities and accelerate collective actions for sustainable resource use and management is also highlighted. The integration of art and sciences has the benefits of interdisciplinary collaborations which can contribute towards making scientific knowledge accessible to the public and local communities for the creation of spaces that support visualisation of scientific issues. Finally, the consolidation of science communication efforts with coastal visual art focused on iconic marine species like billfish holds the promise of fruitful possibilities to advance easily understood messages related to the marine realm and environmental issues that will further support the Sustainable Blue Economy dialogue and policy development processes in Kenya and the WIO (Lesen *et al.*, 2016).

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Supplementary material

Figure S.1. An artist outside their shop in Kilifi County, Kenya.

Table S1. Focus Group Discussion Responses

Questions	Thematic Area	Examples of responses
1. Time spent around ocean	Interactions with the ocean	• Always, when fishing • Often
2. Reasons for being around ocean	Interactions with the ocean	• Local tourism • To collect ocean resources such as driftwood as material for art pieces • Point of sale is at the beach • Part of a fishing crew
3. Challenges facing the ocean	Awareness of human impact on ocean	• Coastal development moving onto beach taking over turtle nesting sites • Plastic pollution • Chemical pollution • Declining species of starfish • Illegal fishing • Climate change • Oil spills
4. Does your art capture trends?	Awareness of human impact on ocean	• Use of recycled and eco-friendly material such as bottles, wood, soda cans • Yes, but subtly using colours • They struggle selling themed art outside of an exhibition context • Designs do not capture trends
5. Knowledge on billfish	Current ocean knowledge	• A fish that can grow bigger than a man • Billfish has been eaten before • Sport fishers measure billfish and commission sculptured moulds made of fibreglass
6. Does the size of fish in art change?	Awareness of human impact on ocean	• They draw the size of the fish as they see them. Tuna and billfish are depicted to be big because that is their actual size. Sardines are small because that is how the artists see them • Yes, it does. Depending on the scene, location, and environment of artists
7. Additional information on ocean market	Ocean art	• There is no demand for environmentally themed art unless pieces are commissioned • Artists need awareness on what materials they are allowed to collect from the ocean for their art

Short communication

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A new report of the stenopodid shrimp *Stenopus devaneyi* Goy, 1984 from the Western Indian Ocean

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Abstract

The rare stenopodid shrimp, *Stenopus devaneyi* Goy, 1984, is reported for the first time in the Western Indian Ocean (WIO), at 60 m depth at Mayotte Island. No specimens were collected but the species is recognized confidently on photographs based on its characteristic colour pattern: two large red spots on the sides of the second abdominal somite. Selected colour photos are presented. Former Indo-West-Pacific records are listed with an updated map for the geographic distribution of this shrimp. A few ecological observations are made.

Keywords: stenopodid shrimp, *Stenopus*, Mayotte Island, Mesophotic zone

Introduction

The present observation was made during the research programme CRUMMA for the study of the biodiversity of the Crustacea observed in the mesophotic zone (50-150 m) off Mayotte Island (Barathieu, 2023). This programme has been undertaken since 2020 by the association Deep Blue Exploration (DBE) to which the authors of this work belong. CRUMMA is actively supported by the French Museum national d'Histoire naturelle, Paris (INPN, Inventaire National du Patrimoine Naturel), with a research grant obtained in 2021. Previous results include the discovery of a rare cavernicolous crab, *Atoportunus dolichopus* Takeda, 2003 (Barathieu *et al.*, 2019) and a photograph inventory of 44 species observed in the mesophotic zone (Poupin *et al.*, 2022). The description of the diving techniques and methodology used can be consulted in these previous contributions.

During a dive of the first two authors on December 31, 2022, on the east slope of Mayotte Island, southern side of S-shaped Pass, 12°52.964' S, 45°16.557' E, at a depth of 60 m, two stenopodid shrimps with red spots on the sides of the second abdominal somite were photographed (Fig. 1). These specimens were determined to be *Stenopus devaneyi* Goy, 1984 by using the colouration-based key in Saito *et al.* (2009). The following entries have been selected: 1) Body with striking colour pattern (not uniform); 2) Carapace not purple; 3) Third maxilliped, fourth and fifth pereopods not blue; 4) Pleon with spots; 6) Third pereopod not blue on proximal segment; 7) rostrum not red; 9) Antennal flagellae white; and 10) Pleon with red spots on second and third somites. In addition, the colour pattern given by Goy and Randall (1986: 89) for the redescription of the species matches that of the specimens photographed very well.

The only specimens of *Stenopus devaneyi* that were collected and deposited in Museum collections are those used for the original description: Marquesas, Nuku Hiva Island (Holotype male Bernice P. Bishop Museum, Honolulu, BPBM S8045, Paratype male BPBM S8496); and Sri Lanka (Donated by Hofmann, Senckenberg Museum, Frankfurt, SMF 12200, male paratype).

This shrimp has been rarely cited in the literature since its description. Sasaki's (2022) compilation of

Figure 1. *Stenopus devaneyi* Goy, 1984 photographed at Mayotte Island, December 31, 2022, 12°52.964' S, 45°16.557' E, 60 m. A) pair of shrimps; B) dorsal view; C) lateral view; D) fronto-lateral view. Photographs G. Barathieu.

bibliographic references indicate only a dozen references, half of them being only for lists or text citation, without new places of observation, colour photographs, and/or additional specimens collected. The core references for this rare species are listed below in chronological order, with mention of specimens, colour photographs, place of collection and/or distribution indicated.

Stenopus devaneyi Goy, 1984 (in Debelius, 1984): 117, diagnosis '*Specimens collected from French Polynesia: Marquesas Islands; and Indian Ocean, Sri Lanka*'.

Stenopus devaneyi – Debelius, 1984: 17, top photo H. Debelius, 2 specimens, '*Pacific*', but probably from Sri Lanka, aquarium; 28, distribution as '*widely distributed and has been seen around both French Polynesia and Sri Lanka*'. –Goy and Randall, 1986: 81, 90, figs 1-4; specimens examined, 2 ♂♂ Marquesas, Nuku Hiva, Taiohae Bay, 6.2-22.9 m, and visual observation at Tahuata, Vaitahu Bay; 1 ♂ Sri Lanka, donated by Hofmann - colour photos pl. 1A, Marquesas, Nuku Hiva, photo JE Randall; pl. 1C, E, 2 specimens, Sri Lanka, aquarium, photo H. Debelius; pl. 1F, Marquesas, Tahuata, photo JE Randall. – Debelius and Baensch, 1994: 552, distribution as '*from Hawaii to the Maldives*'; 553, bottom, 2 specimens, colour photo H. Debelius, no place, but probably from Sri Lanka, aquarium. – Walls and Hunziker, 1995: 74, 78, colour figure and colour description, no specimen. – Debelius, 2001: 116, Ari Atoll, Maldives, 2 specimens photo H. Voigtmann; 117, full page, 2 specimens, no place, probably from Sri Lanka, aquarium, distribution as '*From the Maldives eastward to Hawaii. Not known from East Africa or the Mascarenes*'.

Several colour photos consulted in Debelius (1984, 2001) and Debelius and Baensch (1994) marine guides are not geographically located. However, after a careful examination of the backgrounds landscapes of these photographs they seem to all be from the same place which is 'Sri Lanka, aquarium' as indicated by Goy and Randall (1986: plate 1, captions of photos C and E).

The presence of *Stenopus devaneyi* in Hawaii, is mentioned only in the distributions indicated by Debelius and Baensch (1994) and Debelius (2001), without specimens collected and/or colour photographs. This must be confirmed as there may be confusion due to there being two types specimens from the Marquesas Islands noted as deposited in the Honolulu Museum. Eldredge and DeFelice (2023) have repeated this Hawaiian record but it is neither indicated in the checklist of McLaughlin *et al.* (2005; only three Hawaiian *Stenopus*: *earlei*, *hispidus*, *pyrsonotus*) nor in Hoover (2006: 219, 220; same three *Stenopus*) Hawaiian guide for marine invertebrate supplemented by an Internet update (Hoover, 2023; addition of *S. tenuirostris*). In conclusion, the occurrence of *Stenopus devaneyi* in Hawaii cannot be excluded in the view of the wide Indo-West-Pacific (IWP) distribution of several *Stenopus* species but, for the time being, it seems better to remove it from the Hawaiian fauna.

Figure 2. Geographic distribution of *Stenopus devaneyi* Goy, 1984. Black spots: specimens collected and deposited in Museum collections; white spots: records based on colour photographs and/or field observation; black star: new record documented in this work for Mayotte Island; ?: questionable record; solid line: westward extension range of the species after this work (3 530 km).

A search on the Internet has failed to reveal additional IWP places of occurrence for *Stenopus devaneyi*. A lot of photographs are for the aquarium trade, sometimes without exact indication for the place of collection. It seems, nevertheless, that the species is rather common around the Maldives and Sri Lanka, with a few Internet photographs indicated from these places.

With this new record the geographical distribution of *Stenopus devaneyi* is extended 3 530 km westward, from the Maldives to Mayotte Island (Fig. 2) and this shrimp can now be added to the Western Indian Ocean (WIO) list of Stenopodidean compiled by Goy (2023). Its updated geographic and depth distributions are: IWP, from Mayotte (new record), Maldives, Sri Lanka to French Polynesia, Marquesas Islands (Nuku Hiva, Tahuata), between 6-23 m, extended herein to 60 m.

The marked pairing behavior of this species already seen in the literature consulted is confirmed for Mayotte with two specimens seen in the same crevice. The associated fauna recognized on the photographs examined is composed of: encrusting sponges and/or ascidians; a gastropod *Scutus sp.*; an alpheid shrimp, *Alpheus* sp.; a squat lobster *Trapezionida barbeti* (Galil, 1999); and two fishes *Luzonichthys waitei* (Fowler, 1931) and, probably, the reef goby *Priolepis nocturna* (Smith, 1957). Another stenopodid shrimp, *Stenopus pyrsonotus* Goy and Devaney, 1980, was also photographed in a barrel sponge during the same dive, but not in the same crevice with *S. devaneyi*. Goy and Randall (1986: 91) have indicated that it is a "*very retiring species, having been observed only in recesses of the coral*". They suggest that it is a cleaner shrimp as it was found in close association with the moray eel *Gymnothorax breedeni* (opt. cit. pl. 1F) and that other species of cleaner shrimp, including *Lysmata amboinensis* (De Man, 1888), were often present with *S. devaneyi*. Interestingly, the association in reef crevices between *S. devaneyi*, a moray eel and the reef goby *Priolepis nocturna* is also documented for the Maldives Island in a Japanese blog consulted on the Internet (http://sekainoumi.blog54.fc2.com/blog-entry-3056.html?sp). It is thus possible that the two *Stenopus devaneyi* were sharing their crevice with a moray eel in the Mayotte sighting, but that the eel hid at the sight of the divers.

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Original Article

Antibacterial activity against *Salmonella typhi* and phytochemical screening of seven seagrass species from the coast of Tanzania

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Abstract

Seagrasses in Tanzania have traditionally been used as a remedy against various diseases including stomach problems. This study determined antibacterial activities and phytochemical composition of seven seagrass species against *Salmonella typhi*, a causative agent of typhoid fever. Crude extracts were obtained using methanol, dichloromethane and hexane solvents. All extracts showed antimicrobial activities against *S. typhi*. Hexane extracts showed highest activities with maximum inhibition zone and lowest Minimum Inhibition Concentrations (MIC). The seagrasses *Halodule uninervis* and *Cymodocea serrulata* exhibit strong antimicrobial activity against *S. typhi*, by having the lowest MIC of 0.39 mg/ml. Most extracts were non-toxic, with *Cymodocea rotundata* having the lowest toxicity level (LC_{50} = 2521.31 mg/ml) and *Thalassia hemprichii* the highest (LC_{50} = 0.038 mg/ml). Seven phytochemical groups, namely alkanoids, saponins, tannis, diterpenes, flavonoids, phenolic and cardiac glycosides were detected. There was no significant difference in antimicrobial activity and phytochemical content between leaves and root extracts. This study established for the first time that seagrasses of Tanzania contain promising antibacterial bioactive compounds against *S. typhi*. The results corroborate indigenous knowledge and may be useful in the development of novel antibacterial drugs that will help to solve problems of antibiotic resistance among pathogenic bacteria like *S. typhi.*

Keywords: minimum inhibition concentration, cytotoxicity, typhoid fever, Tanzania

Introduction

Seagrasses are amongst the marine organisms that are rich in secondary metabolites; some of which have important pharmacological properties used as treatments against bacteria diseases, fungal diseases, cancer, arthritis, inflammatory conditions, and viral diseases (Athiperumalsamy *et al.*, 2008; Kumar *et al.*, 2008; Nazar *et al.*, 2009; Yuvaraj *et al.*, 2012; Rengasamy *et al.*, 2013; Goda *et al.*, 2020; Kim *et al.*, 2021). Traditionally, roots and/or leaves of different seagrasses have been used as remedies against various diseases in different parts of the world including in coastal regions of Tanzania (De La Torre-Castro and Rönnbäck, 2004). In Tanzania, *Cymodocea* spp. are used as a remedy for skin diseases, fever, cough and is believed to help during pregnancy as a tranquilizer for babies while *Halophila* spp. are known to have potential against malaria, skin diseases as well as effective in early stages of leprosy (De La Torre-Castro and Rönnbäck, 2004). Additionally, the seagrass *Thalassia ciliatum* is popular for the relief of small pox and fever while *Enhalus acoroides* roots are used as a remedy against stings from different kinds of rays, muscle pain, fever, wounds and stomach problems (De La Torre-Castro and Rönnbäck, 2004), including typhoid.

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Typhoid fever is a potentially severe and occasionally life-threatening bacterial illness caused by the bacterium *Salmonella enterica* serovar *typhi* (commonly known as *Salmonella typhi*). The illness is often characterized by the insidious onset of sustained fever, headache, malaise, anorexia, relative bradycardia, constipation or diarrhea, and non-productive cough. The disease is endemic to areas that are characterized by rapid population growth, increased urbanization, limited safe water and health systems, such as Africa, India, South and Central America (Uneke, 2008). The disease is known to be transmitted through the fecal-oral route via contaminated water and food, especially by food-handling carriers, and human beings are the only known reservoir and host for typhoid fever (Butter, 1992). Typhoid fever is of important socioeconomic impact because it may take several months for a patient to recover and be able to work normally again. According to the World Health Organization (WHO), the morbidity and mortality rate caused by typhoid fever worldwide was estimated to be 11 - 20 million cases leading to 128,000-161,000 deaths every year, and poor communities and vulnerable groups including children are most affected (World Health Organization, 2018). In addition, climatic variables such as rainfall, vapour pressure and temperature have an important effect on the transmission and distribution of typhoid infections in human populations (Kelly-Hope *et al.*, 2007). A study conducted in Vietnam showed that typhoid incidence was seen to increase with temperature, rainfall and river level at time lags ranging from three to five weeks. For example, it was shown that for a 0.1 meter rise in river level, the number of typhoid cases increased by 4.6 % above the threshold of 4.0 meters. On the other hand, with a 1 °C rise in temperature, the number of typhoid cases could increase by 14.2 % (Dewan *et al.*, 2013). Moreover, it was also reported that typhoid incidences varied with geographical and other environmental conditions. For instance, in Zhejiang Province, China, auto-correlation analysis and spatio-temporal scan statistics revealed that high-risk clusters of typhoid fever were located mainly in the coastal regions but scattered across the province (Hua *et al.*, 2017). Along the coast of Tanzania on Pemba Island a rate for typhoid fever of 110 cases/100,000 population/year was reported (Thriemer *et al.*, 2012) while in Singida region (inland), the incidences of 580 - 1,400/100,000 persons were observed (Malisa and Nyaki, 2010). As for other diseases, the biggest challenge for typhoid is the emergence and spread of multidrug-resistant strains of bacteria causing typhoid fever, leading to significant morbidity and mortality (Gupta, 1994). In Tanzania, Msemo *et al.* (2019) reported that 89.9 % of patients diagnosed with *S. typhi* were resistant to amoxicillin, 81.0 % to chloramphenical and 92.1 % to trimethoprim sulfamenthoxazole. Hence, there is a great need to explore and develop new drugs from locally used medicinal plants.

Worldwide, plants have been found to be interesting sources of new drugs to overcome the antimicrobial resistace challenge. It is reported that about 80,000 flowering plants are used in medicine throughout the world (Leamann, 2011). Likewise, various seagrasses have been reported to have phytochemical compounds such as tannins, sterols, terpenoids, steroids, catachols and flavanoids with potential for pharmacological development (Yuvaraj *et al.*, 2012; Regalado *et al.*, 2012; Rengasamy *et al.*, 2013; Goda *et al.*, 2020; Kim *et al.*, 2021). In Tanzania most of the population depend on medicinal plants for their primary health care and about 1200 species of higher plants have been used as medicine (Mahunnah *et al.*, 2012). However, little is known on the potential natural products from the marine environment, in particular seagrasses. In this study the antibacterial activities of the extracts of seven seagrass species were explored against pathogenic bacteria *S. typhi.* These species included *Cymodocea serrulata, Thalassia hemprichii, Halodule uninervis, Thalassodendron ciliatum, Enhalus acoroides*, *Cymodocea rotundata* and *Syringodium isoetifolium* from the Tanzanian coast. The results obtained provides baseline information to corroborate indigenous knowledge and may be useful in development of novel antibacterial drugs that will help to solve the problems of antibiotic resistance of the pathogenic bacteria *S. typhi*.

Materials and methods

Sample collection

Samples of the seagrasses *C. serrulata, T. hemprichii, H. uninervis, T. ciliatum, E. acoroides*, *C. rotundata* and *S. isoetifolium* were handpicked during low tide from the intertidal beaches of Mjimwema (06°50'S, 39°21'E), 4 km south of Dar es Salaam harbor, and Bagamoyo (6°27'32"S, 38°56'E) between Bagamoyo fish landing site and Kaole ruins. Seagrasses were identified in the field using standard identification guide books such as Richmond (2011). The seagrass leaves and roots were separated and transported to the Department of Molecular Biology and Biotechnology (DMBB), University of Dar es Salaam (UDSM) for further laboratory analysis.

Preparation of extraction

The collected seagrass samples were kept on laboratory benches to dry for 10 – 14 days away from direct sunlight until a constant weight was achieved. The dried leaves and roots of seagrasses were then ground to a fine powder using a grinding machine (Laboratory mill model 4).

Extraction of crude extracts from seagrasses

Crude extracts were obtained as described by Rengasamy *et al.* (2010), where 100 g of the seagrass powder was soaked in 500 ml of hexane (non-polar), dichloromethane (less polar) and methanol (polar) by increasing the order of polarity for 48 hours at room temperature in a shaker (Edmund Buhler 7400). The solvents with extracts were concentrated by using a rotary evaporator (BUCHI Rota vapor model R-210). The concentrated crude extract was stored at 4 °C for antibacterial sensitivity assay against *S. typhi* and identification of compounds.

Test organism

S. typhi (ATCC 14028) obtained from the Department of Molecular Biology and Biotechnology (DMBB), University of Dar es Salaam (UDSM), was used as a test organism. The bacteria were grown in nutrient broth and incubated at 37 °C for 24 hours to obtain fresh culture prior to analysis.

Screening of antimicrobial activities of crude extracts from seagrasses species

The antibacterial activities of seagrass extracts against *S. typhi* were determined by the disc diffusion method as explained by Sosovele *et al.* (2012). The test organism was cultured separately on a Muller-Hinton agar plate. A 100 mg/ml concentration of the extracts were made using 99.9 % Dimethyl sulfoxide (DMSO) and loaded onto a sterilized paper disc, dried and placed on agar plates inoculated with freshly grown colonies of the test organism. Thereafter, the diameter of the inhibition zone (IZ) was measured. A Tetracycline disc (1.0 mg/ml) was used as a positive control and a DMSO disc without plant extract was used as a negative control.

Minimum Inhibitory Concentration (MIC) assay

The micro dilution technique using 96-well microtitre plates was used to determine the minimum inhibitory concentration (MIC) of the crude extracts obtained from the seagrass species. The plate was pre-loaded with 25 µl of Muller-Hinton broth in each well, then 25 µl of 100 mg/ml crude extract was added to each well in the first row to make a total volume of 50 µl with a concentration of 50 mg/ml of crude extract. After thorough mixing, serial twofold dilution was carried out by drawing 25 µl from each well of the first row and placed in the next row of wells. The process were repeated downward the columns to the last wells, resulting in concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 and 0.39 mg/ml. Thereafter, 25 µl of *S. typhi* bacterial suspension prepared, equivalent to 0.5 McFarland standard turbidity was added to each well to make a final volume of 50 µl, and halving the concentrations in the first row throughout. Thus, the starting concentration was 25 mg/ml and the final concentration was 0.195 mg/ml (Nondo *et al.*, 2011). The rows that contained only broth and bacterial suspension were used to monitor the growth of bacteria, while the rows containing the 25 µg/ml of tetracycline were used as positive control and rows containing DMSO (10 %) as a negative control. The 96-well microtitre plates were then incubated at 37 °C for 24 hours. The MIC of each tested extract was determined by the addition of 30 µl of 0.02 % p-iodonitrotetrazolium (INT) chloride in each well an hour before reading the results (i.e. 23 hours of incubation). Thereafter the plates were incubated for the remaining incubation period at 37 °C. After 24 hours of incubation, bacterial growth was indicated by the presence of pink coloration. The minimum inhibitory concentration was determined as the lowest concentration which showed no bacterial growth (Nondo *et al.* 2011).

Brine shrimp lethality assay (Cytotoxicity)

The brine shrimp lethality assays of different seagrass crude extracts were carried out at DMBB following standard procedures (Meyer *et al.*, 1982). This test was done to determine whether the seagrass crude extracts possessed toxic compounds that could cause cell damage or death. One teaspoon full of brine shrimp eggs were hatched in 1000 ml filtered seawater in a container and incubated for 24 hours under illumination using an electric bulb (120 Volts). A stock solution of 40 mg/ml concentration of each crude extract was prepared by dissolving the extract with 99.9 % DMSO, from which different concentrations (240, 120, 80, 40 and 24 µg/ml) were made and kept in vials containing ten brine shrimp larvae. Each concentration of the extract was tested in duplicate. The vials containing brine shrimp larvae, DMSO (0.6 %) and seawater were set as a negative control. Incubation was carried out for 24 hours at room temperature after which incubation vials were observed against a light background.

The number of dead larvae were counted and the mean obtained was subjected to analysis using Microsoft Excel. The graph of the brine shrimp percentage mortality rate against log concentration was plotted, and the regression equation was obtained. From the equation, LC_{50} (µg/ml) was determined as described by Throne *et al.* (1995). The obtained data were interpreted as follows: LC_{50} <1 µg/ml were regarded as highly toxic; LC_{50} > 1.0 and < 10.0 µg/ml as toxic; LC_{50} > 10 and <30 µg/ml as moderately toxic; LC_{50} > 30 and < 100 µg/ml as mildly toxic; and LC_{50} > 100 µg/ml as non-toxic (Meyer *et al.*, 1982; Bastos *et al.*, 2009).

Phytochemical analysis

The qualitative test for the identification of phytochemical constituents of alkaloids, glycosides, flavonoids, phenols, tannins, phytosterols, saponins and diterpenes were determined from methanol and dichloromethane extracts only since there were insufficient samples from hexane extracts. The analyses were carried out according to standard procedures described by Junaid and Patil (2020), with minor adjustment. In order to get seagrass extract solutions for phytochemical analysis, the methanol and dichloromethane extracts were dissolved in their respective solvents. The procedures for each constituent is as described below:

Test for Alkaloids: 0.5 ml of extracts was mixed with 1.0 ml dilute HCl. The mixture was filtered before addition of 2 drops of Wagner's reagent. Formation of a brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavonoids: One drop of 10 % ferric chloride solution was added to a drop of extract aqueous solution and appearance of a green precipitate indicated the presence of flavonoids.

Test for Glycosides: One drop of extracts was mixed with one drop of bromine water. Formation of a yellow precipitate indicates the presence of Glycosides.

Test for Phenols: A few drops of seagrass extract aqueous solution was added to a few drops of 5 % ferric chloride solution. The presence of Phenol was observed by a dark green/bluish black colour.

Test for Tannins: 0.4 ml of seagrass extract was mixed with 4.0 ml of 10 % NaOH and shaken well. The presence of tannis was determined by the formation of an emulsion (Hydrolysable tannins).

Test for Saponins: Distilled water (2.5 ml) was added to 0.5 ml of seagrass extract in a test tube and shaken vigorously. The presence of Saponin was determined by the formation of persistent frothing.

Test for Diterpenes: Three drops of seagrass extract was dissolved in distilled water and three drops of copper acetate solution was added. Formation of an emerald green colour showed the presence of diterpenes.

Test for Phytosterols: 0.4 ml of chloroform was treated with the seagrass extract. The mixture was filtered before addition of a few drops of concentrated H_2SO_4, shaken well and allowed to stand to observe if a red color developed in the lower layer in the test tube.

Typhoid data

Typhoid case data (outpatient and inpatient) for all Tanzania mainland regions were obtained from the then Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDEC) in September 2021. Currently, the name of the minitry is the Ministry of Health (https://www.moh.go.tz).

Data analysis

Inhibition zones were presented as the mean plus/minus (±) standard deviation, while minimum inhibition concentration and typhoid fever data were presented as a whole number, with the phytochemical compounds reported as present (+) or absent (-). Statistics were carried out using Graph Pad Instant t_m 1990-1993 software, where a P-Value less than 0.05 was considered significant. Prior to analysis the data were subjected to normality test.

Results

Antibacterial activity of seagrass extracts against *S. typhi*

Extracts obtained using three different solvents (hexane, dichloromethane and methanol) revealed different antibacterial activities against *S. typhi* at a concentration of 100 mg/ml, as shown in Table 1. For each solvent, there were 14 extracts (i.e. from roots and leaves of the seven seagrass species), and hexane showed activity in all extracts, dichloromethane showed activity in five extracts, while methanol showed activities in only one extract. Hexane extracts showed maximum inhibition zone diameters (IZ) ranging from 7.0 ± 0.0 to 10.0 ± 0.0 mm (Table 1). The IZ of negative and positive controls were 0.0 and 22 mm, respectively. Statistically (using repetitive measure ANOVA) the results revealed that hexane extracts had significantly

Table 1. Zones of inhibition (diameter) (IZ) of seagrass extracts at 100 mg/mL concentration against *S. typhi.* The IZ for negative and positive controls were 0.0 and 22.0 mm, respectively.

Seagrass species	Solvents	Zone of inhibition (mm)	
		Roots	Leaves
Cymodocea rotundata	Hexane	10.0 ± 0.0	7.7 ± 0.6
	Dichloromethane	0.0 ± 0.0	8.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Cymodocea serrulata	Hexane	8.5 ± 0.7	7.7 ± 0.6
	Dichloromethane	8.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Halodule uninervis	Hexane	9.0 ± 1.4	8.0 ± 0.0
	Dichloromethane	9.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	7.5 ± 0.7
Thalassia hemprichii	Hexane	8.5 ± 0.7	7.0 ± 0.0
	Dichloromethane	7.0 ± 0.0	7.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Thalassodendron ciliatum	Hexane	8.0 ± 0.0	9.0 ± 0.0
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Syringodium isoetifolium	Hexane	8.0 ± 0.0	9.0 ± 0.0
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0
Enhalus acoroides	Hexane	9.0 ± 0.0	9.5 ± 0.7
	Dichloromethane	0.0 ± 0.0	0.0 ± 0.0
	Methanol	0.0 ± 0.0	0.0 ± 0.0

Key: 0.0 = no zone of inhibition

stronger antimicrobial activities against *S. typhi* than other solvent extracts ($P < 0.0001$). In terms of seagrass species, *H. uninervis* and *T. hemprichii* had more antimicrobial activities than others, whereas out of six extracts, four showed activity, followed by *C. rotundata* and *C. serrulata* which showed activity in three extracts. The least antibacterial activity was shown by *T. ciliatum*, *S. isoetifolium* and *E. acoroides*, and with hexane extracts only (Table 1). However, there were no significant differences in antimicrobial activities against *S. typhi* among the seagrass species ($P = 0.448$). Furthermore, there were no significant differences in antibacterial activities against *S. typhi* between roots and leaves (Wilcoxon matched-pairs signed-ranks test the P value = 0.289).

Out of 42 seagrass extracts only 26 were found to have a MIC at or below the cut off value (3.13 mg/ml), in which for each seagrass species at least one extract was sensitive to *S. typhi* with a MIC at or below the cutoff point (Table 2). Hexane and dichloromethane extracts showed a MIC value within the cut off range in all of the seagrasses while methanol extracts were sensitive at 3.13 mg/ml in only two seagrass species (*H. uninervis* and *T. hemprichii*) (Table 2). The MIC of the positive control (Tetracycline) was below 0.019 mg/ml while the negative control was ≥ 25 mg/ml. Statistically, hexane extracts were extremely sensitive to *S. typhi* ($P < 0.0001$) as compared to dichloromethane and methanol extracts. Furthermore, the seagrass *H. uninervis* and *C. serrulata* extracts were more sensitive to *S. typhi* with the MIC value of 0.39 mg/ml. However, there were no significant differences ($P = 0.663$) in MIC among the seagrasses. In addition, both leaves and roots of all the seagrasses showed more or less similar minimum inhibitory activities in all the tested solvents (Table 2).

Generally, the results showed that most of the seagrass extracts had low a cytotoxicity level (non toxic), with *C. rotundata* having the lowest level (LC_{50} = 2521.31 mg/ml), while *T. hemprichii* extracts had the highest level (LC_{50} = 0.038 mg/ml) (Table 3), regarded as mildly toxic. Statistically, the results revealed significant differences in toxicity among the seagrasses ($P = 0.018$), with the post hoc test Turkey Kramer multiple comparison test showing the differences were between *C. rotundata* and *S. isoetifolium* ($P < 0.05$), and between *C. serrulata* and *S. isoetifolium* ($P < 0.01$). On comparing extracts from different solvents, dichloromethane extracts of all the seagrasses showed a lower cytotoxicity level compared to hexane and methanol extracts (Table 3). Furthermore, on comparing below and above ground parts, all seagrasses had more or less similar trends in cytotoxicity level (Table 3). There were no significant differences ($P > 0.05$) in toxicity among the extracts from different solvents as well as between the roots and shoots.

Table 2. Minimum inhibitory concentrations (MIC) of seagrass extracts against *S. typhi*. The MIC value for negative and positive controls were ≥ 25 and 0.019 mg/ml, respectively.

Seagrass species	Solvent	Minimum inhibitory concentrations (mg/ml)	
		Roots	Leaves
Cymodocea rotundata	Hexane	1.56	3.13
	Dichloromethane	3.13	1.56
	Methanol	12.5	12.5
Cymodocea serrulata	Hexane	1.56	0.39
	Dichloromethane	1.56	3.13
	Methanol	>25	6.25
*Halodule uninervi*s	Hexane	1.56	1.56
	Dichloromethane	3.13	0.39
	Methanol	6.25	3.13
Thalassia hemprichii	Hexane	1.56	0.78
	Dichloromethane	0.78	3.13
	Methanol	>25	3.13
Thalassodendron ciliatum	Hexane	3.13	>25
	Dichloromethane	1.56	6.25
	Methanol	>25	6.25
Syringodium isoetifolium	Hexane	1.56	1.56
	Dichloromethane	1.56	1.56
	Methanol	>25	12.5
Enhalus acoroides	Hexane	3.13	3.13
	Dichloromethane	NT	NT
	Methanol	>25	12.5

Key: NT = Not Tested

The findings revealed that out of eight phytochemical groups examined, seven groups (except phytosterol) were detected in the studied seagrasses (Table 4). Six groups, namely alkanoids, saponins, tannis, diterpenes, flavonoids and cardiac glycosides were found in both the seagrass root and leaf extracts, while the phenolic group was exclusively found in leaf extracts (Table 4). The results further revealed that alkaloids, tannins and diterpenes were the most common groups found in almost all extracts. Among the studied seagrasses, all the seven detected phytochemical groups were found in *C. serrulata* and *T. hemprichii*, six groups were found in *T. ciliatum* and *E. acoroides*, while five groups were found in *H. uninervis*, *C. rotundata* and *S. isoetifolium.*

Data on the typhoid fever records in mainland Tanzania for five years (2016-2020) indicated that there

Table 3. Cytotoxicity of selected seagrass crude extracts to brine shrimp larvae (LC_{50} values in mg/ml).

Seagrass species	Extracts	Toxicity (mg/ml)	
		Leaves	Roots
Cymodocea rotundata	Hexane	0.80	0.11
	Dichloromethane	2521.31	2.80
	Methanol	0.86	1.24
Cymodocea serrulata	Hexane	10.38	0.07
	Dichloromethane	2.93	25.44
	Methanol	0.12	0.05
*Halodule uninervi*s	Hexane	0.24	0.06
	Dichloromethane	3.82	0.14
	Methanol	NT	NT
*Thalassia hemprichi*i	Hexane	0.22	0.04
	Dichloromethane	7.16	16.44
	Methanol	0.16	0.12
*Thalassodendron ciliatu*m	Hexane	0.15	0.84
	Dichloromethane	1.60	2.94
	Methanol	0.06	NT
Syringodium isoetifolium	Hexane	NT	NT
	Dichloromethane	722.39	2.89
	Methanol	NT	NT
Enhalus acoroides	Hexane	0.175	NT
	Dichloromethane	5.767	0.91
	Methanol	NT	NT

Key: NT = Not Tested due to insufficient amount of extract

Table 4. Phytochemical analysis of the selected seagrass methanol and dichloromethane extracts.

Seagrass species		**Alkaloids**	**Flavonoids**	**Saponins**	**Tannins**	**Phenolic**	**Phytosterol**	**Cardiac glycosides**	**Diterpenes**
				Methanol					
Cymodocea rotundata	Leaves	-	-	+	-	-	-	-	-
	Roots	+	-	+	+	-	-	+	-
Cymodocea serrulata	Leaves	-	+	-	+	+	-	-	+
	Roots	-	-	+	+	-	-	+	-
Halodule uninervis	Leaves	-	-	+	-	-	-	+	+
	Roots	-	-	+	-	-	-	+	+
Thalassia hemprichii	Leaves	-	-	-	+	+	-	-	+
	Roots	+	+	+	+	-	-	+	+
Thalassodendron ciliatum	Leaves	-	+	+	+	+	-	-	-
	Roots	+	-	-	-	-	-	-	-
Syringodium isoetifolium	Leaves	+	-	-	+	-	-	-	+
	Roots	+	-	-	+	-	-	-	-
Enhalus acoroides	Leaves	+	+	+	+	-	-	-	+
	Roots	+	-	-	-	-	-	-	+
				Dichloromethane					
Cymodocea rotundata	Leaves	-	-	+	+	-	-	-	+
	Roots	+	-	-	+	-	-	+	-
Cymodocea serrulata	Leaves	-	-	-	+	-	-	+	-
	Roots	+	-	+	+		-	+	-
Halodule uninervis	Leaves	NT	NT	NT	NT	NT	NT	NT	NT
	Roots	+	-	+	+	-	-	+	+
Thalassia hemprichii	Leaves	+	-		-	+	-	-	+
	Roots	+	-	-	+	-	-	+	+
Thalassodendron ciliatum	Leaves	-	-	-	+	+	-	+	-
	Roots	+	-	+	+	-	-	+	-
Syringodium isoetifolium	Leaves	-	+	-	+	+	-	+	-
	Roots	-	-	-	+	-	-	+	-
Enhalus acoroides	Leaves	NT	NT	NT	NT	NT	NT	NT	NT
	Roots	-	-	+	+	-	-	+	-

Key: + = present; – = absent; NT = Not tested

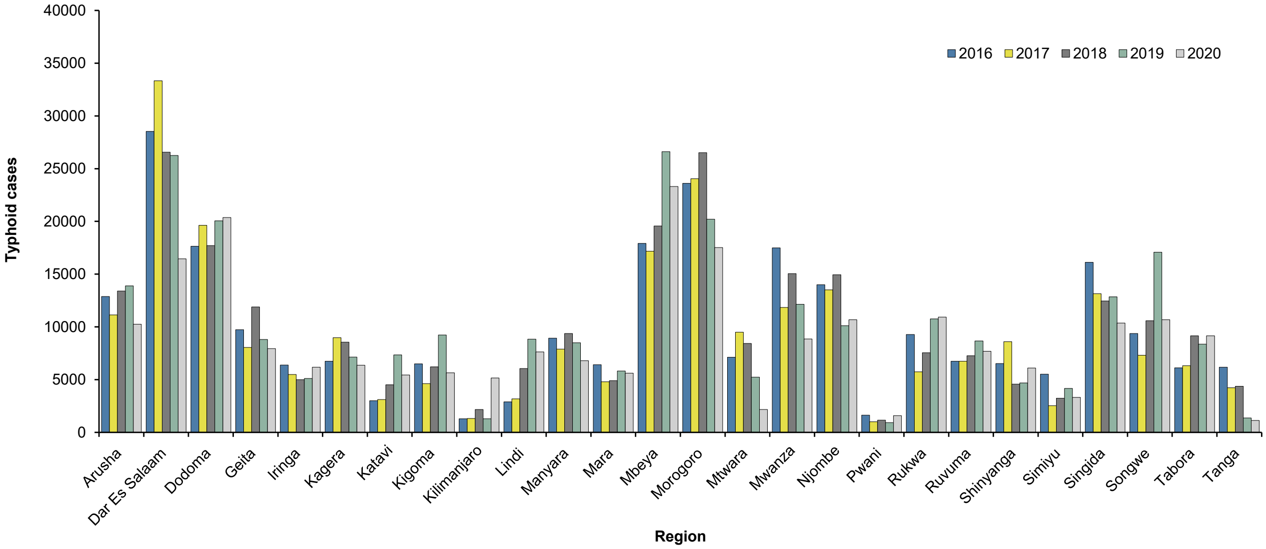

Figure 1. Total typhoid fever cases for each region in Tanzania Mainland from 2016 – 2020.

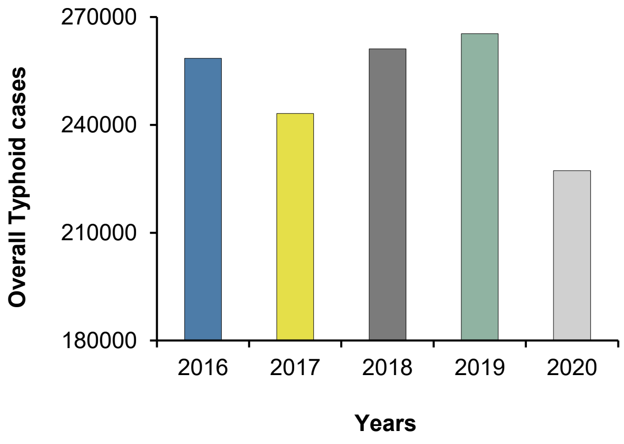

Figure 2. Overall typhoid fever cases for Tanzania Mainland from 2016 – 2020.

is a spatial and temporal fluctuating trend of typhoid occurrence. The numbers of cases were higher in the largest city Dar es Salaam, followed by Morogoro, Mbeya and Dodoma, with the Coast Region (Pwani) having the lowest typhoid cases (Fig. 1). In terms of yearly trends, the total number of typhoid fever cases showed that the disease burden was more or less constant in the five years studied although the numbers decreased from 258,560 cases in 2016 to 243,203 in 2017, followed by an increase in 2019 to 265,355, and finally further down in 2020 to 227,259 cases (Fig. 2). Likewise, the proportion of typhoid from all diagnoses (Out Patient Department/In Patient Department) was more or less constant over the five years (2016-2020) as shown in Table 5.

Discussion

Seagrasses are rich in secondary metabolites; some of which have important pharmacological properties with potential for treatment of human diseases (De La Torre-Castro and Rönnbäck, 2004; Athiperumalsamy *et al.*, 2008; Nazar *et* al., 2009; Regalado *et al.*, 2012; Yuvaraj *et al.*, 2012; Rengasamy *et al.*, 2013; Kim *et al.*, 2021). In the present study, extracts from seven seagrass species, namely *C. serrulata, C. rotundata, T. hemprichii, H. uninervis, T. ciliatum, E. acoroides* and *S. isoetifolium* showed varying antibacterial activities against the tested Gram-negative pathogen *S. typhi*. The findings corroborate similar observations where the three seagrasses species *C. serrulata, Halophila ovalis* and *Zostera capensis* from the South Indian coast were effective against Gram-negative bacteria including *Salmonella* species as well as Gram-positive bacteria (Kumar *et al.*, 2008; Rengasamy *et al.*, 2013). Different studies show that extraction solvents have an effect on the yield (amount of extract) and the constituents of bioactive compounds, thus significantly affecting the biological activity of the extract (Alam *et al.*,

Table 5. Percentage occurrence of typhoid in diagnoses (Out Patient Department/In Patient Department) in Tanzania Mainland Regions.

Region	2016	2017	2018	2019	2020
Arusha	0.9	0.7	0.7	0.7	0.6
Dar es Salaam	0.6	0.6	0.5	0.4	0.3
Dodoma	1.3	1.4	1.0	1.2	1.1
Geita	1.0	0.8	0.8	0.5	0.5
Iringa	0.9	0.7	0.6	0.5	0.7
Kagera	0.4	0.4	0.4	0.3	0.3
Katavi	1.1	1.1	1.1	1.4	1.1
Kigoma	0.4	0.2	0.2	0.3	0.2
Kilimanjaro	0.1	0.1	0.1	0.1	0.3
Lindi	0.3	0.3	0.6	0.7	0.7
Manyara	1.1	0.9	0.9	0.8	0.7
Mara	0.5	0.4	0.4	0.4	0.4
Mbeya	1.8	1.6	1.6	1.8	1.7
Morogoro	1.4	1.3	1.1	0.8	0.7
Mtwara	0.6	0.7	0.6	0.3	0.2
Mwanza	1.1	0.7	0.7	0.5	0.4
Njombe	2.9	2.5	2.3	1.5	1.6
Pwani	0.1	0.1	0.1	0.0	0.1
Rukwa	2.0	1.1	1.2	1.5	1.6
Ruvuma	0.6	0.5	0.5	0.5	0.5
Shinyanga	0.6	0.8	0.4	0.4	0.5
Simiyu	0.8	0.4	0.4	0.5	0.5
Singida	1.6	1.3	1.0	1.0	0.9
Songwe	2.1	1.9	1.9	2.6	1.8
Tabora	0.5	0.5	0.5	0.4	0.4
Tanga	0.3	0.2	0.2	0.0	0.0
Average	0.8	0.7	0.6	0.6	0.8

1994; Sastry and Rao, 1994; Turkmen *et al.*, 2006; Rengasamy *et al.*, 2013; Truong *et al.*, 2019). In this study, hexane extracts had higher potential antimicrobial activities compared to dichloromethane and methanol extracts indicating that the extraction efficiency favoured the non-polar solvents. The results concurred with Sastry and Rao (1994), in which the non-polar extracts had more antibacterial activities than polar. However, they differ from some studies on marine plants that showed methanol (polar) extracts to have higher antimicrobial properties against both Gram-negative and Gram-positive bacteria compared to hexane extract (Alam *et al.*, 1994; Kumar *et al.*, 2008; Rengasamy *et al.*, 2013). Thus polarity of the extraction solvents could cause variations in the level of bioactive compounds of the extract. Apart from solvent, extraction method, temperature, extraction time and phytochemical composition are considered as factors that affect the efficacy of the extraction (Turkmen *et al.*, 2006).

Extracts from *H. uninervis* and *C. rotundata* in all the three solvents showed greater antibacterial activities against *S. typhi* compared to other seagrass species. The results presented here are consistent with some previous studies that different seagrass species might have varied antibacterial activities on different or similar microorganisms. For instance, *C. rotundata* extract was reported to be more effective against several strains of bacteria species as compared to other marine plants (Bhosale *et al.*, 2002), while *Halophila* sp. was reported to have more antimicrobial activity than *Cymodocea* sp. (Kumar *et al.*, 2008). Bushmann and Ailstock (2005) reported that although most seagrasses possess antibacterial compounds, their effects vary between species, locations and seasons.

The presence of the important phytochemical compounds such as tannins, saponins, phenolic, flavonoids, alkaloids, tannins and diterpenes in at least one of the seagrasses is reported, some of which were also reported in previous studies in marine extracts (Kumar *et al.*, 2008; Goda *et al.*, 2020; Kim *et al.*, 2021). As such, the result indicate that seagrasses from the Tanzania coast could be further studied for their active compound potential for the production of drugs against typhoid fever and other diseases.

Most of the studied seagrass extracts had low cytotoxicity properties (non toxic) with the higher value of LC_{50} ranging from 0.11 - 2,521.31 mg/ml. This is in agreement with results obtained from the study by Rengasamy *et al.* (2013) in the Gulf of Mannar, South India which showed low cytotoxicity properties from six seagrass species extracts, with *S. isoetifolium* exhibiting the lowest cytotoxicity level. The observed low toxicity activities of the seagrasses substantiate the usefulness and potential of using seagrasses for the development of pharmaceutical therapy against diseases caused by bacteria.

The typhoid data indicated that the disease is still a public health challenge facing Tanzania. This has also been shown in previous studies in the Singida region (from 2003 to 2007), where there were fluctuations in the incidence of typhoid, and where it was speculated that the reasons could be either water scarcity or lack of access to safe water, improper drainage systems and problems of unhygienic toilets (Uneke, 2008; Thriemer *et al.*, 2012). Despite being close to Dar es Salaam and Morogoro, the Coast Region (Pwani) was found to have a low incidence of typhoid cases. This could possibly be attributed to the use of plant extracts (including from seagrasses) for medical purposes against various illnesses. This may have reduced the number of cases reported in hospitals. As previously reported, people use seagrass as a treatment for fever and stomach ailments on the Tanzanian coast (De La Torre-Castro and Rönnbäck, 2004); symptoms similar to those shown by typhoid fever. Additionally, diet might be an important factor since it was previously reported that most of the coastal communities depend highly on the protein sources from the intertidal seagrass ecosystems (De La Torre-Castro and Rönnbäck, 2004).

In conclusion, the present study reports for the first time that seagrasses of the Western Indian Ocean, specifically Tanzania, contain promising antibacterial bioactive compounds against *S. typhi*. The seagrass *H. uninervis* and *C. rotundata* are of interest due to their strong antibacterial activities and low cytotoxicity levels. Thus they are recommended for further clinical assessment for pharmaceutical production of drugs. The phytochemical analyses of the extracts revealed the presence of phytochemicals such as saponins, tannins, alkaloids, cardiac glycosides, diterpenes and flavonoids that may account for the antibacterial activities observed. The current findings also support the local use of these seagrass for medicine. Further studies are required to corroborate the current findings for the development of a pharmaceutical industry using seagrasses as a therapy against typhoid fever and other diseases.

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Original Article

Combined effects of salinity and temperature on survival and growth during the early life cycle of the rock oyster *Saccostrea cuccullata* (Born, 1778)

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Abstract

Temperature and salinity are among the critical factors affecting the survival and growth of bivalve larvae. The combined effects of temperature and salinity on the embryonic and larval development of the rock oyster *Saccostrea cuccullata* (von Born, 1778) in culture conditions were investigated in a laboratory study on Inhaca Island, Mozambique. A factorial experimental design tested three temperatures (24, 30 and 34 °C) and three salinities (30, 35 and 40 parts per thousand) over a seven-day period. Larval survival and growth (in height and length) were assessed by regular sampling by counting and measurement of larvae under an optical microscope equipped with a micrometric scale. Significantly higher larval survival was observed at the combination of 30 °C and 35 salinity. However, the mid-range temperature (30 °C) and highest salinity (40) resulted in faster growth of the larvae. The lowest temperature (24 °C) negatively affected growth regardless of salinity level and survival decreased linearly with increasing salinities. The present results will aid in the understanding of the environmental factors behind the natural recruitment of spat of *S. cuccullata* and contribute to the optimization of rearing protocols for the larval culture of this oyster species.

Keywords: temperature, salinity, rock oyster, larvae, growth, survival

Introduction

The rock oyster *Saccostrea cuccullata* (Born, 1778) is a widely distributed species in the Indo-Pacific and Eastern Atlantic Oceans and has been introduced into the Eastern Mediterranean Sea (Çevik *et al.*, 2001). *S. cuccullata* is an intertidal species mainly found in brackish water environments between 0 and 5 m depth, where it is usually attached to rocks, roots and trunks of mangrove trees and on pier piles (Dye *et al.*, 1994; Carpenter *et al.*, 1997; Poutiers, 1998). The rock oysters are a popular food consumed throughout its distribution area, but the overexploitation of their natural beds is reducing the catches in different countries, including South Africa (Dye, 1989), Kenya (Tack,

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1999), Mozambique (Everett, 2008) and India (Tenjing, 2020). In Bangladesh, the decline of the rock oyster beds is leading to the collapse of breakwater reefs (Chowdhury, 2019). It is widely acknowledged that shellfish reefs provide relevant ecosystem services such as coastal protection, nursery grounds to enhance fisheries production, contributing to blue carbon capture, and pollutant removal and detoxification (Nagelkerken *et al.*, 2015; Waltham *et al.*, 2020).

S. cucculata is also an interesting species for aquaculture purposes due to its high commercial value, tolerance to extreme environmental conditions and fast growth. Most recent research and development efforts on *S. cuccullata* have focused on improving rock oyster natural populations through the development of improved substrates for spat collection and juvenile growth (Racuyal *et al.*, 2016; Chowdhury, 2019).

However, the culture requirements for *S. cuccullata* during their early life stages are poorly known, which seems to be a major bottleneck for the development of the culture of this species. Major advances thus far on the larviculture of *S. cuccullata* were the production of embryos (Awati and Rai, 1931), and the description of the embryological development and suitable temperature and salinity conditions for the production of straight-hinge veliger larvae (Kalyanasundaram and Ramamoorthi, 1986). Sukumar and Joseph (1988a, 1988b) provided a general insight on the natural breeding cycle of *S. cuccullata*, including gonadal maturation and the description of the factors influencing the spawning cycle. Recently Thanormjit *et al.* (2020) characterized the gametes and early development of *S. cuccullata*.

Temperature and salinity are major environmental factors that affect the survival and growth of bivalve molluscs during early life stages (Robert *et al.*, 1988). Considering the natural occurrence of *S. cucculata* in the intertidal zone, where environmental parameters are likely to reach extreme values, the current research tested a range of water salinity and temperature combinations to evaluate the most suitable conditions for larval culture of the rock oyster.

Materials and methods

Breeding and experimental animals

Adult specimens of *S. cuccullata* were collected manually with a concave iron tool during low spring tide on the rocky shores of Ponta Torres (32° 57 ‘ S, 26° 4’ E) on the island of Inhaca (Maputo, Mozambique). Only specimens larger than 20 mm were selected (Nascimento and Pereira, 1980; Dang *et al.*, 2010). During collection, water temperature (26.5 ± 1.0 °C) and salinity (35.5) were measured. The oysters were placed in a waterless plastic bucket and capped with Rachel mesh to minimize sunlight stress and brought to the Inhaca Marine Biology Station (EBMI). At EBMI, the oyster shells were cleaned using a nylon brush and a knife, washed with distilled water and transferred to a 50 L aquarium with natural seawater at constant temperature (25 ± 2 °C) and salinity (40). Aeration was continuous and illumination was based on natural light and photoperiod.

Ten oysters were randomly selected and their valves were opened through the sectioning of the adductor muscle (Legat *et al.*, 2017; Santos *et al.*, 2020; Thanormjit *et al.*, 2020). The oysters were then washed with filtered (1 µm) and UV-treated seawater to remove microorganisms lodged in the inner layer of the valves. The sex of oysters was identified by gonad biopsy using an optical microscope (Olympus model CK40; magnification of 40 x).

The gonadal tissue was cut with a scalpel and the gametes were collected using a Pasteur pipette. They were then transferred to a 100 mL beaker (male gametes) and a 1 L container (female gametes) filled with filtered (1 µm) and UV-treated seawater (Absher *et al.*, 2000). The viability of the gametes was examined under the microscope, i.e., the sperm cells were mobile and the shape of the oocytes became spherical 20 min after being exposed to water. In vitro fertilization was performed adding 2.5 mL of the sperm solution in 1 L of oocyte suspension. Through microscopic observations, the necessary adjustments were made to establish a ratio of about 3 to 5 spermatozoa for each female gamete to avoid polyspermy. Of the 10 sacrificed adult oysters, only four females and two males were used to obtain the required number of gametes for use in the experiment. Each female contained on average 5 to 10 million eggs and males about 2 million sperm. For fertilization, gametes (spermatozoa and oocytes) were evaluated through microscopic examination according to Helm (2004): mature gametes were considered all ovules normally pear-shaped when removed for the first time and round-shaped in contact with sea water within 20 minutes; the sperm was considered mature when it was motile. Embryos of approximately 40-45 µm were transferred to a 10 L tank filled with 5 L of filtered (1 µm) and UV-treated seawater with moderate aeration at the density of 204 embryos mL^{-1}, estimated using an optical microscope by counting the embryos present in 1 ml. The temperature was set at 28 °C with the use of thermostats (Dophin 50W). The embryos remained under these conditions until the appearance of D-larvae 24h after fertilization. Since the time required to reach the D-larvae stage was previously unknown, the stages of embryonic and larval development were observed every five minutes.

Experimental design

Twenty-seven culture tanks each containing 5 L of filtered (1 µm) and UV-treated seawater were prepared about 24 hours after fertilization. Larvae were reared in the combinations of three temperatures (24, 30 and 34°C) and three salinities (30, 35 and 40), each with three replicates. The D-larvae were directly transferred to those experimental treatments in three replicates per combined temperature x salinity. Approximately 25,000 D-larvae were transferred to each culture tank at an initial density of 5 larvae mL^{-1}. Water volume in each tank was maintained at 5 L. The salinities of 35 and 30 were obtained by diluting seawater (salinity 40) with distilled water. Moderate aeration was provided using air stones. Every 48 h, tank bottoms were siphoned (50 % water volume exchange) to ensure good water quality. To avoid the elimination of live larvae as well as to select the largest diameter larvae, 60 and 40 µm overlapping meshes were used (smaller mesh over the larger one). A density of 400 cells mL^{-1} of the microalgae *Isochrysis galbana* was provided daily to the D-larvae. A reduction of 50 % on the number of microalgae was provided in the days when no water was exchanged. Microalgal density was estimated daily with a Neubauer chamber. Growth was measured daily by sampling 30 larvae according to Hillerbrand *et al.* (1999). An optical microscope equipped with an ocular micrometric scale (Olympus CK40) was used to measure height (maximum distance between the dorsal and ventral regions) and length (maximum distance between the anterior and the posterior regions) (Hu *et al.*, 1993). The mean (± SD) initial length and height of the larvae were 50.28 ± 0.30 µm and 51.30 ± 0.25 µm, respectively. Survival was estimated as the number of larvae alive at the end of the experiment in relation to the initial number. Transparent larvae, which presumably indicated the absence of tissues, were considered dead (Ponis *et al.*, 2003). The trial lasted seven days. The data obtained on length, height and survival of oyster larvae were initially tested for normality and homogeneity of variances by the Kolmogorov-Smirnov (distance) test and the Spearman's test, respectively. The data presented a normal distribution and homogeneous variances ($p<0.05$), therefore they were later submitted to parametric tests. A two-way analysis of variance (ANOVA) was used to verify the influence of temperature and salinity on larval growth and survival. If there were significant differences, the Tuckey test was applied for detailed comparison of the differences (Zar, 2010). A significance level of 5 % ($\alpha=0.05$) was adopted. Statistical treatment was carried out with the aid of the GraphPad Prism V. 8.01.

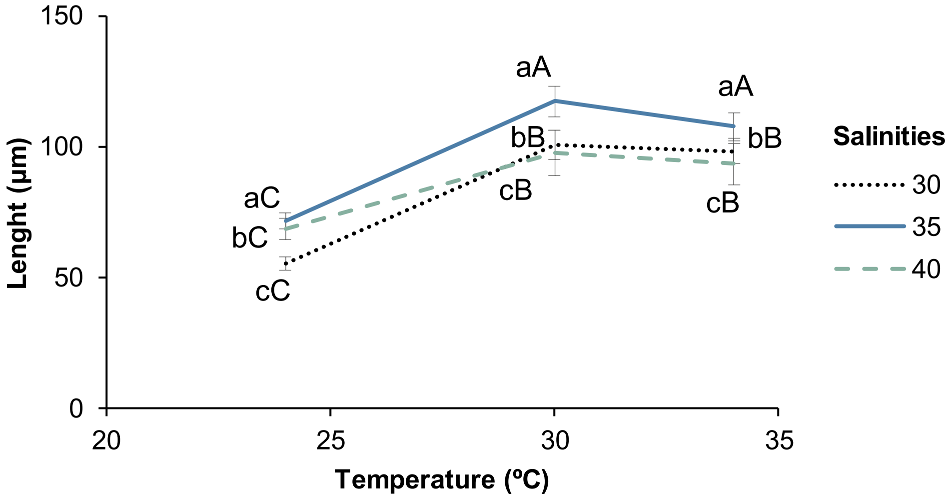

Figure 1. Means (± standard deviation) of length of larvae of the rock oyster, *Saccostrea cuccullata*, reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) for seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

Results

Five minutes after the sperm solution was added to the oocyte suspension, sperm cells were observed surrounding the oocytes. The polar bodies were visible after 20 minutes. The different stages of embryonic development (2, 4, 8, 16 cells, the morula and blastula stages) were all observed within 2 h after fertilization. Trocophora larvae were observed 16 h after fertilization and were characterized by the presence of cilia and circular movements. The D-veliger stage appeared 24 h after fertilization and was characterized by the formation of the D-shaped larval shell.

At the end of the 7 day-long trial, means (± SD) of length (Fig. 1) and height (Fig. 2) of larvae after exposure to the different combinations of salinity and temperature were significantly different with ANOVA $F_{(4,18)}$ = 66.47, p <0.0001 for length and $F_{(4,18)}$ = 380.5, p < 0.0001 for height. The largest larvae (117.30 ± 0.36 µm length and 125.08 ± 0.38 µm height) were observed at 30 °C x 35, while the smaller ones (53.30 ± 0.86 µm length and 54.69 ± 0.40 µm height) were those from treatment 24 °C x 30. The differences in survival rates among treatments were also significant (Fig. 3) with ANOVA $F_{(4,18)}$ = 1309, p <0.0001. The mean (± SD) survival ranged from 26.4 ± 0.4 % in treatment 24 °C x 30 to 35 salinity to 73.2 ± 0.2% in treatment 34 °C x 35 salinity. The highest survival was observed at 34 °C x 35 salinity. All treatments at 24 °C resulted in significantly lower survival rates.

Discussion

The present study suggests that *S. cuccullata* larvae can grow well at 30 °C temperature x 35 salinity to obtain largest larvae, however to obtain higher survival of larvae, they must be grown at 34 °C temperature x 35 salinity. The temperature of 30 °C is well above the average annual temperature of 25 ± 1 °C for the brood stock collection area at Inhaca Island, where (according to Kalk, 1995) the highest average is 27.5 °C and the lowest 21.7 °C. The mean salinity levels at Inhaca are 33 ± 2 (de Boer *et al.*, 2000) with a range from 32 to 42 (Pinto, 1996). This confirms the findings of Kalyanasundaram and Ramamoorthi (1986) that *S. cuccullata* larvae tolerate a wide temperature and salinity variation though with different growth and survival trends.

Temperature and salinity are among the main environmental factors affecting the growth (Robert *et al.*, 1988; His *et al.*, 1989; Manoj Nair and Appukuttan, 2003) and survival of bivalve larvae (Yuan *et al.*, 2016; Verween *et al.*, 2007; Manoj Nair and Appukuttan, 2003; Robert *et al.*, 1988). In this study, embryos and larvae reached all developmental stages defined by Kalyanasundaram and Ramamoorthi (1986). However, due to the distinct culture conditions of the treatments, the life stages were reached at different times.

The highest larval growth was observed at salinity 35, coinciding partly with the results obtained by Kalyanasundaram and Ramamoorthi (1986) which

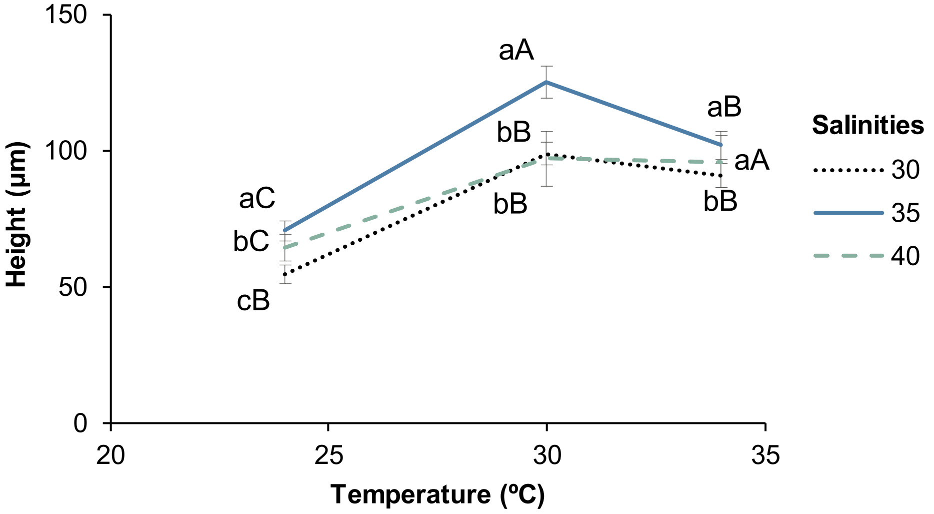

Figure 2. Means (± standard deviation) of height of larvae of the rock oyster, *Saccostrea cuccullata*, reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) for seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

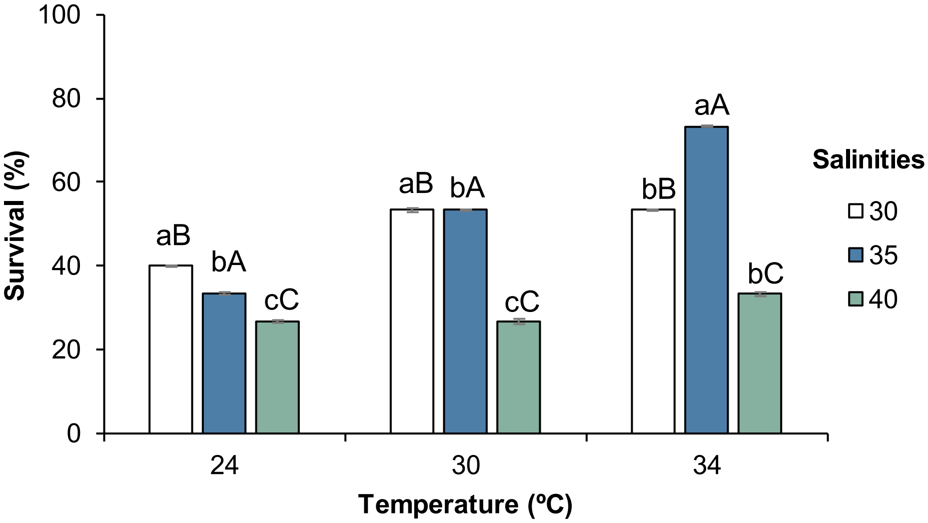

Figure 3. Percentage of survival of rock oyster, *Saccostrea cuccullata* larvae reared at combinations of temperature (24, 30 and 34 °C) and salinity (30, 35 and 40) after seven days. Lowercase letters represent comparison between salinities for the same temperature and capital letters represent comparison between different temperatures for the same salinity ($p < 0.05$).

reported good larval development of *S. cuccullata* at salinities from 20 to 35. In contrast, Sudrajat (1990) determined an optimum salinity of 25 for embryonic development of *S. cuccullata*. In general, oyster larvae tolerate a wide salinity range between 25 and 40 (Lemos *et al.*, 1994). However, this study showed that salinity affects the growth of *S. cuccullata* larvae, being the lowest at salinity 30. It may be possible that osmotic disturbances lead to energy allocations that ultimately impair other functions such as growth (Deaton, 2008). Temperature is regarded as the most important environmental factor influencing bivalve culture (Robert *et al.*, 1988; Helm *et al.*, 2004). Temperature increments accelerate the growth of oyster larvae (Doround *et al.*, 1999). The highest growth recorded here at 30 °C supports previous observations by Kalyanasundaram and Ramamoorthi (1986) that larval growth of *S. cuccullata* is higher at 30 °C. Yukihira *et al.* (2000) reported that the influence of temperature on the metabolism and physiological processes of oysters creates an optimal temperature range for each species providing a maximum rate of growth as well as survival. In fact, a similar trend was observed in the present study, where the maximum temperature tested (34 °C) resulted in reduced larval growth.

The greatest survival (73 %) obtained at the combination of salinity 35 x 34 °C, was higher than the one from the microalgae feeding experiments by Martínez-Fernández and Southgate (2007) in *Pinctada margaritifera* with 70.5 %. It was also greater than the 50 % larval survival obtained by Mafambissa (2009) for *Crassostrea rhizophorae*. Other oyster species such as *Crassostrea gigas* have shown higher larval survival rates of up to 87 % (Ponis *et al.*, 2003).

The results from this study are in partial agreement with Nell and Holliday (1988) who reported higher larval survival of *S. commercialis* in salinities ranging between 23 and 39. However, the current results differ from those obtained by Coeroli *et al.* (1984) where they reported higher survival of *Saccostrea echinata* larvae at salinities ranging from 25 to 30 and temperatures from 25 to 29 °C. Heral and Deslous-Paoli (1990) demonstrated that *C. gigas* larvae tolerate higher salinities (from 45 to 50) with significant mortality above 50. These differences reflect the genetic variability among different species of oysters and their high adaptability to different environmental conditions. The lowest larvae survival in the present study (26.4 %) was achieved at salinity 40, suggesting this is a threshold salinity level for *S. cucculata* larvae.

All treatments at 24 °C resulted in significantly lower survival rates. This seems to be the lower temperature limit tolerated by the larval stages of this species. Another factor to consider is the abrupt submission of the larvae to low temperatures without any previous acclimation, which may have caused a thermal shock and contributed to a decrease in survival rates. Opposite to what was previously reported by Kent *et al.* (1999), the fertilization method used here was very effective as high survival of larvae was observed in treatments where appropriate environmental conditions were provided. Together with the optimal water salinity and temperature for larval rearing established here, this may contribute to the further development of the culture of *S. cuccullata*. These findings related to the environmental variable thresholds for *S. cuccullata* larvae are relevant for future studies on recruitment, as well as to evaluate the direct impact of global warming on this species.

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Original Article

Establishing historical benthic cover levels for coral reefs of the Western Indian Ocean

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Abstract

Data on coral reef health prior to large-scale disturbances are unavailable in most parts of the world including the Western Indian Ocean (WIO). Robust coral reef health baselines could improve the understanding of changes occurring to reefs in the 21st century and prevent the "shifting baseline" phenomenon, enabling researchers and managers to evaluate the success of management measures, and set achievable targets for new interventions. To make this data accessible to the WIO coral reef community, a literature review was conducted to identify and compile data collected prior to 2008 for two principal measures of reef health; hard coral and fleshy algae cover. Baseline hard coral and algae cover levels were calculated using data from selected sites that were known to be in healthy condition prior to (or just after) the 1998 bleaching event. Mayotte had the highest mean hard coral cover with 80.9 % (95 % bootstrap confidence interval (95 % CI) =65.8-95.9 %), followed by Comoros with 62.1 % (95 % CI=53.2-78.8 %) and Madagascar with 55.6 % (95 % CI=49.8-62.5 %). Mean fleshy algae cover varied from 8.4 % in Mayotte (95 % CI=2.4-17.4 %) to 35.4 % in Mozambique (95 % CI=20.6-50.8 %). At a regional scale, mean baseline hard coral cover is estimated to be between 41 and 47 %; reefs were in a coral-dominant state, with more than double the amount of coral compared to algae.

Keywords: hard coral cover, algae cover, coral bleaching, baseline data, shifting baselines, coral reef health

Introduction

Data on coral reef health prior to large-scale degradation (circa 1970s/pre-industrial era) are unavailable in most parts of the world (Knowlton and Jackson, 2008; Souter *et al.*, 2021), and therefore true coral reef health baselines remain difficult to establish, undermining efforts to fully evaluate the changes occurring to reefs in the 21st century.

Reef monitoring data are scarce prior to the 1990s in the Western Indian Ocean (WIO) (Ateweberhan *et al.*, 2011) with most monitoring programmes established in response to the catastrophic first global coral bleaching event in 1998 (Obura, 2013). This phenomenon marked a turning point in the health of coral reefs globally, particularly in the WIO, thereby setting a distinct 'line in the sand' to approximate pre-disturbance conditions (Wilkinson, 2000). Bleaching events prior to 1998 were localised and less severe (Goreau *et al.*, 2000), and pollution and sedimentation caused by urbanisation, industrialisation and land-use change were less widespread compared to present-day (Salm, 1983). Moderate-to-high levels of artisanal fishing caused localised damage in intensely fished areas (McClanahan, 1994), but fishing had not resulted in large-scale impacts on reef functioning, conceivably due to lag-effects (Graham *et al.*, 2007) or low compounding interactions with other stressors, which have since accelerated (Ban *et al.*, 2014; Harvey *et al.*, 2018). Therefore, the limited data from 'healthy' sites before 1998 can be used to estimate consistent and robust hypothetical baseline levels of conditions prior to

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1970, before large-scale disturbance and degradation. These baselines can support multiple research and management applications. Baseline levels can be compared with current conditions to improve the understanding of status and trends in reef health, and evaluate the success of management measures (Bruno *et al.*, 2014). Reliable baselines can also help set ambitious and achievable conservation targets that are within natural ranges, thereby solving issues associated with shifting-baselines (Knowlton and Jackson, 2008).

Recent regional and global reef status reports (Obura *et al.*, 2017; Gudka *et al.*, 2018; Souter *et al.*, 2021) and vulnerability assessments (Obura *et al.*, 2021) framed their analysis around two key benthic indicators of reef health; hard coral cover and fleshy algae cover. These variables have been recognised as Essential Ocean Variables (EOVs) because of their importance as standard measures of coral reef functioning, and their extensive historical monitoring records (Miloslavich *et al.*, 2018; Obura *et al.*, 2019). Hard corals construct the reef framework, but sensitivity to marine heat waves, pollution and sedimentation threatens these reef engineers. Fleshy algae (which may include turf, macro and calcareous algae) are an increasingly important taxonomic group to monitor as the main competitor to hard corals for space, particularly following a disturbance event (Nyström *et al.*, 2008; Jouffray *et al.*, 2015; Mora *et al.*, 2016; Brown *et al.*, 2017).

Long term coral reef monitoring in the WIO began in the 1980s and early 1990s. A significant amount of data from this period were either not digitised, remain unpublished, are archived on personal and institutional databases (principally Non-Government Organizations), or are scattered across the grey literature. Efforts to secure this data in coral reef databases, e.g. Coral Reef Monitoring Database (CoReMo) and the Coral Reef Information System (CRIS), unintentionally led to significant losses in access to historical (early) data in the region as these databases became non-operational (Obura, 2013). These factors have led to very few coral reef datasets from the WIO being freely available and accessible, reducing the utility of the data. Renewed efforts to compile regional data and contribute it into regional and global outputs began around 2015 (Obura *et al.*, 2017; Gudka *et al.*, 2018; Souter *et al.*, 2021; Obura *et al.*, 2021), nevertheless, large data gaps still exist, particularly prior to 1998 (as illustrated in Obura *et al.*, 2017). To make historical data accessible to the wider WIO coral reef research and management community, a literature review was conducted to identify, compile and consolidate available (published) data from sites across the WIO for two key indicators; hard coral and fleshy algae cover. The aim of this exercise is to establish a baseline that reflects the state of reefs around 1970, before widespread degradation occurred in the WIO region. Because of the lack of monitoring data from that time, data collected from reefs known to be healthy prior to 2000 were used to approximate this. The main objectives of this paper are to: a) compile site-level historical hard coral and algae cover data in the WIO into a single dataset; b) estimate pre-disturbance baseline levels of hard coral and fleshy algae cover for countries and ecoregions in the WIO; and c) quantify the magnitude of coral mortality in the WIO due to coral bleaching in 1998 using data from reefs monitored before, during and soon after the event.

Materials and methods

Literature review

A systematic literature review was conducted to locate and extract benthic cover data (percent cover) collected through quantitative coral reef surveys (e.g., Line or Point-Intercept-Transects, photo or visual quadrats) and visual estimates. Data were invariably reported as summarised mean cover values. Sources included grey literature (technical reports, books, and book chapters), scientific journal papers (articles and reviews) and project reports (Fig. S1).

Particular attention was given to extracting live hard coral cover and macro and turf algae cover data due to their importance as principal measures of coral reef health, with hard corals recognised as keystone reef builders and fleshy algae as their main competitors for space. These Essential Ocean Variables (EOVs) are used in national, regional and global biodiversity reporting on coral reefs (Bruno *et al.*, 2014; Jouffray *et al.*, 2015; Mora *et al.*, 2016; Gudka *et al.*, 2018; Miloslavich *et al.*, 2018; Bang *et al.*, 2021). Benthic cover data for the following benthic groups was also extracted: dead coral/recently dead coral (publications recorded these differently); calcareous algae (mainly *Halimeda*) and bleached coral; as well as combinations of these categories where reported and relevant (e.g., rock + algae and dead coral + algae).

Most effort was focused on locating data from as early as possible, ideally prior to 1998. However, since significant data gaps still exist for the early 2000s, data was also compiled from surveys conducted up until 2008. The primary focus was to extract site level data, but data aggregated at broader geographic scales was also included (e.g., "northern Kenya"), or other classes (e.g., "unspecified 9 sites (protected)"). In such cases, the number of sites included was noted. Where available, site coordinates were also included in the compiled dataset.

The systematic literature review was conducted through open-access search engines, Google and Google Scholar, using the following search words and terms: coral cover; status of coral reefs; coral monitoring reports; coral abundance; coral bleaching; coral and 1998 El-Nino; coral mortality; and coral communities. Scientific literature databases such as Scopus were not used due to access constraints, and because a significant amount of literature for this region is in grey literature sources not covered by the international scientific resources. The keywords were further refined by including the following additional search criteria:

- Surveyed areas: Global, Western Indian Ocean, Country (e.g., Tanzania), Location (e.g., Pemba Island) and site (e.g., Misali Island)

- Publication period: documents with data that were published between 1960 to 2008.

Publications were reviewed, and data were obtained from tables or in-line text in the main sections as well as supplementary materials and appendices. During the search process, if the data provided by the document was from secondary sources, the source reference with the primary data was located and used instead. A total of over 70 documents were reviewed, from which 58 documents were found to provide relevant data for this study (Table S3, Supplementary Information), with the other publications either having no useful data or data was provided in a way which could not be extracted efficiently, such as in graphs or figures (e.g. van Katwijk *et al.*, 1993; Ballesteros and Afonso-Carrillo, 1995; Johnstone *et al.*, 1998; Muthiga *et al.*, 1998; Wilkinson *et al.*, 1999; McClanahan, 1999). Data were obtained for 10 WIO countries and territories: Kenya, Seychelles, Tanzania, Mozambique, Comoros, Mayotte, Madagascar, Reunion, Mauritius and South Africa (Table 1). No data from Somalia was available.

After reviewing the literature and compiling the data, data were cross-checked for errors, duplication and missing information. Entries with no coral cover values were deleted and those with incomplete information were updated. Data found in more than one publication were highlighted and cross-referenced to the other sources. Locations were ordered hierarchically by Country, Sector, Site and Station. In some cases, the same site or station may have multiple entries for the same year because of surveys of different reef zones or depths, and this information is provided to enable a distinction to be made. The exact date or year of survey was not clear in a few publications, and this has been recorded in the *Year* column as either combined years, e.g. 1998/99, general time period, e.g. mid - 1990s, or if no information is available, as 'n.d'. The full benthic cover dataset is available online (10.5281/zenodo.7949598).

Establishing baseline cover values

Baseline (pre-disturbance) hard coral and algae cover levels were calculated for each country/territory as well as for 10 ecoregions conceptualised by Obura *et al.*,

Table 1. Summary of the percent cover data extracted through a systematic literature review for 10 Western Indian Ocean countries/territories including the total number of data points, sites, literature sources and time-period.

Territory	Literature sources	Sites	Hard coral data points	Algae data points	Date range
Comoros	5	15	27	15	1997-2002
Kenya	23	84	120	24	1970-2004/05
Madagascar	7	25	41	17	1998-2008
Mauritius	3	43	51	52	1999-2002
Mayotte	2	12	27	6	1998-2001
Mozambique	6	37	62	10	1999-2005
Reunion	9	36	73	40	1985-2004/05
Seychelles	8	28	50	19	1994-2004/05
South Africa	6	10	17	-	1993-2005
Tanzania	9	74	94	10	1987-2004/05

2021 (see Fig. 1 for details; there was no data for Madagascar South). First, specific sites and time points were selected as representative of baseline conditions based on the following criteria:

- sites were characteristic of other reefs in the country/eco-region. This meant data from atypical reef habitats were excluded e.g., deeper algal/invertebrate reefs in Lamu, northern Kenya.
- known to be in healthy condition prior to (or just after) the 1998 mass bleaching event, applying the assumption that there were only minor changes in reef benthic composition before the 1998 bleaching event. Data from sites that were known to have been degraded prior to 1998, as reported in the source study or indicated by the data, were excluded.
- only pre-1998 data were used, unless sites sampled shortly after the 1998 bleaching event (1998-2000) had recent dead coral (and/or bleached) cover data collected and there was confidence that the monitoring was conducted using verified quantitative survey methods, and by experienced surveyors able to accurately distinguish and classify these categories at the necessary resolution. Adding the recent dead coral cover to the living cover values enabled estimation of the pre-1998 hard coral cover level.
- care was taken to ensure that where possible there was equal distribution of sites within a country or ecoregion (herein referred to as a geo-unit) and each site was only represented once in the calculation of the baseline.

Using the filtered dataset, the mean hard coral and algae baseline cover was calculated by averaging across all the selected site mean values within an ecoregion or country/territory (geo-unit). Baseline averages are not disaggregated by reef zone, as zonal information was not reported for several sites, and disaggregating by zone would result in too few data points per zone for some geo-units. Most geo-units did not meet normality and symmetry assumptions

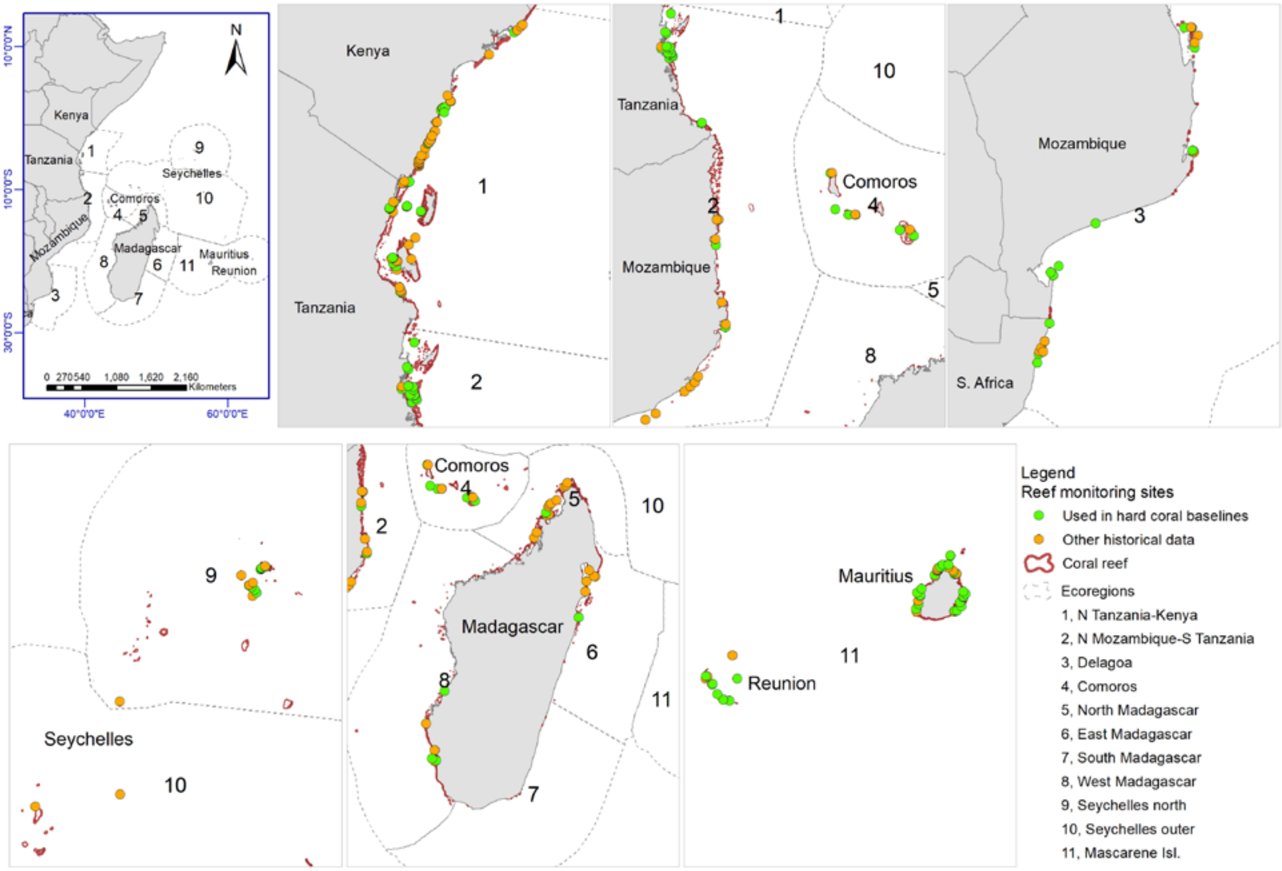

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Figure 1. Monitoring sites across the Western Indian Ocean for which data was extracted from the literature review (orange and green circles). Sites used to calculate baseline averages are shown as green circles. Sites without published location information were assigned approximate coordinates.

and had low sample size, therefore data variability is reported using standard deviation, coefficient of variation (CV, ratio of standard deviation to the mean) and inter-quartile range (Rowland *et al.*, 2021). As an inferential statistic, bootstrap resampling of the indicator mean was performed to provide a 95 % confidence interval using 10,000 iterations (Rowland *et al.*, 2021). The regional mean range (95 % CI) for the WIO was calculated using the same bootstrap resampling method across all the data.

There was less algae data available than hard coral cover, with 162 sites for corals and 101 for algae. All countries/territories and ecoregions (except Madagascar South) had data for hard coral cover calculations, with some of the ecoregions only having a single data point (Madagascar East, and Madagascar North). There was no algae data for South Africa. Where site data was presented as a range (Hardman, 1999), the median value was used for calculations.

For sites with both hard coral and algae data, the algae-coral ratio (ACR) was calculated as ACR = Algae cover/ (Algae + Coral cover) (Bajjouk *et al.*, 2019), and selected site values were averaged to calculate baseline ACR values per geo-unit. This metric provides a useful index for describing the relationship between the competing corals and algae. Using this formula, the values are bound between zero and one, simplifying its interpretation. For this analysis, the percent cover of fleshy algae was a combination of any or all three erect algae types reported in a study (turf, macro and calcareous algae (not including crustose coralline algae)). This follows the regional practice in (Obura *et al.*, 2017; Gudka *et al.*, 2018; Obura *et al.*, 2021) to overcome inconsistencies in the identification and recording of different algae categories across monitoring programmes. Though the algae types have varied ecological functions and interactions with hard coral, with some not always having a competitive role (e.g. algal turfs (< 2 mm)), the combined variable is still considered an important indicator for reef health and productivity, and has been used in other regions (Bachtiar *et al.*, 2019).

Coral mortality during the 1998 El Niño

To calculate the loss in live hard coral cover due to the 1998 bleaching event, sites with hard coral cover data from before and after the 1998 event (up to 2005) were selected. For sites only monitored during or immediately following the bleaching event (1999 and 2000) the pre-98 hard coral cover levels were estimated by adding the bleached and recent dead coral cover to the living cover data (see point 3 in criteria for site selection for baseline estimates). Where sites had more than one data point either before or after the bleaching event, the earliest data (for before) or data collected the soonest after bleaching (for after) was selected. In total, data from 66 sites representing 8 countries and 8 eco-regions across the WIO was compiled.

For each site, the percentage change in hard coral cover was calculated as the *change in cover/the original (pre-98) cover* × *100,* and then all percentage change values were averaged to get national and ecoregional percentage change levels. Additionally, for each period (i.e., before and after bleaching), all site-level hard coral cover data were averaged together to get mean cover levels for each geo-unit. The regional average for the WIO was calculated by averaging across national means of percentage coral cover change to account for biases related to unequal distribution of sites. The results are analysed and presented as geo-unit percentage changes, as well as the number of sites that experienced various levels of coral loss.

Results

For baseline hard coral cover levels (Fig. 2, Table S1, n=162), Tanzania, Mauritius and Kenya had the most data points with 46, 32 and 26 respectively. For eco-regions, Mascarene Islands, N Tanzania-Kenya and N Mozambique-S Tanzania had the most data with 46, 44 and 32 data points respectively (Fig. 3, Table S1). Hard coral cover at individual sites ranged from as low as 7.8 % to as high as 97.2 %.

Mayotte had the highest mean hard coral cover with 80.9 % (n=4, 95 % CI=65.8-95.9 %), followed by Comoros with 62.1 % (n=4, 95 % CI=53.2-78.8 %) and Madagascar with 55.6 % (n=5, 95 % CI=49.8-62.5 %) (Fig. 2). Four countries had hard coral cover levels between 44 and 50 % i.e., Mozambique (n=13), Mauritius (n=32), Reunion (n=14) and Tanzania (n=46). The lowest mean hard coral cover levels in the WIO (below 35 %) were at reef sites in Kenya, Seychelles (n=14) and South Africa (n=4). When aggregated at the eco-regional scale, baseline hard coral cover levels ranged from 30.2 % in Seychelles North (n=10, 95 % CI=24.1-36.5 %) to 71.5 % for Comoros (n=8, 95 % CI= 59.7-84.2 %) (Fig. 3). Mean pre-disturbance hard coral cover is estimated to be between 41.1 % and 46.9 % across the entire WIO.

Fleshy algae data were available for 102 sites, with Mauritius, Kenya and Mozambique comprising 65 % of the

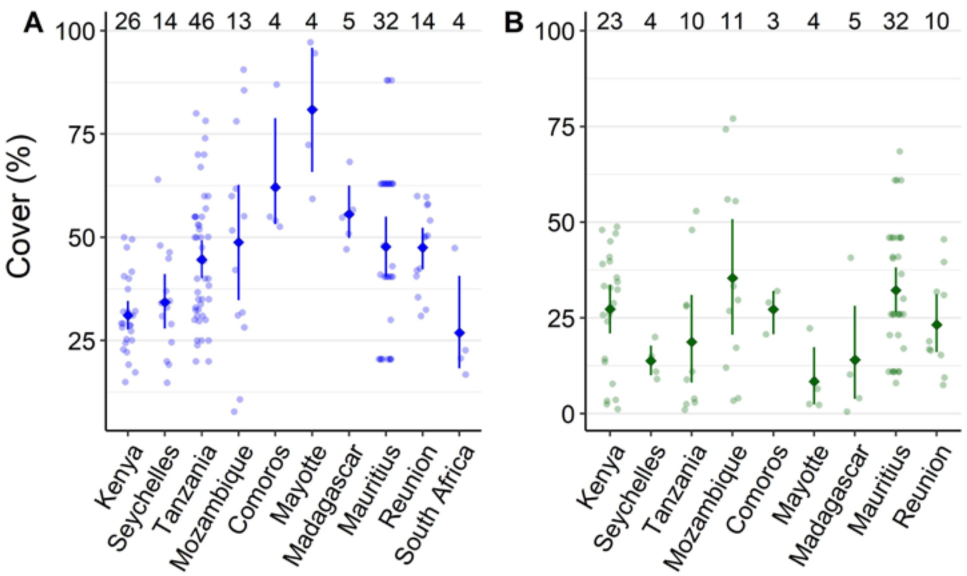

Figure 2. Baseline (pre-disturbance) (A) hard coral cover and (B) fleshy algae cover levels (%) for countries/territories in the Western Indian Ocean (WIO). Error bars represent the upper and lower limit of bootstrapped 95 % confidence intervals of the mean, diamond point represents the mean, and lighter points are individual data points (site means). The number above each bar represents the number of data points. Countries/territories arranged from North to South along x-axis.

data points (Fig. 2, Table S2; n=32, 23, 11 respectively). At the national/territory scale, mean fleshy algae cover varied from 8.4 % in Mayotte (n=4, 95 % CI=2.4-17.4 %) to 35.4 % in Mozambique (n=11, 95 % CI=20.6-50.8 %) (Fig. 2), and aggregated for eco-regions, from 0.5 % in Madagascar East (n=1) to 39.2 % in Delagoa (n=7, 95 % CI=18.4-60.2 %) (Fig. 3). For the WIO, averaging across eco-regional means, mean fleshy algae cover was 18.9 %.

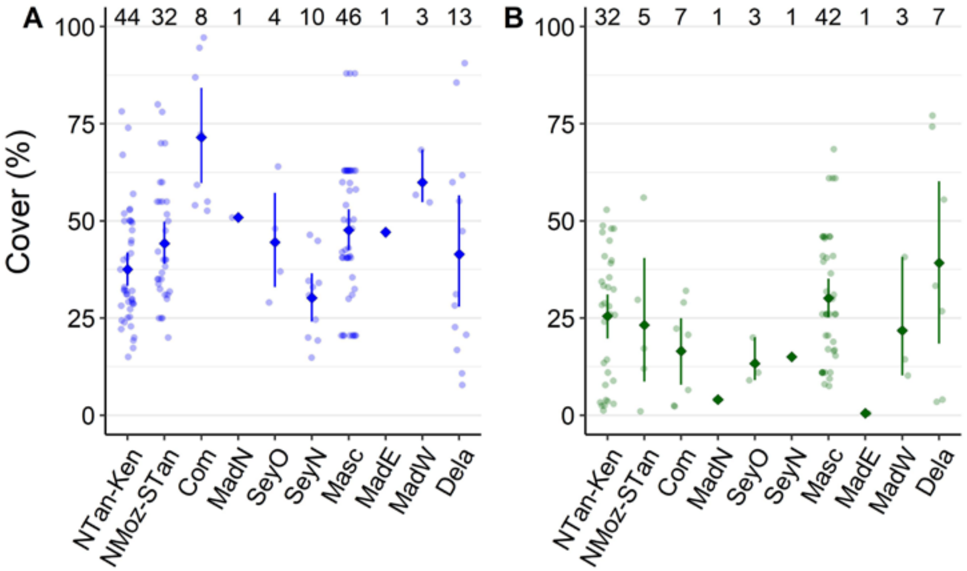

Figure 3. Baseline (pre-disturbance) (A) hard coral cover and (B) fleshy algae cover levels (%,) for 10 eco-regions using sites considered to be in a pre-disturbance state in the Western Indian Ocean (WIO). Error bars represent the upper and lower limit of bootstrapped 95 % confidence intervals of the mean, diamond point represents the mean, and lighter points are individual data points (site means). N represents the number of data points. Eco-regions arranged from North to South along x-axis. Eco-region labels: NTan-Ken – Northern Tanzania-Kenya, NMoz-STan – Northern Mozambique-Southern Tanzania, Com – Comoros, MadN – Madagascar North, SeyO – Seychelles Outer, SeyN – Seychelles North, Masc – Mascarene Islands, MadE – Madagascar East, MadW – Madagascar West, Dela – Delagoa.

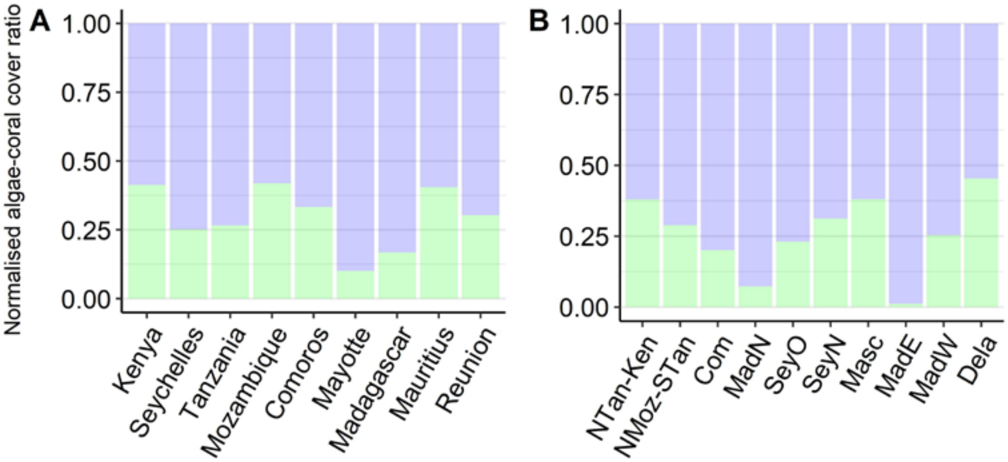

Figure 4. Algae-coral-ratio normalised to the sum of fleshy algae (green) and hard coral (blue) cover for nine countries/territories (A) and 10 eco-regions (B) in the Western Indian Ocean. Calculated using sites considered to be in a pre-disturbance state and with both algae and hard coral data. Countries/territories and eco-regions arranged from North to South along x-axis. Eco-region labels: NTan-Ken – Northern Tanzania-Kenya, NMoz-STan – Northern Mozambique-Southern Tanzania, Com – Comoros, MadN – Madagascar North, SeyO – Seychelles Outer, SeyN – Seychelles North, Masc – Mascarene Islands, MadE – Madagascar East, MadW – Madagascar West, Dela – Delagoa.

When considering the ratio of fleshy algae to hard coral cover, reefs across all geo-units were dominated by hard coral, though algae cover was above 50 % at some sites (Fig. 4, Table S2). Algae-coral ratio ranged from 0.101 (95 % CI=0.024-0.211) in Mayotte (algae cover approximately one tenth that of hard coral), to a highest value of 0.419 (95 % CI=0.252-0.596) in Mozambique, indicating mean algae cover and mean hard coral cover across 11 sites were close to equivalent. For eco-regions, the mean ratio varied between 0.011 for Madagascar East to 0.454 (95 % CI=0.208-0.702) for Delagoa. The mean WIO ratio was 0.258.

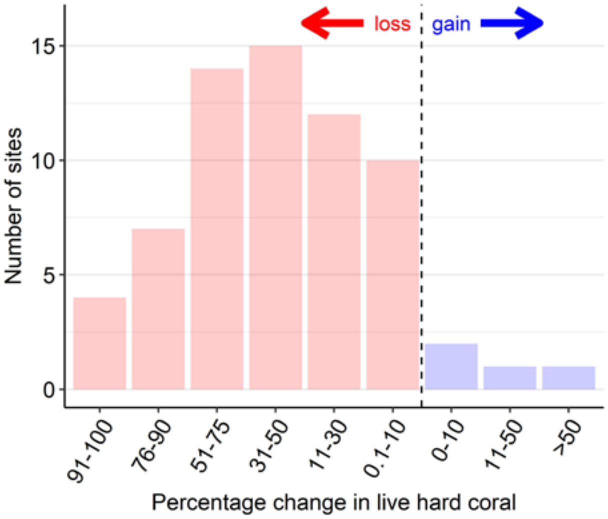

Figure 5. The magnitude of change in live hard coral (%) at sites across the Western Indian Ocean during the mass coral bleaching in 1998. Left of dashed vertical line (red bars) are sites which reported a loss in hard coral cover, and to the right (blue bars) are sites which reported an increase. Total of 66 sites from eight WIO countries/territories.

On average hard coral cover loss in 1998 was 38.6 % (n=66). Thirty-eight percent (38 %) of sites experienced greater than 50 % loss in coral cover, with 16 % of sites experiencing more than 75 % loss and 6 % experiencing more than 90 % loss e.g., the reef flat at Surprise Reef in Mayotte and North St. Pierre in Seychelles (Fig. 5). Overall, 94 % of sites experienced some degradation from the event. Reef sites in Seychelles (n=8), Mayotte (n=6) and Kenya (n=14) experienced the greatest losses in cover, losing on average 70 %, 58 % and 51 % living coral respectively (Table S4, Supplementary Information).

Discussion

Reefs in the WIO have unequivocally been altered over the past 30 to 40 years (Ateweberhan *et al.*, 2011; McClanahan *et al.*, 2014). Increasing pollution, fishing, and temperatures, coupled with inadequate protection have set coral reefs on a path of diminishing returns to society (Cinner *et al.*, 2009; Halpern *et al.*, 2015; Samoilys *et al.*, 2017). The data presented in this paper provides insights into what reef state was likely to have been for different parts of the WIO before various threats began causing widespread degradation.

Reef condition prior to the 1998 El Nino was non-uniform within and across eco-regions, with coral cover varying from below 10 % to above 95 %. Coral dominance was common, with fleshy algae cover less than half that of coral cover in all but 3 eco-regions, with particularly low algae levels in Mayotte, Seychelles, and Madagascar. Reefs in Mayotte had the highest coral cover and the lowest algae cover, though this was from just four sites. When combined with four more sites from Comoros (Eco-region=Comoros), hard coral cover levels for all eight sites were above 50 %. Hard coral cover was highest in the Northern Mozambique channel, reflecting the vibrancy and diversity of this region as the 2nd most biodiverse coral region in the world (Obura, 2012). Baseline estimates use a selection of sites which are assumed to be representative of general reef condition around 1970. Sites were rigorously selected to mainly use data prior to 1998 when reef degradation was less widespread in the WIO, and only sites known or assumed to have had minimal degradation since 1970 were included. This selection of the 'best' sites is an example of space-for-time substitution similar to using pristine or uninhabited reefs as references, and has been used when long-term data is lacking (Sandin *et al.*, 2008; Blois *et al.*, 2013).

There are large differences in the amount of data reported from different parts of the WIO. The most data points were from sites in Kenya (n=120) and the least from South Africa (n=17). Tanzania (n=46), Mauritius (n=32) and Kenya (n=26) had the highest number of sites for which data met the selection criteria to calculate hard coral cover baseline levels. More hard coral cover data was found in published records compared to algae data, an indication of differences in data collection effort between the two taxonomic groups. Confidence in the results (baseline means) varies based on the amount of data and its spread, but encouragingly the Coefficient of Variation (CV) is less than 1 for all hard coral cover and algae-coral ratio estimates, except for the algae-coral ratio for Mayotte (CV =1.168). Delagoa, Seychelles North, Mascarene Islands, N Mozambique-S Tanzania and N Tanzania-Kenya, have the most robust results, due to a sufficient and/or well-represented sample size (in terms of distribution of sites; CV ranging from 0.36-0.66 and n= 10-46). However, ranges could not be established for three of Madagascar's eco-regions due to low or no sample sites. Various methods were tried to establish a range of values to account for the natural variation between sites, and the confidence intervals (95 %) calculated from bootstrapping provide more precise estimates of baseline levels, particularly for geo-units with higher sampling e.g., Kenya. The assertion is that principal coral habitats on the majority of reefs in a geo-unit would have once fallen within this range assuming low to no levels of degradation.

Some caveats exist with the process described in this paper. Although a thorough search for literature containing suitable data was conducted, invariably these efforts were not exhaustive. There are other datasets which exist but remain unpublished, hidden or have only been presented as aggregations in the literature, minimising their suitability for these and other purposes. Baseline averages are not disaggregated by reef zone, as zonal information was not available for some sites, resulting in too few data points for some geo-units. This is consistent with the protocols of other recent regional analyses (Obura *et al.*, 2017; Gudka *et al.*, 2018; Obura *et al.*, 2021). An element of subjectivity with the method comes from determining the sites reflecting pre-disturbance conditions for each geo-unit, and consequently the exclusion of sites that had already degraded prior to 1998. This was mainly based on expert judgement of threatening processes and health of reef sites, and interpretation of the data or site descriptions in the source literature. For example, data from 1970 and 1992 for Diani on the south-coast of Kenya were not included as the sites were damaged by overfishing and high urchin populations in the decades preceding the reference year 1998 (Khamala, 1971; McClanahan and Muthiga, 1988), which is clear from the low hard coral cover of between 1 and 7 %. Data from very specific habitats which are not representative of the regional reef system were also excluded e.g., data collected in 1987 for offshore reefs in Lamu, Kenya by Samoilys (1988). This selection protocol was consistently followed, resulting in some low hard coral cover sites being included as these conditions were deemed to be normal for the site (or area) and not due to prior degradation or disturbance (e.g., Mike's Cupboard in Inhambane, Mozambique). Data were included as much as possible to increase the sample size.

Recent benthic cover values published in regional reports (Table 2), allow for some interesting comparisons to suggest how reef state may have changed over the last two to three decades. At a regional scale, average pre-disturbance hard coral cover is estimated to be between 41 and 47 %. Prior to the 2016 bleaching event, hard coral cover averaged across over 130 sites (though only representing 6 WIO countries) was estimated to be around 41 %, which dropped to 33 % post-bleaching (Gudka *et al.*, 2018). Obura *et al.* (2017) reported mean WIO coral cover at around 30 % between 2010-2015. Though these values are not directly comparable due to differences in methods and data, it tentatively indicates that today's coral cover is lower than what it once was, though with high variation across and within geo-units. Tanzanian reefs have apparently changed the least, with recent levels being similar to baseline levels calculated here, of 30-45 % for hard coral cover and 12-20 % for fleshy algae cover (Table 2). South African reefs show a gradual decline in living hard coral consistent with the regional reports, but inconsistent with some site-based studies (Porter and Schleyer, 2017). Baseline levels are very close to values prior to the 2016 bleaching event for both Seychelles' eco-regions, supporting the notion that reefs had recovered to near pre-98 levels (Robinson *et al.*, 2019). For Reunion, both algae and coral levels ranged between 40-50 % in 2014/15, indicating that hard coral cover had remained quite stable since baselines (though more recent bleaching and extreme-tides have caused significant coral mortality (Nicet *et al.*, 2017)), but algae may be on an upward trajectory. Mauritius had a coral cover of ~50 % with corresponding algae levels of ~20 % in 2015 (Obura *et al.*, 2017), though the data in this study is from an extensive rapid assessment of the entire Mauritius Island, making direct comparisons imprecise. For Madagascar, average hard coral and algae cover was reported as 30 % and ~45 % in 2015 respectively (in Obura *et al.*, 2017), and 45 % and 14 % in 2017, respectively (from 14 sites, W and North Madagascar) (Gudka *et al.*, 2018), indicating an inter-site disparity in current reef health relative to baseline levels. In Kenya, reef benthic state may not have departed drastically from historic conditions, with average hard coral cover ranging from 18-31 %, and algae at around 30 % between 2013-2017. The condition of reefs in Grande Comore and Mohéli (Comoros) in 2010 and 2016 was highly variable, with live coral cover ranging between 6 % and 60 % (Cowburn *et al.*, 2018). The comparisons tentatively indicate that there may have been substantial recovery at several sites across the region after the 1998 bleaching event, though compositional and functional changes in benthic communities are not revealed through the aggregated indicators used in these studies. In order to ascertain clear trends on reef health, it is imperative that data collection standards are improved to collect data at higher taxonomic resolution consistently (e.g. coral genera or species level), and reporting includes non-aggregated indicators.

The impact of the first global bleaching event in 1998 was considerable on WIO reefs, with reefs in

Table 2. Hard coral and fleshy algae cover i) baseline values calculated in this study (95% confidence intervals from bootstrapping), ii) values prior to (Pre-2016) and after (Post-2016) the 2016 bleaching event from regional reports (a-Obura *et al.*, 2017, b- Gudka *et al.*, 2018). All post-2016 records are from Gudka *et al.* (2018).

Geo-unit	Variable	Baseline (95% CI)	Pre-2016	Post-2016
Comoros	Hard coral	62.1 (53.2-78.8)	55 (2007)[b]; 64 (Fore), 40 (back)[a]	55
	Fleshy algae	27.2 (20.7-32.0)		<5
Kenya	Hard coral	31.1 (27.6-34.7)	31[b]; 18-25[a]	25-27
	Fleshy algae	27.3 (20.9-33.5)	30[b]; <20[a]; 34[a]	~30
Madagascar	Hard coral	55.6 (50.1-32.5)	30[a]; ~50[b]	~45
	Fleshy algae	14 (3.8-28.1)	~45[a]; 9[b]	14
Mauritius	Hard coral	47.7 (40.5-54.8)	50 (2002)[a]; 20 (2010) [a]; ~50 (2015)[a]	35
	Fleshy algae	32.2 (26.4-38.2)	~20 (2015)[a]	
Mozambique	Hard coral	48.8 (35-62.8)	~22[a]	
	Fleshy algae	35.4 (20.6-50.8)		
Reunion	Hard coral	47.5 (42.1-52.4)	40-50[a]	
	Fleshy algae	23.2 (16.1-31.2)	40-50[a]	
Seychelles Inner (N)	Hard coral	30.2 (24-36.5)	33[b]; 42[a]	13
	Fleshy algae	15		
Seychelles Outer	Hard coral	44.5 (33-57.2)	44[a]	37
	Fleshy algae	13.3 (9.0-20.0)		
South Africa	Hard coral	26.9 (18.3-40.7)	~18[a]	16.9-20
	Fleshy algae			
Tanzania	Hard coral	44.6 (40.1-49.3)	44[b]; 30-45[a]	40
	Fleshy algae	18.7 (8.1-31.0)	12; ~15-16	15-20

Seychelles, Mayotte and Kenya the most impacted. On average across the WIO, sites lost close to 40 % living coral. This value does not represent an actual mean regional loss in hard coral cover as there is unequal representation of sites across space. However, it does corroborate the severe impact of this event reported elsewhere (Wilkinson, 2000; Ateweberhan *et al.*, 2011; Obura *et al.*, 2017), though the coral loss values vary because of differences in methodologies. Obura *et al.* (2017) estimated the decline to be approximately 25 % based on a relatively small pre-1998 data sample, Ateweberhan *et al.* (2011) estimated a loss of ~45 %, and Wilkinson (2000) stated that some sites lost between 50-80 % live coral resulting in an overall hard coral loss of 16 % in the WIO.

The baseline levels and the wider data compiled for these two key ecological components offers numerous uses for management and research. Baseline levels provide a useful benchmark for current states and combined with time-series data can identify the trajectory of reefs towards or away from possible phase-shifts. Marine Park managers can commission repeat surveys at sites to enable comparisons over decadal timescales enabling evaluations of the effectiveness of past and current interventions. Baseline information can be used to set realistic objectives for new interventions based on historical ecological limits (carrying capacity of community) to ensure they are achievable (McQuatters-Gollop *et al.*, 2019). As demonstrated by the WIO coral reef RLE (Obura *et al.*, 2021), the data can be incorporated into frameworks or other analyses that provide policy or management relevant results.

As reef condition continuously changes, there is heightened need for more, and better-quality data to be made available for management and research. Scaled national investment in long-term, high-resolution monitoring is recommended through regional entities like the Global Coral Reef Monitoring Network (GCRMN) nodes and Nairobi Convention Coral Reef Task Force. Project donors are also encouraged to enforce strict data publishing measures that follow the FAIR principles (Findable, Accessible, Interoperable, and Reusable) (Wilkinson *et al.*, 2016), but researchers are also requested to voluntarily avail data particularly for data-deficient regions as well as other key taxonomic groups such as fish and urchins. Coupled, this will enable historical baseline conditions to be calculated at a higher precision across more ecoregions in the WIO, and using other variables, as well as to trace trends in reef health over time, particularly after acute disturbance events. It is the belief of these authors that the prospects of conservation are greater with open data practices than without them.

Acknowledgements

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Supplementary material

Provided in separate PDF file

Table S1. Baseline (pre-disturbance) hard coral cover levels (%) for 10 countries/territories (top) and 11 eco-regions (bottom) in the Western Indian Ocean (WIO).

Table S2. Baseline (pre-disturbance) fleshy algae cover levels (%) with comparative (same sites) hard coral cover levels (%) and algae-coral-ratios for 9 countries/territories (top) and 11 eco-regions (bottom) in the Western Indian Ocean.

Table S3. List of all publications where data was extracted from.

Table S4. Change in hard coral cover (%) during 1998 bleaching.

Figure S1. Types of publication from which the data was extracted (n=58).

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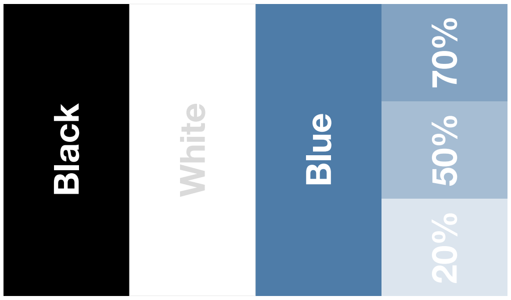

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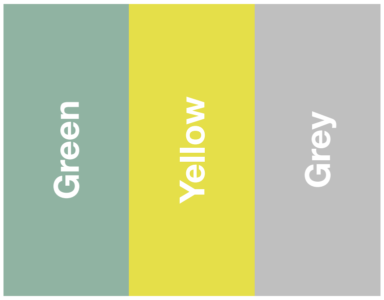

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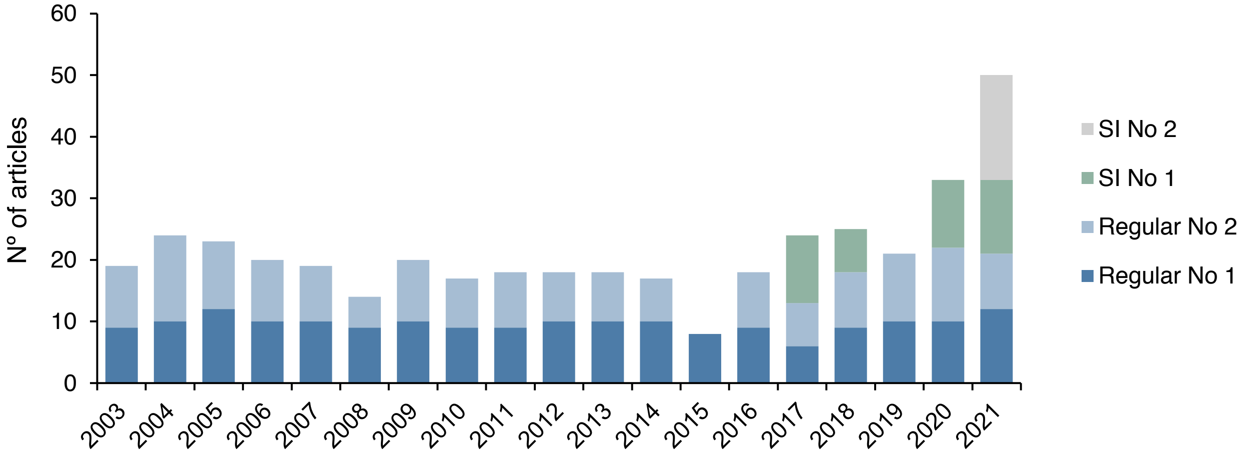

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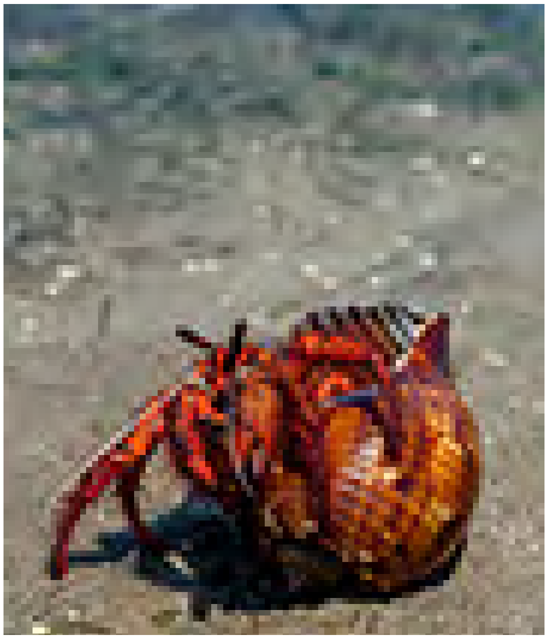
Photographs

Photographs should have good quality/resolution to maximise contrast and detail during printing (15cm longest edge @300 dpi), be focused and well composed.



Example of low resolution

not enough for printing

Example of high resolution

good for printing

Maps

Maps must have good resolution for efficient viewing and good reading

Example of low resolution

not enough for printing

Example of high resolution

good for printing

The Western Indian Ocean Journal of Marine Sciences is the research publication of the Western Indian Ocean Marine Science Association (WIOMSA). It publishes original research papers or other relevant information in all aspects of marine science and coastal management as articles, reviews, and short communications (notes).