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Cover image: Installing fish traps on a shallow seagrass meadow at Inhaca Island (Southern Mozambique) © José Paula, 2009

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Short Communication

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A first assessment of marine litter on a beach of an uninhabited island in the Mozambique Channel

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Abstract

Marine litter is ubiquitous and associated with both ecological and socio-economic consequences. Beaches are major sinks of marine litter and as such its assessment and monitoring are needed. An opportunistic marine litter survey was performed for 12 consecutive days on the island of Juan de Nova in the central Mozambique Channel in February 2007. Plastic dominated the marine beach litter with daily accumulation of plastic positively related to the tide height ($R^2 = 0.768$, $p<0.01$). Annual deposits could reach an average of 1 $t.km^{-1}$, suggesting that regular cleaning of the coastline should be conducted to limit the impact on the local wildlife of this protected area.

Keywords: marine debris, plastic pollution, daily accumulation rate, Indian Ocean, Juan de Nova

Introduction

Marine litter consists of any persistent, manufactured or processed solid items deliberately discarded or accidentally lost, and that end up in the marine environment (UNEP, 2009). Plastics dominate marine litter comprising at least 85 % of total waste (UNEP, 2021). The amount of marine litter and plastic pollution has been growing rapidly and therefore has become one of the biggest challenges in the environment worldwide (Geyer *et al.*, 2017). Emissions of plastic wastes into aquatic ecosystems are projected to reach approximately 12,000 Mt by 2050 without meaningful action (Geyer *et al.*, 2017; UNEP, 2021). The scale of marine litter and plastic pollution are putting the health of the world ocean at risk, and as such their monitoring has become crucial to improve mitigation strategies (Galgani *et al.*, 2019).

Marine beach litter is of great concern as it forms one of the main sinks of marine litter (Onink *et al.*, 2021), it can cause damage to coastal wildlife (Kühn *et al.*, 2015) and has socio-economic consequences (Newman *et al.*, 2015). On beaches, most studies have demonstrated densities in the range of 1 $item.m^{-2}$ except for very high concentration due to local conditions (Galgani *et al.*, 2015). Marine beach litter found at a given inhabited site is often of local origin, but in remote uninhabited islands, debris accumulating on the coast is usually transported over long distances by ocean currents (Onink *et al.*, 2021; Vogt-Vincent *et al.*, 2023). The regular monitoring of litter accumulation on beaches is the most common approach to reveal long-term patterns, cycles of accumulation and its origin (Ryan *et al.*, 2009; Pieper *et al.*, 2021).

Varying methods are used to investigate and quantify marine litter, which makes it difficult to draw a quantitative global picture of beach litter distribution (Galgani *et al.*, 2015; Smith and Turell, 2021) and the total amount of beached plastic debris (Onink *et al.*, 2021). At the scale of the beach, depending on the characteristics of various abiotic factors like wind or tide, the accumulation of the different components of the litter can vary drastically (Cesarano *et al.*, 2023).

In addition, the sampling frequency can significantly influence estimates of the rate of litter accumulation (Smith and Markic, 2013; Ryan *et al*., 2014; Barnardo *et al*., 2020).

Marine litter is not limited to areas that are densely populated, but it reaches the most remote parts of the world, including uninhabited islands (Ryan and Watkins, 1988; Barnes *et al*., 2009; Onink *et al*., 2021). Due to its ubiquity in oceanic and coastal ecosystems, efforts to document marine litter are necessary to contribute to a better understanding of its sources, sinks and characteristics, and to predict trends, and ecological and socio-economic impacts. The objective of this study was to contribute to the global effort of marine beach litter survey by documenting its accumulation on a beach of a remote uninhabited coralline island of the central Mozambique Channel, in the southwest Indian Ocean.

Material and methods

Study site

During a field trip at Juan de Nova (Fig. 1) for seabird monitoring, an opportunistic and exploratory marine litter survey was conducted for 12 consecutive days, between the 10th and the 21st of February 2007, during the third quarter and the new moon phases, in the morning at the timing of the low tide in this isolated and uninhabited island. Juan de Nova lies in the central Mozambique Channel (17°03.15S and 42°43.24E), 150 km west of Madagascar and 300km east of Mozambique, with an area of 5 km^2, 200 km^2 of coral reef and a maximal elevation of 12 m. The island is located in the narrowest part of the Mozambique Chanel, where large-scale flows are strong but highly variable leading to the formation of mesoscale eddies (Ridderinkhof and de Ruitjer, 2003), which can favour the temporal retention of waters and the aggregation of particles. The tide is semi-diurnal and the amplitudes are

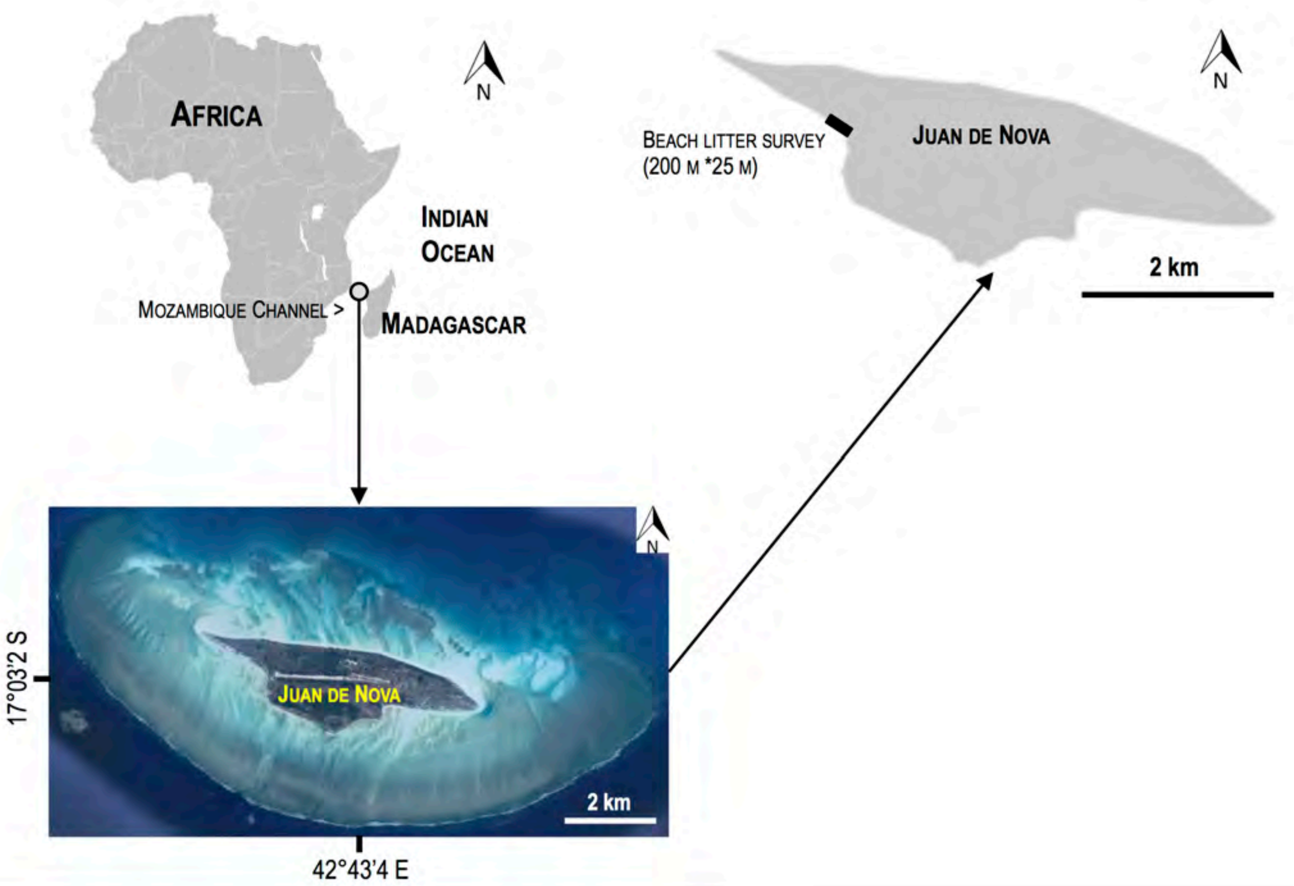

Figure 1. Map of the study area, with the location of Juan de Nova island in the Mozambique Channel, satellite view of Juan de Nova with the coral reef barriers surrounding the island, and position of the transect of marine beach litter survey.

high in the region (Chevane *et al.*, 2016) and this leads to large tidal movements. Juan de Nova constitutes, along with Europa, Glorioso archipelago, Tromelin and Bassas da India, the Scattered Islands, a district of the French Southern and Antarctic Lands (TAAF) administration. The island has been largely uninhabited for the last 50 years, except for military and scientific personnel (<20 persons), which highly limits local sources of pollution, as all wastes are regularly evacuated from the island by boat and plane (TAAF, 2015).

Sampling

After initial cleaning (i.e. Day Zero clean-up) along a 200 m transect located on the west coast of the island in front of the largest part of the coral reef (2.2 km), all manufactured items that could be found at the surface of the sand (above 5 mm in size) were collected between the lower limit of the beach and the mark of the higher tide (~25 m). All collected pieces of litter were classified in 2 main categories (artificial polymer materials and metals) and 19 subdivisions according to their nature and size. This protocol was close to the standard protocols developed for the macro-litter monitoring along shorelines for African marine litter monitoring (Barnardo *et al.*, 2020).

Data analyses

The standing-stock was calculated as the total number of items per 100 meters of beach. Then the daily accumulation rates in number and density of items per 100 m^2 and 100 meters of beach were then calculated for the different categories of wastes and their subdivisions. For this, the daily number of items collected was divided by the transect surface (5000 m^2) and length (200 m), and then reported both by 100 m^2 and 100 meters to standardize the results and allow comparison with other studies (Barnardo *et al.*, 2020). The mean daily accumulation rate in number of items was related to the high tide height during the survey using a linear model.

The annual amount of marine beach litter deposit was estimated at the scale of the island, based on the daily minimum and maximum of items collected during the survey along the transect. As the tide and the ocean currents in the vicinity of the island had an influence on the deposition of items all along the shoreline, two scenarios were considered, with a uniform deposition rate and with a non-uniform deposition rate. In this last scenario, the same deposition rate as in the uniform deposition scenario was used for half of the shoreline (6 km) and half of the deposition rate was used for the other part of the island (6 km). These values were extrapolated to the whole island to obtain a daily number of items for the island, considering 12 km of shoreline. Then the daily mass of litter for the island was estimated with an average mass of marine beach litter item of 15.6 g (Smith and Turell, 2021; Meakins *et al.*, 2022). Finally, this daily estimate was used to calculate a theoretical annual deposit.

Results and discussion

At day zero the standing-stock was of 7 items.100 m^{-1} of beach and the number of daily items collected during the 11 other days ranged from 0.5 to 42.5 items.100 m^{-1}. As the standing-stock fell within the range of daily items, data from day zero were added in all following analyses. A total of 436 items of marine beach litter were collected during the 12 days survey (Table 1), with a daily range of 1-85 items and a mean (± s.d.) daily number of 36.33 ± 30.60 items. The mean daily density for all categories of items (0.727 ± 0.612.100 m^{-2}) and for each item were lower than the densities generally observed for the marine beach litter (Galgani *et al.*, 2015). With two exceptions (rubber ball and aluminium foil), the litter was exclusively composed of plastic items of different size, nature and use. Plastic fragments of different sizes dominated the litter and plastic caps, plastic strapping bands, and plastic fishing net and ropes were regularly collected. All other items were rarely collected, with less than one item accumulated per day. Most collected items were eroded, suggesting that they spent several days at sea, and that they were not from a local land-based source. The very low standing-stock confirmed that there was no local source for this litter. The dominance of plastic in marine beach litter is common and support global data reports (UNEP, 2021), but also regional surveys both in Eastern Africa (Okuku *et al.*, 2021; Meakins *et al.*, 2022) and islands of the Western Indian Ocean (Duhec *et al.*, 2015; Bouwman *et al.*, 2016; Dunlop *et al.*, 2020; Mulochau *et al.*, 2020). The overall density of items in Juan de Nova was low compared to beaches of South Africa (Meakins *et al.*, 2021) and Kenya (Okuku *et al.*, 2021), and slightly lower than in the Seychelles (Duhec *et al.*, 2015; Dunlop *et al.*, 2020) and Saint Brandon Rocks in Mauritius (Bouwman *et al.*, 2016). This lower density of debris on the beach of Juan de Nova can be explained by the fact that the island is uninhabited, the survey was conducted in 2007 earlier than in other sites at a period when plastic pollution was slightly lower, and the circulation in the Mozambique Channel with the mesoscale eddies, which could trap and concentrate marine litter, therefore possibly limiting their deposit on beaches of Juan de Nova.

Table 1. Summary of marine beach litter composition, amount and daily accumulation rate of the number of items and density per 100 m and 100 m^{-2} during the study period.

	Total Number	Mean daily number ± s.d. (items.100 m^{-1})	Mean daily density ± s.d. (items.100 m^{-2})
TOTAL	**436**	**18.16 ± 15.30**	**0.727 ± 0.612**
Artificial polymer materials	**435**	**18.12 ± 15.35**	**0.725 ± 0.612**
Plastic fragments (<1cm)	137	5.71 ± 7.67	0.228 ± 0.307
Plastic fragments (1-5 cm)	145	6.04 ± 5.38	0.242 ± 0.215
Plastic fragments (>5-10 cm)	59	2.46 ± 2.52	0.098 ± 0.100
Plastic fragments (> 10 cm)	1	0.04 ± 0.14	0.001 ± 0.005
Plastic bottles	4	0.17 ± 0.25	0.006 ± 0.009
Plastic caps	21	0.87 ± 1.17	0.035 ± 0.047
Plastic strapping bands	12	0.50 ± 1.30	0.020 ± 0.052
Plastic fishing net and ropes < 50 cm	22	0.92 ± 0.95	0.037 ± 0.038
Plastic containers	7	0.29 ± 0.33	0.011 ± 0.013
Plastic bags	4	0.17 ± 0.25	0.007 ± 0.009
Plastic flip-flops	7	0.29 ± 0.50	0.012 ± 0.019
Plastic syringes	1	0.04 ± 0.14	0.001 ± 0.005
Plastic pens	1	0.04 ± 0.14	0.001 ± 0.005
Plastic pen caps	4	0.17 ± 0.32	0.006 ± 0.013
Plastic dowels	1	0.04 ± 0.14	0.001 ± 0.005
Plastic cutlery	1	0.04 ± 0.14	0.001 ± 0.005
Foamed polystyrene fragments < 50 cm	6	0.25 ± 0.5	0.010 ± 0.020
Rubber balls	1	0.04 ± 0.14	0.001 ± 0.005
Metal	**1**	**0.08 ± 0.29**	**0.001 ± 0.005**
Aluminium foil	1	0.08 ± 0.29	0.001 ± 0.005

There was a strong positive relationship between the accumulation rate of marine beach litter during the survey and the tide height (Fig. 2). Marine beach litter accumulation reflects the long-term balance between inputs, land and ocean-based sources and outputs from various forms of export. Among factors influencing the densities of beach debris is the tide (Ryan *et al.*, 2009; Galgani *et al.*, 2015), though the relation between accumulation of marine litter on beach and tide is not always directional. In the case of Juan de Nova, the absence of land-based sources and standing stock in the sand suggests that the tide has a strong influence on the deposition of marine litter. The large coral reef ecosystem surrounding the island could act as a barrier for marine litter during lower tide height. In addition, during lower tide height, the surface of beach covered by the sea is smaller leading to a smaller surface for deposition.

The annual deposition of marine litter on the shore of Juan de Nova ranges between 0.25 and 29 t, based on the daily accumulation recorded during the survey, and depending of the deposition rate scenario (Table 2). This estimate is based on a limited number of observations that did not cover the full range of tidal heights, does not consider any other environmental factors that influence marine litter deposit, and does not consider possible resuspension rates. Onink *et al.*, (2021) simulated global marine plastic transport and highlighted coastlines and coastal waters as important reservoirs of marine plastic debris with limited transport of this debris between coastal zones and the open ocean. This suggests a limited loss of debris from the coastal zone of Juan de Nova, and increases the probability of debris being ultimately stranded, though some could remain trapped in the coral reefs (Mulochau *et al.*, 2020). In addition, daily sampling has been proposed to be a good approach for accurate quantification of available debris in coastal habitats and to estimate their accumulation on beaches (Smith and Markic, 2013; Barnardo *et al.*, 2020). Thus, this study provides a realistic order of magnitude estimate of the annual amount of marine litter stranded on Juan de Nova in 2007.

Conclusion and perspectives

This was a first assessment of marine beach litter on a small island of the central Mozambique Channel, in a region where few studies have been conducted (Connan *et al.*, 2021) and in which the human population and economic development are rapidly increasing. In addition, the western part of the Indian Ocean is suspected to be a major concentration area in an

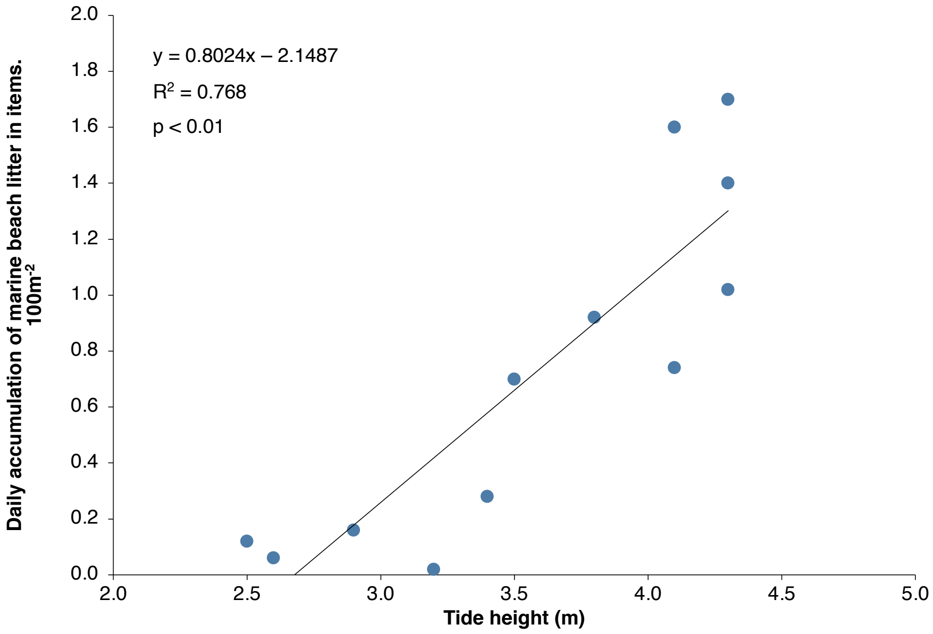

Figure 2. Relation between the tide height and the daily accumulation on marine beach litter at Juan de Nova Island in February 2007.

ocean basin that could harbour the second largest concentration of plastic of the world ocean (Erisken *et al.*, 2014; van der Mheen *et al.*, 2019). On the uninhabited island of Juan de Nova, marine beach litter was present and dominated by plastic. Although the survey was conducted in 2007, the estimated annual amount of debris stranded on the island was already significant for an uninhabited and isolated small island, with an average of ~1 $t.km^{-1}$. Most of marine litter in the western Indian Ocean originates from Asia (Vogt-Vincent *et al.*, 2023). As release of plastics in the sea has increased and should continue to increase in the future in Asia (UNEP, 2021), this suggests that the amount of marine litter deposited on Juan de Nova would increase in the future. As the island is a protected area with remarkable biodiversity, this marine litter likely has consequences on species both at sea and on land, which should be evaluated. In the future, it will be useful to monitor the marine beach litter based on standard methods to allow comparison with other studies, evaluate the long-term evolution of deposits and to clean up beaches to remove this pollution from the natural environment to limit it impacts on biodiversity. The TAAFs administration should strengthen controls on compliance with the regulations on waste management by fishing and transport vessels to limit the illegal dumping of waste at sea in the Economic Exclusive Zone of Juan de Nova as debris associated with fisheries and transport are a major risk in the western Indian Ocean (Vogt-Vincent *et al.*, 2023). New monitoring at the same site and at other uninhabited islands of the region will help to better understand the dynamics of plastic pollution in the western Indian Ocean.

Table 2. Estimation of the annual deposit of marine beach litter on the 12 km of shoreline of Juan de Nova Island assuming a uniform and a non-uniform rate of deposition around the island (see method).

	Uniform deposition rate	Non-uniform deposition rate
Daily minimum number of item deposit	60	45
Daily maximum number of item deposit	5 100	3 825
Daily minimum mass of item deposit (kg)	0.936	0.702
Daily maximum mass of item deposit (kg)	79.560	59.670
Annual minimum mass of item deposit (ton)	0.342	0.256
Annual maximum mass of item deposit (ton)	29.034	21.779
Annual mean mass of item deposit (ton)	12.299	9.224

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Original Article

Age, growth and mortality of the thumbprint emperor (*Lethrinus harak*) in Zanzibar

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Abstract

The growth parameters of *Lethrinus harak* from Zanzibar were studied from 308 samples collected between June 2019 and May 2020 at Unguja Ukuu and Mkokotoni landing sites. Growth rings counted on whole otoliths formed annually, with opaque margins generated in December to February and hyaline rings in March to November. Ring counts revealed that most fish were between three and four years old, with mean lengths of 17.7 and 21.4 cm, respectively. Some specimens were five to seven years old, measuring 24.2 to 28.2 cm, on average. The growth parameters were estimated as L_∞ = 34.22 cm, K = 0.25 year^{-1}, and t_0 = 0.00. The total mortality (Z) was estimated as 0.68 year^{-1}, natural mortality (M) was 0.65 year^{-1} using Pauly's equation, and fishing mortality (F) was 0.03 year^{-1}. The low estimate of exploitation rate (E) of 0.04 year^{-1} was almost certainly an underestimate, resulting from the absence of older (>8 years) large individuals, and younger (<3 years) small individuals in samples.

Keywords: *Lethrinus harak*, growth parameters, mortality rate

Introduction

Coastal communities in developing nations rely heavily on coral reef fisheries for their livelihood and food security (Johnson *et al.*, 2013). Coral reef habitats are, however, threatened by human activities and climate change (Cinner *et al.*, 2012; Lachs and Oñate-Casado, 2020). In East Africa, human-induced pressure on coral reefs has been well documented (Wilkinson, 2008; Hoegh-Guldberg, 2011) and the associated loss of ecosystem services linked to the coral reefs is of concern (Cinner *et al.*, 2011). Inadequate monitoring and sparse scientific data on key exploited species hinder management and conservation efforts in the region.

Local communities in Zanzibar are heavily dependent on small-scale fisheries for their livelihoods (Jiddawi and Khatib, 2007; Jeppesen and Richmond, 2016). Small-scale fisheries of Unguja Island are representative of the multi-species artisanal fisheries common along the eastern African coastline, with few commercial fleets present near the coast. Fisheries are characterized by monsoon cycles, and inshore fisheries using multiple gear types land mostly reef-associated species (e.g., Siganids, Lethrinids, Octopus), small pelagic fishes (e.g., Engraulids) and larger pelagic species (Scomberids) (Jiddawi and Öhman, 2002). Depletion of fishery resources in Tanzania has been attributed to overfishing and use of destructive fishing methods such as beach seine, stick and spear fishing and poison, locally known as *juya*, *mkuki/kijiti* and *utupa* respectively (Jiddawi and Öhman, 2002; Khatib and Jiddawi, 2010).

Landings of emperors have increased annually in parts of the Western Indian Ocean, because of high demand (Kulmiye *et al.*, 2002; Vasanthrajan *et al.*, 2012). The commercial importance of Lethrinids have resulted in several studies of their life history (Grandcourt, 2002; Kulmiye *et al.*, 2002; Pilling *et al.*, 2003; Ebisawa, 2006; Ebisawa and Ozawa, 2009; Currey *et al.*, 2013; Trianni, 2016). Despite global efforts to assess the stocks of Lethrinid species, such information is lacking in Tanzania.

The thumbprint emperor (*Lethrinus harak*) is a widely distributed tropical reef species (Midway *et al.*, 2018) and an important food source in Tanzania (Kimirei *et al.*, 2013; Midway *et al.*, 2018). Improved management is however required to facilitate sustainable fishing practices. Age, growth rate, and mortality rate are three of the most influential life history characteristics controlling the productivity of fish populations (Mendoza, 2006). This information is important in assessments of stock status, on which management strategies typically rely (Ebiswa and Ozawa, 2009). The aims of this study were to use catch-at-length data and analysis of otolith ring counts to estimate the age distribution of *L. harak*, growth and mortality rates (Pilling *et al.*, 2003; Vieira, 2023).

Methodology

Study site and sampling

This study was conducted in Zanzibar (Zanzibar archipelago, Tanzania) (Fig. 1). Fish samples were obtained at landing sites on the southern (Unguja Ukuu landing site) and northern coasts (Mkokotoni landing site) of Unguja Island. A total of 308 *L. harak* specimens were randomly purchased from fishers over a 12-month period from June 2019 to May 2020. Fish samples were immediately frozen after purchase and transferred to a laboratory for analysis.

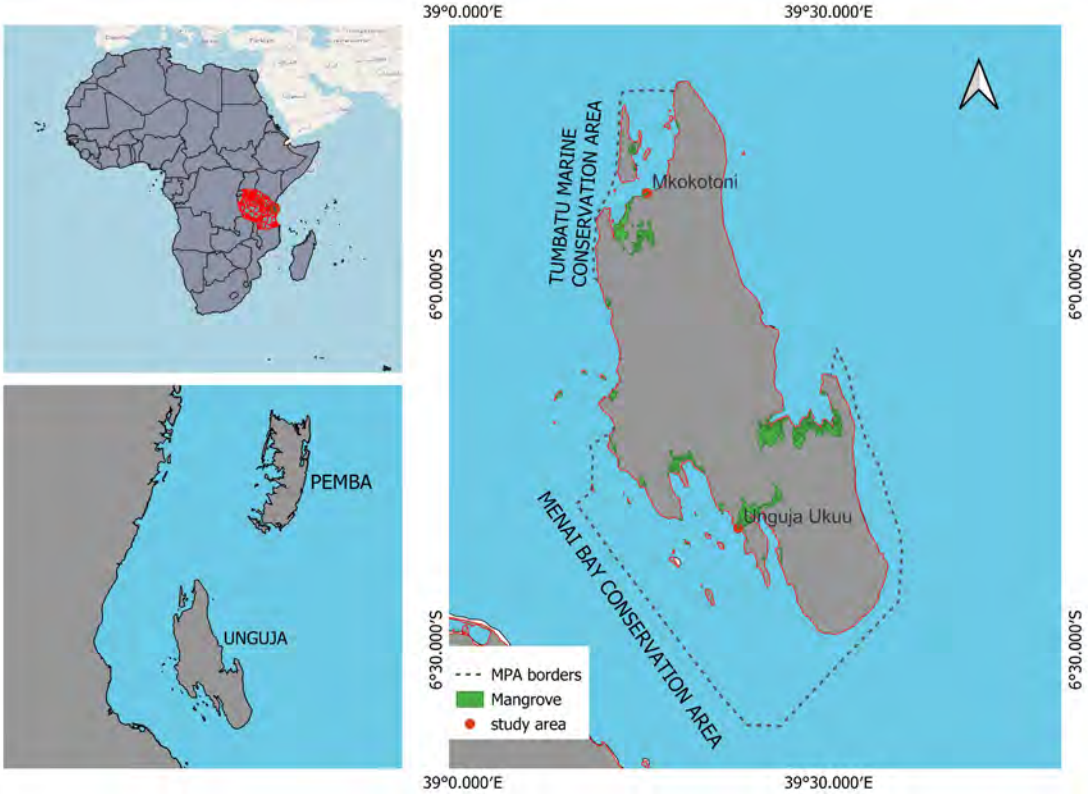

Figure 1. Map of Unguja Island (Zanzibar archipelago, Tanzania) showing the main sampling sites.

Measurements of length and weights

The total length (TL ± 1 cm) and total body weight (TW ± 1 g) of each fish was measured using a measuring board and balance. Otoliths were extracted via a horizontal cranial incision, cleaned, and stored in 70 % ethanol (Matta and Kimura, 2012). In the laboratory, otoliths were dried through air-exposure, and length (± 0.1 mm) and weight (± 0.001 g) measurements determined with a vernier caliper and electronic balance. The yearly age rings (annuli) were visualized using a light microscope equipped with a C-P8 opticam PRO8 digital camera. The age readings were carried out by one person after calibration. For fish larger than 25 cm TL, the otoliths were polished using P500 and P800 polish papers to identify the growth annuli clearly. To determine if hyaline and opaque rings were formed each year, the edges of otolith samples collected monthly were examined, with hyaline margins appearing as dark and opaque margin as white under reflected light.

Data analysis

Age determination

Age was determined from otolith measurements and length frequency analysis. Growth rings were counted using a reflected light stereoscopic microscope. To analyse the cohorts using length frequency data, a modal progression analysis (MPA) was performed by the separation of normal distributed components (NORMSEP) method using FiSAT-II software (Gayanilo *et al*., 2005). Length data of *L. harak* were available by month and grouped into 2-cm intervals.

Length weight relationship and sex ratio

The R-core team (2020), software version (4.0.3) was used to determine the potential relationships between fish TW and TL using the power function:

$$W = a{*}TL^{b}$$

Where *W* is wet weight (g), *TL* is total length (cm), *a* is the multiplicative factor and *b* is the exponent ($b > 1$). When $b = 3$, the growth pattern is isometric, positive allometric when $b > 3$, and negative allometric when $b < 3$ (Heydarnejad, 2009, Phillips *et al.,* 2018). The parameters *a* and *b* were estimated using the non-linear least square estimation procedure. The relationship between otolith weight and fish age was described using a linear relationship (Heydarnejad, 2009)

Growth rate

A growth model provides information about fish age in relation to their length and weight and is often viewed as the end product of growth analysis (Jones, 2000). The Von Bertalanffy Growth Formula (VBGF) was used:

$$Lt = L_{\infty} [1-e^{-k\,(t-t0)}]$$

Where Lt = mean length at age t, L_{∞} = asymptotic length, K = growth coefficient, determining the rate of change in the length increment, t = age of the species, and t_0 = the hypothetical age at which the length is zero. The growth parameters (L_{∞}, K and t_0) were estimated for males and females separately, and for both sexes combined, using length-at-age data obtained from otolith measurements. The growth performance index, Phi-prime (Ø), was calculated from Munro and Pauly (2012) as follows:

$$Ø = \log K + 2\log L_{\infty}$$

Where L_{∞} and K are asymptotic length and rate constants from VBGF respectively.

Mortality rate

The total mortality (*Z*) estimate includes natural mortality and fishing mortality which is calculated as the sum of fishing mortality (*F*) and natural mortality (*M*) (Jayasankar, 2014), given as:

$$Z = M + F$$

Where *Z* is total mortality, *M* is natural mortality and *F* is fishing mortality. Total mortality (*Z*) was calculated using a length converted catch curve using the FiSAT II software with age-based data. The model explains the relationship between the total mortality coefficient and the mean length of the exploited portion of the fish stock which was calculated as:

$$Z = K\,(L_{\infty} - Lmean) / (L_{\infty} - L')$$

L mean is the mean length of a sample representing a steady state in a population, and *L'* is a cut-off length or the lower limit of smallest length class included.

Natural mortality (M)

Natural mortality was obtained using Paulys' equation (Pauly, 1980) expressed as:

$$\text{Log } M = 0.654 \log K - 0.28 \log L_{\infty} + 0.463 \log T$$

Where *T* is the environmental temperature.

Fishing mortality (F)

Fishing mortality provides a value for the level of depletion in a fish stock that results from fishing activities, obtained from:

$$F = Z - M$$

Exploitation rate (E)

The exploitation rate was calculated as the ratio between the fishing mortality coefficient (*F*) and total mortality coefficient (*Z*), which is equal to the fraction of the stock taken by fishing activities over time.

$$E) = F/Z$$

For optimally exploited stocks, $E = 0.5$ year^{-1}

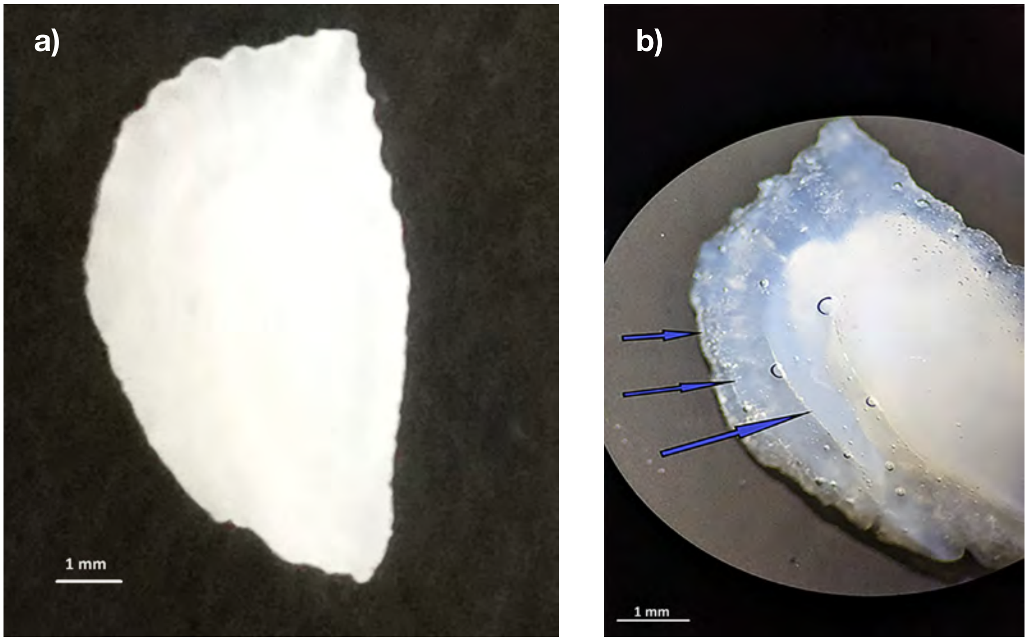

Figure 2. Otolith image concave side up. (a) without microscope and (b) with microscope under reflected light with dark background (2.0 magnification); arrows indicate annulus rings.

Results

Of 308 individuals investigated, 180 (58.4 %) were males and 128 (41.6 %) were females, with the smallest and largest lengths being 14.3 and 29.6 cm TL respectively. The smallest otolith was 0.051 mm long and weighed 0.028 g, and the largest otolith measured 0.88 mm and weighed 0.048 g. The otoliths were generally white in colour and elongated in shape, concave toward the anterior apex and semi rounded in shape toward the posterior apex (Fig. 2a). The whole otoliths showed clear rings when visualized with a reflected light stereoscopic microscope with a dark background (Fig. 2b).

Age estimates from otolith readings

From the yearly otolith annuli readings of the 308 fishes, 129 (41.9 % - comprising 100 males and 29 females) were three years old, 104 (33.8 % - 66 males and 38 females) were four years old, 33 (10.7 % - 7 males and 26 females) were five years old, 29 (9.4 % - 5 males and 24 females) were six years old and 13 individuals (4.2 % - 2 males and 11 females) were seven years old. Individuals younger than three and older than seven years were absent. The mean lengths at ages three to seven years were 17.7 cm, 21.0 cm, 24.2 cm, 25.9 cm and 28.2 cm, respectively. The marginal observation of otoliths showed that the opaque and hyaline rings were formed throughout the year with monthly variations. Individuals with opaque margins were dominant in December, January and February and hyaline margins during the remainder of the months (March to November).

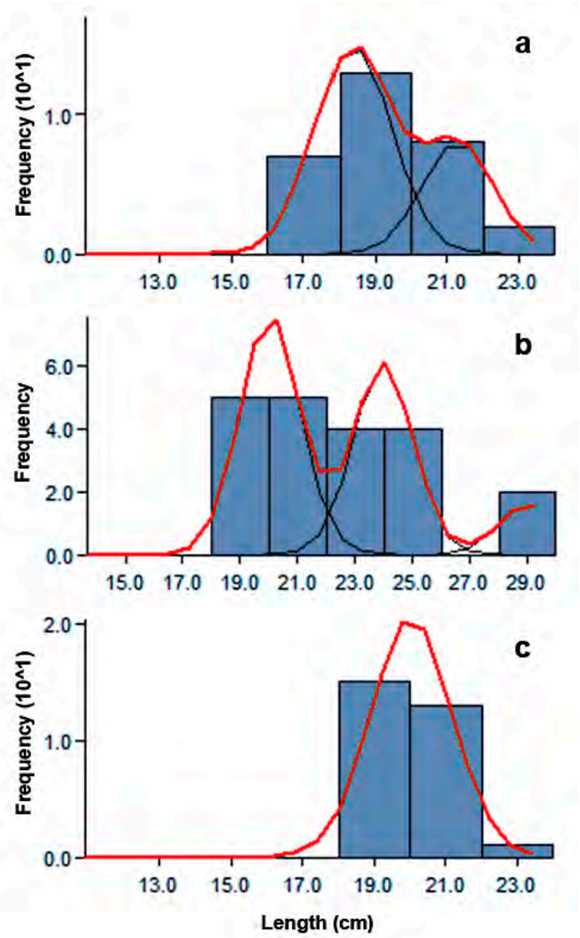

Figure 3. NORMSEP model showing population cohorts of *Lethrinus harak*. (a) two cohorts in January; (b) three cohorts in November; (c) one cohort in December.

Age group from length frequency data

The results of the NORMSEP model assessed using FiSAT II showed two cohorts in June to October 2019, and in January, February and May 2020. In November 2019 and March 2020 three cohorts were detected, and one in December and April, respectively (Fig. 3).

Length-weight relationship

The length-weight relationship indicated that *L. harak* exhibits an isometric growth pattern (Fig. 4a, b and c) for both sexes, and for males and for females respectively, where both length and weight of fish increased at the same rate. Otolith weight was strongly correlated to age (p < 0.0001, R² = 0.5503, r =0.7418) (Fig. 4d). Otolith radius showed strong positive correlation to fish length (p < 0.000 1, R² = 0.6571, r = 0.81095) (Fig.4e). Key length/size at age for both sexes were also established corresponding to one year (June 2019-May 2020) (Table 1).

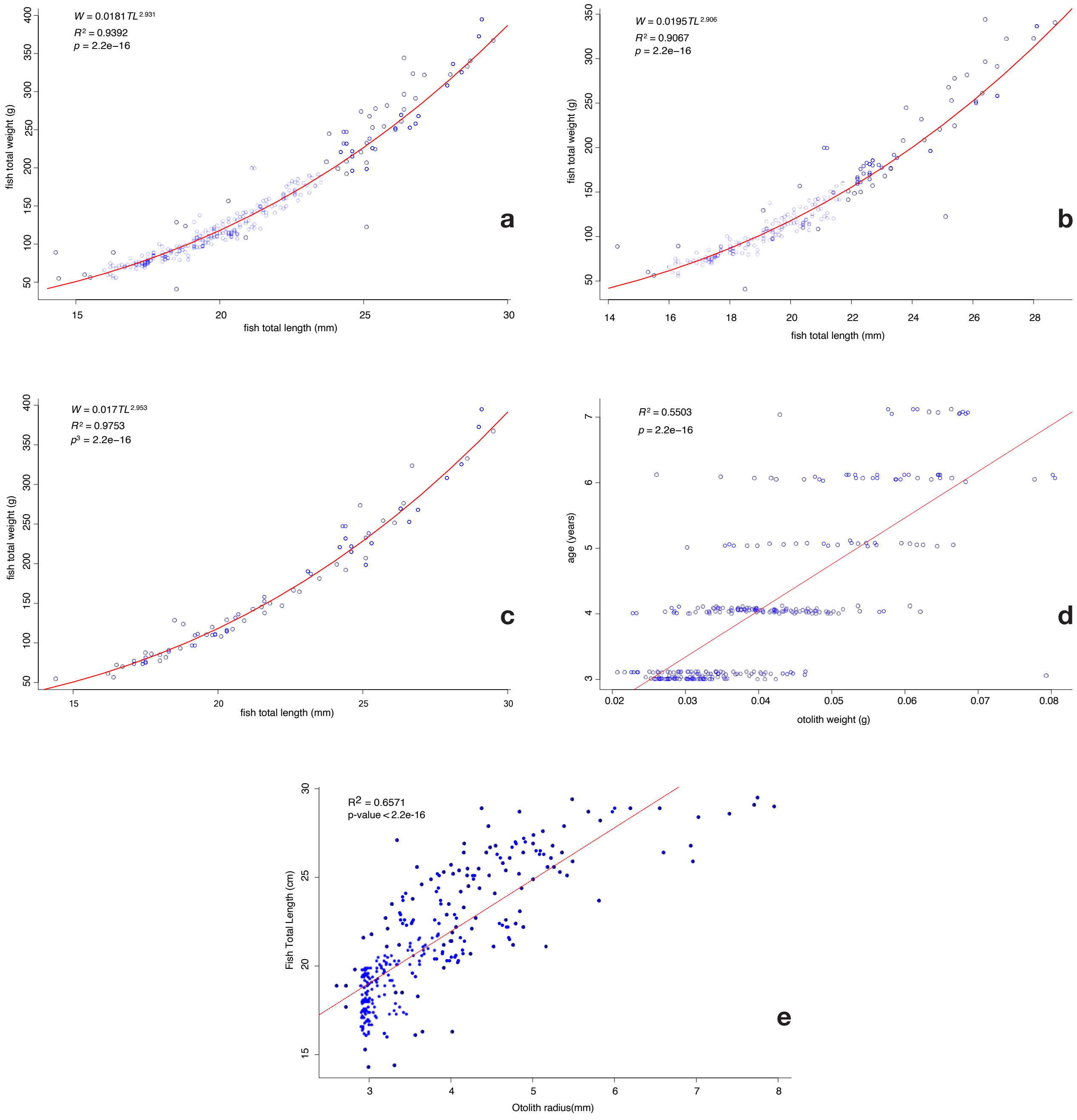

Figure 4. Isomeric growth curves for (a) both sexes, (b) males, and (c) females of *Lethrinus harak*. Linear regressions between (d) otolith weight and fish age and (e) otolith radius and fish length.

Table 1. Key length/size (cm) according to the age class for both sexes together of *Lethrinus harak,* corresponding to one year (June 2019-May 2020).

Total length	Age Classes							
TL in cm	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	
14			2					
15			2					
16			17					
17			31					
18			38					
19			39					
20				48	1			
21				23	2			
22				20	1			
23				7	3			
24				6	9			
25					13	11		
26					4	14	2	
27						2		
28						1	5	
29						1	6	
Mean Length (cm)			17.69	21.04	24.18	25.86	28.15	
Sd (cm)			1.17	1.21	1.47	0.95	1.07	
Total (N)			129	104	33	29	13	308
Percentage			41.88	33.77	10.71	9.42	4.22	100.00

Growth rate

Growth parameters calculated from length-at-age data (Table 2) found the asymptotic length $L\infty$ from data at both sampling sites to be larger than the maximum observed length (29.6 cm). Females had a higher asymptotic length (34.2 cm) than males (30.6 cm) which corresponded to a lower K value for females ($K = 0.25$) than males ($K = 0.30$) (Fig. 5a-c).

Mortality rates

The instantaneous total mortality rate (Z) calculated from the length converted catch curve (LCC) was 0.68 year^{-1} (95% C.I. of 0.38 - 0.99 and $r^2 = 0.99$) (Table 3), M was 0.65 year^{-1} and F was 0.03 year^{-1}. The exploitation rate was 0.04 year^{-1} (Table 3).

Discussion

The results obtained from this study indicate that the *Lethrinus harak* captured in Zanzibar waters are between three to seven years of age, although the fishery is dominated by fish of three and four years of age (75.7 %). The data on fish length do not deviate from Carpenter and Niem (2001) which showed *L. harak* individuals to be frequently fished at lengths between 20 to 30 cm (equivalent to 3 and 4 years of age). *L. harak* in the present study were all below seven years of age and the largest individual measured 29.6cm TL. *L. harak* can grow to a maximum of approximately 50 to 54 cm TL (Carpenter and Allen, 1989; Matthews *et al*., 2019), equivalent to an age of 12 to 15 years (Lasi, 2003; Trianni, 2016). The current analyses were therefore based on only a part of the population.

Like many coral reef fish species, *L. harak* was expected to have opaque and translucent zones formed during warmer and cool seasons respectively (Fowler, 2009). Such changes are associated with physiological change, growth rate differences and reproductive cycles (Fowler, 2009). For example, six Scaridae species develop opaque rings indicating high growth rates in summer (Choat *et al*., 1996). However, the current data showed the presence of both zones (opaque and hyaline) throughout the year; however, they dominated in different months. The opaque zones were observed in December, January and February, following the summer season and the hyaline zones during the rest of the months with full occurrence between July and September. This suggests that *L. harak* grows quicker in December to February, possibly associated with a change in water temperature and increase in primary production, caused by upwelling from north westerly winds. Coastal upwelling brings cold, nutrient-rich waters to the ocean surface from depth, increasing primary productivity (Hammond *et al*., 2022; Horii

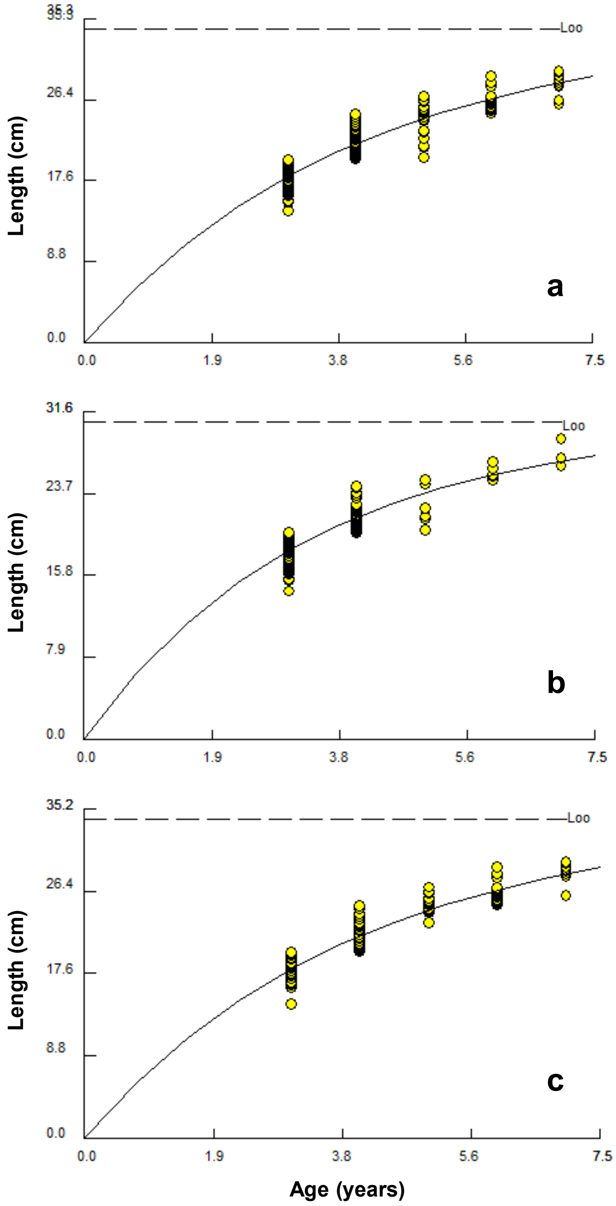

Figure 5. Von Bertalanffy Growth Function (VBGF) graphs for (a) both sexes, (b) males, and (c) females of *Lethrinus harak*.

et al., 2022). This tropical summer growth is contrary to other parts of the world where the opaque band in *Lethrinus* (incl. *L. harak*) occurred in August to December (Hilomen, 1997). Likewise, *Pargus auratus*, had less growth of opaque bands during winter (Ferrell *et al.*, 1992), compared to *L. nebulosus* in northern Great Britain where they had greater opaque zones that formed during the winter period (McPherson *et al.*, 1988). It should not be assumed that the opaque and translucent nature of bands in different species will be similar; though the pattern of wide and narrow banding tends to be consistent. The opacity and translucency of these bands varies considerably with species, light source and methodology. The ring deposition might also be affected by environmental or physiological stress such as spawning, migration, environmental fluctuations, hydro-meteorological conditions and age of fish (Hüssy and Mosegaard, 2004; Putnis and Korņilovs, 2008; Fablet *et al.*, 2011).

The values of *K* and L_∞ observed in this study suggested slow growth rates of *L. harak* in Zanzibar. This suggests that the *L. harak* do not get the opportunity to reach the asymptotic length, resulting in the removal of smaller individuals from the ecosystem, which can lead to detrimental effects. Decline in spawning biomass can cause negative effects to the point where recruitment is significantly impaired (Vasilakopoulos, 2011; Abdulrahman *et al,* 2021). Furthermore, domination of relatively young fish in catches, between three and four years of age, results in a population length structure that is heavily shifted toward younger age classes because of positive selection toward larger fish in the recent past; such size-selective fishing may have evolutionary consequences and may be difficult to reverse (Hsieh *et al.*, 2009). The observed values of *K* and L_∞ are closer to those presented by Ebisawa and Ozawa (2009), Trianni (2016) and Midway *et al.* (2018). The differences in both values between the current study and other studies may be attributed to geographical location, variation in recruitment pattern, seasonal changes in growth and size dependent selection (Hufnagl *et al.*, 2013). *L. harak* of age 12 years are commonly fished elsewhere (Lasi, 2003; Ebisawa and Ozawa, 2009), and therefore 3-year old fishes are still relatively young. Over-exploitation can result in scarcity of larger individuals, a likely scenario for *L. harak* in Zanzibar (Ali *et al.*, 2003; Beverton and Holt, 2012).

Table 2. The growth parameters of *Lethrinus harak* based on the Von Bertalanffy Growth Function (VBGF). Asymptotic length (L_∞) in cm, growth rate (*K*) in year^{-1}, and Phi prime Ø is an index of growth performance.

Species	Method	Sex	L∞	K	t0	Ø
L. harak	VBGF Plots	Both	34.25	0.25	0.00	2.46
	VBGF Plots	Males	30.57	0.30	0.00	2.44
	VBGF Plots	Females	34.22	0.25	0.00	2.46

Table 3. Mortality parameters, including the Exploitation rate (*E*), of *Lethrinus harak* in Zanzibar waters and from other studies. LCC = Length converted catch curve.

Model	Total mortality (Z) year^{-1}	Natural mortality (M) year^{-1}	Fishing mortality (F) year^{-1}	Exploitation rate (E)	Source	Place
LCC Age based	0.68	0.65	0.03	0.04	Present study	Tanzania/ Zanzibar
LCC	1.52	0.75	0.77	0.51	Lasi 2003	Fiji. Suva lagoon
Age based CC	0.239	0.381	-	-	Hilomen 1997	Lizard Island GBR
LCC	0.326-0.867	0.00-0.90	0.02-0.08	0.082-0.341	Trianni 2016	Saipan lagoon

The value of total mortality observed is less than that reported by Lasi (2003), higher than Hilomen (1997) and closer to Trianni (2016). Since the estimates of total mortality rate (*Z*) in this study show high contribution of natural mortality (*M*) and low contribution of fishing mortality (*F)*, total absence of older individuals could lead to biased estimates. The higher value of *M* could be attributed to sampling individuals of a smaller size, as they are known to have higher natural mortality from predation (Pauly, 1980). Hence the absence of older, larger individuals resulted in underestimation of Z and E. Theoretically, optimally exploited stocks have similar M and Z values, and close to 0.5 of its biomass (Pikitch *et al*., 2014). Even compared to smaller species with high recruitment variability and lower exploitation rates (E = 0.2; Pauly, 1984), the E of 0.04 year^{-1} estimated in this study is unrealistically low.

Overall, the relatively small asymptotic length in relation to the maximum length reported for *L. harak*, combined with the absence of individuals older than seven years of age from the sample (the lifespan of the species is estimated to be 15 years) may have led to over-estimation of M and an under-estimation of F. Thus, although the analysis suggested optimum exploitation (low F and low E), the absence of older, larger specimens in samples may actually reflect very high exploitation rates of *L. harak* in Zanzibar waters, something the model failed to show.

Conclusions

Lethrinus harak in Zanzibar waters is harvested at the age of three to four years, and no specimens aged between 8 and 15 years were available in the sample. Consequently, mortality estimates are biased, and the model output of low exploitation rate is in conflict with the general perception that *L. harak* is overexploited in Zanzibar. Future studies sampling deeper offshore reefs are required to investigate whether older individuals occur deeper, or whether they are completely absent from Zanzibar. Following that, a re-analysis of data, representing all size categories is recommended, to provide information for management of the species.

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Original Article

The viability of seagrass ecosystems for supporting dugong recovery in Kenya

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Abstract

Seagrasses are the primary source of food for dugongs and a good indicator of marine ecosystem health. The East African dugong (*Dugong dugon*) population is listed as critically endangered under the IUCN Red List. This study aimed to document the status of seagrass beds and evaluate their potential for supporting dugong recovery in Kenya. A cross-sectional survey was conducted in December 2016 to March 2017, with data gathered through desktop reviews, interviews, beach surveys and aerial surveys. Seven seagrass species were found at sampled sites, namely *Syringodium isoetifolium*, *Thalassodendron ciliatum*, *Halophila ovalis, Zostera capensis*, *Thalassia hemprichii*, *Cymodocea serrulata*, and *Halodule uninervis*. *Halodule* and *Halophila* seagrass species are important in the diet of dugongs. Two dugongs were sighted during the aerial survey. The spread of sea urchins, unplanned infrastructure development, Illegal, Unreported and Unregulated fishing, and boat anchors negatively affected seagrass ecosystems and hence dugong distribution in Kenya.

Keywords: seagrass, dugong, *Dugong dugon*, conservation, Kenya

Introduction

Dugongs (*Dugong dugon*) are the only herbivorous marine mammals in the world and the only extant member of the family Dugongidae (Grech and Marsh, 2008; Marsh and Saalfeld, 1990; Tol *et al.,* 2016). Over the years, there has been a sharp decline in the population of dugongs globally, leading to their listing as 'vulnerable to extinction' under the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Zedan, 2004) and critically endangered in East Africa (IUCN, 2022) necessitating their protection and conservation. The diet of dugongs is predominantly seagrass, whose ecosystems are located between mangrove ecosystems and coral reefs (Meidina *et al.*, 2023). Seagrass are marine flowering plants found in shallow coastal water where they flower, pollinate, seed, and germinate into new plants forming meadows (Akbar *et al.*, 2021; de la Torre-Castro and Rönnbäck, 2004; Tol *et al.*, 2016; Valentine and Duffy, 2007). They are habitats and foraging areas for many marine organisms including dugongs (Meidina *et al.*, 2023). The IUCN Red List attributes approximately 27 % of threatened marine species, including dugongs, to loss of seagrass and degradation of seagrass beds (Akbar *et al.*, 2021; Zedan, 2004).

Seagrass species are grouped into two major groups based on their life forms. The first group comprises short-lived, 'pioneering' species which include *Halophila ovalis*, *Halophila minor*, *Cymodocea rotundata*, *Halodule uninervis*, *Halodule wrightii*, *Halophila stipulacea*, *Syringodium isoetifolium* and *Zostera capensis*. The second group consists of long-lived, 'climax' species which include *Enhalus acoroides*, *Thalassia*

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hemprichii, and *Thalassodendron ciliatum*. Species in both groups exhibit typical zonation that is dependent on the water's depth, exposure to air and sunlight during low tide, and soil structure (Meidina *et al.*, 2023). None of the species is widespread, with all limited to the tropics except for *Zostera* spp. (Awadh *et al.*, 2021). Twelve species of seagrass occur in Kenya (Eklöf *et al.*, 2008) and cover approximately 75 % of the reef surface of Kenyan coastal waters, with pioneer species occurring closer to the coastal areas (Abuodha, 1998; Ochieng and Erftemeijer, 2003) while climax species occur in the deeper areas (Awadh, 2021).

Dugongs forage different genera of seagrasses in different locations with preferences varying due to extremes of weather conditions (Marsh and Saalfeld, 1990). Adulyanukosol *et al.* (2010) ranked dugong's preference for seagrasses based on the regularity of feeding, the relative abundance of different seagrasses, and signs of previous feeding. *Halophila ovalis* has been reported to be the most preferred species of seagrass by dugongs followed by *Halodule uninervis* thin-leaf morph, *Syringodium isoetifolium*, *Halodule uninervis* broad-leaf morph, and finally *Zostera muelleri* (Adulyanukosol and Poovachiranon, 2006; Adulyanukosol *et al.*, 2010; Marsh and Saalfeld, 1990; Tol *et al.*, 2016). The preferred order of seagrasses by dugongs varies across ranges and times (Anderson, 1998).

The absence of dugongs in seagrass meadows is increasingly being associated by scientists with a decline in dugong populations partly attributed to the loss and destruction of meadows (Hays *et al.*, 2018; Valentine and Duffy, 2007). Various human factors such as netting, hunting, trawling, vessel traffic, poor-quality terrestrial runoff, and biological factors such as sea urchin outbreaks have been noted to have the potential of adversely affecting the well-being of dugongs and their seagrass habitat (Grech and Marsh, 2008). Understanding dugong feeding behaviour and threats to the health of seagrasses in Kenya will assist in conservation strategies to conserve and even recover the population of dugongs. This analysis aimed to document the status of seagrass and evaluate its potential for supporting dugong recovery in Kenya.

Materials and methods

Study Design

This cross-sectional analysis of the status of seagrasses on the Kenyan coast was carried out using data from a main study that assessed the distribution, status, threats, and strategies for dugong conservation in Kenya since the 1960s (Awadh, 2021). The triangulation method using desktop reviews, interviews, beach surveys, and aerial surveys was used in the main study. For the current analysis, data was collected between December 2016 and March 2017. Approval to conduct the study was granted by the Kenya Wildlife Service (KWS).

Study procedure

Data for this analysis was collected through aerial surveys and beach seagrass meadow surveys. Additional information on dugong sightings was gathered through interviews with fishers.

Seagrass meadow surveys

Rapid ecological surveys were used to sample dugong habitats along the coast of Kenya. The location for the sampling of seagrass meadows was selected following a review of literature, a beach cast survey, and in consultation with local conservationists and fishers on locations of dugong sightings. Six fishing villages adjacent to the beach with a history of anecdotal, scientific or documented information on dugong sightings and seagrass meadows were selected as sites for the survey. Three of the selected villages were in the south of Kenya's coast; specifically Vanga, Shimoni, and Msambweni, while three were in the north; Matondoni, Kizingitini, and Kiunga. Systematic beach surveys were used to track seagrass species and beach-cast incidence to identify the availability of suitable seagrass habitat for dugongs. These surveys were carried out during low tide through snorkelling. Sampled sites were about 1-2m below sea level during low tide. For every sampled site, three random 50 m^2 (5 m width by 10 m length) plots divided into nine quadrants of 1 m^2 were sampled at an interval of 10 m along the beach. In total, 18 plots were sampled in this study. The total number of seagrass stems and species in each quadrant was counted and recorded following recorded procedures (McKenzie, 2003)(Fig. 1). Whole samples of seagrass were collected for species identification (FishCORAL Project, 2015). Underwater photographs were taken to show the density or level of degradation of seagrass meadows in study sites.

Aerial surveys

Aerial surveys were used to complement data on the distribution of seagrass along the Kenya coast, and for locating dugongs. The procedure used in the aerial surveillance was as described by Findlay *et al.* (2011). An aircraft was flown at a series of East to West or West to East 12-mile transects during the day in a progressive North to South direction at an altitude of 137m and speed of approximately 80 knots. A total of 37 transects were established in the north, extending from the Somali border at Kipini while sixteen transects were established in the south, from Diani Beach to Jimbo on the Tanzanian border. The aerial surveys were conducted for 3 days in December 2016 and another three days in March 2017. A total of 847 aerial sightings of the ocean were made. The survey was done at a time when the ocean was calm, with less turbulence and good visibility.

The Arc GIS 9.3 software was used to plot and map data from the aerial survey to indicate the location of dugong sightings and the distribution of seagrass in the study area.

Seagrass data analysis

The normality, distribution, and skewness of collected data were determined using the Kolmogorov – Smirnov test. The ability of different seagrass species to predict the presence of dugongs was determined using linear regression. For this analysis, species of seagrasses were the predictor variable while the presence of dugong was the dependent variable. Species with statistically significant correlation to dugong were included in a multivariate regression analysis model (Alexopoulos, 2010). Seagrass species with a low correlation coefficient that was not statistically significant were not included in the predictive model. The statistical level of significance was set at p=0.05.

Results

Seagrass ecosystem

The results of the seagrass species and densities in each of the study sites and the estimated dugong population are presented in Table 1. The stem counts and densities of seagrass per site are indicated. Seven species of seagrass were found occurring in Kenyan coastal waters, namely. *Syringodium isoetifolium*, *Thalassodendron ciliatum*, *Halophila ovalis*, *Zostera capensis*, *Thalassia hemprichii*, *Cymodocea serrulata*, and *Halodule uninervis*.

S. isoetifolium was the most ubiquitous seagrass species in the study sites. Vanga had the highest densities of S. *isoetifolium* at 67.9 stems/m^2 while Kiunga had the lowest densities at 12.0 stems/m^2. *Halophila ovalis* and *T. ciliatum* species occurred across all sites. Kiunga had a fair coverage of *H. ovalis* at 69.9 stems/m^2 and *H. uninervis* at 72.1 stems/m^2 as well as having the highest density of seagrass. *Cymodocea serrulat*a was only recorded in Shimoni while *Z. capensis* species was recorded in four sites with densities of less than 11.3 stems/m^2 in each site.

Thalassia hemprichii spp. only occurred in study sites located on the northern part of the Kenyan coast. Five (5) seagrass species were recorded in five out of the six study sites. The existence of *C. serrulate*, *Halodule* spp. and *S. isoetifolium* seagrasses varied slightly in their stem count densities. Overall, *S. isoetifolium*, *H. ovalis* and *T. ciliatum* occurred across all study sties while *C. serrulata* and *T. hemprichii* were the least common species in all study sites.

Correlation between seagrass species and dugong presence

From the linear regression, only *Halodule* and *Halophila* spp. showed statistically significant association to dugong and were included in the multivariate analysis. These two seagrass species were found to predict the presence of dugong with *Halodule* spp. being stronger predictors of dugong presence compared to *Halophila*

Table 1. Densities of seagrass per site and current dugong estimates.

Landing site	Seagrass Specie cover (stem count (density/m2))								Estimates of dugong population
	SI	HO	ZC	TC	TH	HU	CS	Total count	
Kiunga	180(12.0)	620(68.9)	53(5.9)	359(39.9)	482(53.6)	649(72.1)	0	**2343**	10
Kizingitini	268(29.7)	543(60.3)	102(11.3)	473(52.6)	104(11.5)	0	0	**1490**	1
Matondoni	400(44.4)	74(8.2)	77(8.6)	238(26.4)	282(31.3)	0	0	**1069**	1
Msambweni	333(37.0)	29(3.2)	0	263(29.2)	0	83(9.2)	0	**708**	1
Shimoni	459(51)	187(20.8)	0	398(44.2)	0	71(1.42)	332(6.64)	**1447**	1
Vanga	611(67.9)	39(4.3)	20(2.2)	449(54.4)	0	46(5.1)	0	**1165**	0

KEY: SI- *Syringodium isoetifolium;* HO- *Halophila ovalis;* ZC- *Zostera capensis;* TC- *Thalassodendron ciliatum;* TH- *Thalassia hemprichii;* HU- *Halodule uninervis;* CS- *Cymodocea serrulate*

Table 2. Regression analysis results of predicting dugong by seagrass species.

Regression Statistics	Halophila ovalis	*Halodule uninervis*
Correlation coefficient.	0.832783	0.905105
R Square	0.693528	0.819214
Adjusted R Square	0.61691	0.774018
Standard Error	2.337823	1.795553
Observations	6	6

ANOVA						
		df	SS	MS	F	Significance F
Halophila ovalis	Regression	1	49.47166	49.47166	9.051761	0.039604
	Residual	4	21.86167	5.465418		
Halodule uninervis	Regression	1	58.43729	58.43729	18.12564	0.01308
	Residual	4	12.89605	3.224012		

		Coefficients	Standard Error	t Stat	P-value
Halophila ovalis	Intercept	-1.80931	1.67536	-1.07995	0.340932
		0.35132	0.116771	3.008614	0.039604
Halodule uninervis	Intercept	-0.292	0.95791	-0.30483	0.775697
		0.323782	0.076051	4.257422	0.01308

spp. Statistically significant regression equations were generated for the two seagrass species with that of *H. ovalis* being significant at (F (1,4) = 9.052, p = 0.039) with an R^2 of 0.69 while the equation for *H. uninervis* was (F (1,4) = 18.126, p = 0.013) with an R^2 of 0.82.

The equation to predict dugong presence using *Halodule* spp. of seagrass was calculated as y= -0.292+0.324 + 1 while the predictive equation for *Halophila* spp. was calculated as y= -1.81+0.351 + 1. This model can be explained by 69.4 % of *Halophila* spp. and 81.9 % of *Halodule* spp. Thus, if things remain constant, the dugong population will increase 0.35 times for every increase in *Halophila* spp. per square meter of seagrass while it increases 0.32 times for every increase in *Halodule* spp. (Table 2).

Dugong's sighting

Two dugongs were sighted during the aerial survey. The first one was sighted in Kiunga at latitude (-4.541705°S 39.473034°E) while the second was sighted between Shimoni and Msambweni at latitude (-1.986209°S 41.142696°E) (Fig. 1). From the interviews with fishers, an estimated 14 dugongs were thought to occur in the study area with the majority occurring in Kiunga. Locations where fishers reported to have sighted dugongs included Doa, Sii island, Mwarembo, Mbayai, and Funzi creeks in the south and from the Siyu channel through Kiwayuu to Kiunga at Pezali, Konani, Ndia-mbili, Mlango-wa-huseni and Kui on the north coast. The estimated number of dugongs per site as given by fishers is indicated in Table 1.

Threats to seagrass

Observed threats to seagrass health and thus dugong survival were categorized into two; anthropogenic and natural threats.

Habitat loss and degradation caused by human activities (Anthropogenic threats)

A combination of frequent seine nets, gill nets, ring nets fishing, and the use of boat anchors in fishing zones, were noted to lead to the uprooting of seagrasses; moreover, these activities did not give time for the meadows to recover. These activities contributed to patchy, shorter, and degraded seagrass beds in studied areas. Figure 2 (A and B) shows two photos from the underwater ecological survey. Figure 2 (A) is a healthy seagrass bed photo taken in Kiunga which hosts a conserved marine national reserve; Figure 2 (B) is a degraded seagrass bed taken from Kizingitini. The healthy seagrass bed is dense, with at least three species of seagrasses visible while the degraded seagrass bed is patchy with sand visible.

These human activities have negatively affected seagrass meadows thus threatening the survival of dugongs. The threats were highest in Vanga, while they were average in Kizingitini, Matondoni, and Msambweni. Vanga is a fishing village at the Kenya-Tanzania border characterized by migrant fishers who use illegal and harmful methods in fishing that could be contributing to seagrass degradation. Kiunga and Shimoni reported slightly less habitat loss and degradation; this is likely because fishers' access to these areas is restricted by the presence of the conservation area.

Natural threats to seagrass

During the seagrass surveys, seagrass beds were regularly found to have high densities of sea urchins, across study sites. However, some sites, e.g., one out of three sample plots in Kiunga and one out of three in Shimoni, did not record any sea urchins. Kiunga and Shimoni had the lowest (2) and second-lowest (4) mean sea urchin occurrence per square meter. The number of sea urchins per meter square was highest in Vanga with a mean of 22 urchins per m^2 followed by Msambweni, Matondoni, and Kizingitini as indicated in Table 3.

Discussion

Status of seagrass

This analysis aimed to document the status of seagrass and evaluate its potential for supporting dugong recovery in Kenya. Seven species of seagrass were found to occur in the study areas. The distribution and densities of the seagrass varied between the study sites. Two dugongs were sighted in the study area during the aerial survey. Kiunga which was reported to have higher numbers of dugong than other sites had higher densities of seagrass compared to other sites. This area also had high densities of *Halodule* spp., and *Halophila* spp. that are reported as the preferred species of seagrass by dugongs. *Halodule* spp., and *Halophila* spp. were found to predict the presence of dugongs indicating that their restoration could result in the recovery of dugongs. On the other hand, areas with low densities of seagrass were also noted to have corresponding low numbers of dugongs as reported by fishers.

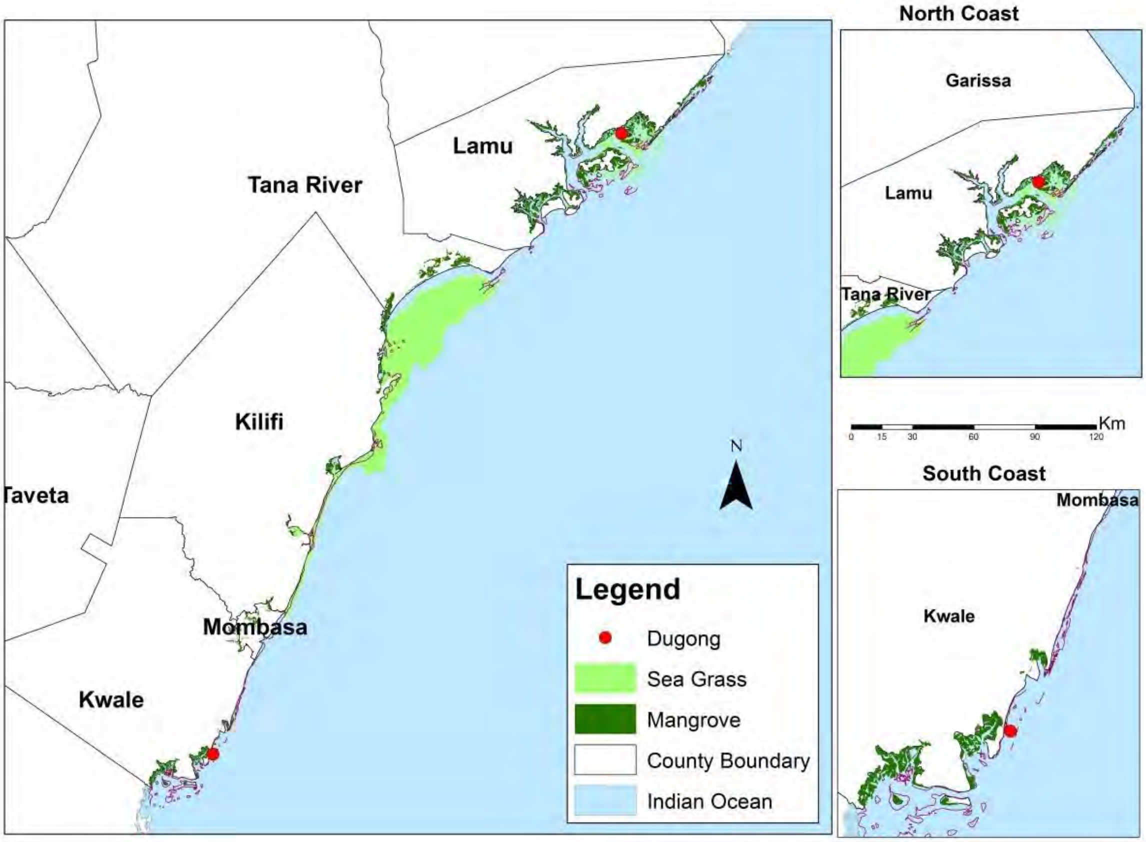

Figure 1. Map of Kenya coastline indicating the locations of dugong sightings during the aerial survey conducted between December 2016 and March 2017.

Seagrass decline and loss have been reported worldwide (Nadiarti *et al.*, 2021). Species of seagrass preferred by dugong are scarce in Kenyan waters. Seven

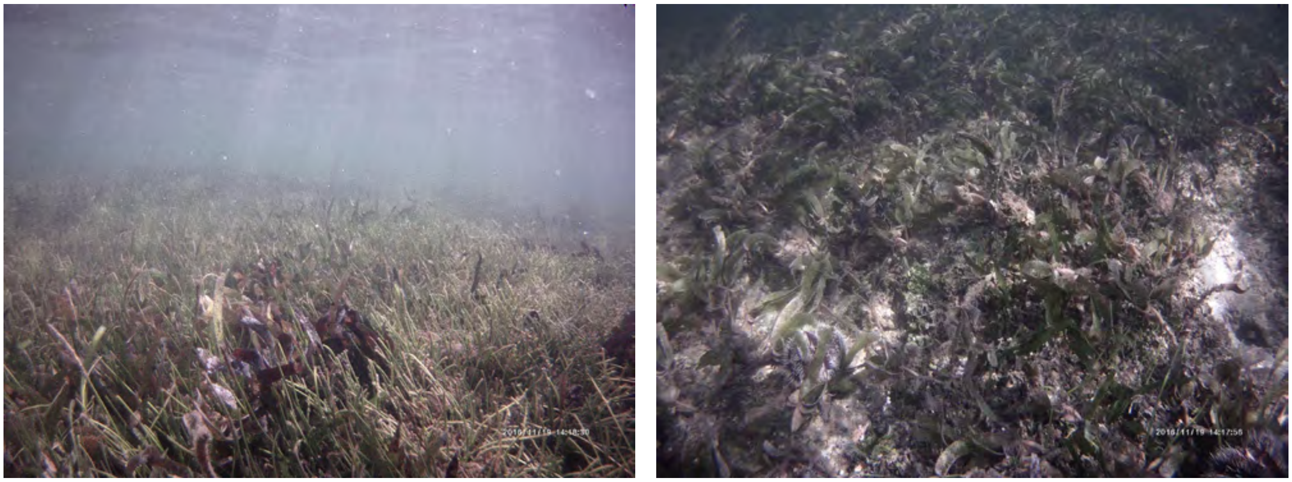

Figure 2. Photograph of a dense and healthy seagrass bed (A), versus a weak and a degraded seagrass bed (B).

of the twelve species of seagrass recorded along the Kenyan intertidal were identified in the six sample sites. Preen and Marsh (1995) and Adulyanukosol *et al.* (2010) reported dugongs to prefer *Halodule* spp., *Halophila* spp., *Thalassia* spp., and *Syringodium* spp. of seagrass. The findings from the present agree with this observation as the locations of dugong sighting were found to have high densities of these species. In this study, the coverage for *Halodule* and *Halophila* was low in five sites and fair in Kiunga. This correlates with dugong sightings in this area during aerial surveys and reports of higher dugong populations from fishers when compared to other studied areas. *Thalassia* coverage was fair in Matondoni and poor in five sites, while *Syringodium* was fair in Matondoni Shimoni Msambweni and Vanga and poor in Kizingitini and Kiunga. Hence, the study area has the potential to host dugongs and may offer ideal conditions for their population to thrive and recover.

Halophila spp. and *Halodule* spp. of seagrass were associated with dugongs as well as being predictors of dugong presence. These two species were fairly common in Kiunga and low in the rest of the study area. Kiunga is thought to host the highest populations of dugongs in Kenyan waters. Even so, the densities of these seagrasses are not high enough to support large populations of dugongs due to their heavy foraging. These findings agree with Meidina *et al.* (2023) and Preen (1995) that dugongs target *H. uninervis* and *H. ovalis* species of seagrass. Preen (1995) and Preen and Marsh (1995) suggested that dugongs target *H. uninervis* and *H. ovalis* for food, likely due to these species' low fibre and high nitrogen content. The finding from the present study also agree with Ochieng and Erftemeijer (2003) who found vast meadows of *H. uninervis* and *H. ovalis* to be uncommon on Kenya's south coast, explaining the apparent scarcity of dugongs in the south of Kenya. The relationship between *Halodule* spp. coverage and presence of dugongs calls for its restoration for success in dugong conservation. Dugong sites in Kenya have decreased from at least 28 sites to about ten areas. The diminishing number of dugongs and the low coverage of dugong's preferred seagrass species in the study area present the risk of dugong habitat shrinking further.

Threats to the health seagrass meadows

Anthropogenic and natural threats were identified that result in the degradation of seagrass meadows and thus affect dugong distribution and recovery. Human activities related to fishing practices were observed to

Table 3. Count of sea urchins per m^2 per study site.

	Kiunga	Kizingitini	Matondoni	Msambweni	Shimoni	Vanga
Plot 1	0	163	216	124	102	112
Plot 2	22	40	190	305	0	297
Plot 3	34	102	34	87	7	191
Site total	56	305	440	516	109	600
Plot mean	18.67	101.67	146.67	172.00	36.33	200.00
#Urchins per m^2	2.07	11.30	16.30	19.11	4.04	22.22

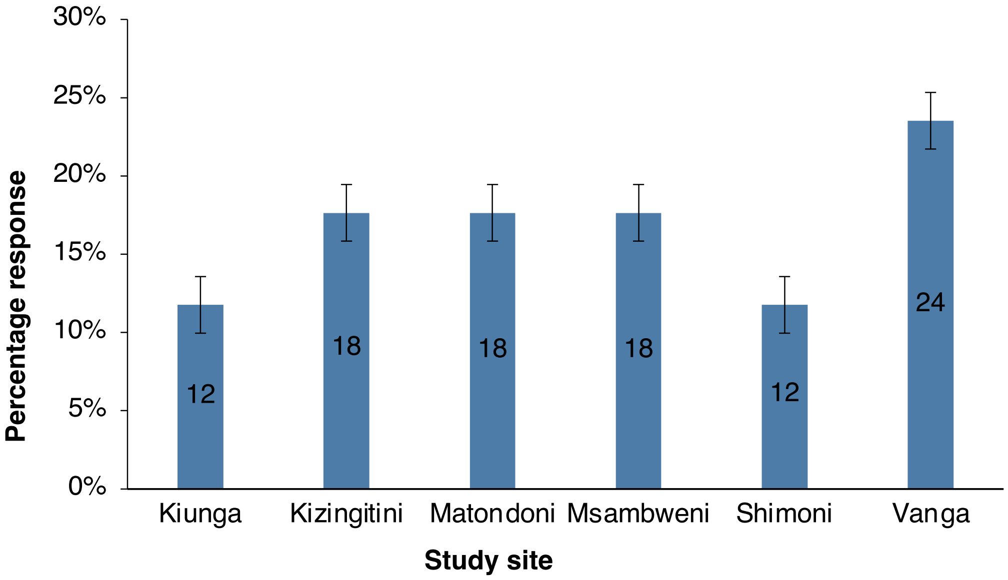

Figure 3. Densities of sea urchins by study site along the Kenya coast.

cause the degradation of seagrass beds. This being an activity that is repeated very frequently, it was noted to also affect the recovery of degraded meadows. A contrast was observed in the densities and health of seagrass when comparing sites that were protected from fishing within marine parks and unprotected sites. Protected sites had more as healthy seagrass beds compared to unprotected areas. Moreover, protected areas were thought to have higher numbers of dugongs compared to unprotected areas with high fishing activities. Urchins were a major natural threat observed to affect seagrass in the study area. The presence of urchins corresponded with high human actives with areas having high human activity such as Vanga which has a port having higher urchin densities per meter square compared to other sites. Besides urchin infestation destroying seagrass meadows, they are also known to affect dugong feeding as dugongs avoid foraging in areas with high densities of urchins.

Seagrass plays a vital role in the coastal ecosystem as is an indicator of the overall health of the ecosystem. The well-being of seagrass meadows can reveal changes in the surrounding environment, which may be due to natural causes and other anthropogenic activities (Hemminga and Duarte, 2000). While seagrass beds are in fair condition in the surveyed locations, threats leading to their decline still exist. Sea urchins, unplanned infrastructure development, IUU fishing and boat anchors were some of the factors found to destroy seagrasses in the study site. The government of Kenya has enacted legislation to protect dugongs as well as adopting articles 61, 63, and 64 of the United Nations Convention on the Law of the Sea (UNCLOS) that takes into consideration the protection and management of marine mammals inclusive of dugongs (Awadh *et al.*, 2021; Samoilys *et al.*, 2017). Protected areas had higher densities of seagrasses with greater diversity compared to unrestricted areas. This reinforces the need for conservationists and stakeholders to meet COP 15 biodiversity targets. More effort needs to be put in place to conserve dugong feeding grounds and seagrass if dugong populations are to be restored.

Conclusion

Seven species of seagrass were found occurring in Kenyan coastal waters. The densities of these seagrass species varied across the coast. The densities of seagrass can support dugongs but are not enough to support dugong recovery. *Halodule* spp., and *Halophila* spp. of seagrass were highly associated with dugongs and can be used to predict their presence. Both human activities such as harmful fishing practices, and natural threats such as urchin inundation, were found to negatively affect seagrass meadows. Reducing human activities in areas known to host dugongs could lead to an increase in the densities of seagrasses and recovery of dugongs.

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Original Article

Effects of climate change on mangrove-dependent livelihoods in Lamu County, Kenya

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Abstract

The effects of climate change on mangrove-dependent livelihoods were examined in Lamu County, Kenya. Climatic instrumental, household survey, key informant interview, and focus group discussion data were collected from August to December 2021. Data analysis indicated a significantly increasing trend in annual air temperature between 1985 and 2020, with minimum and maximum temperatures increasing by 0.034 °C and 0.0281 °C, respectively. Rainfall during the seasonal long rains declined, but not significantly. In contrast, the seasonal 'short rains' increased significantly. The mean sea level rose significantly, from 7066 mm in 1985 to 7150 mm in 2020. The perception data showed an increasing effect of climate change on mangrove-dependent livelihoods in the last 10 years. Critical livelihood aspects that were affected included the destruction of property, displacement, the prevalence of waterborne infections, reduced mangrove products, increased salinity in underground waters, and destruction of fish habitats.

Keywords: climate, mangroves, temperature, rainfall, sea level, livelihood

Introduction

Climate change is a long-term change in the average weather patterns that define the Earth's global, regional, and local climates (Bruine de Bruin *et al.*, 2021). Climate change factors including sea level rise, increased storminess, altered precipitation regime, and increasing temperature impact mangroves at global, regional, and national scales. Mangrove-dependent livelihoods refer to people who live in the mangrove ecosystem (Youshao, 2021). The mangrove ecosystem provides a means of sustaining the lives of the population living around it. Mangroves sustain the lives of the coastal population through their products (i.e., mangrove trees for wood, fuel, and medicines), their preservation of the coastal ecosystem (i.e., protecting the coastline from strong waves), and their provision of habitat for fishes and seafood (Fongzossie *et al.*, 2022). Climate change is causing destructive impacts on mangrove ecosystems, leading to a reduction in the availability of mangrove products and the destruction of recreational sites and beaches (Mung'ong'a *et al.*, 2019). Mangrove-dependent societies are being affected by climate change impacts, and there is a need for community education on climate change impacts and livelihood diversification strategies.

Globally, the total surface area of mangroves has decreased by 1.04 million ha between 1990 and 2020 and thus livelihoods depending on mangroves have been at stake (Friess *et al.*, 2020). Empirical studies have closely linked variations in climate to mangrove biodiversity change (Rogers *et al.*, 2019; Cameron *et al.*, 2021). Indeed, livelihoods depending on mangrove

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ecosystems globally are exposed to a massive decline in mangroves and therefore their means of survival. Ward *et al.* (2016) established that sea level rise and storminess were likely to have a greater impact on mangroves in North and Central America, Asia, Australia, and East Africa than in West Africa and South America. Coastal communities in Pakistan, Bangladesh, and Sri Lanka that depend on the mangrove ecosystem for trade and fishery have negatively been exposed to climate change with the mangrove ecosystem deteriorating and associated mangrove products and fish reducing in supply (Salik *et al.*, 2016). In agreement with Salik *et al.* (2016), Wilson (2017) showed that sea level rise that causes saline intrusion, coastal erosion and destruction of primary habitat is currently the most immediate climate-related threat to mangroves in Caribbean Small Islands (SIDS).

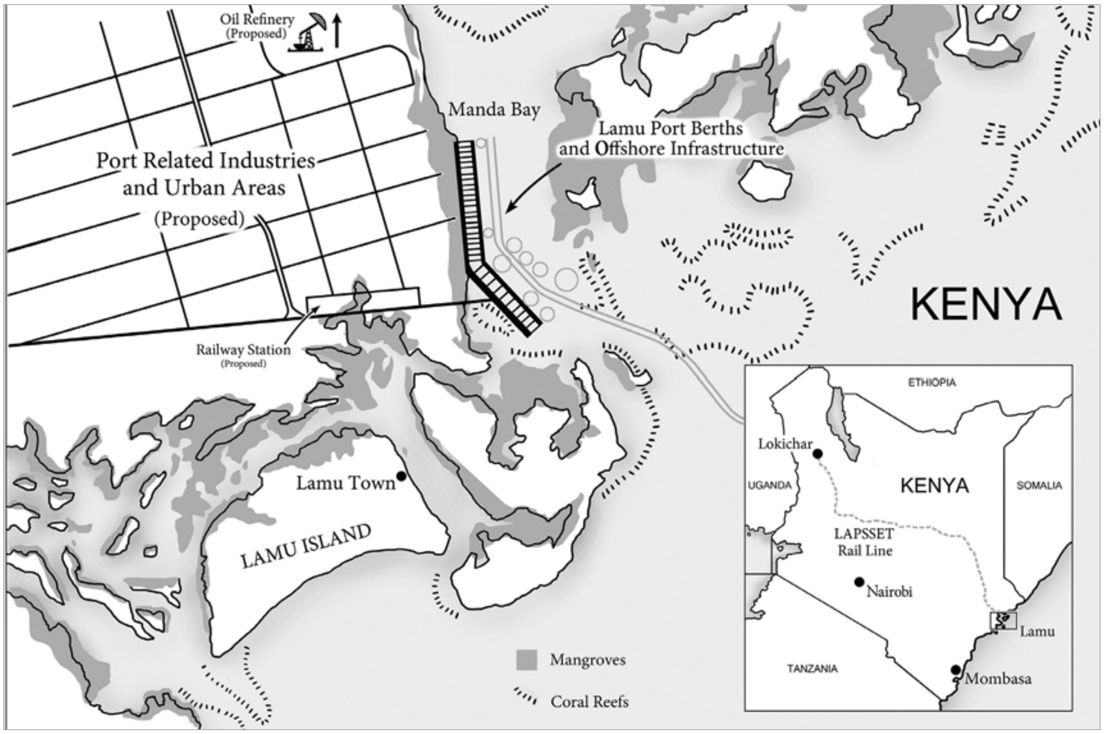

Figure 1. Lamu County Map (prepared by S. Njenga)

Climate change and its impacts on mangrove-dependent livelihoods are also apparent in Africa. Indeed, it is an emerging threat to mangrove ecosystems in Africa (Scales *et al.*, 2018). In Cameroon, Fongnzossie *et al.* (2022) found that irregular rainfall patterns and increasing temperature have left the households on Manoka Island vulnerable to the impacts of climate change such as floods, and rain storms that have destroyed mangroves supporting lives. In the East African mangrove ecoregion, climate change has also affected the mangrove-dependent communities across the coastlines of Mozambique, Tanzania, Kenya, and southern Somalia by undermining their capacity to access mangrove ecosystem resources (Bandeira *et al.*, 2018). Overall regional decline of mangrove surface area with a net loss of 984 sq. km between 1975 and 2013 has resulted in socio-economic vulnerability among the mangrove-dependent communities, with various mangrove ecosystem benefits being impaired, such as reduced catch of shrimp and fish (Bandeira *et al.*, 2018). Mung'ong'o *et al.* (2019) noted the vulnerability of fisher-mangrove-dependent communities in the Rufiji Delta in Tanzania climate change impacts with reduced catch from fisheries in the mangroves are experienced. Maina *et al.* (2021) identified sea level and macroclimate as the main drivers of the present-day ecological condition of mangroves in the Western Indian Ocean. Nicholson (2019) showed that rainfall seasonality is quite complex, changing within tens of kilometres showing strong links to the El Nino-Southern Oscillation (ENSO) phenomenon in the Eastern Africa Region.

In Kenya, climate change is negatively affecting the coastal regions with climate change variables such as sea level rise and flooding displacing coastal indigenous populations and destroying property (Kogo et al., 2020). Mangrove cover in Kenya is estimated at 50,000-60,000 hectares and has significantly declined by almost one-fifth between 1985 and 2020 (Maina *et al*., 2021). During the same period, climate variables have also become erratic with significant changes in climate being observed (Yvonne *et al*., 2020). Indeed, previous studies in Kenya have since established that climate variables such as rainfall, temperature, sea level rise, and sea storminess have severe consequences for ecosystem-dependent communities i.e., mangrove-dependent livelihoods (Okello *et al*., 2015; Yvonne *et al*., 2020). Given that over 70 % of Lamu's people partially depend on mangroves and fisheries for trade and livelihood (Wanderi, 2019), it is becoming clear that these livelihoods are now threatened by climate change. Even though the link between climate change and mangrove-dependent livelihoods has been well-studied elsewhere, few studies have been undertaken in the context of Lamu, Kenya. This study sought to fill this gap in information.

Materials and methods

The study area

Lamu is one of six coastal counties in Kenya. It is situated on the north coast with Lamu Town as the county capital. The county shares boundaries with Somalia to the northeast, Tana River County to the southwest, Garissa County to the north, and the Indian Ocean to the south. The Lamu Archipelago, which consists of more than 65 islands, and the county's mainland together make up the county's 6,273.1 km^2 (2,422.1 sq. mi) of territory. The coastline's overall length is 130 km (81 mi), while the total area of land and water is 308 km^2 (119 sq. mi) (Fig. 1). Lamu County's biophysical characteristics present a vulnerability to climate change impacts, given that it is generally flat and lies between 0 and 50 m above sea level (Gichenje *et al*., 2019). Further, the soils in Lamu are generally shallow and prone to water logging. The area is thus susceptible to flooding from excessive rains, sea level rise, and sea storminess (Zachary, 2023). Further, the mangroves that form a significant element of biodiversity in the area are vulnerable to erratic rainfall and sea level rise resulting in flooding suffocation, and death of mangroves (Andreetta *et al*., 2014). The death of mangroves from climatic factors implies that mangrove-dependent livelihoods are impaired, thus endogenous groups in Lamu such as the Boni that practice subsistence agriculture, hunting, and gathering of edible forest products are exposed to climate change variables (i.e., erratic rainfall, sea level rise, and increasing temperatures (Olsen *et al*., 2020). Further, a key economic activity in Lamu is tourism with visitors attracted by recreational beaches and sport fishing in the mangrove ecosystem. Climate change impacts the mangrove ecosystem which is a tourist attraction leading to a loss of income for livelihoods depending on tourism activities such as tour guiding (Njoroge *et al*., 2020). The findings from this study and the data generated are critical for climate change adaptation and mitigation efforts for the benefit of mangrove-dependent livelihoods in Lamu, and Kenya at large.

Conceptual framework

This study employed a conceptual framework as an analytical tool to evaluate the effect of climate change on mangrove-dependent livelihoods in Lamu County-Kenya (Fig. 2). The key independent variable was climate change. The sub-independent variables included rainfall, temperatures, and sea level rise. The temperature aspect captured was the annual minimum and maximum in degrees centigrade. The rainfall aspect captured was mean annual rainfall and mean seasonal rainfall in millilitres. The sea level rise aspect captured was the mean annual sea level in

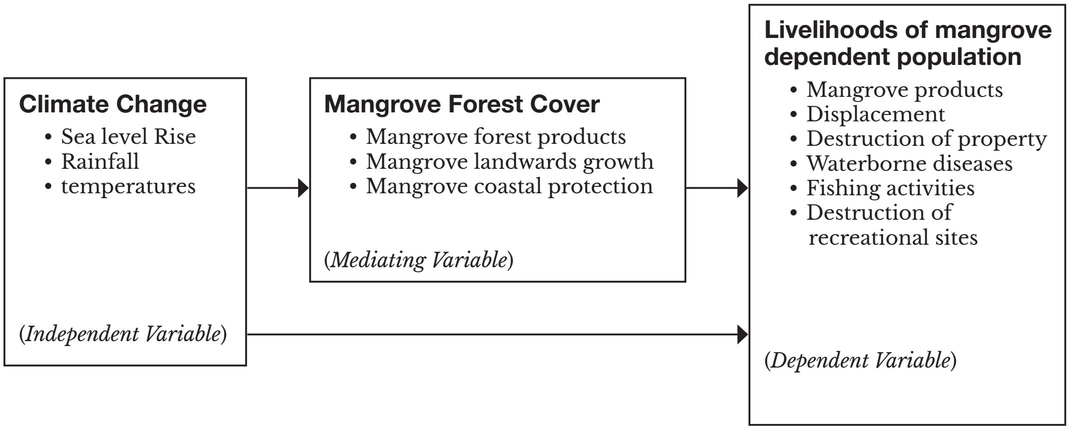

Figure 2. A conceptual framework for the study.

millimeters. The changes in climatic variables were conceptualized as directly and indirectly impacting mangrove-dependent livelihoods. The direct effect of climate change occurred without a mediating factor while the indirect effects occurred through mangrove forest cover as the mediating factor. The mangrove forest cover in Lamu County, a mediating variable, was captured in terms of mangrove products, its coastal protection feature, and its capacity to protect people and animals from flooding. The change in the mangrove forest cover due to the impact of climate change was expected to affect mangrove-dependent livelihoods. The dependent variable was therefore mangrove - mangrove-dependent livelihoods. The livelihoods affected included the supply of mangrove forest resources, human population displacement, destruction of property and infrastructure, destruction of recreational sites and beaches, the emergence of human and livestock diseases, and interference with fishing activities.

Data collection and analysis

The National Oceanic and Atmospheric Administration (NOAA) provided data on the atmospheric temperature (SST), while the Kenya Meteorological Department (KMD)-Lamu Station provided rainfall data, and KMFRI and the University of Hawaii Sea Level Centre (UHSLC) provided the sea level rise (SLR) data. These data were used to quantify climate change variables in Lamu. Further, key informant interviews (KII), household (hh) surveys, and focus group discussions (FGDs) were used to gather perception data. The household survey was employed among 380 hh sampled from the target population of 37,963 hh in Lamu County (Balaton-Chrimes, 2021). The hh were distributed over nine divisions (Witu, Amu, Hindi, Mkumbini, Mpeketoni, Basuba, Faza, Kiunga, and Kizingitini). The sample size was determined based on formulae proposed by Kothari, 2004)

$$\mathbf{n} = \frac{z2pqN}{e2(N-1) + z2pq}$$

Where e = error taken as 5 %; p= population reliability taken as p=0.5; q= (1-p), z is the normal distribution at 0.05 level of significance such that z =1.96, N is the target population and n is the sample size.

$$\mathbf{n} = \frac{1.96*1.96*0.5*0.5*37{,}963}{0.05*0.05\ (37963-1) + 1.96*1.96*0.5*0.5}$$

n = 380.32
n = 380

Further, using stratified random sampling, the sample size was stratified into sub-counties and then administrative divisions and then hh from each stratum were randomly selected from a list prepared for each administrative division. The head of the hh automatically participated in the study and in cases where they were absent, the representative of the head of the hh participated in the study. During the hh survey, questionnaires were administered to 380 sampled hh of which 353 filled the questionnaire, giving a return rate of 92.8 %. The majority of the hh heads that participated in the study were males with 227 (64.3 %) against 126 (35.7 %) females. The majority of the HH heads that participated in the study were aged 35 years and above and hence were old enough to answer questions on climate change and how it has impacted their livelihood. Most respondents in the survey had the highest education level between primary and Certificate/Diploma. Further, most (86.3 %) of hh heads had stayed in Lamu for over thirty years and hence had stayed long enough to answer questions on climate change within Lamu. The findings also showed that most respondents were fishermen, fisherfolk, and mangrove cutters/licensees who are presumably more susceptible to climate change (Yvonne *et al.*, 2020).

The questionnaire sought information on how climate has changed in the last 10 years and how that change was impacting mangrove-dependent livelihoods in Lamu. The local research assistants involved in the administration of the hh survey questionnaire were identified and trained before the study date. The role of the researcher during the survey was that of monitoring the work of research assistants through phone calls and random visits to places where research assistants were collecting data. The survey questionnaire was in English and Kiswahili language with research assistants reading for those respondents who were illiterate. The data collected in the survey were recorded on survey forms filled out by the research assistants. Personal information such as names were not collected, with codes being adopted to represent the respondents.

Key informant interviews were conducted with a range of specialists and representatives from various institutions (including the Lamu County Government, Kenya Forest Service (KFS), Kenya Marine and Fishery Research Institute (KMFRI), National Environmental Management Authority (NEMA), grassroots and not-for-profit organizations, and the Lamu Division of Climate Change) selected purposively. The study interviewed twenty (20) respondents based on a prepared KII guide. The interview guide was administered in English language given that the respondents were conversant with English. The KII was undertaken at the place of work of respondents between 5th September 2021 – 10th October 2021. The researcher made short notes as the interview progressed. The names of the interviewees were not recorded, with codes being adopted to represent them. Finally, purposive sampling was used to choose the participants in FGDs drawn from various representatives including Save Lamu civil society organization, mangrove cutter license holders, Beach Management Units (BMUs) managers, women group members, youth group members and artisanal fishermen. Each FGD session lasted 60 minutes and had a total of ten (10) participants (excluding the principal investigator). There were five FGD sessions altogether undertaken between 11th September 2021 to 18th September 2021 in Shella, Mpeketoni, Faza, Kiunga and Kizingitini. Each FGD session had a mix of genders, ages, and groups represented. The FGDs were spearheaded by a trained local leader who acted as a research assistant. The FGD sessions were undertaken in English and Kiswahili language with a voice recorder being adopted to capture discussions and moderations. The role of the researcher during the FGD was that of taking short notes as the discussion progressed. Personal information such as the names of respondents were not captured during the FGD to protect their identity.

The study's quantitative data were analysed using the Statistical Package for Social Scientists (SPSS) version 23 and Microsoft Excel 2021. Mean, minimum, maximum, and graphs were among the descriptive statistics employed in the study. Regression analysis was used to determine whether time was a predictor of rainfall, temperature, and sea level. To investigate the significance of the slope of the trend of climatic variables such as rainfall, temperature, and sea level rise, a Mann-Kendall non-parametric test was used. The content analysis approach was used to transcribe and evaluate qualitative data. The analysis entailed identifying themes derived from study questions and story answers from FGD and KII participants. The synthesis was based on triangulation, in which data from quantitative and qualitative sources were combined. The study was approved by the Kenya National Council for Science and Technology (NACOSTI). Further, all participants in the study provided formal consent (written and verbal) before participating in the study which took place between September 2021 and March 2022.

Results

Temperature variation and trend analysis

The minimum values for the annual maximum and annual minimum temperatures in Lamu were 30.5 °C and 23.2 °C, respectively. Further, the maximum yearly maximum and minimum temperatures were 32 °C and 25 °C, respectively (Fig. 3). The regression analysis revealed that yearly minimum and maximum temperatures in Lamu were rising at the rate of 0.034 °C and 0.0281 °C respectively. The coefficient of determination (R^2) showed that time (in years) accounts for 62.6 % and 55.7 % of the total variation in the annual maximum and annual minimum temperature, respectively (Fig. 3). Further, the Mann-Kendall non-parametric test (Table 1) revealed that Kendall's tau_b was positive and statistically significant for the maximum annual and minimum annual temperature (Kendall's tau-b = 0.626, $p < 0.05$; Kendall's tau-b = 0.627, $p < 0.05$) respectively. These findings implied that the trend for annual maximum and minimum temperature from 1985-2020 in Lamu was rising and that time was a predictor for temperature experienced. Further, Kendall's tau_b was positive and statistically significant for the correlation between maximum and minimum annual temperature (Kendall's tau-b = 0.599, $p < 0.05$)

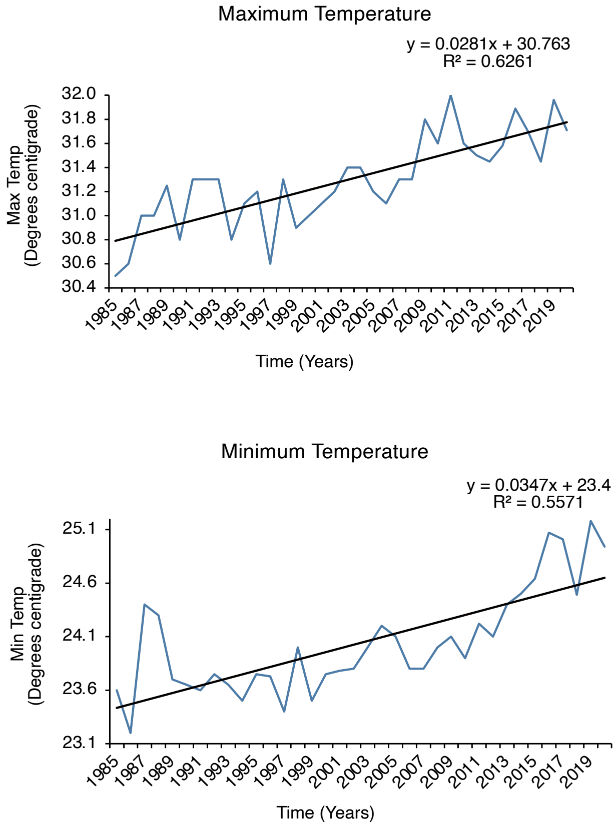

Figure 3. Annual maximum and minimum temperature (1985-2020) in Lamu (Climate Data Services, KMD, 2020).

Table 1. Mann-Kendall test for temperature trends in Lamu (1985-2020).

			Year	MaxTemp	MinTemp
Kendall's tau_b	Year	Correlation Coefficient	1.000	0.626**	0.627**
		Sig. (2-tailed)	0.0	0.000	0.000
	MaxTemp	Correlation Coefficient	0.626**	1.000	0.599**
		Sig. (2-tailed)	0.000	0.0	0.000
	MinTemp	Correlation Coefficient	0.627**	.599**	1.000
		Sig. (2-tailed)	0.000	0.000	0.0

**At the 2-tailed threshold of 0.01, the correlation is significant.

depicting that as the maximum temperature rises, so does the minimum temperature in Lamu.

Rainfall variation and trend analysis

Figure 4 presents the lowest and the highest annual rainfall at 600 mm and 2250 mm, respectively over the study period. The coefficient of determination showed that time in years explained 2.2 % of the trend in Lamu County's average annual rainfall. Furthermore, the Mann-Kendall non-parametric test showed that Kendall's tau_b was positive but not statistically significant (Kendall's tau-b =.147, $p > 0.05$) hence time (in years) and was not a significant predictor of the annual average rainfall in Lamu. Further, Figure 5 presents seasonal rainfall trends with Lamu receiving a bimodal rainfall distribution (long rain and short rain seasons) throughout the year. Long rain season is rainfall received between mid-April to the end of June (Naeku, 2020) while the short rain season comes in November and December, decreasing rapidly to a minimum in January and February (Kairo *et al*., 2021). The trend includes the minimum and maximum rainfall for the long rain season and short rain season from 1985 to 2020. Further, Kendall's tau_b (Table 3) showed that long rain seasons had a negative but not statistically significant trend (Kendall's tau_b = -0.048, $p > 0.05$). In contrast, the short rain season showed a positive and statistically significant trend (Kendall's tau-b =0.445, $p < 0.05$). Therefore, the finding suggests that the time factor accounted for more of the short rain season's rainfall than the long rain season's rainfall.

Seal level variation and trend analysis

Figure 6 shows the trend analysis for annual sea level including the minimum and maximum at 7 023 and 7 151 millimetres, respectively. The coefficient of determination (R^2) showed that time explained 49.07 % of the total change in mean sea level throughout the research period. The Mann-Kendall test (Table 4) revealed that Kendall's tau_b was positive and statistically significant (Kendall's tau-b = 0.577, $p <0.05$), indicating that time in years described the trend for annual mean sea level at Lamu from 1995 to 2018 and these findings corroborates that of Guerry (2022). Time was therefore a predictor of Lamu's mean sea level.

People's perception of climate change

Perception of temperature

Figure 7 showed that 87.8 % of respondents in the hh survey perceived that the temperature was higher now than it had been in the previous ten years. This contrasted with only 12.2 % who perceived differently. In terms of temperature variance, 87.5 % of those surveyed said it had changed significantly during the preceding 10 years. The FGD also found a considerable temperature change, with participants showing that the temperature in Lamu has been rising for quite some time. A respondent [a tour guide from Kiunga] in the FGD revealed that,

> "Lamu temperatures have recently risen. The beach area becomes warm at 11 a.m. and stays so throughout the day. Most local tourists are afraid of the hot weather and would often rest until late in the day before returning to the beach. I have never seen temps climb this high in my 20 years on Lamu."

Table 2. Mann-Kendall test for average annual rainfall trends in Lamu (1985-2020).

			Year	Rainfall (mm)
Kendall's tau_b	Year	Correlation Coefficient	1.000	0.147
		Sig. (2-tailed)	0.0	0.218
	Rainfall (mm)	Correlation Coefficient	0.147	1.000
		Sig. (2-tailed)	0.218	.

Source: Kenya Meteorological Department, Lamu (2020)

Perception of Rainfall

Figure 8 shows the frequency distribution of respondents' opinions on the volume and distribution of rainfall (2010-2020). Most respondents (90.1 %) believed that recent rainfall was low compared to 10 years ago. In terms of rainfall distribution, most respondents (91.8 %) believed that rainfall was unequally distributed over the seasons as compared to 10 years ago. Indeed, a respondent [a farmer from the Faza area] in the FGD stated,

> "I have noticed that the long rains that used to start in April now arrive as late as June and often end earlier as well." There is presently little distinction between "long" and "short" showers…Rainfall has been irregular and inconsistent throughout the season, including in the Mkokoni and Ndau districts."

Perception of Sea Level Rise

Figure 9 shows that the sea level is higher currently than it was 10 years ago, as supported by 86.7 % of respondents. The finding suggests that the sea level in Lamu County has risen during the last 10 years. The respondents in the FGD from the Faza area of Lamu reported having experienced an increase in sea level. They noted floods and property devastation, disruption of fishing activities, loss of agriculture due to inundation, and increasing coastline erosion. One of the respondents [the beach leader in Faza] in the FGD stated,

> "The sea level has been rising for as long as I can remember, and most of the places where I used to play as a child have now been submerged by rising waters." Rising water levels have engulfed the majority of the neighbouring areas. The mangroves near the shore have also gotten inundated, with most mangrove trees standing deep in water and drying out."

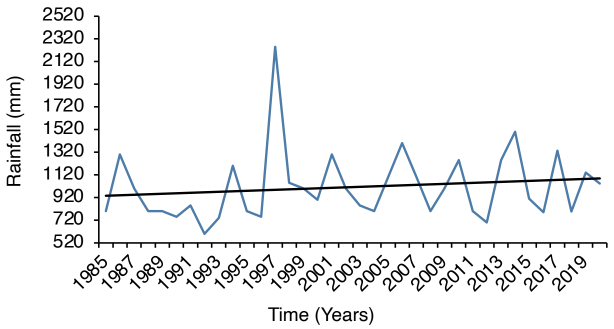

Figure 4. Average annual rainfall trend analysis for Lamu County [1985-2020] (Climate Data Services KMD, 2020).

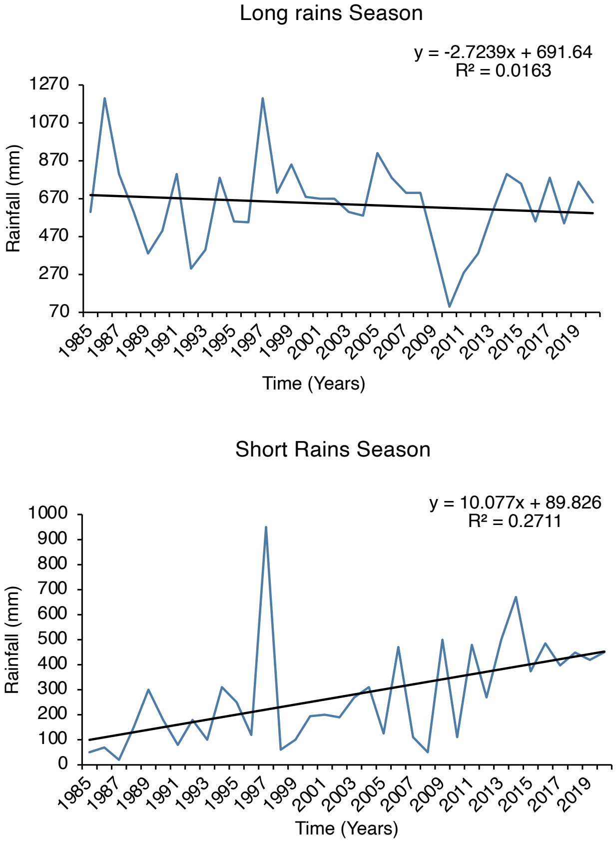

Figure 5. The trend in average season rainfall in Lamu County [1985–2020] (Climate Data Services, Kenya Meteorological Department, 2020).

Table 3. Mann-Kendall test for average seasonal rainfall trends in Lamu (1985-2020).

			Year	Long Rains	Short Rains
Kendall's tau_b	Year	Correlation Coefficient	1.000	-0.048	0.445**
		Sig. (2-tailed)	0.0	0.682	0.000
	Long Rains	Correlation Coefficient	-0.048	1.000	-0.063
		Sig. (2-tailed)	0.682	0.0	0.594
	Short Rains	Correlation Coefficient	0.445**	-0.063	1.000
		Sig. (2-tailed)	0.000	0.594	0.0

** At the two-tailed significance threshold of 0.01, the correlation is significant.

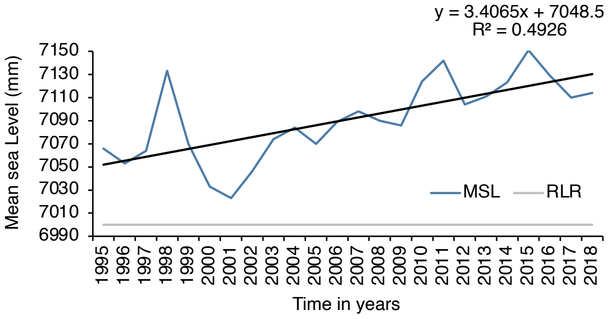

Figure 6. Annual mean sea level (1995-2020) for Lamu (Hawaii Sea Level Centre [UHSLC]) (Caldwell, 2020)

Perceived impact of climate change on mangrove-dependent livelihoods in Lamu

The information in Table 5 reveals several impacts of climate change variables on mangrove-dependent livelihoods. Most (96 %, 95.8 %) of respondents in the hh survey were of the perception that rainfall and sea level rise have been destroying property and infrastructure respectively. The respondents in the FGD recalled the Tsunami event in 2004, during which the sea level in villages such as Faza and Mpeketoni rose significantly destroying homes, fishing boats, and nets. One respondent [resident of Mpeketoni] stated: "*Climate change has not been forgiving especially around here in Faza, the erratic rainfall has destroyed most of the marram roads built through CDF and County government, and even houses have never been spared in the wake of floods.*" Furthermore, most of the respondents in the survey revealed that rainfall (94.1 %) and sea level rise (100 %) were the major causes of displacement of populations from ancestral lands in Lamu. The KII with the respondent from KFS revealed, *"The heavy downpours are associated with increased soil erosion landwards and transported and deposited in the mangrove. The soil improves mangrove sediments inclination thereby leading to increased landwards growth of mangrove thereby displacing population from ancestral land."*

Most (94.6 % and 97.2 %) of the respondents in the survey were of the perception that climate change through erratic rainfall and sea level rise had resulted in a rise in waterborne infectious diseases respectively. In addition, respondents in the FGD stated most residents in the Faza and Mkokoni areas of Lamu depended on rainwater as the main source of drinking water. Once it is depleted, they are forced to beg for it or use stagnant water from nearby pools leading to a rise of water-borne diseases. The stored water also sometimes has been contaminated leading to waterborne diseases such as Bilharzia. One respondent [a member of Save Lamu] in the FGD stated, "*Children and adults in Lamu's terror-prone Boni forest are often affected by outbreaks of bilharzia and diarrhoea due to drinking contaminated water. The most affected families are those from Kiangwe, Mangai, Milimani, and Basuba villages inside the dense Boni forest….*"

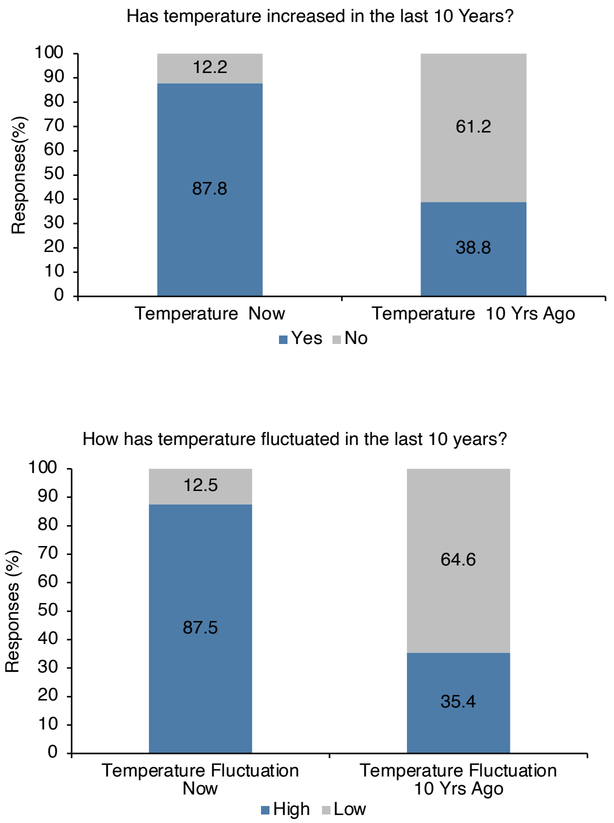

Figure 7. Perception of temperature.

Table 4. Mann-Kendall test for annual mean sea level trends in Lamu.

			Year	MSL (mm)
Kendall's tau_b	Year	Correlation Coefficient	1.000	0.577**
		Sig. (2-tailed)	0.0	0.000
	MSL (mm)	Correlation Coefficient	0.577**	1.000
		Sig. (2-tailed)	0.000	0.0

**Correlation is significant at the 0.01 level (2-tailed)

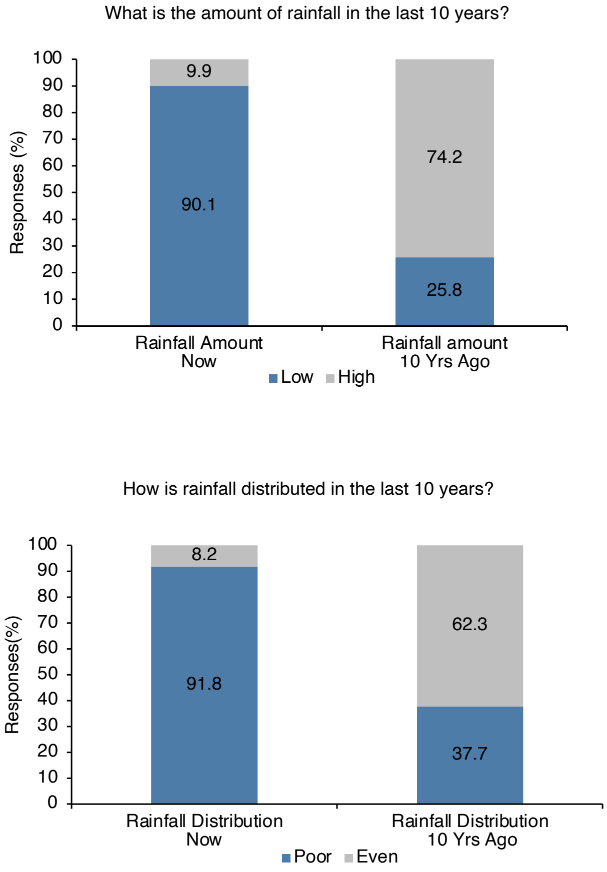

Figure 8. Perception of rainfall amount and distribution in the last 10 years.

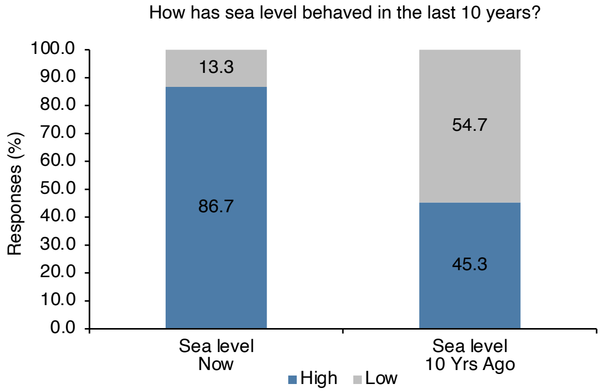

Figure 9. Sea level now and 10 years ago.

The majority (100 %, 100 %, and 94.1 %) of the respondents in the hh survey noted that rainfall, temperature, and sea level rise respectively were contributing to reduced availability of mangrove products like wood, timber, medicine, and honey. Additionally, in the interview with a marine expert from KMFRI, the respondent thought that mangroves in Lamu, just like in other tropical areas, were particularly susceptible to sea level rise given that they commonly have limited opportunity to move landward due to terrestrial space constraints, sediment poor environments and existing human structures and land uses in the coastal zone. The expert stated further, "*Changes in SLR can cause sediment erosion, inundation stress, and increased salinity in mangrove habitat leading to the death of mangrove.*"

The majority (95.8 %) of the respondents in the HH survey also perceived increased salinity in the underground fresh water. Additionally, one respondent from Mkokoni who participated in the FGD stated,

> "There is a shortage of drinking water as most wells have become salty...climate change has worsened the water situation in some villages such as Faza." Further, another respondent noted, "The Island does not have any piped water. Residents rely primarily on rainwater for their domestic needs as most wells have either dried up or become salty...."

The study also noted that the majority (97.5 %, 97.7 %, and 96.8 %) of the respondents in the hh survey

Table 5. Perceived impact of climate change on mangrove-dependent livelihoods.

	Rainfall		Temperature		Seal Level	
Perceived Impact of Climate Change on Mangrove-dependent Livelihood	**Not True** %	**True** %	**Not True** %	**True** %	**Not True** %	**True** %
Destruction of property and infrastructure	4.0	96.0	-	-	4.2	95.8
Displaced populations from their ancestral lands	5.9	94.1	-	-	0.0	100.0
Rise in waterborne infectious diseases	5.4	94.6	-	-	2.8	97.2
Reduced mangrove products like wood, timber, and honey.	0.0	100.0	0.0	100.0	5.9	94.1
Increased salinity in underground water	-	-	-	-	4.2	95.8
Destruction and interference with recreational beaches	2.5	97.5	2.3	97.7	3.2	96.8
Destruction of fish habitat hence low fish catch	-	-	4.5	95.5	-	-

revealed that climate change through rainfall, temperature, and sea level rise respectively, was responsible for either destruction of or interference with recreational beaches. In an FGD with a respondent from the Manda area, the respondent stated,

> "The rising sea level is slowly swallowing up most of the beaches that tourists like basking on. I am worried that in the future, all our sandy beaches will be underwater. Our lives revolve around tourism and fishing and if the tourists stop coming because there are no beaches for them to bask and swim, I foresee a difficult life for most of us..."

Finally, most of the respondents in the hh survey were of the perception that temperature (95.5 %) variability was responsible for the destruction of fish habitat, and hence low fish catch. Based on the FGD, respondents observed that high temperatures were affecting fishing and crop production activities. High temperatures have affected the breeding areas of fish, thus reducing quantities in the ocean.

Discussion

The study findings showed that Lamu's mean annual temperatures were on the rise, a finding that is in line with Yvonne *et al.* (2020), and Dzoga *et al.* (2019). The maximum rainfall occurred in 1997-1998, coinciding with El Niño while the driest year was 1992 when the country as a whole was experiencing La Niña effects including Lamu County, which experienced severe drought (Kemarau and Eboy, 2021; Generoso *et al.*, 2020). The current findings also revealed that the longer rainfall season was declining while the short rain season rainfall was increasing, hence the seasonal rains were becoming unpredictable with frequent drought situations in between flooding, a result that is supported by Yvonne *et al.* (2020), Maina, *et al.* (2021), and Yvonne *et al.* (2020). The study also noted that the sea level in Lamu was on the rise, a finding that was in line with Ward *et al.* (2016) and Zachary *et al.* (2023).

The climate change experienced in the Lamu area (i.e., sea level rise, increasing temperature, and erratic seasonal rainfall) was linked to the vulnerability of mangrove-dependent livelihoods in Lamu County. The findings showed that erratic rainfall, increasing temperature, and sea level rise contributed to the reduced availability of mangrove products like wood, timber, medicine, and honey. The findings noted that mangroves in Lamu, as in other tropical areas, are susceptible to sea level rise as they often have limited opportunity to move landward due to terrestrial space constraints and existing human structures and land uses in the coastal zone. The changes in SLR result in sediment erosion, inundation stress, and increased salinity in mangrove habitats leading to the death of mangroves (Cameron *et al.*, 2021; Fongnzossie *et al.*, 2022; Maina *et al.*, 2021; Mung'ong'o *et al.*, 2019).

These findings also revealed that erratic seasonal rainfall and sea level rise have been destroying property and infrastructure (i.e., roads, homes, fishing gear), displacing populations from their ancestral lands, and submerging coastal sandy beaches. The displacement of the local population happens via two major mechanisms. First, the floods from erratic rainfall and sea level rise submerge lands next to the ocean therefore displacing their owners (McMichael *et al.*, 2020; Nicholls *et al.*, 2021). Secondly, the deposition of soils in the mangroves from erosion has resulted in increased landwards growth of mangroves thereby displacing the population from ancestral land (Ghosh *et al.*, 2019; Ward *et al*, 2021). Increasing sea levels have also swallowed most of the land forming recreational beaches hence interference with tourism activities (i.e., basking, swimming) and lives revolving around tourism and fishing (López-Dóriga *et al.*, 2019; Athanasiou *et al.*, 2020).

The study noted erratic rainfall and sea level rise were associated with a rise in waterborne infectious diseases. The use of contaminated stagnant waters left in pools after floods in Faza, Mkokoni, Kiangwe, Mangai, Milimani, and Basuba areas in Lamu was associated with a rise of water-borne diseases such as Bilharzia and diarrhoea due to drinking contaminated water (Cissé *et al.*, 2019; El-Sayed *et al.*, 2020). Further, the increasing temperature was responsible for interference with fish breeding and the destruction of fish habitat, resulting in low fish catch in Lamu. High temperatures have affected the breeding areas of fish, thus reducing quantities in the ocean as fish migrate away from traditional fishing grounds. The reduced fish catch for the population living around the ocean implies that their livelihoods are impaired (Dzoga *et al.*, 2019; Lindmark *et al.*, 2022). Climate change has therefore resulted in increased vulnerability of mangrove-dependent communities in Lamu. These findings call for climate actions and programmes aimed at improving the resilience of vulnerable populations in Lamu via mitigation and adaptation.

In terms of contribution to theory and practice in this field of study, the rising annual mean sea level and erratic seasonal rainfall and associated flooding have implications for decision-makers regarding disaster early warning, relocation, and building of dykes to minimize damage to properties and infrastructure. Further, given the significant impact of climate change on the mangrove ecosystem, the population depending on the mangrove ecosystem is increasingly becoming vulnerable calling for increased revenue allocation to programmes aimed at achieving climate justice. Such increased allocation should enable the full implementation of various climate change-related policies aimed at mitigation and adaptation. The vulnerable mangrove-dependent communities should also seek local solutions to climate change such as joining formal and informal groups to enable them to source funds among themselves to finance adaptation programmes such as purchasing alternative cooking fuels, modern fishing gadgets, and relocation where necessary. The increasing temperature and erratic rainfall have implications for livelihoods related to small-scale farming regarding decisions around planting early maturing and drought-resistant crop verities. The findings on sea level rise are critical for the Lamu County government and disaster management authorities globally.

Conclusions

The study concludes that there is a declining long rainy season (March–June) and an increasing short rainy season (October–December) in the study area and this implies that the rainfall in the longer rainy season has become unreliable with consequences such as drought and flooding. Further, increasing temperatures and rising sea levels in Lamu County have impacted mangrove-dependent livelihoods in Lamu (i.e., fisherfolk, mangrove cutters, mangrove product harvesters, and boat makers) in terms of interference with fish availability, reducing incomes from mangrove products, and impaired tourism activities when beaches become submerged. The study also concludes that the rising sea level for Lamu in the study period has resulted in adjacent lands to the ocean being submerged with the displacement of the local population living around coastal lands. The climate change variables (i.e., sea level rise and associated flooding) have also been linked to the destruction of property and infrastructure such as road networks, fishing gear, and shelter, among others. This now means the livelihoods that depend on mangroves are more vulnerable because of these alterations in climate. Though the current study examined the trend in climate change variables (temperature, rainfall, and sea level rise), the scope of the study did not examine the causal factors of the climate variables. Future studies should go a step further by examining causal factors behind climate change variables in Lamu using the most updated data sets.

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Original Article

Improved accessibility and changing dynamics of small-scale fisheries and aquaculture activities in southwest Madagascar

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Abstract

Small-scale fishing and aquaculture activities in the village of Andrevo in southwest (SW) Madagascar was investigated to assess changing dynamics during a period of improved road infrastructure and accessibility. Socio-economic surveys and fisheries monitoring were undertaken using a simple random sampling strategy. Stakeholders involved in the small-scale fisheries and aquaculture sectors were sampled. The main types of fishing gear were hook-and-line (including longline), harpoon, speargun, gill nets, bottom seine nets, mosquito and shark nets (called ZZ or *Jarifa*). Subsectors of the fishery were boat fishers (74 %), foot fishers (18 %), and combined (8 %). On average, 236 boats fish on a daily basis, with an overall catch rate (all gears combined) of 7.5 kg/trip/gear and total catch of approx. 47 tonnes/month. Mariculture of seaweed and sea cucumber farming constitute alternative income-generating activities. The production of dry seaweed varied from 9-70 tonnes/year, and sea cucumber production depended on the number of juveniles delivered. An ecosystem-based approach to managing fisheries and aquaculture at Andrevo is recommended, using locally-based measures such as co-management and marine protected areas (MPAs). The dynamics of the small-scale fishery and aquaculture activities are also discussed in this paper in relation to improved road infrastructure and accessibility in SW Madagascar.

Keywords: southwest Madagascar, small-scale fisheries, fishing gear, mariculture of seaweed, sea cucumber aquaculture

Introduction

Madagascar is the fourth largest island in the world with a coastline of about 5000 km and an Exclusive Economic Zone (EEZ) of approximately 1,140,000 km^2 (Rabenevanana *et al*., 1990). Madagascar relies heavily on its coastal resources for food security, trade and tourism (Le Manach *et al*., 2012; Cooke, 2003; Gabrié *et al*., 2000). The fisheries sector plays an important role in the economy as an income generating activity and a source of animal protein for coastal communities (FAO, 2016; Le Manach *et al*. 2012; Donner and Potere, 2007; Newton *et al*. 2007).

Madagascar's shelf is broadest along the west coast where it borders on the Mozambique Channel. In southwest (SW) Madagascar near Toliara city, the seascape includes coral reefs (Grand Récif and Ifaty/Ranobe Reef; Thibaud, 2012) and mangroves, making it rich in marine fauna and flora, and attractive to fishers (Rejela, 1993). Fishing is a fundamental activity in SW Madagascar, known for its traditional or "small-scale" fishing practices (Noniarilala, 2010; Bemiasa, 2009). The expansion of aquaculture activities on sea cucumbers (holothuriculture) and seaweed (algoculture) in SW Madagascar provides alternatives to fishing and an additional income stream (Todinanahary *et al.*, 2017). Together, these activities generate direct and indirect employment opportunities while contributing a substantial proportion of animal protein to the diets of coastal communities (Rakotonaivo, 2012; Mills et *al.*, 2011; Belle et *al.*, 2009).

The transportation of fishery products to markets has been a challenge in SW Madagascar, because of inadequate preservation and transportation methods, and poorly maintained access roads. The Road Infrastructure Development Project (PAIR) on National Road 9 (RN9) has improved road access to SW Madagascar thereby promoting trade and regional development (FAD, 2013). A key aspect of the present study was to understand how the improved road infrastructure in SW Madagascar has affected fishing and aquaculture activities in coastal communities. The operational aspects of these activities are an important component of developing sustainable management practices in a shared seascape in SW Madagascar.

In light of the contextual background, a study was undertaken to examine the dynamics of fisheries and describe the development of aquaculture activities in SW Madagascar, with a focus on Andrevo village. Specific aims were to: (1) undertake a socio-economic survey to identify stakeholders in the fishing and aquaculture sectors; (2) evaluate the small-scale fishery, focusing on gears used, fishing effort, catch composition and domestic markets; and (3) review the recent development of the aquaculture industry. Trends in the small-scale fishery and aquaculture activities are discussed in relation to improved road infrastructure and accessibility in SW Madagascar.

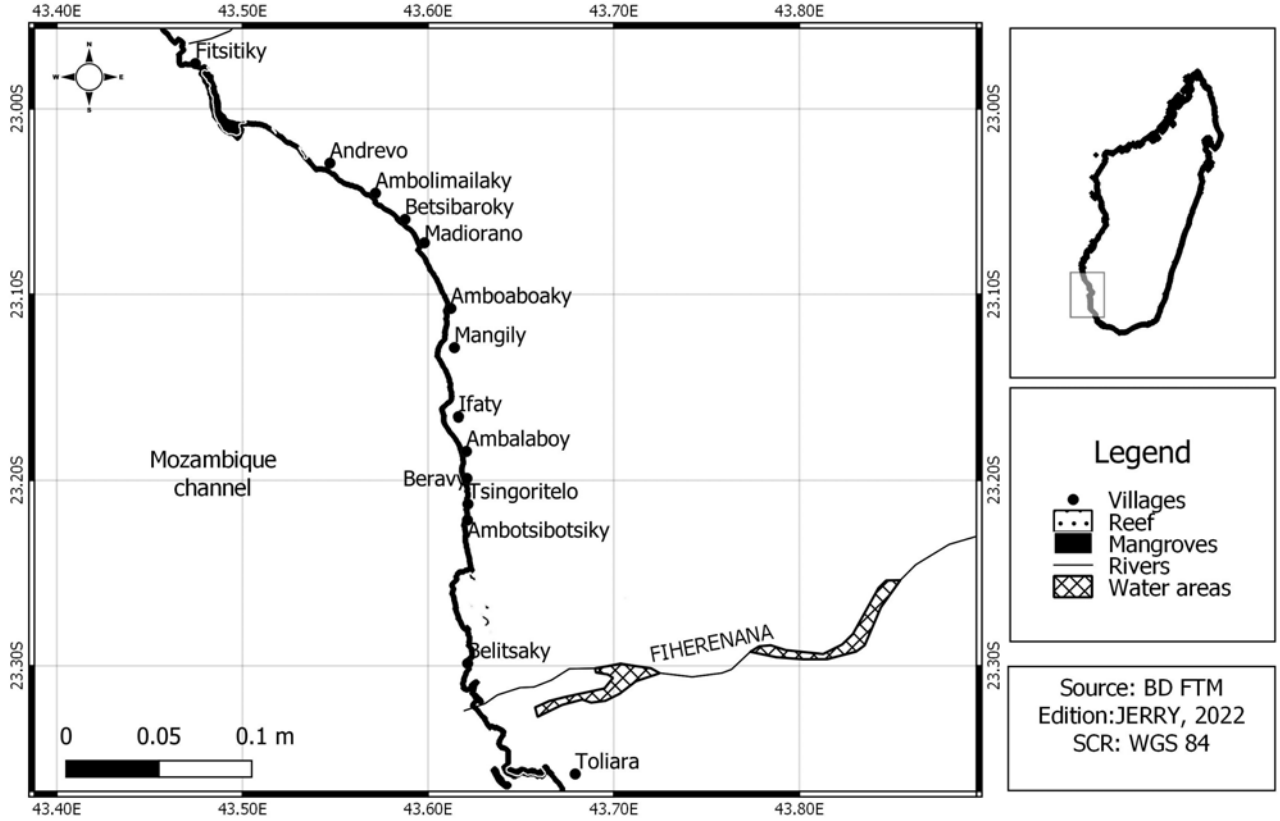

Figure 1. Location of the village of Andrevo, Madagascar.

Material and methods

Study area

SW Madagascar has a dry climate with high reliance on fishing (McClanahan *et al.*, 2014). Thirteen fishing villages surround Ranobe Bay: Belitsaky, Ambotsibotsiky, Tsingoritelo, Beravy, Ambalaboy, Ifaty, Mangily, Amboaboaky, Madiorano, Betsibaroky, Ambolomailaky, Andrevo and Fitsitiky (Belle *et al.*, 2009; Davies *et al.*, 2009) (Fig.1). These villages supply Toliara city and other inland markets with marine fisheries products. Andrevo village was chosen for this study because of its importance as a small-scale fishing village that supplies Toliara with fish products, the geographical representativeness of the area, the existence of aquaculture activities that generate income for the coastal population, and ease of access.

The Vezo ethnic group is predominant among fishing communities (Laroche *et al.*, 1997) in Ranobe Bay. Vezo ethnicity relates to performing maritime activities rather than to ancestry or place of origin (Astuti, 1995). The Vezo practice traditional fishing or 'small-scale fishing' (Lemahieu *et al.*, 2018; McClanahan *et al.*, 2014), mainly in Ranobe Bay, using fishing gear such as hook-and-line, gill- and beach seine nets and spear guns (McClanahan *et al.*, 2014).

Fieldwork and data analysis

Field work took place between 28 October and 20 December 2017 and was divided into (1) a socio-economic survey and (2) a fisheries survey to obtain information on fishing methods, effort and catches.

Socio-economic survey

Semi-structured interviews were carried out in fishermen households to generate qualitative and quantitative insights into the Vezo lifestyle (Fauroux, 2002). Samplers were first introduced to the Chef de Fokontany (village head) to explain the purpose of the research and ask for guidance and suitable survey respondents, before conducting surveys. Each interview took place in a location chosen by the informant and was carried out in the Vezo dialect (Garth and Charlie, 2016; Huguenin and Richard, 2014; Barnes-Mauthe *et al.*, 2013).

For the socio-economic data, the quantitative values (e.g., age of the fishers) were grouped into classes, to facilitate the statistical representation of the data, whereby class intervals were calculated according to the Sturge formula: Number of classes = 1 + (3.3 log n) where log n represents the logarithm to the base 10 and n represents the number of people in the sample. The number of classes is rounded up (Sturges, 1926). Dividing the range of variation in weight (the difference between the largest and smallest value of weight) by the number of classes found gives an order of magnitude of the class range:

$$\textbf{Class interval} = \frac{\textit{Maxim value - minimal value}}{\textit{number of classes}}$$

The data was processed and analysed in Excel.

Fisheries survey

Two main types of fishing strategy were sampled: (1) foot fishing using bare-handed collection and bottom seining as fishing techniques, with boats used only for transporting fishing products; and (2) boat fishing, in which fishing boats are used for fishing, including for transport of products. Fishing effort was expressed as the number of boats going to sea on a particular day (for boat fishing with gillnets, mosquito nets, spear guns, lines and harpoons) or as the number of foot fishers active per day or undertaking bottom seine fishing. The location of fishing sites was obtained from the fishermen and cross-checked against geographical coordinates using a GPS.

The average fishing effort per trip was calculated by dividing the total number of boat trips (or number of foot fishers) over the 30 days of monitoring. The calculation involved dividing the given value by the number of trips per day, considering the total days of monitoring. The average duration of fishing trip per fishing gear was also recorded to calculate the Catch Per Unit Effort (CPUE) (Lee *et al.*, 2010).

CPUE (catch/effort) for boat fishers was expressed as kg/boat/trip for each gear, and for foot fishers it was expressed as kg/fisherman/trip. Data were available for total catch, the number of fishing boat and fishermen on board, and the average duration of a trip.

Results

Socio-economic characteristics

The village of Andrevo has 275 households composed of 1280 inhabitants (4.7 persons/household) of which 82 % (861 inhabitants) of the active population (1050 inhabitants) belong to fisher households. Of these, 180 fishers belonging to 151 registered households (54.9 %) were surveyed representing 14.02 % of the fisher population.

Most of the surveyed fishers were Vezo (91.7 %), nomadic fishers native to SW Madagascar, with small proportions of Masikoro (6.1 %), Antandroy (1.7 %), Mahafaly (0.6 %) and Bara (0.47 %). Others were traditional farmers and migrants to the coastal area (Table 1).

Most fishers were 24 to 38 years old (39.4 %), with 20 % of fishers aged 31-38 yrs and 19.4 % aged 21-31 yrs (Table 1). Children learn to fish from the age of 7 yrs, by following their parents, or by using discarded pieces of fishing gear in sea grass areas. The population of SW Madagascar is increasingly oriented towards fishing.

Table 1. Descriptive statistics of socio-economic data.

Age		Level of study			Ethnic group				Fishery											Secondary activity						
									BF				FF		BF/FF											
Class	Percentage	I	P	C	V	M	An	Ma	G	L	S	H	D	H	G	L	S	H	D	None	A	F	Ho	Co	B	Bm
[10;17[	2,22	0	3	1	4	0	0	0	2	0	1	0	0	0	1	0	0	0	0	4	0	0	0	0	0	0
[17;24[	16,11	1	22	6	29	0	0	0	14	9	0	4	0	1	1	0	0	0	0	25	1	0	0	0	2	1
[24;31[	19,44	3	26	6	32	0	2	1	21	7	1	2	0	3	0	0	0	1	0	23	0	1	5	1	4	1
[31;38[	20	1	33	1	34	1	0	0	19	0	0	2	1	13	0	0	0	0	0	30	3	0	0	0	1	1
[38;45[	15	9	17	1	22	5	0	0	14	0	0	1	5	7	0	0	0	0	0	20	1	2	0	0	2	2
[45;52[	14,44	6	19	2	24	2	1	0	16	2	0	1	2	6	0	0	0	0	0	19	4	0	2	0	2	0
[52;59[	7,78	11	2	1	12	2	0	0	7	2	0	2	1	2	0	0	0	0	0	11	2	1	0	0	0	0
[59;66[	3,89	5	1	1	6	1	0	0	5	0	0	0	1	1	0	0	0	0	0	4	2	0	0	0	1	0
[66;73[	1,11	2	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Total	99,99	38	123	19	165	11	3	1	99	21	2	12	10	33	2	0	0	1	0	138	13	4	7	1	12	5
Percentage		21,1	68,3	10,6	91,7	6,1	1,7	0,6	55,0	11,7	1,1	6,7	5,6	18,3	1,1	0,0	0,0	0,6	0,0		7,2	2,2	3,9	0,6	6,7	2,8
	100	100,0			100,0				100,0											76,7	23,3					
																					100,0					

A: Algaculture
An: Antandroy
B: Breeding
BF: on Boat Fishing
Bm: Boat manufacturing
C: College
Co: Commerce
D: Draoto (small mesh net)
F: Farming
FF: on Foot Fishing
G: Gilnet
H: Harpoon
Ho: Holothuriaculture
I: Illiterate
L: Line (Handline)
M: Masikoro
Ma: Mahafaly
P: Primary
S: Speargun
V: Vezo

The education level of fishers in Andrevo was low, with only 10.6 % having reached secondary school, 68.3 % not exceeding primary school and 21.1 % illiterate (Table 1). There was no secondary school in the village, insufficient funding and a lack of awareness of the importance of school education.

Among the fishers surveyed, 76.7 % had no other activities apart from fishing (Table 1). Some 23.3 % of fishers had more than one occupation, although fishing remained the main activity. Agriculture is practiced by 6.7 % of fishers during the rainy seasons (Table 1). Other secondary occupations include 'dockers' that offload fishing boats, boat-building, trade, sea cucumber and algae farming.

Small-scale fisheries

Fishing at Andrevo covers a large area and different sites are visited depending on season, weather, means of transport, fishing gear and species targeted. Of 32 potential fishing sites, six were visited most frequently due to their proximity to town. However, regardless of the distance to the site, fishing duration lasted between 4 and 6 hrs.

As in other fishing villages in SW Madagascar, the fishers at Andrevo use a pirogue with outrigger. This traditional fishing boat is made from *Givotia madagascariensis* wood, locally called farafatsy. Propulsion is through a rectangular sail of variable size (1.75 m long for large *Lay be* and 1.0 m for small *Ana-day*).

Of 180 fishermen surveyed, 133 (74 %) were boat fishers, 33 (18 %) were foot fishers, and 14 (8 %) participated in both boat and foot fishing. Eight fishing gears were identified: handline, harpoon, gill net, speargun, bottom seine, mosquito net, shark net (ZZ or *Jarifa*) and longline, of which the first six were the most used (Ramahatratra, 2014) (Table 2). Most of the fishing gears were self-made and assembled with materials bought in Toliara (e.g., nylon thread or wood paste). Gill nets (46 %) were the most used gear in Andrevo; they are efficient, easy to use and cheaper than shark nets or fish guns (Fig. 2) Bottom-set seine nets or 'draoto' (3 %) were only used by the Masikoro ethnic group, mainly farmers who moved to the coast for fishing (Table 2).

Table 2. Description of fishing gear and percentage usage.

Gear	Construction and use	Percentage of use (%)	Illustration
Gillnet	- made of nylon thread, - 100 m length or even more and 4 m wide in general but also vary according to the requirements of the fishermen, -catch fish.	46	
Harpoon	- a kind of spear whose pointed head may or may not have one or more hooks, - length of the wooden stick depends on fisher's preference and use, - catch octopus, fish, lobsters, shrimp, sea cucumbers, bivalves, and sea urchins on the reef shelf and even offshore underwater.	29	
Longline	- made from a 5 m long nylon or Talirano line, - a rectangular wood 14 cm long, 5 cm thick and 8 cm wide serves as a support for the line on which it is wound, - A hook for the squid line and a hook for the fish line are attached to the end of the line.	16	
Bottom seine or 'Draoto'	- similar to beach seine but small in size and does not have a pocket, - 100 m long and 75 cm wide, with a 400 m rope on both ends.	3	
Mosquito net	- a very small mesh net, (2 mm) known locally as *Makarakara,* - 100 m length and as wide according to the prefence of the fishermen, with a large bag 4 m long and two wings at each side of the net, - catch small pelagics such as *Spratelloides delicatulus* (*Varilava*), *Stolephorus indicus* (*Tove*) and *Herklotsichtys quadrimaculatus* (*Geba*) (seasonal use).	3	
Speargun	- consists of a carved wooden handle on which a nail blocks the arrow, - catch fish.	1	
ZZ net and *Jarifa*	- made with a thick rope of about 6 mm, - larger mesh nets measuring 8 to 10 fingers (*nendry*), can be up to 200 m long and 7 m high, - catch shark, sea turtle and fish.	2	

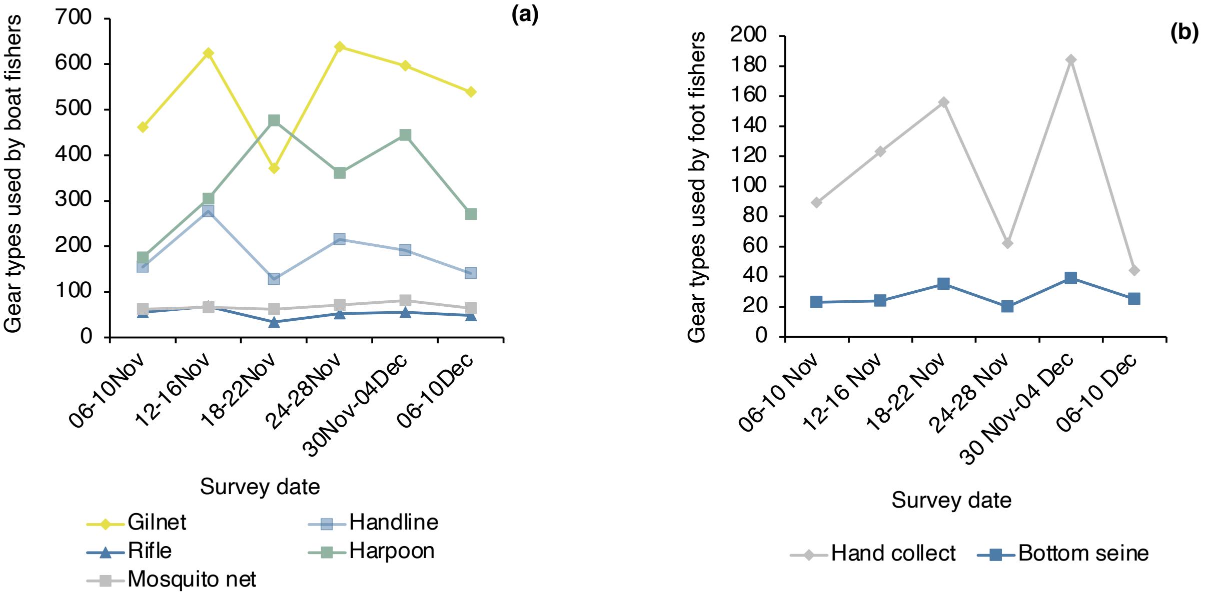

Figure 2. Weekly trends in gear types used by (a) boat fishers and (b) foot fishers in Andrevo over the sampling period

The average catch was 7.5 kg/trip ranging from 2.3 to 15.9 kg/trip (all gears combined) (Fig. 3). Daily catches varied by gear as follows: 11.2 kg/boat for gillnets, 4.8 kg/boat for mosquito nets, 3.3 kg/boat for harpoons, 1.9 kg/boat for lines, and 1.1 kg/boat for spear guns. Bottom seines (13.3 kg/trip) and foot fishing (16.9 kg/trip) were the most productive in terms of quantity.

Overall, catches comprised of 50 species belonging to 35 families (Fig. 4), including fish (50.5 %), edible shellfish (18.2 %), holothurians (19.3 %), cephalopods (7.5 %), sea urchins (2.3 %) and crustaceans (2.1 %). The composition of catches from boat fishing was dominated by Gerreidae (15.7 %), Carangidae (14.5 %) and Sphyraenidae (11.4 %). Catches by foot fishers included Holothuridae (32.2 %) and Arcidae (29.9 %), and for bottom seining Labridae (23.3 %) was most common (Fig. 4).

Based on the average number of fishing days per month over the course of a year (est. 26.5 days) the monthly and annual production of Andrevo village was estimated. The average daily catch (7.5 kg/trip all

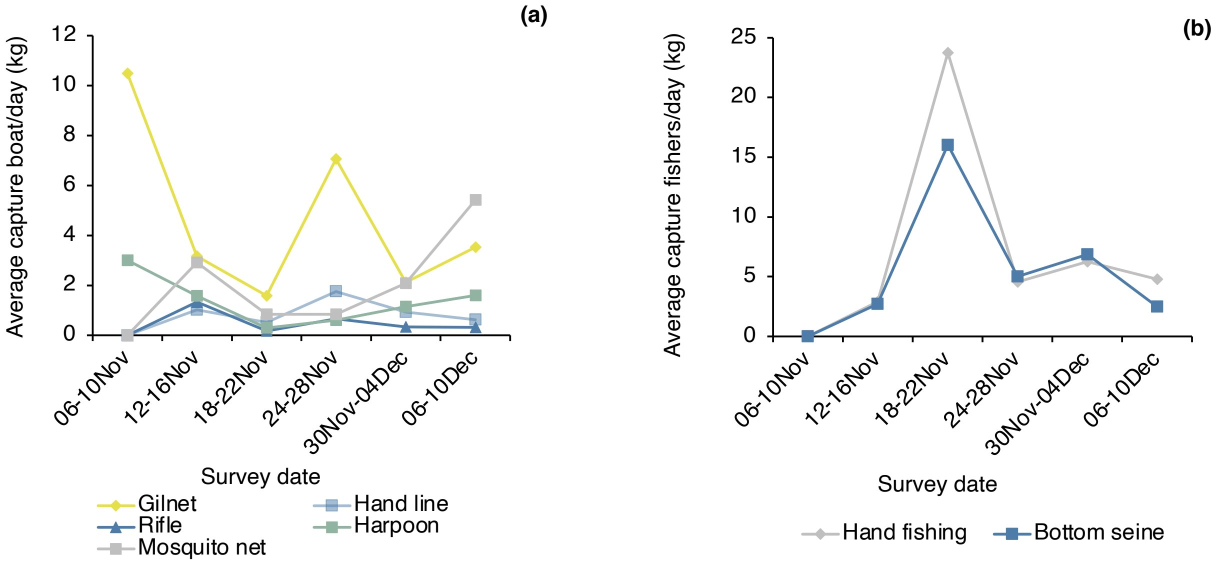

Figure 3. Weekly variation in catch per trip by fishing type, (a) boat fishers and (b) foot fishers

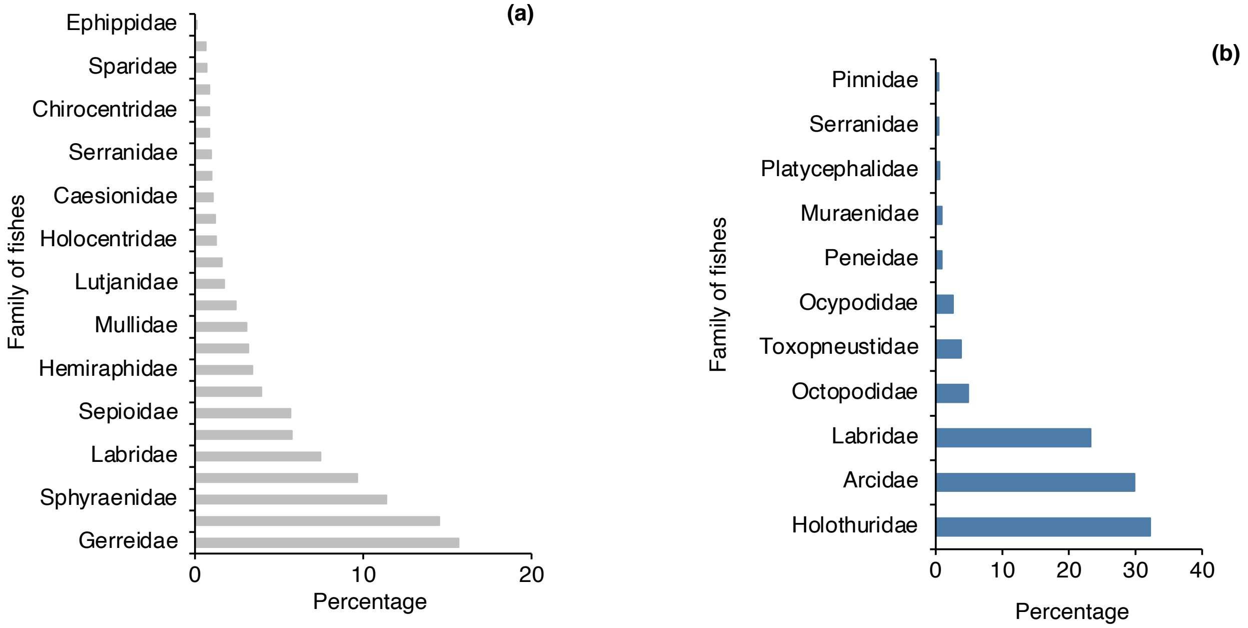

Figure 4. Main families caught by (a) boat fishers and (b) foot fishers.

gears combined) was multiplied by the average fishing effort (236.3 boats/day). The monthly production of Andrevo (i.e., 7.5 kg x 236.3 boats x 26.5 days) was estimated as 46.9 tonnes and the annual production was estimated at 563.6 tonnes. Most of the catch was sold, and the remainder consumed as food.

Discussion

Trends in small-scale fisheries

Le Manach *et al.* (2012) and Davies *et al.* (2009) reported that small scale fisheries were fundamentally important in SW Madagascar, where agriculture is largely unviable because of the dry climate. During this study, 180 fishers were surveyed, 91 % of whom were Vezo, similar to the findings of Davies (2009) and Astuti (1995). The west coast of Madagascar is home to most of the country's fishers, and therefore experiences the highest fishing pressure (Laroche *et al.,* 1997; Guidicelli *et al.*, 1984; Lamahieu *et al.*, 2018), while the eastern part of the island has the highest overall human population density (Le Manach *et al.*, 2012). The demographics in the coastal area of SW Madagascar have changed dramatically over the last twenty years, following migrations to the coast and an increase in both fishing villages and the number of fishers (ECN, 2012). The fishing community now comprises of several ethnic groups (Table 1).

Eight fishing gears were identified from this survey, similar to the findings of Ramahatratra, (2014) and Razanoelisoa (2008) except for the addition of bottom seines, a newly introduced technique in Andrevo, used by immigrant fishers. This shows an evolution in the use of fishing gear, when confronted with scarcity of resources and increasing demand. Several fishing areas were frequented by fishers suggesting rotation among areas when catch rates decline (Natale *et al.*, 2015; Johnson *et al.*, 2017).

There is some evidence for local depletion caused by overfishing. Species that were initially discarded have now become target species, including various shellfish, sharks, moray eels and gametes of the sea urchin *Tripneustes gratilla*. According to fishers, popular species such as *Atherinomorus* and *Sphyraena* have become rare or absent from catches. Octopus were initially dried and sold in the highlands (Fianarantsoa and Antananarivo) but the advent of international markets (e.g., Mauritius, Reunion, EU) have increased the demand for octopus. Overall, high unemployment and a lack of alternative employment in SW Madagascar have resulted in increased fishing effort and the trade in marine products, with fishing becoming a remedy for unemployment, rather than a traditional occupation.

Six buyers (fishmongers) monopolise the purchase of fish in Andrevo. Each has their own clients (fishermen) with prices negotiated according to abundance of products. Fishmongers re-sell to retailers (Fig. 5) who transport catches to markets, mainly at Ankilimaliniky, Ankililoaky, Ambolomailaky and Toliara. No sorting by species or quality takes place. There are no door-to-door sales, even when catches are high. A similar system is used to supply hotels and restaurants,

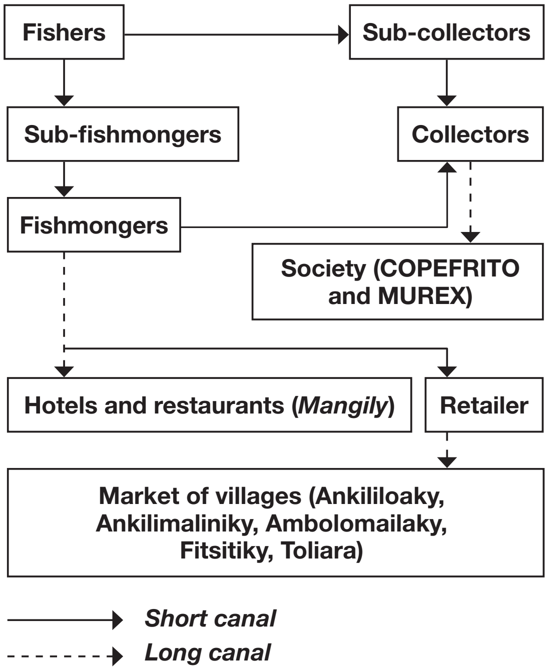

Figure 5. Market chain for fishery products.

with each fishmonger supplying their own clients. The sale is done fresh and the prices vary according to fish abundance and size. Ice is used to preserve fresh fish during transport, which is now more consistent and faster because of improved road infrastructure.

Prior to asphalting the main access road (RN9), Andrevo was less accessible and a larger proportion of fish catches were consumed locally. The remainder were processed in smoked, salted or dried form for sale in the interior, at Ankililoaky, Ankilimaliniky, Fitsitiky or even Toliara. After the asphalting of the RN9, the fresh fish products are distributed, with wholesalers and sub-collectors using ice to maintain the fish quality. Two groups of species are highly targeted by Andrevo fishermen: fish and cephalopods. The dominance of Murex and COPEFRITO in the octopus market, the primary octopus distributors in the SW Madagascar, plays a significant role in the income of this sector (Raberinary, 2015). It provides a stable environment, ensuring a consistent supply and potentially reducing market volatility. However, it may lead to limited competition, fixed prices at lower levels and reduced choices for consumers.

Development of coastal aquaculture – seaweed farming

Seaweed farming (algaculture) through wild seaweed collection cultivation in SW Madagascar creates opportunities for diversification, employment and increased income (Chaboud, 2006). The cultivation of red algae *Eucheuma striatum* began in 1991 in Madagascar, initiated by the Institut Halieutique et des Sciences Marines (IH SM) in Toliara, in association with the Biomad company.

Seaweed farming in Andrevo was initiated in 2012 by *Projet d'Appui aux Communautés des Pêcheurs de Toliara* (PACPT). By 2017, there were 116 (89 active) seaweed farmers organized into groups of villagers and fishermen. Specialized technicians trained by Ocean Farmers Society supervise farming activities. Farmers are contractors of the Society to whom they sell their harvests. The Society provides the infrastructure and buys materials.

A fixed elevation system is used for seaweed farming, with ropes or nets stretched by stakes or rock blocks. The system can be adapted to available space and depth as follows:

- "long line": 30 to 40 m long rope, immersed in water between 1 and 1.2 m deep.
- "mini long line": rope of 10 to 20 m in length and a water depth of less than 1.2 m;
- "off bottom": 2 to 5 m long rope in shallow water between 0.5 and 1 m

The system is cost-effective, easy to maintain and insensitive to surface weather conditions as the seaweed remains submerged, even at low tide. Cuttings of *E. striatum* (fragments of 70 to 100 g) are fixed to

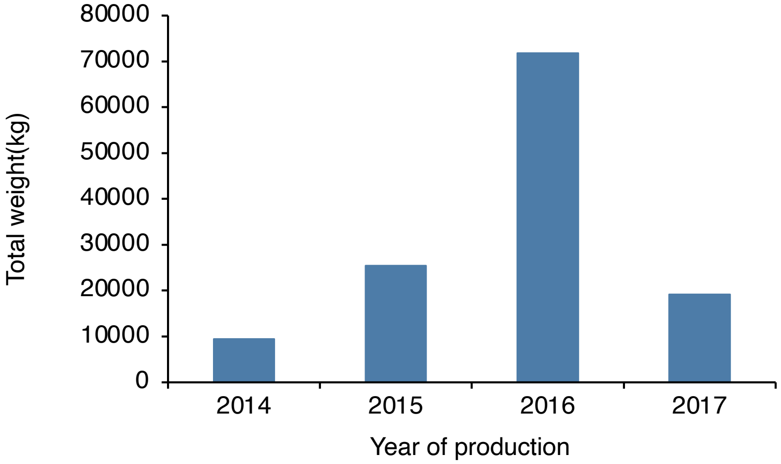

Figure 6. Annual variation of algae production in the village of Andrevo from 2014 to 2017 (COPEFRITO, 2016; pers.com.).

a monofilament nylon thread of 1-3 mm in diameter and maintained perpendicular to the current, by fixing them to stakes in the sediment.

The annual production of Andrevo varies from 9.4 to 71.8 tonnes, including 2 harvests a year or less. These values are due to the parallel increase in the number of wild seaweed farmers and the number of bags of seaweed supplied by these farmers (Fig. 6).

Sea cucumber farming

Indian Ocean Trepang (IOT) was the first industrial sea cucumber farm in Madagascar. It has a hatchery for producing larvae and post larvae and a pre-growth farm for producing juveniles (both located in Ankaloaha just behind Toliara airport) and a juvenile grow-out farm in Belaza and in the seagrass beds at Ankoronga, Toliara (SW Madagascar). IOT has been active since 2014 and has expanded its grow-out farm to Andrevo. Apart from the technical feasibility study of this expansion project in Andrevo, an environmental and social impact study was carried out in March 2017 and the Ministry of Fisheries Resources and Fisheries (MRHP) and the Ministry of Environment supported their selection.

Sea cucumber farming in Andrevo is undertaken by 25 farmers divided into 18 households, increasing from 14 farmers (7 teams of fisherman and wife) at the onset. Rearing is done in the natural environment, without addition of feed, at a density of 500 individuals/enclosure of 90 m^2. The density can be sustained by the nutritional regeneration capacity of the sediment in the enclosure. Juveniles are bought from IOT. Although the number of juveniles delivered to each farmer team is similar, the difference in production of adult sea cucumbers is determined by the technical and breeding maintenance of each team.

The prices of sea cucumbers in the Toliara region fall within a fairly wide range, depending on the quality and species (Randrianarivelo, 2008). The technical and commercial partner at Andrevo prefers to buy sea cucumbers fresh, to avoid poor quality products. The purchase price of sea cucumbers from fishermen varies according to weight.

Agencies supporting aquaculture

'*Vondronolona Ifotony*' (VOI) is an association of fishermen established in 2002. In Andrevo, it works closely with a local association called *Fikambanana Soan'Andrevo* (FIKASOA) with 232 members. FIKASOA works with similar associations around the Ranobe Bay to facilitate management of natural resources and to promote income-generating activities for improved living standards.

VOI / FIKASOA promotes seaweed and sea cucumber farming, reed farming or vondro and mangrove reforestation or *ala honko* as income generating activities for the local population. Among others, non-governmental organisations (NGOs) and fishing companies provide support for the promotion and management of fisheries and aquaculture.

Conclusion and Recommendations

The study of the dynamics of small-scale fishing and aquaculture development in Andrevo village generated important insights related to the general organisation of the villagers, the socio-economic characteristics of the fishers and especially knowledge about fishing effort and catches. No modernisation of dugout canoes or fishing gear was observed. The fishing areas frequented are extensive, from the beach to the reef fronts; only shark or *Jarifa* nets are set offshore. The development of coastal aquaculture, targeting export products such as red algae and sea cucumbers, support livelihoods. The presence of favourable shallow areas for culture and a buoyant market through exporting companies such as IOT, COPEFRITO, makes further expansion possible. Andrevo is the third largest producer of seaweed in the region, with annual production increasing from 9.4 to 71.8 tonnes between 2014 and 2016. Logistic hurdles for small-scale fishers and aquaculture development in Andrevo are insufficient materials, scarcity of resources and low prices for products. Use of destructive techniques, market monopolization, and disrespect for local regulations have been reported. NGOs assist fishers with technical support for development and sustainable management. Spatial conflict between small-scale fishing and aquaculture appears to be negligible. Asphalting of the main road (RN9) has improved accessibility to markets, to which freshly caught fish kept on ice can now be rapidly and consistently distributed. This has in turn affected fish processing priorities and local consumption patterns in Andrevo. Improved access has also enhanced opportunities for aquaculture, including investment, technical expertise and access to markets. Care should be taken that fishing effort does not increase rapidly as a result of improved accessibility.

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Original Article

Fish species, families and guilds recorded in selected estuaries of Mozambique

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Abstract

This review documents the occurrence of fish species in a range of estuaries from Mozambique. Altogether 217 fish species were recorded, belonging to 77 families, and dominated in terms of species richness by Gobiidae, Carangidae and Mugilidae. A guild analysis was conducted to compare the occurrence and degree of estuary-association by the various species in tropical Mozambique, with that recorded from nearby predominantly subtropical and warm-temperate estuaries in South Africa. The major difference in guild occurrence between the two countries centered on the higher representation of marine stragglers in Mozambique estuaries, a probable result of the wide mouths and macrotidal exchange of some of the larger systems along this coast. Estuarine lakes and lagoons within the Mozambique coastal region showed increasing reduction in marine connectivity with channel distance from the sea, and a concomitant increase in freshwater fish species domination of these incipient coastal lakes and lagoons. Once these systems lose all connectivity with the sea, they become freshwater coastal lakes and lagoons, and all estuary-associated marine fish species disappear.

Keywords: estuarine fish, species list, fish guilds, African estuaries

Introduction

Although several fisheries related studies have been conducted in some Mozambique estuaries (e.g., Costa *et al.*, 2020), little biological or ecological information is available on the ichthyofauna when compared to the extensive work that has been completed on the fishes in a wide range of South African estuaries. The end result is that estuarine fish assemblages in Mozambique, apart from selected fisheries taxa, are relatively poorly known, and understanding of the structure and functioning of estuarine fish communities in this region is still in its infancy. Indeed, there is no comprehensive list of species that have been recorded from estuaries in the country – hence the attempt here to compile such a list. Future ichthyological studies will benefit from this synthesis of information on what taxa are likely to occur in estuaries in this important tropical region on the eastern coast of Africa. Different species are also placed in appropriate fish guilds in order to compare guild composition in Mozambique with that recorded in the predominantly subtropical and warm-temperate estuarine systems of South Africa.

Study area

The Mozambique coast comprises three broad regions based on geomorphological characteristics (Macamo *et al.*, 2016). The dune coast extends from Ponta do Oura in the south to Bazaruto (Fig. 1). Based on available spatial data from Mozambique waterways and Google Earth approximately 18 functional estuaries are found within this region. These include typical

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river estuaries and bays but there are also extensive high dunes, behind which estuarine and coastal lakes have developed. The central zone extending north to Angoche (Fig. 1) is a swamp coast, with at least 50 estuaries in this region, many formed by the confluence of more than one river at the coast. This zone has the highest number of river inflows and is characterized by muddy sediments and turbid waters. The coral coast starts north of Angoche, characterized by lower river runoff, reduced sediment input and clean water. Although apparently characterized by an abundance of estuaries (93), most of these are small systems fed by coastal streams and groundwater seeps. Mangroves may be present, and even extensive in typical large permanently open river valley type estuaries, but they are also widespread along deltaic sections of the coast where no immediate estuaries are present (e.g., to the north and south of the Zambezi River Estuary) (Fig. 1).

Figure 1. Map of Mozambique showing the three major coastal regions and estuarine systems mentioned in the text.

All the estuaries of Mozambique may be categorized as tropical and most belong to two main types; namely estuarine lakes and lagoons, and permanently open estuaries. An example of an estuarine lake is Lagoa Poelela in southern Mozambique (Fig. 1). This segmented estuarine lake system has a large and deep lake (65 km^2 in area and an average depth of 13.7 m) at its top end, a fairly uniform lake salinity of 8 ppt, and is connected to the marine environment by a 75 km long channel that intersects a number of estuarine lagoons en route to the sea (Hill *et al.*, 1975). There is little tidal regime within the Poelela system, and no tidal changes within the upper lake. In contrast, an example of a permanently open estuary is the Bons Sinais system in central Mozambique (Fig. 1) that has a semi-diurnal tidal regime of approximately 4 m during spring tides. This estuary receives a highly seasonal river flow, is 28 km in length, and has channel depths ranging from less than 2 m to more than 20 m in places (Hoguane *et al.*, 2020). The Morrumbene Estuary (Fig. 1) is a similar type of system entering Inhambane Bay. This 20 km long estuary has a channel that is about 10 m deep at the mouth, decreasing to 1-2 m in the middle reaches at low tide (Day, 1974).

One of the largest estuaries in Mozambique is the Zambezi system, a river mouth type of estuary that is dominated by outflowing freshwater from a very large catchment area (1 390 000 km^2). This river flow usually averages about 3 000 m^3 s^{-1} and creates a freshwater plume that enters the coastal ocean and is sensitive to winds on diurnal and synoptic time scales (Nehama and Reason, 2015). Nevertheless, lateral channels and small lagoons on the Zambezi floodplain are frequently influenced by saline tidal inputs during reduced river inflow periods and therefore offer nursery habitats for estuary-associated marine fish species (Bills, 1999). Indeed, there are many mangrove-dominated coastal inlets to the north and south of the Zambezi River Estuary but these inlets, although providing food and shelter for estuary-associated marine fish species, are not estuarine because there is no riverine or land-based freshwater influence on their functionality.

There are also examples of Mozambique estuarine lakes/lagoons that are in the process of becoming completely isolated from the sea (e.g. Lake Piti in southern Mozambique), at which stage they will become coastal lake/lagoon systems that have lost all marine connectivity, such as has occurred at Lake Sibaya in northern KwaZulu-Natal, South Africa (Allanson *et al.*, 1966). Some of these lagoon systems are bounded by longitudinal dunes that separate the water bodies from the sea and will be strongly influenced by climate change and sea level rise in the future (Miguel *et al.*, 2019). Access by marine fish to intermittently open estuaries is limited when the sand berm at the mouth is closed, thus restricting any fish movement to or from the adjacent estuarine system. However, heavy precipitation in the river catchments of these systems, sometimes associated with cyclonic rainfall events, usually breaches the sand berm and restores estuarine-marine connectivity for a period that is determined primarily by the duration of river outflow and scouring action in the mouth.

Methods

Information on the occurrence of fish species from a range of estuaries in Mozambique was collected from both published and unpublished sources (Table 1). Because of the wide variety of gear used in the various studies cited in this table, no attempt has been made to quantify catches, or compare species occurrence between different systems or regions along the coast. Detailed information on the life cycles of most fish species occurring in Mozambique estuaries is scarce. However, by using very broad estuary-associated categories (Table 2), it was possible to place the recorded species into one of seven guilds.

Using data from Whitfield (2019), a similar fish species list and guild classification was compiled for those taxa recorded in South African estuaries to allow for comparisons between the relative composition of life-history categories of estuary-associated fish species in Mozambique and South Africa (Table 3). However, the primary aim of this review was to compile a preliminary list of species and families found in Mozambique estuaries, thus supporting future work on the estuarine ichthyofauna within this important biogeographical region.

Results

Although preliminary, Table 1 indicates a potentially rich ichthyofauna associated with the estuaries of Mozambique. In terms of species richness, Mozambique estuaries have diverse fish assemblages, comprising a total of at least 217 species and belonging to 77 families (Table 1). This compares to the 170 species belonging to 60 families recorded in South African estuaries (Appendix).

A comparison between the occurrence of fish families between estuaries in Mozambique and South Africa revealed a high degree of similarity in terms of species richness (Table 3). Eight of the top ten families were shared between the two countries, with the Gobiidae and Mugilidae being in the top three places from both regions. Most of the families were shared between Mozambique and South Africa and many of the species were the same in both regions (Table 1; Appendix).

An analysis of fish guild composition in estuaries from the two countries revealed some differences in

Table 1. Fish species recorded from Mozambique estuarine systems ([1]Unpublished records from AW Paterson; [2]Costa *et al*., 2020; [3]Mugabe *et al*., 2021; [4]Mocuba *et al*., 2023; [5,6]Unpublished records from SP Weerts; [7]Day, 1974; [8]Published and unpublished records from the SAIAB Fish Collection, including Smith and Heemstra, 1986; Bills 1999, 2001). For a description of the estuarine guild categories see Table 2.

Fish families	Fish species	South African common names	Estuarine guilds	[1]Moebase, Molócuè, Ligonha	[2,3,4] Bons Sinais	[5] Muladi	[6] Phungwe	[7] Morrumbene	[8] SAIAB
Acanthuridae	*Acanthurus triostegus*	Convict surgeonfish	MS	+					
Ambassidae	*Ambassis ambassis*	Longspine glassy	ER	+				+	+
Ambassidae	*Ambassis dussumieri*	Malabar glassy	EM	+		+	+	+	+
Ambassidae	*Ambassis natalensis*	Slender glassy	EM			+	+		+
Anguillidae	*Anguilla bicolor*	Shortfin eel	CM						+
Anguillidae	*Anguilla labiata*	African mottled eel	CM						+
Anguillidae	*Anguilla marmorata*	Giant mottled eel	CM						+
Anguillidae	*Anguilla mossambica*	Longfin eel	CM						+
Apogonidae	*Apogonichthyoides uninotatus*	Onespot cardinal	MS					+	
Apogonidae	*Foa brachygramma*	Weed cardinalfish	MS					+	
Apogonidae	*Ostorhinchus quadrifasciatus*	Two-stripe cardinal	MS	+					
Ariidae	*Arius africanus*	African sea catfish	MI		+				
Ariidae	*Plicofollis dussumieri*	Blacktip sea catfish	MI						+
Atherinidae	*Atherinomorus lacunosus*	Hardyhead silverside	MI					+	+
Atherinidae	*Hypoatherina barnesi*	Slender silverside	MI	+					
Belonidae	*Strongylura leiura*	Banded needlefish	MI			+			+
Belonidae	*Tylosurus crocodilus*	Hound needlefish	MI					+	+
Blenniidae	*Antennablennius bifilum*	Horned blenny	EM						+
Blenniidae	*Omobranchus elongatus*	Cloister blenny	EM					+	
Blenniidae	*Omobranchus ferox*	Gossamer blenny	ER						+
Blenniidae	*Omobranchus punctatus*	Muzzled blenny	EM					+	
Blenniidae	*Petroscirtes mitratus*	Floral blenny	EM					+	
Blenniidae	*Petroscirtes variabilis*	Variable sabretooth blenny	EM					+	
Bothidae	*Bothus pantherinus*	Leopard flounder	MS			+			+
Bothidae	*Engyprosopon natalense*	Natal flounder	MS					+	
Caesionidae	*Caesio xanthonota*	Yellowback fusilier	MS						+
Callionymidae	*Callionymus marleyi*	Sand dragonet	MS					+	
Callionymidae	*Synchiropus marmoratus*	Marbled dragonet	MS					+	
Carangidae	*Alectis indicus*	Indian mirrorfish	MS	+					
Carangidae	*Carangoides armatus*	Longfin trevally	MS					+	
Carangidae	*Caranx heberi*	Blacktip kingfish	MS			+			+
Carangidae	*Caranx ignobilis*	Giant kingfish	MI					+	
Carangidae	*Caranx melampygus*	Bluefin kingfish	MI					+	
Carangidae	*Caranx papuensis*	Brassy kingfish	MI	+		+			
Carangidae	*Caranx sexfasciatus*	Bigeye trevally	MI			+		+	
Carangidae	*Craterognathus plagiotaenia*	Barcheek kingfish	MS	+					
Carangidae	*Megalaspis cordyla*	Torpedo scad	MS	+					
Carangidae	*Platycaranx malabaricus*	Malabar kingfish	MS	+					
Carangidae	*Scomberoides commersonnianus*	Talang queenfish	MI						+
Carangidae	*Scomberoides lysan*	Doublespotted queenfish	MI			+		+	+
Carangidae	*Scomberoides tala*	Barred queenfish	MI					+	+
Carcharinidae	*Carcharinus leucas*	Zambezi shark	MI						+
Centriscidae	*Aeoliscus punctulatus*	Speckled shrimpfish	MS					+	
Chanidae	*Chanos chanos*	Milkfish	MI			+			+
Cichlidae	*Coptodon rendalli*	Redbreast tilapia	FI			+			+
Cichlidae	*Oreochromis mossambicus*	Mozambique tilapia	FI	+	+			+	+
Cichlidae	*Oreochromis placidus*	Black tilapia	FI						+
Cichlidae	*Pseudocrenilabrus philander*	Southern mouthbrooder	FI						+
Cichlidae	*Tilapia sparrmanii*	Banded tilapia	FS						+
Clariidae	*Clarias gariepinus*	Sharptooth catfish	FI		+				+
Clupeidae	*Gilchristella aestuaria*	Estuarine roundherring	ER						+
Clupeidae	*Hilsa kelee*	Kelee shad	MI	+	+		+		+
Clupeidae	*Pellona ditchela*	Indian pellona	MI	+	+				
Clupeidae	*Sardinella albella*	White sardinella	MS		+				
Congridae	*Uroconger lepturus*	Longtail conger	MS	+					
Cynoglossidae	*Cynoglossus durbanensis*	Durban tonguesole	MS					+	
Cynoglossidae	*Paraplagusia bilineata*	Doublelined tonguesole	MS					+	
Cyprinidae	*Enteromius annectens*	Broadstriped barb	FS	+					
Cyprinidae	*Enteromius paludinosus*	Straightfin barb	FS						+

Fish families	Fish species	South African common names	Estuarine guilds	[1]Moebase, Molócuè, Ligonha	[2,3,4] Bons Sinais	[5] Muladi	[6] Phungwe	[7] Morrumbene	[8] SAIAB
Cyprinidae	*Enteromius radiatus*	Beira barb	FS	+					
Dasyatidae	*Himantura uarnak*	Reticulate whipgray	MI					+	
Dasyatidae	*Maculabatis ambigua*	Baraka's whipray	MS				+		
Dasyatidae	*Maculabatis gerrardi*	Sharpnose stingray	MS	+					
Dasyatidae	*Pastinachus sephen*	Feathertail stingray	MS	+					
Dorosomatidae	*Sardinella melanura*	Blacktip sardinella	MS					+	
Drepaneidae	*Drepane longimana*	Concertina fish	MS						
Eleotridae	*Butis koilomatodon*	Mud sleeper	EM	+					
Eleotridae	*Eleotris fusca*	Dusky sleeper	ER						+
Eleotridae	*Eleotris mauritiana*	Widehead sleeper	ER						+
Eleotridae	*Eleotris melanosoma*	Broadhead sleeper	ER			+			+
Elopidae	*Elops machnata*	Skipjack	MI						+
Engraulidae	*Engraulis japonicus*	Japanese anchovy	MI	+					
Engraulidae	*Stolephorus commersonnii*	Commerson's anchovy	MI					+	+
Engraulidae	*Stolephorus holodon*	Thorny anchovy	MI	+		+			
Engraulidae	*Stolephorus indicus*	Indian anchovy	MI		+	+		+	
Engraulidae	*Thryssa setirostris*	Longjaw glassnose	MI	+	+				
Engraulidae	*Thryssa vitrirostris*	Orangemouth glassnose	MI	+	+		+		+
Ephippidae	*Platax orbicularis*	Orbicular batfish	MS	+				+	
Ephippidae	*Tripterodon orbis*	Spadefish	MS	+				+	
Epinephelidae	*Epinephelus coioides*	Orange-spotted grouper	MS						+
Epinephelidae	*Epinephelus malabaricus*	Malabar grouper	MI			+	+		
Epinephelidae	*Epinephelus tauvina*	Greasy grouper	MI					+	
Fistulariidae	*Fistularia petimba*	Red cornetfish	MS					+	
Gerreidae	*Gerres filamentosus*	Threadfin pursemouth	MI	+				+	
Gerreidae	*Gerres longirostris*	Strongspine pursemouth	MI			+		+	+
Gerreidae	*Gerres oblongus*	Slender pursemouth	MI					+	
Gerreidae	*Gerres oyena*	Slenderspine pursemouth	MI	+					
Gobiidae	*Aulopareia ocellata*	Taileyed goby	EM				+		
Gobiidae	*Awaous aeneofuscus*	Freshwater goby	FI			+			+
Gobiidae	*Coryogalops sordidus*	Epaulette goby	EM					+	
Gobiidae	*Croilia mossambica*	Naked goby	ER						+
Gobiidae	*Drombus triangularis*	Brown drombus	EM					+	
Gobiidae	*Favonigobius melanobranchus*	Blackthroat sandgoby	EM			+			
Gobiidae	*Glossogobius callidus*	River goby	ER	+					+
Gobiidae	*Glossogobius giuris*	Tank goby	ER	+		+		+	+
Gobiidae	*Istigobius ornatus*	Ornate sandgoby	EM			+			
Gobiidae	*Mugilogobius mertoni*	Chequered mangrove goby	ER						+
Gobiidae	*Oligolepis acutipennis*	Sharptail goby	ER	+		+			
Gobiidae	*Oxyurichthys keiensis*	Kei goby	ER	+		+			
Gobiidae	*Oxyurichthys ophthalmonema*	Eyebrow goby	EM	+		+			
Gobiidae	*Pandaka silvana*	Dwarf goby	EM						+
Gobiidae	*Paratrypauchen microcephalus*	Comb goby	ER	+					
Gobiidae	*Periophthalmus argentilineatus*	Barred mudskipper	ER				+	+	
Gobiidae	*Periophthalmus kalolo*	Common mudskipper	ER	+		+			+
Gobiidae	*Psammogobius biocellatus*	Sleepy goby	ER	+		+			
Gobiidae	*Redigobius balteatus*	Bull goby	EM	+		+			
Gobiidae	*Redigobius dewaali*	Checked goby	ER						+
Gobiidae	*Silhouettea sibayi*	Barebreast goby	ER						+
Gobiidae	*Stenogobius kenyae*	Africa rivergoby	FI						+
Gobiidae	*Yongeichthys nebulosus*	Shadow goby	EM	+					+
Grammistidae	*Belonoperca chabanaudi*	Arrowhead soapfish	MS	+					
Haemulidae	*Diagramma pictum*	Painted sweetlips	MS					+	
Haemulidae	*Plectorhinchus playfairi*	Whitebarred rubberlip	MS					+	
Haemulidae	*Pomadasys commersonnii*	Spotted grunter	MI						+
Haemulidae	*Pomadasys furcatus*	Banded grunter	MS						+
Haemulidae	*Pomadasys kaakan*	Javelin grunter	MI	+	+	+	+		
Haemulidae	*Pomadasys maculatus*	Saddle grunter	MI	+	+			+	
Haemulidae	*Pomadasys multimaculatus*	Cock grunter	MI	+				+	
Hemiramphidae	*Hemiramphus far*	Spotted halfbeak	MI	+				+	
Hemiramphidae	*Hyporhamphus affinis*	Tropical halfbeak	EM						+
Hemiramphidae	*Hyporhamphus capensis*	Cape halfbeak	EM						+

Fish families	Fish species	South African common names	Estuarine guilds	[1]Moebase, Molócuè, Ligonha	[2,3,4] Bons Sinais	[5] Muladi	[6] Phungwe	[7] Morrumbene	[8] SAIAB
Hemiramphidae	*Hyporhamphus improvisus*	Shortfin halfbeak	EM	+					
Kuhliidae	*Kuhlia mugil*	Barred flagtail	MI						+
Labridae	*Stethojulis strigiventer*	Three-ribbon wrasse	MS					+	
Leiognathidae	*Deveximentum ruconius*	Pugnose soapy	MS	+					
Leiognathidae	*Deviximentum insidiator*	Slender soapy	MS	+					
Leiognathidae	*Gazza minuta*	Toothed soapy	MI	+		+		+	
Leiognathidae	*Leiognathus equula*	Slimy	MI	+		+		+	+
Lethrinidae	*Lethrinus nebulosus*	Spangled emperor	MS					+	+
Lobotidae	*Lobotes surinamensis*	Tripletail	MI						+
Lutjanidae	*Lutjanus argentimaculatus*	Mangrove snapper	MI	+		+		+	
Lutjanidae	*Lutjanus fulviflamma*	Dory snapper	MI	+		+		+	
Lutjanidae	*Lutjanus fulvus*	Blacktail snapper	MI					+	+
Lutjanidae	*Lutjanus monostigma*	Onespot snapper	MS	+					
Lutjanidae	*Lutjanus sanguineus*	Humphead snapper	MS					+	
Megalopidae	*Megalops cyprinoides*	Oxeye tarpon	MI	+					+
Mochokidae	*Synodontis zambezensis*	Zambezi squeaker	FS	+					
Monocanthidae	*Paramonacanthus frenatus*	Wedgetail filefish	MS					+	
Monocanthidae	*Stephanolepis aurata*	Porky	MS					+	
Monodactylidae	*Monodactylus argenteus*	Round moony	MI	+				+	
Monodactylidae	*Monodactylus falciformis*	Oval moony	MI						+
Mugilidae	*Chelon dumerili*	Grooved mullet	MI					+	+
Mugilidae	*Chelon melinopterus*	Giant-scale mullet	MI	+			+		+
Mugilidae	*Crenimugil buchanani*	Bluetail mullet	MI					+	+
Mugilidae	*Crenimugil seheli*	Bluespot mullet	MI					+	+
Mugilidae	*Ellochelon vaigiensis*	Squaretail mullet	MI	+					
Mugilidae	*Moolgarda cunnesius*	Longarm mullet	MI	+			+		
Mugilidae	*Mugil cephalus*	Flathead mullet	MI		+				+
Mugilidae	*Osteomugil robustus*	Robust mullet	MI				+	+	+
Mugilidae	*Planiliza alata*	Diamond mullet	MI						+
Mugilidae	*Planiliza macrolepis*	Large-scale mullet	MI	+			+	+	
Mugilidae	*Planiliza subviridis*	Greenback mullet	MI						+
Mullidae	*Upeneus sulphureus*	Sunrise goatfish	MS	+					
Mullidae	*Upeneus vittatus*	Yellowbanded goatfish	MS	+				+	
Muraenesocidae	*Muraenesox bagio*	Pike conger	MI	+			+		
Opichthidae	*Brachysomophis crocodilinus*	Crocodile snake eel	MS						+
Opichthidae	*Pisodonophis boro*	Rice-paddy eel	MI					+	
Opichthidae	*Pisodonophis cancrivorus*	Longfin snake-eel	MI					+	
Ostraciidae	*Lactoria cornuta*	Longhorn cowfish	MS					+	
Ostraciidae	*Ostracion cubicum*	Yellow boxfish	MS					+	
Paralichthyidae	*Pseudorhombus arsius*	Largetooth flounder	MS	+				+	
Pegasidae	*Pegasus volitans*	Longtail seamouth	MS					+	
Pinguipedidae	*Parapercis robinsoni*	Smallscale grubfish	MS					+	
Platycephalidae	*Papilloculiceps longiceps*	Tentacled flathead	MS					+	
Platycephalidae	*Platycephalus indicus*	Bartail flathead	MI	+				+	
Platycephalidae	*Thysanophrys celebica*	Celebes flathead	MS					+	
Plotosidae	*Plotosus lineatus*	Striped eel catfish	MI					+	
Polynemidae	*Polydactylus plebeius*	Striped threadfin	MI	+					
Polynemidae	*Polydactylus sextarius*	Sixfinger threadfin	MI	+					
Pomacentridae	*Abudefduf saxatalis*	Sergeant-major	MS					+	
Priacanthidae	*Priacanthus hamrur*	Moontail bullseye	MS					+	
Pristidae	*Pristis microdon*	Smalltooth sawfish	MI						+
Pristidae	*Pristis zijsron*	Longcomb sawfish	MI						+
Pseudochromidae	*Pseudochromis natalensis*	Natal dottyback	MI					+	
Rhinobatidae	*Acrotariobatus leucospilus*	Greyspot guitarfish	MS	+					
Samaridae	*Samaris cristatus*	Cockatoo flounder	MS					+	
Scaridae	*Leptoscarus vaigiensis*	Marbled parrotfish	MS					+	
Sciaenidae	*Johnius amblycephalus*	Bearded croaker	MS		+				
Sciaenidae	*Johnius dorsalis*	Small croaker	MI	+					
Sciaenidae	*Otolithes ruber*	Tigertooth croaker	MI		+				
Scorpaenidae	*Dendrochirus brachypterus*	Dwarf lionfish	MS					+	
Scorpaenidae	*Parascorpaena aurita*	Golden scorpionfish	MS					+	
Scorpaenidae	*Pterois volitans*	Red lionfish	MS						+

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Siganidae	*Siganus rivulatus*	Marbled spinefoot	MS					+	
Sillaginidae	*Sillago sihama*	Silver sillago	MI	+	+			+	+
Soleidae	*Solea turbynei*	Blackhand sole	MI					+	+
Solenostomidae	*Solenostomus cynopterus*	Ghost pipefish	MS					+	
Sparidae	*Acanthopagrus vagus*	Estuarine bream	MI	+				+	+
Sparidae	*Argyrops spinifer*	King soldierbream	MS					+	
Sparidae	*Crenidens crenidens*	Karanteen seabream	MI					+	
Sparidae	*Diplodus capensis*	Blacktail	MI						+
Sparidae	*Rhabdosargus holubi*	Cape stumpnose	MI						+
Sparidae	*Rhabdosargus sarba*	Tropical stumpnose	MI					+	+
Sphyraenidae	*Sphyraena acutipinnis*	Sharpfin barracuda	MI					+	
Sphyraenidae	*Sphyraena barracuda*	Great barracuda	MI						+
Sphyraenidae	*Sphyraena jello*	Pickhandle barracuda	MI					+	+
Sphyraenidae	*Sphyraena pinguis*	Yellowstripe barracuda	MS	+					
Syngnathidae	*Acentronura tentaculata*	Shortpouch pipehorse	MS					+	
Syngnathidae	*Hippichthys cynospilos*	Blue-spotted pipefish	EM					+	
Syngnathidae	*Hippichthys heptagonus*	Belly pipefish	EM						+
Syngnathidae	*Hippichthys spicifer*	Bellybarred pipefish	EM	+					
Syngnathidae	*Hippocampus camelopardalis*	Giraffe seahorse	MI					+	
Syngnathidae	*Hippocampus kuda*	Spotted seahorse	MS					+	
Syngnathidae	*Syngnathoides biaculeatus*	Alligator pipefish	MS						
Synodontidae	*Saurida gracilis*	Gracile lizardfish	MI	+				+	
Terapontidae	*Pelates quadrilineatus*	Fourlined terapon	MI					+	+
Terapontidae	*Terapon jarbua*	Thornfish	MI	+				+	+
Tetraodontidae	*Amblyrhynchote honckenii*	Evileye pufferfish	MI	+					
Tetraodontidae	*Arothron hispidus*	Whitespotted pufferfish	MI					+	
Tetraodontidae	*Arothron immaculatus*	Blackedged pufferfish	MI						+
Tetraodontidae	*Canthigaster solandri*	False-eye toby	MS						
Tetraodontidae	*Chelonodontops laticeps*	Bluespotted pufferfish	MI	+					+
Tetraodontidae	*Chelonodontops patoca*	Milkspotted pufferfish	MS						+
Tetraodontidae	*Lagocephalus guentheri*	Blackback pufferfish	MS	+					
Tetraodontidae	*Torquigener hyselogeneion*	Orange-spotted toadfish	MS					+	
Tetrarogidae	*Ablabys binotatus*	Redskinfish	MS					+	
Trichiuridae	*Trichiurus lepturus*	Cutlass fish	MS	+					

Table 2. Categorization of the major fish guilds utilizing Mozambique estuaries (modified from Whitfield, 1999).

Fish guilds	Description of categories
Marine immigrants (MI)	Marine fish species that usually breed at sea with the juveniles and/or adults making use of the estuarine environment. The juveniles of many of these species show varying degrees of association with estuaries as nursery areas.
Marine stragglers (MS)	Marine fish species that breed at sea, with only a small proportion of the overall population ever entering or making use of estuaries. Most marine stragglers are confined to the lower estuarine reaches where they occur in very low numbers.
Estuarine residents (ER)	Fish species, usually of marine origin, that breed and are able to conduct their life cycle within the estuarine environment. Some estuarine resident species may also have marine or freshwater breeding populations.
Estuarine migrants (EM)	Fish species, usually of marine origin, that breed in estuaries but have a marine or freshwater aspect to their life cycle. Estuarine migrants often have marine or freshwater breeding populations.
Freshwater immigrants (FI)	Freshwater fish species that are often recorded in estuaries, retreating into catchment rivers when conditions become unfavourable. Some of these species may also breed in estuaries when conditions are suitable.
Freshwater stragglers (FS)	Freshwater fish species that sometimes enter estuaries when conditions are favourable. Freshwater stragglers are usually confined to the low salinity upper estuarine reaches where they occur in low numbers.
Catadromous migrants (CM)	Species that spawn at sea but use freshwater catchment areas during the juvenile and subadult life stages.

Table 3. The 10 most species rich fish families recorded in the estuaries of Mozambique compared to those of South Africa. The numbers in brackets refer to the number of species documented for each of the families.

Estuary-associated fish families in Mozambique	Estuary-associated fish families in South Africa
Gobiidae (23)	Gobiidae (24)
Carangidae (13)	Mugilidae (14)
Mugilidae (11)	Sparidae (13)
Tetraodontidae (8)	Carangidae (9)
Haemulidae (7)	Syngnathidae (8)
Syngnathidae (7)	Clupeidae (5)
Blenniidae (6)	Gerreidae (5)
Engraulidae (6)	Haemulidae (5)
Sparidae (6)	Engraulidae (4)
Eleotridae (5)	Tetraodontidae (4)

composition (Fig. 2). Marine immigrants (40 %) and marine stragglers (34 %) were dominant in Mozambique estuaries, with marine immigrants (52 %) and marine stragglers (16 %) also dominant in South African estuaries but showing a different proportional representation. The other major guilds in the estuaries of both Mozambique and South Africa were estuarine species. In the former country, estuarine migrants comprised 11 % of the species richness and estuarine residents 8 %. These proportions were the reverse for South African estuaries, with estuarine residents comprising 16 % and estuarine migrants 8 % (Fig. 2).

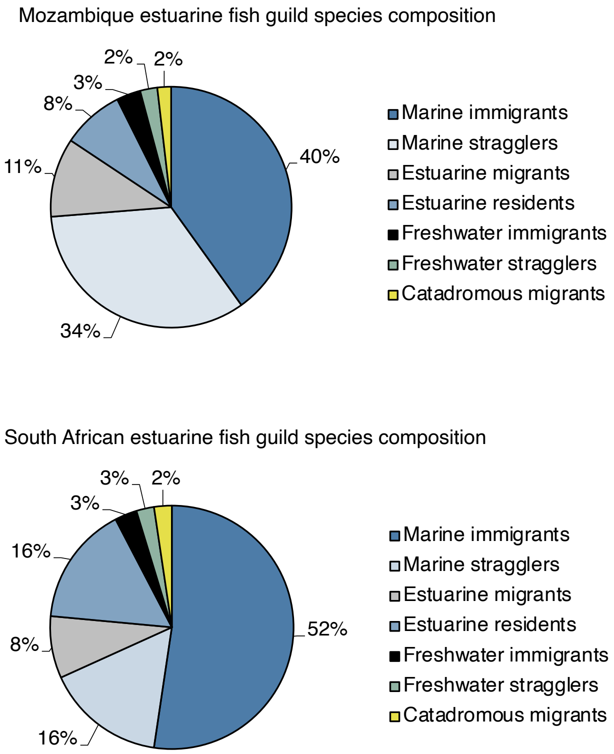

Figure 2. Estuarine fish guild composition in Mozambique and South African estuaries based on number of species. For a description of the guild categories, refer to Table 2.

Considering actual rather than relative number of species in different fish guilds, the most striking difference between the two countries was in the markedly higher numbers of marine stragglers reported from Mozambique systems, 73 species in Mozambique compared to 35 in South African estuaries.

Discussion

In terms of species richness, the dominant fish family found in both Mozambique and South African estuaries was the Gobiidae, with more than 22 species from each country. This statistic reflects the diverse nature of this taxon, with representatives from marine, estuarine and freshwater goby species. Mugilidae ranked second in South African estuaries (14 species) and third in Mozambique estuaries (11 species). The higher species richness in the south can be attributed to the additional presence of three endemic taxa (*Chelon richardsonii*, *Chelon tricuspidens* and *Pseudomyxus capensis*) in temperate and sub-tropical but not tropical estuaries. Similarly, the Sparidae were more diverse (13 species) in South Africa than Mozambique (6 species), primarily due to the absence of temperate members of this family from the more tropical northern estuaries (Whitfield and Mann, 2023). Conversely, the tropical Carangidae were more diverse in Mozambique (13 species) than the more temperate South African (9 species) estuaries (Table 3).

This latitudinal diversity gradient is reflected in the total number of species. Altogether 170 species were recorded in South African and 217 species in Mozambique estuaries. The higher species richness in the latter systems is because of the exclusive tropical nature of estuaries in Mozambique, which conforms to temperate/tropical global trends recorded for estuary-associated fish diversity (Harrison and Whitfield, 2022). In addition, a number of Mozambique estuaries have broad and deep mouths that promote the occurrence of marine stragglers within these systems, particularly during flood tides. The Morrumbene is an example, with a mouth 3 km wide at high tide, and the fish data of Day (1974) showed it to be a system very rich in marine stragglers (Table 2). Added to this is the scale and diversity of tropical habitats such as coral reefs and seagrasses in the adjacent nearshore waters. Large areas of coral reef and seagrass occur adjacent to the Morrumbene, and species with known affinities with these habitats are a source of larvae and juveniles in estuarine waters (e.g., Mocuba *et al*., 2023). This accounts for the higher abundance of Lutjanidae, Tetraodontidae, Sphyraenidae, Syngnathidae, Scorpaenidae, Scaridae, Pomacentridae and Labridae species in Mozambique estuaries such as Morrumbene.

The fish species richness in coastal habitats adjacent to Mozambique estuaries is considerably higher than in the estuarine waters, even where similar habitats are present in both systems, e.g. seagrass beds. For example, 249 species from 62 fish families were identified from seagrass beds in the Quirimano Archipelago of northern Mozambique but only one of the six dominant species (*Gerres oyena*) was abundant in both estuaries and the coastal zone in that region (Gell and Whittington, 2002). A similar major differential between the South African coastal fish assemblage species richness off the Maputaland coast and the adjacent estuarine systems was recorded by Whitfield (1980). A good example of marine stragglers entering estuaries from the species rich tropical Delagoa Bioregion is provided by the Kosi estuarine system. More than 150 coastal fish species that are normally absent from estuaries have been reported from a small beachrock reef inside the mouth of this system (Blaber and Cyrus, 1981; Dennis King, unpublished fish photographs).

The influence of poor marine connectivity is also well illustrated in Mozambique estuarine fish assemblages. The origins and morphologies of many estuarine and coastal lakes and lagoons in Mozambique stem from long term sea level transgression in southeastern Africa (Miguel *et al*., 2019). The evolution of fish assemblages in these systems follows a series of characteristic changes as the system becomes increasingly isolated from the sea. Initially, the estuarine lake or lagoon may have a permanent and strong connection to the sea, and the ichthyofauna will reflect a dominance of marine estuarine-opportunist and marine estuarine-dependent fish species. As the link with the ocean becomes intermittent due to the temporary closure of the mouth by a sand berm, the number of marine species found in the estuary declines but the number of freshwater species increases (Whitfield *et al*., 2017). Estuarine resident species representation remains the same and may even dominate the fish assemblage in temporarily isolated estuarine lakes or lagoons that have been isolated from the sea for extended periods (Schutte *et al*., 2020). Once these systems become permanently cut-off from the sea, the salinity declines considerably, marine species disappear after two decades of closure, and freshwater fish species then dominate the newly created coastal lake or lagoon, with some estuarine resident fish species also remaining relatively abundant in the now isolated coastal water body (Allanson *et al*., 1966).

An example of a lake system that is only marginally estuarine due to very infrequent linkages with the sea is the oligohaline Lagoa Piti. Few, if any, marine fish species are present within this lake but there is still a strong representation of estuarine fish species such as *Awaous aeneofuscus*, *Croilia mossambica*, *Eleotrus fusca*, *Gilchristella aestuaria*, *Hyporhamphus affinis*, *Hyporhamphus capensis*, *Redigobius dewaali* and *Silhouettea sibayi*. Conversely, freshwater fish species such as *Clarias gariepinus*, *Enteromius paludinosus*, *Enteromius viviparus*, *Lacustricola katangae*, *Lacustricola myaposae*, *Micropanchax johnstoni, Oreochromis mossambicus*, *Pseudocrenilabrus philander* and *Tilapia sparrmanii* are in the process of becoming completely dominant in this increasingly isolated coastal lake system (Bills, 2001).

Estuarine lagoon or lake systems that have long channels linking the major water body to the sea are also dominated by freshwater fish species. For example, the mesohaline Lagoa Poelela has a 75 km long channel linking it to the Indian Ocean and is therefore dominated by freshwater cichlid fish species, primarily *Coptodon rendalli*, although some estuarine resident fish species were also recorded (Hill *et al*., 1975). In Lagoa Quissico and Massava, which are part of the same overall segmented lake system but closer to the sea, juvenile marine mugilids (e.g. *Planiliza macrolepis*) were abundant and freshwater fish species scarce (Hill *et al*., 1975).

A comparative fish guild analysis between the species composition in Mozambique and South Africa showed that estuaries in both countries were dominated by marine taxa but the proportions differed, i.e. 47 % of the fish species in South African estuaries were marine immigrants wereas this figure was 40 % for Mozambique (Fig. 2). Conversely marine stragglers were 20 % of the fish species in South Africa and 34 % in Mozambique, with the high representation in the latter region an indication of the broad mouths and strong influence of macrotidal conditions on some estuaries in this region (Whitfield *et al.*, 2023). These conditions may also have contributed to the higher representation of estuarine migrants in Mozambique estuaries when compared to estuarine residents (Fig. 2).

There is no doubt that the fish faunas, and indeed other biological components in Mozambique estuaries are undersampled and understudied. Estuaries here are subject to different driving forces than the well studied systems of South Africa, especially with respect to tidal regimes, which range from micro- to macrotidal. There are also the issues relating to global warming and sea level rise that will impact the fish fauna of Mozambique in various ways, e.g. higher estuarine water temperatures, increased river flooding due to more extreme cyclonic precipitation events, and erosion of coastal dune systems that presently isolate certain systems from the sea. Little attention is being paid to the plight and conservation measures required for certain overexploited fish species in Mozambique estuaries. All the above knowledge gaps currently present a significant constraint to current undertanding of the ecological functioning of these systems. Given the importance of estuaries in the region to sustaining coastal livelihoods, these gaps should be addressed as a matter of urgency. The current review presents a first attempt to compile a list of fish species and families found in Mozambique estuaries and therefore lays a foundation for more detailed ichthyological studies in the future.

Acknowledgements

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Appendix

Table A.1. Fish species recorded from South African estuarine systems. For a description of the fish guild categories used, please see Table 2.

Fish families	Species names	Common names	Fish guilds
Ambassidae	*Ambassis ambassis*	Longspine glassy	ER
Ambassidae	*Ambassis dussumieri*	Malabar glassy	EM
Ambassidae	*Ambassis natalensis*	Slender glassy	EM
Anguillidae	*Anguilla bicolor*	Shortfin eel	CM
Anguillidae	*Anguilla labiata*	African mottled eel	CM
Anguillidae	*Anguilla marmorata*	Giant mottled eel	CM
Anguillidae	*Anguilla mossambica*	Longfin eel	CM
Antennariidae	*Antennarius striatus*	Striped angler	MS
Ariidae	*Galeichthys feliceps*	White sea catfish	MI
Atherinidae	*Atherina breviceps*	Cape silverside	ER
Atherinidae	*Atherinomorus duodecimalis*	Tropical silverside	MS
Atherinidae	*Atherinomorus lacunosus*	Hardyhead silverside	MS
Belonidae	*Strongylura leiura*	Banded needlefish	MI
Blenniidae	*Omobranchus woodi*	Kappy blenny	EM
Blenniidae	*Parablennius pilicornis*	Ringneck blenny	ER
Bothidae	*Bothus pantherinus*	Leopard flounder	MS
Carangidae	*Caranx heberi*	Blacktip kingfish	MS
Carangidae	*Caranx ignobilis*	Giant kingfish	MI
Carangidae	*Caranx melampygus*	Bluefin kingfish	MI
Carangidae	*Caranx papuensis*	Brassy kingfish	MI
Carangidae	*Caranx sexfasciatus*	Bigeye trevally	MI
Carangidae	*Lichia amia*	Leervis	MI
Carangidae	*Scomberoides commersonnianus*	Talang queenfish	MI
Carangidae	*Scomberoides lysan*	Doublespotted queenfish	MI
Carangidae	*Scomberoides tala*	Barred queenfish	MI
Carcharinidae	*Carcharinus leucas*	Zambezi shark	MI
Chanidae	*Chanos chanos*	Milkfish	MI
Cichlidae	*Coptodon rendalli*	Redbreast tilapia	FI
Cichlidae	*Oreochromis mossambicus*	Mozambique tilapia	FI
Cichlidae	*Pseudocrenilabrus philander*	Southern mouthbrooder	FI
Cichlidae	*Tilapia sparrmanii*	Banded tilapia	FS
Clariidae	*Clarias gariepinus*	Sharptooth catfish	FI
Clinidae	*Clinus spatulatus*	Estuary klipfish	ER
Clinidae	*Clinus superciliosus*	Super klipfish	EM
Clupeidae	*Etrumeus whiteheadi*	Redeye roundherring	MS
Clupeidae	*Gilchristella aestuaria*	Estuarine roundherring	ER
Clupeidae	*Hilsa kelee*	Kelee shad	MI
Clupeidae	*Herklotsichthys quadrimaculatus*	Blueline herring	MS
Clupeidae	*Sardinops ocellatus*	South African pilchard	MS
Dasyatidae	*Dasyatis chrysonata*	Blue stingray	MS
Dasyatidae	*Gymnura natalensis*	Backwater butterflyray	MS
Dasyatidae	*Himantura uarnak*	Reticulate whipgray	MI
Drepanidae	*Drepane longimana*	Concertina fish	MS
Eleotridae	*Butis butis*	Duckbill sleeper	ER
Eleotridae	*Eleotris fusca*	Dusky sleeper	ER
Eleotridae	*Eleotris mauritiana*	Widehead sleeper	ER
Eleotridae	*Eleotris melanosoma*	Broadhead sleeper	ER
Elopidae	*Elops machnata*	Skipjack	MI
Engraulidae	*Engraulis capensis*	Cape anchovy	MI
Engraulidae	*Stolephorus holodon*	Thorny anchovy	MI
Engraulidae	*Thryssa setirostris*	Longjaw glassnose	MI
Engraulidae	*Thryssa vitrirostris*	Orangemouth glassnose	MI
Epinephelidae	*Epinephelus andersoni*	Catface rockcod	MS

Fish families	Species names	Common names	Fish guilds
Epinephelidae	*Epinephelus malabaricus*	Malabar rockcod	MS
Epinephelidae	*Epinephelus marginatus*	Yellowbelly rockcod	MS
Fistulariidae	*Fistularia commersonii*	Smooth flutemouth	MS
Galaxiidae	*Galaxias zebratus*	Cape galaxias	FS
Gerreidae	*Gerres filamentosus*	Threadfin pursemouth	MI
Gerreidae	*Gerres longirostris*	Strongspine pursemouth	MI
Gerreidae	*Gerres methueni*	Evenfin pursemouth	MI
Gerreidae	*Gerres oblongus*	Slender pursemouth	MI
Gerreidae	*Gerres oyena*	Slenderspine pursemouth	MI
Gobiidae	*Awaous aeneofuscus*	Freshwater goby	FI
Gobiidae	*Caffrogobius gilchristi*	Prison goby	EM
Gobiidae	*Caffrogobius natalensis*	Baldy	EM
Gobiidae	*Caffrogobius nudiceps*	Barehead goby	EM
Gobiidae	*Croilia mossambica*	Naked goby	ER
Gobiidae	*Favonigobius melanobranchus*	Blackthroat goby	ER
Gobiidae	*Favonigobius reichi*	Spotted sandgoby	ER
Gobiidae	*Glossogobius callidus*	River goby	ER
Gobiidae	*Glossogobius giuris*	Tank goby	FM
Gobiidae	*Oligolepis acutipennis*	Sharptail goby	ER
Gobiidae	*Oxyurichthys keiensis*	Kei goby	ER
Gobiidae	*Oxyurichthys ophthalmonema*	Eyebrow goby	EM
Gobiidae	*Pandaka silvana*	Dwarf goby	EM
Gobiidae	*Paratrypauchen microcephalus*	Comb goby	ER
Gobiidae	*Periophthalmus argentilineatus*	Barred mudskipper	ER
Gobiidae	*Psammogobius biocellatus*	Sleepy goby	ER
Gobiidae	*Psammogobius knysnaensis*	Speckled sandgoby	ER
Gobiidae	*Redigobius bikolanus*	Bigmouth goby	ER
Gobiidae	*Redigobius dewaali*	Checked goby	ER
Gobiidae	*Silhouettea sibayi*	Barebreast goby	ER
Gobiidae	*Stenogobius polyzona*	Chinestripe goby	ER
Gobiidae	*Taenioides esquivel*	Bulldog eelgoby	ER
Gobiidae	*Trypauchenopsis intermedia*	Bearded eelgoby	ER
Gobiidae	*Yongeichthys nebulosus*	Shadow goby	ER
Haemulidae	*Plectorhinchus gibbosus*	Harry hotlips	MS
Haemulidae	*Pomadasys commersonnii*	Spotted grunter	MI
Haemulidae	*Pomadasys kaakan*	Javelin grunter	MI
Haemulidae	*Pomadasys multimaculatus*	Cock grunter	MI
Haemulidae	*Pomadasys olivaceus*	Piggy	MI
Hemiramphidae	*Hemiramphus far*	Spotted halfbeak	MI
Hemiramphidae	*Hyporhamphus capensis*	Cape halfbeak	EM
Kuhliidae	*Kuhlia mugil*	Barred flagtail	MS
Kuhliidae	*Kuhlia rupestris*	Rock flagtail	MI
Leiognathidae	*Leiognathus equula*	Slimy	MI
Lethrinidae	*Lethrinus nedulosus*	Blue emperor	MS
Lobotidae	*Lobotes surinamensis*	Tripletail	MI
Lutjanidae	*Lutjanus argentimaculatus*	Mangrove snapper	MI
Lutjanidae	*Lutjanus fulviflamma*	Dory snapper	MI
Megalopidae	*Megalops cyprinoides*	Oxeye tarpon	MI
Monocanthidae	*Stephanolepis aurata*	Porky	MI
Monodactylidae	*Monodactylus argenteus*	Round moony	MI
Monodactylidae	*Monodactylus falciformis*	Oval moony	MI
Mugilidae	*Chelon dumerili*	Grooved mullet	MI
Mugilidae	*Chelon melinopterus*	Giant-scale mullet	MI
Mugilidae	*Chelon richardsonii*	Southern mullet	MI
Mugilidae	*Chelon tricuspidens*	Striped mullet	MI
Mugilidae	*Crenimugil buchanani*	Bluestail mullet	MI
Mugilidae	*Crenimugil crenilabis*	Fringelip mullet	MS
Mugilidae	*Crenimugil seheli*	Bluespot mullet	MI
Mugilidae	*Moolgarda cunnesius*	Longarm mullet	MI
Mugilidae	*Mugil cephalus*	Flathead mullet	MI

Fish families	Species names	Common names	Fish guilds
Mugilidae	*Osteomugil robustus*	Robust mullet	MI
Mugilidae	*Planiliza alata*	Diamond mullet	MI
Mugilidae	*Planiliza macrolepis*	Large-scale mullet	MI
Mugilidae	*Planiliza subviridis*	Greenback mullet	MI
Mugilidae	*Pseudomyxus capensis*	Freshwater mullet	MI
Muraenesocidae	*Muraenesox bagio*	Pike conger	MI
Muraenidae	*Strophidon sathete*	Slender giant moray	MI
Myliobatidae	*Myliobatis aquila*	Eagleray	MI
Opichthidae	*Ophisurus serpens*	Sand snake-eel	MI
Opichthidae	*Pisodonophis boro*	Estuary snake-eel	MI
Paralichthyidae	*Pseudorhombus arsius*	Largetooth flounder	MS
Platycephalidae	*Platycephalus indicus*	Bartail flathead	MI
Polynemidae	*Polydactylus plebeius*	Striped threadfin	MI
Polynemidae	*Polydactylus sextarius*	Sixfinger threadfin	MI
Pomatomidae	*Pomatomus saltatrix*	Elf	MI
Priacanthidae	*Priacanthus hamrur*	Moontail bullseye	MS
Pristidae	*Pristis zijsron*	Longcomb sawfish	MI
Pseudochromidae	*Pseudochromis natalensis*	Natal dottyback	MI
Rhinobatidae	*Acrotariobatus annulatus*	Lesser guitarfish	MS
Sciaenidae	*Argyrosomus japonicus*	Dusky kob	MI
Sciaenidae	*Johnius dorsalis*	Small croaker	MI
Sciaenidae	*Otolithes ruber*	Tigertooth croaker	MI
Scorpaenidae	*Pterois volitans*	Red lionfish	MS
Siganidae	*Siganus sutor*	Whitespotted rabbitfish	MS
Sillaginidae	*Sillago sihama*	Silver sillago	MI
Soleidae	*Solea turbynei*	Blackhand sole	MI
Sparidae	*Acanthopagrus vagus*	Estuarine bream	MI
Sparidae	*Crenidens crenidens*	Karanteen seabream	MI
Sparidae	*Diplodus capensis*	Blacktail	MI
Sparidae	*Diplodus hottentotus*	Zebra	MI
Sparidae	*Lithognathus lithognathus*	White steenbras	MI
Sparidae	*Lithognathus mormyrus*	Sand steenbras	MI
Sparidae	*Rhabdosargus globiceps*	White stumpnose	MI
Sparidae	*Rhabdosargus holubi*	Cape stumpnose	MI
Sparidae	*Rhabdosargus sarba*	Tropical stumpnose	MI
Sparidae	*Rhabdosargus thorpei*	Bigeye stumpnose	MI
Sparidae	*Sarpa salpa*	Strepie	MI
Sparidae	*Sparodon durbanensis*	White musselcracker	MI
Sparidae	*Spondyliosoma emarginatum*	Steentjie	MI
Sphyraenidae	*Sphyraena barracuda*	Great barracuda	MI
Sphyraenidae	*Sphyraena jello*	Pickhandle barracuda	MI
Syngnathidae	*Acentronura tentaculata*	Shortpouch pygmy pipehorse	MS
Syngnathidae	*Hippichthys heptagonus*	Belly pipefish	EM
Syngnathidae	*Hippichthys spicifer*	Bellybarred pipefish	EM
Syngnathidae	*Hippocampus capensis*	Knysna seahorse	ER
Syngnathidae	*Microphis brachyuras*	Short-tail pipefish	FS
Syngnathidae	*Microphis fluviatilis*	Freshwater pipefish	FS
Syngnathidae	*Syngnathus temmincki*	Longsnout pipefish	EM
Syngnathidae	*Syngnathus watermeyeri*	Estuarine pipefish	EM
Terapontidae	*Pelates quadrilineatus*	Fourlined terapon	MI
Terapontidae	*Terapon jarbua*	Thornfish	MI
Tetraodontidae	*Amblyrhynchote honckenii*	Evileye pufferfish	MI
Tetraodontidae	*Arothron hispidus*	Whitespotted pufferfish	MI
Tetraodontidae	*Arothron immaculatus*	Blackedged pufferfish	MI
Tetraodontidae	*Chelonodontops laticeps*	Bluespotted pufferfish	MS
Torpedinidae	*Torpedo fuscomaculata*	Blackspotted electric ray	MI
Torpedinidae	*Torpedo sinuspersici*	Marbled electric ray	MI
Trichiuridae	*Trichiurus lepturus*	Cutlass fish	MS

Original Article

Meiofauna as bioindicators of organic and inorganic pollution of estuarine sediments in Kenya

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Abstract

Meiofaunal density, diversity, and community assemblages were studied at the highly contaminated Tudor Creek and the less contaminated Mida Creek in Kenya to assess their potential as bioindicators of marine pollution. Sampling during the dry (January/February 2017) and wet (November/December 2017) seasons indicated a significantly greater total organic matter content at Mida (23.7 and 23.9 %) than at Tudor Creek (6.6 and 5.9 %) in the dry and wet seasons. Heavy metal concentrations were always greater at Tudor Creek. Meiofaunal densities were greater at Mida (2729 and 2804 ind.10 cm^{-2}) than Tudor Creek (612 and 183 ind.10 cm^{-2}) during both seasons. Meiofauna at Mida Creek (10 and 7 taxa in the dry and wet seasons) were dominated by nematodes, copepods, and turbellarians. Meiofauna at Tudor Creek (8 and 6 taxa) were dominated by nematodes, turbellarians and ostracods. Meiofaunal diversity was greater at Tudor Creek, but dominance was highest at Mida Creek. Community dissimilarities between the two sites were shown in a Bray-Curtis cluster analysis. There is a high likelihood that heavy metals affect meiofauna density and diversity in the sediments of the two studied creeks in Kenya.

Keywords: pollution, heavy metals, meiobenthos, Dabaso, Mikindani

Introduction

Marine pollution arises when harmful chemicals find their way into the ocean. The chemicals can have non-point sources, such as surface runoff from farms and urban roads and buildings, and point sources, such as sewage treatment works discharges, factories, and oil refineries. Pollution by metals results from anthropogenic activities, causing them to increase to toxic levels for organisms under certain circumstances. Metals are important pollutants arising from industrial and residential areas in urban and peri-urban environments (Prüss-Ustün *et al.,* 2014). In most developing countries, between 80 % and 90 % of domestic sewage in coastal urban centers is discharged without treatment (Labadi, 2017). This can be linked to urbanization, increased infrastructure development, and food production as a result of the rising world population, posing serious environmental risks to marine ecosystems (Mayorga *et al.,* 2010; Thompson *et al.,* 2017). This also influences water quality in terms of dissolved oxygen concentration, biological oxygen demand, turbidity, and conductivity, directly affecting marine life.

In Mombasa County, Kenya, anthropogenic activities, such as industrial plant development, fishing, construction of houses, wastewater discharge, crop production, and the disposal of agrochemical containing wastes, are major causes of pollution in Tudor Creek (Okuku *et al.,* 2011). The use of metals by different industries makes them important toxicants in many effluents. They are also released through sewage and surface runoff. Metals such as mercury (Hg) and arsenic (As) often bio magnify in the food chain (Okuku *et al.,* 2019). They

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affect organism reproduction and survival rates and can be toxic at high concentrations. An increase in their concentration above acceptable levels results in serious environmental health risks (Prüss-Ustün *et al.,* 2014).

There is, therefore, a need for biomonitoring to assess the impacts from these types of pollution (El Zrelli *et al.,* 2015). Biomonitoring has proved to be more effective than certain other methods of environmental monitoring since the level of toxicity of a substance is judged by its biological effects, not its concentration in the environment alone. Biota provide direct information about pollution hazards occurring over a long period, while chemical monitoring provides results based on the sampling time and does not provide a measure of bioavailability. Current research focused on the optimization of biological organisms, mostly meiofauna, to conduct biomonitoring programmes while including measurement of physio-chemical properties of the sediments and metal analysis to render the study more robust. Biological indicators can be defined as communities, organisms, or species whose presence or absence is indicative of environmental condition. Meiofauna are usually believed to be vulnerable to stress in the marine environment and can be excellent biological indicators. Some meiofauna are highly sensitive, while others are tolerant of high pollution levels. They are, therefore, effective indicators of environmental change (Orlando-Bonaca *et al.,* 2008).

Meiofauna are good bioindicators because of their small size, fast generation, widespread distribution, direct benthic development, and high diversity and density (Zeppilli *et al.,* 2015; Balsamo *et al.,* 2012). Their community structure reflects oxygen levels, organic matter content, and contaminant concentrations at a site (Moreno *et al.,* 2008b). Additionally, being constrained to the sediment through their life cycle (Sutherland *et al.,* 2007) and being susceptible to pollutants (Danovaro *et al.,* 2009), they are good for assessing environmental quality (Moreno *et al.,* 2008a). A few taxa (tolerant/opportunistic) dominate the community while those that are less tolerant become rare or disappear as a result of high organic matter content and low oxygen levels (Dal Zotto *et al.,* 2016). Meiofauna are assumed to have the highest level of sensitivity to disturbance in soft sediments (Penha-lopes *et al.,* 2014). This study was carried out to identify the potential of meiofaunal communities as indicators of pollution in estuaries at Dabaso and Mikindani in Kenya, which have different levels of anthropogenic disturbance.

Materials and methods

Study area

The study was conducted at two sites perceived to have different levels of contamination by virtue of their location relative to urban settlements, namely Mikindani in Tudor Creek and Dabaso in Mida Creek (Okuku *et al.,* 2011).

Tudor Creek, located at 4°2' S, 39°40' E, borders Mombasa Island on the northwest side. Three seasonal streams (Mtsapuni, Kombeni, and Tsatu) enter the Creek near Mariakani, roughly 32 km north-west of the port (Fig. 1). Sediments of Tudor Creek are predominantly mud and some parts are covered with sand (Kamau *et al.,* 2015). A mangrove forest composed of *Rhizophora mucronata* and *Avicennia marina* covers approximately 8 km^2 of the Creek. *A. marina* covers the mid zone while *R. mucronata* covers the landward zone (Kirui *et al.,* 2013). The mangrove forest is extremely polluted by raw sewage that is mainly discharged into the Creek from surrounding settlements at Mikindani, Tudor, and Old Town. The Creek is bordered by a large human population residing in settlements at Mikindani, Bangladesh, Burukenge, Mishomoroni, Changamwe, Tudor, Kibarani, Kongowea, Moroto, Kenya Meat Commission, and the Old Town.

Mida Creek, located at 3°20'S, 39 ° 58' E in Watamu, Kilifi County, stretches inland from the sea to the Arabuko Sokoke forest (Fig. 1). It is an extensive area of 31.6 km^2, consisting of a tidal inlet composed of sand and mud flats, located near Dabaso village (Kairo *et al.,* 2002). The estimated terrain elevation is 6 m above sea level. The Creek is characterized by muddy and shallow sandy soils (Wafula *et al.,* 2019). *R. mucronata, A. marina* and *Ceriops tagal* are the dominant mangrove species. The Creek provides a feeding and development area for sea turtles, birds, and fish amongst other biota. The area is less populated by humans than Tudor Creek and its surroundings, and there are thus fewer anthropogenic activities that result in pollution.

Sampling strategy

Sampling was conducted at Dabaso (3° 20' 41.77' S, 39° 59' 19.79'' E) and Mikindani (4° 0' 25.59'' S, 39° 38' 16.29'' E) during low tide in the dry (January/February 2017) and wet (November/December 2017) seasons (Fig. 1). The study collected eight samples along a transect perpendicular to the shoreline within the intertidal zone at each site. At Mikindani, the transect was laid alongside a sewage discharge channel, while at Dabaso, the transect was near the tidal inlet. The samples were tested for grain size, total organic matter (TOM), dissolved oxygen (DO), biological oxygen demand (BOD), metal, and meiofauna parameters.

Sample collection

Sediment for grain size and TOM analysis was sampled by inserting a 6.4 cm inner diameter corer into the sediment to a depth of 10 cm. The sediment was stored in Ziploc bags in a cooler box containing ice until transfer to the laboratory, to prevent degradation of the organic matter. Interstitial water for DO and BOD analysis was collected by excavating a shallow hole in the sediment and allowing pore water to drain into the hole. The water was transferred to Winkler bottles, 2 mL of concentrated sulfuric acid added to the sample surface and shaken several times. The sample was thereafter stored frozen prior to laboratory analysis (Helm *et al.,* 2012). Sediment for metal analysis was collected by inserting a 3.6 cm inner diameter corer into the sediment to a depth of 10 cm and the sediments stored in Ziploc bags. Meiofauna samples were collected using a 3.6 cm inner diameter corer inserted into the sediment to a depth of 10 cm. The sediment was placed in sample bottles and preserved using 8 % buffered formaldehyde.

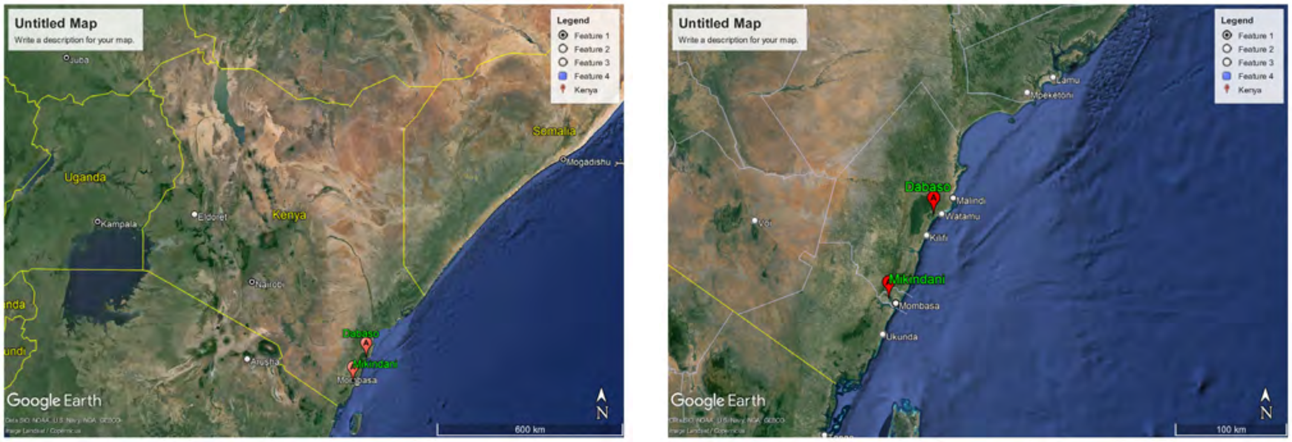

Figure 1. Map showing the Kenyan coastline with sampling sites - Dabaso (3° 20’ 41.77’ S, 39° 59’ 19.79” E) (Mida Creek) and Mikindani (4° 0’ 25.59” S, 39° 38’ 16.29” E) (Tudor Creek).

Sediment grain size analysis

One hundred grams of oven dried sediment was transferred to an electric shaker with a stack of 63 µm, 125 µm, 250 µm, 500 µm, 1000 µm and 2000 µm mesh size sieves for 10 minutes. The fraction retained in each sieve was weighed on a microbalance and the proportion of the total start weight was used to calculated the proportion falling into each sediment grain size classes: granule (>2000µm), very coarse sand (1000µm - 2000µm), coarse sand (500µm - 1000µm), medium sand (250µm - 500µm), fine sand (125µm - 250µm), very fine sand (63µm - 125µm) and silt and clay (pan) (<63µm).

Total organic matter analysis

Samples were analyzed for organic matter using a ‘loss on ignition’ method as described by Hoogsteen *et al.* (2018). A 25 g portion of sediment from each replicate sample was placed into a porcelain dish and ashed in a furnace at 600 °C for 6 h, cooled, and weighed. The organic matter content was calculated as the percentage of the weight loss after ashing (% OM).

Dissolved oxygen (DO) and biological oxygen demand (BOD) analysis

DO was analyzed by titrating 200 ml of the sample with sodium thiosulfate to a pale straw colour. The titrate was slowly added into the solution using a pipette while stirring continuously. A solution of 2 ml of starch was added to form a blue color. Titration continued until the sample turned clear. The DO concentration in the sample was equal to the amount of titrant (sodium thiosulfate) used in milliliters.

To determine the BOD, the initial DO concentration was determined using the Winkler protocol (Helm *et al.,* 2012). The samples were then incubated in 300 ml incubation bottles with buffered dilution water dosed with seed microorganisms. The samples were stored in the dark for 5 days at 20 ^{0}C. The final DO concentration was determined as:

BOD (mg/L) = (Initial DO-Final DO) ÷ Volume of sample/Volume of bottle

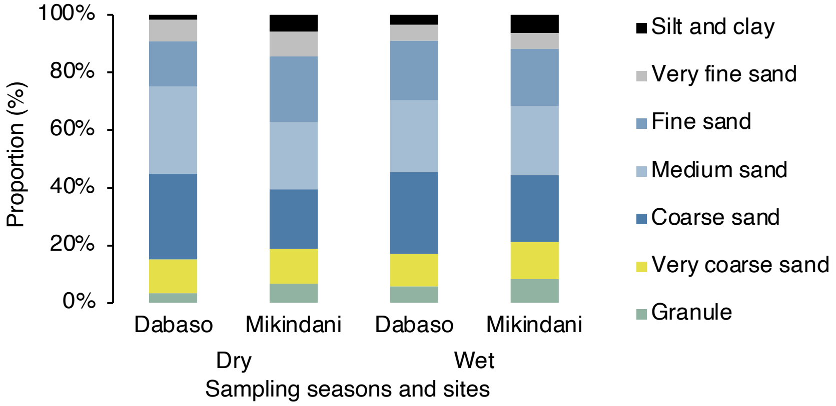

Figure 2. Grain size composition of sediment at Dabaso (Tudor Creek) and Mikindani (Mida Creek) in the dry and wet seasons.

Metal analysis

The samples were first air-dried for two weeks, and then oven-dried at 60 °C for eight hours to remove any remaining moisture. The dried samples were crushed into fine powder and sieved to grain sizes less than 60 µm. Approximately 1.4 g of the fine sample was mixed with 0.4 g of cellulose to achieve a 20 % dilution. Three pellets, each weighing approximately 350 mg, were prepared using a hydraulic press from each sample, and were ready for Energy-dispersive X-ray fluorescence (EDXRF) analysis.

Meiofauna extraction and identification

In the laboratory, sediment samples were washed through a 1 mm mesh size sieve and retained on a 38 µm mesh size sieve to extract the meiofauna. The contents retained by the 38 µm mesh size sieve were centrifuged three times (Hodda and Abebe, 2006) using magnesium sulphate ($MgSO_4$) of 1.28 specific density at 6000 rpm for ten minutes. The supernatant was passed through a 38 µm sieve, rinsed using filtered tap water, preserved using a 4 % buffered formaldehyde solution, and stained with Rose Bengal overnight. The samples were observed under a dissecting microscope at magnification x10. Meiofauna were identified to the highest taxonomic level.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to test for differences in TOM, DO, BOD, metals, and meiofauna amongst sites. The PAST Statistical Programme was used to determine Shannon Wiener diversity, dominance, and evenness indices for meiofauna (Hammer *et al.*, 2001). Meiofauna densities were square root transformed for community assemblage analysis using Multidimensional Scaling (MDS) on Plymouth Routines in Multivariate Ecological Research (PRIMER v5.2.9) software (Clarke and Warwick, 2001). Pearson's Product-Moment correlation analysis was conducted using Statistica to determine the correlation between abiotic factors and meiofauna densities.

Results

Abiotic Factors

Coarse and medium sand contributed more to the sediment weight at Dabaso (29.7 %, 30.3 %) than at Mikindani (20.7 %, 23.3 %) in the dry season (Fig. 2). Other grain size classes had a higher contribution at Mikindani than Dabaso. In the wet season, coarse, medium, and fine sand had the highest contribution at both sites, but this was slightly higher at Dabaso (28.3 %, 25.0 %, 20.6 %) compared to Mikindani (23.3 %, 23.9 %, 19.7 %). Granules and very coarse sand had a higher contribution at Mikindani compared to Dabaso. Silt and clay made the lowest contribution at both sites, although this was slightly higher at Mikindani than at Dabaso (Fig. 2).

DO concentrations were significantly higher ($p < 0.05$) at Dabaso compared to Mikindani in the dry and wet seasons combined (Table 1). The DO concentration was significantly higher at Dabaso in the wet season ($p = 0.02$) and at Mikindani in the dry season ($p = 0.02$).

The BOD was significantly higher ($p = 0.039$) at Mikindani compared to Dabaso in the dry season, while in the wet season it was significantly higher ($p = 0.041$) at Dabaso (Table 1). When concentrations within sites are compared between seasons, a slight increase from the dry (3.4 ± 0.10mg/L) to wet (3.5 ± 0.03mg/L) season was recorded at Dabaso, but the difference was not significant ($p = 0.5$). In contrast, at Mikindani there was a significant decrease ($p = 0.003$) from the dry (4.8 ± 0.2mg/L) to wet (2.8 ± 0.03mg/L) season.

Table 1. Physio-chemical parameters (mean ± standard error) for sediment porewater at Mikindani and Dabaso in the dry and wet seasons. DO = dissolved oxygen, BOD = biological oxygen demand, TOM = total organic matter.

Parameter	Site	Dry season	Wet season
DO (mg/L)	Mikindani	3.8 ± 0.02	2.1 ± 0.04
	Dabaso	4.0 ± 0.1	5.3± 0.03
BOD (mg/L)	Mikindani	4.8 ± 0.23	2.8± 0.03
	Dabaso	3.4 ±0.10	3.5 ± 0.03
TOM (%)	Mikindani	6.6 ± 0.2	5.9 ± 0.1
	Dabaso	23.7±0.7	23.9± 0.03

The TOM content was significantly higher ($p < 0.05$) at Dabaso compared to Mikindani in the dry and wet season combined. TOM content in the sediment was not different between seasons at either site (Table 1).

Metal concentrations

Seven metals were analysed in the sediment, namely iron (Fe), titanium (Ti), zirconium (Zr) manganese (Mn), rubidium (Rb), zinc (Zn), and lead (Pb), in that order in terms of concentration. All metals exhibited higher concentrations at Mikindani compared to Dabaso in both seasons (Table 2). Iron, Ti, Zr, Rb and Zn concentrations were nearly threefold higher at Mikindani compared to Dabaso. Manganese and Fe concentrations significantly increased from the dry to wet season at Dabaso, but not Ti ($p = 0.342$), Zr ($p = 0.132$), Rb ($p = 0.295$), Zn ($p = 0.262$), or Pb ($p = 0.402$). All metals increased significantly in concentration from the dry to wet season at Mikindani apart from Zn ($p = 0.920$) (Table 2).

Meiofaunal density and distribution

Overall, meiofaunal density was significantly higher at Dabaso (2729 ± 387 ind.10 cm^2, 2804 ± 11 ind.10 cm^2) compared to Mikindani in the dry and wet seasons (604 ± 114 ind.10 cm^2, 183 ± 30 ind.10 cm^2) respectively (Fig. 3). The density was particularly low at Mikindani in the wet season, being more than 10 times lower than at Dabaso. The density at Dabaso was slightly higher in the wet season, but the difference was not significant ($p = 0.081$). In contrast, at Mikindani the density was significantly higher in the dry compared to wet season ($p = 0.048$).

The meiofauna community was comprised of 10 and 7 taxa at Dabaso and 8 and 6 taxa at Mikindani in the dry and wet seasons respectively. Nematodes dominated in dry and wet seasons at Dabaso (85.4 % and 90.4 %) and Mikindani (76.6 % and 53.7 %) respectively. Other taxa were less abundant, contributing <10 % of the total density at both sites in each season. Copepods

Table 2. Metal concentrations (mg/kg dry weight; mean ± standard error) in Mikindani and Dabaso sediments in the dry and wet seasons. Fe = iron, Ti = titanium, Zr = zirconium, Mn = manganese, Rb = rubidium, Zn = zinc, Pb = lead.

Metals	Sites	Dry season	Wet season
Fe	Mikindani	17147±585.0	21633±886.2
	Dabaso	4146±183.1	5240±257.2
Ti	Mikindani	2677±122.0	3133±86.28
	Dabaso	897.0±10.4	807.0±65.0
Zr	Mikindani	870.0±36.3	1294±96.7
	Dabaso	309.0± 5.2	350.0±29.1
Mn	Mikindani	164.1±8.9	325.8± 17.2
	Dabaso	127.5±0.4	153.6±12.3
Rb	Mikindani	85.6±3.0	103.3±0.8
	Dabaso	15.3±0.2	32.2±1.3
Zn	Mikindani	70.5±3.7	70.0±7.6
	Dabaso	16.4±2.3	12.5±0.7
Pb	Mikindani	25.1±3.3	36.6±4.8
	Dabaso	15.2±3.8	22.7±1.5

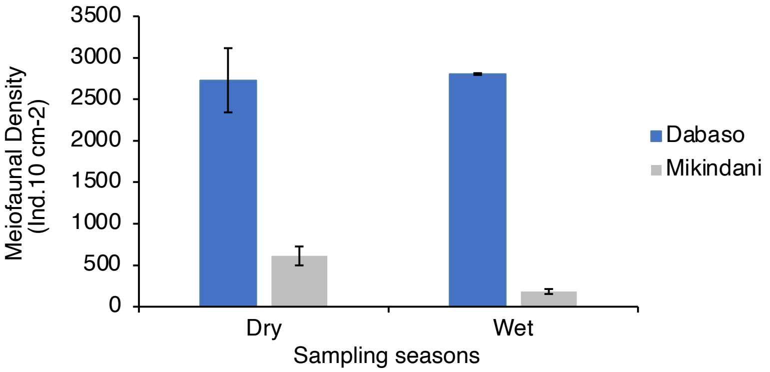

Figure 3. Meiofaunal densities (mean ± standard error) at Dabaso (Tudor Creek) and Mikindani (Mida Creek) in the dry and wet seasons.

contributed 7.9 % and 5.4 % of the total density at Dabaso in the dry and wet seasons respectively but were much less abundant at Mikindani in the dry (4.3 %) and wet (0.1 %) season. Ostracods were only present at Mikindani, at 6.8 % and 4.7 % contribution in the dry and wet seasons respectively (Fig. 4). Other meiofauna, like Isopods, Kinorhynchs, Tunicates, Halacaroids, Sipunculids, and Amphipoda, contributed <2 %. Isopods were only recorded at Dabaso while Halacaroids were recorded only at Mikindani.

Meiofaunal diversity

Taxa richness (S) at Dabaso (10, 7) and Mikindani (8, 6) was higher in the wet compared to dry season respectively (Table 3). Dominance and evenness were higher at Dabaso compared to Mikindani in both seasons. Simpson and Shannon Wiener diversity indices indicated higher species diversity in Mikindani compared to Dabaso in both seasons (Table 3), but there was no significant difference between sites and season for all indices.

Meiofaunal Community structure

Non-Metric Multidimensional scaling (nMDS) on meiofauna communities in the dry season produced two major clusters (Fig. 5a). Four samples from Mikindani were distinctively dissimilar to the other four samples, and the eight Dabaso samples. Analysis of Similarity (ANOSIM) showed a significant difference between Mikindani and Dabaso samples ($p < 0.05$). On the other hand, Dabaso samples clustered together, showing similarity of the meiofaunal communities ($p > 0.05$).

In the wet season, Bray-Curtis cluster analysis of the major meiofaunal taxa produced two major clusters, cluster 1 that had communities from Dabaso and cluster 2 that had communities from Mikindani (Fig. 5b).

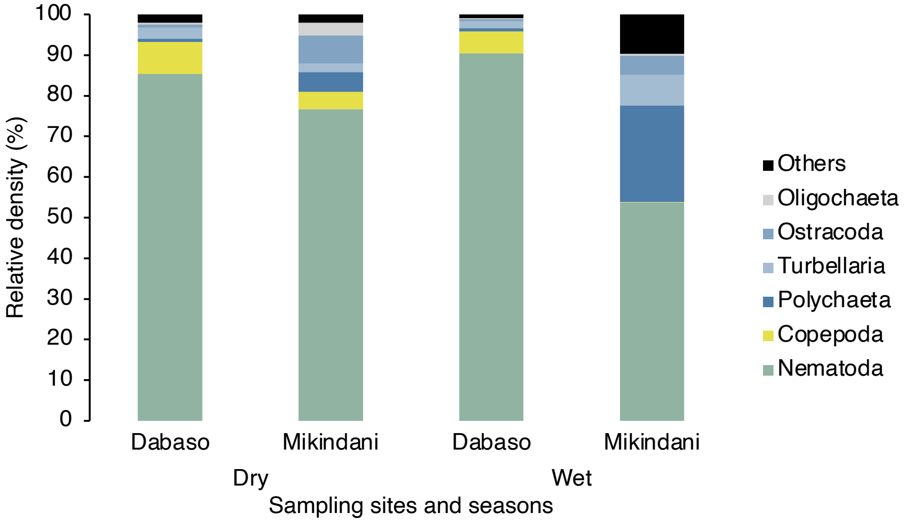

Figure 4. Relative density of meiofaunal taxa at Dabaso (Tudor Creek) and Mikindani (Mida Creek) in the dry and wet seasons.

Table 3. Meiofauna taxa, dominance, Simpson and Shannon Weiner diversity indices, and evenness at Dabaso (Tudor Creek) and Mikindani (Mida Creek) in the dry and wet seasons.

	Dry season		**Wet season**	
	Dabaso	**Mikindani**	**Dabaso**	**Mikindani**
Taxa	10	7	8	6
Dominance	0.68	0.55	0.81	0.40
Simpson	0.32	0.45	0.19	0.60
Shannon	0.71	0.97	0.45	1.16
Evenness	0.22	0.43	0.21	0.56

This indicated that the meiofaunal composition at each site was similar, but different between sites. The samples at Mikindani were highly separated from one another, showing high dissimilarity. Analysis of Similarity (ANOSIM) showed high similarity of samples within the groups (p = 1.2 %), while the percentage similarity (SIMPER) analysis showed that dissimilarity between the sites was high (72 %).

Correlation between abiotic factors and meiobenthos

At Dabaso, no meaningful correlation was found between meiofaunal density and any abiotic parameter. At Mikindani, meiofaunal density was significantly positively correlated to TOM (r = 0.497**) and DO (r = 0.404**). Density displayed a significant negative correlation with Mn (r = -0.657**), Rb (r = -0.440*), Zr (r = -0.476**) and Pb (r = -0.416*). Density was negatively correlated with Ti (-0.080) and Zn (-0.242) and positively correlated with Fe (0.265), but the correlations were not significant. Density was positively, but not significantly, correlated with BOD. Different metals also showed positive but not significant correlations with one another; Ti with Zr (r = 0.353), Ti with Rb (r = 0.392), Mn with Zr (r = 0.496), Mn with Rb (r = 0.417), and Zr with Pb (r = 0.314) (Table 4).

Discussion

Previous studies have shown the Mikindani area to be polluted (Okuku *et al.*, 2011; Kamau *et al.*, 2015) while the Dabaso area was semi-pristine (Kairo *et al.*, 2002; Waweru *et al.*, 2022). The present study highlighted the occurrence of meiobenthic fauna in relation to physio-chemical parameters and metal pollution. The findings show higher metal concentrations at Mikindani (near a sewage disposal point) than at Dabaso (pristine environment).

Physico-chemical parameters showed differences between Mikindani and Dabaso. In Dabaso, coarse and medium sand contributed the most to the sediment size, which may be due to dense mangrove forest stands acting as filters to trap sediments. Mangroves trap sediments like sand but they are less efficient in trapping mud compared to mudflats (Van Santen *et al.*, 2007). Conversely, at Mikindani the higher proportion of fine sand, very fine sand, and silt/clay could be attributed to over a decade of runoff from a large residential estate with a population of more than 20,000 inhabitants (Kamau *et al.*, 2015).

The dissolved oxygen concentration in sediment porewater was higher at Dabaso compared to Mikindani in

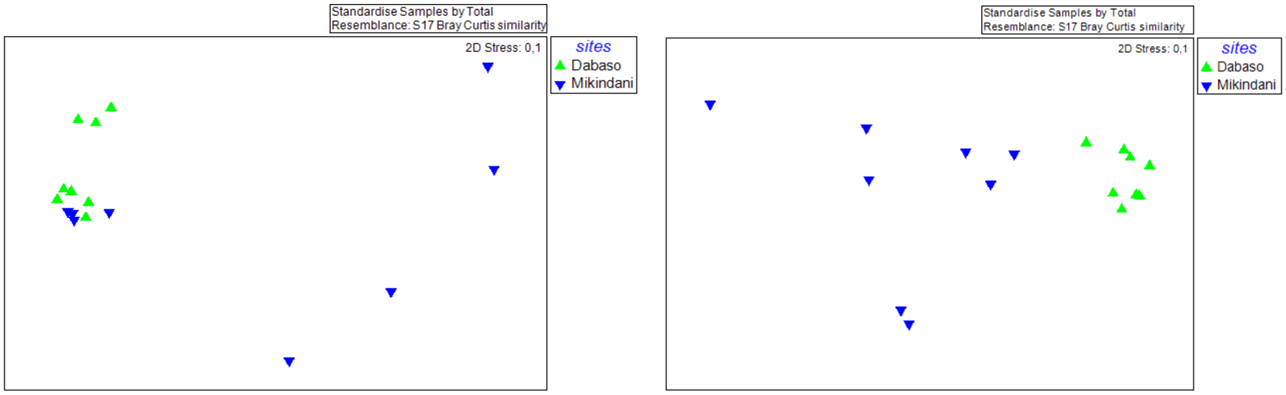

Figure 5. Non-Metric Multidimensional scaling plot of meiofauna community assemblages based on square root transformed data for Dabaso (Tudor Creek) and Mikindani (Mida Creek) in the dry (a) and wet (b) seasons.

Table 4. Pearson correlation coefficients for abiotic factors and meiofauna densities at Mikindani and Dabaso in the dry and wet seasons. Values represent R values for eight replicates. TOM = total organic matter, DO = dissolved oxygen, BOD = biological oxygen demand, Ti = Titanium, Mn = Manganese, Fe = Iron, Zn = zinc, Rb = rubidium, Zr = zirconium, Pb = lead, MeioD = Meiofauna density. Asterisks indicate significance levels (*P<0.05, **P<0.01).

	TOM	DO	BOD	Ti	Mn	Fe	Zn	Rb	Zr	Pb
a. Dabaso										
TOM										
DO	0.022									
BOD	0.067	0.311								
Ti	0.006	-0.233	-0.349							
Mn	-0.040	0.295	-0.216	0.481**						
Fe	0.115	0.674**	0.028	0.027	0.421*					
Zn	0.128	-0.192	-0.182	-0.152	-0.320	0.069				
Rb	-0.049	0.803**	0.050	0.110	0.548**	0.788**	-0.362*			
Zr	-0.161	-0.100	-0.318	0.806**	0.401*	0.001	-0.308	0.236		
Pb	-0.213	0.237	0.126	-0.230	-0.069	0.105	0.205	0.018	-0.254	
MeioD	-0.219	0.030	-0.251	0.201	0.246	-0.188	-0.146	0.080	0.202	0.194
b. Mikindani										
TOM										
DO	-0.078									
BOD	-0.159	0.964**								
Ti	0.134	-0.451**	-0.538**							
Mn	-0.340	-0.702**	-0.621**	0.346						
Fe	-0.014	0.049	0.073	-0.543**	-0.240					
Zn	-0.268	-0.018	0.044	-0.022	0.264	-0.019				
Rb	0.118	-0.527**	-0.514**	0.392*	0.417*	-0.442*	0.059			
Zr	0.455**	-0.402*	-0.330	0.353*	0.496**	-0.117	0.027	0.072		
Pb	-0.015	-0.666**	-0.619**	0.291	0.518**	-0.233	-0.200	0.394*	0.240	
MeioD	0.497**	0.404*	0.273	-0.080	-0.657**	0.265	-0.242	-0.440*	-0.476**	-0.416*

both seasons. This could be attributed to the fact there are limited land-based sources of pollution at Dabaso, while Mikindani had a nearby channel discharging sewage from surrounding settlements. In the wet season, the DO concentration increased at Dabaso, probably as a result of the inflow of oxygenated freshwater (Abril *et al.*, 2010). At Mikindani, the low DO concentration in the wet season could be due to high loads of oxygen-demanding wastes flushed from land to the mangroves. Such oxygen reducing agents include ferrous iron, some oxidizable substances, H_2S, ammonia, and nitrite (Verma and Saksena, 2010).

Biological oxygen demand was statistically higher at Mikindani in the dry season, probably caused by the high oxygen-demanding wastes from sewage and algal decomposition elevating the demand (Okuku *et al.,* 2011). In addition, Vaquer-Sunyer and Duarte (2008) showed that excessive production of OM (from organic waste) increased oxygen demand in coastal ecosystems, hence high BOD levels. Low BOD at Mikindani in the wet season resulted from dilution effect of rainwater. Higher BOD at Dabaso, mostly in the wet season, could be caused by the higher accumulation of OM from mangrove litter fall. Dense mangrove forest stands at Dabaso contributed to litter fall as a source of TOM (Omollo and Dharani, 2021) while at Mikindani mangrove forests were scattered and smaller in size, reducing autochthonous processes, while allochthonous processes were limited to effluent from the land.

Metals are usually immobilized in sediment (Wan *et al.,* 2018). This study showed that different metals (Fe, Mn, Ti, Pb, Zr, Zn, Rb) were present at a higher concentration in sediment at Mikindani than at Dabaso. This could be attributed to the high inflow of anthropogenic pollutants in Mikindani Creek from the neighboring human community and industries in Mikindani village. However, it is paramount to normalize geochemical parameters of metals around the vast area of Mikindani and Dabaso in relation to chemical composition and chemical changes in the sediments to understand the huge differences reported. This study agreed with the study by Okuku *et al.* (2019) that showed high concentrations of metals in Tudor Creek. High Zn concentrations could be associated with dissolution of zinc anodes used in leisure boats as well as the use of galvanized metals and automobile tyres within the urban area. The deposited metals accumulate in street dust and later find their way into the aquatic systems through stormwater runoff (Hwang *et al.,* 2016). High Mn concentrations at Mikindani during the wet season could be attributed to discharge from the adjacent cement factory while high Pb concentrations could be associated with remnants of Pb in the sediment from boats used along the Creek and vehicles from the nearby villages.

Different correlations were observed between metals and other physio-chemical parameters. There was a significant positive correlation between DO-Rb and DO-Fe in Dabaso. This could be attributed by the fact that both DO, RB and Fe are influenced by similar environmental factors, such as water, temperature and salinity (Liu *et al.*, 2022). The negative correlation between biological oxygen demand and certain metals could be attributed to benthic organisms consuming metals for minerals intake (Xing *et al.,* 2022).

Meiofaunal densities showed seasonal variability. Density at Dabaso was higher than at Mikindani, which can be attributed to pollution levels, TOM and DO, which influence the composition of communities at a site (Schmid-Araya and Schmid, 1995). According to Ingels *et al.* (2014) and Carvalho *et al.* (2017), food availability in the form of organic matter plays an important role in regulating the density of meiofauna, as exhibited by the results of this study where Dabaso with high OM had higher density than at Mikindani. Additionally, the densities decreased at Mikindani in the wet season as a result of extreme reduction in oxygen concentrations (hypoxic conditions), caused by increased pollution and hence the disappearance of many sensitive taxa (Rabalais *et al.,* 2002). High inputs of metals can decrease the density of highly sensitive species, such as copepods (Pascal *et al.,* 2010), as at Mikindani. Nonetheless, this is not always the case as the densities may not change in a short period of time due to the dominance of highly tolerant nematodes, as described by De Troch *et al.* (2013).

Copepods, which are found in coarse sandy sediments and are sensitive to pollution (Uriarte and Villate, 2005; Moore and Bett, 2008) were present only at Dabaso. Additionally, they are the meiofauna most sensitive to low oxygen concentration (De Troch *et al.,* 2013), hence low density at Mikindani during the dry season and absent during wet season due to increased release of domestic and industrial effluents which caused low oxygen levels. This study showed copepods could act as good biological indicators when in significantly low densities or absent in polluted estuarine sediments. Turbellaria, ostracods and oligochaetes were more numerous at Mikindani compared to Dabaso because they are more resilient to pollution. A few halacaroids were found at Mikindani because they are well adapted to highly polluted and hypoxic environments and can be used as positive indicators of pollution (Bonaglia *et al.,* 2020). Navarro-Barranco *et al.* (2020) found that amphipods can be used as bioindicators of pollution which aligned with the current results where Mikindani lacked the presence of these meiofauna group compared to Dabaso, and are thus good indicators of pollution. However, the identification of nematodes to genus level could give better clarity to pollution indicators since some genera have been found to opportunistic or sensitive to organic or inorganic pollution.

Meiofauna diversity was higher at Mikindani compared to Dabaso in both seasons, which can be attributed to high opportunistic species contributing to the high diversity (Somerfield and Warwick, 1996). This study conforms with the results of previous studies by Schratzberger and Ingels (2018) and Pusceddu *et al.* (2013) that link increased diversity to the low dominance of opportunistic taxa. Mohamed *et al.* (2018) found a higher meiofaunal diversity in coarse and medium sized sediments, which was not the case for Dabaso with similar sediment size composition. High dominance at Dabaso during both seasons can be linked to very high density of nematodes (Mohamed *et al.,* 2018).

Excessive pollution in marine ecosystems is detrimental to meiofaunal density, diversity, composition, and community assemblages. It was evident that the differences observed in biotic factors were partly influenced by abiotic parameters (DO, TOM, BOD, grain size, metals), which were caused by the heavy inflow of pollutants at Mikindani. According to the results of this study, the sites were greatly affected by inorganic pollution. The meiofauna assemblages were attributed to sediment characteristics and food availability. Some taxa, like amphipods, showed sensitivity to contamination by being absent in Mikindani. The detrimental conditions due to high effluent discharge make it very difficult for some taxa to survive in these habitats.

Further studies should focus on a wider range of metals at Mikindani to ascertain if the higher concentrations here are attributed to the sediments grain size or to effluent discharge. Additionally, nematodes should be identified to the genus or species level to provide more information on opportunistic or sensitive taxa and to calculate a maturity index, thus providing insights on the sediment status at Dabaso and Mikindani.

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Original Article

Isolation, culture trials, and biochemical composition of microalga *Tetraselmis* from coastal waters of Tanzania

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Abstract

Microalgae are primary producers in aquatic ecosystems. Their high nutritional value make them suitable for applications in aquaculture and biotechnology. Serial dilution techniques were used to isolate the green microalga *Tetraselmis* sp. from water samples collected from the Ruvu Estuary in Tanzania. Laboratory culture trials were undertaken at varying salinity and light intensity levels, followed by biochemical analysis. Intermediate salinities (15 and 35) favoured cell accumulation, and light intensity significantly influenced *Tetraselmis* biochemical composition. Low light intensity (2.9 ± 1.6 Klux) and early harvests (day 7) increased the protein, lipid, carbohydrate and fibre content, whereas high light intensity (5.5 ± 3.3 Klux) led to greater ash accumulation. The day 16 harvest contained higher levels of minerals, with calcium, potassium, and magnesium being prominent. Trace minerals, including selenium, were present in safe quantities. The potential of *Tetraselmis* sp. from coastal Tanzania for aquaculture and biotechnological applications is highlighted.

Keywords: microalgae, *Tetraselmis* sp., Tanzania coastal waters, growth conditions, biochemical composition

Introduction

Microalgae are primary producers and play an important role in aquatic ecosystems as they support food webs. Microalgae have gained increasing attention for their potential applications in aquaculture, biotechnology, and environmental restoration. Due to their high-quality nutritional profile, they are extensively cultured and utilized as natural food sources in hatcheries for the rearing of fish, molluscs, and crustacean larvae (Brown, 2002; Sirakov *et al.*, 2015). These natural foods improve the growth, health and survival of fish and shellfish larvae. However, the nutritional value and growth can be influenced by various factors, including light intensity, nutrient limitation, salinity, temperature, and pH (Creswell, 2010). Among these factors, salinity and light are particularly emphasized as primary factors for marine species, as they can be adjusted and maintained at desirable levels without causing adverse effect on the cultured organism (Hotos and Avramidou, 2021). Salinity affects growth of microalgae through acting directly on the osmoregulation process. Although all marine species can survive in wide ranges of salinity, high salinity can inhibit the growth and alter chemical composition of some species (Khatoon *et al.*, 2018). Likewise, light is paramount and a critical factor to microalgae as it directly affects photosynthesis from which growth and biomass production is ensured. Depending on microalgae species, light intensity increases growth rate, but extreme intensity up to saturation point may lead to photo-inhibition (Metsoviti *et al.*, 2019).

Despite the pivotal role that microalgae play in marine ecosystems and their potential industrial applications, a significant gap exists in the knowledge of the microalgal composition within the coastal waters of Tanzania. The microalgae *Tetraselmis*, known for their diverse biochemical composition and potential use in aquaculture and biotechnology, remain largely un-investigated in this region. Limited studies have explored the aquaculture potential of microalgae in Tanzania, with a focus on specific species such as the cyanobacteria *Arthrospira fusiformis* in the Momella Lakes (Michael *et al.*, 2019; Mulokozi *et al.*, 2019), and a freshwater green algae *Chlorella vulgaris* (Mtaki *et al.*, 2021). However, comprehensive investigations into the cultivation and nutritional profiles of uni-algal marine species in the Western Indian Ocean remain notably limited. Most of the available research has concentrated on the distribution, composition and abundance of microalgae in this region (Kyewalyanga, 2002; Moto *et al.*, 2018; Sekadende *et al.*, 2021). With the growing demand for seafood and increasing attention towards the biotechnological applications of microalgae, there is a need to explore potential microalgae species for aquaculture and other industrial uses. This study addresses the pressing need for comprehensive research on *Tetraselmis* sp. in Tanzanian coastal waters by aiming to achieve three critical objectives: isolate *Tetraselmis* strains from the local environment; conduct laboratory culture trials to assess growth in terms of cell density in response to variations in salinity and light intensity; and analyse the biochemical composition of the isolated strain.

Materials and methods

Water sampling and microalgae isolation

Water sampling for microalgae isolation was carried out along the gradient of the Ruvu Estuary, Bagamoyo, Tanzania (6.384°S, 38.86°E) during both the Southeast monsoon and Northeast monsoon periods to increase the chance of encountering the microalgae with aquaculture potential. Samples were collected at brackish and full-strength salinity stations using a 10 litre bucket. Three sampling points were established at each sampling station. The *Tetraselmis* strain (Fig. 1B) used in the current study was obtained during the Southeast monsoon. At each sampling point, 100 litres of water were concentrated through a plankton net with 20 µm mesh size, and these samples were then combined to create one sample. The concentrated crude samples were transferred into glass bottles, and immediately enriched with a few drops of the standard laboratory culture medium, Guillard's F/2 (Kang *et al.*, 2011). The crude sample was exposed to ambient light near the windows for five days, with gentle mixing in the laboratory at the University of Dodoma. This technique was primarily conducted to exclude certain microorganisms with limited mobility. During this time, the flagellated algae concentrated themselves on one side of the flask in response to the light, making it easy to collect them using a pipette. The motile algae were then transferred into other flasks where the serial dilution technique with Guillard's F/2 medium was applied until the uni-algae strain of *Tetraselmis* sp. was obtained. Using the serial dilution technique, ten test tubes, each containing 9 ml of the culture medium, were prepared. After incubation for two days, 1 ml of the microalgae culture from one test tube was transferred to another test tube containing fresh culture medium. This transfer process was continuously repeated accompanied by regular microscopic checks. To ensure aseptic conditions, all flasks and the culture media used in the isolation process were sterilized before use. The identification of the isolated *Tetraselmis* strain was based on morphological features observed under a compound light microscope.

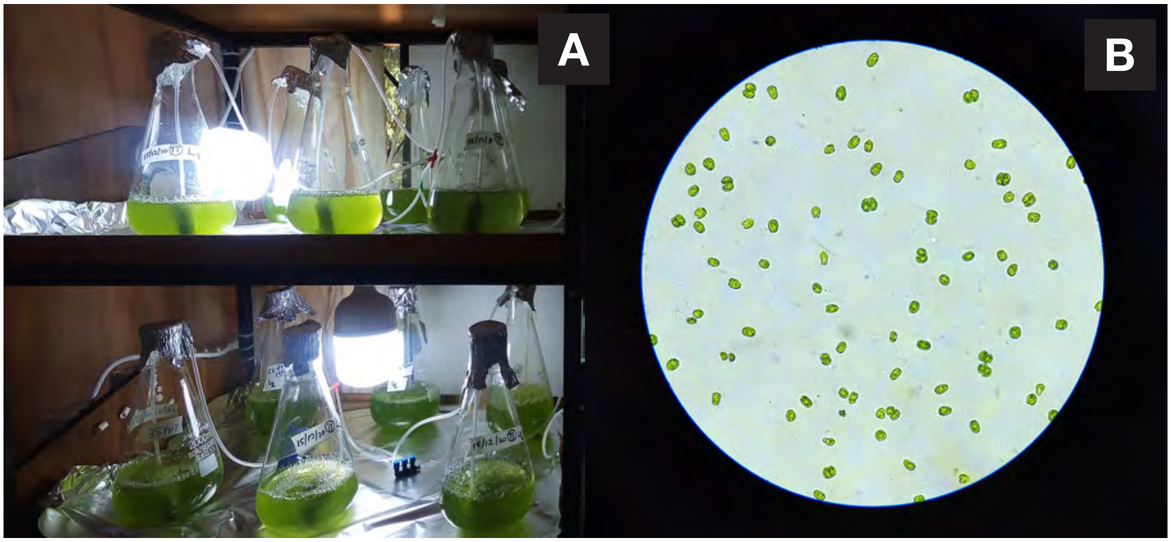

Figure 1. Laboratory set up for *Tetraselmis* sp. culture (A). Cells of *Tetraselmis* sp. under microscope, at magnification of 100X (B).

Experimental design

The successfully isolated strain of *Tetraselmis* sp. (Fig. 1B) was tested for growth, and accumulation of biochemical composition under different levels of salinity and light intensty to determine the optimal laboratory culture conditions. Three different experiments were carried out, with the first two lasting for 14 days each and the third extending to 16 days. The initial experiment focused on evaluating the effect of different light intensities on *Tetraselmis* sp. cell density at a salinity level of 35, reflecting the conditions in which the strain was originally collected from full-strength salinity water. Experiment 2 aimed to assess how different salinity levels influence the accumulation of *Tetraselmis* sp. cell density under top-light conditions. The final experiment analysed the biochemical composition of a microalga cultured under varying light intensities while maintaining a constant salinity of 35. Commencing each experiment involved obtaining a sub-culture sample from the mother culture and inoculating it with a fresh culture medium of Guillard's F/2 medium. The microalgae culture (100 ml) was inoculated with 1900 ml of the culture medium in an Erlenmeyer flask and placed in the growth chamber (Fig. 1A) at a temperature of 28-29 °C while being gently aerated. The culture was illuminated with artificial light provided by white LED lamps at a photoperiod of 12/12 hours for day and night.

Assessment of growth performance under varying light intensity and salinity levels

To evaluate the effect of varying light intensity on the growth of *Tetraselmis* sp., the culture was illuminated by the artificial light positioned either at the top or on the side of the culture flasks. The light was quantified by a VXLM_636 Vertex light meter. The top light supplied an average intensity of 5.5 ± 3.3 Klux while the side light supplied an intensity of 2.9 ± 1.6 Klux to the culture. For testing the effect of salinity on the growth of *Tetraselmis* sp., five levels of salinities i.e. 5, 10, 15, 35 and 40 were set under the top light. Different salinities used in the testing of growth were prepared by either diluting the ocean water, or in the case of preparing the salinity of 40, table salt was added to the seawater. The growth was determined in terms of cell density which was counted daily using a haemocytometer under a compound microscope (Olympus, BM 190, Japan). One millilitre of a sample was taken from the main culture, fixed with lugol solution and placed on the haemocytometer for observation and counting at a magnification of 100 X.

Assessment of biochemical composition of *Tetraselmis* sp.

The experiment was conducted under both top-light and side-light laboratory conditions, with light intensities ranging from 5.5 ± 3.3 Klux to 2.9 ± 1.6 Klux, respectively. Biomass for analysing the biochemical composition of *Tetraselmis* sp. was obtained by harvesting the culture on day 7 and 16 of the experiment. This strategy aimed to examine potential shifts in the biochemical composition of *Tetraselmis* sp. across different stages of its growth cycle. At day 7, a subsample (100 ml) was taken from the main culture while at the end of experiment the entire culture was harvested. The harvested sample was left to settle for about two hours and the supernatant was decanted and discarded. The concentrate was then centrifuged (Model FC5706, Ohaus Corporation, Germany) for 5 minutes at 4430 rpm to obtain the wet biomass. The obtained biomass was spread thinly on the plates then placed in the oven for 2 hours to dry to constant weight at a temperature of 105 °C. The dry biomass was then ground to powder and kept in a cool place until required for the analysis of proximate composition and mineral contents.

The proximate analysis of crude protein, lipid, fibre, ash and soluble carbohydrates in the *Tetraselmis* sp. biomass was conducted following standard analytical methods. The crude protein was determined using the semi-micro Kjeldahl digestion and indophenol blue colorimetric methods (Emteryd, 1989; Quarmby and Allen, 1989). On the other hand, lipid content was analysed according to Bligh and Dyer (1959). This method involved the addition of the mixture of three organic solvents (chloroform, methanol, and water) in specific ratios to the polyethylene vial containing 2 g biomass of *Tetraselmis* sp. After the extraction, the filtrate was transferred into a graduated separating funnel where the upper alcoholic layer of methanol and water was discarded while the mixture of chloroform and lipid was retained. The chloroform was then evaporated under vacuum by using rotavapor (Heidolph, Germany) at a temperature of 40 °C, and the resulting residue, which contained lipids, was oven-dried for three hours at 50 °C followed by weighing to determine the percentage lipid composition.

The crude fibre content was determined gravimetrically after acid-alkali hydrolysis (Allen, 1989; Quarmby and Allen, 1989). In this method, 1 g of *Tetraselmis* sp. powder was added into boiling sulphuric acid (1.25 % v/v) then washed with boiling water. The extracted sample was added to 1.25 % sodium hydroxide for further extraction and then washed with boiling water while suction pumping to separate it from the alkali. After oven drying the extracts at 105 °C for 2 hours, the sample was weighed followed by calculation to obtain the composition of fibre in the biomass. Total soluble carbohydrate of the *Tetraselmis* sp. biomass was extracted by hot water and estimated by calorimetric procedures after reacting with anthrone reagent (Allen, 1989).

The total mineral content of the biomass, termed as ash, was determined by combusting 1 g of the sample at 550 °C in the muffle furnace for 2 hours. The ash was then weighed and the percentage composition calculated. The determination of the concentration of individual mineral in the biomass followed the procedures described by Emteryd (1989). The dry sample (0.1 g) was oxidized using a mixture of acids (1 ml per-chloric acid and 5 ml nitric acid) followed by reading the concentration in the Atomic Absorption Spectrophotometer (AA240 Varian, USA). The analysed minerals were the macro elements calcium, magnesium, potassium and phosphorus, and the two trace minerals iron and selenium (antioxidant).

Data analysis

All collected data were analysed using SPSS software (IBM SPSS, version 20) while the graphs were drawn using R software (version 4.03). The variation in the growth performance under different conditions and biochemical compositions in *Tetraselmis* sp. biomass was analysed using the general linear model (GLM). Tukey Honest Significant Difference (THSD) test was used for multiple comparisons of salinity means when a significant difference existed between the salinity levels. A p-value of less than 0.05 was considered significant.

Results

Effect of light on cell density

Tetraselmis sp. showed an exponential growth trend in both light orientations (Fig. 2). There was no statistical variation between the two lights (F = 1.093; p = 0.301) although the top light seemed to surpass the side light. However, significant variation was observed among the culture time (F = 11.103; $p < 0.05$) whereas the organism accumulated more cell density as time went on. The maximum cell density for both of the light orientations was recorded at day 12 with the density reaching 4.5 ± 0.728 x10^6 cells/ml for top light and 3.5 ± 2.133 x10^6 cells/ml for side light. The only culture which entered a stationary phase was that of side light from day 12 to 14, while the culture with top light orientation did not display that trend.

Influence of salinity on cell density

The growth of *Tetraselmis* sp. was slow for all salinity levels in the first three days of the experiment as the organism was adapting to the culture environment (Fig. 3). After the take-off, a good growth trend was observed in the salinity of 25 and 35 while poor growth was observed in the extreme salinity levels of 5 and 40. The microalgae continued to grow without showing either stationary or death phases for the

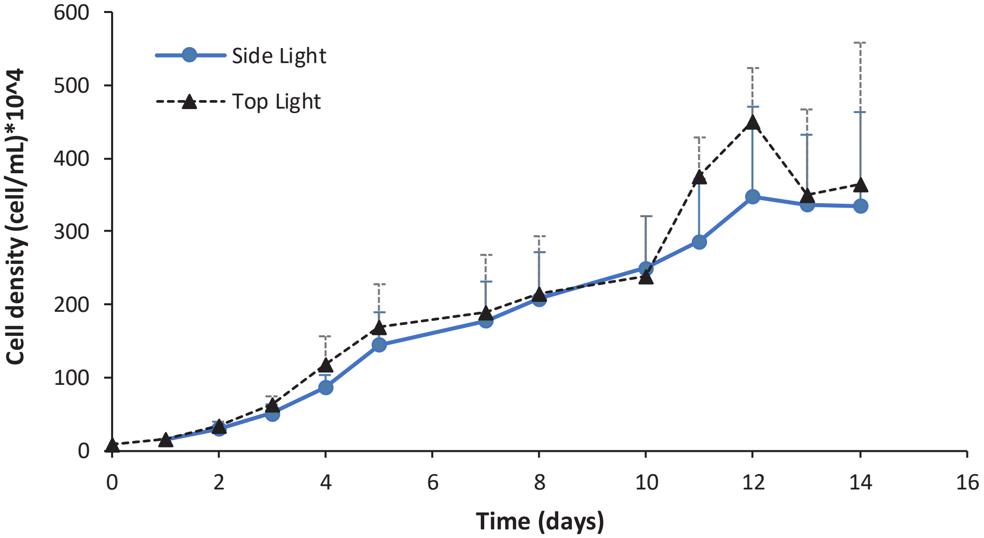

Figure 2. Growth performance of *Tetraselmis* sp. (± SD) at different light orientations during the culture experiment (days).

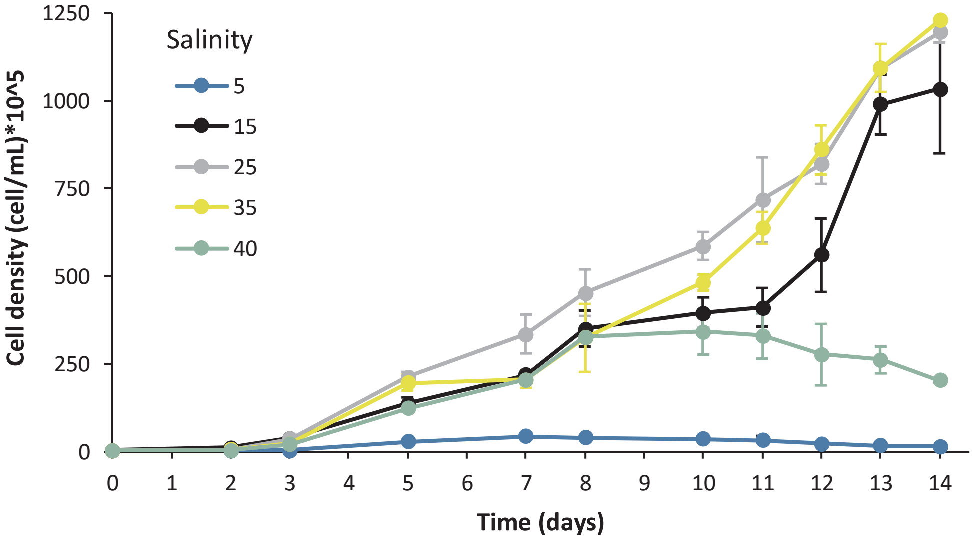

Figure 3. Growth performance of *Tetraselmis* sp. (± SD) at different salinity levels during the culture experiment (days).

salinities of 15 to 35. There was a significant difference in cell densities between salinity levels (F = 371.511; $p < 0.05$). The Tukey multiple comparisons test revealed significant variations in cell density between all salinity levels except the salinity of 25 and 35 (Table 1). The highest mean cell density (1230.5 ± 2.263 x10^5 cells/ml) was attained at day 14 in a salinity of 35, while on the same day salinity 5 attained the minimum density (15.1±1.555 x10^5 cells/ml). There was also a significant effect of culture time (F = 294.241; $p < 0.05$) and combined effect of salinity and time (F = 38.203; $p < 0.05$) on the growth of *Tetraselmis* sp. Exponential growth of the organism occurred for all salinity levels from day 3 except salinity 5 which behaved differently, and salinity 40 which showed a decline in cell density from day 11 of the experiment.

Influence of light on biochemical composition

There was a significant effect of light intensity (F = 91.29; $p < 0.05$), harvesting day (F= 188.83; $p < 0.05$) and interactive effect of light intensity and harvesting day on the protein content of *Tetraselmis* sp. There was a high

Table 1. Tukey HSD multiple comparisons of cell density between salinity levels.

Salinity	Salinity	Mean Difference Cells/ml	P-value
5	15	-355*	< 0.001
	25	-474*	< 0.001
	35	-438*	< 0.001
	40	-168*	< 0.001
15	5	355*	< 0.001
	25	-119*	< 0.001
	35	-83*	< 0.001
	40	187*	< 0.001
25	5	474*	< 0.001
	15	119*	< 0.001
	35	36	0.116
	40	305*	< 0.001
35	5	438*	< 0.001
	15	83*	< 0.001
	25	-36	< 0.001
	40	269*	< 0.001
40	5	168*	< 0.001
	15	-187*	< 0.001
	25	-305*	< 0.001
	35	-269*	< 0.001

Table 2. Comparative total mean of proximate composition between the light intensities.

Composition (%)	Light intensity		P-value
	2.9 ± 1.6 Klux	5.5 ± 3.3 Klux	
Crude protein	67.91 ± 2.27	64.67 ± 7.31	P < 0.05
Crude lipid	11.16 ± 1.25	9.38 ± 0.57	P = 0.941
Carbohydrate	5.95 ± 0.24	5.45 ± 1.16	P = 0.402
Ash	2.73 ± 0.18	6.65 ± 2.17	P = 0.010
Crude fibre	1.34 ± 0.05	1.25 ± 0.12	P = 0.830

average protein content (67.91 ± 2.27 %) in the biomass cultivated in low light intensity compared to 64.67 ± 7.31 % recorded in high light intensity (Table 2). Similarly, the biomass accumulated a large quantity of protein on day 7 of growth compared to day 16 (Table 3).

The composition of crude lipid, carbohydrate, fibre and ash was also affected significantly by light intensity, harvesting time and the interactive effect of the two parameters. In a similar scenario to protein, a high quantity of crude lipid, carbohydrate, and fibre was recorded in the biomass cultivated with low light intensity (Table 2), harvested on day 7 (Table 3). The content of ash was contrary to other proximate components where a high average quantity (6.65 ± 2.17 %) was recorded in the high light intensity, and in the biomass harvested at day 16 (8.63 ± 2.17) of the culture experiment.

The contents of all quantified minerals except phosphorus were significantly high in the biomass harvested on day 16 (Table 4). The biomass harvested on day 16 was composed of a large quantity of calcium, followed by potassium and magnesium while the tracer minerals were in lower quantities. Comparing the influence of light, *Tetraselmis* sp. accumulated more mineral content when cultivated with high light intensity, whereas calcium contributed the highest amount (Table 4). On the other hand, the mineral phosphorus was abundant in the biomass cultivated at the low light intensity, although it was preceded by calcium.

Discussion

Tetraselmis sp. is a marine microalga widely used in aquaculture as a natural food for fish and shellfish larvae due to its valued nutritional content and ease of culture under hatchery conditions (Meseck *et al.*, 2005). In this study, *Tetraselmis* sp. exhibited optimal growth in intermediate salinities of 15 and 35, while extreme salinities of 5 and 40 led to comparatively poor growth. This trend indicates *Tetraselmis*' sp. preference for moderate salinity ranges, which is in line with the requirements of many marine species. Salinity variation influences microalgae growth performance, physiological as well as biochemical processes (Pal *et al.*, 2010). Depending on species, salinity causes stress to microalgae when it is above the survival range and may have significant effects on the growth and other body processes (Pandit *et al.*, 2017). Despite originating from a marine environment, *Tetraselmis* sp. exhibited poor growth in a salinity of 40 under laboratory conditions, deviating from previous findings (Pugkaew *et al.*, 2019; Hotos and Avramidou, 2021) that reported good performance even at salinity above 40. This variation in findings may be linked to differences in the strain used and the species' adaptation to its natural environment. The observed limited growth at a salinity of 40 could be attributed to the organism's adaptation to the chemical composition of surface water in the coastal waters of Tanzania, where the average salinity is reported to be 35 (Mahongo and Shaghude, 2014; Painter *et al.*, 2021). Conversely, the observed underperformance and limited growth at

Table 3. The proximate composition of *Tetraselmis* sp. biomass harvested at different days. Mean ± SD, n = 6.

Composition (%)	Harvesting day		P-value
	Day 7	Day 16	
Crude protein	68.62 ± 2.97	63.97 ± 6.56	P = 0.143
Crude lipid	11.45 ± 1.4	9.09 ± 0.57	P = 0.003
Carbohydrate	6.49 ± 0.24	5.40 ± 1.16	P = 0.048
Ash	2.67 ± 0.18	8.63 ± 2.17	P = 0.001
Crude fibre	1.38 ± 0.05	1.20 ± 0.12	P = 0.016

Table 4. Quantity of minerals in *Tetraselmis* sp. biomass at different light orientation, and harvesting time. Mean ± SD

Mineral	Quantity of minerals (mg/100g)			
	Day 7	Day 16	Top-light	Side-light
Calcium	543.73±236.47	3847.25±784.12	2520.72±2248.09	1870.26±1382.35
Magnesium	349.98±123.01	403.06±52.43	408.73±58.65	344.30±116.79
Potassium	761.07±635.22	1223.34±10	1176.19±11	808.20±58
Phosphorus	1400.31±673.29	1245.89±129.42	956.71±187.36	1689.49±356.51
Iron	64.87±6.59	80.09±8.14	79.20±9.12	65.76±7.57
Selenium	0.002±0.000	0.013±0.019	0.003±0.002	0.012±0.02

salinity of 5 align with expectations, considering the marine nature of the species. On the other hand, the good performance of the organism at lower salinities of 15 and 25, compared to full strength, aligns with its natural environment. The estuary experiences periodic freshwater inflows, which correspondingly influences the organism capacity to thrive. The growth trend where no stationary phase was exhibited by *Tetraselmis* sp. indicates that the organism can be cultivated under laboratory conditions for an extended period. This unique characteristic opens avenues for diverse applications, enhancing its utility across various fields. Similar trend was reported by Hotos and Avramidou (2021) who also recorded an exponential growth without a stationary phase even at day 17 for the salinity of 20 and 40.

The findings regarding the effect of light on *Tetraselmis* sp. growth suggest that the organism was equally affected by light orientation. However, the highest cell density was achieved at higher light intensity (5.5±3.3 Klux). Light quantity and quality are important factors in regulating the productivity of photosynthetic organisms. Optimal growth is achieved when these organisms are exposed to light of optimal wavelengths (Schulze *et al.*, 2016). Lower light intensities below the saturation point have limiting effect on microalgae growth, which in turn, affects the synthesis of body composition (Hotos and Avramidou, 2021; Metsoviti *et al.*, 2019). The study conducted by Hotos and Avramidou (2021) reported a high growth rate of *Tetraselmis* sp., reaching up to 9.6x10^6 cells/ml at a light intensity of 8.0 Klux. In contrast, at 2 Klux the maximum density attained was 2.2x10^6 cells/ml (Hotos and Avramidou, 2021). A similar pattern was observed by Montes-González *et al.* (2021) in *Tetraselmis suecica,* where a high density of 26x10^6 cells/ml was recorded under high light intensity compared to low intensity. Despite the similar trend in the findings, the cell density recorded in the current study is lower compared to previous research studies (Hotos and Avramidou, 2021; Montes-González *et al.*, 2021). The variation in the number of cells obtained might be due to differences in the strain used, and also other laboratory conditions including the type and quantity of nutrient, photoperiod, pH and salinity. When comparing the growth trend to other microalgae, such as *Chlorella vulgaris* (Metsoviti *et al.*, 2019), *Desmodesmus* sp. and *Scenedesmus obliquus* (Nzayisenga *et al.*, 2020), it was observed that growth increased with higher light intensity. The similarity in growth trends shows that *Tetraselmis* sp., like other photosynthetic microalgae, undergo rapid cell division at higher light intensities, resulting in higher cell count.

In terms of body compounds, it was found that low light intensity, coupled with early harvesting during the phase of steady growth, resulted in higher levels of protein, lipid, carbohydrate, and fibre content. This suggests that light plays a crucial role not only in growth but also in shaping the nutritional profile of *Tetraselmis* sp. biomass. This trend was also observed in other microalgae species, such as *Chlorella vulgaris* (Metsoviti *et al.*, 2019) where the quantities of crude protein and fibre remained constant as light intensity increased. However, in contrast to previous research (Nzayisenga *et al.*, 2020; Maltsev *et al.*, 2021), the findings regarding lipid production differed. Higher lipid content was recorded in *Chlorella* and *Scenedesmus* species under higher light intensities (Nzayisenga *et al.*, 2020; Maltsev *et al.*, 2021). In other species of *Tetraselmis*, previous research reported protein content within a range of 31 to 59 % (Schwenzfeier, 2013; Khatoon *et al.*, 2018; Wang *et al.*, 2021), which is lower than reported in this study. However, the values of carbohydrate (20 %) and lipid (22 %) reported by Schwenzfeier (2013) are much higher than those recorded in the current study. The content of lipid in the current study is also lower than the 28.3 % reported by Kim *et al.* (2016). The variation in the body composition of microalgae depends on various factors, including the purpose of cultivation and the culture conditions. Previous studies have reported that microalgae tend to accumulate high levels of carbohydrate or lipids when the culture is deficient of nitrogen (Markou *et al.*, 2012; Teo *et al.*, 2014; Kim et *al.*, 2016). In the current study, the microalgae were cultivated in a commercial medium (Guillard's F/2) without any manipulation of ingredients, explaining the variation in the contents of carbohydrates and lipids compared to previous studies.

Minerals are essentials for the normal processes and health of animal bodies though required in small quantities. In the current study, the biomass harvested on day 16 contained notably higher levels of minerals, particularly calcium, potassium, and magnesium. This observation indicates that longer cultivation periods may enhance the mineral content of *Tetraselmis* sp., potentially extending its nutritional value for aquaculture and other applications. In terms of total minerals (ash), the recorded amount is approximately half of the 15.2 % reported by Pereira *et al.* (2019) for *Tetraselmis* sp. produced at commercial scale. Despite the lower composition of ash content, the values fall within the comparable range (1.9 to 37 %) observed in most microalgae species (Liu, 2017). On the other hand, the high content of calcium, potassium, and magnesium in the biomass of *Tetraselmis* sp. harvested on day 16 implies the abundance of those minerals in the surrounding water. However, the variation of phosphorus levels in combination with other minerals can be explained in the context of the effectiveness of *Tetraselmis* sp. in removing phosphorus from the water (Patel *et al.*, 2012). The study by Patel *et al.* (2012) reported higher uptake rates of phosphorus from wastewater on day 8 compared to day 16, which is a similar time frame to the current study. The levels of trace minerals in the biomass of *Tetraselmis* sp. were low, as they are required in low quantity. Selenium plays an essential role as a strong antioxidant, protecting living cells against oxidative effects. In the human body, it slows aging, prevents cell damage, and boosts the immune system (Gojkovic *et al.*, 2015). However, the benefits of selenium are observed at lower doses, while high concentrations can generate reactive oxygen species that damage body cells (Sun *et al.*, 2014). For instance, Watanabe *et al.* (1997) recommended a range of 0.15 to 0.5 mg/kg of selenium per dry weight as suitable for proper fish growth. The presence of selenium in *Tetraselmis* sp. biomass in lower amouts is a good sign that the microalga is safe for consumers. Moreover, in aquaculture it indicates that when *Tetraselmis* sp. is used as a natural food, there is no need of fortifying the feeds with synthetic antioxidants.

Conclusions

The study revealed that *Tetraselmis* sp. exhibited optimal growth in moderate salinity levels of 15 and 35, highlighting its adaptability to local conditions within the Western Indian Ocean. Light intensity significantly influenced body composition, favouring higher protein, lipid, carbohydrate, and fibre content under low light and early harvest. Extended cultivation periods enhanced mineral accumulation, notably calcium, potassium, and magnesium. The study demonstrated that the local strain of *Tetraselmis* sp. can be kept for culture under laboratory conditions, and the establishment of optimal culture conditions can be set depending on the cultivation objectives. Therefore, this study concludes that, with a specific focus on *Tetraselmis* sp., there is significant potential for isolating and cultivating this local marine microalga for industrial applications.

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Original Article

Enzymatic and antimicrobial potential of Actinomycetota species from mangrove sediments in Tanzania

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Abstract

The enzymatic and antimicrobial potential of Actinomycetota species present in mangrove sediments at Bagamoyo in Tanzania was explored. Ten strains were isolated from sediments and identified based on morphological and biochemical characteristics. All isolates were Gram positive and catalase positive. Eight isolates tested positive for amylase production. Crude extracts from five isolates showed antimicrobial activities in at least one of the five tested microorganisms, with MIC values ranging from 10 to 2.5 mg/mL. The 16S rRNA gene region of the five isolates was sequenced, and DNA barcoding revealed that the isolates belong to the genera *Streptomyces* (3 strains), *Micrococcus* (1 strain) and *Hoyosella* (1 strain). The *Hoyosella* isolate is reported here for the first time from Western Indian Ocean mangrove systems. The identified Actinomycetota have demonstrated capabilities to ferment sugars, produce enzymes and secondary metabolites with antimicrobial properties, with potential application in pharmaceutical, agricultural and biotechnological industries.

Keywords: mangrove, Actinomycetota, enzymatic properties, antimicrobial activity

Introduction

The microbes inhabiting different extreme environments such as the mangroves, deserts, hot springs and salt lake ecosystems have been a focus in search for novel pharmaceutical compounds to treat existing and emerging diseases (Khadayat *et al.,* 2020). Marine ecosystems are well defined for their unique biotic and abiotic components. Even though the number of marine ecosystems is actively debated, most ecologists agree on six; namely, estuaries, salt marshes, mangrove forests, coral reefs, open ocean and the deep-sea ocean (Duarte *et al.,* 2020). Mangroves are groups of trees and shrubs that grow in the coastal intertidal zone of marine environments. In the Western Indian Ocean (WIO) region, mangroves are estimated to cover approximately 1.0 million ha, constituting about 5 % of global mangrove coverage (Maina *et al.,* 2021). Ninety per cent of mangroves in the WIO region occur in deltas and estuarine in the four countries of Mozambique, Madagascar, Tanzania and Kenya, in decreasing order of coverage (Mangora *et al.,* 2016). The mangrove ecosystems are among the world's most productive environments that provide a huge but scarcely explored source of microbes, including Actinomycetota, with high potential to produce bioactive secondary metabolites such as antimicrobial, anticancer and anticardiovascular agents (Salimi *et al.,* 2018; Siddharth and Rai, 2019; Cera *et al.*, 2022).

Actinomycetota is a phylum of Gram-positive bacteria characterized by the presence of high guanine and cytosine content in their DNA. The first discovery of species in this group of bacteria was made in 1877 (Goodfellow, 2012). Actinomycetia is the common class of phylum Actinomycetota comprising of 16 orders including order Actinomycetales which is often called Actinomycetes, with most of its species having been reported to produce secondary metabolites with biomedical properties (Ranjani *et al.,* 2016). The most common genera of Actinomycetales are *Streptomyces*, *Nocardia* and *Micromonospora* (Ranjani *et al.,* 2016). Actinomycetota occur either as spores or vegetative forms in terrestrial habitats such as soil, plant litter and composts as well as in aquatic environments such as marine and freshwater.

Ecologically, species in phylum Actinomycetota play a vital role in various biological processes such as bioremediation (Adenan and Ting, 2022) and promotion of plant growth (Soumare *et al.,* 2021). Over decades, marine Actinomycetota have been used as an outstanding source of enzymes and bioactive metabolites. Physiological parameters such as pH, temperature, salt concentration and atmospheric conditions are some of the factors that affect their production of enzymes and biomedically important metabolites (Sadikiel *et al.,* 2022). Enzymes produced by most acidophilic Actinomycetota are hydroxylases, transferases and esterase (López-Mondéjar *et al.*, 2019), while those produced by thermophilic and alkaliphilic species include amylases, proteases, keratinases, xylanase and dextranase (López-Mondéjar *et al.,* 2019; Lema *et al.,* 2022). Actinomycetota from the WIO region have been found to exhibit significant enzymatic diversity with biotechnological potential (Sarkar and Suthindhiran 2022). These enzymes include proteases, lipases, cellulases, amylases and keratinases (González *et al.,* 2020). Moreover, research on marine Actinomycetota from the WIO coastline revealed their significance as producers of compounds, emphasizing their pharmaceutical importance (Kamjam *et al.,* 2017). Therefore, this study was designed to explore the enzymatic and antimicrobial potential of Actinomycetota species inhabiting the mangrove sediments on the Bagamoyo coast of Tanzania to provide baseline information useful in the development of enzyme-derived biotechnologies and novel antibacterial agents.

Materials and methods

Sample collection

Sampling was carried out in the mangrove forest in the intertidal zone of Indian Ocean coast of Bagamoyo District, Pwani region, Tanzania at approximately Longitude 38° 53’ 55.772” E and Latitude 6° 25’ 46.548” S (Fig. 1). The sediments samples were collected in January 2022 using a syringe barrel from 10 points at a depth of 10 cm within a 100 m^2 area of the mangrove. The sediments were placed into sterile polythene bags, mixed well and then taken to the laboratory at the Department of Molecular Biology and Biotechnology (DMBB) at the University of Dar es Salaam (UDSM) for further analysis.

Isolation of Actinomycetota

One gram (1.0 g) of the sediments samples was suspended in 9 ml of sterile sea water and serially diluted to 10^{-4} as previously described by Sosovele *et al.* (2012). The diluted samples were placed on to a set of selective isolation plates in triplicates. The selective isolation plates contained starch casein agar (SCA) media constituting of 10.0 g starch, 1.0 g casein, 15.0 g agar, 500 mL sea water (Obtained from the sampling site) and 500 mL distilled water adjusted to pH 7.0 ± 0.1. Media was autoclaved at 121 °C for 15 minutes then supplemented with nalidixic acid (20 µg/mL) and nystatin (50 mg/mL) antibiotics to suppress the growth of bacteria and fungi, respectively without affecting the growth of Actinomycetota (Sosovele *et al.,* 2012). The inoculated plates were incubated at 28 °C for 2 weeks. Isolation was carried out by randomly picking developed colonies from selected dilution plates based on colony morphology followed by repeated serial dilution and streaking techniques until pure colonies were obtained.

Morphological identification of the Actinomycetota isolates

Morphological characterization of Actinomycetota isolates was carried out macroscopically by observing colony colour, aerial mycelium, texture, elevation as well as pigment production and microscopically by observing cell morphology and Gram’s staining test (Lema *et al.,* 2022).

Biochemical characterization of the Actinomycetota isolates

Actinomycetota isolates were subjected to carbohydrate fermentation, catalase and amylase production as hereunder described.

Carbohydrate fermentation test

The isolated Actinomycetota were screened for their ability to ferment D-glucose, sucrose and starch in broth media (10.0 g peptone, 5.0 g NaCl, 1.0 g beef extract, 0.018 g phenol red, 10.0 g carbon source, 1 L distilled water. pH 10) (Remya and Vijayakumar, 2008). The isolates were placed in sterile test tubes containing sterilized broth media and incubated at 37 °C. Observation was conducted after 48 hours through examining the colour change of the broth.

Catalase test

A slide method catalase test was conducted as described by Reiner (2010), where a sterile wire loop was used to collect a small amount of organism from a pure culture and placed onto a sterile microscope slide. Observation was made immediately after addition of 1 drop of 3 % H_2O_2 on a microscope slide containing a bacteria smear.

Amylase test

The isolated bacteria were tested to determine if they could hydrolyze starch by producing amylase enzyme. According to Mahboobeh *et al.* (2016), starch agar media (2.0 g soluble starch, 5.0 g peptone, 0.5 g yeast extract, 0.5 g beef extract, 5.0 g sodium chloride, 1000 mL distilled water, pH 7.4) was used to grow the isolates, whereby each isolate was spotted on the media and left to grow for 48 hours at 37 °C. After incubation, the surface of the plate was flooded with iodine solution using a dropper for 30 seconds then observed to check if there was a clear zone formed around the bacteria isolates or not.

Figure 1. A map showing location of the sampling site at Bagamoyo, Tanzania.

Production of secondary metabolites

Each of the selected Actinomycetota isolate's pure culture was cultivated in 15 L production broth (24 g soluble starch, 3.0 g meat extracts, 5.0 g yeast extracts, 3.0 g peptone, 1.0 g glucose, 4.0 g $CaCO_3$ 1000 mL distilled water, pH 7.0). After 14 days, bacterial extract was filtered followed by the addition of equal volume of ethyl acetate for metabolites extraction. The mixture was shaken vigorously for 15 minutes then subjected to a separating funnel and the organic layer comprising the secondary metabolites was collected. Solvent contained in the crude extracts was removed using a rotary evaporator at 40 °C. The recovered extracts were stored in the refrigerator until required for antimicrobial assays.

Test microbial strains

Testing strains, *Escherichia coli* ATCC-8739, *Salmonella typhi* ATCC-14028, *Bacillus subtilis* ATCC-6633, *Staphylococcus aureus* ATCC-25923 and *Candida albicans* ATCC-10231 were obtained from the microbial strains' library at the DMBB laboratory. The bacteria and yeast were streaked on nutrient agar (NA) and potato dextrose agar (PDA), respectively then incubated at 37 °C for 24 hours. After incubation, pure cultures were picked and transferred in test tubes containing sterile saline (0.85 %) then centrifuged. The turbidity of bacterial cells observed was compared with 0.5 McFarland standard.

Screening for antimicrobial activities

Disc diffusion assay

Disc diffusion assay was used to screen for antimicrobial activities of the crude extracts from Actinomycetota isolates against the test microorganisms as previously described by Chanthasena *et al.* (2022) Twenty microliter (20 µl) of the isolate's crude extracts (300 mg/mL) were impregnated in sterile 6 mm discs prepared from Whatman No. 1 filter paper. Test bacteria suspensions were swabbed on the sterile nutrient agar plates followed by the addition of the negative control discs containing DMSO, discs soaked with test samples and finally discs with chloramphenicol (1 mg/mL) and fluconazole (1 mg/mL) as positive control for the bacteria and yeast respectively. The petri dishes were incubated for 24 hours at 37 °C. Results were obtained by measuring the diameter of the inhibition zone (mm), if any.

Minimum inhibitory concentration of the extracts

Minimum inhibitory concentration (MIC) of the active crude extracts was evaluated using a 96-well microtiter plate assay method following the procedure previously described by Masalu *et al.* (2020), with modifications. Fresh cultured test bacteria were inoculated in 100 mL of Mueller Hinton and incubated for 24 h at 37 °C prior to the experiment. Using sterile 96 microtiter plates, two-fold serial dilution was carried out to obtain 100 µL per well of the following concentrations: 10, 5.0, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 mg/mL. Then, 100 µL of Mueller Hinton broth inoculated with standardized 0.5 McFarland test organisms was added into each well to make a total of 200 µL per well. Microtiter wells with broth only were set for sterility control. Chloramphenical and Fluconazole were used as positive controls for bacteria and fungal respectively. Microtiter plates were incubated at 37 °C for 24 h. The MIC results were determined using a micro plate reader (ELISA plate analyser).

Molecular identification of the selected Actinomycetota isolates

The five Actinomycetota isolates whose extracts showed antimicrobial activities were selected for molecular identification using 16S rRNA gene sequencing. DNA extraction and purification was done by using Quick-DNA™ Fungal/Bacterial Miniprepkit as per manufacturer's protocol (Cat No. D6005, Zymoresearch Corp. USA). Quality and quantity of the DNA obtained (ng/µL) (A260/A280) were measured using a UV-Vis NanoDrop spectrophotometer. 16S rRNA gene was amplified using the universal eubacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Sengupta *et al.*, 2015). All PCR amplifications were performed at a total volume of 25 µl containing 12.5 µl of master mix, 1.0 µl forward primer, 1.0 µl of reverse primer, 3 µl template DNA and 7.5 µl of nuclease-free water. DNA was amplified by using Veriti™ 96-Well Fast Thermal Cycler (ThermoFisher Scientific, USA) (Stach *et al.*, 2003). The Amplification programme was initiated by denaturation at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 50 seconds, and extension at 68 °C for 1 minute. A final extension was performed at 68 °C for 5 minutes.

The PCR products were sequenced at a commercial genomics facility, Inqaba Biotec in South Africa, using the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 (Nimagen) according to the manufacturer's instructions. The products were then cleaned by following the protocol of the ZR-96 DNA Sequencing Clean-up Kit (Cat No. D4053, Zymoresearch Corp. USA). The purified products were inserted on the Applied Biosystems™ ABI 3500xL Genetic Analyzer (Cat No. 4406016, ThermoFisher Scientific) and forward sequenced only with a 50 cm array, using POP-7™ as compatible polymer. Sequence chromatogram analysis was performed using FinchTV analysis software v1.4.0.

Phylogenetic analysis

Quality control check of the identified sequences was performed using Chromas Version 2.6.6 (Technelysium Pty Ltd., Australia) prior to analysis. Sequence alignment was carried out using AliView Version 1.26 (Uppsala Universitet) by the MUSCLE method. The alignment analyses were later crosschecked with Molecular Evolutionary Genetics Analysis X (MEGAX) version 10.0.5, the phylogenetic tree was also constructed using the MEGAX programme with the Tamura-Nei distancing model, maximum likelihood as statistical method, Nearest-Neighbor-Interchange as maximum likelihood Heuristic Method tree inference option (Kumar *et al.*, 2018).

Results

Isolation of Actinomycetota

Observation of the inoculated samples on SCA selective media done after 14 days of incubation showed growth of distinct bacteria colonies, some of which showed powdery consistency and firmly attached to the agar surface projecting a tangled mass of mycelium resembling fungi, while some had a smooth

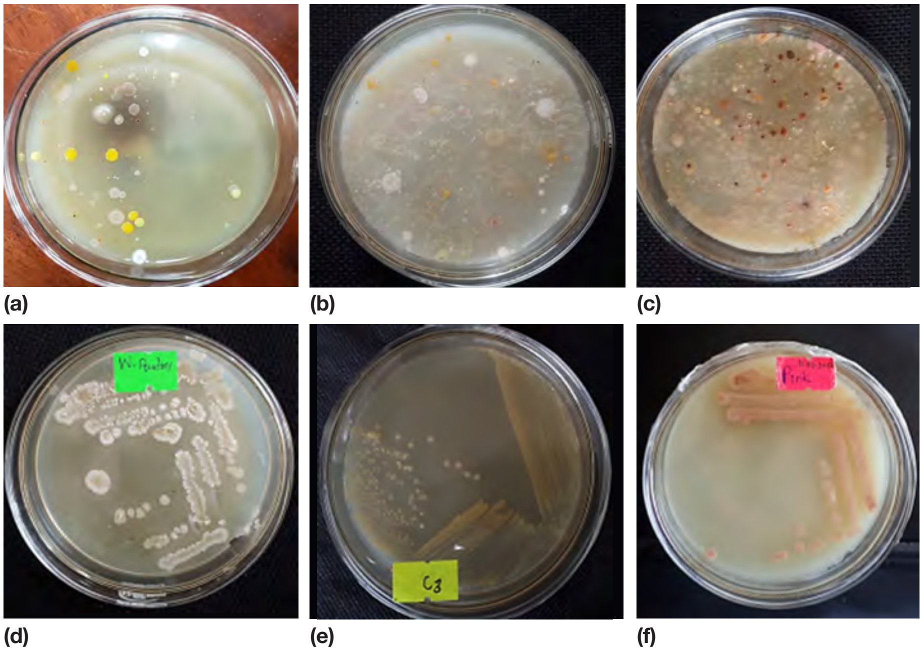

Figure 2. Representative petri dishes with Actinomycetota colonies: mixed isolates from sediment samples (a–c); pure isolates (d–f).

waxy appearance with different colours such as white, yellow, brown, grey, pink and cream (Fig. 2 a-c).

The observed cultures were highly suspected to be Actinomycetota as most of the features such as the cream colony colour for *Hoyosella* species and mycelia projection for *Streptomyce*s species were as described in various related literature (Jurado *et al.,* 2009; El-Naggar *et al.,* 2016). The colonies were randomly picked based on their colour, texture, pigmentation and elevation from the mixed cultures and were sub-cultured until a single strain grew in each plate as presented in Figure 2 d-f. Upon repeated transfers to new media, 10 isolates were selected for morphological, biochemical and antimicrobial characterization.

Morphological and biochemical characteristics of the selected Actinomycetota isolates

The 10 selected isolates were first characterized based on morphology and the results are shown in Table I. The isolated colonies had varying colour, texture, pigmentation and elevation. Some colonies were hairy

Table 1. Morphological characteristics of the selected Actinomycetota isolates.

TABLE S Isolate Code	Colony Color	Aerial mycelium	Cell Morphology	Texture	Pigmentation	Elevation	Probable genera	Reference
WP27F	White	White	Rod	Rough	Brown	Raised	*Streptomyces*	Hao *et al.,* 2009
WZ27F	White	White	Rod	Rough	Nil	Raised	*Streptomyces*	El-Naggar *et al.,* 2016
WS27F	White	Nil	Rod	Smooth	Nil	Raised	*Nocardioides*	Siddharth and Rai 2019
C27F	Cream	Nil	Rod	Smooth	Cream	Raised	*Hoyosella*	Jurado *et al.,* 2009
CS27F	Cream	Nil	Spiral	Rough	Nil	Flat	*Streptacidiphilus*	Cho *et al.,* 2012
G27F	Brown	Nil	Spiral	Smooth	Nil	Raised	*Rhodococcus*	Kumar and Jahangir 2018
B27F	Brown	Nil	Spiral	Smooth	Brown	Raised	*Rhodococcus*	Kumar and Jahangir 2018
P27F	Pink	Pale red	Spiral	Rough	Cream	Raised	*Streptomyces*	Mohamed *et al.,* 2017
Y27F	Yellow	Nil	Coccus	Smooth	Nil	Raised	*Micrococcus*	Greenblatt *et al.,* 2004
PY27F	Pale yellow	Nil	Coccus	Smooth	Nil	Raised	*Micrococcus*	Greenblatt *et al.,* 2004

Table 2. Biochemical characteristics of the selected Actinomycetota isolates.

SN	Isolate Code	Carbon fermentation test				Catalase Test	Amylase Test
		Glucose	Sucrose	Starch	Gas production		
1	WP27F	+	+	+	+	+	+
2	WZ27F	+	+	+	+	+	+
8	WS27F	+	+	+	+	+	+
3	C27F	+	+	+	+	+	+
5	CS27F	+	+	-	+	+	-
4	G27F	+	+	+	+	+	+
6	B27F	+	+	+	+	+	+
7	P27F	+	+	+	+	+	+
9	Y27F	+	+	+	+	+	+
10	PY27F	+	+	-	-	+	-

with aerial mycelium projections while others had a smooth appearance. Microscopically, all isolates were Gram positive and majority of them were rod or spiral with few (2) being cocci shaped. Variation in colour and colony texture of different isolates has been used in other studies for the grouping of Actinomycetota (Hasani *et al.*, 2014). As such, these findings show that the isolates belonged to 6 different genera with *Streptomyces* (isolates WP27F, WZ27F and P27F) being in the majority (Table 1).

Results of the biochemical tests (Table 2) revealed that the majority of the selected Actinomycetota species fermented sugars. All isolates were able to ferment D-glucose and sucrose sugars and tested positive for catalase enzyme production. Of the 10 isolates, eight were able ferment starch and tested positive for amylase enzyme production.

Antimicrobial activities of the extracts

Out of the 10 tested isolates' extract, five coded WP27F, WZ27F, C27F, P27F and Y27F showed significant antimicrobial activity against all tested microorganisms as presented in Table 3. The highest inhibition zone was 20.7 mm exhibited by the extracts of WP27F strain against *C. albicans* (Fig. 2a). This strain (WP27F) also showed high activity (18.3 mm) against Gram-negative bacteria *E. coli* followed by the extracts from P27F strain which showed inhibition zones of 16.0 mm against both *E. coli* and *S. typhi* (Table 3, Fig. 3c).

The extracts from isolates WP27F, WZ27F, C27F, P27F and Y27F were subjected to determination of the minimum inhibitory concentrations (MICs) against the test microorganisms, the results of which are shown in Table 4. The MIC values ranged from 2.5 to 10 mg/mL.

Molecular identification of the selected Actinomycetota isolates

16S rRNA gene sequencing of the isolates whose extracts showed significant antimicrobial potential possessed percentage similarities of 99-100 to their

Table 3. Antimicrobial activities (inhibition zones, mm) of extracts from Actinomycetota isolates.

SN	Isolate Code	Inhibition zone (mm)				
		B. subtilis	*S. aureus*	*S. typhi*	*E. coli*	*C. albicans*
1	WP27F	11.7	11.0	14.7	18.3	20.7
2	WZ27F	12.0	7.00	7.00	7.70	10.0
3	WS27F	NA	NA	NA	NA	NA
4	C27F	13.0	8.00	13.0	11.0	12.7
5	CS27F	6.70	7.00	10.0	NA	NA
6	G27F	NA	NA	NA	NA	NA
7	B27F	10.0	NA	NA	NA	NA
8	P27F	11.3	16.0	16.0	13.0	16.0
9	Y27F	10.3	10.0	12.0	9.70	12.3
10	PY27F	NA	NA	NA	NA	NA

KEY: NA = Not Active

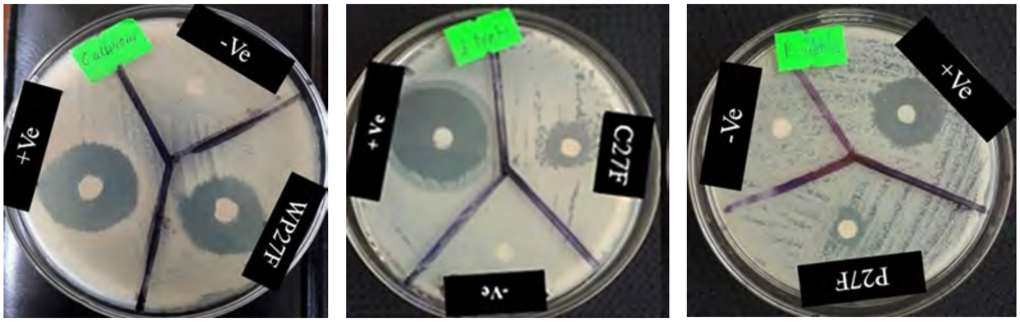

Figure 3. Representative plates showing inhibition zones, (a) WP27F extracts against *Candida albicans*, (b) C27F extracts against *Salmonella typhi*, (c) P27F extracts against *Bacillus subtilis*.

conspecifics in the genbank (Table 5). The phylogenetic reconstruction of partial 16S rRNA gene sequences of the five selected Actinomycetota strains with the related strains from the database are shown in Figure 4 with their accession number in brackets. Three of the five isolates belonged to genus *Streptomyces* (strains WP27F, ON954769, WZ27F, ON954770 and P27F, ON955761), one to genus *Micrococcus* (strain Y27F, ON955268) and the other one to genus *Hoyosella* (strain C27F, ON954771). Since they were 99-100 % similar to already known type strains (*S. chumphonensis* (100 %), *S. fradiae* (99.4 %), *M. luteus* (99.7 %) *H. altamirensis* (100 %)), and *S. tumenensis* (100 %), the identified isolates will here be referred as *S. chumphonensis* WP27F (ON954769), *S. fradiae* WZ27F (ON954770), *S. tumenensis* P27F (ON955761), *M. luteus* Y27F (ON955268) and *H. altamirensis* C27F (ON954771).

Discussion

Morphological and biochemical characteristics of the isolated Actinomycetota

Morphological characterization revealed that the majority of the isolates formed white colonies similar to the study conducted by Rahman (2008). Isolates with white colour and mycelia projections were highly suspected to be *Streptomyces* species as described by Khadayat *et al.* (2020), however, there are some studies that have reported on the *Streptomyces* species with other colours such as pink and grey (Mohamed *et al.,* 2017). The colony features of strain WP27F (ON954769) and P27F (ON955761) resembled those of *S. chumphonensis* (AB738400) and *Streptomyces* sp. (LC427864), respectively (Phongsopitanun *et al.,* 2014; Khadayat *et al.,* 2020). The morphological features of isolate WZ27F (ON954770) including the colony colour and the inability to produce pigments is similar to that of *S. fradiae* NEAE-82 (El-Naggar *et al.,* 2016).

The non-*Streptomyces* species displayed different colony colours; the literature suggests the strains with cream colour and smooth appearance to be of genus *Hoyosella* (Jurado *et al.,* 2009) and cream with rough texture to be of genus *Streptacidiphilus* (Cho *et al*. 2012). The morphological description of isolate C27F (ON954771) fits that of *Hoyosella altamirensis* (FJ179485) with the colony appearing cream, circular and smooth as reported by Jurado *et al.* (2009). Isolates Y27F (ON955268) and PY27F macroscopic features resembled those of *Micrococcus luteus* (Greenblatt *et al.,* 2004; Shahin *et al.,* 2022). The brown colonies with a rough texture belonged to genus *Rhodococcus* (Cho *et al.,* 2012) while those that were white with a smooth texture were from the genus *Nocardioides* (Siddharth and Rai, 2019).

These results showed that all *Streptomyces* species were able to ferment sugars agreeing with the study conducted by Charousová *et al.* (2017). The biochemical characteristics of strains WP27F (ON954769), P27F (ON955761) and WZ27F (ON954770) such as the ability to hydrolyze starch has been reported from *Streptomyces*

Table 4. MIC (in mg/mL) of the extracts from the selected Actinomycetota isolates.

Isolate	*B. subtilis*	*S. aureus*	*S. typhi*	*E. coli*	*C. albicans*
WP27F	10.0	10.0	5.0	2.5	2.5
WZ27F	10.0	NA	NA	NA	10.0
C27F	5.0	NA	5.0	10.0	10.0
P27F	NA	5.0	5.0	10.0	5.0
Y27F	NA	NA	10.0	NA	10.0

KEY: NA = Not Active

Table 5. BLASTn results for sequences of isolated Actinomycetota strains and their closest matches in GenBank.

Isolate (AN)	Size of isolate sequence (bp)	Closest match (AN)	Size of query sequence (bp)	% Sequence similarity	Source	Reference
WP27F (ON954769)	1196	*Streptomyces chumphonensis* (AB738400)	1475	100.00	Marine sediments, Thailand	(Phongsopitanun et al. 2014)
		Streptomyces chumphonensis (ON430586)	1470	99.92	Mangrove sediment, China	(Miao and Mo 2022)
WZ27F (ON954770)	1163	*Streptomyces fradiae* (KJ467538)	1508	99.40	Soil, Egypt	(El-Naggar et al. 2016)
		Streptomyces sp. (KC179807)	1553	99.22	Marine sediment, Korea	(Manivasagan el al. 2013)
C27F (ON954771)	1183	*Hoyosella altamirensis* (KX146464)	1483	100.00	Cave biofilm, Spain	(Jurado et al. 2009)
		Hoyosella rhizosphaerae (NR152654)	1483	98.06	Saline soil, China	(Li et al. 2016)
WP27F (ON955761)	1183	*Streptomyces tumenensis* (KC122242)	1395	100.00	Rhizosphere soil, India	(Rai and Singh 2012)
		Streptomyces tumenensis (AM180560)	1485	99.24	Soil, China	(Chen et al. 2005)
Y27F (ON955268)	1228	*Micrococcus luteus* (MG421013)	1520	100.00	biofilm of boat hull, India	(Balan et al. 2019)
		Micrococcus luteus strain (KU707915)	1498	100.00	Polluted water, India	(Nandi et al. 2019)

Key: % sequence similarity = percentage sequence, AN = accession number

chumphonensis (AB738400), *Streptomyces* sp. MARS-17 and *S. fradiae* (KJ467538) respectively, with both strains originating from the sediment samples (Rahman, 2008; Phongsopitanun *et al.*, 2014; Gopikrishnan *et al.*, 2016). *Streptomyces* species are well known for the production of amylase and catalase enzymes (Taha *et al.*, 2021). In the current study, all *Streptomyces* species were capable of producing amylase and catalase enzymes.

Biochemical analysis of species in genera *Hoyosella* reveal that these species are capable of fermenting sugars. Hamada *et al.* (2016) reported on the glucose and inositol utilizing *H. altamirensis* species. In the current study, isolate C27F was able to ferment glucose, sucrose and starch sugars. Moreover, it showed the ability to produce amylase and catalase enzymes similar to the observations made in other related studies (Hamada *et al.*, 2016; Lema *et al.*, 2022)

Genus *Streptacidiphilus* is among the rare genera of phylum Actinomycetota (Kim *et al.*, 2012). Some of species in this have been reported to degrade various forms of carbohydrates (Malik *et al.*, 2020). In this study, isolate WS27F was observed to ferment glucose, sucrose and starch sugars.

Nocardioides species have been reported to ferment arabinose, fructose, galactose, lactose, maltose, mannose and sucrose (Abdulla, 2009). Isolate CS27F showed the ability to ferment glucose and sucrose but tested negative for starch hydrolysis, similar to the study reported by Wang *et al.* (2016) on *Nocardioides*

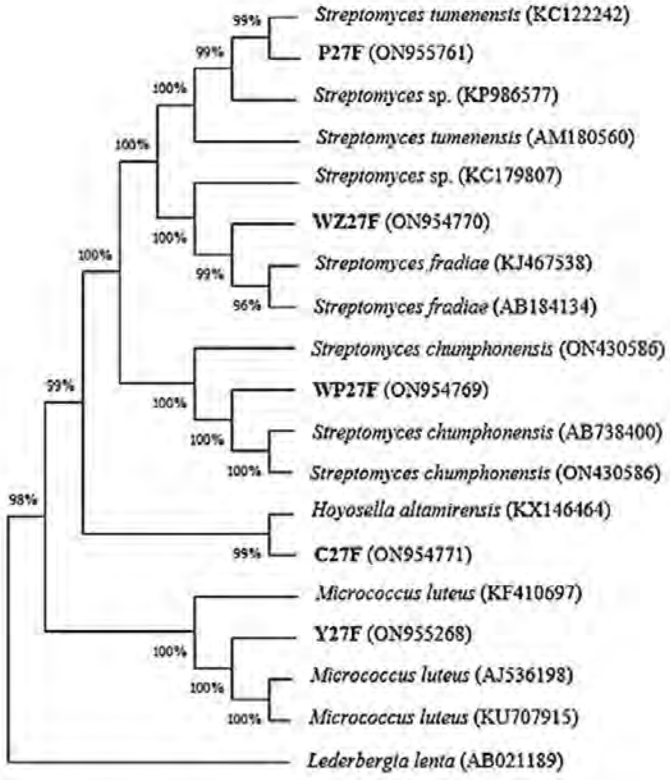

Figure 4. Phylogenetic affiliations of partial (≈1200 bp) 16S rRNA gene sequences of Actinomycetota isolates retrieved from Bagamoyo mangrove sediments and the strains of the most closely related genera. The inference tree was constructed through MEGAX using the Tamura-Nei as a substitution method, maximum likelihood as a statistical method, and nearest-neighbor interchange as a maximum likelihood heuristic method. Bootstrap values are expressed as percentages, based on 1 000 resampling of data. Bootstrap values, >50 % are shown at branch points. *Lederbergia lenta* (AB021189) was used as an outgroup to position the root of the tree.

rotundus species isolated from deep sea water. The ability of isolate C27F to produce catalase enzyme and amylase enzymes has also been observed in other *Nocardioides* species (Kubota *et al.,* 2005).

The studied *Rhodococcus* species (G27F and B27F) tested positive for fermenting all carbon sources agreeing with the study conducted by Fei *et al.* (2015). The isolates tested positive for the production of catalase enzyme as observed for strain *Rhodococcus fascians* (Gesheva *et al.,* 2010) but isolate B27F tested negative for the production of amylase enzyme. The inability of the strain B27F to produce amylase enzyme is in contrast with the observations made in the study conducted by Ghimire *et al.* (2021) on the endophytic *Rhodococcus* species. Such variations might be contributed to by inter-species differences.

Micrococcus species showed the ability to ferment the tested carbon sources with variations between the similar species. Isolate Y27F was able to utilize all carbon sources unlike isolate PY27F which was unable to utilize starch. Isolates Y27F and PY27F showed the ability to produce catalase enzyme similar to the *Micrococcus* species studied by Bannerman and Peacock (2006). For amylase production, isolate Y27F tested positive agreeing with the study reported by Fan *et al.* (2009) while isolate PY27F tested negative. These interspecies variations have also been reported in other *Micrococcus* species (Singh *et al.,* 2014). The catalase enzyme produced by *M. luteus* has been reported to significantly increase under stress (Ravikumar *et al.,* 2007).

The identified species from Bagamoyo mangroves have demonstrated their capability to ferment sugars and produce enzymes which can be applied in various fields including pharmaceutical, agricultural and biofuel industries, reminiscent of those observed in related studies (Gesheva *et al.,* 2010; Loux *et al.,* 2015).

Molecular identification of the selected Actinomycetota isolates

The phylogenic characteristics of the five selected strains based on 16S rRNA gene sequencing showed that the majority of isolates belonged to the genus *Streptomyces*. The dominance of *Streptomyces* species in soil including mangrove sediments has been reported in numerous studies (Ranjani *et al.,* 2016; Law *et al.,* 2019). Strain WP27F (ON954769) was 100 % related to *Streptomyces chumphonensis* (AB738400) isolated from marine sediments in Thailand (Phongsopitanun *et al.,* 2014). In Tanzania, a similar strain (2BI (MT192564)) to this species has been isolated from Momela Soda Lakes, Arusha (Sadikiel *et al.,* 2022). Isolate P27F (ON955761) showed a 100 % similarity with *S. tumenensis* (KC122242) isolated from rhizosphere and non-rhizosphere soil of cotton fields in India (Rai and Singh, 2012) and 99.24 % resemblance to *S. tumenensis* (AM180560) isolated from soil in China (Chen *et al.,* 2005). Little is known on the presence of this species in WIO regions.

Isolate Y27F (ON955268) showed a 100 % resemblance with *Micrococcus luteus* (MG421013) and *M. luteus* (KU707915) isolated from biofilm from a boat hull and polluted water, respectively, both in India (Balan *et al.,* 2019; Nandi *et al.,* 2019). Moreover, the strain from the current study showed a 100 % resemblance with *Micrococcus* sp. (MT328139) isolated from Momela Lakes in Arusha, Tanzania (Sadikiel *et al.,* 2022). A similar strain (*M. luteus* (MT249420)) to this species with 99.40 % resemblance has also been reported from Kenyan mangrove ecosystems (Muwawa *et al.,* 2020)

Isolate C27F (ON954771) showed a 100 % similarity with *Hoyosella altamirensis* (FJ179485) isolated from cave biofilm in Spain (Jurado *et al.,* 2009). Other studies have exposed the isolate of *Hoyosella* species from caves and lake environments (Jurado *et al.,* 2009; Lema *et al.,* 2022), while it's presence in mangrove ecosystems indicates another source of this species.

Antimicrobial activities of the extracts

Several authors have reported on the antimicrobial activities of the extracts from Actinomycetota species (Khadayat *et al.,* 2020; Cera *et al.,* 2022). In this study, extracts from five isolates belonging to genus *Streptomyces* (3), *Hoyosella* (1) and *Micrococcus* (1) showed the most bioactivity against all tested microorganisms, whereas, two isolates belonging to genus *Rhodococcus* and *Nocardioides* showed activity to at least one of the five test micro-organisms. On the other hand, three isolates of genus *Streptacidiphilus* (1), *Rhodococcus* (1) and *Micrococcus* (1) did not show any activity at the tested concentration.

Species in genera *Streptomyces* are well known for the production of bioactive extracts comprising of various classes of secondary metabolites including alkaloids, dilactones, flavonoids and diketopiperazines that have been reported to possess antimicrobial and antitumor properties (Martín and Liras, 2022). The production of secondary metabolites by *S. chumphonensis* is reported by Phongsopitanun *et al.* (2021). In this study, extracts from strain *S. chumphonensis* WP27F (ON954769) showed the most bioactivity against *C. albicans* (2.5 mg/mL) and the gram-negative bacteria, *E. coli* (2.5 mg/mL) and *S. typhi* (5.0 mg/mL). When the extracts were screened against gram-positive bacteria, *B. subtilis* and *S. aureus*, moderate activity with MIC of 10.0 mg/mL was recorded. This indicates that extracts from *S. chumphonensis* WP27F have high potential to act against fungus and the gram-negative bacteria which calls for further exploration. The extracts from the isolate *S. tumenensis* P27F (ON955761) showed moderate activity against *S. aureus* (5 mg/mL), *S. typhi* (5 mg/mL), *C. albicans* (5.0 mg/mL) and *E. coli* (10.0 mg/mL) but did not show any activity against *B. subtilis* at the concentration < 10 mg/mL. *Streptomyces* sp. (LC427864) that was 95.78 % related to the isolate of *S. tumenensis* P27F (ON955761) from the present study was reported to have moderate antimicrobial activity against *S. aureus*, *E. coli*, and *S. typhi* (Khadayat *et al.,* 2020). The inhibition zone of the extracts from *S. tumenensis* P27F (ON955761) against *E. coli* was 13.0 mm which is comparable to that of *Streptomyces* sp. (LC427864) (10 – 13 mm) against 12 strains of *E. coli* (Khadayat *et al.,* 2020). Extracts from the isolate from *S. fradiae* WZ27F (ON954770) showed antimicrobial activity against pathogenic microbes *B. subtilis*, *S. aureus*, *S. typhi*, *E. coli* and *C. albicans* with inhibition zones between 7.0 to 12.0 mm at the concentration of 300 mg/mL. The MIC of *S. fradiae* WZ27F extracts was 10 mg/mL against *B. subtilis* and *C. albicans* and > 10.0 mg/mL against *S. aureus*, *S. typhi* and *E. coli*. The observed antibacterial activity of the extracts against gram-positive and gram-negative bacteria has also been reported from the extracts of *Streptomyces* sp. GB-2 that showed 97.64 % similarity with *S. fradiae* WZ27F (ON954770), which is reported to exhibit microbial activity against *Bacillus cereus* and *E. coli* with inhibition zones of 29 and 30 mm, respectively (Lu *et al.,* 2009). In Tanzania, the secondary metabolites isolated from *S. fradiae* in 1986 were found to be active against gram-positive bacteria including *B. subtilis* and *B. brevis* as well as displaying anticancer properties against stem cells of murine L1210 leukemia (Drautz *et al.,* 1986).

Hoyosella species also produced extracts with significant antimicrobial activities against both bacteria and fungi. The extracts from *H. altamirensis* C27F (ON954771) showed moderate antimicrobial activities against the gram-positive bacteria, *B. subtilis* with MIC of 5.0 mg/mL, gram-negative bacteria *S. typhi* and *E. coli* with MIC values of 5.0 mg/mL and 10.0 mg/mL, respectively, as well as against the fungi, and *C. albicans* with MIC of 10.0 mg/mL. The MIC of *H. altamirensis* C27F extracts against *S. aureus* was observed to be above 10 mg/mL. Little is known on the bioactivity of the extracts from *Hoyosella* species, however, Lema *et al.* (2022) reported on the bioactivity of this species with comparable observations against *E. coli* and *S. aureus*.

Extracts from *M. luteus* Y27F (ON955268) showed moderate antimicrobial activities against all tested microorganisms unlike those from isolate PY27F which did not show any activity at the tested concentration. The MIC of Y27F (ON955268) extracts against *S. typhi* and *C. albicans* was 10.0 mg/mL whereas against *B. subtilis*, *S. aureus* and *E. coli* was observed to be above 10.0 mg/mL. The bioactivities of *M. luteus* Y27F (ON955268) against various pathogens including *C. albicans* has been previously reported (Shahin *et al.,* 2022). Moreover, the pigments produced by *M. luteus* isolated from the marine environment in India have been reported to possess antimicrobial activities against *P. aeruginosa, K. pneumoniae, E. coli* and *Aspergillus niger* with inhibition zones of 12, 9, 14 and 17 mm, respectively (Balan *et al.,* 2019).

In the current study, the majority of the non-*Streptomyces* species showed weak or no bioactivity against the pathogenic microbes, however, there are some studies reporting on the antimicrobial activities of such other species belonging to genera *Nocardioides* (Siddharth and Rai, 2019), *Streptacidiphilus* (Yu *et al.,* 2021) and *Rhodococcus* (Zampolli *et al.,* 2022). Overall results of antimicrobial screening in this study revealed that the extracts from the isolates belonging to genera *Streptomyces, Hoyosella* and *Micrococcus* exhibited activity against the test pathogenic microbes.

Conclusions

This study aimed at isolating and identifying Actinomycetota species inhabiting the sediments of Bagamoyo mangroves in Tanzania in the WIO region with the ultimate goal of unravelling their enzymatic and antimicrobial potentials. Ten strains of Actinomycetota were isolated and identified to belong to six genera, namely *Streptomyces*, *Nocardioides*, *Hoyosella*, *Streptacidiphilus*, *Rhodococcus*, and *Micrococcus*. The identified species demonstrated their capability to produce enzymes which can be applied in various fields including pharmaceutical, agricultural, biofuel and biotechnological industries. Crude extracts from the selected five strains that were finally molecularly identified as *Streptomyces chumphonensis* WP27F (ON954769), *Streptomyces fradiae* WZ27F (ON954770), *Streptomyces tumenensis* P27F (ON955761), *Micrococcus luteus* Y27F (ON955268) and *Hoyosella altamirensis* C27F (ON954771) portrayed biomedical potency against pathogenic microbes. The study marks the first report on the identification of *Streptomyces tumenensis* species in Africa and the first work on the isolation of *Hoyosella* species from the WIO region. The findings warrant further explorations of the secondary metabolites from such ecosystem as source of biomedical and other industrially important agents.

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Original Article

Population genetic status of endangered whitespotted whipray *Maculabatis gerrardi* (Gray, 1851) in Tanzania

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Abstract

The whitespotted whipray *Maculabatis gerrardi* is exploited in Tanzania for its meat, skin and cartilage, and is classified as an endangered species by the IUCN. A mitochondrial COI gene fragment from 105 *M. gerrardi* individuals obtained from four unprotected and one protected marine area in Tanzania was used to determine the present genetic diversity, demographics, and effective population size of whiprays. Lower levels of nucleotide and haplotype diversity and mean mutational effective population size were apparent in unprotected than in protected areas. Analysis of molecular variance (AMOVA) identified a significant genetic difference between subpopulations of *M. gerrardi* and hierarchical AMOVA identified separate genetic stocks, indicative of high levels of philopatry or individual sedentarity in *M. gerrardi*. The importance of marine protected areas to conserve genetic diversity of whiprays is highlighted.

Keywords: genetic diversity, effective population size, genetic monitoring, elasmobranch, gene flow

Introduction

Rays are economically important to the people living along the coastline in terms of food security, nutrition, source of income and employment opportunities (Barrowclift *et al.*, 2017). Unfortunately, their numbers are dwindling and they are more likely to be overfished due to their overall body shape and benthic habits, which make them vulnerable to capture in net and trawl operations (Schluessel *et al.*, 2010). Rays of various sizes from Tanzania's coastline are typically caught for dried filets and liver oils, and their stomachs are cleaned, boiled, and turned into soup (Dulvy *et al.*, 2014). Elasmobranchs generally display slow growth, late maturity and low fecundity which causes a low rate of increase of the species (Compagno *et al.*, 1990; Dulvy *et al.*, 2014). Rays have been receiving less attention when compared to other elasmobranches (sharks); this makes this group more threatened and currently their extinction risk is substantially higher than most other vertebrates with only one-third of them being considered safe (Jorgensen *et al.*, 2022).

Maculabatis gerrardi formerly known as *Himantura gerrardi*, dwells in shallow waters and feeds on a diverse range of species, most notably crustaceans, followed by polychaetes and teleosts (Rastgoo *et al.*, 2018). They are ovoviparous, bearing live young developed from eggs retained within the mother's body, implying that dispersal occurs through juvenile or adult migration (Borsa *et al.*, 2012). The biological productivity of sharpnose stingray, *M. gerrardi* is constrained. Nonetheless, the meat is regarded as being of high quality, their skins are used to make leather and it is consumed locally and traded globally. The International Union for Conservation of Nature (IUCN) categorizes *M. gerrardi*s pecies under the endangered red list due to overfishing through bycatch in trawl, gillnet, and longline fisheries. The *M. gerrardi* population is believed to have decreased by 50–79% over the last 75 years as a result of overexploitation (Sherman, 2020).

There have been a number of initiatives to conserve and manage marine species, including the creation of marine protected areas (MPAs). In order to safeguard stocks of marine species, particularly fish and their habitats, the Marine Parks and Reserves Unit in Tanzania was established under the Marine Parks and Reserves (MPRU) Act 29 of 1994. The MPAs serve as a refuge for fish communities, increasing their diversity and density. Extractive resource usage is permitted within the park but is more restricted than in the nearby areas where fishing is typically practiced (Kamukuru *et al.*, 2004). However, different marine species are still threatened by various factors that include habitat degradation, overfishing, bycatch and climate change (Trathan *et al.*, 2015).

Fisheries experts should be able to use techniques that can help to precisely identify fish populations or units in order to manage and conserve marine species. Also, they must be capable of monitoring and reporting on migration trends and effective population sizes. By doing this, they can protect important areas and influence action plans. For example, the data can be applied for creation of fishing quotas and spatiotemporal closures. In order to monitor and evaluate the stock structure and improve the protection and conservation of diverse marine species, several researchers have been employing various approaches. Nevertheless, the majority of marine monitoring and evaluation is based on conventional methods that are time consuming and provide only a partial picture of the ecological situation. The advancement of genetic technologies has the potential to fundamentally alter how marine life is conserved and protected. For instance, determining effective population sizes, introgression across species, and gene flow among populations all benefit from the knowledge of genetic diversity at neutral loci (Van Oppen and Coleman, 2022). The design of MPA networks may be improved by using data on neutral and adaptive genetic diversity in recovery plans for threatened species. Genetic and genomic approaches can be employed in the context of conservation and restoration to direct and improve conventional conservation measures as well as to design more modern aided evolution techniques (Van Oppen *et al.*, 2015). The use of genetic information has facilitated the detection of genetically diverse groups, the measuring of genetic connectivity and the identification of risks related to inbreeding and demographic change (Ekblom and Wolf, 2014). Unfortunately, genetic data has not been extensively used in management plans for a number of marine species, including rays. Understanding genetic connectivity and genetic diversity are important for effective conservation and management of rays (O'Dwyer *et al.*, 2021). There are very few genetic studies available for the Western Indian Ocean (WIO) coastline, making it impossible to draw broad generalizations regarding the patterns of connection of marine fish species (Muths *et al.*, 2012). The consequences of the decline in the populations of marine fish species on genetic diversity have drawn increasing attention (Domingues *et al.*, 2018). There is an urgent need for more population genetic studies to report on ray species since only few studies describe the genetic diversity of rays (Domingues *et al.*, 2018). Currently, only 10 % of ray species have been investigated in terms of their population genetic structure, genetic diversity and demographic history (Marion *et al.*, 2014). Despite the considerable dispersal capacity of some marine animals with large body sizes, recent research has demonstrated that elasmobranch species in the Indo-Pacific have high levels of genetic differentiation (Arlyza *et al.*, 2013). This can be a sign that certain elasmobranch species need particular management strategies. The objective of this study is to employ molecular genetic methods to support the management and conservation of *M. gerrardi* through population monitoring.

Materials and methods

Sampling

Maculabatis gerrardi samples were collected from five different locations along Tanzania's coastline. The five locations were the Deep Sea-Tanga, Kivukoni-Dar es Salaam, Bweju-Mafia, Bandarini-Mtwara, and Malindi-Unguja (Fig. 1). Bweju-Mafia was selected as the control location since it is an MPA. A sample of *M. gerrardi* fin tissue was cut and immediately stored in 99.9% ethanol.

From its border with Kenya (4°49'S) to its border with Mozambique (10°28'S), Tanzania's coastline is more than 1424 km long (URT 2015). The East African Coast Current (EACC), which always moves in a northerly direction, is the main ocean current in this area. The other ocean currents that influence the gene flow of marine species in the area are the eastward-movingSouth Equatorial Countercurrent (SECC) and the westward-moving South Equatorial Current (SEC) (Fig. 1).

DNA extraction

DNA was extracted from about 25mg of tissue. Total DNA was recovered for mtDNA analysis following the instructions of the tissue DNA extraction kits (ZYMO Research Inc, California, USA). Extracted DNA was kept in the freezer for further investigation. The quality of the DNA extracts was evaluated using agarose gel electrophoresis.

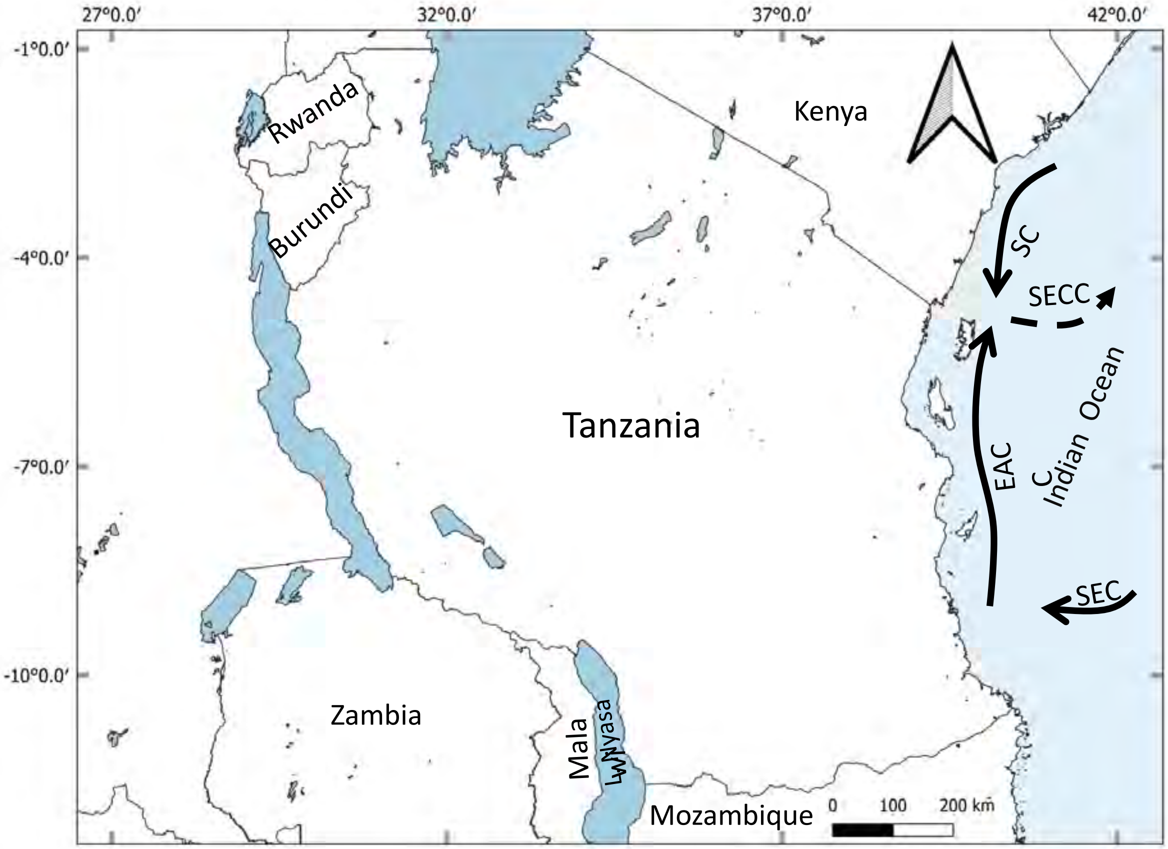

Figure 1. Sampling area of *Maculabatis gerrardi* along the Tanzania coastline (White circles indicate sampling sites). Black arrows signify the Somali Current (SC), East African Coast Current (EACC), South Equatorial Countercurrent (SECC) and South Equatorial Current (SEC).

Amplification of mtDNA and sequencing

A fragment of the COI gene was amplified using the primers FishF1: 5'-TCAACCAACCACAAAGATTGGCAC-3' and FishR1: 5'-TAGACTTCTGGGTGGCCAAATCA-3' (Ward *et al.*, 2005). A BIORAD T100 thermocycler was used for polymerase chain reaction (PCR). The reaction was carried out in a 35 µl volume with 11.7 µl RNase-free water, 17.5 µl Mastermix, 1.75 µl BSA, 0.3µM of each primer, and 2 µl DNA templates. Initial denaturation was set at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 54 °C for 45 seconds, extension at 72 °C for 1 minute, and final extension at 72 °C for 15 minutes. PCR products were electrophoresed using 0.8g agarose gels. Using an ABI 3770XL automated sequencer (Applied Biosystems, Foster City, USA), 105 samples were successfully sequenced using the primer FishF1 and FishR1.

Mitochondrial DNA (mtDNA) analysis

The sequences were initially edited using the Chromaspro v. 1.5 (Technelysium) programme, and then the species identity was verified by comparing the edited sequences to the sequences present in GenBank using the BLAST programme. Squint Alignment Editor v. 1.02 was used to determine the frequency of stop codons, which indicate sequencing mistakes or pseudogenes (Goode and Rodrigo, 2007). CLUSTAL W implemented in the software MEGA 6 was used to do multiple alignments of the sequences (Thompson *et al.*, 1994; Tamura *et al.*, 2013). The sequences were collapsed into haplotypes using the online FaBox 1.41 Collapse programme. The statistical parsimony approach, as implemented in the software PopArt, was used to investigate the genetic relationship between the samples from the five landing sites (Leigh and Bryant, 2015). Arlequin v.3.5.2.2 was used to calculate nucleotide and haplotype diversity (Excoffier and Lischer, 2010). The same programme was used to determine the historical demographics as well as the neutrality indicators, Tajima's D test, and Fu's Fs (Tajima, 1989; Rogers and Harpending, 1992, Rogers, 1995; Fu, 1997). Fu's Fs is sensitive in detecting demographic expansion and genetic hitchhiking, whereas Tajima's D test is effective in detecting selective sweeps (Fu, 1997). Analysis of molecular variance was used to investigate the differences between populations (AMOVA) (Excoffier *et al.*, 1992). Pairwise Fst values were calculated among sample sites using the same method, and Bonferroni correction was applied to the significance P-values sequentially. The spatial analysis of molecular variance (SAMOVA 2.0) strategy was used to identify populations that are geographically homogeneous and maximum dissimilar from one another (Dupanloup *et al.*, 2002). K values ranged from 2 to 3, with the initial condition set to 400. The configuration with the highest variance among clusters (Fct) was deemed to be the best population grouping.

In order to determine whether population genetic structure was present in the dataset, hierarchical AMOVA was carried out using the findings of SAMOVA, pairwise Fst-values and haplotype network. The mtDNA sequence data were estimated using the MIGRATE v.3.116 programme. The programme was used to estimate the boundaries Θ (2*Nefμ*) where *Nef* stands for effective population size of females and μ for the mutation rate per generation per locus (Bradic *et al.*, 2012). The Bayesian search strategy and Brownian mutation model was applied. The Metropolis sampling algorithm was used to sample from the prior distributions and generate posterior distributions. The exponential prior distribution was used to estimate Θ (range = 0–50) and M (range = 0–400). The initial runs were carried out in three replicates, each of which included a single lengthy chain with 50,000 recorded steps and 50 increments. Burn-in was 100,000 and the sampling parameter value was 2,500,000. The final runs were a single, lengthy chain of 50,000 recorded steps, 50 increments, four replicates, 2,500,000 sampled parameter values, and a burn-in value of 100,000. A static heating scheme with four chains and temperatures set at 10, 4, 2, and 1 was used.

Results

Haplotype and nucleotide diversities

The overall nucleotide and haplotype diversity values for populations were 0.00301 ± 0.0019 and 0.7714 ± 0.0403 respectively. High value nucleotide diversity 0.0045 ± 0.0028 as well as high haplotype diversity 0.889 ± 0.0640 were recorded in Mafia while the lowest nucleotide diversity 0.0009 ± 0.0009 and haplotype diversity 0.2842 ± 0.1284 were recorded in Unguja (Fig. 2). The alignment of 105 sequences with 628 bp length was obtained. The sequences were deposited at the GenBank (accession numbers OQ455827 - OQ455931). A total number of 27 haplotypes were identified from COI sequences. Among the 27 haplotypes detected, 16.19% were haplotypes seen only once in a group of samples. The dominant

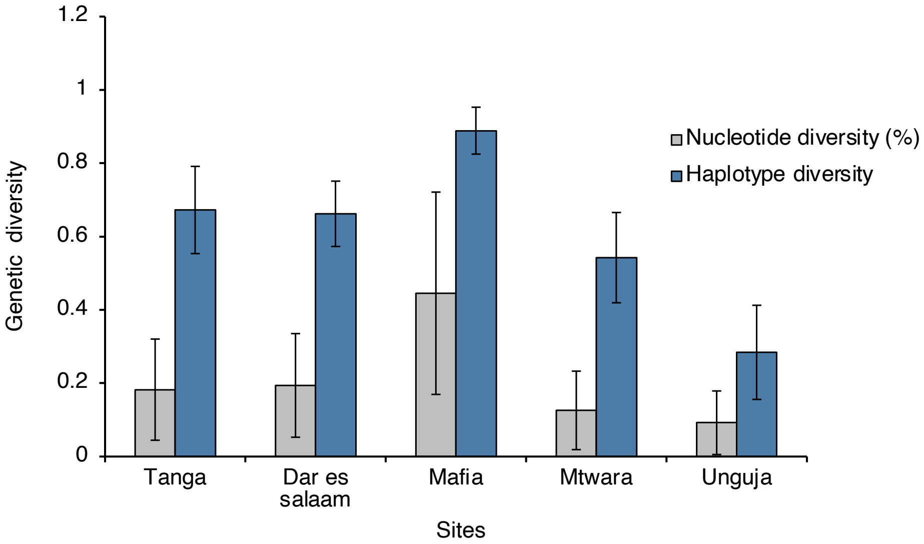

Figure 2. Nucleotide and haplotype diversities of *Maculabatis gerrardi* along the coastline of Tanzania in the Western Indian Ocean.

Table 1. Number of haplotype distributions for each sampled landing site. Abbreviations: N = number of individuals; Nh = number of haplotypes.

Sites	Code	N	Haplotype distribution																											Nh
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Tanga	Ta	19	11			2			1	1														1	1	1	1			8
Dar es salaam	Da	30	17	1	1	5	2	1	1	1	1																			9
Mafia	Ma	18										1	6	2	1	1	1	2	1	1	1	1								11
Mtwara	Mt	18	3			1	2																12							4
Unguja	UN	20	17																						1			1	1	4
	Total	105	48	1	1	8	4	1	2	2	1	1	6	2	1	1	1	2	1	1	1	1	12	1	2	1	1	1	1	27

haplotype (h1) accounted for 46% of all individuals and was present in four populations out of five, except the Mafia population, whereas 17 individuals were from Dar, 17 from Unguja, 11 from Tanga and 3 from Mtwara. The second most frequent haplotype (h21) had 12 individuals all from Mtwara. The third frequently occurring haplotype (h4) had 8 individuals of which 5 were from Dar, 2 from Tanga and 1 from Mtwara. The fourth most frequently occurring haplotype (h11) had 6 individuals all from Mafia (Table. 1).

Population genetic structure

The genetic differentiation across all groups was significant (Fst = 0.248, P < 0.001). All pairwise AMOVA that involved samples from Mafia and Mtwara demonstrated significant genetic differentiation after sequential Bonferroni correction (Table 2). The haplotypes seen only once in a group of samples occurred in high frequencies with a starlike network (Fig. 3).

Three groups of genetic subpopulation clustering were identified by SAMOVA. The three best groups

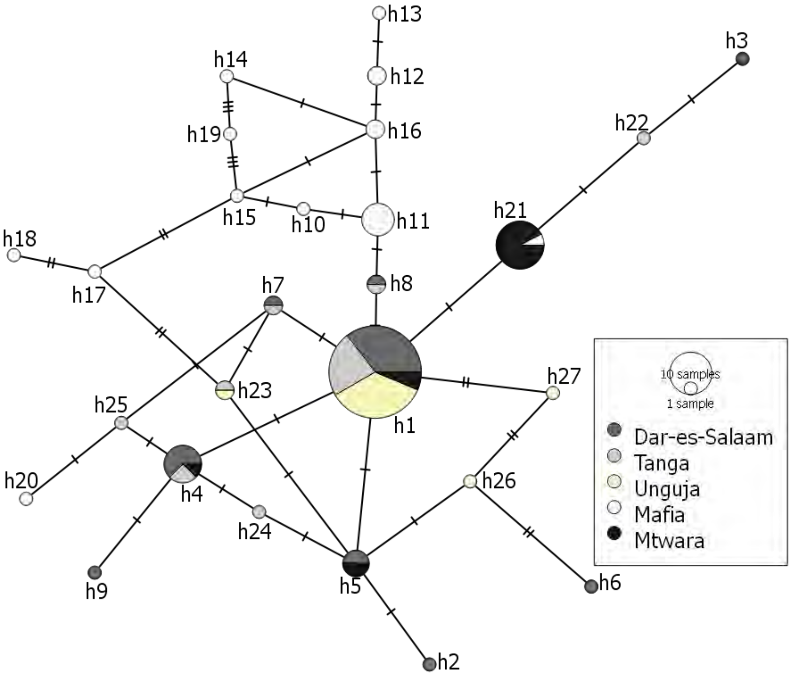

Figure 3. Minimum spinning network indicating haplotype distribution of *Maculabatis gerrardi* based on cytochrome oxidase subunit I sequences. The central haplotype network indicates percentage of haplotypes from all sampled subpopulations except Mafia. The central haplotype represents 48 individuals. The size of the other circles corresponds to the number of individuals as indicated at the bottom right side of the haplotype network.

Table 2. Pairwise F_{st}-values of *Maculabatis gerrardi* subpopulations from the Tanzanian coastline based on cytochrome oxidase subunit I sequences (for site codes see Table 1).

CODES	TA	DAR	MA	MT	UN
TA	0.00000				
DAR	-0.0251	0.00000			
MA	0.2202*	0.2334*	0.00000		
MT	0.3224*	0.3159*	0.2843*	0.00000	
UN	0.0548	0.0757	0.4215*	0.5232*	0.00000

Statistically significant differences of adjusted P-values after sequential Bonferroni (p < .001) are indicated by *

of subpopulations found by SAMOVA are the Mafia subpopulation, the Mtwara subpopulation, and the Tanga, Dar es Salaam, and Unguja subpopulations (Fct = 0.494). The Hierarchical AMOVA revealed that the Mafia subpopulation was genetically different from Tanga, Dar es Salaam, Mtwara and Unguja populations (Fct = 0. 0.241, p < 0.001). The Mtwara subpopulation was also genetically different from Tanga, Dar es Salaam and Unguja subpopulations (Fct = 0.211, P < 0.05).

The average mean mutation-scaled effective population size recorded was 0.027 ± 0.0027. The estimate of effective population size indicated the highest mean mutation-scaled effective population size for samples collected at Mafia (0.0091) and the lowest for the samples collected from Unguja Island (0.0027) (Table 3).

Mismatch distribution and demographic history

All populations of *Maculabatis gerrardi* showed negative Fu's Fs-values, with samples from Tanga, Dar es Salaam and Mafia being significantly different from zero while Mtwara and Unguja had non-significant values. All populations from five landing sites showed non-significant negative Tajima's D-values, with the exception of samples from Dar es Salaam which had significant negative D-values. The population of Tanga and Dar es Salaam had non-significant values for the SSD and Raggedness index analysis while the population of Mafia, Mtwara and Unguja showed significant values for SSD and Raggedness index analysis (Table 4). Mismatch distribution analysis displayed a unimodal distribution with a poorly pronounced nature of the curve (Fig. 4).

Discussion

Haplotype and nucleotide diversities

The present study's analysis of COI sequences reveals that *M. gerrardi* along the Tanzanian coastline has low to moderate nucleotide and high haplotype diversities as well as the occurrence of an excess of private haplotypes. In comparison to the results of the current study, a recent investigation on the queen mackerel revealed very similar nucleotide diversity but reduced haplotype diversity (Rumisha *et al.*, 2023). The majority of the mitochondrial COI gene investigations that have been carried outin the WIO to date have revealed low to moderate genetic and haplotype diversities (Nehemia and Kochzius, 2017; Nehemia *et al.*, 2017). The high haplotype diversity and low nucleotide diversity is an indication of a genetic bottleneck caused by stochastic extinction of the majority of haplotypes and population growth (Alves *et al.* 2001). So, the results of this study may suggest that the *M. gerrardi* population has experienced a bottleneck that has led to stochastic extinction of most haplotypes, followed by population expansion. The genetic diversity found at Mafia was high compared to that obtained at other sites. In comparison to other locations, Mafia sites also had a large number of haplotypes and higher

Table 3. Estimated effective population size using the programme MIGRATE (mean, 2.5% and 97.5 % confidence interval) in *Maculabatis gerrardi* from the Tanzanian coast on the Western Indian Ocean. Θ: mutation-scaled effective population size (for site codes see Table 1).

	Θ		
Codes	mean	2.5%	97.5%
TA	0.0062	0.0093	0.0127
DA	0.0064	0.0021	0.0178
MA	0.0091	0.0021	0.0178
MT	0.0027	0.0000	0.0065
UN	0.0029	0.0000	0.0065

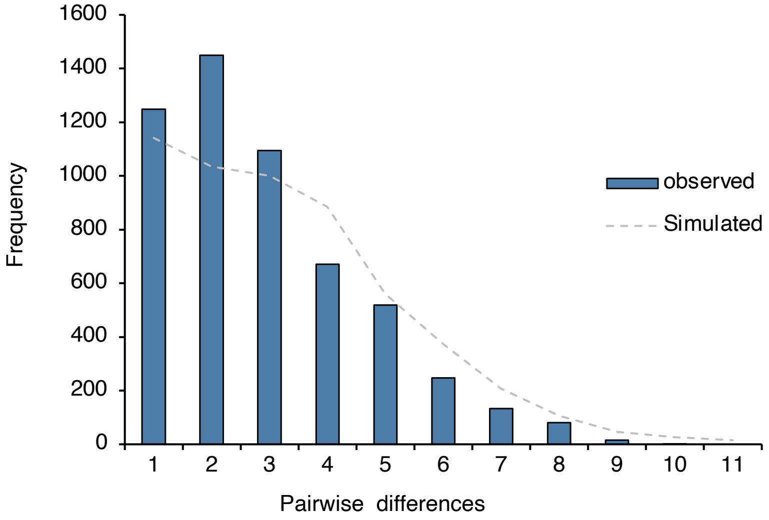

Figure 4. The observed (bars) and expected (dotted line) mismatch distributions of COI sequences for *Maculabatis gerrardi* under the sudden expansion model.

effective population size. Mafia Island's higher genetic diversity may be related to the larger effective population size that has been found for this subgroup. A positive correlation between effective population size and genetic diversity has been observed (Tringali and Bert, 1998; Rieman and Allendorf, 2001). However, there are other aspects, like biological, ecological, and evolutionary history traits of the species, which should not be disregarded, that are also connected to genetic diversity (Petit-Marty *et al.*, 2022).

The mafia sample collection site is located in a MPA with fewer human activities. Mafia Island's high genetic diversity might be a sign that a MPA could be useful for managing and conserving marine resources. A gear restriction and fishing exclusion zone management system is used to regulate the fishery in the Mafia MPA. Because of restrictions on the minimum mesh size for nets and the minimum size of the individual fish collected, this management strategy tends to reduce the fisherman's catch. Fish that can be fished must be mature and have spawned at least once. It might also be a sign that the Mafia subpopulation is more resilient to environmental changes than other subpopulations and is therefore more stable. Genetic diversity increases species' ability to adapt to environmental changes and increases population resilience (Faulks *et al.*, 2011). The findings might also point to a limitation of Mafia subpopulation interactions with other subpopulations along Tanzania's coastline.

Population genetic structure

The Analysis of Molecular Variance (AMOVA) indicates significant genetic differences among the subpopulations of *M. gerrardi* studied. SAMOVA and hierarchical AMOVA supported existence of genetic groupings of populations along the Tanzanian coast. These findings are also supported by pairwise AMOVA and the haplotype network. The research conducted in the Coral Triangle region revealed the presence of population genetic groups in stingrays, *N. kuhlii* species, which are distributed in a parapatric manner as a result of sedentarity or philopatry (Arlyza *et al.*, 2013). The population genetic groupings revealed in

Table 4. Neutrality tests and mismatch distribution for cytochrome oxidase subunit I sequences in *Maculabatis gerrardi* from the Tanzanian coastline (for site codes see Table 1).

Codes	Fu's Fs	p	Tajima's D	p	SSD	p	Raggedness index	p
TA	-4.562	0.000	-1.076	0.165	0.011	0.400	0.064	0.550
DA	-4.353	0.005	-1.806	0.017	0.005	0.400	0.060	0.490
MA	-4.827	0.004	-0.136	0.500	0.000	0.000	0.000	0.000
MT	-0.650	0.275	-0.257	0.437	0.000	0.000	0.000	0.000
UN	-1.237	0.069	-1.408	0.066	0.159	0.000	0.668	0.000

the present study may be due to the sedentarity or philopatry of *M. gerrardi*, as the stingray species share some behaviors. The influence of ocean currents and eddies and isolation by distance (IBD) can help to describe the genetic structuring of marine species (White *et al.*, 2010; Nehemia *et al.*, 2019). Most research carried out along Tanzania's coast demonstrates that there is significant gene flow among the subpopulations of most marine species (Nehemia *et al.*, 2019; Bugota and Rumisha, 2023; Rumisha *et al.*, 2023). But most of these studies concentrated on species that have a pelagic larval phase. Fish species whose eggs develop in females until they reach the juvenile stage have less potential for gene flow, whereas those with a pelagic larval phase have greater potential (Mitton *et al*., 1989). The genetic connectivity observed in *M. gerrardi* between the subpopulations of Dar es Salaam, Tanga, and Unguja may be attributed to the East African Coastal Current (EACC), which runs northward between the coasts of Tanzania and Kenya. It is possible that this current is distributing juveniles of this species among these subpopulations (Fig. 1). The limited interaction between the Mafia subpopulation and other subpopulations may indicate that the Mafia MPA is unlikely to sustain the species' existing mtDNA genetic diversity or act as an effective source population (Schwanck *et al*., 2023). MPAs are predicted to increase genetic diversity, but to be effective, gene flow between protected and non-protected areas is required (Allendorf *et al.*, 2008).

Mismatch distribution and demographic history

All samples from five landing sites of Tanga, Dar es Salaam, Mafia, Mtwara and Unguja showed negative Fu's Fs-values, with samples from Tanga, Dar es Salaam and Mafia being significantly different from zero. All the five landing sites show populations having non-significant but negative Tajima's D values, perhaps supporting the hypothesis of neutrality of the COI marker. In addition to that, all sampled populations have significant negative Fu's Fs values, suggesting rapid population expansion. According to Fu (1997) Fu's Fs has been regarded as more powerful than Tajima's D in signifying traces of past population expansion. The presence of a star-like topology in the haplotype network, which may also reflect population bottlenecks and the recolonization of new habitats after the sea level rose, supports the idea of population growth as does the unimodal distribution for mismatch analyses observed. Similar observations have been made for a wide range of species in the region (Kochzius and Nuryanto, 2008; Nehemia *et al.*, 2017; Rumisha *et al.*, 2023).

Conclusion

The results of this study show that the endangered species *M .gerrardi* has low to high nucleotide and haplotype diversity, and a high effective population size for samples from MPAs. However, three genetic group subpopulations of *M. gerrardi* were discovered on the Tanzanian coastline, which may indicate that there is less chance of gene flow in fish species whose eggs grow in females until they reach the juvenile stage. Despite being an endangered species, the high genetic diversity and large effective population size at Mafia Island may be indicative of the effectiveness of the MPA in protecting, conserving, and managing marine species. It is recommended that countries take initiatives to curb destructive fishing and expand MPAs. The results of this study will broaden the body of knowledge that may be used to make marine management decisions in the WIO by increasing the information that is now available in the area. Future research should take into account the use of nuclear loci since the maternal genetic mtDNA markers have been criticized for only reflecting one locus and reflecting only the maternal population. In order to improve marine species conservation, future research studies should focus on additional aspects that influence the genetic diversity and gene flow of marine species, such as environmental factors, selection efficiency, and mutation rate.

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Short communication

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The occurrence of ambergris in sperm whales caught by Soviet whaling flotillas

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Abstract

Ambergris is a solid waxy secretion of the digestive system of sperm whales, historically used in the perfume industry. The original documentation of 29 cruises made by the scientific groups of the Soviet whaling flotillas “Slava”, “Soviet Ukraine” and “Yuri Dolgoruky”, between 1961 and 1978 were analyzed for the presence of ambergris. In total, 59 814 sperm whales (*Physeter macrocephalus*) were examined, with ambergris found in 240 specimens (0.4 %), of which 159 were males (0.44 %, n = 35 994) and 81 females (0.34 %, n = 23 820). The average weight of ambergris was 18.3 kg (17.9 kg in males and 19 kg in females). Two isolated sperm whale herds, south of Madagascar and south of New Zealand and Tasmania, contained the largest ambergris secretions.

Keywords: Ambergris, sperm whales, *Physeter macrocephalus*, distribution, whale database, Soviet whaling

Introduction

Ambergris (lat. *Ambrosiaca grisea*) is a specific waxy substance, which is formed in the intestines of, mostly, sperm whales. The nature and history of ambergris has been discussed in many books and papers (e.g., Schafer, 1981; Tomilin, 1937, 1957, 1962; Clarke, 1954, 2006).

The purpose of this short paper is to present unique original data collected by biologists during whaling of 3 Soviet flotilias. The data are not complete, with data from only three flotilias used. Even for these some information (e.g., ambergris weight) was not always present. It is therefore only possible to produce a preliminary analysis and formulate some hypotheses, which should be confirmed or refuted by further studies, when more data is available.

Materials and methods

“Whale inspection logs”, “Whale passports”, “Haul reports” by biologists of scientific groups of whaling flotillas “Slava” (5 cruises), “Soviet Ukraine” (15 cruises) and “Yuri Dolgoruky” (9 cruises), from the period from 1961 to 1978, were processed. Unfortunately it was not possible to find data about ambergris for other Soviet flotilias, e.g. “Aleut”, “Vladivostok”, “Far East” and “Soviet Russa”. Also, even for examined whales some ambergris could be undetected and discarded, because the search for ambergris was not required, so the occurrences below provide only the lower margin.

Table 1 presents all occurrences with whale sex, length and coordinates, and ambergris weight, if it was weighted.

Maps of the distribution of caught whales were compiled, and weights of ambergris were analyzed when available.

Results

In total, 59,814 sperm whales were examined during this period, including 35,994 males and 23,820 females. Ambergris was found in 240 whales, which is 0.4 % of the total number of sperm whales caught. Ambergris was found in 159 males (0.44 %), and 81 females (0.34 %).

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Table 1. Ambergris occurrence.
Fleet codes: SL – Slava, SU – Soviet Ukraine, UD – Yuri Dolgoruky.
Negative latitudes mean south, positive – north. Negative longitudes mean west, positive – east. Latitudes and longitudes are in degrees.

Fleet	Date	Latitude	Longitude	Sex	Length, m	Ambergris Weight, kg
SL	12/25/1961	-54	-61	M	15.8	
SL	12/25/1961	-54	-62	M	14.2	
SL	2/5/1963	-64.8833	149.7667	M	13.5	21.3
SL	2/6/1964	-61.4	83.88333	M	15.4	3
SL	2/18/1964	-63.6	152.7	M	15.1	1.5
SL	2/19/1964	-64.0833	157.1	M	15.8	
SL	3/5/1964	-48.1167	170.2	M	15	12
SL	3/13/1964	-43.4167	-178.717	M	14.2	
SL	3/17/1964	-43.1833	172.9667	M	15.7	
SL	3/18/1964	-43.1833	172.9667	M	13.5	
SL	3/18/1964	-43.1833	172.9667	M	13.5	
SL	3/22/1964	-42.5667	169.8167	M	14.8	7
SL	3/23/1964	-43.8667	168.3667	M	16.4	
SL	4/18/1964	-43.8	147.3333	M	12.3	2.5
SL	4/19/1964	-44.2833	146.2833	M	11.7	
SL	4/19/1964	-44.2833	146.2833	M	11.6	
SL	4/20/1964	-42.8833	145.0167	M	11.6	20
SL	4/20/1964	-42.8833	145.0167	M	13	20
SL	4/20/1964	-42.8833	145.0167	M	16.1	30
SL	4/20/1964	-42.8833	145.0167	M	13.7	1
SL	4/21/1964	-42.1333	144.3	M	15.3	10
SL	4/21/1964	-42.1333	144.3	M	15	
SL	4/21/1964	-42.1333	144.3	M	10.2	
SL	4/22/1964	-42.3833	144.75	F	11.7	15
SL	4/22/1964	-42.3833	144.75	F	11.6	20
SL	4/22/1964	-42.3833	144.75	F	10.6	20
SL	4/23/1964	-42.6667	144.8333	M	16	10
SL	4/25/1964	-41.6667	144.3	F	11	
SL	12/6/1964	7.833333	73.16667	F	9.7	
SL	12/7/1964	5.9	74.16667	M	10.8	37
SL	12/13/1964	-2	74	M	14.4	0.52
SL	12/24/1964	-31.25	81	F	9.2	9
SL	1/14/1965	-32	93	M	10.7	21
SL	2/8/1965	-40.75	143.3833	M	16	90
SL	2/8/1965	-40.75	143.3833	M	14.5	33
SL	2/9/1965	-41.1667	142.1667	F	11.1	5
SL	2/9/1965	-41.1667	142.1667	F	10.6	53
SL	2/9/1965	-41.1667	142.1667	F	11	5
SL	2/10/1965	-42.6333	141.5333	M	11.1	0.25
SL	2/11/1965	-41	142	F	11.6	1.5
SL	2/13/1965	-43	144	F	10.5	0.5
SL	2/15/1965	-44	148	F	11.1	5
SL	2/15/1965	-44	148	F	10.6	8
SL	2/16/1965	-43	149	M	11.7	0.2
SL	2/16/1965	-43	149	M	15.6	0.2
SL	2/16/1965	-43	149	M	12.7	16
SL	2/20/1965	-45	148	M	12.1	0.3
SL	2/20/1965	-45	148	M	12.3	0.3

Fleet	Date	Latitude	Longitude	Sex	Length, m	Ambergris Weight, kg
SL	2/26/1965	-60	151	M	13.7	0.7
SL	3/18/1965	-44	147	M	12.2	2
SL	3/19/1965	-44	148	F	11.4	1.2
SL	3/19/1965	-44	148	M	11.1	23
SL	3/19/1965	-44	148	M	13.5	86
SL	3/26/1965	-41	149	F	11.1	0.3
SL	3/29/1965	-21	149	M	12.5	1.3
SL	3/29/1965	-41	149	M	10.9	1
SL	3/30/1965	-43	145	F	10.6	10
SL	3/30/1965	-43	145	M	10.6	0.9
SL	3/30/1965	-43	145	M	10.6	0.3
SL	3/31/1965	-43	145	M	16.1	7.1
SL	4/9/1965	-43	145	F	10.8	3.5
SL	11/12/1965	-14.7333	51.65	F	9.4	9
SL	11/12/1965	-14.7333	51.65	F	8.9	1
SL	12/9/1965	-33.5	45.83333	M	14.7	0.7
SL	12/10/1965	-32.0667	44	F	10.2	3.8
SL	12/10/1965	-32.0833	44.03333	F	10.4	0.26
SL	12/17/1965	-32.6667	44.46667	F	10.3	70
SL	12/21/1965	-31.7667	42.3	M	14.2	1.6
SL	1/30/1966	-55.9333	58.86667	M	13.3	0.77
SL	4/12/1966	-46.8167	165.9833	M	12.6	27
SL	4/12/1966	-46.9667	166.0333	M	14	2.5
SL	4/12/1966	-47	165.45	M	14.3	0.6
SU	11/27/1962	-41.3333	-56.8	M	13.1	2.4
SU	1/25/1963	-63.9	102.7167	M	14.7	
SU	1/25/1963	-63.9	102.7167	M	15	
SU	2/2/1963	-64.9	118.25	M	15	2.75
SU	2/2/1963	-64.6	118.4	M	14.7	0.68
SU	2/27/1963	-65.1667	15.005	M	16.3	6.2
SU	2/27/1963	-64.65	155.9	M	14.7	4.95
SU	2/28/1963	-64.8	158.4	M	15.2	0.6
SU	2/8/1964	-60.15	14.01167	M	15	
SU	4/27/1964	-43.3833	144.5333	M	12.6	100
SU	10/26/1964	10.43333	47.53333	M	10.2	
SU	10/26/1964	10.43333	47.53333	M	9.7	
SU	10/26/1964	-11.4667	47.53333	M	13	
SU	10/27/1964	13.11667	46.21667	M	10.2	
SU	10/27/1964	13.11667	46.21667	M	8.3	
SU	11/16/1964	-3	52.16667	M	13.3	20.5
SU	11/19/1964	0.383333	52.23333	M	13.5	20
SU	11/19/1964	0.383333	52.23333	M	10.5	20
SU	2/13/1965	-49	147.5	M	14.4	90
SU	2/15/1965	-41.3333	149	M	11.6	1.5
SU	2/15/1965	-41.5	148.5	M	11.8	27
SU	2/15/1965	-41.5833	148.5833	F	9.1	50
SU	2/15/1965	-41.6667	148.6667	F	9.2	1.5
SU	2/16/1965	-42.4167	148	F	9	
SU	2/16/1965	-42.5	148.4167	F	10.1	
SU	2/27/1965	-42.5833	148.5	M	14	3
SU	12/10/1965	-36.3	111.3667	M	14.1	37

Fleet	Date	Latitude	Longitude	Sex	Length, m	Ambergris Weight, kg
SU	1/5/1966	-62.0833	15.01	M	15.4	
SU	1/12/1966	-64.6667	174.75	M	14.2	
SU	1/20/1966	-67.9667	179.4167	M	14.5	
SU	1/24/1966	-64.8333	163.2333	M	14.8	26
SU	11/15/1966	21.5	69.95	M	11.4	
SU	12/27/1966	-64.1333	112.3667	M	15.7	
SU	12/27/1966	-64.1167	113.1333	M	14.6	1.5
SU	12/27/1966	-69.8333	-155	M	14.8	
SU	3/23/1967	-66.2333	162.7667	M	16	0.7
SU	4/10/1967	-45.8833	166.0667	M	15.4	86
SU	4/10/1967	-47	165.3333	M	15.4	
SU	4/10/1967	-46.9833	165.0333	F	11.6	
SU	12/18/1967	-32.9167	44.86667	M	13.8	32
SU	2/4/1968	-67.1667	174.3333	M	15.7	
SU	2/6/1968	-69.15	178.4333	M	16.1	
SU	4/1/1967	-68.05	178.7667	M	14.9	2
SU	4/1/1967	-68.5	173.9667	M	15.3	25
SU	11/23/1968	-31.3833	0.816667	M	13.3	1
SU	11/23/1968	-31.1333	0.716667	M	12.9	48
SU	1/20/1969	-61.75	-61.15	M	16.3	
SU	4/8/1969	-58.1833	148.4833	M	14.9	0.5
SU	4/9/1969	-59.0833	148.25	M	15	1.1
SU	4/12/1969	-42.2333	149.05	M	11.6	5
SU	4/13/1969	-42.25	148.8333	M	11.1	42
SU	4/18/1969	-44.0333	143.5167	M	11.1	0.75
SU	4/24/1969	-33.8	127.6833	M	12.2	
SU	4/29/1969	-34.6833	120.6667	M	11.2	27
SU	4/29/1969	-34.8333	120.6667	F	10.9	10
SU	5/3/1969	-31.5	114.8333	M	11.7	5
SU	12/25/1970	-34.6333	4.033333	M	13.1	64
SU	1/9/1970	-35.5667	12.28333	F	11.4	0.11
SU	2/8/1970	-47.05	54.33333	M	12.2	3.54
SU	4/6/1970	-42.5	49.66667	M	11.6	80
SU	4/6/1970	-41.15	50.16667	M	14	0.8
SU	4/13/1970	-35.6833	45.21667	F	10.2	
SU	4/15/1970	-32.4667	43.33333	M	10.4	10
SU	4/5/1971	-36.3	58.53333	M	12.5	
SU	4/12/1971	-35.0333	45.36667	F	10.8	
SU	4/12/1971	-34.3333	45.41667	F	10.6	
SU	4/12/1971	-34.2667	45.28333	M	12.5	50
SU	4/12/1971	-34.2667	45.28333	F	8.9	
SU	4/13/1971	-34.2667	45.28333	F	11.7	
SU	4/13/1971	-33.15	45	F	10.5	
SU	4/15/1971	-32.4833	44.88333	F	10.2	94
SU	2/7/1972	-53.9	-2.41667	M	14.7	65
SU	2/24/1972	-49.8333	49	M	13.6	
SU	4/19/1972	-31.1333	4.333333	F	8.8	20
SU	4/26/1972	-30.2	38.3	F	10	15
SU	5/8/1972	-27.75	41.45	F	9.5	
SU	12/4/1972	-58.2667	45.25	M	14.9	80
SU	4/24/1973	-33.9667	12.53333	F	10.4	1.85

Fleet	Date	Latitude	Longitude	Sex	Length, m	Ambergris Weight, kg
SU	4/4/1974	-36.65	155.0667	F	9.8	40
SU	4/4/1974	-36.65	155.0667	F	9.7	45
SU	11/15/1975	-27.3333	13.98333	F	10.5	
SU	11/22/1976	-28.8333	13.5	M	13.6	3
SU	12/1/1976	-33.6667	45.26667	F	10.5	20
SU	12/7/1977	-31.95	30.81667	M	11.1	
UD	22628	-62.9333	115.7333	M	14.1	6
UD	23322	-35.25	18.46667	M	9.9	
UD	23326	-35.9333	-5.3	M	15.3	60
UD	23429	-64.5333	67.51667	M	13.7	28
UD	23475	-36.6	70.28333	F	10.3	0.65
UD	23481	-34.15	64.51667	F	10.2	5.3
UD	23460	-48.1667	71.18333	M	12.3	0.6
UD	23468	-35.8	70.73333	M	10.8	5.39
UD	23380	-48.3333	70.83333	M	14.8	28
UD	23831	-35.1167	123.7833	F	10.7	130
UD	23821	-35.1667	115.5	M	11.9	
UD	23824	-35.4833	116.6167	M	12.7	3.55
UD	23822	-35.4167	115.3833	M	11.9	
UD	23822	-35.35	115.8333	M	11.7	
UD	23823	-35.55	117.1667	F	10.6	0.88
UD	23823	-35.55	116.8333	M	11.3	23
UD	23823	-35.6	116.8833	M	9.5	18
UD	23825	-33.55	116.2667	M	12.2	20
UD	23826	-35.3	119.5833	M	10.8	0.46
UD	23825	-35.5	118.0833	F	10.6	
UD	23825	-35.75	117.55	F	9.6	20
UD	23823	-35.55	117.1667	F	10.4	65
UD	23823	-35.5333	116.8333	F	10.6	40
UD	23828	-35.9	119.2833	M	12.2	19
UD	23822	-35.1	115.3333	M	12.5	13
UD	23842	-34.3333	132.25	M	11.3	
UD	23827	-34.9167	119.1667	M	13.1	
UD	23827	-35.2167	120.95	F	10.7	
UD	23830	-35.2667	118.75	F	10.5	27
UD	23830	-34.9833	120.9833	M	9.7	
UD	23829	-35.1167	118.6833	F	9.9	20
UD	23830	-35.1167	119.0667	F	10.3	1.5
UD	23832	-34.75	123.4667	F	9.5	2.89
UD	23838	-33.6667	130	M	9.5	
UD	20553	-35	132.5333	F	10.5	3.1
UD	23840	-35.5	133.8333	M	10.5	2.2
UD	23841	-35.55	134.5833	M	12.2	1.5
UD	23841	-35.5833	134.6667	M	11.7	
UD	23840	-35.4167	133.7333	F	11.1	28
UD	23841	-35.5	135	M	12.2	0.75
UD	23841	-35.4	134.6	M	12.4	12.9
UD	23840	-35.0667	134.6	M	12.3	9.5
UD	23841	-35.3167	133.6833	M	10.6	0.25
UD	23847	-35.5	115	M	11.3	
UD	23847	-35.5833	115.5833	M	11.8	

Fleet	Date	Latitude	Longitude	Sex	Length, m	Ambergris Weight, kg
UD	23845	-33.6	127.1333	F	10.3	
UD	23836	-34.4333	126.25	F	10.7	21
UD	23837	-35.7	129.3333	F	10.4	4
UD	23837	-33.6	129.95	F	10.3	
UD	23838	-33.85	130.5	F	9.8	11.7
UD	23838	-33.6667	130	F	9	
UD	23859	-29.2667	113.7167	F	10.4	60
UD	23859	-29.0833	113.3	F	10.9	
UD	23860	-28.5	113.25	F	10.5	
UD	23861	-27.3	112.3167	F	10.6	8
UD	23833	-34.7833	125.3167	F	10.5	0.18
UD	23833	-33.7833	126.2667	F	10.5	19
UD	23834	-33.8333	126.3667	F	9.4	16
UD	23834	-34	127.25	F	11	2
UD	23852	-32.45	164	F	10.9	1
UD	23854	-32.7667	113.95	F	10.7	2.25
UD	23855	-31.8333	115.25	F	10.8	
UD	23847	-35.6	114.6667	M	11.7	1
UD	23847	-35.6167	115	M	13.1	
UD	23856	-32.0667	113.95	F	10.5	
UD	23726	-56.6167	44.3	M	12.9	9.7
UD	23837	-32.8333	114.6667	M	12	4.2
UD	23870	-26.5333	110.6333	F	10	12.3
UD	23870	-25.7	110	F	9.8	3
UD	24083	-55.4	6.5	M	14.7	2
UD	23805	-55.5833	19.33333	M	14.7	2
UD	24422	-33.4167	-4.26667	M	12.3	3
UD	24476	-62.9667	28.55	M	15.3	203
UD	25552	-55.75	23.33333	M	12.6	1.9
UD	25552	-55.6667	23.41667	M	12.6	12.6
UD	25651	-45.7333	149	M	10.8	6.2
UD	25677	-31.7833	114.4667	F	10.2	60
UD	25674	-28.6167	113.2167	F	10.9	15
UD	25604	-64.7167	59.53333	M	14.6	2
UD	25649	-47.6833	148.9833	M	13.8	4
UD	25591	-60.85	101.3333	M	13.5	
UD	25910	-34.6667	-3.05	M	13.6	4
UD	25987	-54.5333	-45.6167	M	14.5	3.5
UD	26310	-54.3333	-57.2667	M	13.2	3.3

The distribution of sperm whales caught by the flotillas is illustrated by the map (Fig. 1).

As can be seen, sperm whales were caught in all the oceans of the Southern Hemisphere with relatively the same density. But sperm whales with ambergris were overwhelmingly found in the Indian Ocean (Fig. 2).

In 171 cases, the discovered ambergris was weighed - 112 times in males, and 59 times in females. It should be noted that some whales had multiple nodules of ambergris, and Table 1 shows their total weight. The average weight of ambergris was 18.3 kg, for males – 17.9 kg, for females – 19 kg.

Figure 3 shows the places of occurrence of ambergris concretions weighting more than or equal to 50 kg (Fig. 3).

The map reveals two “local herds” (two populations) of sperm whales; one cluster was located south of Madagascar along the meridian to Antarctica, while the

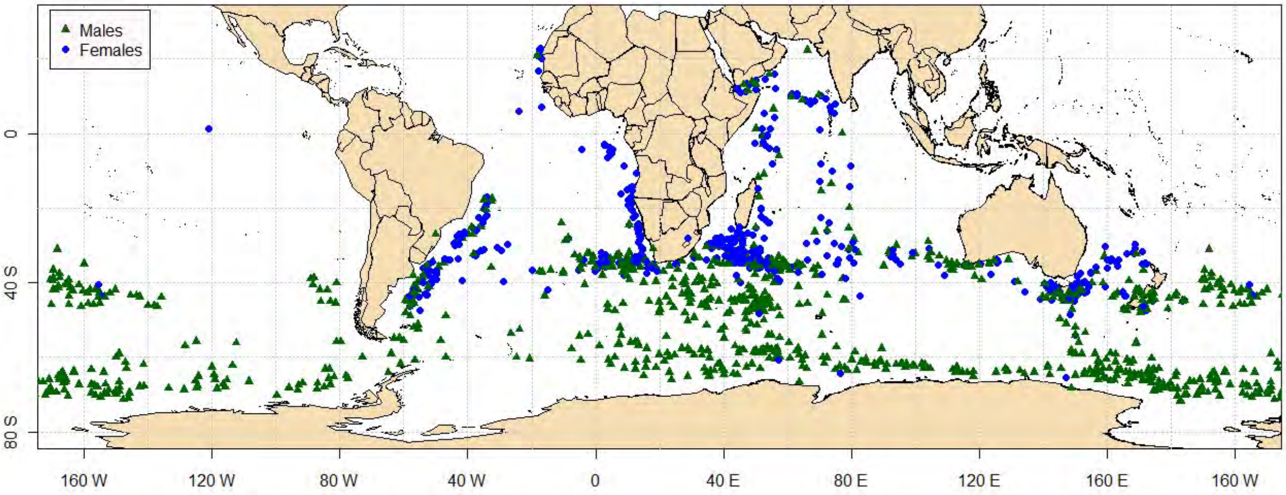

Figure 1. Distribution of sperm whales caught by flotillas "Slava", "Soviet Ukraine" and "Yuri Dolgoruky" for the period 1961-1978.

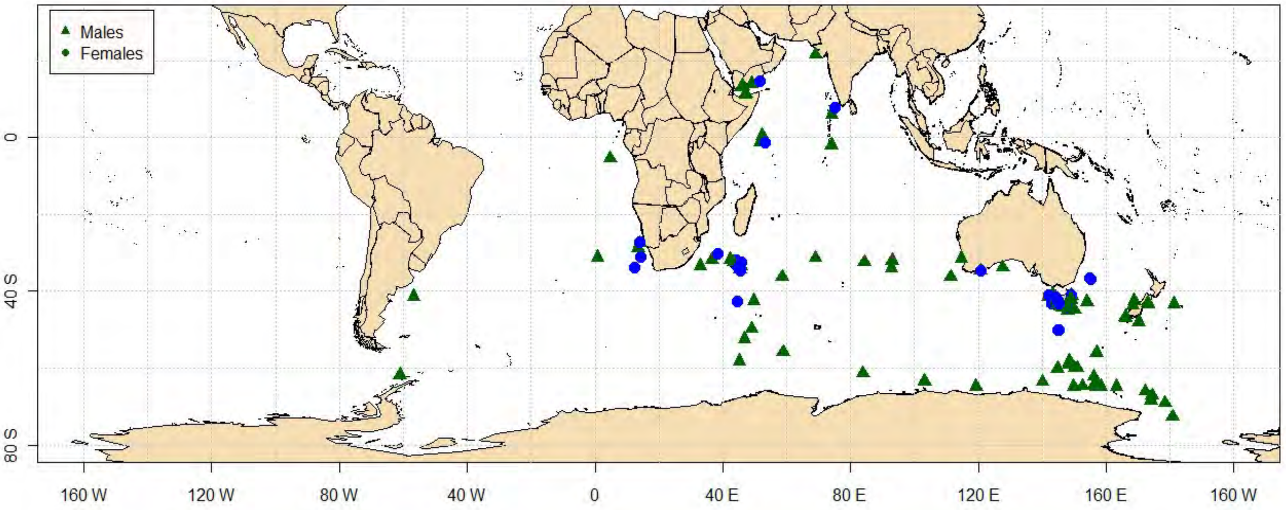

Figure 2. Distribution of caught sperm whales in which ambergris was found.

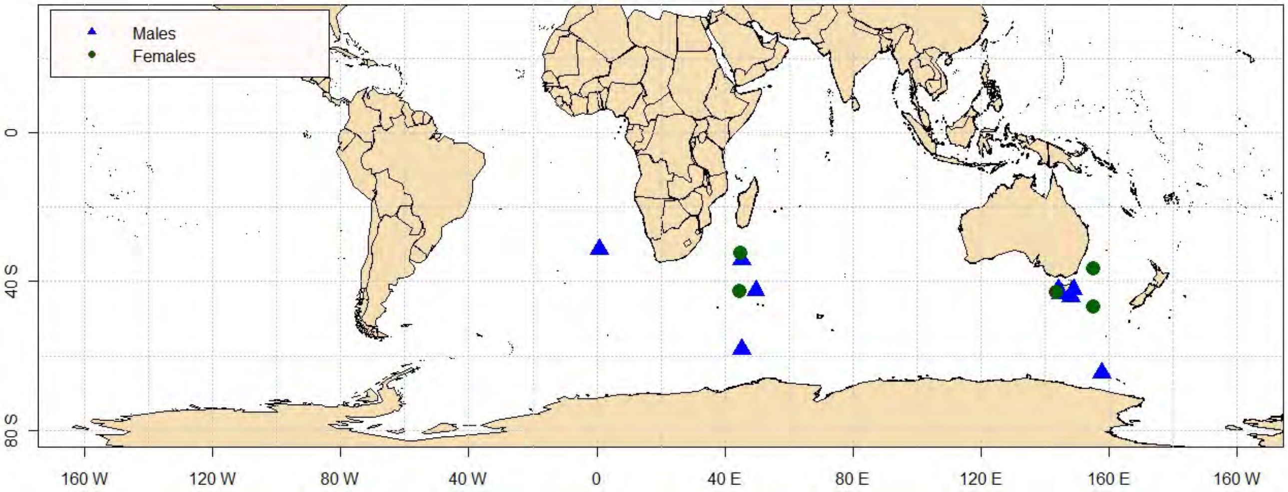

Figure 3. Distribution of sperm whales with large ambergris boulders.

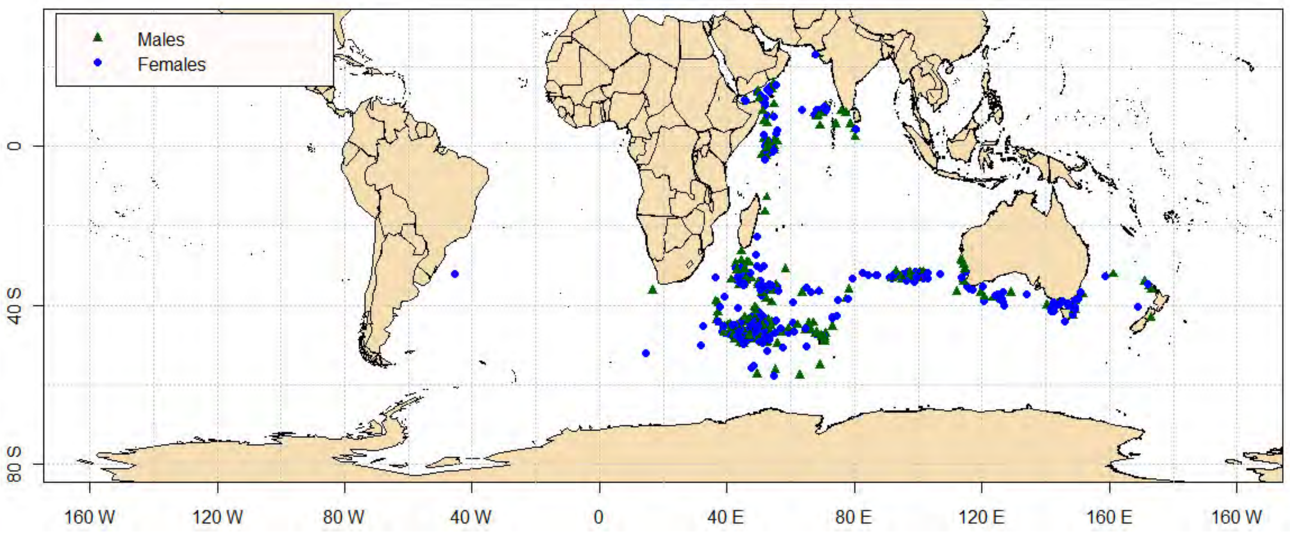

Figure 4. Distribution of pygmy blue whales (*Balaenoptera musculus brevicaudis*, Ichihara, 1961).

second was from Australia and Tasmania, south to the Balleny Islands.

Discussion

In a number of studies it is stated that ambergris is found in male sperm whales, while in females it is either completely absent or extremely rare, and ambergris concretions are smaller than in males (Schwediawer, 1783; Hardy, 1949; Ivashin, 1966). The current data indicates that ambergris is found in both male and female sperm whales (Table 1). In percentage terms, the occurrence of ambergris in females is just a little lower (0.34 % of cases) than in males (0.44 % of cases). Also the weight of ambergris found in females is almost the same as that in males.

It was noticed that the zone of ambergris occurrences practically coincided with the range of pygmy blue whale (*Balaenoptera musculus brevicauda*, Ichihara, 1961) (Fig. 4).

The reasons for the coincidence of the distribution of sperm whales with ambergris and the range of pygmy blue whales are not yet clear. To clarify this issue, separate studies will be required, with a thorough analysis of the trophic relationships in these two species of whales, as well as the ecological situation in the Indian Ocean region and adjacent waters.

Conclusions

In the period from 1961 to 1978, 59,814 sperm whales were caught during 29 whaling seasons by the whaling flotillias Slava, Soviet Ukraine and Yuri Dolgoruky; 35,994 of these were males and 23,820 females. The constructed whale distribution map showed that in the Southern Hemisphere, sperm whales were caught in all oceans (the Atlantic, Indian, Pacific and Southern Arctic). Ambergris was found in 240 sperm whales, which was 0.40 % of the whales examined. In males, the percentage of ambergris found was 0.44 %, and 0.34 % in females.

The distribution map of sperm whales with ambergris showed that the vast majority of them were from the Indian Ocean.

Ambergris was weighed in 171 cases. The average weight of ambergris in males was 17.9 kg, and in females it was 19 kg.

The distribution map of sperm whales with large ambergris boulders (weighing more than 50 kg) revealed the presence in the southern part of the Indian Ocean of two isolated local concentrations (populations) of sperm whales - Madagascar and Australian-Tasmanian.

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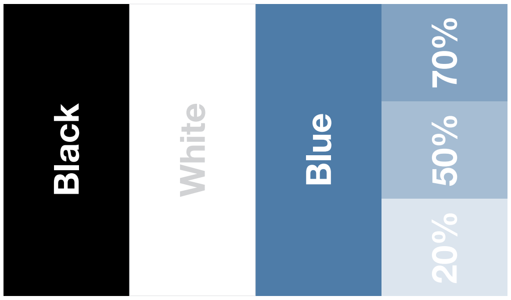

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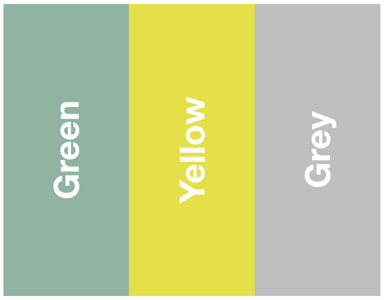

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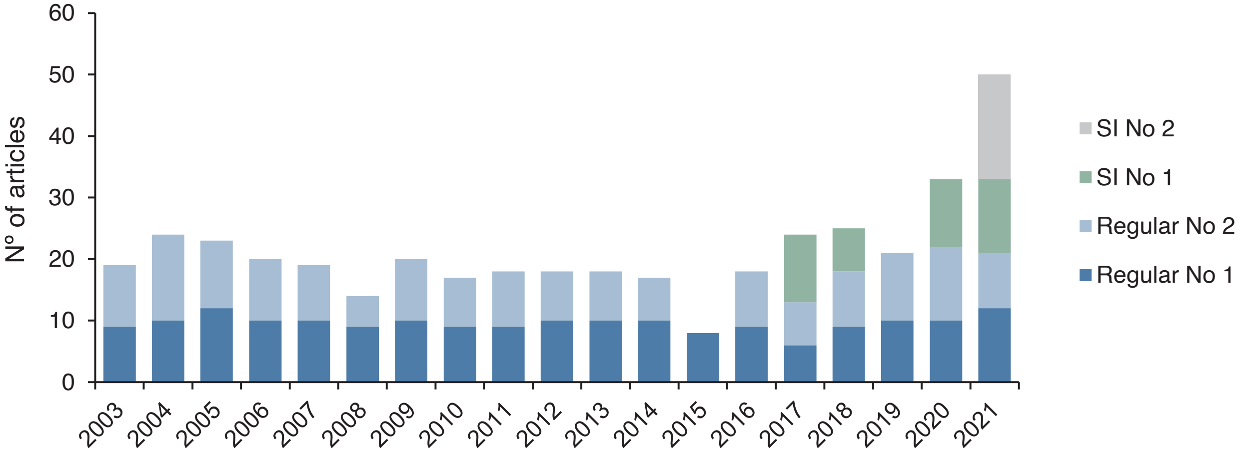

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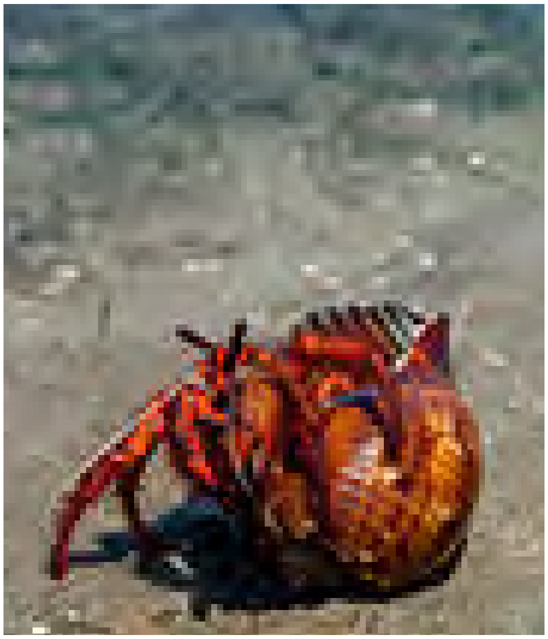

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