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Cover image: Sea waves shaping rock. © José Paula, 2024

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Original Article

Influence of Covid-19 restrictions on the status of mangrove vegetation in coastal Kenya

Mumini Dzoga*[1], Cosmas Munga[1], Fathima A. Badurdeen[2], Kevin Tole[3], Clarice Kombe[4], Ali Shee[1]

[1] School of Applied and Health Sciences, Technical University of Mombasa, Kenya

[2] School of Humanities and Social Sciences Technical University of Mombasa, Kenya

[3] Institute of Computing and Informatics, Technical University of Mombasa, Kenya

[4] Community Action for Nature Conservation, Kenya

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*** Corresponding author:**
mdzoga2014@tum.ac.ke

Abstract

Mangrove forest ecosystems provide essential services to tropical coastal communities, including as a source of food, fuel and building materials. During the Covid-19 pandemic human movements were restricted at all levels, including locally in coastal communities. The impact of these measures on marine ecosystems such as mangroves were not well understood. A household survey was conducted in a mangrove-dependent community at Mtwapa Creek to evaluate their perceptions on the rate of mangrove degradation, factors contributing to mangrove degradation, and species that were most exploited during the pandemic period. Multiple Correspondence Analysis (MCA) was used to determine the association of Covid-19 with economic activities. Due to the restrictions on human interactions and travel during the pandemic, some natural ecosystems thrived, but in contrast accelerated destruction of mangrove forests occurred along Mtwapa Creek. The MCA indicated significant association between mangrove degradation rate and unemployment, charcoal burning, and reduced farming activities. A Chi-square test confirmed significant degradation of mangrove forests. Loss of income sources during the pandemic led to an increase in exploitation of *Rhizophora mucronata, Ceriops tagal* and *Avicennia marina*. These species were mainly harvested for construction and charcoal burning. Mangrove management measures could not be effectively enforced during the pandemic.

Keywords: Post Covid-19 pandemic, mangrove conservation, mangrove degradation, Mtwapa Creek, tropical coastal communities

Introduction

The infancy of Covid-19 virus was characterised with increased frequency of human infection and life losses. Within the first four weeks, about three million infections and 200, 000 deaths were reported worldwide (CCSA, 2020) which proliferated tension for human interaction at local, national, and international scale (Nicola *et al.,* 2020). As a result, measures to control human movement and social gatherings were effected (Diffenbaugh, *et al.*, 2020) leading to economic disruption as well as ecological impacts (Nicola *et al.,* 2020; Akinsorotan *et al.,* 2021). Whereas humanity endured physical and emotional health impacts, some ecological systems were observed to recover. Stokes *et al,* (2020) in their succinct report observed that the measures implemented by governments during the pandemic resulted to an increase of inland fisheries in 79 countries. Also, due to limited industrial activities and minimal travels by road, air and sea, a reduction in the emmissions of carbon dioxide (CO_2) and nitrogen oxides (NO_x) was observed (CCSA, 2020).

As part of the ecological systems on coastal shorelines located in tropical regions, mangrove forests are important carbon sequesters. In the global perspective, mangroves have a sequestration capacity of 21 gigatons

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of CO_2 (Mark and Marice, 2021) providing the basis for climate change mitigation measures (Chow, 2017). Mangrove root systems are nesting and breeding habitats for juvenile fishes (Gambo *et al.,* 2019). A thriving mangrove forest increases the small-scale fisheries productivity for the wellbeing of the dependent local communities through provision of protein and income. Mangroves have other socio-economic benefits which include medicinal and chemical uses, fodder for livestock and food for humans. Nevertheless these ethnobiological uses of mangroves have been reported to reduce over time (Dahdouh-Guebas *et al.*, 2021). Despite these benefits, mangrove forests are under pressure worldwide (Kairo *et al.,* 2002). The decline in mangrove forests is associated with increased human settlement in coastal areas and exploitation of mangrove products for timber, construction, fuel wood, and agriculture (Romañach *et al.,* 2018; Dahdouh-Guebas *et al.,* 2000).

In order to protect mangrove ecosystems from overexploitation, the Government of Kenya has established several management measures. Mangrove forests are protected by the Kenyan law as natural resources. All mangroves in Kenya are designated government reserve forests under the Legal Notice No. 174 of 1964 (Government of Kenya, 2017). The Forest Conservation and Management Act of 2016 provide funds for management and conservation of forests including mangrove forests. Such management should safeguard sustainable development through participation of local communities (Government of Kenya, 2016b). In addition, the Wildlife Conservation and Management Act of 2013 as well as the Fisheries Management and Development Act of 2016 ensure sustainable exploitation of mangrove forests. Also, non-government actors such as the World Wide Fund for Nature (WWF) and Western Indian Ocean Marine Science Association (WIOMSA) contribute immensely in management and conservation of natural resources within East Africa. They support local communities and civil society organisations mainly through funding efforts in natural resources management and conservation, restoration of degraded ecosystems, community sensitization on sustainable exploitation of natural resources, advocacy and campaigns for policy change and capacity building (Prosperi *et al.,* 2021).

The impact of anthropogenic activities on aquatic and terrestrial ecosystems has been a subject of many scientific studies (Dzoga *et al.,* 2019; Mark and Marice, 2021) especially during the Covid-19 pandemic era. However, there is limited information on the state of mangrove ecosystems during this period. Like in many parts of the world the Kenyan population encountered restrictions on human interactions, movement, and social gatherings during this period. As much as these measures improved the wellbeing of humanity, their implications on community dependent ecosystems like mangrove forests were not well known. To fill this gap, the Mtwapa Creek mangrove ecosystem community in particular was selected for this study to determine the status of the mangrove forest after the Covid-19 pandemic. Based on these findings, the current work recommends measures which could be implemented in order to protect mangrove ecosystems and other natural resources during pandemics.

Materials and methods

Study area

Mtwapa Creek is located in Kilifi south sub-County at Latitude -3.886234S and Longitude 39.675178E (Fig. 1). The creek is about 13.5 km long and borders the Indian Ocean (Mutua *et al.,* 2004). The patches of mangrove forests in the area are named after the adjacent villages; namely Lutsanga, Kidutani and Timbetimbe. The creek is fed by three seasonal streams (i.e., Ndovu, Kashani and Kidutani). In Kenya, there are nine species of mangroves of which eight species are located within the study area (Mirera and Mtile, 2009). Lutsanga, Kidutani, and Timbetimbe villages have a population size of 495, 815, and 470, respectively. The three villages are within Mtwapa sub-County with a population size of 127, 377 people (Government of Kenya, 2019).

Sample size and sampling procedure

Purposive sampling was applied to select Lutsanga, Kidutani, and Timbetimbe villages for this study within the the Mtwapa Creek mangrove forests ecosystem. The Cochrans formula for infinite population size ($z = 1.96, e = 0.05, p = 0.5, q = 1 - 0.5 = 0.5$) was used to estimate the initial sample size which generated 384 required participants. Based on the actual population size of the three villages (495, 815, and 470), the sample size was then corrected with Cochrans correction formula (n = new sample size, n_0 = initial sample size, N = actual population of the villages) for small sample sizes. Hence a final sample size of 316 participants was used. Proportional samples for each village were determined by the ratio of population size of each village divided by the overall population of the three villages, then multiplied by 316. Thus the respondents for Lutsanga, Kidutani, and Timbetimbe were 88, 145, and 83, respectively. Data was collected through semi-structured interviews using questionnaires designed to gather information on economic activities, household size, factors hindering mangrove conservation, estimated income of the respondents and types of mangrove species exploited among other variables. Respondents for the semi-structured interviews were selected through a simple random process.

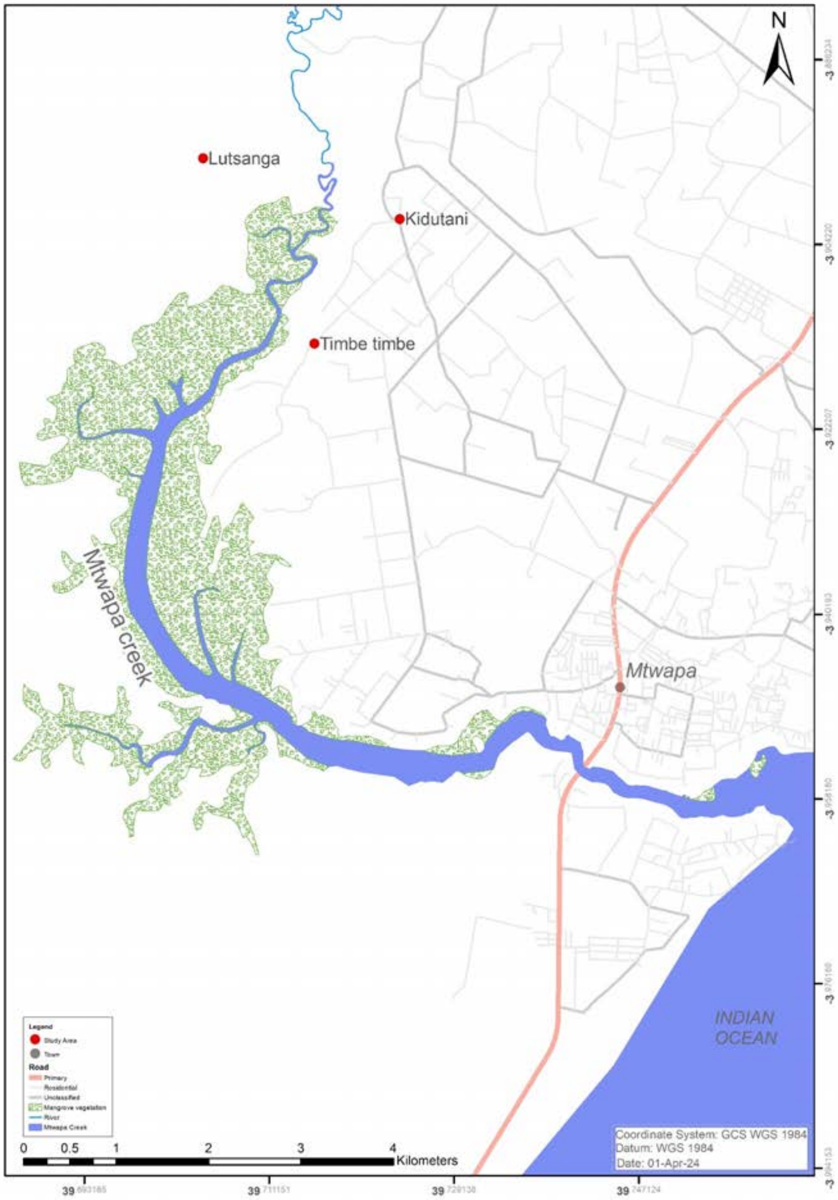

Figure 1. A map of Mtwapa Creek showing the distribution of mangroves from the mouth and upper parts of the creek and the study sites of Lutsanga, Kidutani and Timbetimbe.

Data analysis

Frequency distribution of variables was determined by SPSS v16 software. Microsoft Excel was used for descriptive statistics on mangrove species exploitation and factors that hinder mangrove conservation and restoration. Multiple Correspondence Analysis (MCA) was performed to determine the association between degrees of mangrove degradation with economic activities. The Chi-square test was conducted to determine the significance level of mangrove degradation during Covid-19.

Results

Income categories of mangrove dependent communities

As indicated in Figure 2, the majority (35 % and 29 %) of the Mtwapa Creek community were earning less than USD 35 per month. Only 2 % of the residents

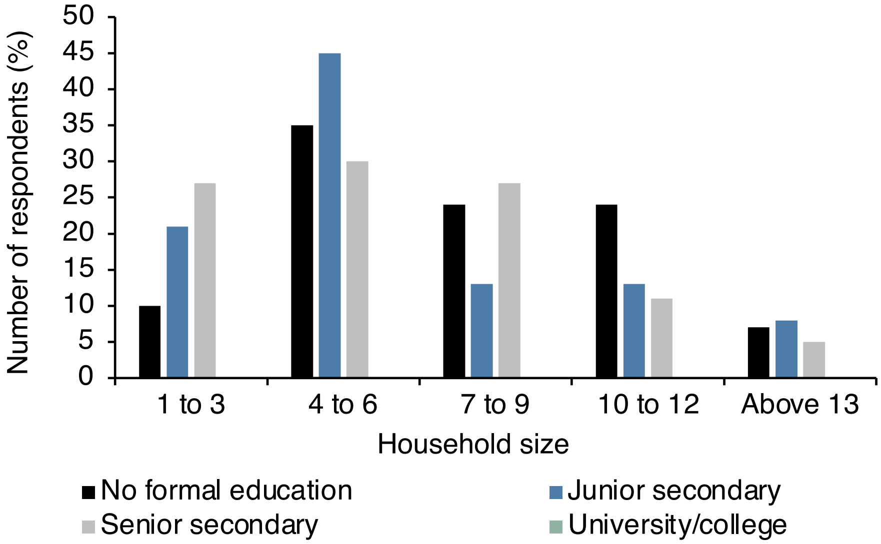

Figure 2. Comparison between household size and level of education attained.

earned above USD 140 per month. Across all the household sizes with an exception of household size of 7 to 9 members, most community have attained junior secondary education while others have no formal education (Table 1).

Status of mangrove degradation during Covid-19 era

The degradation of mangrove forests was observed to be highest during the Covid-19 period by the majority (84 %) of the mangrove dependent community (Table 2).

About 11 % of the residents reported inadequate knowledge of mangrove exploitation. For these residents, they reported to have no direct contact or interaction with mangroves in the study area. Their interaction with mangroves was mainly through use of mangrove products e.g., timber, charcoal and firewood. These products are sold within the study area as well as in the nearby commercial centers in Kilifi and Mombasa. Mtwapa Creek depicted widespread mangrove degradation as indicated in Figure 3.

Levels of association of mangroves degradation with economic activities during the Covid-19 period

As shown in Figure 4, a high rate of mangrove degradation (Covid-19-impact-High) was highly associated with unemployment, charcoal burning, selling firewood, fishing and lack of farming economic activities. A moderate rate of mangrove degradation (Covid-19-impact-Moderate) was highly associated with farmers and employed people, and those who did not participate in charcoal burning, fishing, and selling of firewood. Results of the Chi square test showed significant levels of mangrove degradation during the Covid-19 era ($\chi = 1093.9$, $P < 0.05$).

Table 1. Income categories of the mangrove dependent community in Mtwapa Creek.

Estimated monthly income (USD)	Frequency	Percent (%)
Below 7	90	29
7-35	109	35
35-70	75	24
70-105	25	8
105-140	5	2
Above 140	12	2
Total	316	100

Table 2. Status of mangrove degradation during Covid-19 period in Mtwapa Creek, Kilifi County based on perceptions of respondents during the study.

Degree of mangrove degradation	Frequency	Percent (%)
High	266	84
Moderate	9	3
Low	5	2
Do not know	36	11
Total	316	100

Levels of mangrove species exploitation in Mtwapa Creek

The mangrove species *Rhizophora mucronata* was observed to be the most exploited mangrove species followed by *Ceriops tagal* and *Aveccinia marina*. The least exploited species were *Heritiera littoralis* and *Brugeuira gymnorrhiza* (Fig. 5).

Factors affecting mangrove conservation in Mtwapa Creek

The majority of the mangrove dependent community indicated lack of funding, insufficient knowledge and skills, insecurity, and lack of government support as the greatest challenges impeding mangrove conservation efforts (Fig. 6).

Figure 3. Status of mangrove degradation at Mtwapa Creek in Kilifi County, Kenya.

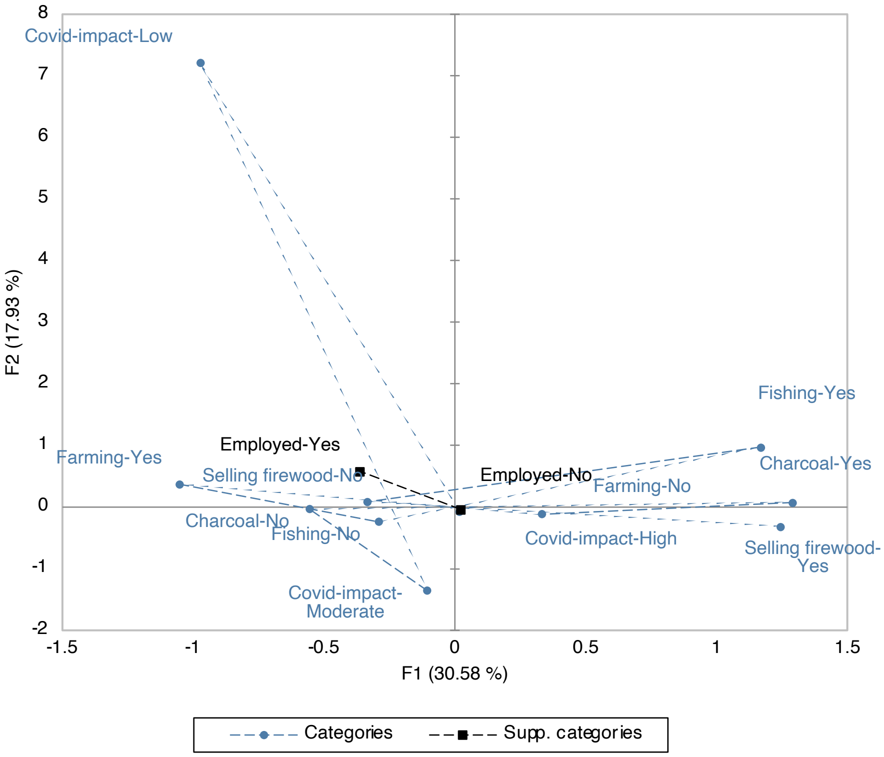

Figure 4. Multiple Correspondence Analysis (MCA) indicating association of the rate of mangroves degradation during the Covid-19 period with economic activities. Categories (axes F1 and F2: 48.51 %).

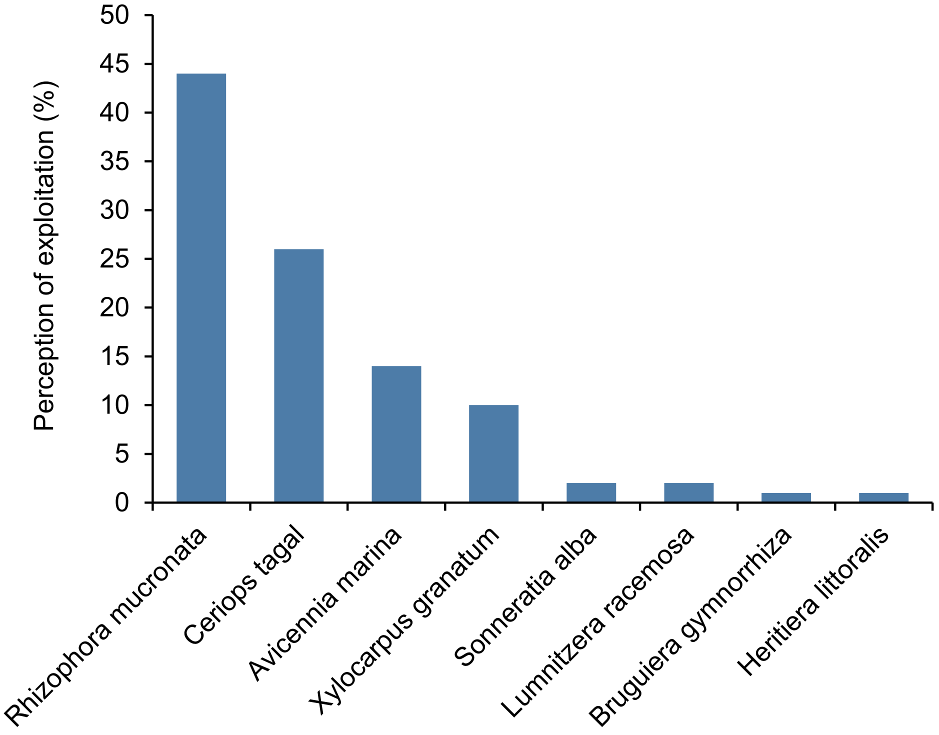

Figure 5. Perceptions of degree of exploitation of mangrove species at Mtwapa Creek over the study period.

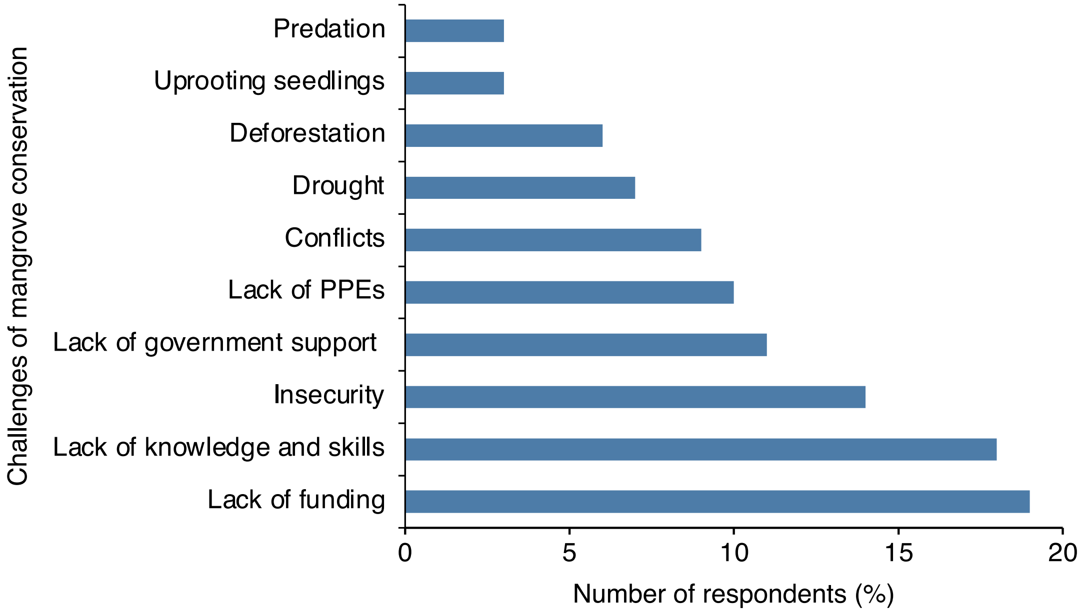

Figure 6. Challenges facing the mangrove dependent community for mangrove conservation at Mtwapa Creek in Kilifi County, Kenya over the study period.

Discussion

Dwindling mangrove forest ecosystems has been observed in many coastal countries globally (Das *et al.*, 2020). Degradation of mangrove forests is intricately connected with natural and anthropogenic factors (Romañach *et al.*, 2018). While natural factors are more pronounced and widely studied with regards to mangrove forest deterioration (Paul *et al.*, 2017), human interventions play a critical role in restoration and conservation of these ecosystems. Anthropogenic factors have, however, caused severe destruction to mangrove ecosystems. The destruction of mangroves is associated with economic activities including charcoal burning, cutting of wood for fuel, fishing, and unemployment (Mark and Marice, 2021). This study has shown that man-made activities accelerate the destruction of mangroves forests. In Mtwapa Creek, the mangrove forest ecosystem is a source of livelihood for most residents. Most of the residents lack professional education necessary for stable jobs from the government and private sector (see Fig. 2). Thus, they sustain their livelihoods from casual work e.g., fishing, and charcoal burning. Unemployment and lack of stable income as well as lack of entrepreneurial knowledge and skills have increased reliance on mangrove resources by the majority of Mtwapa Creek residents.

The Covid-19 pandemic period led to not only loss of human lives but also to dwindling of economic fortunes. As humanity suffered, the natural resources such as fisheries were reported to flourish (Akinsorotan *et al.*, 2021). Contrary to this, mangrove forest ecosystems experienced immense destruction as shown in this study. The restriction on movement, social gatherings, and prolonged lockdowns led to loss of sources of income and increased frequency of human illness (Ede *et al.*, 2021). This intensified mangrove forest destruction for charcoal burning and selling of wood fuel.

Legal frameworks and regulations are important in the management of natural resources such as mangrove forests. While these frameworks outline procedures for management and conservation of mangroves and associated resources (Government of Kenya, 2013, 2016a, 2016b), their enforcement and implementation were a challenge especially during the pandemic period. Priorities over this period changed to support the wellbeing of humanity over natural resources. Natural resources became a means for prosperity as well as survival, resulting to overexploitation. This condition was aggravated by lapses in organisations mandated to manage natural resources. However, this observation is contrary to a study by Akinsorotan *et al.*, (2021) which indicated progressive protection of the natural resources across the globe. This study has observed that there was minimal government support to residents in terms of monitoring, enforcement of regulations and financial aid. While sustainable natural resource management is effective through the involvement of the community (Hamza *et al.,* 2023), the lack of government support during the pandemic was indicated by most residents as among the major challenges for mangrove forest restoration and conservation during this period.

Protection of mangrove species is critical not only for the health of marine and coastal ecosystems but also for human nutrition and health. *Rhizophora mucronata, Ceriops tagal and Aveccinia marina* were the most exploited mangrove species. These species are the most dominant and abundant in Kenya. They are exploited mainly for construction, wood fuel and charcoal burning. As currently indicated, their exploitation rates exceed their regeneration. This will ultimately hinder the ecosystem services. Therefore, urgent restoration and conservation measures should focus on the selection of these species for planting and re-planting. Furthermore, there is a need to train and instil skills and knowledge to members of the Mtwapa Creek community on mangrove forest conservation, restoration and sustainable exploitation. This would enhance restoration and conservation measures. Since the exploitation of mangroves is mainly for economic reasons, provision of alternative sources of livelihoods to residents (especially during pandemics) will be vital in the conservation and restoration efforts.

Conclusions

Mangrove degradation in Mtwapa Creek is a result of anthropogenic factors rather than environmental conditions. During the Covid-19 period, mangrove degradation was highly associated with charcoal burning, unemployment, and lack of alternative sources of livelihood such as farming. The mangrove dependent community of Mtwapa Creek are characterised by low-income earnings of less than USD 7 per month. Low level of income earnings coupled with limited alternatives for livelihoods during the pandemic period compelled the local community to rely on charcoal burning as the main source of income. As a result, the mangrove forest of Mtwapa Creek was highly degraded during this period. Restoration and conservation measures have not been successful due to the lack of government support in terms of monitoring and financial provision. Furthermore, lack of knowledge and skills to manage mangrove forests has led to continuous degradation. Frequent monitoring, financial support, provision of alternative livelihoods, especially during pandemics, and training on mangrove skills and knowledge will be critical in reviving the mangrove ecosystems of Mtwapa Creek in Kilifi County, Kenya.

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References

Akinsorotan OA, Olaniyi OE, Adeyemi AA, Olasunkanmi AH (2021) Corona virus pandemic: Implication on biodiversity conservation. Frontiers 3: 5

CCSA (2020) How COVID-19 is changing the world: a statistical perspective. UNCTAD. 87 pp

Chow J (2017) Mangrove management for climate change adaptation and sustainable development in coastal zones. Journal of Sustainable Forestry 37 (2): 139-156 [doi:10.1080/10549811.2017.1339615]

Dahdouh-Guebas F, Mathenge C, Kairo JG, Koedam N (2000) Utilization of mangrove wood products around Mida Creek (Kenya) amongst subsistence and commercial users. Economic Botany 54 (4): 513-527

Das CS, Mallick D, Mandal RN (2020) Mangrove forests in changing climate: A global overview. Journal of the Indian Society of Coastal Agricultural Research 38 (2): 104-124

Diffenbaugh NS, Field CB, Appel EA, Azevedo IL, Baldocchi DD, Burke M, Burney AJ, Ciais P, Davis SJ, Fiore AM, Fletcher SM, Hertel TW, Horton DE, Hsiang SM, Jackson RB, Jin X, Levi M, Lobell DB, MCKinley GA, Moore FC, Montgomerry A, Nadeau KC, Pataki DE, Randerson JT, Schtnell M, Seneviratne SI, Singh D, Wong-Parodi A (2020) The COVID-19 lockdowns: a window into the Earth System. Nature Reviews 1 (9): 470-481

Dzoga M, Simatele MD, Munga CN (2019) Climate variability and small-scale fisheries in Kenya: Characterization of current socio-economic conditions of artisanal fishing communities in Ungwana Bay and the Lower Tana Delta. International Journal of Environmental Sciences and Natural Resources 17 (3): 555971

Ede CI, Masuku MM, Jili NN (2021) Implications of COVID-19 lockdown on South African business sector. International Journal of Financial Research 12 (4): 12-23

Gamboa GZ, Ratunil VB, Escobal EC, Ebarsabal GA (2019) Diversity and vegetation analysis of mangroves. Journal of Agricultural Sciences and Fisheries 1 (1): 19-27

Government of Kenya (2013) Wildlife Conservation and Management Act, 2013. Government of Kenya. 74 pp

Government of Kenya (2016a) The Fisheries Management and Development Act. Government of Kenya. 120 pp

Government of Kenya (2016b) The Forest Conservation and Management Act. Government of Kenya. 227 pp

Government of Kenya (2017) National Mangrove Ecosystem Management Plan: 2017-2027. Government of Kenya. 102 pp

Government of Kenya (2019) 2019 Kenya Population and Housing Census. Government of Kenya. 251 pp

Hamza AJ, Esteves LS, Cvitanović M, Kairo, JG (2023) Sustainable natural resource management must recognise community diversity. International Journal of Sustainable Development and World Ecology 30 (7): 727-744 [doi:https://doi.org/10.1080/13504509.2023.2192006]

Kairo JG, Dahdouh-Guebas F, Gwada P, Ochieng C, Koedam N (2002) Regeneration status of mangrove forests in Mida Creek, Kenya: a compromised or secured future? Ambio 31 (7/8): 562-568 [http://www.vub.ac.be/APNA/staff/FDG/pub/pub.html]

Mark SD, Marice L (2021) The state of the world's mangroves 2021. Global Mangrove Alliance. 41 pp

Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, Agha M, Agha R (2020) The socio-economic implications of the coronavirus pandemic (COVID-19): A review. International Journal of Surgery 78: 185-193

Paul AK, Ray R, Jana S, Kamila A (2017) Mangrove degradation in the Sundarbans. In: Finkl CW, Makowski C (eds) Coastal wetlands: Alteration and remediation. Springer Nature. 357-392 pp

Prosperi J, Musili P, Lang'at KS, Komu H, Williamson D (2021) Mangrove ecosystem conservation manual - A focus on Kenya. FSPI Mikoko Conservation and resilience of Kenyan mangrove forest. Mikoko Project Team. 104 pp

Romañach SS, DeAngelis DL, Koh HL, Li Y, Teh SY, Barizan RS, Zhai L (2018) Conservation and restoration of mangroves: Global status, perspectives, and prognosis. Ocean and Coastal Management 154: 72-82 [https://doi.org/10.1016/j.ocecoaman.2018.01.009]

Stokes GL, Lynch AJ, Lowe BS, Funge-Smith S, Valbo–Jørgensen J, Smidt SJ (2020) COVID-19 pandemic impacts on global inland fisheries. PNAS 117 (47): 29419-29421

Original Article

Seasonal difference in marine litter along the coast of Dar es Salaam, Tanzania

Florence J. Peter[1,2], Anale M. Rocha[3]

[1] Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Brussels, Belgium

[2] Institute of Marine Sciences, University of Dar es Salaam, PO Box 668 Buyu, Zanzibar, United Republic of Tanzania

[3] Nipe-Fagio, PO Box 11747, Dar es Salaam, Tanzania

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*** Corresponding author:**
frolencejovenary@gmail.com

Abstract

Marine litter accumulation poses a significant threat to marine ecosystems and biodiversity. Marine litter and the influence of river discharge on marine litter accumulation during wet and dry seasons along the coast of Dar es Salaam was investigated. Litter samples collected between 2019 and 2021 from transects on beaches and by nets spanned across two rivers were counted, dried and weighed, and litter counts and weight (kg) calculated by area (litter/m^2) and volume (litter/m^3). Over three years, a total of 127,658 marine litter items weighing 470 kg were sampled from beaches, and 1,365 items from rivers, in nine different litter categories. Most of the litter (70 %) was produced locally and the marine litter density decreased by 61 % over the three years of the study. Litter densities at beaches and rivers were significantly greater during the wet than the dry season, attributed to the transport of litter in rivers to the ocean, especially plastics, which had a higher density than other riverine litter. It is concluded that high local plastic production and poor waste disposal systems require an intervention, and that a policy to minimize and reduce marine litter should be developed and implemented.

Keywords: marine litter, pollution, Dar es Salaam, plastics

Introduction

Marine litter is a global problem due to poor waste disposal management (Smail *et al.*, 2019) and has been associated with growth in industrial production (Salgado-Hernanz *et al.*, 2021). Schmidt *et al.* (2017) estimate that approximately 12 million tons of marine litter enters the oceans each year. It is also estimated that 80 % of the marine debris originates from the land and is transported to the ocean via surface runoff (Meijer *et al.*, 2021) and the rest originates from fisheries activities, shipping, ocean currents and tourism (GESAMP, 2015). This has severely impacted the environment worldwide, resulting in a deterioration in water quality and aquatic and terrestrial ecosystems, which impacts biodiversity (Barnes *et al.*, 2009). A significant proportion of marine litter is dominated by plastic debris (ranging from 50 % – 80 %) in comparison to other litter types (Cressey, 2016; Okuku *et al.*, 2021). The negative effects of plastic pollution in the environment are further exacerbated by its comparatively high persistence in nature, prolonging its negative effects on ecosystems (Lebreton *et al.*, 2017; Worm *et al.*, 2017).

Global industrial production of plastic has increased from 322 million tons in 2014 to 368 million tons in 2019 (Plastics Europe, 2021). This further complicates efforts to manage the ever-increasing resultant waste, particularly regarding single-use plastics (Barboza *et al.*, 2018). However, during the COVID-19 pandemic between 2019 to 2021, plastic production decreased by approximately 0.27 %, to about 367 million tons,

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due to a global decline in production across all sectors (Plastics Europe, 2021). In 2019, Tanzania became one of the few countries in the world that has imposed a ban on the use of disposable plastics (URT, 2019). Poor policy and lack of a marine litter action plan, however, has delayed the implementation of the ban in mainland Tanzania, while the Tanzanian Island of Zanzibar introduced a ban in November 2006 (Maione, 2021). However, the management of single-use plastic waste remains a major challenge in the Global South, including Tanzania and neighbouring countries (Ammendolia *et al.*, 2021). A recent brand audit conducted on marine litter in Kenya revealed that about 16 % of the litter originated from Tanzania (Okuku *et al.*, 2021).

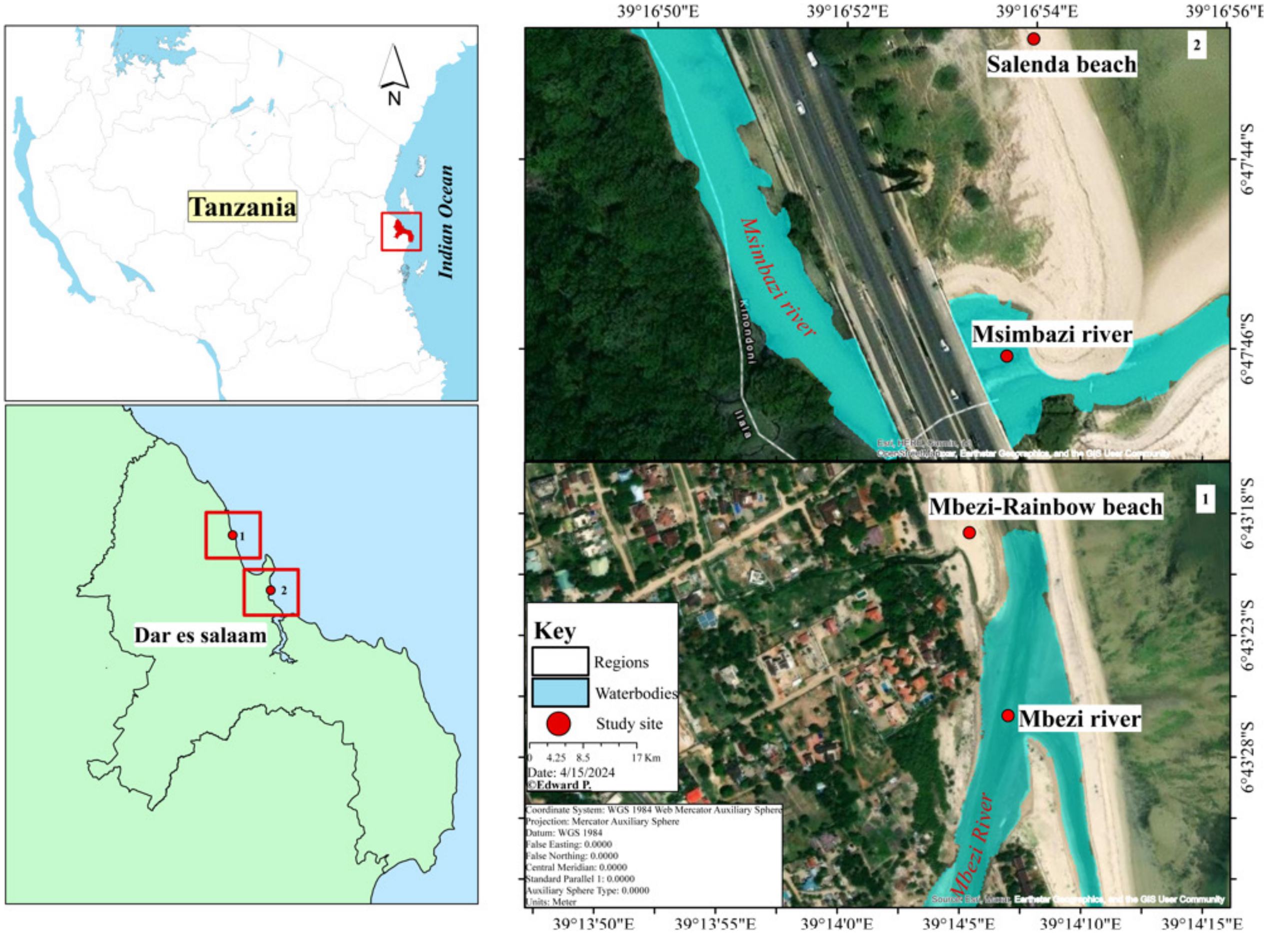


Figure 1. Study areas in Dar es Salaam, with sampling sites at Mbezi Rainbow Beach and Salenda Beach, and adjacent Mbezi and Msimbazi Rivers.

According to the United Nations Environmental Programme, marine litter may be defined as "*any persistent, manufactured or processed solid materials discarded, disposed of or abandoned in the marine and coastal environment. Marine litter consists of items that have been made or used by people and deliberately discarded into the sea or river or on beaches; brought indirectly to the sea with rivers, sewage, stormwater, or winds; accidentally lost, including material lost at the sea in bad weather; or deliberately left by people on the beaches and shores*" (UNEP, 2021). Marine litter occurs in different forms, including paper, cardboard, hygiene products, processed wood, glass, rubber, clothing, plastics, fishing gear, metal, foam, construction material, and pottery (Potts and Hastings, 2011; Barnardo and Ribbink, 2020). Plastic waste accounts for 75 % of marine litter, representing the highest proportion in comparison to other litter types (Galgani *et al.*, 2019). Plastic debris is grouped into three categories based on size, viz. macroplastic (> 25 mm), mesoplastic (5-25 mm), and microplastic (< 5 mm) (Barnes *et al.*, 2009; Barnardo and Ribbink, 2020). Additional categories referring to megaplastic and nanoplastic have recently been described to include plastic debris > 1 m (Smail *et al.*, 2019) and < 1μm (Ramkumar *et al.*, 2022) in diameter respectively. Currently, approximately 75 % of marine plastic pollution studies are focusing on microplastics worldwide, which implies macro and mesoplastic pollution remain comparatively under-investigated. Most of these studies (approximately 80 %) are executed in the Global North (Blettler *et al.*, 2018).

The accumulation of marine litter contributes significantly to the deterioration of marine ecosystems and their biodiversity (Hastings and Potts, 2013). The impact of marine litter has been worsening progressively, resulting in a myriad of negative impacts on marine organisms. These impacts include entanglement (Potts and Hastings, 2011), ingestion, destruction of breeding grounds, reproduction problems (Laist, 1987), and developmental impairment (Fowler, 1987), therefore, making unconducive environments for organisms to live in. This is attributable to its non-biodegradable nature, which makes it highly persistent in the environment over long periods of time (Galgani *et al.*, 2019). Additionally, plastic litter often fragments into minute particles (micro- and nanoplastics), that can have major, sustained impacts throughout marine food webs through bioaccumulation and bioconcentration (Miller, *et al.*, 2020).

Several beach clean-ups along the Dar es Salaam coast have helped reduce the marine litter problem, but the rate of riverine discharge of litter and discards by coastal communities exceeds the rate of clean-up (Rangel-Buitrago *et al.*, 2018). Therefore, this study aimed at the quantification of marine litter at selected sites along the Western Indian Ocean coast in Dar es Salaam, Tanzania to bring the concerned ministries together for effectively policy implementation (URT, 2019).

Material and methods

Study area

The study was conducted in Dar es Salaam (Fig. 1), a city with diverse economic activities, including a major port, large markets and shopping centers, railway, construction, industries and factories, and small enterprises. It is among the fastest-growing cities in Africa, ranked third in 2010 (Mkalawa and Haixiao, 2014). Dar es Salaam is densely populated, with an estimated population of 7 million people and an annual growth rate of about 5.2 % (Dar Es Salaam Population, 2021). Samples were collected from four sampling sites, two on beaches and two in rivers, namely: Mbezi Rainbow Beach and Salenda Beach (Salenda bridge), and the adjacent Mbezi and Msimbazi Rivers. These are the major rivers traversing different localities in Dar es Salaam, flowing into the Indian Ocean.

Sampling

Beaches

The survey design was based on the accumulation and standing-stock survey method, in a 5 000 m^2 transect (250 m length, 20 m width) (Fig. 2) (Barnardo and Ribbink, 2020). Sampling was done during low tide. Two transects were set on each beach. The sampling was based on each category of marine litter count. The data were collected in two seasons, viz. the dry (July) and wet (April) season, over a period of three years (2019 to 2021). Before sampling the beaches were cleaned two to three days before the survey and the accumulation survey was carried out on 10 consecutive days. Two sections of the beach were sampled, the intertidal and supratidal zone. Litter from the intertidal zone was dried, sorted based on the category of the litter, counted, and weighed. Litter from the supratidal zone was also sorted according to litter type, counted, but not dried before weighing. A waste audit and brand audit (WABA) was implemented to identify the source (company, country) and nature of the litter (Barnardo and Ribbink 2020). This was done by looking at the label which indicates the manufacturer details and in which country the item was made.

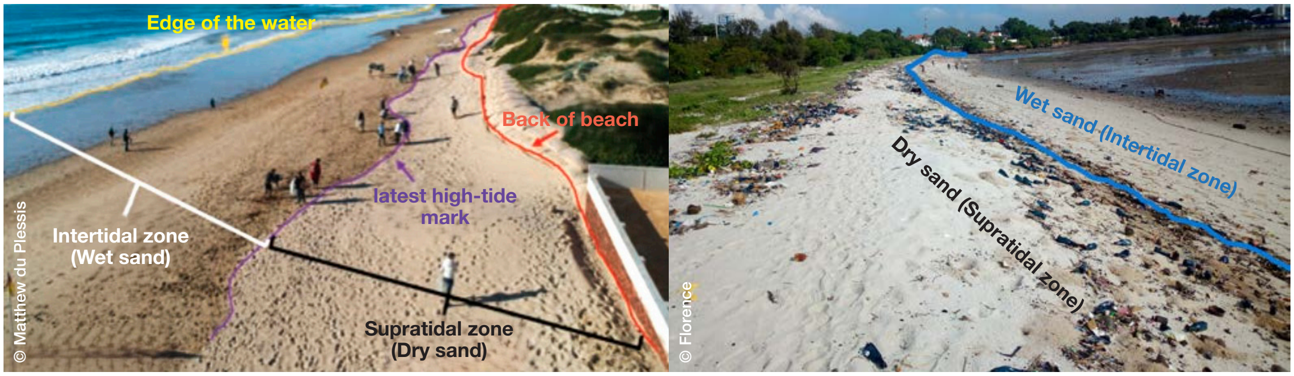


Figure 2. The supratidal and intertidal zone where transects of 250 m length and 20 m width were placed for marine litter sampling.

Rivers

A net of 2 mm mesh size fixed to a metal frame of 2 m length, 1 m height, and 1 m width, was used to collect litter in the rivers (Barnardo and Ribbink, 2020). The length, width, and height of the net allowed the calculation of litter/m^3. The net was fixed with ropes under a bridge perpendicular to the current flow (Fig. 3). To estimate the current velocity, the width of the bridge was measured. A stopwatch was then used to measure the time a plastic object moved under the bridge on three occasions, and the average velocity was calculated. Velocity was grouped in three categories: low velocity (0.1 m/s to 0.6), moderate velocity (0.6 m/s to 2 m/s), and high velocity (2 m/s). The net was lifted four times (duplicates); every 30 minutes each from the river, 15 minutes each for taking data and setting the net back into the river for a period of three hours. Data in each duplicate was sorted per litter type, summed and averaged to get total count as 1 sample of a day. This procedure was carried out on 10 consecutive days. Therefore, the samples (the litter type count) for each of the 10 days were summed to get the total count as one large sample of a given season. 1 sample (total count) was obtained from each river per season, which makes a total of 4 samples for two seasons every year and a total of 12 samples in three years. The lower part of the net was lifted first to keep the entangled marine litter in the net. The material collected by the net from the river was counted, dried, and weighed (Barnardo and Ribbink 2020).

Data analysis

The mean density of litter on the beaches in each season per year was calculated as counts of items per area (m^2), and for litter in the rivers items/m^3 (Smail *et al.*, 2019). Litter in the rivers and on beaches for both dry and wet seasons were summed, then divided by volume and the area to get the mean density of marine litter for each year respectively. Marine litter density by weight per area was calculated as kg/m^2 and litter from the river was calculated in kg/m^3 (Edyvane *et al.*, 2004). The percentage composition of marine litter was also calculated (Okuku *et al.*, 2020, 2021).

Descriptive statistics were computed using Microsoft Excel and R-Studio (Barnardo and Ribbink2020). The mean current velocity in the river was expressed as m/s (Li *et al.*, 2020). One-way ANOVA was used to test for significant differences in the marine litter contribution between seasons for both river and beach respectively (Okuku *et al.*, 2021). Two-way ANOVA was used to test for significant differences between seasons and years on beaches.

Cluster (Principle component analysis and Agglomerative hierarchical clustering) was used to investigate the relationship between marine litter composition and abundance in seasons and zones respectively (Asensio-Montesinos *et al.*, 2019). Analysis of Similarity (ANOSIM) was also used to test the similarity in marine litter abundance between seasons. (Pham *et al.*, 2014).

Results

Marine litter composition and its trend along the coast of Dar es Salaam

A total of 127,658 marine litter items, equivalent to a total weight of 470 kg, were sampled in the three years on the beaches. The items were composed of clothing and fishing gear, and hygiene, metal, foam, paper, wood, plastic, and rubber objects. The density of litter decreased from 5.7 ± 2.09 items/m^2 in 2019 to 2.2 ± 0.06 items/m^2 in 2021 (Fig. 5). However, two-way ANOVA showed no significant difference between the Mbezi Rainbow Beach and Salenda Beach and between the rivers (Fig. 4). Locations (Mbezi and Salenda) showed no significant differences (F(1, 106) = 2.63, p = 0.11.

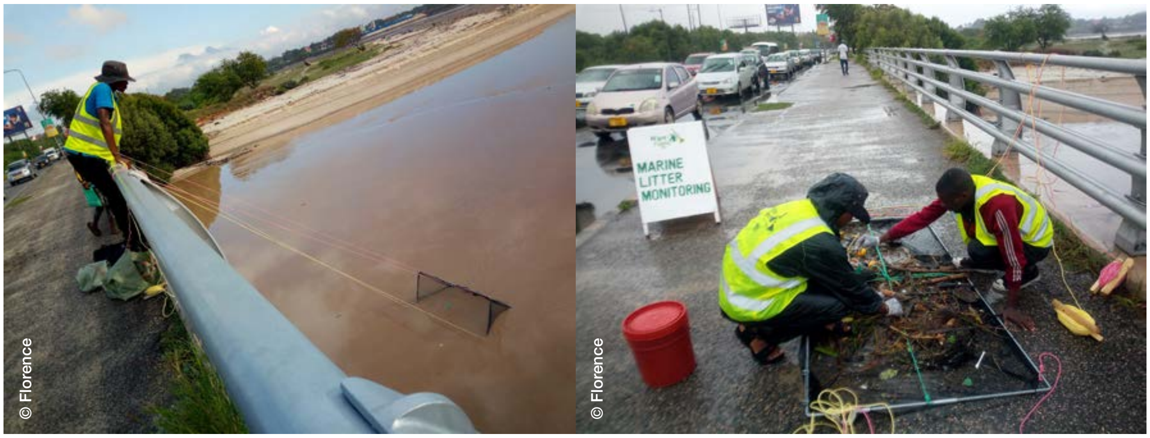


Figure 3. Placement of the net used to sample litter in the rivers.

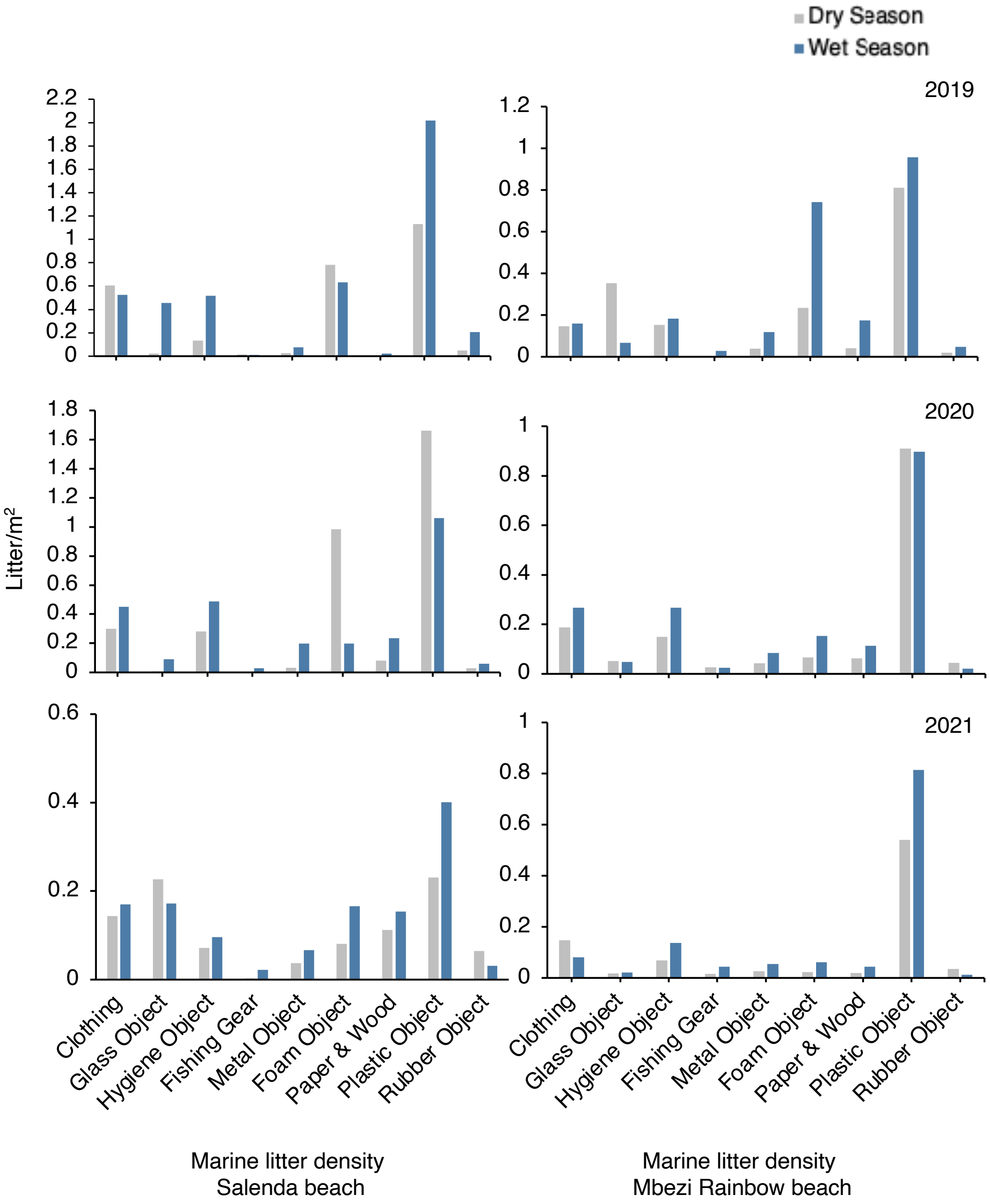


Figure 4. Marine litter composition at the two beach sites during dry and wet seasons.

Also, the interaction between year and seasons showed no significance difference F(1, 106) = 0.12, p = 0.72. However, one-way ANOVA showed significant differences between years F (2, 106) = 3.58, p = 0.03.

Marine litter distribution during dry and wet seasons

Agglomerative Hierarchical Clustering (AH) identified two major clusters (Fig. 6). The first cluster includes dry season samples DSM19, DSS19 and DSS20, and one wet season sample WSS19. The second cluster has two sub-clusters of dry and wet season samples. The dry season samples included DSS21 and DSSM21, while the wet season divides further into two clusters, including WSS21 and WSM21 and another cluster of

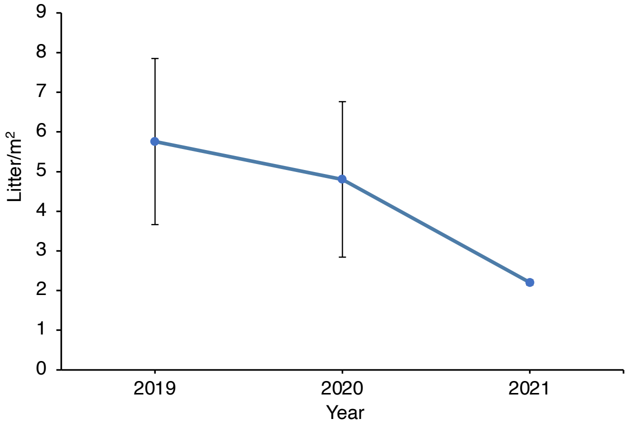
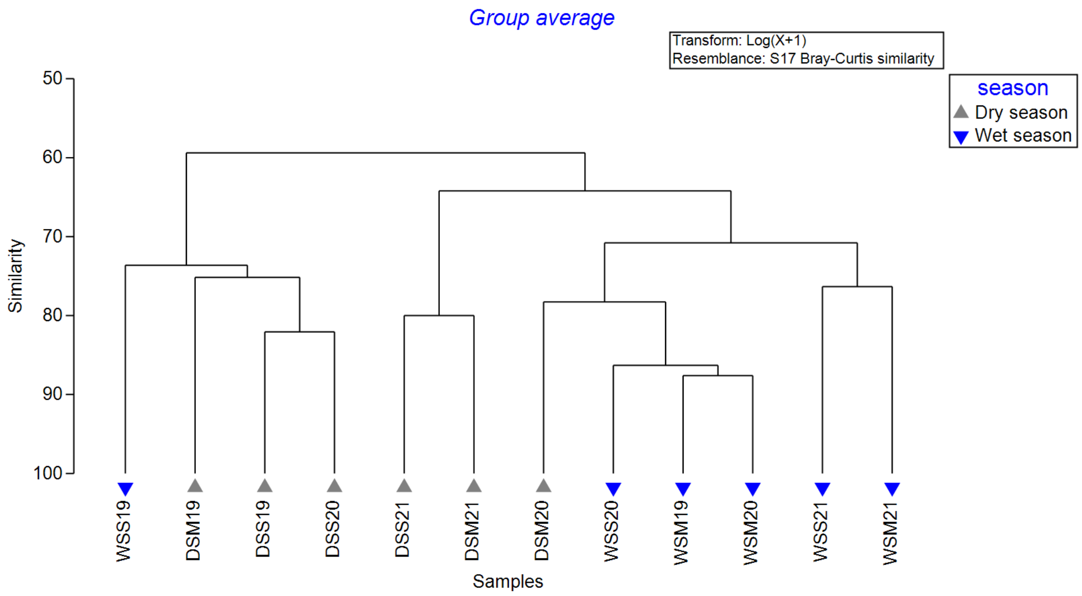


Figure 5. Decrease of marine litter density from 2019 to 2021.



Key: DSM and DSS – dry season Mbezi Beach and Salenda Beach, WSM and WSS – wet season Mbezi and Salenda, while 19, 20 and 21 are years 2019, 2020 and 2021 respectively.

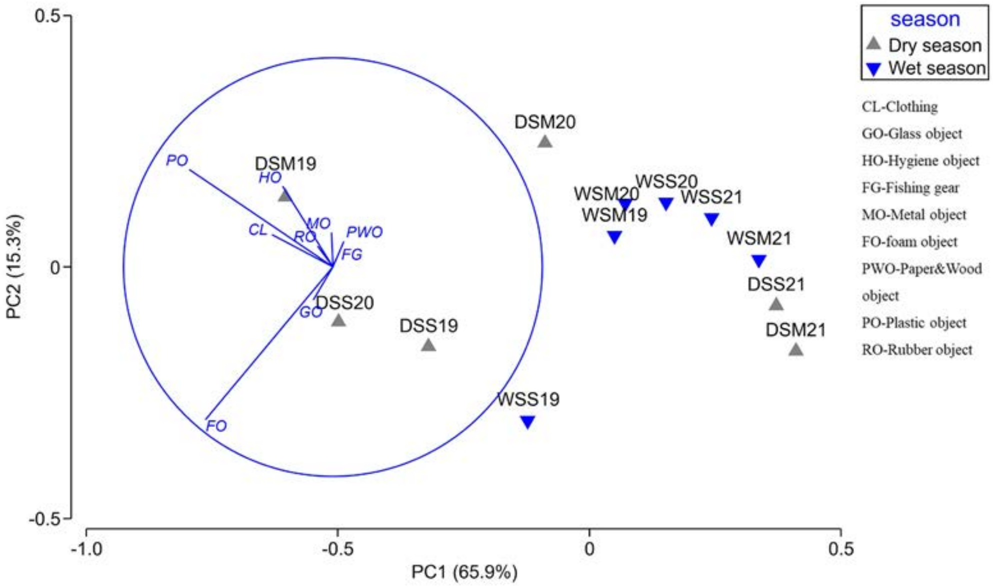
Figure 6. Bray-Curtis similarity of litter density during the wet and dry season in three years per location.

WSM19, WSM20 and WSS20 and DSM20 (Fig. 6). These cluster divisions are based on Bray-Curtis similarities in marine litter densities.

PCA shows that there was a higher density of foam and plastic objects in DSM19, DSS20, DSS19, and WSS19 than FG, HO, MO, RO, CL, PWO and GL. However, PO, FO, HO, CL, RO show low density abundance in DSS21, DSSM21, WSM21, WSS21 and WSS20. PO and FO show the relationship between them (Fig. 7). With ANOSIM test, years tested significantly different and these differences were between 2019 and 2021, as well as 2020 and 2021 except 2019 and 2020 (R = 0.3889, p = 0.013).

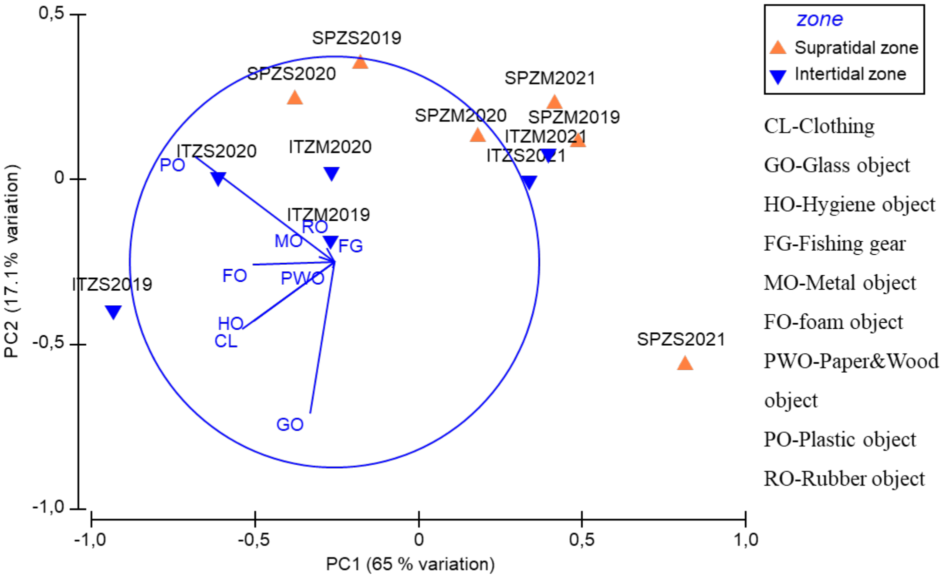
Marine litter composition and distribution in the supratidal and intertidal zone

The cluster analysis (PCA) shows higher density of PO, RO, FO, MO, and HO in ITZS2019, ITZM2019, SPZS2019, ITZS2020, ITZM2020, and SPZS2020, but low density in SPZM2019, SPZM2020, SPZM2021,



Key: DSM and DSS – dry season Mbezi Beach and Salenda Beach, WSM and WSS – wet season Mbezi Beach and Salenda Beach, while 19, 20 and 21 are years 2019, 2020 and 2021 respectively.

Figure 7. Principle Component Analysis of litter during the wet season and dry season from 2019 to 2021.



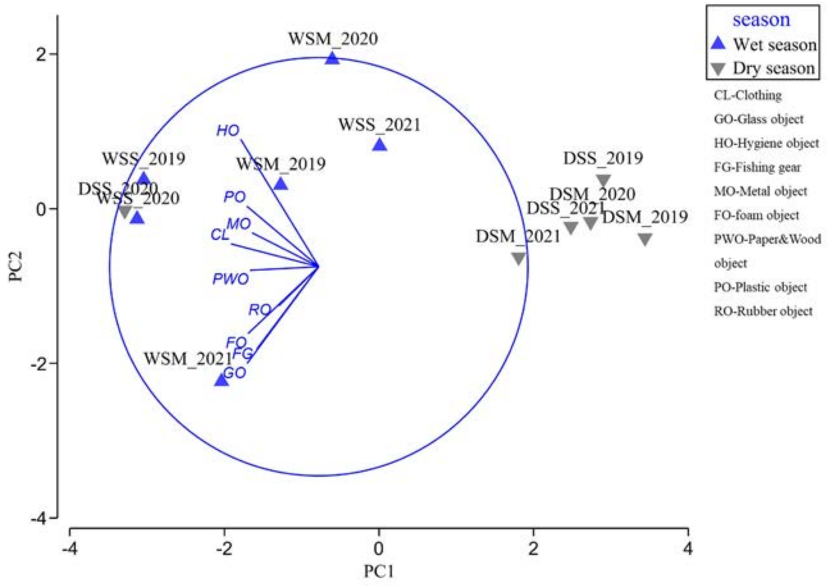
Key: ITZS – Intertidal zone at Salenda Beach, ITZM – Intertidal zone Mbezi Beach, SPZS – Supratidal zone at Salenda Beach and SPZM - Supratidal zone at Mbezi Beach.

Figure 8. Principle Component Analysis of litter density in the zone from Mbezi Rainbow Beach and Salenda Beach.

ITZM2021, ITZS2021 and SPZS2021. However, CL, PWO, FG and GO showed low density in SPZM2019, SPZM2020, SPZM2021, ITZM2021, ITZS2021 and SPZS2021 but have moderate abundance in ITZS2019, ITZM2019, SPZS2019, ITZS2020, ITZM2020, and SPZS2020. The density of marine litter gave a total of 82.1 % variation as PC1 encountered 17.1% of variation while PC2 encountered 65.0 % variation (Fig. 8).

Contribution of the rivers to marine litter

A total of 1,365 items of litter were sampled in the rivers from 2019 to 2021, and nine categories of litter as mentioned above. PCA, riverine litter showed high density during the rainy season compared to the dry season, but the density of riverine litter during the rainy season varied among locations. Plastic objects, metal objects, clothing, hygiene objects, paper and



Key: WSS – Wet season sample at Msimbazi River , WSM – Wet season sample at Mbezi River, DSS – Dry season sample at Msimbazi, and DSM -Dry season sample at Mbezi River in respective years.

Figure 9. PCA of litter per season from Mbezi and Msimbazi Rivers.

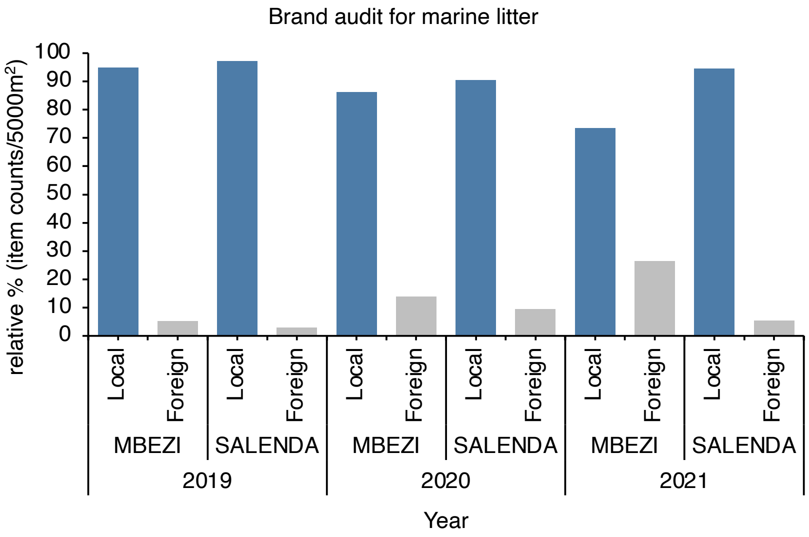


Figure 10. Proportion of marine litter branded by local and foreign manufacturers.

wood objects had a high density in seasonal samples WSS_2019, WSM_2019, WSS_2020, RSM_2020 and DSS_2019 while glass objects, fishing gear, and foams had a higher density in WSM_2021. However, riverine litter had a lower density in dry season samples except in sample DSS_2020 (Fig. 9). ANOSIUM shows no significant difference between the rivers Mbezi and Msimbazi (R = 0.34, p = 0.25). Also, ANOSIM analysis shows significant difference between the rainy and dry season (R = 0.387, p = 0.02).

Brand Audit

Local and foreign contributions to marine litter

Twenty-six brands were identified in the litter, involving local and foreign manufactured objects. Only 27,321 objects were assigned for brand audit among 127,658 objects sampled in total. Local brands contributed 70 % and foreign brands 30 %, which is equivalent to 19,124 and 8,196 objects, respectively. This shows that there is higher production and/or usage in the country than what is introduced from outside (Fig. 10). However, 30 % contributed by foreign branded objects included 21 % from China and 9 % from Kenya.

Contribution to marine litter accumulation at the local level and main manufacturers

At the local level, about 40 % of plastic items were manufactured by the Melt Group Ltd., followed by about 20 % by Azam and 10 % by Plastic Production Co. Ltd. Other manufacturers contributed <10 % (Fig. 11).

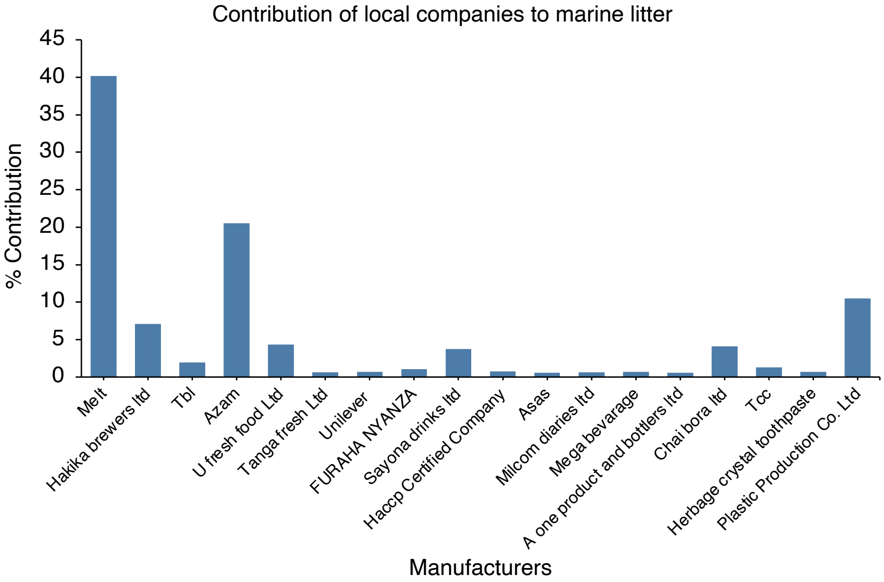
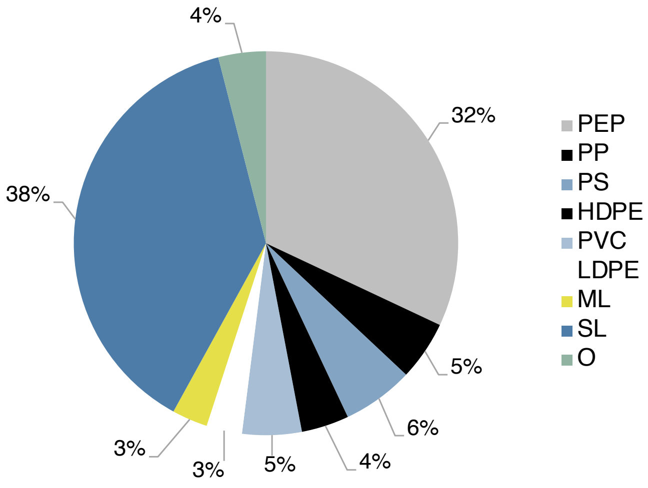


Figure 11. Manufacturers whose items contributed to marine litter along the Dar es Salaam coast.



Key: PET-Polyethylene, PP-Polypropylene, PS-Polystyrene, HDPE-High-density polyethylene, PVC-Polyvinyl, and LDPE-Low-density Polyethylene, ML-Multilayer, SL-Single layer, O - others.

Figure 12. Types of plastic contributing to marine litter.

Type of plastics that contributed to marine litter

Different forms of plastics were identified during sampling (Fig. 12). These included Polyethylene (PET), Polypropylene (PP), Polystyrene (PS), High-Density Polyethylene (HDPE), Polyvinyl (PVC), and Low-Density Polyethylene (LDPE), Multilayer (ML), Single layer (SL), and other (O). Plastic objects showed higher diversity in all zones and seasons than other litter. SL and PET contributed more (38 % and 32 %, respectively) compared to other forms of plastics, while the contribution of ML and LDPE (each 3 %) was lower.

Discussion

Marine litter accumulation and composition on the beaches

A higher density of marine litter was found in most wet season samples compared to dry season samples. This was probably due to runoff that collects litter from towns, households, cities, and roads and unorganised dumping grounds into the river and then into the marine environment (Santos *et al.*, 2020). On the one hand, the decreasing trend of marine litter, especially in plastics, was due to the single-use plastic ban policy and law that was made in 2019, and other initiatives (URT, 2019). Each town council is required to develop a strategy to keep the environment clean, such as recycling plastics (URT, 2019). Some waste collecting trucks were bought in to collect waste from households. Different initiatives to minimise marine litter and waste in general involve zero waste, zero plastic, and a single-use plastic free East African Community (EAC) (Huaxia, 2021; GAIGA, 2022), world ocean day, world environment day, marine litter action plans and weekly clean-ups along the coast of Tanzania as well as Dar es Salaam. The active engagement and involvement of stakeholders, including children from primary and secondary schools, universities, ministries, coastal communities, organisations, political leaders, and musicians has contributed to the decrease in marine litter accumulation along the Dar es Salaam and other parts of the coast (Pettipas *et al.,* 2016).

Litter accumulation and composition in the intertidal and supratidal zone

The higher density of plastics in the intertidal and supratidal zones than other marine litter is because, during low tide, when the intertidal zone is exposed to the sun and dries out, some light waste from the intertidal zone can be blown by strong winds to the supratidal zone (Barnardo *et al.*, 2021). This might be the reason why foam objects had a higher density in the supratidal zone than in the intertidal zone. However, the decrease in litter in the intertidal and supratidal zones, especially between years 2019 and 2021 as well as 2020 and 2021, might be due to the introduction of the single-use plastic ban and other efforts, including weekly clean-ups, that have been made to reduce waste in the marine and terrestrial environment in Tanzania (URT, 2019).

Brand Audit

The higher proportion of locally produced objects in the litter might be due to industrial investments made in the country (Salgado-Hernanz *et al.*, 2021). However, it is apparent that the industries producing plastics do not consider environmentally friendly alternatives that are biodegradable, resulting in materials accumulating in the oceans (Hastings and Potts, 2013). The Melt Group, Azam Group, and Hakika Breweries Ltd. manufacture more than one product, accounting for the higher contribution of litter from these compared to other companies. Bakhresa food products Co. Ltd is also one of the top three manufacturers (23.8 %) that contributes to waste accumulation along the coast of Kenya (Okuku *et al*. 2021). There is a higher percentage (32 %) of Polyethylene (PET) in the environment, because most beverages are sold in PET bottles. PET bottles are very light, allowing them to be transported easily in water, accounting for the higher accumulation compared to other litter (Shilla, 2019).

Contribution of the rivers to marine litter accumulation on beaches

The transport of waste in rivers in the wet season to the ocean, especially plastic that has a higher density than other riverine litter, is due to poor waste disposal and high plastic production in the country. The current study revealed that marine litter increases during the wet season, because runoff collects wastes from land and discharges it into the ocean (Silva-Cavalcanti *et al.,* 2009; Silva *et al.,* 2016; Santos *et al.,* 2020). A study in the South East Pacific suggests that rivers are a major agent of marine litter accumulation in the ocean (Rech *et al.,* 2014).

Conclusions

The wet season produced a higher density of marine litter than in the dry season, and plastic is highly abundant in both seasons compared to other marine litter. Plastics showed a higher density in the intertidal zone than in the supratidal zone. This was also the case for riverine litter, where plastic objects showed higher density than other riverine litter. It can be concluded that local manufacturers are the main producers of most litter discarded into the ocean compared to foreign products. This is greatly influenced by industries' production, poor waste management, and lack of a marine litter action plan. The change in people's behaviour, reduction in industrial production, particularly of plastics, as well as a proper policy enforcement, will help to reduce the pollution of litter in marine environments. This will not only improve marine life, but will protect the health of humans in general. Several entities, including government, industries, traders, neighbouring countries, organisations and local people should continue to implement the single-use plastic ban. Therefore, stakeholders should come up with innovative packaging that is recyclable or reusable and introduce penalties and punishment that will change people's behaviour. In terms of further research, litter quantification in the offshore marine environment is required.

Reference

Ammendolia J, Saturno J, Brooks AL, Jacobs S, Jambeck JR (2021) An emerging source of plastic pollution: Environmental presence of plastic personal protective equipment (PPE) debris related to COVID-19 in a metropolitan city. Environmental Pollution 269: 116160 [doi: 10.1016/j.envpol.2020.116160]

Asensio-Montesinos F, Anfuso G, Randerson P, Williams AT (2019) Seasonal comparison of beach litter on Mediterranean coastal sites (Alicante, SE Spain). Ocean and Coastal Management 181: 104914 [doi: 10.1016/j.ocecoaman.2019.104914]

Barboza LGA, Cózar A, Gimenez BCG, Barros TL, Kershwa PJ, Guilhermino L (2018) Macroplastics pollution in the marine environment, Second Edition. World seas: An environmental evaluation Volume III: Ecological issues and environmental impacts. Second Edition. Elsevier Ltd. 17: 305-328 [doi: 10.1016/B978-0-12-805052-1.00019-X]

Barnardo T, Niekerk TMV, Pichegru L, Marlin D (2021) The first baseline estimation of marine litter in Port Elizabeth, South Africa. Marine Pollution Bulletin 172: 112903 [doi: 10.1016/J.MARPOLBUL.2021.112903]

Barnardo T, Ribbink AJ (2020) African marine litter monitoring manual. 152 pp [www.sst.org.zaForanyqueries, emailinfo@sstafrica.org]

Barnes DKA, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences 364 (1526): 1985-1998 [doi: 10.1098/RSTB.2008.0205]

Blettler MCM, Abrial E, Khan FR, Sivri N, Espinola LA (2018) Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Research 143: 416-424 [doi: 10.1016/j.watres.2018.06.015]

Cressey D (2016) The plastic ocean. Nature 536 (7616): 263-265

Dar Es Salaam Population 2021 (Demographics, Maps, Graphs) (no date) [https://worldpopulationreview.com/world-cities/dar-es-salaam-population]

Edyvane KS, Dalgetty A, Hone PW, Higham JS, Wace NM (2004) Long-term marine litter monitoring in the remote Great Australian Bight, South Australia. Marine Pollution Bulletin. 48 (11-12): 1060-1075 [doi: 10.1016/j.marpolbul.2003.12.012]

Fowler C (1987) Marine debris and northern fur seals: a case study. Marine Pollution Bulletin 18 (68): 326-335

GAIGA (2022) Illegal trade and smuggling of plastic bags in the east African community bloc. An investigative report. 31 pp

Galgani L, Beiras R, Galgani F, Panti C, Borja A (2019) Editorial: Impacts of marine litter. Frontiers in Marine Science 6 (4): 4-7 [doi: 10.3389/fmars.2019.00208]

GESAMP (2015) Sources, fate and effects of microplastics in the marine environment: Part 2 of a global assessment. Reports and Studies. GESAMP (90). 96 pp [file:///C:/Users/BACHEL~2/AppData/Local/Temp/sources-fate-and-effects-of-microplastics-in-the-marine-environment-part-2-of-a-global-assessment-en.pdf%0Awww.imo.org]

Hastings E, Potts T (2013) Marine litter: Progress in developing an integrated policy approach in Scotland. Marine Policy 42: 49-55 [doi: 10.1016/j.marpol.2013.01.024]

Huaxia (2021) Four east African countries join hands to fight plastic pollution. Xinhua, Xinhua [http://www.news.cn/english/africa/2021-12/12/c_1310366676.htm].

Laist D (1987) Bio effects of lost and discarded plastics on marine biota. Marine Pollution Bulletin 18: 319-326

Lebreton LCM, Zwet JVD, Damsteeg J, Slat B, Andrady A, Reisser J (2017) River plastic emissions to the world's oceans. Nature Communications 8: 1-10 [doi: 10.1038/ncomms15611]

Li D, Dong Z, Wang C, Liu J, Yao H (2020) Calculation method for the early warning index of sudden water pollution based on the linear variation assumption of the substance concentration in the river network. Water Resources Management 34 (9): 2821-2835 [doi: 10.1007/s11269-020-02584-7]

Maione C (2021) Quantifying plastics waste accumulations on coastal tourism sites in Zanzibar, Tanzania. Marine Pollution Bulletin 168: 112418 [doi: 10.1016/J.MARPOLBUL.2021.112418]

Meijer LJJ, van-Emmerik T, van-der-Ent R, Schmidt C, Lebreton L (2021) More than 1000 rivers account for 80 % of global riverine plastic emissions into the ocean. Science Advances 7 (18): 1-14. [doi: 10.1126/sciadv.aaz5803]

Miller ME, Hamann M, Kroon FJ (2020) Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. PLOS ONE15 (10): e0240792 [doi: 10.1371/JOURNAL.PONE.0240792]

Mkalawa CC, Haixiao P (2014) Dar es Salaam city temporal growth and its influence on transportation. Urban, Planning and Transport Research, 2 (1): 423-446 [DOI: 10.1080/21650020.2014.978951]

Okuku EO, Kiteresi LI, Owato G, Mwalugha C, Omire J, Otieno K, Mbuche M, Nelson A, Gwada B, Mulupi L (2020) Marine macro-litter composition and distribution along the Kenyan Coast: The first-ever documented study. Marine Pollution Bulletin 159: 111497 [doi: 10.1016/j.marpolbul.2020.111497]

Okuku EO, Kiteresi LI, Owato G, Otieno K, Omire J, Kombo MM, Mwalugha C, Mbuche M, Gwada B, Wanjeri V, Nelson A, Chepkemboi P, Achieng Q, Ndwiga J (2021) Temporal trends of marine litter in a tropical recreational beach: A case study of Mkomani beach, Kenya. Marine Pollution Bulletin 167: 112273 [doi: 10.1016/j.marpolbul.2021.112273]

Pettipas S, Bernier M, Walker TR (2016) A Canadian policy framework to mitigate plastic marine pollution. Marine Policy 68: 117–122 [doi: 10.1016/j.marpol.2016.02.025]

Pham CK, Ramirez-LIodra, Alt CHS, Amaro T, Bergmann M, Canals M, Company BJ, Daves J, Duineved G, Galgani F, Howell LK, Huvenne VIA, Isidro E, Jones DOB, Lastras G, Morato T, Gomes-Pereira JN, Purser A, Stewart H, Tojeira I, Tubau X, Rooij DV, Tyler PA (2014) Marine litter distribution and density in European seas, from the shelves to deep basins. PLoS ONE 9 (4): e95839 [doi: 10.1371/journal.pone.0095839]

Plastics Europe (2021) Plastics Europe Association of Plastics Manufacturers: Plastics—the facts 2021 - An analysis of European plastics production, demand and waste data. Plastics - the Facts 2021. pp 34 [https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/]

Potts T, Hastings E (2011) Marine litter issues, impacts and actions. Scottish Association for Marine Sciences. 139 pp

Ramkumar M, Balasubramani K, Santosh M, Nagarajan R (2022) The plastisphere: A morphometric genetic classification of plastic pollutants in the natural environment. Gondwana Research 108 (May): 4–12 [doi: 10.1016/j.gr.2021.07.004]

Rangel-Buitrago N, Gracia A, Vélez-Mendoza A, Mantilla-Barbosa E, Arana VA, Trilleras J, Arroyo-Olarte H (2018) Abundance and distribution of beach litter along the Atlantico Department, Caribbean coast of Colombia. Marine Pollution Bulletin 136 (August): 435-447 [doi: 10.1016/j.marpolbul.2018.09.040]

Rech S, Macaya-Caquilpan V, Pantoja JF, Rivadeneira MM, Madariaga DJ, Thiel M (2014) Rivers as a source of marine litter - A study from the SE Pacific. Marine Pollution Bulletin 82 (1-2): 66-75 [doi: 10.1016/j.marpolbul.2014.03.019]

Salgado-Hernanz PM, Bauza J, Alomar C, Compa M, Romero L, Deudero S (2021) Assessment of marine litter through remote sensing: recent approaches and future goals. Marine Pollution Bulletin 168 (November 2020): 112347 [doi: 10.1016/j.marpolbul.2021.112347]

Santos AA, Nobre FSdN, Ribeiro F, Nilin J (2020) Initial beach litter survey in a conservation unit (Santa Isabel Biological Reserve, Sergipe) from northeast Brazil. Marine Pollution Bulletin 153 (November 2019): 111015 [doi: 10.1016/j.marpolbul.2020.111015]

Schmidt C, Krauth T, and Wagner S (2017) Export of plastic debris by rivers into the sea. Environmental Science and Technology 51 (21): 12246-12253 [doi: 10.1021/acs.est.7b02368]

Shilla DJ (2019) Status updates on plastics pollution in aquatic environment of Tanzania: Data availability, current challenges and future research needs. Tanzania Journal of Science 45 (1): 101-113 [doi: 10.4314/tjs.v45i1]

Silva-Cavalcanti JS, Barbosa dA MC, Ferreira dCosta M (2009) Plastic litter on an urban beach - A case study in Brazil. Waste Management and Research 27 (1): 93-97 [doi: 10.1177/0734242X08088705]

Silva ML, Sales AS, Martins S, Castro RO, Araújo FV (2016) The influence of the intensity of use, rainfall and location in the amount of marine debris in four beaches in Niteroi, Brazil: Sossego, Camboinhas, Charitas and Flechas. Marine Pollution Bulletin 113 (1-2): 36-39 [doi: 10.1016/j.marpolbul.2016.10.061]

Smail E, Campbell J, Takaki D, Plag H, Garello R, Djavidnia S, Mountinho J, Serafy G E, Giorgettu A, Vinci M, Jack MEM, Larkin K, Wright JD, Galgani F, Topouzelis K, Bernard S, Bowser A, Colangeli G, Pendleton L, Segui LM, Canals LM, Simpson M, Marino A, Zabaleta I (2019) A global platform for monitoring marine litter and informing action. Geoblueplanet.Org (December). 66 pp [https://geoblueplanet.org/wp-content/uploads/2020/03/Marine-Litter-White-Paper-Draft_07Mar2020.pdf]

UNEP (2021) From pollution to solution: a global assessment of marine litter and plastic pollution. New Scientist. 148 pp [https://www.unep.org/resources/pollution-solution-global-assessment-marine-litter-and-plastic-pollution]

URT (2019) (Subsidiary legislation): Kanuni za usimamizi wa mazingira za kupiga marufuku mifuko ya plastiki 100 (20): 1-9

Worm B, Lotze KH, Jubinville I, Wilcox C, Jambeck J (2017) Plastic as a persistent marine pollutant. Annual Review of Environment and Resources 42. pp 1-26 [doi: 10.1146/annurev-environ-102016-060700]

Original Article

Seasonal Dynamics of *Trichodesmium* and Phytoplankton Communities in the Eastern Arabian Sea: Winter Vs Spring-inter monsoon

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Anima Tirkey[1,2]*, Hitesh Solanki[1], Aswathy V. Krishna[2],
Mini Raman[2], Syed M. Ali[2], Arvind Sahay[2]

[1] Department of Botany, Gujarat University, Ahmedabad, India

[2] Space Application Centre, Ahmedabad, India

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*** Corresponding author:**
animatirkey20@gmail.com

Abstract

The filamentous marine cyanobacterium *Trichodesmium* forms extensive blooms in tropical seas, where it plays an important role in nitrogen fixation. This study investigated *Trichodesmium*-associated phytoplankton in the Eastern Arabian Sea (EAS) during bloom and non-bloom conditions. *Trichodesmium* blooms over the continental shelf primarily comprised of *T. erythraeum* and *T. thiebautii* during the Spring Inter Monsoon (SIM) and *T. erythraeum* during the Winter Monsoon (WM). *T. erythreaum* blooms have rarely been reported from the north-east Arabian Sea during WM. Trichomes comprised of 143-1552 cells during the WM, with filaments ranging from 1339 to 13163 µm in length. Non-diazotrophic groups like diatoms and dinoflagellates were associated with *Trichodesmium* during both seasons within the bloom waters. The study found a novel symbiotic relationship between the heterocystous diazotroph *Richelia intracellularis* and *Nitzschia sicula* during WM blooms. This research expands knowledge of lesser-known phytoplankton species and their associations in the EAS.

Keywords: Arabian Sea, Phytoplankton associations, *Trichodesmium* bloom

Introduction

Trichodesmium, a marine cyanobacterium is an important nitrogen-fixer in the sea. It is regarded as the most prominent marine diazotroph and has intriguing traits such as nitrogen fixation without heterocyst formation (Bergman *et al*., 2012). These blue-green algae fix nitrogen, which gets into the marine food web by excretion, grazing, sinking, and decomposition. The marine cyanobacteria's ability to fix nitrogen (N_2) is crucial for the global biogeochemical cycles of nitrogen and carbon. *Trichodesmium* transform N2 into bioavailable ammonium ($NH4^+$), which can support non-diazotrophic phytoplankton in oligotrophic oceans (Chen *et al*., 2011; Oyeku and Mandal, 2020) and help primary and secondary production (Carpenter *et al*., 1999; Sohm *et al*., 2011). *Trichodesmium* accounts for more than 30 % of algal blooms, making them important players in the global biogeochemical cycle (Dugdale *et al*., 1961; Capone *et al*., 1997). *Trichodesmium* is one of the most common bloom-forming phytoplankton in tropical and subtropical oceans, particularly in the eastern Pacific and Arabian Sea (Dugdale *et al*., 1961; Capone *et al*., 1997). It typically forms a prominent cluster or colony, and it regularly produces blooms that are patchy in appearance. The spatial distribution of *Trichodesmium* blooms is often correlated with the physical change of the water body, because the influence of winds can disrupt the organization of bundles which make its distribution uneven and patchy. Warm waters, calm weather conditions, low wind speed, lack of grazers and highly transparent waters are the necessary conditions required for the formation of the *Trichodesmium* blooms (Carpenter and Price, 1976; Lewis *et al*., 1988; Gianesella-Galvao *et al*., 1995; Capone *et al*., 1997, Sellner, 1997; Sarangi *et al*., 2004, Siqueira *et al*., 2006; Blondeau-Patissier *et al*., 2018).

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Past literature revealed the co-existence of diatoms like *Rhizosolenia* sp., *Chaetoceros* sp., *Bacteriastrum* sp., *Asterionella glacialis*, *Leptocylindrus danicus*, *Guinardia cylindrus*, *Thalassionema nitzschiodes*, *Nitzschia longissima, Thalassiosira eccentrica*, *Hemialus hauckii* and *Skeletonema costatum* with *Trichodesmium* during bloom in both the Bay of Bengal and Arabian Sea (Satpathy *et al*., 2007; Jabir *et al*., 2013). Additionally, *Trichodesmium* has been found in association with dinoflagellates like *Scrippsiella* sp., *Gymnodinium* sp., *Ceratium* sp., and *Gonyaulax pacifica* during blooms in the Arabian Sea (Matondkar *et al*., 2006). Diazotrophic cyanobacteria are autotrophic, or symbiotically associated with autotrophs like diatoms and dinoflagellates, and live in the upper euphotic region of the sea (Carpenter and Capone, 2008). The cyanobacteria, *Richelia intracellularis* and *Calothrix rhizosoleniae*, were observed in symbiotic association with diatom genera such as *Rhizosolenia*, *Hemiaulus*, *Bacteriastrum*, *Guinardia* and *Chaetoceros* in warm tropical and subtropical oligotrophic water during *Trichodesmium* blooms (Alldredge and Silver, 1982; Villareal *et al*.,1992; Ferrario *et al*., 1995; Gomez *et al*.,2005; Padmakumar *et al*., 2010; Jabir *et al*., 2013; Madhu *et al*., 2013). The Arabian Sea during the Spring Inter Monsoon (SIM) period is known for its stratified state, resulting in oligotrophic water. *Trichodesmium* releases fixed nitrogen in the form of ammonium or dissolved organic nitrogen, which is then available to a community of nutrient-starved non-diazotrophic phytoplankton (Poulton *et al*., 2009).

Numerous occurrences of *Trichodesmium* blooms in Indian waters between February to May have been reported especially on the west coast (Prabhu *et al*., 1965; Qasim, 1970; Devassy *et al*., 1978; Devassy *et al*., 1987; Shetty *et al*., 1988; Koya and Kaladharan, 1997; Sarangi *et al*., 2004; Krishnan *et al*., 2007; Padmakumar *et al*., 2010; Parab and Matondkar, 2012; D'Silva *et al*., 2012; Martin *et al*., 2013; Jabir *et al*., 2013) as compared to the east coast (Ramamurthy *et al*., 1972; Adhakary and Sahu, 1992; Santhanam *et al*., 1994; Jyothibabu *et al*., 2003; Mohanty *et al*., 2010; Shetye *et al*., 2013). In an attempt to enhance the understanding of the seasonality in *Trichodesmium* and phytoplankton community dynamics, the present study focused on: i) identifying phytoplankton communities within the study area during WM and SIM; ii) quantifying cells per trichome within non-bloom and bloom waters using microscopy; iii) understanding the relationship between *Trichodesmium* and other phytoplankton communities in bloom and non-bloom conditions; and iv) comprehending diazotroph-diatom symbiotic association within blooms.

Materials and methods

Study area

The Eastern Arabian Sea (EAS), part of the tropical basin of the northern Indian Ocean, experiences strong seasonality with semi-annually reversing monsoon winds. Therefore, surface circulation along the EAS reverses every six months. The West Indian Coastal Current (WICC) flows poleward during the WM (November to February) and towards the equator during the summer monsoon (June- September) (Shetye *et al*., 1991). In summer, the WICC transports high-salinity water from the North Arabian Sea southward along the shelf slope region. In winter, the southern EAS is characterized by the occurrence of thermal inversions (Thadathil and Gosh, 1992; Durand *et al*., 2004), while the northern part of the EAS is affected by winter convection which leads to winter blooms (Madhupratap *et al*., 1996; Prasanna Kumar and Prasad, 1996). During the spring-summer transition (April-May), a small warm pool develops in the southern EAS, promoting the formation of the gyre with monsoon onset (Joseph, 1990; Hareesh Kumar *et al*., 2009: Nyadjro *et al*., 2012).

Data collection

An oceanographic cruise was conducted in the Arabian Sea during the SIM from April 15th to May 5th 2017 onboard (FORV-359 Sagar Sampada). Sample collection for microscopic analysis and other parameters was carried out from the southern to the northern Arabian Sea. Massive blooms of *Trichodesmium sp.* were observed in the EAS on May 2nd 2017. A Total of 13 stations (named SS1 to SS13) were covered during this SIM cruise. Another oceanographic cruise was carried out during the WM period in the Arabian Sea from December 15th 2019-January 6th 2020 on board (ORV-364 Sagar Kanya). The cruise track for sample collection started from Goa, moving towards the northern Arabian Sea and further moving southwards up to 4 °N along 64 °W and then diagonally heading towards Goa. A total of 14 sampling points were covered during this cruise named SK1 to SK14. A *Trichodesmium* bloom was also encountered during the WM cruise in the EAS on December 17th 2019. The blooms of *Trichodesmium sp.* observed during both cruises were floating on the surface aligned approximately parallel to the shoreline and distributed over the continental shelf of the EAS which made surface waters appear pale brown to green in colour. Samples were collected from bloom and non-bloom stations for microscopic examinations. The cruise tracks with the station locations are shown in Figure 1.

Hydrographic and meteorological measurements

Hydrographic measurements, such as temperature, salinity and conductivity were obtained using a Conductivity-Temperature-Depth profiler (CTD Sea-bird 911 plus) equipped with a Niskin rosette sampler used for water sample collection from the surface to euphotic depth. The water samples were analysed for phytoplankton taxonomy. Meteorological parameters like wind speed, wind direction, and air temperature was obtained from Automated Weather Station (AWS) on board respective cruises.

Diversity and Evenness analysis

A diversity index is a measure of species diversity in a community that consists of a simultaneous population of several different species, whereas an evenness index shows the relative abundance of species within a selected region. Species richness was calculated using Simpson's diversity index (Simpson, 1949) and species evenness was calculated using Pielou's evenness index (Pielou, 1966) for the purpose of understanding the phytoplankton community within the sampled area during both the seasons.

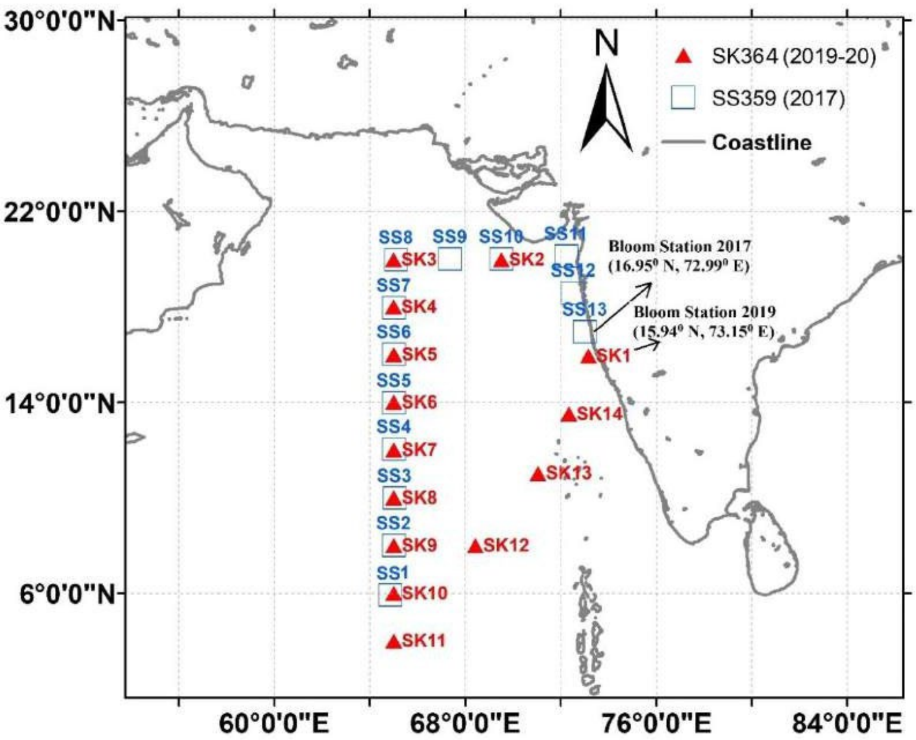

Figure 1. Study area showing the sampling points during SS359 (SS1 to SS13) and SK364 (SK1 to SK14). SS13 and SK1 are the bloom stations in respective seasons.

Phytoplankton identification and enumeration

For phytoplankton identification and enumeration, 1-5 litres of water were fixed with 1 % Lugol's Iodine and preserved with 4 % formaldehyde solution and further stored under dark and cool (4 °C) conditions until microscopic analysis. Samples were concentrated approximately up to 10-15 ml by siphoning the top layer of the sample carefully with a tube. 1ml of concentrated sample was transferred to the Sedgwick-Rafter slide for identification and counting using a Zeiss® Observer A1 AX10 inverted microscope. Phytoplankton samples were enumerated and identified to the lowest possible taxonomic level using a standard taxonomic key (Tomas, 1997).

Simpson's Diversity Index:

$$\boldsymbol{D = 1 - \frac{\sum ni(ni-1)}{N(N-1)}}$$

D: Simpson Diversity Index
ni: Number of individuals belonging to i species
N: Total number of individuals

Pielou's Evenness Index:

$$\boldsymbol{J' = H'/H'max}$$

J': Pielou evenness index
H': The observed value of Shannon index H'max: lnS
S: Total number of species

Results

Hydrographic characteristics of EAS during Spring Inter Monsoon and Winter Monsoon

During the SIM, *in-situ* measured sea surface temperature (SST) was high, ranging from 28-30 °C. The SST at bloom station SS13 was 30 °C. The salinity values ranged from 34.31- 36.73 PSU at the surface with 35.54 PSU at bloom station SS13. Dissolved oxygen (DO) concentration in the surface waters ranged from 3.53-4.13 ml/l. DO value was observed to be highest at the bloom station (4.13 ml/l). During the WM season, SST was relatively lower, ranging from 26-29.52 °C. SST at the bloom station SK1 was observed to be 28.64 °C. Sea surface salinity ranged from 34.73-36.35 PSU, with the lowest value of 34.73 PSU observed at the bloom station SK1. Dissolved oxygen (DO) concentration was comparatively higher during the WM, ranging from 4.19-4.40 ml/l at the surface, with the highest value of 4.40 ml/l observed at the bloom station SK1. During both seasons, the sky appeared to be clear at the bloom stations and wind speeds were observed to be 4 m/s at SS13 and 4.8 m/s at SK1.

Phytoplankton community structure of EAS during SIM and WM

During the SIM, 432 phytoplankton species were identified in the EAS using microscopy. A total of 217 species of diatoms, 198 species of dinoflagellates and 17 species belonging to other algal communities, including *Trichodesmium sp.* were observed. The phytoplankton diversity in the EAS was relatively higher during the WM. Overall, 513 phytoplankton species were identified out of which 280, 212 and 21 species were the respective contributions of diatoms, dinoflagellates and other algal groups including *Trichodesmium sp.* Microscopic analysis showed the dominance of diatoms during both the seasons within the study area except for the bloom stations SS12, SS13 and SK1, where approximately 75-90 % was contributed by *Trichodesmium* sp. Tables 1 and 2 summarize the distribution of the most abundant phytoplankton species observed within the study area during both seasons. During the SIM pennate diatoms such as *Pseudo-nitzschia sp.*, *Nitzschia sp.*, *Asterionellopsis sp.*, *Thalassionema sp.*, *Fragillariopsis sp.* and *Navicula sp.* were dominating phytoplankton species. However, centric diatoms such as *Chaetoceros sp. Bacteriastrum sp. Dactyliosolen sp. Hemiaulus sp. Rhizosolenia sp. Guinardia sp.* and *Leptocylindrus sp.* were the major contributing phytoplankton during the WM throughout the study area.

Taxonomic study of *Trichodesmium* sp. in bloom waters

Massive blooms of *Trichodesmium sp.* were observed to be floating on the surface aligned approximately parallel to the shoreline and distributed over the continental shelf off Ratnagiri during SIM (Fig. 2a) and the continental shelf off Malvan during the WM (Fig. 2b). Surface waters of the bloom station SS13 appeared pale brown to green in colour during the SIM while superficial brownish patches were observed in the surface waters of SK1 during the WM. Microscopic analysis revealed a mixed bloom of *T. erythraeum* and *T. thiebautii* during the SIM contributing 75 % of the total phytoplankton population with cell density 9.13 x 10^6 trichomes l^{-1} at the bloom station SS13, and the remaining 25 % phytoplankton population were predominantly composed of diatoms, dinoflagellates and other algae. *T. erythraeum* was observed to be the major constituent of the bloom out of these two species with cell density 7.86 x 10^6 trichomes l^{-1}, whereas *T. thiebautii* contributed 1.27 x 10^6 trichomes l^{-1} at the bloom station. Two types of colonies were observed; *T. erythraeum* trichomes with parallel straight tuft colonies with attenuated end cell (Fig. 3a), which were composed of 12-55 trichomes, and *T. thiebautii* with radial puff colonies (Fig. 3b), composed of 27-168 trichomes per colony. Each trichome had a length range of 55.55-1209.9 µm and was formed by cells ranging from 12-98 per trichome. During the WM it was observed that the bloom was caused only by *T. erythraeum* with cell density 4.26 x 10^6 trichomes l^{-1}. *T. erythraeum* contributed 67 % of total phytoplankton population at the bloom station SK1 whereas diatoms constituted 31 %, and dinoflagellates and other algae both contributed 1 % of the total phytoplankton population. Results showed that the bloom was comprised of by both individual trichomes (Fig. 3c) and the colonial form (Fig. 3d). Colonies were composed of 25-30 trichomes. It was observed that individual trichomes were found to be more numerous as compared to the colonial form with the very long trichome with length ranging from 1339-13163.49 µm and width of 35-38 µm. Trichomes were found to be comprised of 143-1552 cells per trichome. Thus, the comparative analysis between the seasons show that the bloom waters of the WM were comprised of longer trichomes and the number of trichomes existing individually were higher.

Diazotroph-diatom symbiotic association within blooms

Diazotrophs-diatoms symbiotic association (DDAs) were more diverse during the WM bloom. However, it was observed that during the SIM *Richelia*

Table 1. Distribution of the most abundant phytoplankton species in EAS during Spring Inter Monsoon.

	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13
Centric Diatoms													
Chaetoceros lorenzianus	√	√	√	√	√	√	√	√	√	√	√	√	√
Lauderia annulata	x	√	√	√	√	√	√	√	√	√	√	√	√
Rhizosolenia imbricata	√	√	√	√	√	√	√	√	√	√	√	√	√
Bacteriastrum delicatulum	√	√	√	√	√	√	√	√	√	√	√	√	√
Guinardia striata	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassiosira eccentrica	√	√	√	√	√	√	√	√	√	√	√	√	√
Rhizosolenia styliformis	√	√	√	√	√	√	√	√	√	√	√	√	√
Chaetoceros compressus	√	√	√	√	√	√	√	√	√	√	√	√	√
coscinodiscus radiatus	√	√	√	√	√	√	√	√	√	√	√	√	√
Hemiaulus hauckii	√	√	√	√	√	√	√	√	√	√	√	√	√
Proboscia alata	√	√	√	√	√	√	√	√	√	√	√	√	√
Ditylum brightwellii	x	√	√	√	√	√	√	√	√	√	√	√	√
Pennate Diatoms													
Pseudo-nitzschia australis	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudo-nitzschia delicatissima	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudo-nitzschia heimii	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassionema nitzschiodes	√	√	√	√	√	√	√	√	√	√	√	√	√
Navicula directa	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassiothrix longissima	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudo-nitzschia liniola	√	√	√	√	√	√	√	√	√	√	√	√	√
Fragilariopsis oceanica	√	√		√	x	√	x	√	√	x	√	√	√
Thalassionema bacillare	x	√	√	√	x	√	√	√	√	√	√	√	√
Nitzschia sicula	√	√	√	√	√	√	√	√	√	√	√	√	√
Nitzschia longissima	√	√	√	√	√	√	√	√	√	√	√	√	√
Dinaflagellates	√	√	√	√	√	√	√	√	√	√	√	√	√
Gonyaulax spinifera	√	√	√	√	√	√	√	√	√	√	√	√	√
Ceratium fusus	√	√	√	√	√	√	√	√	√	√	√	√	√
Ceratium vulture	√	√	√	√	√	√	√	√	√	√	√	√	√
Prorocentrum micans	√	√	√	√	√	√	√	√	√	√	√	√	√
Ceratium furca	√	√	√	√	√	√	√	√	√	√	√	√	√
Dinophysis acuminate	√	√	√	√	√	√	√	√	√	√	√	√	√
Alexandrium tropicales	√	√	x	√	√	x	√	√	√	√	√	√	√
Prorocentrum micans	√	√	√	√	√	√	√	√	√	√	√	√	√
Protoperidinium brevipes	√	√	√	√	√	√	√	√	√	√	√	√	√
Scrippsiella trochoidea	√	√	√	x	√	√	√	√	√	√	√	√	√
Protoperidinium pallidum	√	√	x	√	x	√	x	√	√	x	√	√	√
Gymnodinium sanguineum	x	x	√	√	√	x	√	√	x	√	x	√	√
Other Algae													
Dictyocha fibula	√	√	√	√	√	√	√	√	√	√	√	√	√
Octatis octanaria	√	√	√	√	√	√	√	√	√	√	√	√	√
Meringosphaera sp.	√	x	√	√	√	√	√	√	√	√	√	√	√
Dictyocha octanaria	√	√	√	√	x	√	x	√	x	√	√	√	√
Calcidiscus leptoporus	√	√	x	√	x	x	√	x	x	x	√	√	√
Brachiomonas submarina	√	√	x	x	√	√	x	x	√	√	x	√	√
Nephroselmis sp.	√	√	x	x	x	x	√	x	x	√	x	x	x
Trichodesmium erythraeum	√	x	x	x	x	√	x	x	x	√	x	√	√
Trichodesmium thibautii	√	x	x	x	x	x	x	x	x	√	x	√	√
Richelia intracellularis	x	x	x	x	x	x	x	x	x	x	x		√

Table 2. Distribution of the most abundant phytoplankton species in the EAS during the Winter Monsoon.

	SK1	SK2	SK3	SK4	SK5	SK6	SK7	SK8	SK9	SK10	SK11	SK12	SK13	SK14
Centric Diatoms														
Bacteriastrum delicatulum,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Bacteriastrum elongaltum,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Chaeotoceros curvisetus,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Chaetoceros compressus,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Chaetoceros lorenzianus	√	√	√	√	√	√	√	√	x	√	√	√	√	√
Chaetoceros messanensis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Climacodium frauenfeldianum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Coscinodiscus radiates	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Coscinodiscus marginatus	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Coscinodiscus centralis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Dactyliosolen phuketensis,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Dactyliosolen fragilisimus	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Guinardia delicatula	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Guinardia striata	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Guinardia flaccida	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Helicotheca tamesis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Hemiaulus hauckii	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Hemiaulus sinensis	√	√	√	√	x	√	√	x	√	√	√	√	√	√
Leptocylindrus mediterraneus	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Rhizosolenia borealis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Odontella aurita	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Odontell mobiliansis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassiosira gravida	√	√	x	√	√	√	√	√	x	√	√	√	√	√
Rhizosolenia hebtata	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Rhizosolenia imbricata,	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Rhizosolenia styliformis	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Proboscia alata	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassiosira eccentric	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pennate Diatoms														
Lioloma pacificum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassionema nitzschiodes	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassithrix gibberula	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Thalassiothrix longissima	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudo- nitzschia dlicatissima	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Pseudo-nitzschia seriata	√	√	√	√	√	√	x	√	√	√	√	√	√	√
Pseudo-nitzschia subpacifica	√	√	√	√	x	√	√	√	√	√	√	√	√	√
Dinoflagellates														
Protoperidinium depressum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Protoperidinium pallidum	x	√	√	√	√	√	√	√	√	√	√	√	√	√
Protoperidinium pellucidum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Protoperidinium oceanicum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Ceratium furca	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Ceratium trichoceros	√	√	√	√	√	x	√	√	√	√	√	√	√	√
Ceratium vulture	√	√	√	√	√	√	√	x	√	√	√	√	√	√
Ceratium fusus	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Gonyaulax polygrama	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Gonyaulax spinifera	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Nocticula scintillans	√	√	x	√	√	√	√	√	√	√	√	√	√	√
Ornithocercus magnificus	x	√	√	√	√	√	√	√	√	√	√	x	√	√
Phalacroma argus	√	x	√	√	√	√	√	√	√	√	√	√	√	√
Phalacroma rotudatum	√	√	√	√	√	√	√	√	√	√	√	√	√	√

	SK1	SK2	SK3	SK4	SK5	SK6	SK7	SK8	SK9	SK10	SK11	SK12	SK13	SK14
Podolampas bipes	√	√	x	√	√	√	x	√	√	√	√	√	√	√
Prorcentrum micans	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Prorocentrum gracile	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Oxytoxum scolopax	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Protoperidinium divergens	√	x	√	√	√	x	√	x	√	√	√	√	√	√
Scrippsiella trochoidea	√	√	√	√	√	√	√	√	√	√	√	x	√	√
Other Algae														
Trichodesmium erythraeum	√	√	x	√	x	√	√	x	√	√	x	x	√	√
Trichodesmium thiebautii	√	√	x	√	x	√	√	x	√	√	x	√	√	√
Dictyocha fibula	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Dictyocha octanaria	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Octatis octanaria	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Dictyocha speculum	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Hillea fusiformis	x	√	√	√	√	x	√	√	√	x	√	√	√	√
Phaeocystis globosa	√	√	x	√	x	√	√	√	√	√	x	√	√	√
Meringosphaera	x	√	√	√	√	√	√	x	√	√	√	x	√	√
Richelia intracellularis	√	√	√	√	x	x	√	x	x	x	x	x	√	x

intracellularis was associated endosymbiotically with *Guinardia cylindrus* (Fig. 4a) with two trichomes per cell and a single trichome in *Rhizosolenia hebetata* (Fig. 4b) at the bloom station SS13. Trichomes of *R. intracellularis* were made up of 9-17 vegetative cells, with one terminally located heterocyst in *G. cylindrus* and at both ends of the trichomes in *R. hebetata* which was spherical and wider than vegetative cells. While it was noted that during the WM, *R. intracellularis* was living as an endosymbiont inside *R. hebetata* (Fig. 4c-4e), *R. styliformis* (Fig. 4f), *Hemiaulus hauckii* (Fig. 4g), *H. sinensis* (Fig. 4h) and *G. cylindrus* (Fig. 5a) was found in the bloom waters SK1 and non-bloom waters (SK2, SK3, SK4, SK7, SK13, SK14). *R. intracellularis* was observed to be associated endosymbiotically with *Nitzschia sicula* (Fig. 5b-5c) only at the bloom station.

Moreover, *R. intracellularis* was observed to be associated epiphytically with *Chaetoceros decipiens* (Fig. 5d) at SK1. *Calothrix rhizosoleniae* was observed to be associated epiphytically with *Chaetoceros compressus* (Fig. 5e-5f) at non bloom stations like SK3, SK7. Bunches of free living and solitary trichomes of R. intracellularis were also observed at SK1 (Fig. 5g). 1-3 trichomes of *R. intracellularis* were observed inside *R. styliformis* cells, which occurred in single or double form at one or both ends of the host. A single trichome was observed inside *N. sicula*, located at the centre of the valve. The endosymbiotic association between *R. intracellularis* and *N. sicula* has not been reported earlier. Trichomes of *R. intracellularis* were made up of 6 - 16 vegetative cells, with a terminally located heterocyst at one end or both ends of the trichomes, which were

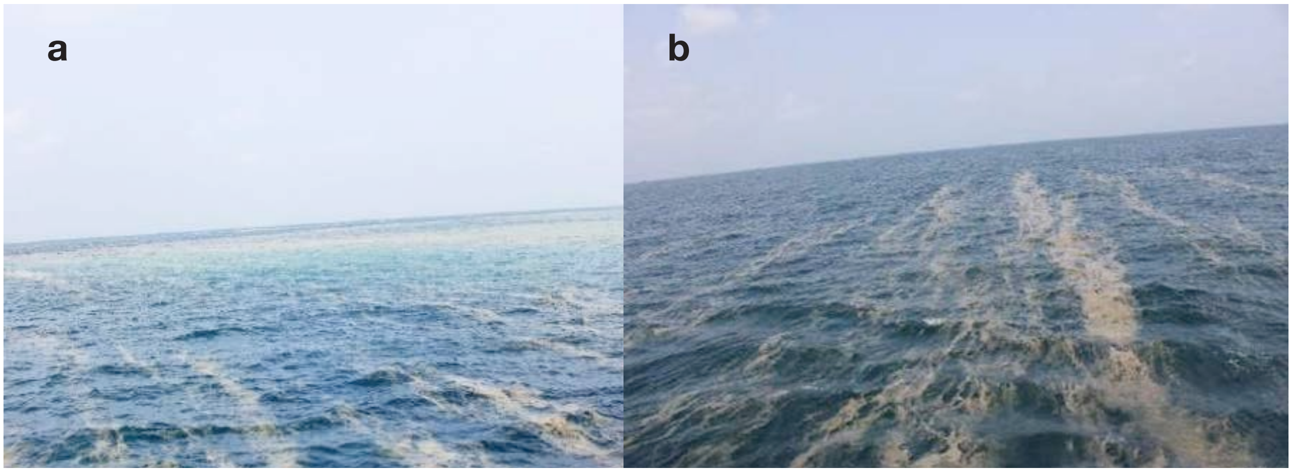

Figure 2. Field photograph of *Trichodesmium* bloom (a) off Ratnagiri during Spring Inter Monsoon (May 2nd 2017) captured on-board FORV-Sagar Sampada 359, and (b) during the Winter Monsoon (December 17th 2019) captured on-board ORV-SK 364.

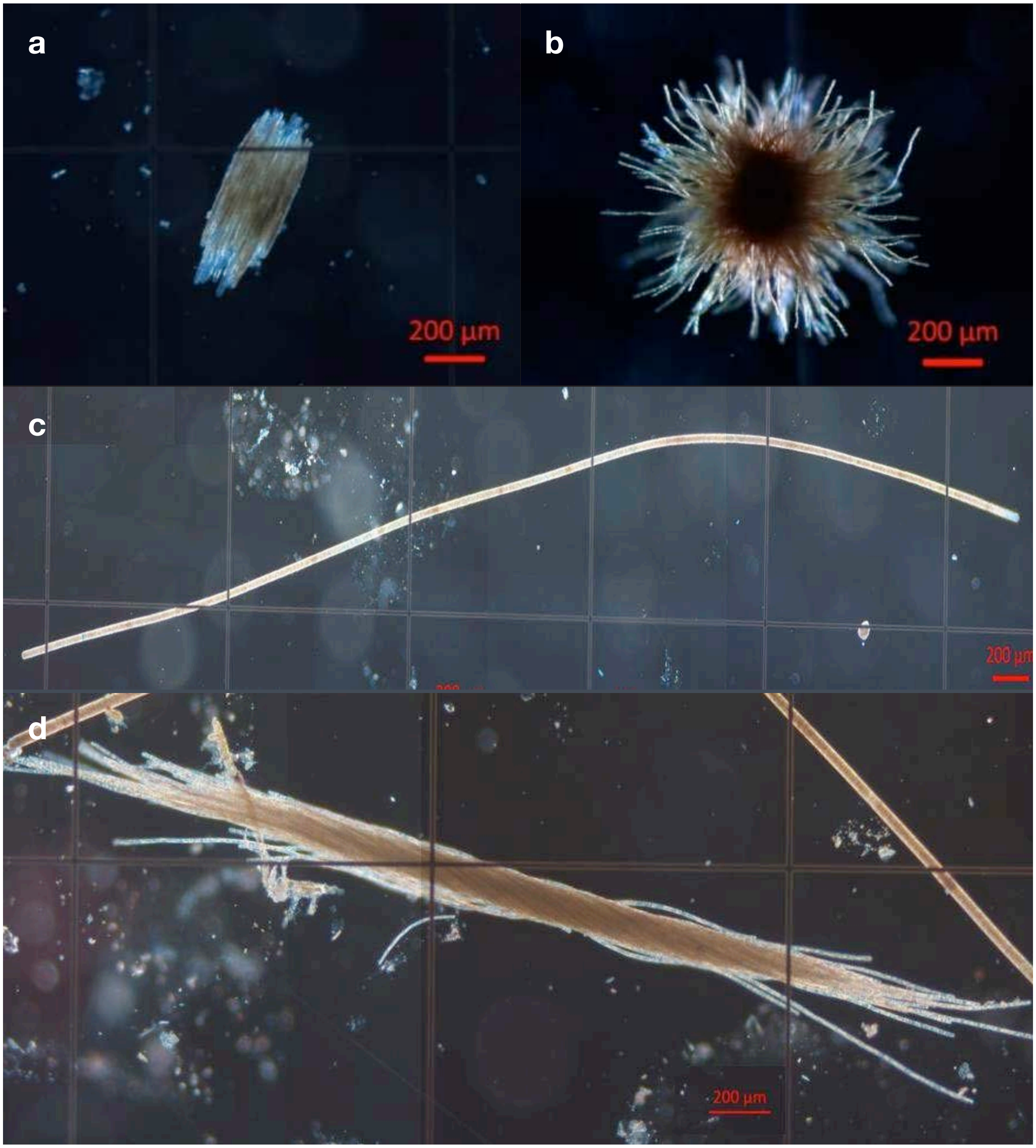

Figure 3. (a) T. erythraeum tuft colony, (b) T. thiebautii puff colony, (c) Single trichome of T. erythreaum, (d) Tuft colony of T. erythreaum (a,b – SIM; c,d – WM).

spherical and wider than vegetative cells. Free living trichomes were observed to have one terminally located heterocyst.

Trichodesmium sp. versus other phytoplankton community

Phytoplankton community structure showed variability with respect to the abundance of *Trichodesmium sp.* It is observed from Figure 6 that the diatoms and dinoflagellate abundance increased with the increase in number of *Trichodesmium* trichomes until it exceeds the limit and form blooms. The abundance of other algal communities followed irregular trends with respect to the number of *Trichodesmium* trichomes (Fig. 6d).

Table 3 shows that in general during the SIM, the number of species of diatoms, dinoflagellates and other algae increased with the increase in the number of *Trichodesmium* trichomes. Simpson's diversity index showed maximum species diversity (D=0.95) at station SS5 which is in open waters and minimum

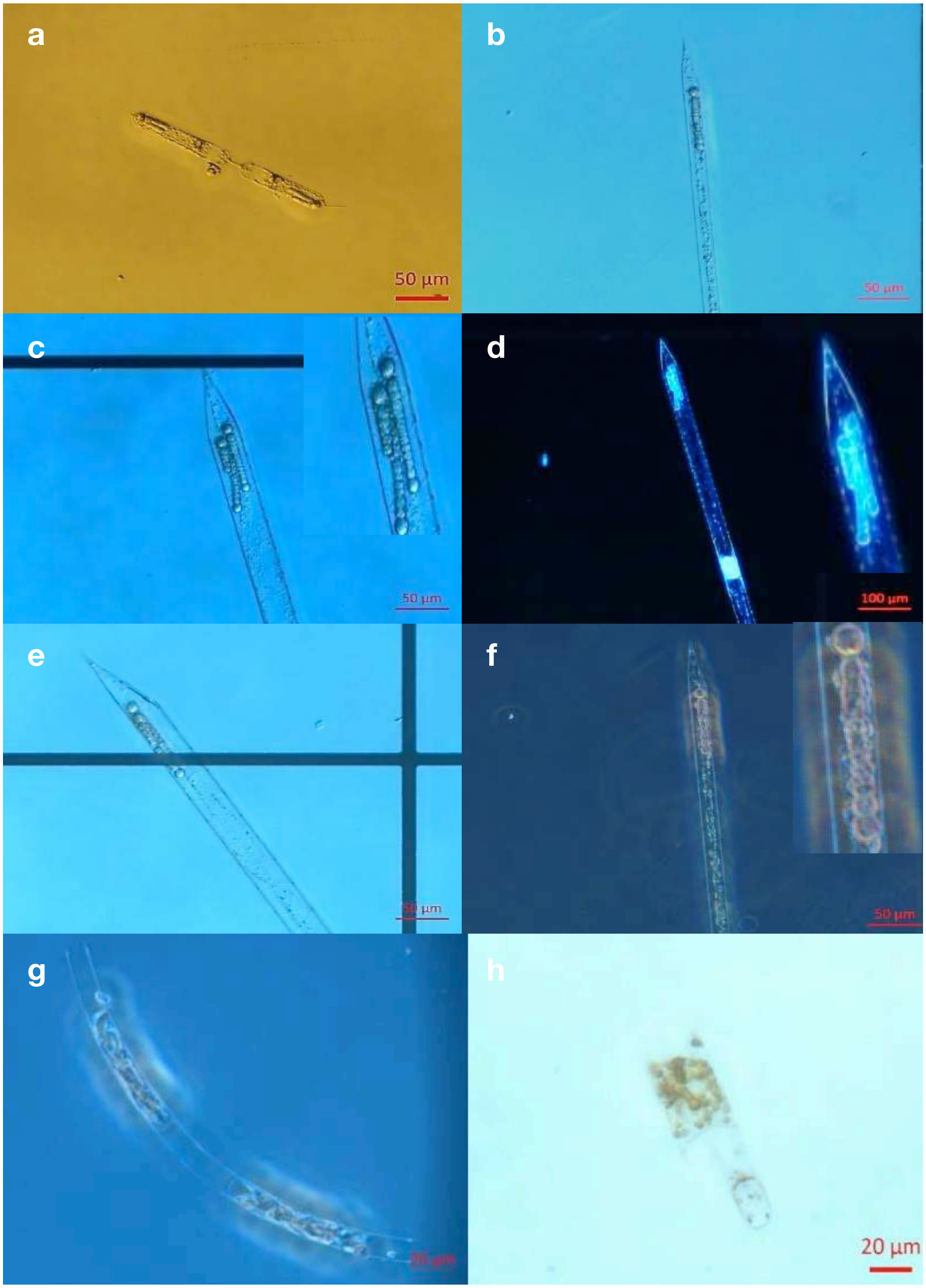

Figure 4. *Richelia intracellularis* as an endosymbiont (a) in *Guinardia cylindrus,* (b-e) in *Rhizosolenia hebetata*, (f) in *R. styliformis*, (g) in *Hemiaulus hauckii,* and (h) in *Hemiaulus sinensis*. (a-b – during SIM; c-h – during WM).

Simpson's index (D=0.41, 0.46) was found in the eastern part of continental shelf at SS12 and SS13 where the bloom was encountered. Species evenness was observed to be highest at SS10 (J'= 0.94), SS3 (J'=0.93), and SS5 (J'=0.9) while minimum evenness was noticed at SS12 (J'= 38) and SS13 (J'=0.31) which were the bloom stations.

Table 4 summarizes the diversity of phytoplankton species during the WM. The highest species diversity

Figure 5. DDAs during the WM - *Richelia intracellularis* as an endosymbiont (a) in *Guinardia cylindrus*, (b-c) in *Nitzschia sicula*, (d) *R. intracellularis* epiphytically attached to *Chaetoceros decipiens*, (e-f) *Calothrix rhizosoleniae* epiphytically attached to *Chaetoceros compressus*, (g) free trichomes of *R. intracellularis*.

according to Simpson's diversity index was observed at SK7 and the lowest was encountered at SK1, which was the bloom station. Overall the species diversity of phytoplankton in terms of Simpson's index (D) ranged between 0.54- 0.98. Pielou's species evenness (J') ranged between 0.42-0.86, which shows that the phytoplankton species were evenly distributed throughout the study area during the WM with an exception at the bloom station SK1 (J'= 0.42).

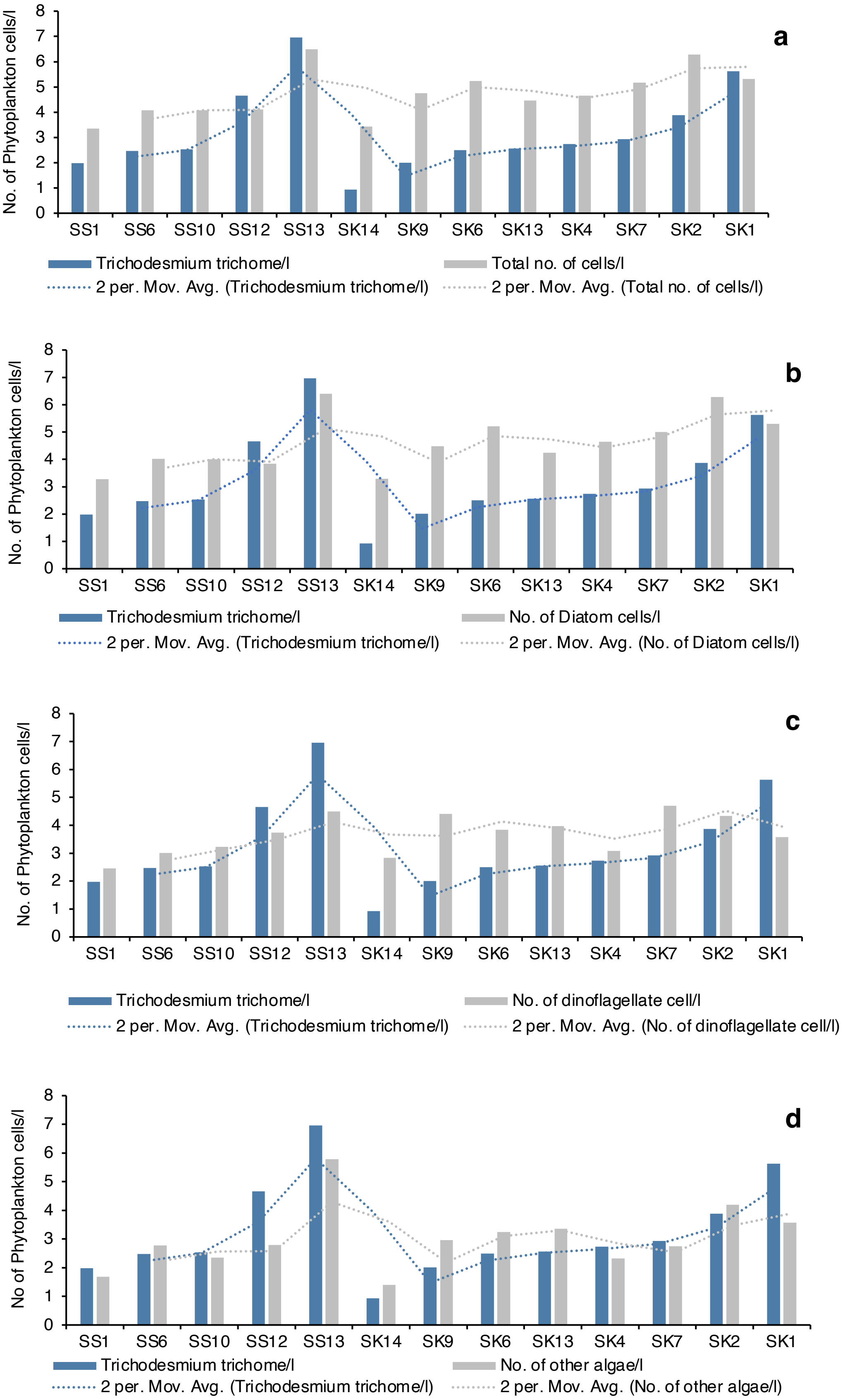

Figure 6. Comparison of number of *Trichodesmium* trichomes with (a) total number of cells/l, (b) No of diatoms cells/l, (c) no of dinoflagellates, and (d) no of other algal groups.

Table 3. Comparison of *Trichodesmium*, diatoms, dinoflagellate and other algal abundance along with Evenness and Richness index at various sampling sites during the Spring Inter Monsoon.

Station ID.	*Trichodesmium* tricomes/l	No. of Diatom sp./l	No. of Dinoflagellate Sp./l	No. of Other algae sp./l	Simpson's diversity index (D)	Pielou's evenness index (J')
SS1	$9.52x10^{1}$	$1.8x10^{1}$	$1.5x10^{1}$	8	0.79	0.74
SS2	0	$1.8x10^{1}$	0	4	0.81	0.84
SS3	0	$1.7x10^{1}$	0	2	0.84	0.93
SS4	0	$2.1x10^{1}$	$1.1x10^{1}$	5	0.72	0.71
SS5	0	$2.9x10^{1}$	$1.4x10^{1}$	0	0.9	0.9
SS6	$2.96x10^{2}$	$3.0x10^{1}$	$1.4x10^{1}$	8	0.81	0.74
SS7	0	$3.5x10^{1}$	$1.8x10^{1}$	0	0.84	0.73
SS8	0	$5.1x10^{1}$	$2.0x10^{1}$	0	0.87	0.88
SS9	0	$4.8x10^{1}$	$2.5x10^{1}$	0	0.87	0.89
SS10	$3.36x10^{2}$	$6.7x10^{1}$	$2.7x10^{1}$	7	0.95	0.94
SS11	0	$8.2x10^{1}$	$2.5x10^{1}$	0	0.88	0.74
SS12	$4.53x10^{4}$	$1.01x10^{2}$	$2.9x10^{1}$	$1.1x 10^{1}$	0.41	0.38
SS13	$9.13x10^{6}$	$1.19x10^{2}$	$3.0x10^{1}$	$1.3x10^{1}$	0.46	0.31

Correlation between hydrographic parameters and phytoplankton groups

Principal Component Analysis (PCA) was performed to understand the relation between different hydrographic parameters and the phytoplankton groups. The results of PCA for the SIM and the WM are depicted in Fig. 7a and 7b. During the SIM, it was observed that other algae (excluding *Trichodesmium*) and *Trichodesmium* trichomes were positively correlated with SST and negatively correlated with salinity (Fig. 7a). Diatoms and dinoflagellates were positively correlated with DO and salinity and negatively correlated with SST (Fig. 7a). Also, it was observed from Fig. 7a that the *Trichodesmium* trichomes and other algae (that excludes *Trichodesmium*) had a very strong positive correlation with DO.

During the WM, *Trichodesmium* trichomes were positively correlated with SST and DO, while it had a negative correlation with salinity (Fig. 7b). Diatom and dinoflagellates showed a strong negative correlation with SST and a positive correlation with salinity and DO (Fig 7b).

Table 4. Comparison of *Trichodesmium*, diatoms, dinoflagellate and other algal abundance along with Evenness and Richness index at various sampling sites during the Winter Monsoon.

Station ID.	*Trichodesmium* tricomes/l	No. of diatom sp./l	No. of dinoflagellate sp./l	No of Other algal sp. /l	Simpson's diversity index (D)	Pielou's evenness index (J')
SK1	$4.26x10^{6}$	$6.9x10^{1}$	$1.2x10^{1}$	$1.0x10^{1}$	0.54	0.42
SK2	$7.50x10^{3}$	$9.0x10^{1}$	$2.2x10^{1}$	7	0.97	0.82
SK3	0	$8.7x10^{1}$	$1.1x10^{1}$	9	0.98	0.86
SK4	$5.40x10^{2}$	$8.9x10^{1}$	$4.2x10^{1}$	4	0.96	0.75
Sk5	0	$6.1x10^{1}$	$1.9x10^{1}$	8	0.93	0.74
SK6	$3.14x10^{2}$	$7.0x10^{1}$	$3.4x10^{1}$	$1.0x10^{1}$	0.96	0.79
SK7	$8.45x10^{2}$	$9.7x10^{1}$	$7.4x10^{1}$	$1.0x10^{1}$	0.98	0.81
SK8	0	$8.4x10^{1}$	$3.6x10^{1}$	$1.4x10^{1}$	0.95	0.74
SK9	$1.01x10^{2}$	$3.7x10^{1}$	$2.5x10^{1}$	8	0.93	0.72
SK10	0	$5.4x10^{1}$	$3.0x10^{1}$	$1.0x10^{1}$	0.97	0.83
SK11	0	$3.1x10^{1}$	$2.5x10^{1}$	6	0.92	0.73
SK12	0	$4.1x10^{1}$	8	6	0.96	0.86
SK13	$3.60x10^{2}$	$2.6x10^{1}$	$2.5x10^{1}$	8	0.95	0.82
SK14	8.48	$5.0x10^{1}$	$2.2x10^{1}$	$1.2x10^{1}$	0.97	0.86

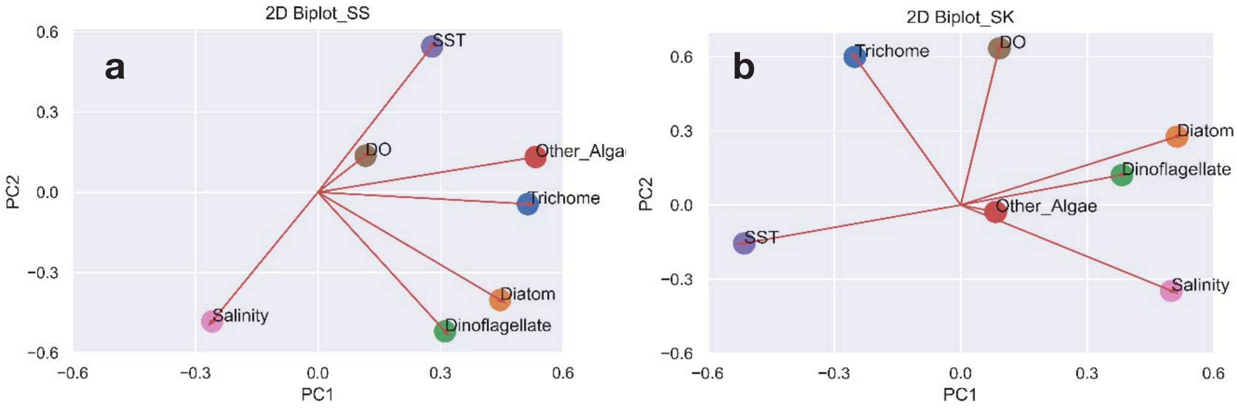

Figure 7. Principal Component Analysis (PCA) showing the inter-relationship between different hydrographic parameters and the phytoplankton groups during the SIM (a) and the WM (b).

Discussion

The EAS is a highly dynamic basin, where seasonal variations in the biological and physico-chemical characteristics has been a recurring phenomenon. The hydrographical features observed during the SIM and WM reveals the seasonality within the study area. Phytoplankton biodiversity is mainly affected by the sea water temperature and salinity (Sugie *et al.*, 2020). The observations from this study shows that water temperature was higher in the bloom regions during both the seasons. Temperature has been recognized as a key factor that controls *Trichodesmium* blooms (Marumo and Nagasawa, 1976; Carpenter, 1983; Satpathy *et al.*, 2007; Oyeku and Mandal, 2020). Usually, blooms of non-heterocystous filamentous cyanobacteria occur during the warm season (Ramamurthy *et al.*, 1972), as cyanobacteria need comparatively high temperatures for their optimal growth compared to other phytoplankton (Suvapepant, 1992; Sellner, 1997). *Trichodesmium* is a stenohaline cyanobacteria, which requires salinity greater than 33 PSU for optimum growth and abundance and cannot withstand low salinity (Ramamurthy *et al.*, 1972; Krishnan *et al.*, 2007). The study also revealed that the salinity in the bloom waters were higher than 34 PSU. Also, it was observed that DO values at the bloom stations were higher during both the seasons which might be because of photosynthetic release of oxygen by the dense blooms of *Trichodesmium* filaments. Similar observations hav also been reported on the east and west coast of India (Satpathy *et al.*, 2007; Mohanty *et al.*, 2010; Capone *et al.*, 1997; Padmakumar *et al.*, 2010) during *Trichodesmium* blooms.

According to Prabhu *et al.*, 1965; Qasim, 1970; Devassy *et al.*, 1978; Devassy *et al.*, 1987; Shetty *et al.*, 1988; Koya and Kaladharan, 1997; Sarangi *et al.*, 2004; Krishnan *et al.*, 2007; Padmakumar *et al.*, 2010; Parab and Matondkar, 2012; D'Silva *et al.*, 2012; Martin *et al.*, 2013; Jabir *et al.*, 2013; Jyotibabu *et al.*, 2017; Ahmed *et al.*, 2017; Dias *et al.*, 2020; Ramesh *et al.*, 2021, *Trichodesmium* blooms usually occur every year between February-May along the west coast of India when temperature is elevated with intense sunlight and when salinity is more stable (Jyothibabu *et al.*, 2003). However, during this study, blooms were also witnessed in the WM. High SST (28.64) favours water column stratification, probably leading to nutrient-poor environments that support the existence of extensive blooms of filamentous *T. erythaeum* in the study area during the WM. *Trichodesmium* bloom occurrence during WM in the Arabian Sea is not often observed so can be considered quite unusual. Also, the observed length of *Trichodesmium* filaments or trichomes and number of cells per trichome within this study are of great interest as this long filamentous *Trichodesmium* has not been recorded earlier from the Arabian Sea (Length: 1339-13163.49 µm). As *Trichodesmium* blooms occur in a nutrient poor environment (Devassy *et al.* 1978; Padmakumar *et al.*, 2010), the long filaments and great numbers of cells per trichome observed during the study period could possibly occur to optimize the nutritional and other physiological requirements.

As a diazotrophic cyanobacteria, *Trichodesmium* fix atmospheric nitrogen and make this available for other phytoplankton communities in a usable form. This study suggests that non- diazotrophic community growth increased with an increase in the number of *Trichodesmium* filaments. However, the abundance of other phytoplankton decreased when the number of *Trichodesmium* cells surpassed the normal limits, i.e., under bloom condition (Fig. 6). This phenomenon was observed during both cruises, in spite of its occurrence during different seasons. Thus, it clearly indicates the positive relationship between *Trichodesmium* and other phytoplankton species unless it overconsumes the available nutrients and flourishes to form blooms. Similar trends of abundance were observed for diatoms and dinoflagellates (Fig. 6b-6c), but variability was noted in the abundance of other algal groups (Fig. 6d).

Symbiotic association of diazotrophs-diatoms helps diatoms to fix atmospheric nitrogen in oligotrophic waters with the help of epiphytic or endosymbiont diazotrophs, as they are the only organisms capable of converting molecular N_2 into NH_4, a more readily assimilated form of dissolved nitrogen (Karl *et al*., 2002). Diazotrophs are significant in environments where nitrogen limits primary production. In the present study, it was observed that the diazotroph, *Richelia intracellularis* was found to be endosymbiotically associated with diatoms (DDAs) restricted within the bloom waters of the EAS during the SIM, while such associations were found in bloom and non-bloom regions during the WM. Past studies also emphasized the importance of DDAs on phytoplankton nutrient and energy budgets in the Palk Bay (Madhu *et al*., 2013) as well as in the oligotrophic waters of the Arabian Sea (Jabir *et al*., 2013) during the SIM. Additionally, DDAs were more abundant within the bloom waters of the EAS during the WM as well as with varying diatom species. Therefore, bloom waters with diazotrophs like *Trichodesmium* and *Richelia* not only support the proliferation of a non-diazotrophic phytoplankton community through regenerated ammonium produced from these nitrogen fixers (Kulkarni *et al*., 2010), but also through a significant contribution to the nitrogen cycle and the budget of oligotrophic seas during blooms.

Phytoplankton community composition showed distinct variation in bloom and non-bloom stations. At the bloom stations, certain phytoplankton species were observed to be abundant. These include *Trichodesmium erythraeum*, *Chaetoceros curvisetus*, *Bacteriastrum elongatum*, *Chaetoceros dichaeta*, *Pseudo-nitzschia liniola*, *Chaetoceros compressus*, *Richelia intracellularis*, *Protoperidinium parthenopes*, *Ceratium furca*, *Chaetoceros decipience*, and *Gonyaulax spinifera*. This suggests that these species thrive and are present in significant quantities during phytoplankton blooms. On the other hand, at the non-bloom stations, different phytoplankton species were observed in higher numbers. These include *Pseudo-nitzschia pungens*, *Pseudo-nitzschia delicatissima*, *Chaetoceros lorenzianus*, *Chaetoceros messanensis*, *Bacteriastrum delicatulum*, *Dactyliosolen fragilissimus*, *Chaetoceros compressus*, *Nitzschia longissima*, *Navicula delicatula*, *Thalsssiothrix gibberula*, *Lioloma pacificum*, *Protoperidinium conicum*, *Protoperidinium oceanicum*, *Gymnodinium breve*, and *Podolampas palmipes*. This indicates that these species exhibit higher population densities in environments that are not experiencing phytoplankton blooms. Overall, the study provides insights into the distribution and abundance of phytoplankton species in different ecological contexts, highlighting the variations between bloom and non- bloom stations.

During the SIM, it was observed that pennate diatoms such as *Pseudo-nitzschia sp*., *Nitzschia sp*., *Asterionellopsis sp*., *Thalassionema sp*., *Thalsssiothrix sp*., *Fragillariopsis sp*., and *Navicula sp*. were the dominant phytoplankton species. This means that these types of diatoms were the most abundant and influential in the phytoplankton community during this period. In contrast, during the WM, centric diatoms such as *Chaetoceros sp*., *Bacteriastrum sp*., *Dactyliosolen sp*., *Hemiaulus sp*., *Rhizosolenia sp*., *Guinardia sp*., and *Leptocylindrus sp*. took over as the major contributing phytoplankton throughout the study area. This implies that these centric diatoms became the most significant and impactful contributors to phytoplankton composition during the WM period, replacing the previously dominant pennate diatoms.

Conclusions

Trichodesmium sp. association with other phytoplankton communities during the SIM and WM were studied within the EAS. Similar trends were observed between *Trichodesmium* trichomes and other phytoplankton community abundance except at bloom stations. Microscopic analysis revealed that a mixed bloom of *T. erythraeum* and *T. thiebautii* occurred during the SIM period in the EAS off the coast of Ratnagiri, while the bloom witnessed during WM on the continental shelf off Malvan comprised only of *T. erythraeum*. The observed length of *Trichodesmium* trichomes and number of cells per trichome during WM have not been observed before in the EAS. Symbiotic associations of *Richelia intracellularis* with diatoms like *G. cylindrus*, *R. hebetata*, *R. styliformis*, *N. sicula* and *C. decipience* were observed during bloom period. Free living trichomes were also reported during the WM bloom at the bloom station. Endosymbiotic association of *R. intracellularis* with *N. sicula* is a novel observation at the bloom station during the WM. Overall, the distribution of diatoms and dinoflagellates were observed to have a negative correlation with SST while an inverse relation was found for *Trichodesmium.*

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References

Adhikary SP, Sahu J (1992) Studies on the *Trichodesmium* bloom of Chilka Lake, east coast of India. Phykos 30: 101-107

Ahmed A, Gauns M, Kurian S, Bardhan P, Pratihary A, Naik H, Shenoy DM, Naqvi SWA (2017)

Nitrogen fixation rates in the eastern Arabian Sea. Estuarine, Coastal and Shelf Science 191: 74-83

Alldredge AL, Silver MW (1982) Abundance and production rates of floating diatom mats (*Rhizosolenia castracanei* and *R. imbricata* var. *shrubsolei*) in the Eastern Pacific Ocean. Marine Biology 66: 83-88

Bergman B, Sandh G, Lin S, Larsson J, Carpenter E J (2012) *Trichodesmium*- a widespread marine cyanobacterium with unusual nitrogen fixation properties. FEMS Microbiology Reviews 37 (2013): 286-302

Blondeau- Patissier D, Brando VE, Lønborg C, Leahy SM, Dekker AG, (2018) Phenology of *Trichodesmium* spp. blooms in the Great Barrier Reef lagoon, Australia, from the ESA-MERIS 10-year mission. PLoS ONE 13 (12): e0208010 [https://doi.org/10.1371/journal.pone.0208010]

Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter E J (1997) *Trichodesmium*, a globally significant marine cyanobacterium. Science 276 (5316): 1221-1229

Carpenter EJ, Price CC (1976) Marine *Oscillatoria* (*Trichodesmium*): explanation for aerobic nitrogen fixation without heterocysts. Science 191: 1278-1280

Carpenter EJ (1983) Nitrogen fixation by marine *Oscillatoria* (*Trichodesmium*) in the world's oceans. In: Carpenter EJ, Capone DG (eds) Nitrogen in the marine environment. Academic Press, New York. pp 65-103

Carpenter EJ, Montoya JP, Burns J, Mulholland MR, Subramaniam A, Capone DG (1999) Extensive bloom of a N_2-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. Marine Ecology Progress Series 185: 273-283

Carpenter EJ, Capone DG (2008) Nitrogen fixation in the marine environment. Nitrogen in the marine environment, 2nd ed. Capone DG, Bronk DA, Mulholland MR, Carpenter EJ (eds). Academic Press/Elsevier, San Diego, CA. pp 141-198

Chen YLL, Tuo SH, Chen HY (2011) Co-occurrence and transfer of fixed nitrogen from *Trichodesmium* spp. to diatoms in the low-latitude Kuroshio Current in the NW Pacific. Marine Ecololgy Progress Series 421: 25-38 [doi:10.3354/meps08908]

Dias A, Kurian S, Thayapurath S (2020) Optical characteristics of colored dissolved organic matter during blooms of *Trichodesmium* in the coastal waters off Goa. Environmental Monitoring and Assessment 192, 526 (2020) [https://doi.org/10.1007/s10661-020-08494-w]

Devassy VP, Bhatrarhiri PMA, Qasim SZ (1978) *Trichodesmium* Phenomenon. Indian Journal of Marine Sciences 73: 168-186

Devassy VP, Bhattathiri PMA, Qasim SZ (1979) Succession of organisms following *Trichodesmium* phenomenon. Indian Journal of Marine Sciences 8: 89-93

Devassy VP (1987) *Trichodesmium* red tides in the Arabian Sea. In: Rao TSS (ed) Contributions in marine sciences: A special volume to felicitate Dr SZ Qasim Sastyabdapurti on his sixtieth birthday. National Institute of Oceanography, Dona Paula, India. pp 61-66

D'Silva MS, Anil AC, Naik RK, D'Costa PM (2012) Algal blooms: a perspective from the coasts of India. National Hazards 63 (2): 1225-1253

Dugdale RC, Menzel DW, Ryther JH, (1961) Nitrogen fixation in the Sargasso Sea. Deep Sea Research (1953) 7 (4) pp 297-300 [https://doi.org/10.1016/0146-6313(61)90051-X]

Durand F, Shetye SR, Vialard J, Shankar D, Shenoi SSC, Ethe C, Madec G (2004) Impact of temperature inversions on SST evolution in the southeastern Arabian Sea during the pre-summer monsoon season. Geophysical Research Letters. 31: L01305 [doi:10.1029/2003GL018906]

Ferrario ME, Villafane V, Helbling W, Holm-Hansen O (1995) The occurrence of the sym-biont *Richelia* in *Rhizosolenia* and *Hemiaulus* in the North Pacific. Revista Brasileira de Biologia 55 (3): 439-443

Gianesella-Galvao SM F, Costa MPF, Kutner MBB (1995) Bloom of *Oscillatoria* (*Trichodesmium*) *erythraeum* (Her.) Kutz. in coastal waters of the Southwest Atlantic. Publication **Español** do Instituto Oceanográfico, São Paulo 11: 133-140

Gomez F, Furuya K, Takeda S (2005) Distribution of the cyanobacterium *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean. Journal of Plankton Research 27 (4): 323-330

Hareesh Kumar, Joshi VHM, Sanilkumar KV, Rao AD, Anand P, Kumar KA, Rao CV (2009) Growth and decay of the Arabian Sea mini warm pool during May 2000: Observations and simulations. Deep Sea Research 56, Part I: 528-540 [doi:10.1016/j.dsr.2008.12.004]

Jabir T, Dhanya V, Jesmi, Y, Prabhakaran MP, Saravanane N, Gupta GVM, Hatha AM (2013) Occurrence and distribution of a diatom-diazotrophic cyanobacteria association during a *Trichodesmium* bloom in the Southeastern Arabian Sea. International Journal of Oceanography 350594. 6 pp [http://dx.doi.org/10.1155/2013/350594]

Joseph P V (1990) Warm pool over the Indian Ocean and monsoon onset. Tropical Ocean Atmosphere News Letters 53: 1-5

Jyothibabu R, Madhu NV, Murukesh N, Haridas PC, Nair KKC, Venugopal P (2003) Intense blooms of *Trichodesmium erythraeum* (Cyanophyta) in the open waters along east coast of India. Indian Journal of Marine Science 32: 165-167

Jyotibabu R, Karan C, Jagadeesan L, Arunpandi N, Pandiarajan RS, Muraleedharan KR, Balachandran KK (2017) *Trichodesmium* blooms and warm-core ocean surface features in the Arabian Sea and the Bay of Bengal. Marine Pollution Bulletin 121 (1-2): 201-215

Karl D, Michaels A, Bergman B, Capone D, Carpenter E, Letelier R, Lipschultz F, PaerlH, Sigman D, Stal L (2002) Dinitrogen fixation in the world's oceans. Biogeochemistry 57: 47-98

Koya KPS, Kaladharan P (1997) *Trichodesmium* bloom and mortality of *Canthigaster margaritatus* in the Lakshadweep Sea. Marine Fisheries Information Service Technical and Extension Series 147: 14

Krishnan AA, Krishnakumar PK, Rajagopalan M (2007) *Trichodesmium erythraeum* (HER) bloom along the southwest coast of India (Arabian Sea) and its impact on trace metal concentrations in seawater. Estuarine, Coastal and Shelf Science 71: 641-646

Kulkarni VV, Chitari RR, Narale DD, Patil JS, Anil AC (2010) Occurrence of cyanobacteria-diatom symbiosis in the Bay of Bengal: implications in biogeochemistry. Current Science 99 (6): 736-737

Kumar SP, Prasad T G (1996) Winter cooling in the northern Arabian Sea. Current Science 71: 834-841

Lewis MR, Ulloa O, Platt T (1988) Photosynthetic action, absorption, and quantum yield spectra for a natural population of *Oscillatoria* in the North Atlantic. Limnology and Oceanography 33: 92-98

Madhu NV, Meenu P, Ullas N, Ashwini R, Rehitha TV (2013). Occurrence of cyanobacteria (*Richelia intracellularis*)- diatom (*Rhizosolenia hebetata*) consortium in the Palk Bay, southeast coast of India. Indian Journal of Geo-Marine Sciences 42: 453-457

Madhupratap M, Prasanna Kumar S, Bhattathiri PMA, Kumar MD, Raghukumar S, Nair KKC, Ramaiah N (1996) Mechanisms of the biological response to winter cooling in the northeastern Arabian Sea. Nature 384: 549-552

Martin GD, Jyothibabu R, Madhu NV, Balachandran KK, Nair M, Muraleedharan KR, Arun, PK, Haridevi CK, Revichandran C (2013). Impact of eutrophication on the occurrence of *Trichodesmium* in the Cochin backwaters, the largest estuary along the west coast of India. Environmental Monitoring and Assessment 185 (2): 1237-1253

Marumo R, Nagasawa S (1976) Seasonal variation of the standing crop of a pelagic blue-green alga, *Trichodesmium* in the Kuroshio water. Bulletin of the Plankton Society of Japan 23: 19-25

Matondkar SGP, Parab SG, Desa E, Dwivedi RM (2006) Basin scale distribution of *Trichodesmium* spp. in the Arabian Sea using Oceansat I/ OCM. Remote Sensing of the Marine Environment Proceedings of SPIE: International Society for Optical Engineering 6406V-I [doi: 10.1117/12.693687]

Mohanty AK, Satpathy KK, Sahu G, Hussain KJ, Prasad MVR, Sarkar SK (2010) Bloom of *Trichodesmium erythraeum* (Ehr.) and its impact on water quality and plankton community structure in the coastal waters of southeast coast of India. Indian Journal of Marine Science 39 (3): 323-333

Nyadjro ES, Subrahmanyam B, Murty VSN, Shriver J F (2012) The role of salinity on the dynamics of the Arabian Sea mini warm pool. Journal of Geophysical Research 117: C09002 [doi:10.1029/2012JC007978]

Oyeku OG, Mandal SK (2021) Historical occurrences of marine microalgal blooms in Indian peninsula: Probable causes and implications. Oceanologia 63: 51-71

Padmakumar KB, Smitha BR, Thomas LC, Fanimol CL, SreeRenjima G, Menon NR, Sanjeevan VN (2010) Blooms of *Trichodesmium erythraeum* in the South Eastern Arabian Sea during the onset of 2009 Summer Monsoon. Ocean Science Journal 45 (3): 151-157 [doi10.1007/s12601-010-0013-4]

Parab SG, Matondkar SGP (2012) Primary productivity and nitrogen fixation by *Trichodesmium* spp. in the Arabian Sea. Journal of Marine Systems 105: 82-95

Pielou EC (1966) The measurement of diversity in different types of biological collections. Journal of Theoretical Biology 13: 131-144

Poulton AJ, Stinchcombe MC, Quartly GD (2009) High numbers of *Trichodesmium* and diazotrophic diatoms in the southwest Indian Ocean. Geophysical Research Letters 36: 15: L15610 [doi:10.1029/2009GL039719]

Prabhu MS, Ramamurthy S, Kuthalingam MDK, Dhulkheid MH (1965) On an unusual swarming of the planktonic blue green algae *Trichodesmium* spp. off Mangalore. Current Science 34: 95

Prasanna Kumar S, Prasad TG (1996) Winter cooling in the northern Arabian Sea. Current Science 71: 834-841

Qasim SZ (1970) Some characteristic of a *Trichodesmium* bloom in the Laccadives. Deep Sea Research 17: 655-660

Ramamurthy VDR, Kumar AS, Bhargava RMS (1972) Studies on the blooms of *Trichodesmium erythraeum* (EHR) in the waters of the Central west coast of India. Current Science 41: 803-805

Ramesh C, Koushik S, Shunmugaraj T, Ramana Murthy MV (2021) Massive blooms of Cyanobacteria *Trichodesmium erythraeum* and *Synechococcus* sp. in Mandapam Group of Islands, Southeast Coast of India, Tamil Nadu 57 (3): 32-36 [doi: 10.1615/HydrobJ.v57.i3.30]

Sanilkumar KV, Kumar PVH, Joseph JK, Panigrahi JK (2004) Arabian Sea mini warm pool during May 2000. Current Science 86: 10-105

Santhanam R, Srinivasan A, Ramadhas V, Devaraj M (1994) Impact of *Trichodesmium* bloom on the plankton and productivity in the Tuticorin Bay, Southeast coast of India. Indian Journal of Marine Science 23: 27-30

Sarangi RK, Chauhan P, Nayak SR (2004) Detection and monitoring of *Trichodesmium* blooms in the coastal waters off Saurashtra coast, India using IRS-P4 OCM data. Current Science 86: 1636-1641

Satpathy KK, Mohanty AK, Sahu G, Prasad MVR, Venkatesan R, Natesan U and Rajan M, (2007) On the occurrence of *Trichodesmium erythraeum* (Ehr.) bloom in the coastal waters of Kalpakkam, east coast of India. Indian Journal of Science and Technology 1 (2):1-9 [http://www.indjst.org]

Sellner KG (1997) Physiology, ecology and toxic properties of marine cyanobacteria blooms. Limnology and Oceanography 42: 1089-1104

Shetty HPC, Gupta TRC, Kattai RJ (1988) Green water phenomena in the Arabian Sea off Mangalore. Proceedings of the first India Fisheries Forum. pp 339-346

Shetye SR, Gouveia AD, Shenoi SSC, Michael GS, Sundar D, Almedia AM, Santanam K (1991) The coastal current off western India during the northeast monsoon, Deep Sea Research 38, Part A: 1517-1529

Simpson EH (1949) Measurement of diversity. Nature 163: 688

Siqueira A, Kolm HE, Brandini FP (2006) Offshore distribution patterns of the cyanobacterium *Trichodesmium erythraeum* Ehrenberg and associated phyto- and bacterioplankton in (on/off?) the southern Atlantic coast (Paraná, Brazil). Brazilian Archives Biology and Technology 49 (2): 323-337

Sohm JA, Subramaniam A, Gunderson T E, Carpenter E J, Capone D G (2011) Nitrogen fixation by *Trichodesmium* spp. and unicellular diazotrophs in the North Pacific Subtropical Gyre. Journal of Geophysical Research 116: G03002

Sugie K, Fujiwara A, Nishino S, Kameyama S, Harada N (2020) Impact of temperature, CO2 and salinity on phytoplankton community composition in western Arctic Ocean. Frontier in Marine Sciences 6: 821

Suvapepant S (1992) *Trichodesmium* blooms in gulf of Thailand. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. Kluwer. pp 343-348

Thadathil P, Gosh AK (1992) Surface layer temperature inversion in the Arabian Sea during winter. Journal of Oceanography 48: 293-304

Tomas CR (1997) Identifying marine phytoplankton. Academic Press, New York, NY, USA. 858 pp

Villareal TA (1992) Marine nitrogen-fixing diatom-cyanobacteria symbioses. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other diazotrophs. NATO ASI Series 362. Springer, Dordrecht. pp 163-175 [https://doi.org/10.1007/978-94-015-7977-3_10]

Original Article

Remote sensing of coral reef habitats in Madagascar using Sentinel-2 satellite images

Aina L. Nomenisoa[1,2], Gildas Todinanahary[1], Hubert Z. Edwin[1], Toky Razakarisoa[1], John Bunyan Israel[1], Saverio Raseta[1], Henitsoa Jaonalison[1], Jamal Mahafina[1], Igor Eeckhaut[2]

[1] Institut Halieutique et des Sciences Marines (IH.SM), University of Toliara, PO Box 141, Toliara, Madagascar

[2] Laboratoire de Biologie des Organismes Marins et Biomimétisme, University of Mons, PO Box 7000, Mons, Belgium

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*** Corresponding author:**
ainaledon294@gmail.com

Abstract

Publicly available Sentinel-2 satellite imagery was used to map the coral reef systems of Toliara in Madagascar, to standardize methods for monitoring reef health and guiding management decisions. Fieldwork conducted between March and December 2021 used georeferenced photoquadrats to assess benthic structure. The satellite image classification was based on the Object-Based Image Analysis (OBIA) and machine learning algorithms, with k-NN achieving the highest overall accuracy at 83 %, followed by the Bayes classifier (79 %), DT (68 %), RT (67 %) and SVM (42 %). The analysis identified distinct surface areas occupied by seagrass (21 km^2), sand (73 km^2), rubble (21 km^2), coral (10 km^2) and algae (6 km^2). Comparative assessment with the Allen Coral Atlas underscored the importance of aligning satellite image analysis with *in-situ* data. The study emphasized the role of selecting appropriate classifier algorithms for precise mapping and stressed the importance of local data collection for accurate habitat mapping. It also showcased the successful application of OBIA with satellite imagery and field data for coral reef mapping, providing insights into habitat health and spatial changes essential for effective conservation.

Keywords: remote sensing, coral reef, Madagascar, Sentinel-2, OBIA, Machine learning algorithms

Introduction

Coral reefs protect shorelines against storms, serve as fish nurseries, and provide socio-economic benefits when associated with tourism and recreation, shoreline protection, fisheries, and biodiversity services (Eakin *et al.*, 2010). However, these ecosystems face significant threats on both global and local scales, primarily due to climate change and anthropogenic pressures (Xu and Zhao, 2014). Nearly half of the world coral reefs have been destroyed or badly damaged in the last 30 years (Wilkinson, 2008). Current trends suggest that between 70 % to 90 % of global coral reefs are at risk of extinction within the foreseeable future (Foo and Asner, 2019), a fate that extends to the reefs of Madagascar as well (van Hooidonk *et al.*, 2016). Effective management and conservation efforts requires comprehensive monitoring strategies that encompass both spatial and temporal dimensions, focusing on the distribution of species on the benthos as well as their associated substrates (Nurlidiasari and Budiman, 2010). Such measures require a reliable method that can efficiently process continuous data into manageable spatial units (Kennedy *et al.*, 2021). The European Space Agency (ESA) has made Sentinel-2 images freely available since 2015 (ESA's Sentinel-2 team, 2015) offering a 10 m spatial resolution (pixel size), which significantly enhances the utility of these images for coral reef mapping. Remote sensed mapping of coral reefs is particularly important for developing countries, where 80 % of the world's coral

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reefs occur (UNEP-WCMC, WorldFish Centre, WRI, TNC, 2021). Therefore, it is critical to determine the efficacy of mapping using freely available satellite images from Sentinel-2 (Hedley *et al.*, 2012, 2018; Wouthuyzen *et al.*, 2019; Yunus *et al.*, 2019).

To achieve comprehensive mapping of coral reefs, it is crucial to derive maps of geomorphic zones and benthic communities at various scales (Phinn, *et al.*, 2012). Numerous initiatives have been undertaken globally to map coral reefs and understand their distribution, including coral reefs from Madagascar. However, existing datasets, such as those from the United Nations Environment Program-World Conservation Monitoring Centre (UNEP-WCMC) and the Allen Coral Reef Atlas, have limitations such as irregular updates, and unstable accuracy, hindering their applicability in dynamic monitoring. Although efforts to study coral reefs from Madagascar have provided valuable insights, further studies are necessary to fully comprehend these ecosystems and their changing habitats.

Efficient and cost-effective methods using remote sensing data are needed to delineate comprehensive reef coverage, geomorphic zoning, and benthic composition in Madagascar. Despite numerous studies on mapping coral reefs, variations in processing schemes, data characterization, and classification methods pose challenges, emphasizing the necessity for a tailored methodology. Therefore, there is a need for a comprehensive methodology tailored to coral reefs from Madagascar, encompassing standardized fieldwork data collection and image processing workflows. This study focuses on the barrier reef in Toliara, commonly known as the 'Grand Récif de Toliara' (GRT), and the reefs of Ankilibe and Sarodrano, where local coral reef geomorphology has been conducted

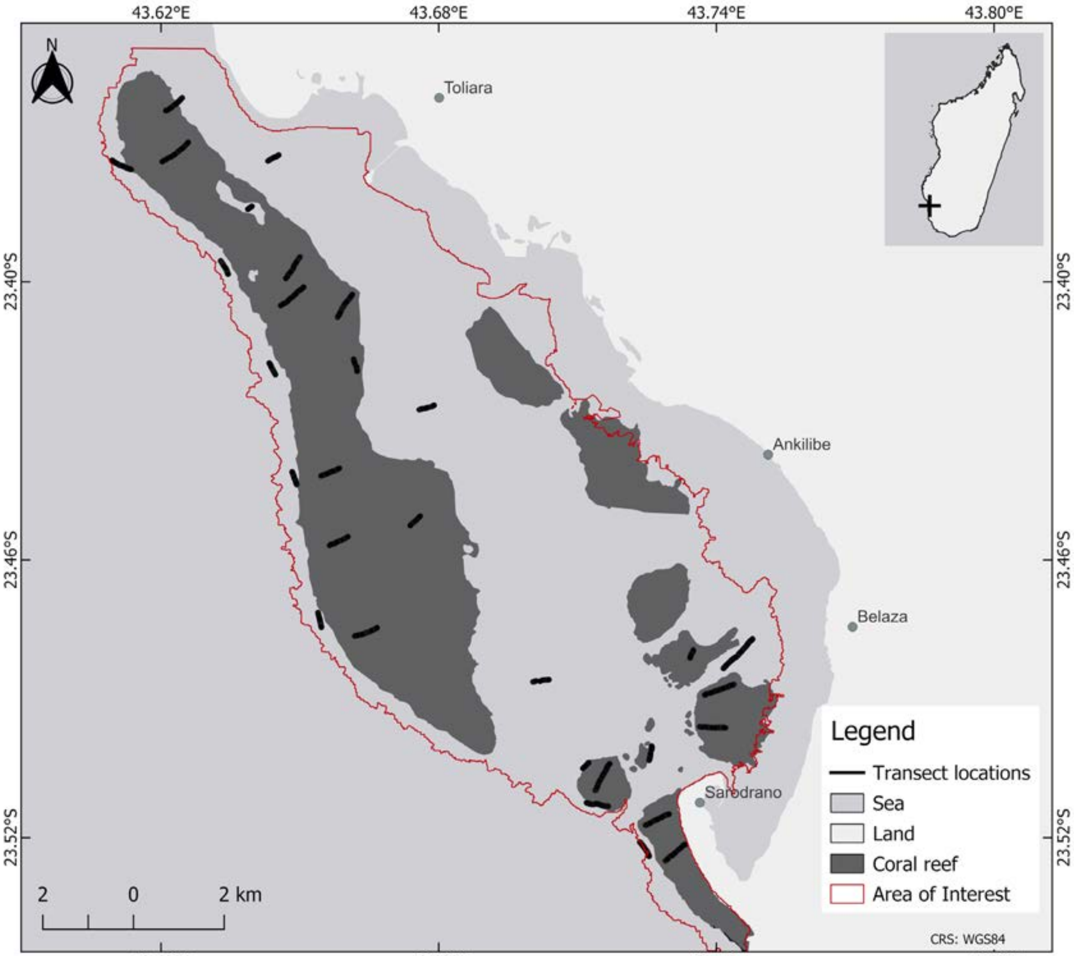

Figure 1. Study area with the placement of the transect lines.

(Pichon, 1972; Battistini *et al.*, 1975; Andréfouët *et al.*, 2013), and several studies focusing on the bio-ecology of coral reefs have been realized (Todinanahary, 2016; Razakandrainy, 2018; Botosoamananto *et al.*, 2021). Undertaking a comprehensive study at the scale of the Toliara region is essential for coral reefs of Madagascar, necessitating standardized methodologies for future data collection and processing. The primary aim of this research was to develop a methodology for coral reef mapping in Madagascar, using freely accessible satellite imagery and advanced remote sensing techniques. Specifically, the study aimed to assess geomorphic zonation and benthic coverage along the barrier reef and fringing reefs of Toliara, using Object-Based Image Analysis (OBIA) applied to Sentinel-2 satellite data, alongside fieldwork data acquisition. This study seeks to establish a foundational understanding of nearshore benthic communities in Madagascar, serving as a vital resource for marine scientists to effectively track ecosystem changes. Furthermore, it aims to furnish policymakers with essential data for monitoring the health of these reefs and formulating sustainable management strategies over the long term.

Materials and methods

Study area, field data collection and processing

The study was carried out within the Bay of Toliara, and was focused on the fringing reef of Sarodrano, the fringing reef of Ankilibe, and the barrier reef of Toliara (Fig. 1). The selection of these sites was based on several factors: (1) accessibility, (2) ecological variability, encompassing both degraded and healthy areas to facilitate the discernment of local stressors, and (3) ecological complexity, given the co-existence of a barrier reef, a fringing reef, and patch reefs within the same coral reef system. Fieldwork was conducted between March and December 2021, during low spring tides to optimize the time available for in-water surveying. It consisted of collecting georeferenced photoquadrats (in-water images) along transect lines (Fig. 1). Due to the limitation of GPS signal penetration underwater, the GPS device was secured in a floating airtight bag at the surface. This device was programmed to record new geographic positions every second. Specifically, one diver captured benthic images every 3-5 meters, guided by a compass to maintain course, while a second diver at the surface maneuvered the GPS-containing bag, moving in synchronization with the diver below (Fig. 2). Each dive averaged 30 minutes, covering approximately 500 meters of transect where current conditions allowed; however, in instances of stronger currents, transects were shortened accordingly. Initial and final GPS coordinates of each transect were logged on a diving slate to facilitate subsequent GPS data integration. Approximately 250 photos were collected per transect, totaling 4187 photos across the 30 transects. Ground-truthing data collection was confined to depths shallower than 20 meters. Subsequently, the photos were linked to GPS data to assign specific geographic positions to each photoquadrat using the software GPS Photo Manager (Roelfsema *et al.*,

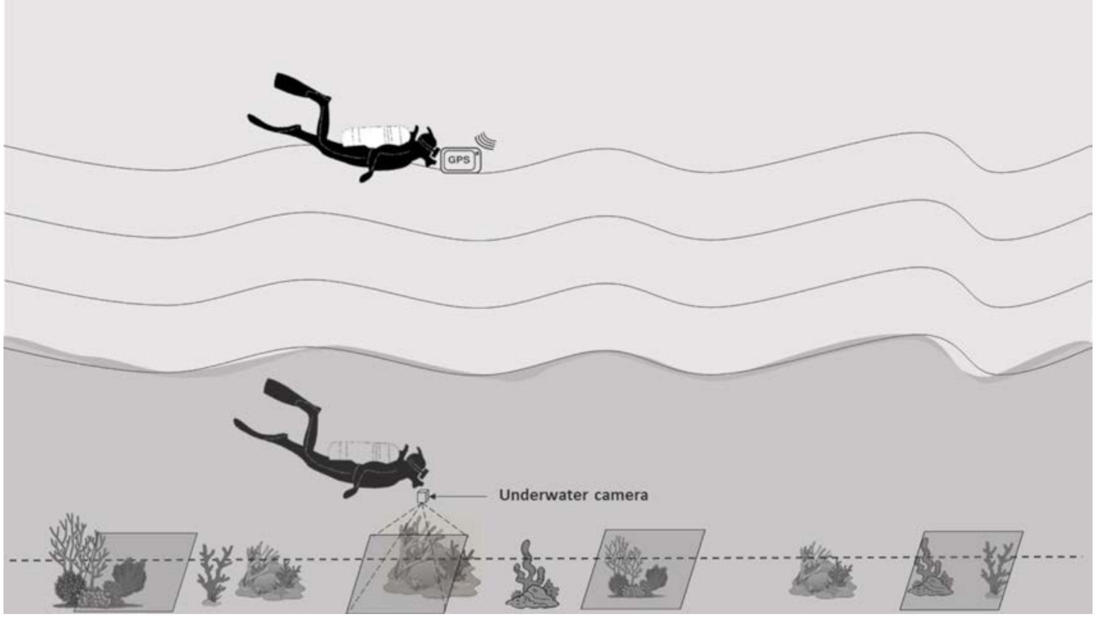

Figure 2. Method of photoquadrat data collection.

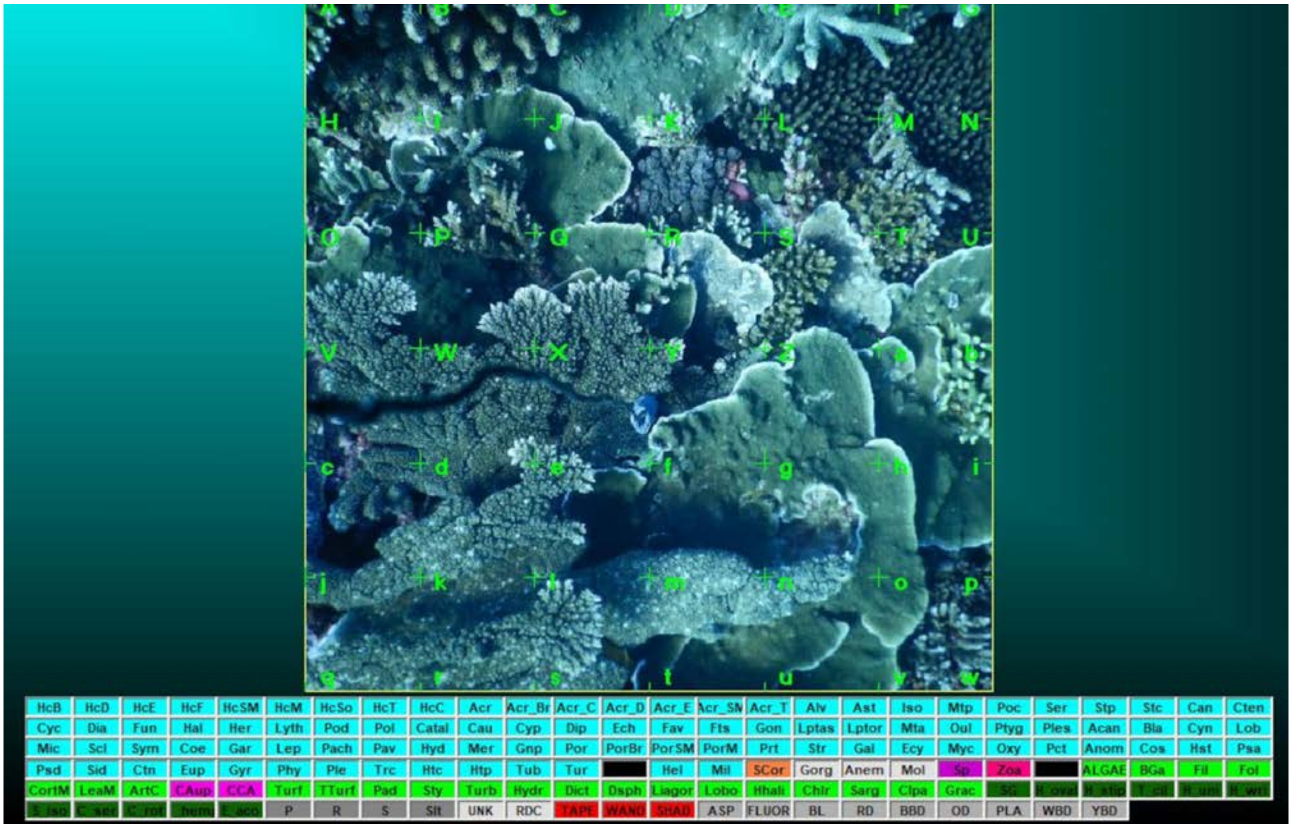

Figure 3. 49 stratified points (7 points per lines) on CPCE.

2019). This integration facilitated the visualization of photos and associated benthic attributes within a Geographic Information System (GIS) interface. All the GIS operations in this study were performed using the QGIS software, version 3.34.

Assessment of benthic cover from geotagged photos

Benthic cover within the transect lines was obtained from each geotagged photo by analyzing photoquadrats using the Coral Point Count with Excel extension (CPCe) software (Kohler and Gill, 2006). A total of 49 stratified points were distributed on each photo and the substrates corresponding to these points were identified using a predefined code name "CPCe benthic codes 41 Madagascar" provided with the software (Fig. 3). Based on their knowledge and the 41 CPCe benthic codes, the users attributed specific classes to each of the 49 stratified points. This number of stratified points per photoquadrat was sufficient to identify down to the level of benthic habitat classes. The benthic cover of the photoquadrat was then automatically estimated by the software as a function of the number of points occupied by each category of substrate. Once the analysis was finished, the software exported the data in an Excel file.

Conversion of the CPCE data into calibration and validation sample points

The data points produced by the CPCe software spans a 1x1 meter area, whereas the pixel size of a Sentinel-2 image measures 10 meters by 10 meters. To ensure comparability between CPCe data points and Sentinel pixels, the 41 benthic CPCe codes were refined into five classes: Coral (i.e., live corals), Algae, Rubbles, Sand, and Seagrass. These data points were then overlaid onto the Sentinel-2 image layer. A new sample was then manually created and assigned a pixel category based on the predominant benthic cover depicted in the CPCe data pie chart (Fig. 4). From the 4187 photos, 1243 control points were derived, where 75 % were used for calibrating the machine learning algorithms to classify the satellite image, while the remaining 25 % served as validation points to assess their accuracy.

Satellite image processing

Pre-processing

Sentinel-2A data were accessed from the Copernicus server (https://scihub.copernicus.eu/dhus/#/home). The Sentinel-2A image collected on 21-08-2021 was used in this study (Table 1). The Sentinel-2 satellite was launched in 2015 and offers 10 m spatial resolution for the visible and the Near Infra-Red (NIR)

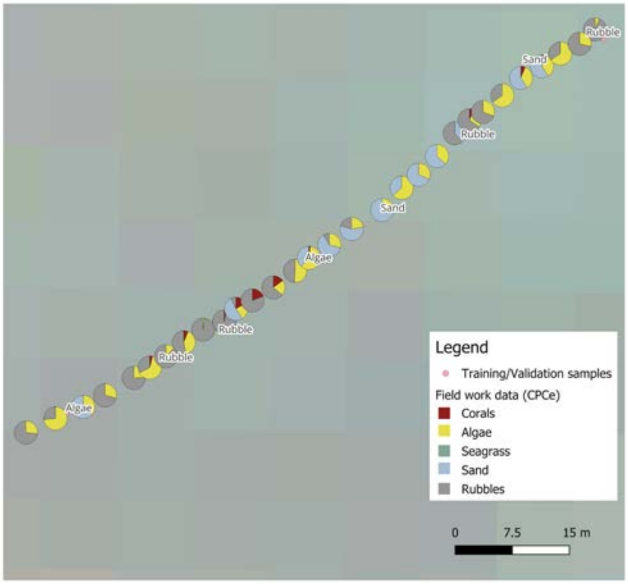

Figure 4. Calibration and validation sample points.

bands (ESA's Sentinel-2 team, 2015). The most appropriate images for coral reef habitat mapping are those that contain the least cloud cover and sun glint and are acquired during the spring low tide period. This last requirement is crucial in the analyses as the characterization of the benthos is complicated especially when they are submerged in the water column. The raw downloaded Sentinel-2 image was first corrected for the effect of the atmosphere. For this correction, the sen2cor atmospheric Correction Processor algorithm was used from the SNAP software or Sentinel Application Platform (Louis *et al.*, 2016). This approach consisted of transforming the Level-1C image (surface reflectance measured at the top of the atmosphere) into Level-2A (bottom-of-atmosphere reflectance). Next, as the benthos was submerged underwater, the water column effect needed to be corrected. The water column correction algorithm of Lyzenga (1981) was used for this purpose. This was processed using the sen2coral module of the SNAP software. As a result, three bands of the depth invariant index (DII) were generated, each composed of different combinations of spectral bands (Table 1): blue and green (DII_B2B3), blue and red (DII_B2B4), and green and red (DII_B3B4). Additionally, the Normalized Difference Vegetation Index (NDVI) was computed (Zoffoli *et al.*, 2020). These newly generated layers were added to the atmospheric corrected image for the geomorphic and benthic image classification.

Image classification

Coral reef habitat classification serves as an important tool for surveying and understanding these marine ecosystems. The process involves leveraging raw input data—such as videos or images—captured from coral sites (Nguyen *et al.*, 2021). Through this data, distinctive features of the seabed, referred to as classes, are extracted and categorized, including corals, sands, rubbles, seagrass, etc. To undertake this mapping,

Table 1. Sentinel-2 image specification used in this study.

Name	Sentinel-2A			
Correction level	Level 1A			
Date of acquisition	21-08-2021			
Radiometric resolution	12 bit/pixel			
Swath width	290 km at nadir			
	Band number	**Spectral Bands**	**Central wavelength (nm)**	**Spatial resolution (m)**
Multispectral Bands	1	Coastal Aerosol	442.7	60
	2	Blue	492.7	10
	3	Green	559.8	10
	4	Red	664.6	10
	5	Vegetation Red Edge	704.1	20
	6	Vegetation Red-Edge	740.5	20
	7	Vegetation Red-Edge	782.8	20
	8	Near Infra-Red (NIR)	832.8	10
	8A	Narrow NIR	864.7	20
	9	Water vapor	945.1	60
	10	Short Wave Infra-Red (SWIR)-Cirrus	1373.5	60
	11	Short Wave Infra-Red (SWIR)	1613.7	20
	12	Short Wave Infra-Red (SWIR)	2202.4	20

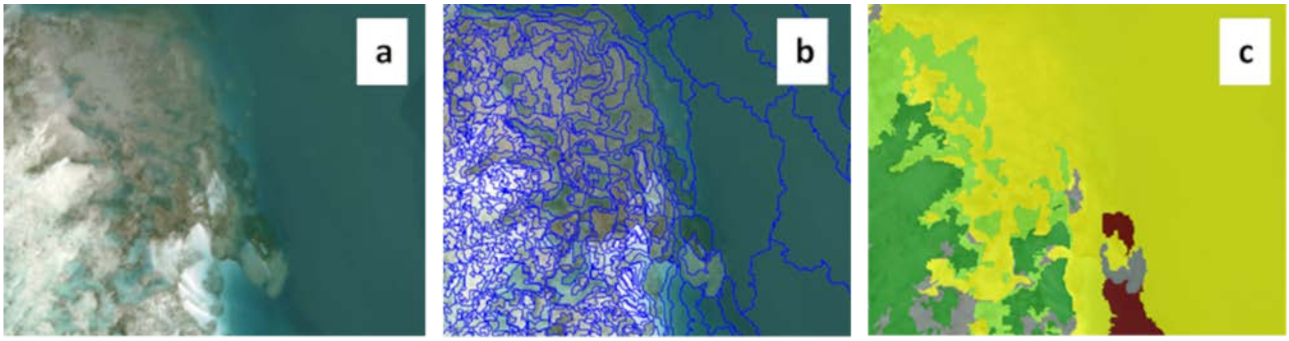

Figure 5. Object based image analysis (OBIA)process. a) Acquired image, b) Image segmentation, c) Image classification.

there are two approaches used: manual extraction of features, which offers high accuracy but is laborious and time-intensive, or the use of machine-learning algorithms for rapid processing and replicability, albeit with a potential risk of misclassification. While manual methods ensure precision, the advent of machine learning presents an efficient alternative, albeit with a need for careful validation and refinement to minimize errors in classification. For this, the OBIA method (Object-Based Image Analysis) was applied using eCognition Developer Software version 10.3 (Trimble Germany GmbH, 2022). This approach consists of grouping similar pixels (form, colour, texture, etc.) into segments (Fig.5), and then attributing classes to those segments (Hedley *et al.*, 2016).

Geomorphic zonation

The concept of geomorphic zonation in coral reefs involves identifying features that are relatively stable over time. In this study, the extraction of several key geomorphic features was focused on, including Internal Reefs, Lagoon, Reef Flat, Enclosed Basins, Reef Crest, Reef Front, and Reef Slope, following the definitions provided by Battistini *et al.* (1975). A lagoon is a naturally occurring depression with varying depths and sizes, typically found either behind a barrier reef or completely enclosed by reef structures. Enclosed basins, on the other hand, are smaller, shallower depressions or pools nestled within the reef structure on the reef flat. The reef slope constitutes the submerged front portion of a reef, sloping seaward with differing inclinations. It comprises coral formations and sedimentary deposits primarily of biogenic origin. The reef front delineates the outer edge of the reef flat at low tide, particularly during spring tides. Meanwhile, the reef flat is a horizontally oriented platform atop a reef structure, often reaching or surpassing sea level. It may exhibit material accumulations and surface incisions. The reef crest is primarily composed of coarse elements and is situated on the anterior part of the reef flat, manifesting in various shapes such as domes, ramparts, or scattered accumulations. Internal reefs are positioned within a lagoon, frequently separated from the open ocean by a barrier reef. They exhibit diverse sizes and shapes and are typically surrounded by shallow lagoon waters. These internal reefs consist of lagoonal coral patches, some of which extend to the surface and larger lagoon reefs, which are substantial coral formations within the lagoon, either partially exposed or submerged. These larger formations often display distinctive zoning patterns akin to those observed on reef flats.

After the image segmentation, these features were extracted by the visual photo-interpretation method using the built-in manual classification tools within the Ecognition Developer software.

Benthic image classification

For this purpose, the processed satellite image comprised: (i) the 10-meter resolution spectral bands from Sentinel-2 images (Table 1) which were atmospherically corrected, (ii) the calculated depth invariant bottom indexes (DII_B2B3, DII_B2B4, DII_B3B4), and (iii) the NDVI layer. Several satellite imageries, classification techniques, typologies and machine learning algorithms have been globally adopted to gather data on benthic coverage of coral reefs (Burns *et al.*, 2022). However, determining the most effective approach presents challenges due to inconsistencies in various factors, including the spectral and spatial resolutions of satellite images, methodologies for *in situ* reference data collection, the diversity and quantity of benthic classes mapped, and protocols for accuracy assessment. In this study, five prominent machine learning classifiers commonly employed in mapping coral reef benthic habitats were assessed with the goal of identifying the best performer tailored to environmental conditions, fieldwork data characteristics, and the particular benthic classes under study. These findings

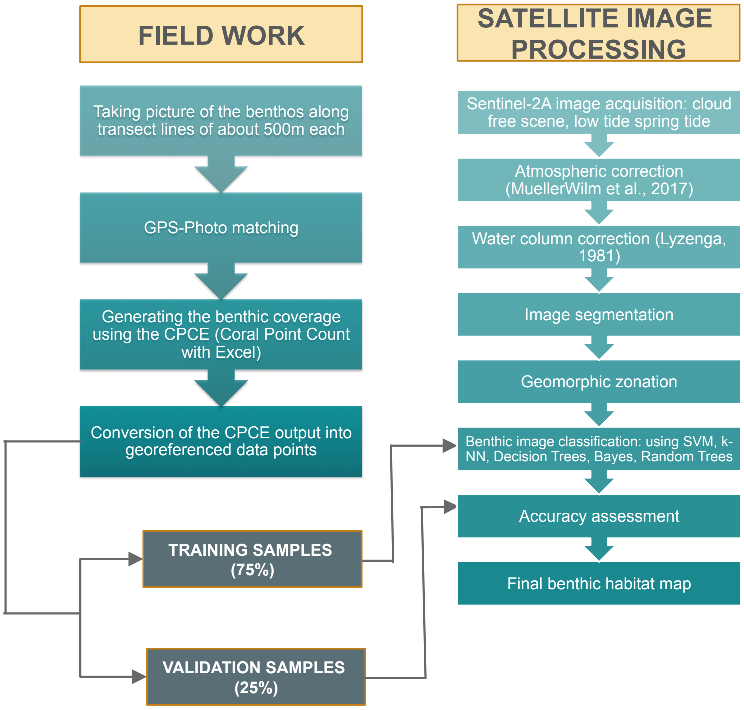

Figure 6. Image processing workflow.

will inform future investigations, guiding parameter choices, and streamlining classification processes, minimizing trial-and-error efforts. The five machine learning classifiers were:

Support Vector Machine (SVM). This assigns class labels to segmented objects by determining an optimal hyperplane, guided by feature vectors extracted from objects' attributes such as spectral values, texture, and spatial relationships. This hyperplane maximizes the margin between classes while minimizing misclassifications, with a focus on support vectors to define the boundary effectively (Mountrakis *et al.*, 2011).

Decision Tree (DT). The DT algorithm recursively partitions the dataset into subsets based on feature conditions, constructing a tree where each internal node represents a feature test and each leaf node denotes a class label. It employs a divide-and-conquer approach to classify instances, following a path from the root to a leaf node determined by feature conditions (Dietterich, 2000).

Random Trees (RT). This is a combination of multiple tree-based classifiers to produce a single classification, an ensemble of decision trees, where each single tree contributes a vote for the assignment of the most popular class to the input data (Xie and Niculescu, 2021).

k-Nearest Neighbour (k-NN). The k-NN algorithm classifies segmented objects based on the class most represented by their k nearest neighbours. K is a user-defined parameter that is the number of nearest neighbouring objects that are included in the majority voting process (Burns *et al.*, 2022) Bayes.

The Bayesian algorithm assigns classes to segmented objects by calculating the probability of each class given the object's features. It uses Bayes' theorem to compute the conditional probability of each class, incorporating prior knowledge and assuming feature independence to make informed decisions (Lewis, 1998). The flow chart of the image processing is provided in Fig. 6.

Benthic class description

The coral class (Fig. 7a) refers to a category with a hard underlying framework that is typically composed of coral-derived limestone, although non-carbonate materials can also be present. This class includes living corals. The rubble class (Fig. 7b) pertains to any area featuring loose, cylindrical to irregularly shaped fragments of bedrock or clasts of corals, bivalves, and coralline algae. This category encompasses limestone reef matrix and underlying areas of coral sand cemented together. The macroalgae class (Fig. 7c) is composed of large, multicellular marine plants that typically thrive in shallow waters surrounding coral reefs. Macroalgae are often observed on top of dead

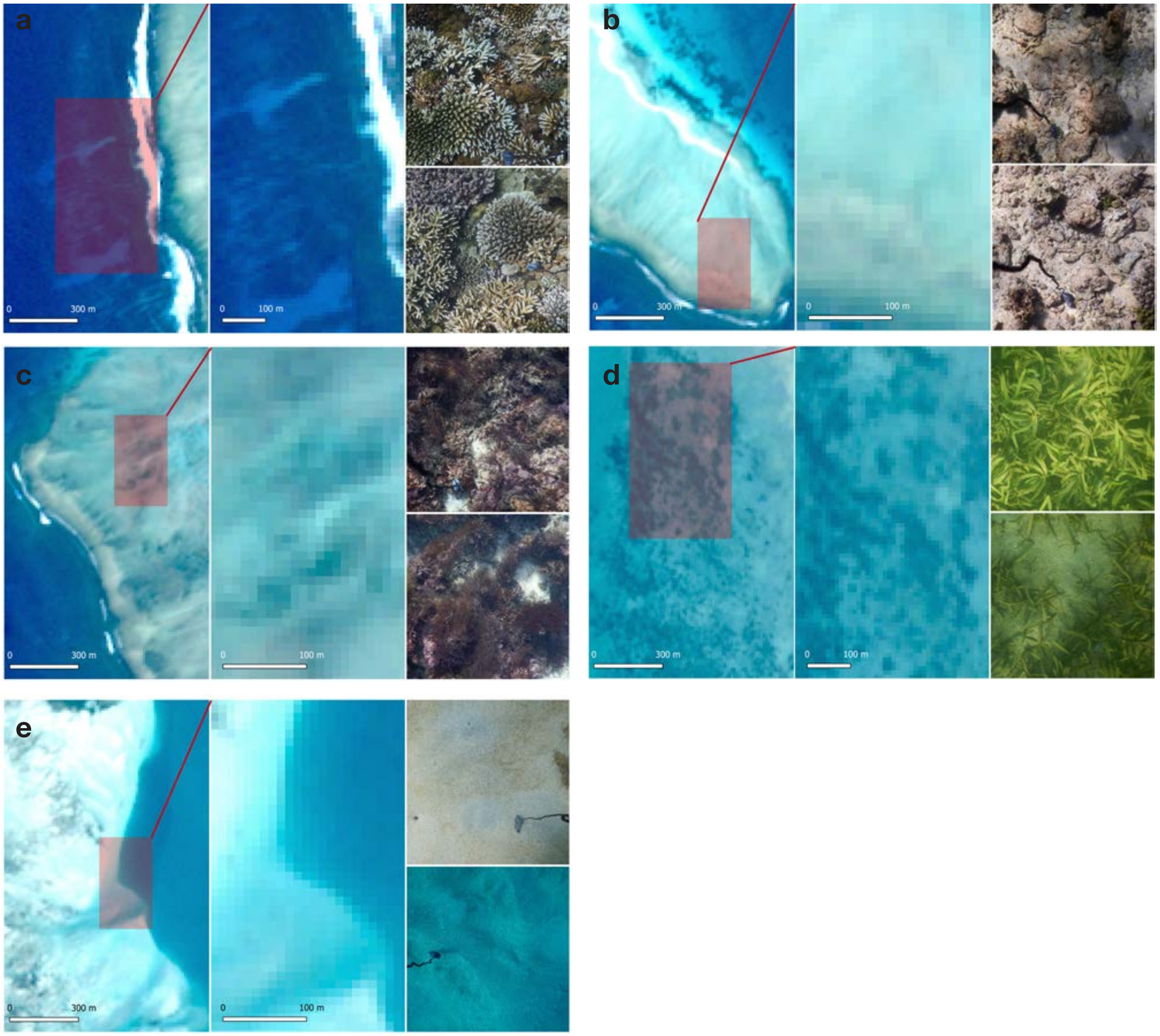

Figure 7. Benthic Classes Across Multiple Scales: 300 m (Sentinel-2 image), 100 m (Sentinel-2 image), and 1 m scale (photoquadrat). (a) Coral, (b) Rubbles, (c) Algae, (d) Seagrass, (e) Sand.

corals, in areas with clear water and abundant sunlight. The seagrass class (Fig. 7d) pertains to a soft-bottomed environment that is mainly characterized by the prevalence of a single or a combination of different species of seagrass. This classification also encompasses sparser or spatially confined seagrass as long as it forms the dominant benthic class. The sand class (Fig. 7e) pertains to soft-bottomed reef regions where fine unconsolidated granular material prevails. This granular material is finer than coral rubble but coarser than mud and thickly overlays any underlying bedrock. Sparse algae, scattered rocks, or small, isolated coral heads may also occur in the sand class. This class also encapsulates areas that are covered by a layer of fine-grained sediment that is mostly composed of organic matter and inorganic particles.

Accuracy assessment of the benthic habitat mapping

The validity, or usefulness, of any interpretation or classification map may be determined with an accuracy assessment that compares the created map with the field work data (Yamano, 2013). Accuracy assessment is commonly derived using an error matrix (also called confusion matrix), which tabulates the level of agreement between the thematic class at a location in the image-based map and the same location in the reference data (Yamano, 2013). The accuracy of each mapping category is described by the individual class accuracies, or according to the user's accuracy (UA) and producer's accuracies (PA) and Overall accuracy (OA), which are all derived from the error matrix. This is generated by using the built-in accuracy assessment tool in the eCognition Developer software. These metrics adhere to the definitions provided by Congalton and Green (2008). OA focuses on assessing the general performance of a classification algorithm across all classes in a dataset. It provides a holistic measure of the classifier's accuracy by considering all classes simultaneously. PA represents the proportion of correctly classified pixels or features for a specific class in relation to the total number of pixels or features that belong to that class on the ground. It focuses on how accurately the algorithm identifies and maps the pixels or features that truly belong to a specific class on the ground. UA represents the proportion of correctly classified pixels or features for a specific class in relation to the total number of pixels or features classified as that class by the classifier. It focuses on how accurately the algorithm assigns pixels or features to a particular class, regardless of whether they truly belong to that class or not. Five classifier algorithms (SVM, DT, RT, Bayes, k-NN) were executed on the image and their accuracy evaluated. Using the classifier algorithm that demonstrated the highest overall accuracy, the benthic classification outcome was refined by merging similar classes and eliminating small misclassified objects, thus improving the clarity and coherence of the final result.

Accuracy assessment of the Allen Coral Atlas

To assess the effectiveness of the benthic cover results for the reefs surrounding Toliara, a comparison with vector data from the Allen Coral Reef Atlas (ACA) (https://allencoralatlas.org) was conducted, acquired in August 2021. This data was cropped to match the scale of the current study, enabling meaningful comparisons. The ACA has the great advantage that it covers reefs around the world, so it is easy to refer to this atlas for a first approximation of benthic coverage of coastal and reef habitats (ACA, 2020).

Andréfouët (2008) mentioned the necessity of alignment of the extent of the ground truth data with the spatial resolution of the sensors. For a meaningful multi-sensor comparison accompanied by objective accuracy assessment, it is imperative that ground truth observations and typology align with the spatial resolution of the sensors. Given this, the raw CPCe data from this study, derived from 1 m x 1 m photoquadrat (with new pictures captured every 3-5 meters), offers a suitable basis for evaluating the accuracy of the ACA data rather than the readapted training and validation data (Fig. 4) that was used to match with the pixel size of the Sentinel-2 image. The ACA data is sourced from Planet Dove satellite images, a commercial satellite that provides a spatial resolution of 3-5meters (Safyan, 2020).

The benthic cover of the reefs of the ACA was composed by 6 categories: "Coral/Algae", "Microalgal Mats", "Rock", "Rubble", "Sand", and "Seagrass". To facilitate this comparison, the field work dataset was reorganized to mirror the typology of the ACA for the accuracy assessment. Specifically, classes such as "Coral" were changed to "Coral/Algae" and "Algae" to "Coral/Algae" in order to align with the ACA data. Classes such as "Rock" and "Microalgal mats" were also removed, as they were absent in the typology of raw data from the current study. However, classes like "Seagrass", "Sand", and "Rubbles" remained unaltered to maintain consistency across datasets.

Results

Accuracy of the image classifications

The overall accuracies of the classifier algorithms used for classifying benthic habitats of coral reefs in Toliara are depicted in Figure 8. The k-NN algorithm showed the highest Overall Accuracy (OA) at 83 %, followed by the Bayes classifier, DT, RT, and SVM, with OA values of 79 %, 68 %, 67 %, and 42 % respectively. Table 2 provides an in-depth analysis of classifier performance in categorizing various benthic habitat samples. It highlights the challenges faced by the Support Vector Machine (SVM) algorithm in accurately classifying Algae and Seagrass samples, with low values of Producer's Accuracy (PA) and User's Accuracy (UA). Other classifiers, such as Naive Bayes, DT, k-NN, RT, showed more robust performance. Naive Bayes and DT achieved high PA values for Rubble

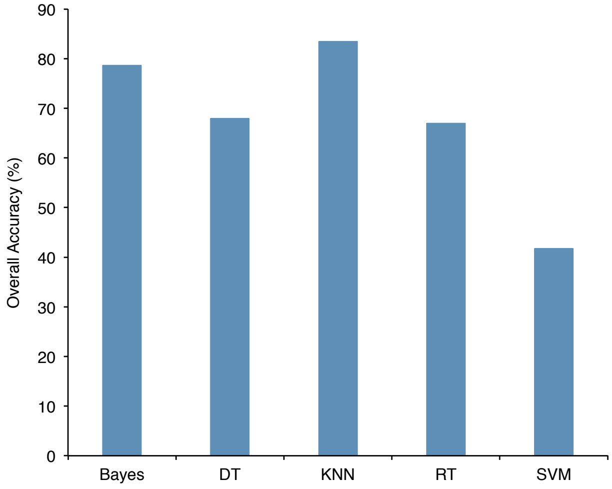

Figure 8. Overall accuracies of the classifiers algorithms used for benthic image classification.

Table 2. Confusion matrix of the five classifiers algorithms.

Classifier Algorithm	User Class \ Sample	Algae	Coral	Rubble	Sand	Seagrass
Bayes OA = 79%	Algae	9	0	0	0	0
	Coral	0	6	0	0	0
	Rubble	2	0	23	5	3
	Sand	1	0	1	20	2
	Seagrass	0	0	2	6	23
	PA	0,75	1	0,885	0,645	0,821
	UA	1	1	0,697	0,833	0,742
	User Class \ Sample	**Algae**	**Coral**	**Rubble**	**Sand**	**Seagrass**
DT OA = 68%	Algae	9	0	2	1	5
	Coral	0	6	0	0	0
	Rubble	0	0	22	7	9
	Sand	3	0	1	23	4
	Seagrass	0	0	1	0	10
	PA	0,75	1	0,846	0,742	0,357
	UA	0,529	1	0,579	0,742	0,909
	User Class \ Sample	**Algae**	**Coral**	**Rubble**	**Sand**	**Seagrass**
k-NN OA = 83%	Algae	11	0	2	2	2
	Coral	0	6	0	0	0
	Rubble	0	0	19	1	1
	Sand	1	0	4	28	3
	Seagrass	0	0	1	0	22
	PA	0,917	1	0,731	0,903	0,786
	UA	0,647	1	0,905	0,778	0,957
	User Class \ Sample	**Algae**	**Coral**	**Rubble**	**Sand**	**Seagrass**
RT OA = 67%	Algae	10	0	3	2	3
	Coral	0	6	0	0	0
	Rubble	0	0	16	6	6
	Sand	2	0	4	23	5
	Seagrass	0	0	3	0	14
	PA	0,833	1	0,615	0,742	0,5
	UA	0,556	1	0,571	0,676	0,824
	User Class \ Sample	**Algae**	**Coral**	**Rubble**	**Sand**	**Seagrass**
SVM OA = 42%	Algae	0	0	0	0	0
	Coral	3	6	0	2	0
	Rubble	4	0	12	1	0
	Sand	4	0	2	9	12
	Seagrass	1	0	12	19	16
	PA	0	1	0,462	0,290	0,571
	UA	undefined	0,545	0,706	0,333	0,333

and Seagrass, respectively, while k-NN demonstrated effectiveness in identifying Coral and Algae habitats. RF performed well in classifying Algae and Sand but struggled with Seagrass. In contrast, SVM struggled with Algae and Seagrass classification, with notably low PA values across multiple classes and variable UA values. Compared to other classifiers evaluated in this study, SVM exhibited lower OA, UA and PA for most habitat classes, indicating its potentially unsuitability for benthic habitat mapping applications under the given data calibration types and benthic environment. Figure 9 depicts various outputs of benthic habitat classification, highlighting the variability in results despite employing identical calibration data and image pre-processing techniques. This shows the importance of selecting the appropriate classifier algorithm, as it profoundly impacts the outcomes of benthic coverage assessment and the precise evaluation of each benthic habitat's surface area.

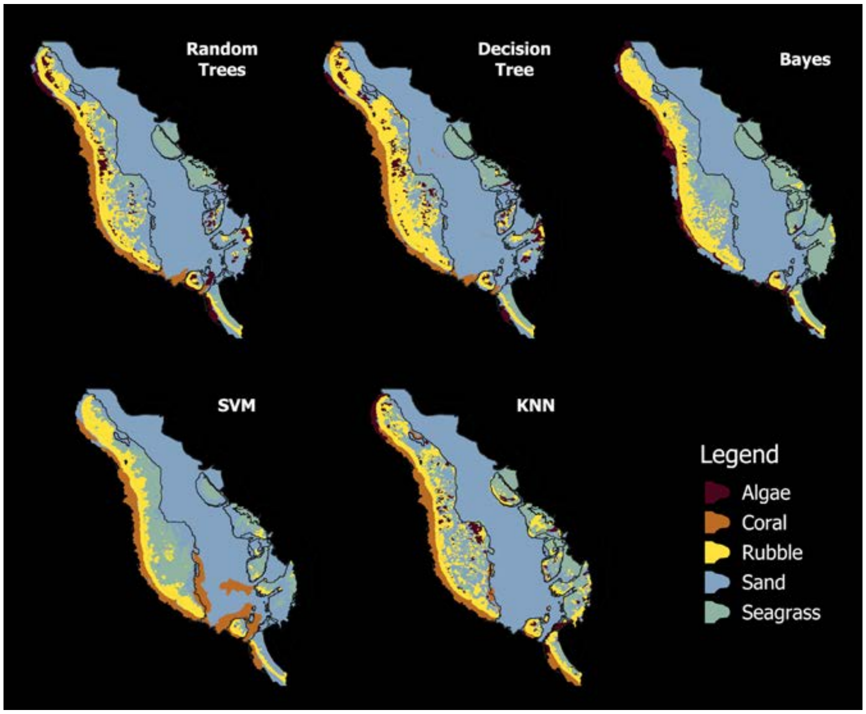

Figure 9. Outputs of different machine learning algorithms to map the benthic coverage of Toliara's coral reefs.

In reference to ACA, Table 3 presents the outcome of the accuracy assessment for its benthic coverage data. Compared with the 4187 CPCe data, the ACA achieves an overall accuracy of 50 %, failing to meet the 60 % minimum standard outlined in Yamano (2013). Additionally, a very low overall accuracy (OA) and user accuracy (UA) was noticed across all classes, particularly for rubbles and seagrass habitats.

Benthic coverage extent of the reefs of Toliara

The benthic coverage of the Toliara reefs was evaluated by using the k-NN classifier, which demonstrated the highest accuracy compared to the four other algorithms. Each habitat type is categorized based on its location within the reef system (Fig. 10). The internal reefs, along with their associated habitats, primarily span the area between Ankilibe and Sarodrano, as illustrated in Fig. 12c. The surface area measurements are presented for five benthic classes: Rubble (21 km^2), Coral (10 km^2), Algae (6 km^2), Seagrass (22 km^2), and Sand (73 km^2). Internal reefs exhibit substantial surface area coverage, particularly for Seagrass (8 km^2) and Rubble (4 km^2). Coral and Algae also contribute significantly to the internal reef ecosystem, with surface areas of 0.4 km^2 and 1.46 km^2 respectively. The Lagoon habitat features smaller surface areas compared to internal reefs. Notably, Sand is the predominant substrate in the lagoon, covering a substantial area of 57 km^2. Unlike Rubble (0.4 km^2), Coral (0.3 km^2) and Algae (0.5 km^2) that represent less significant surface coverage, Seagrass emerges as the next dominant feature in this habitat type, spanning an area of 5 km^2 indicating its importance as a habitat within the lagoon environment. Reef flat habitats showcase considerable surface area coverage, particularly for Seagrass (8 km^2) and Rubble (11 km^2) and Sand (12 km^2).

Table 3. Confusion matrix of the benthic coverage of the Allen Coral Atlas data.

OA= 50%	Coral/Algae	Rubble	Sand	Seagrass
Coral/Algae	596	35	155	54
Rubble	181	617	408	281
Sand	2	53	180	15
Seagrass	206	140	103	241
User accuracy (%)	0.6	0.7	0.2	0.4
Producer accuracy (%)	0.7	0.4	0.7	0.3

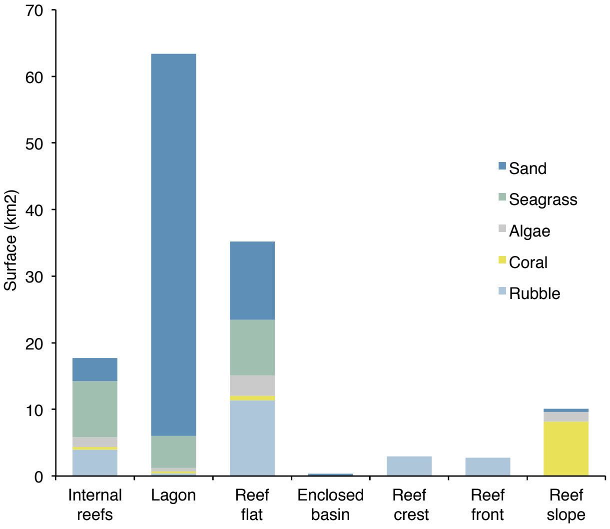

Figure 10. Surface of coral benthic cover per reef geomorphology.

Enclosed basin habitats exhibit relatively smaller surface area coverage compared to other habitat types. Sand dominates this habitat type, covering 0.2 km^2, while Coral (0.04 km^2), Algae (0.004 km^2) and Seagrass (0.006 km^2) habitats exhibit minor surface area coverage. Both Reef Crest and Reef Front habitats exhibit minimal surface area coverage for all habitat classes, indicating their relatively limited extent within the reef system. These habitats are predominantly composed of Rubble which both represents about 3 km^2.

Reef slope habitats demonstrate unique characteristics with significant surface area coverage for Coral (8 km^2). Algae (1 km^2) and Sand (0.5 km^2) classes also exhibit notable coverage, while Seagrass (0.017 km^2) and Rubble classes are present in smaller amounts.

Discussion

Evaluating global coral reef mapping initiatives

Recent initiatives aimed at enhancing global coral reef mapping, such as the Allen Coral Atlas (Allen Coral Atlas, 2020) and the Global Distribution of Coral Reefs (UNEP-WCMC, WorldFish Centre, WRI, TNC, 2021), provide publicly accessible datasets facilitating easy access to information on coral reef geomorphology and benthic coverage. However, caution is warranted when using such data for national coral reef management strategies, restoration programmes, or economic valuations of these ecosystems. A comparison of the coral reef data generated by the UNEP-WCMC, the ACA geomorphology and the present study is provided in Figure 12. A total surface of 162 km^2 for the total extent of coral reef systems surrounding Toliara was calculated in the present study, while ACA provides a total of 126.8 km^2 and the UNEP-WCMC coral reef data totaled 61.1 km^2. It is also worth noting that this later dataset misses the fringing reef of Sarodrano (Fig. 12.a) which is present in both Figure 12.b and Figure 12.c. Figure 12.b also shows that the enclosed basin, locally

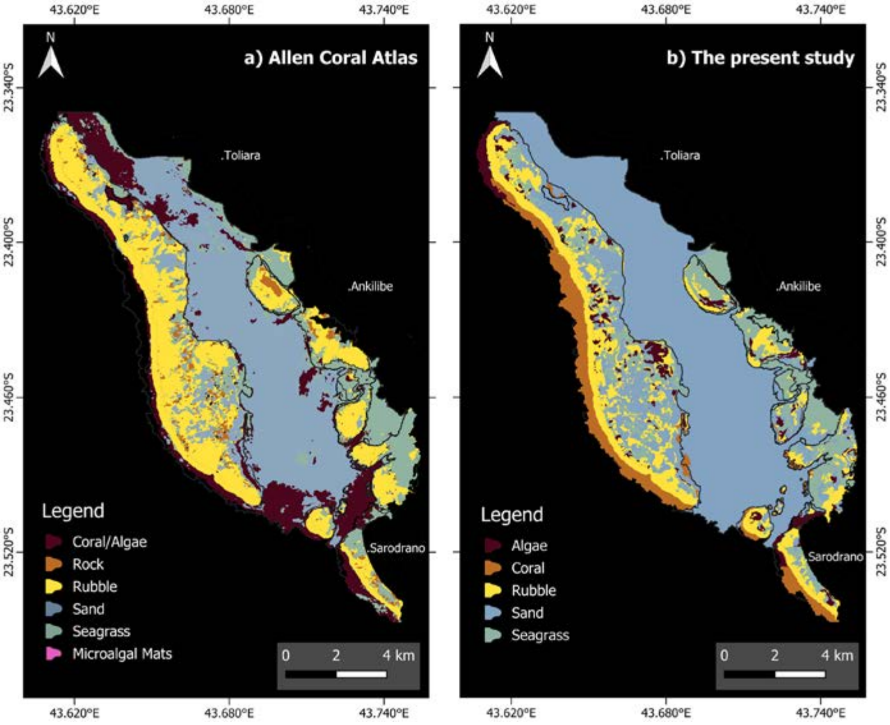

Figure 11. Comparison of benthic coverage provided by the ACA data and the present study.

known as "*Grande Vasque*" is erroneously interpreted as reef slope. This figure also showcases several hard reef structures within the lagoon which are misrepresented as reef slopes where it is clearly just a deep lagoon. Furthermore, concerning the benthic coverage of the coral reef system in Toliara, the current analysis reveals surface areas occupied by seagrass, sand, rubble, coral, and algae, amounting to 21 km², 73 km², 21 km², 10 km², and 6 km² respectively. Had marine scientists or policymakers used the ACA dataset, their calculations would have shown 22 km² for seagrass, and 48 km² for sand, 21 km² for Coral/Algae, and 28 km² for Rubble. The findings from the present study align with those reported by Botosoamananto *et al*. (2021), who conducted localized surveys within this coral reef system. The outer reef slope is predominantly characterized by robust hard corals, as highlighted, with the highest concentration of macroalgae observed in its northern section. Additionally, the authors noted substantial hard coral coverage within the reef patches of Sarodrano and the southern segment of the inner slope of the "*Grand Récif de Toliara*" (GRT). They also observed a significant presence of rubble on the reef flats as illustrated in Figure 11, a phenomenon documented by Bruggemann *et al.* (2012) and Andréfouët *et al.* (2013). These studies describe this particular section of the barrier reef, marked by an accumulation of dead coral and rubble on the reef flat.

Insight and considerations to enhance coral reef mapping

Assessment of benthic cover of coral reefs requires special attention, as variations in methodology can lead to inconsistencies in coral reef mapping and classification. The total surface area of coral reefs in Madagascar has been reported differently across studies, with UNEP-WCMC, WorldFish Centre, WRI, TNC (2021) reporting a total surface area of about 3,100 km² and the Allen Coral Atlas (Allen Coral Atlas, 2020) showing a total of 5,076 km² for the coral reefs of Madagascar. These variations do not indicate changes in coral reef extent but rather the different methodologies used to assess them. Coral reefs are complex and diverse ecosystems, with different species, morphology, and spatial arrangement depending on local environmental conditions. Inaccurate coral reef mapping and classification can have serious consequences for conservation efforts, leading to misinformed policy and management decisions. Overestimating coral cover can lead to inappropriate land-use decisions such as coastal development or tourism which can result in coral reef degradation and loss. Conversely, underestimating coral cover can result in inadequate protection or management measures, putting these important ecosystems at risk. Therefore, when using remote sensing techniques, a consistent and standardized methodology for assessing marine habitats is essential for accurate and effective conservation

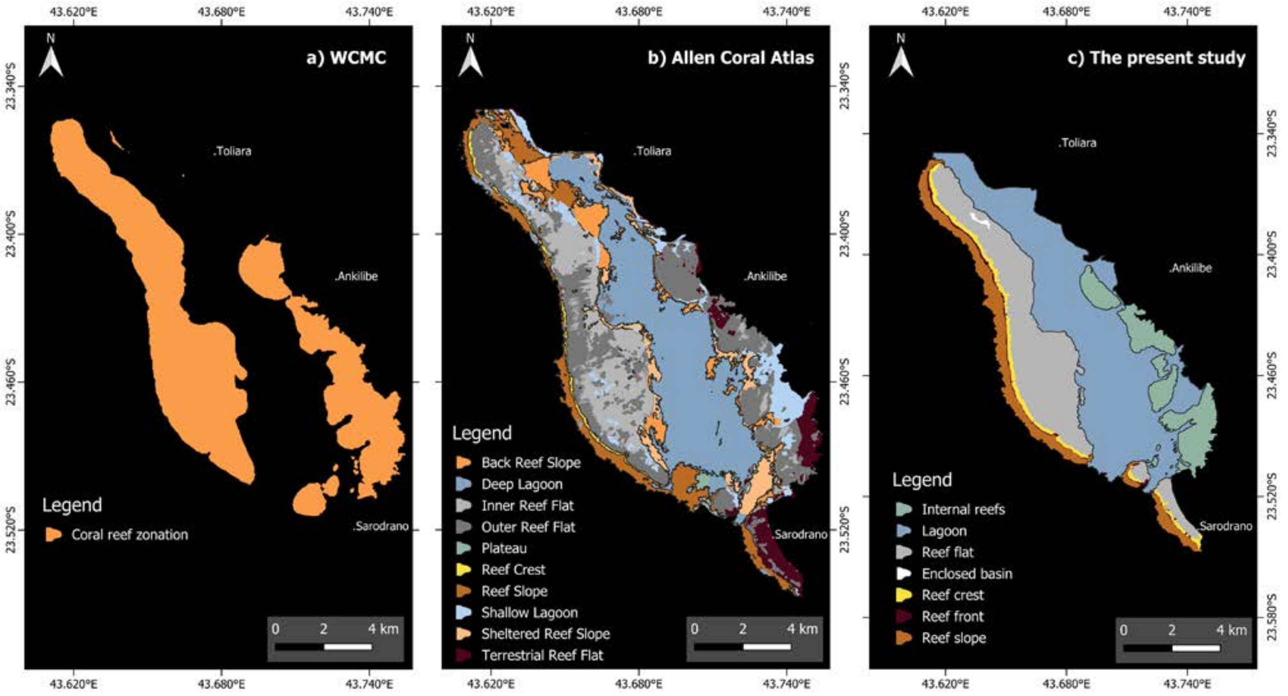

Figure 12. Comparison between the available data of coral reef geomorphology in Toliara.

and management. Validation and calibration of these techniques using *in-situ* data are necessary to ensure their accuracy and reliability. Overall, remote sensing can be a powerful tool for marine habitat assessment but it must be used with caution and care to avoid the negative consequences of methodology diversity. This study of the Toliara coral reef system underscores the significance of integrating multiple fieldwork datasets into the model training process, resulting in a notably enhanced mapping accuracy. This emphasizes the crucial role of *in situ* data collection in refining remote sensing techniques for more precise benthic habitat mapping. Across the globe, various classification algorithms are used for coral reef classification, with their effectiveness contingent upon regional characteristics, available field data, and the spectral and temporal resolution of satellite imagery. While SVM, k-NN, and RT algorithms are prevalent in object-based coral reef benthic mapping publications (Burns *et al.*, 2022), studies such as those by Mountrakis *et al.*, (2011) indicate that SVM often yields higher accuracy values compared to other techniques. However, the current research reveals that in this specific context, the k-NN algorithm outperforms SVM in terms of accuracy. This highlights the importance of selecting the most suitable algorithm tailored to local conditions when mapping coral habitats. Moreover, the performance of classification algorithms is subject to variations in image quality and resolution, training data set size, class types, and algorithm-specific parameter tuning. Hence, it is imperative to identify the most effective approach for this region based on these factors.

Conclusion

This study highlights the successful application of the OBIA method in conjunction with *in-situ* data to map coral reefs at local scales, using freely available high-resolution satellite images from Sentinel-2. This approach provides a replicable methodology for coral reef mapping projects using the same types of image and field data and lays the foundation to assess long-term changes in coral reef habitats, spatial observations of coral reef resilience, evaluation of seagrass distribution, and assessment of habitat health for herbivorous fishes. The use of georeferenced photographs not only establishes a formal linkage between the image and field data but also presents a valuable opportunity for informing stakeholders, managers, and other interested parties on the capabilities of satellite imaging for mapping and measuring reef features. Over 4,000 georeferenced photographs were used as reference data to produce a highly accurate map of the 18 km-long barrier reef of Toliara and the nearby reef systems. The efficacy of this mapping approach relies on both the quantity and quality of fieldwork data used to train the classifier algorithms for identifying features within satellite images. The greater the availability of comprehensive field data, the higher the accuracy of the resultant map. This explains why the ACA does not perform optimally in areas with limited field data. While global data may offer an initial reference for evaluating the extent or likelihood of coral reef occurrence, solely depending on such information for decision-making concerning coral reef management or restoration programmes entails considerable risks. Therefore, nations should prioritize local data collection and national-level satellite image processing to ensure precise assessments. This study illustrates that, even with freely available satellite imagery such as Sentinel-2 and basic logistical resources for field work data collection, sufficient accuracy can be attained to produce maps of coral reef geomorphology and benthic habitats.

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References

Allen Coral Atlas (2020) Imagery, maps and monitoring of the world's tropical coral reefs [doi: 10.5281/zenodo.3833242]

Andréfouët S (2008) Coral reef habitat mapping using remote sensing: A user vs producer perspective, implications for research, management and capacity building. Journal of Spatial Science 53: 113-129 [doi: 10.1080/14498596.2008.9635140]

Andréfouët S, Guillaume MMM, Delval A, Rasoamanendrika FMA, Blanchot J, Bruggemann, JH (2013) Fifty years of changes in reef flat habitats of the Grand Récif of Toliara (SW Madagascar) and the impact of gleaning. Coral Reefs 32: 757-768 [doi: 10.1007/s00338-013-1026-0]

Battistini R, Bourrouilh F, Chevalier JP, Coudray J, Denizot M, Faure G, Ficher JC, Guilcher, A, Harmelin-Vivien M, Jaubert J, Laborel J, Montaggioni L, Masse JP, Mauge LA, Peyrot-Clausade M, Pichon M, Plante R, Plaziat JC, Plessis YB, Richard G, Salvat B, Thomassin BA, Vasseur P, Weydert P (1975) Éléments de terminologie récifale indopacifique. Téthys 7: 1-111

Botosoamananto RL, Todinanahary G, Razakandrainy A, Randrianarivo M, Penin L, Adjeroud M, (2021) Spatial patterns of coral community structure in the Toliara region of southwest Madagascar and implications for conservation and management. Diversity 13: 486 [doi:10.3390/d13100486]

Bruggemann, JH, Rodier M, Guillaume MMM, Andréfouët S, Arfi R, Cinner JE, Pichon M, Ramahatratra F, Rasoamanendrika F, Zinke J, McClanahan TR (2012) Wicked social–ecological problems forcing unprecedented change on the latitudinal margins of coral reefs: the case of southwest Madagascar. Ecological Society 17: 4-21 [doi:10.5751/ES-05300-170447].

Burns C, Bollard B, Narayanan A (2022) Machine-learning for mapping and monitoring shallow coral reef habitats. Remote Sensing 14: 2666 [doi:10.3390/rs14112666]

Congalton RG, Green K (2008) Assessing the accuracy of remotely sensed data: Principles and practices. Mapping Science. CRC Press, Boca Rotan FL., Second Edition. 183 pp

Dietterich TG (2000) An experimental comparison of three methods for constructing ensembles of decision trees: Bagging, boosting, and randomization. Machine Learning 40: 139-157

Eakin CM, Nim CJ, Brainard R, Aubrecht C, Elvidge C, Gledhill DK, Muller-Karger F, Mumby PJ, Skirving W, Strong AE, Wang M, Weeks S, Wentz F, Ziskin D (2010) Monitoring coral reefs from space. Oceanography 23:119-133

ESA's Sentinel-2 team (2015) The story of Sentinel-2. European Space Agency 161: 4-9

Foo SA, Asner GP (2019) Scaling up coral reef restoration using remote sensing technology. Frontiers in Marine Science 6: 1-8 [doi:10.3389/fmars.2019.00079]

Hedley JD, Roelfsema CM, Phinn SR, Mumby PJ (2012) Environmental and sensor limitations in optical remote sensing of coral reefs: Implications for monitoring and sensor design. Remote Sensing 4: 271-302 [doi: doi:10.3390/rs4010271]

Hedley JD, Roelfsema CM, Chollet I, Harborne AR, Heron SF, Weeks S, Skirving W, Strong AE, Eakin CM, Christensen TRL, Ticzon V, Bejarano S, Mumby PJ (2016) Remote sensing of coral reefs for monitoring and management: A review. Remote Sensing 8: 1-40

Hedley JD, Roelfsema C, Brando V, Giardino C, Kutser T, Phinn S, Mumby PJ, Barrilero O, Laporte J, Koetz B (2018) Coral reef applications of Sentinel-2: Coverage, characteristics, bathymetry and benthic mapping with comparison to Landsat 8. Remote Sensing of the Environment 216: 598-614 [doi:10.1016/j.rse.2018.07.014]

Kennedy EV, Roelfsema CM, Lyons MB, Kovacs EM, Borrego-Acevedo R, Roe M, Phinn SR, Larsen K, Murray NJ, Yuwono D, Wolff J, Tudman P (2021) Reef Cover, a coral reef classification for global habitat mapping from remote sensing. Scientific Data 8: 1-20 [doi:10.1038/s41597-021-00958-z]

Kohler KE, Gill SM (2006) Coral point count with Excel extensions (CPCe): A Visual Basic programme for the determination of coral and substrate coverage using random point count methodology. Computers & Geosciences 32: 1259-1269 [doi:10.1016/j.cageo.2005.11.009]

Lewis DD (1998) Naive (Bayes) at forty: The independence assumption in information retrieval, In: Nédellec C, Rouveirol C (eds) Machine Learning: ECML-98, Lecture Notes in Computer Science. Springer Berlin, Heidelberg pp 4–15 [doi:10.1007/BFb0026666]

Louis J, Debaecker V, Pflug B, Main-Knorn M, Bieniarz J, Mueller-Wilm U, Cadau E, Gascon, F (2016) Sentinel-2 Sen2Cor: L2A processor for users. In: Proceedings of the Living Planet Symposium 2016. ESA SP-740, Prague, Czech Republic. 8 pp

Lyzenga DR (1981) Remote sensing of bottom reflectance and water attenuation parameters in shallow water using aircraft and Landsat data. International Journal of Remote Sensing 2: 71-82 [doi:10.1080/01431168108948342]

Mountrakis G, Im J, Ogole C (2011) Support vector machines in remote sensing: A review. ISPRS Journal of Photogrammetry and Remote Sensing 66: 247-259 [doi:10.1016/j.isprsjprs.2010.11.001]

Nguyen T, Liquet B, Mengersen K, Sous D (2021) Mapping of coral reefs with multispectral satellites: A review of recent papers. Remote Sensing 13: 4470 [doi:10.3390/rs13214470]

Nurlidiasari M, Budiman S (2010) Mapping coral reef habitat with and without water column correction using Quickbird image. International Journal of Remote Sensing and Earth Sciences 2:45-56 [doi:10.30536/j.ijreses.2005.v2.a1357]

Phinn SR, Roelfsema CM, Mumby PJ (2012) Multi-scale, object-based image analysis for mapping geomorphic and ecological zones on coral reefs. International Journal of Remote Sensing 33: 3768-3797 [doi:10.1080/01431161.2011.633122]

Pichon M (1972) Les peuplements à base de sléractiniaires dans les récifs coralliens de la Baie de Tuléar

(Sud-Ouest de Madagsacar), Station Marine d'Endoume. ed. Marseille, France. pp 135-154

Razakandrainy A (2018) Mécanismes de maintien des populations de coraux dans la région de Toliara, sud-ouest de Madagascar (Mémoire DEA en Océanographie Appliquée). Université de Toliara, Institut Halieutique et des Sciences Marines (IH.SM), Toliara, Madagascar. 54 pp

Roelfsema CM, Markey K, Kennedy EV, Kovacs EM, Borrego-Acevedo R, Fox HE, Bambic B, Free B, Rice K, Phinn SR (2019) Protocol for georeferenced benthic psotoquadrat surveys. Remote Sensing and Research Centre, School of Earth and Environmental Sciences. University of Queensland, Brisbane, Australia. 17 pp

Safyan M (2020) Planet's Dove Satellite Constellation. In: Pelton, JN (ed) Handbook of small satellites. Springer International Publishing, Cham. pp 1-17 [doi:10.1007/978-3-030-20707-6_64-1]

Todinanahary GGB (2016) Evaluation du potentiel biologique, économique et social de la coralliculture dans le sud-ouest de Madagascar (Thèse de Doctorat). Université de Mons (Belgique), Faculté des Sciences, Biologie des Organismes Marins et Biomimétisme.

Trimble Germany GmbH (2022) eCognition Developer.

UNEP-WCMC, WorldFish Centre, WRI, TNC (2021) Global distribution of coral reefs, compiled from multiple sources including the Millennium Coral Reef Mapping Project. Version 4.1, updated by UNEP-WCMC. Includes contributions from IMaRS-USF and IRD (2005), IMaRS-USF (2005) and Spalding *et al.* (2001). UN Environment Programme-World Conservation Monitoring Centre, Cambridge, UK [doi: 10.34892/t2wk-5t34]

van Hooidonk R, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo L, Williams G, Heron, SF, Planes S (2016) Local-scale projections of coral reef futures and implications of the Paris Agreement. Scientific Reports 6:1-8 [doi:10.1038/srep39666]

Wilkinson C (2008) Status of coral reefs of the world. Global Coral Reef Monitoring Network (GCRMN). Townsville, Australia. 294 pp

Wouthuyzen S, Abrar M, Corvianawatie C, Salatalohi A, Kusumo S, Yanuar Y, Darmawan, Samsuardi Y, Arrafat MY (2019) The potency of Sentinel-2 satellite for monitoring during and after coral bleaching events of 2016 in the some islands of Marine Recreation Park (TWP) of Pieh, West Sumatra. Earth and Environmental Science 284: 012028 [doi: 10.1088/1755-1315/284/1/012028]

Xie G, Niculescu S (2021) Mapping and monitoring of land cover/land use (LCLU) changes in the Crozon Peninsula (Brittany, France) from 2007 to 2018 by machine learning algorithms (Support Vector Machine, Random Forest, and Convolutional Neural Network) and by Post-classification Comparison (PCC). Remote Sensing 13: 3899 [doi:10.3390/rs13193899]

Xu J, Zhao D (2014) Review of coral reef ecosystem remote sensing. Acta Ecologica Sinica 34: 19-25 [doi:10.1016/j.chnaes.2013.11.003]

Yamano H (2013) Chapter 3: Multispectral applications. In: Coral reef remote sensing: A guide for mapping, monitoring and management. Springer, Dordrecht, Netherlands. pp 51-78

Yunus AP, Jie D, Song X, Avtar R (2019) High resolution Sentinel-2 images for improved bathymetric mapping of coastal and lake environments (preprint). Earth Sciences [doi:10.20944/preprints201902.0270.v1]

Zoffoli ML, Gernez P, Rosa P, Le Bris A, Brando VE, Barillé A-L, Harin N, Peters S, Poser K, Spaias L, Peralta G, Barillé L (2020) Sentinel-2 remote sensing of *Zostera noltei*-dominated intertidal seagrass meadows. Remote Sensing of Environment 251: 112020 [doi:10.1016/j.rse.2020.112020]

Original Article

Economic viability of seaweed and sea cucumber culture using integrated multitrophic aquaculture systems in Zanzibar, Tanzania

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Markus Bernard[1,2], Hafzur Rahman[1,2], William Mangile[1,2,3],
Flower E. Msuya[4], Andreas Kunzmann[1,2,*]

[1] Leibniz Centre for Tropical Marine Research, Fahrenheitstraße 6, 28359 Bremen, Germany

[2] University of Bremen, Bibliothekstraße 1, 28359 Bremen, Germany

[3] School of Aquatic Science and Fisheries, University of Dar es Salaam, Kunduchi, Dar es Salaam, Tanzania

[4] Zanzibar Seaweed Cluster Initiative, Malindi Rd, Zanzibar, Tanzania

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*** Corresponding author:**
andreas.kunzmann@leibniz-zmt.de

Abstract

The viability of co-culturing seaweed and sea cucumbers in Zanzibar, Tanzania, was assessed using integrated multitrophic aquaculture (IMTA) systems with oyster and recirculating aquaculture systems with milkfish. Seaweed production was affected by the ice-ice disease, resulting in specific growth rates of 1.32 to -1.96 % d^{-1}. Nitrogen content in the seaweed thallus and inorganic nutrient in the water indicated that seaweed could potentially be used as a biofilter. An economic analysis showed the economic viability of IMTA systems, co-culturing seaweed *Kappaphycus alvarezii*, sea cucumber *Holothuria scabra* and oyster *Pinctada margaritifera*, achieving a maximum benefit-cost ratio of 1.61 over one year. Interviews with local farmers on Zanzibar showed willingness to accept new aquaculture systems, but also highlighted constraints, including a lack of infrastructure, funds and expertise.

Keywords: aquaculture, integrated polyculture, cost-benefit ratio, RAS, social viability, questionnaire

Introduction

Due to the projected rise of global human population (United Nations, 2019) and the subsequent increased demand for fisheries products (FAO, 2020), many more wild fish stocks will be overexploited or depleted in a few years. Aquaculture, playing a vital role to meet the ever increasing demand for food, has however developed a controversial reputation, due to high density operations, environmental degradation and water pollution (Hall *et al.*, 2011; Ahmed and Turchini, 2021). Major changes are necessary to control aquaculture production – more resilient and innovative practices need to be developed if this sector is to become the most efficient and responsible food production system of the future (Soto, 2009; Troell *et al.*, 2009; Ahmed *et al.*, 2019).

There are several promising systems that address the issue of effluent from aquaculture operations. Recirculating aquaculture systems (RAS) as one possible solution, are based on the recirculation of water with various kinds of filters, such as biofilters, solid filters or protein skimmers (Badiola *et al.*, 2012; van Rijn, 2013). This way, water can be recycled and the amount of fresh water needed and waste water produced greatly reduced. Instead of conventional biofilters with bacteria, biofilters can also comprise of different animals or plants used to filter the water. However, RAS is expensive and technologically challenging and therefore not appropriate for aquaculture in many developing countries (Badiola *et al.*, 2018; Engle *et al.*, 2020). For aquaculture systems to work in developing countries they need to be low-tech and low-cost, which can

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easily be built in spite of the lack of infrastructure and high expertise. Another solution could be Integrated Multitrophic Aquaculture (IMTA), a sustainable aquaculture practice through co-culturing of species from different trophic levels. In IMTA, faeces, uneaten food materials, and nutrients from higher trophic species such as finfish or shrimps become food for lower trophic species such as detritivores or filter feeders. Dissolved inorganic nutrients can be taken up by plants or algae, acting as biofilters (Chopin *et al.*, 2001; Troell *et al.*, 2003; Neori *et al.*, 2004; Ren *et al.*, 2012). IMTA not only aims to promote an ecologically sustainable and social approach (Ertör and Ortega-Cerdà, 2015), but it also can provide economic stability for aquaculture producers by providing farmers with a more diversified set of crops compared to monoculture (Knowler *et al.*, 2020).

Recent literature shows many good examples of IMTA practices in tropical countries (Putro *et al.*, 2015; Ahmed and Glaser, 2016; Felaco *et al.*, 2020; Franchini *et al.*, 2020; Putro *et al.*, 2020), including seaweed (Largo *et al.*, 2016; Shpigel *et al.*, 2018). In Tanzania the focus has been on the combination of sea cucumbers with seaweed (Namukose *et al.*, 2016; Kunzmann *et al.*, 2018; Fabiani *et al.*, 2023; Kunzmann *et al.*, 2023). Few of these studies integrated filter feeders such as bivalves, and economic aspects were not considered in many of them.

Seaweed farming is gaining worldwide popularity, reaching a global production of 13.5 million tonnes in 2020 (Buschmann *et al.*, 2017; FAO, 2022). The seaweed culture cycle is relatively short compared to other aquaculture species, and farming techniques are low cost. As the global market for seaweed has expanded the economic returns from seaweed aquaculture have risen (García-Poza *et al.,* 2020).

Aquaculture on Zanzibar includes the farming of fish, mud crabs, pearl oysters, sea cucumbers, seaweed and sponges (Msuya *et al.*, 2016; Charisiadou *et al.*, 2022). In Tanzania seaweed farming began in 1989, initially focused on two macroalgae species, *Eucheuma denticulatum* (spinosum) and *Kappaphycus spp* (cottonii) (Msuya *et al.*, 2007; Msuya, 2020). The industry is the third largest sector in terms of value, contributing 7.6 % to the GDP of Zanzibar in 2011 (Msuya and Hurtado, 2017). Seaweed farming faced multiple challenges in the past decades (Msuya and Porter, 2014), including the ice-ice disease, which is caused by infestation of microbes and facilitated mainly by high temperatures (Ward *et al.*, 2022). There have been attempts to overcome this issue by moving the seaweed farms into deeper waters (Msuya, 2020).

In Tanzania, sea cucumber fishing is an important economic and subsistence activity for local communities and is extensively practiced (Eriksson *et al.*, 2012). Aquaculture production of sea cucumbers has primarily focused on the most valuable species *Holothuria scabra*, which are sold and exported mainly to China for high prices.

Shellfish farming for pearl production in Zanzibar started in 2004, with an estimated production in 2012 of about 1,000 pearls, valued at 10 to 20 US$ each (Mmochi, 2015). The potential of pearl oysters for bioremediation has been positively evaluated and pearl production could be a viable economic activity for coastal communities in East Africa (Southgate *et al.*, 2006; Ishengoma *et al.*, 2011).

Finfish production in Tanzania is mainly focused on the species milkfish *Chanos chanos* and flathead grey mullet *Mugil cephalus*. Traditionally wild-caught fingerlings are reared in larger earthen ponds, connected to the sea, or in smaller earthen ponds directly in the mangroves until market size and then sold mainly on local markets (Msuya *et al.*, 2016).

The success of sustainable aquaculture systems can contribute to farmers' food security and income, but requires the development and testing of new sustainable aquaculture systems. Most systems used, are outdated and can have severe impacts on the surrounding environments. This study therefore aims to compare biomass production and economic viability of different land-based IMTA approaches, including the cultivation of seaweed *Kappaphycus alvarezii* and sea cucumbers *H. scabra* with pearl oysters *Pinctada margaritifera* or milkfish *C. chanos*.

Materials and methods

Study site and species collection

Research was conducted in 2022 at the KOICA-RGoZ Mariculture Hatchery Zanzibar, Tanzania (6°07'01.0"S 39°12'42.0"E). Seaweed (*K. alvarezii*) was collected from a small group of local farmers in Muungoni (6°19'11.7"S 39°24'43.2"E). Sea cucumbers (*H. scabra*) were collected from a farmer in Unguja Ukuu (6°19'01.3"S 39°22'16.4"E) and pearl oysters (*P. margaritifera*) from the farms in Nyamanzi (6°16'03.5"S 39°14'55.9"E). The milkfish (*C. chanos*) were taken from brackish water ponds near Bumbwini (5°56'42.6"S 39°12'09.4"E). All animals were kept in a separate tank without any sediment and feed, to ensure an empty gut when weighing, before stocking. Marine sand/sediment was taken during low tide from near Fukuchani (5°50'09.5"S 39°17'01.7"E) and the bottom of the tanks was covered with 5 cm of this, acting as the source of organic nutrients for the sea cucumbers.

Experimental design

Two distinct experimental designs were developed for the co-culture trials. One system (IMTA) combined seaweed, sea cucumbers and sea oysters in one tank, while in another system (RAS), seaweed, sea cucumbers and milkfish were integrated in separate tanks. Both experiments were conducted simultaneously from October to December/January 2022/2023. The IMTA systems ran for 90 days, with two cycles of 45 days each, whereas the RAS system ran for 70 days with one cycle only.

The IMTA system consisted of four treatments (A, B, C and D) with four replicates each (4x4), i.e., 16 tanks. Because of logistic restraints, of the 16 tanks, seven were 490 liter fiberglass tanks, three 500 liter fiberglass tanks and six 1000 liter plastic tanks. Nylon ropes were tied on two sides over the tanks, on which 50 g of fresh seaweed fronds were tied at 20 cm intervals and suspended 20 cm below the surface; sea oysters were suspended in a rectangular cage. Figure 1 shows the setup of tanks at the hatchery for all treatments with their respective replicates. All four treatments had discrete stocking densities (g m^{-2}), the ratio of seaweed, sea cucumbers and pearl oysters were 1:0:0, 1:1:1, 2:1:1 and 1:2:2 for treatments A, B, C and D, respectively. Treatment A was stocked only with seaweed at a density of 200 g m^{-2}. In treatment B all three species were stocked at 200 g m^{-2}. Treatment C was stocked with 400 g m^{-2} seaweed and both sea cucumbers and pearl oysters at 200 g m^{-2}, while treatment D was stocked with 200 g m^{-2} seaweed and 400 g m^{-2} sea cucumbers and pearl oysters.

The RAS, labelled treatment E, consisted of one 1000 liter tank for fish, one 1800 liter tank for sea cucumbers and one 500 liter tank for seaweed, which were set up in a row, shown graphically in Figure 2. The tanks were connected by two U-shaped water pipes each and due to height difference and gravitational force, water flowed from the fish through the sea cucumber to the seaweed tank. A submersal pump (DC Runner 2.2 Aqua Medic) in the seaweed tank pumped the water back to the fish tank at a rate of 1200 L h^{-1}. Seaweed fronds were suspended the same way as in the IMTA at 795 g m^{-2} initial stocking density. Sea cucumbers had an initial stocking density of 105.85 g m^{-2} and milkfish, in two batches, on day 0 and day 39, were stocked at a density of 2013.33 g m^{-3}. The fish were fed every day with 4 % bodyweight day^{-1} of Koudijs Tilapia broodstock feed 3.0 mm (Vietnam), containing minimum 36.0 % crude protein. In addition to the system containing seaweed, a control

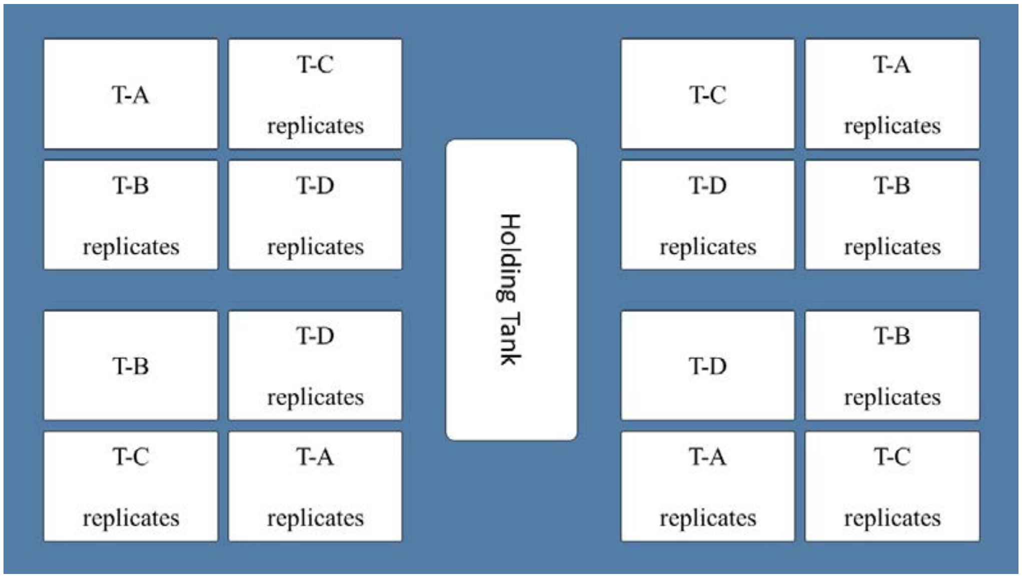

Figure 1. Layout of all tanks for treatments A (1:0:0 – seaweed, sea cucumbers and pearl oysters), B (1:1:1), C (2:1:1) and D (1:2:2) with each of the 4 replicates. The holding tank in the middle was used to keep stock of seaweed. Seven tanks were round 490 litre fiberglass tanks (0.7 m x 0.7 m x 1 m), three square 500 liter fiberglass tanks (1 m x 1 m x 0.5 m) and six square 1000 liter plastic tanks (1 m x 1 m x 1 m).

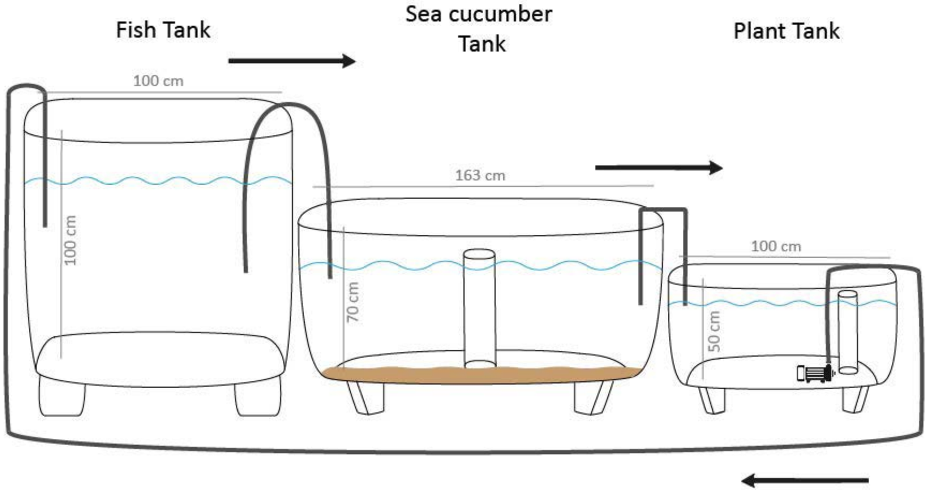

Figure 2. Sketch of the experimental RAS set-up with squared fish (1000 liter plastic, 1 m x 1 m x 1 m), sea cucumber (1600 liter fiberglass, 1.63 m x 1.63 m x 0.5 m) and seaweed (500 liter fiberglass, 1 m x 1 m x 0.5 m) tanks. The arrows indicate the water flow and the grey lines indicate water pipes and hoses.

system was constructed, which did not include any seaweed or artificial biofilter and therefore required regular water changes.

Growth of all species was monitored three times during the entire experimental period (initial, half way and at the end). Specific growth rates (SGRs), measuring the percentage increase in fish weight per day, were calculated by using the formula according to (Dawes *et al.*, 1993):

$$SGR = \frac{(ln(W_d) - ln(W_0))}{d} * 100$$

Where SGR indicates the specific growth rate (% d^{-1}); W_0: Weight at day 0, W_d: Weight at day d.

Water parameter measurements and N content

Temperature, salinity, pH, and DO, were measured at 14-day intervals in the IMTA system and twice a week in the RAS using a YSI ProQuatro Multiparameter sensor (USA) and measurements taken between 09h00 and 12h00. Water samples of 11 ml were taken three times a week from the milkfish and seaweed tank of the RAS, and stored frozen at -20 °C. During the analysis, the samples were thawed and inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}) were analysed with spectrophotometry using a Microplate reader infinite 200Pro (TECAN, Austria) following the procedure of Strickland and Parsons (1972). The data were graphically and statistically processed using the statistical software RStudio and R version 4.0.5.

Seaweed samples from the IMTA were collected on the first and last day of each cycle (Day 0 and 45), dried in an oven at 60 °C for 24 hours, and then stored at room temperature. 1 to 2 mg of the ground and homogenized samples from each treatment were put in pre-combusted (500 °C, 3 hours) tin cups and then the nitrogen content (%) of dry weight was determined by using an EURO EA 3000 CN elemental analyser, following a similar approach to Kennedy *et al.* (2005). The data were transformed logarithmically (Logan, 2011), and checked for normality with the Shapiro-Wilk test and for homogeneity of variance with the Levene-Test. A t-test was applied to determine the differences in nitrogen between batch one and batch two of seaweed and a two-way ANOVA was carried out to determine the effect of stocking density on different days.

Economic viability and questionnaire on farmers' perception

Buying and selling records (species-wise) were maintained to perform the financial analysis. Seaweed was collected directly from farmers, at a price of 0.43 US$ per kg wet weight (1 kg = 1000 TZS). Sea cucumbers and sea oysters were also collected from farmers and the costs per individual were 0.19 US$ (450 TZS) and 0.2 US$ (470 TZS), respectively. The price of milkfish per kilogram was 0.84 US$ (2000 TZS). According to Msuya (personal communication), in December 2022, the price of a kilogram of dry seaweed (*Kappaphycus*) in Zanzibar was 0.86-0.95 US$ per kg (2000-2200 TZS), and full-grown milkfish was sold for 2.15 US$ per kg (5000 TZS). Gutted sea cucumber price was approximately 15.07 US$ per kg (35,000 TZS/kg, according to ministry of fisheries, Zanzibar) and sea oyster were sold at current market price of 10.55 US$ per kg (24500 TZS/kg).

For the estimation of total costs, the following components were considered per square meter: variable costs, such as human labour, transportation, feed, ropes, pipes and species, as well as fixed costs, including tanks and pumps. Estimation of total production, gross return (sales value of total production), and benefit-cost ratio (BCR) were used to examine the economic performance of the treatments (Rahman *et al.*, 2017; Magondu *et al.*, 2022). BCR is used to compare the ratio of benefits, in this case the gross return, and the costs and is calculated as follows:

$$BCR = \frac{|PV\ [Benefits]|}{|PV\ [Cost]|}$$

Where PV is the Present Value, being the current value of the sum of benefits or costs in this case. The annual estimates of variable costs (including tank repairing costs), return, and BCR value were used to approximate the long-term profit analysis. Whereas fixed costs were considered an initial investment and not annualized.

For the questionnaire survey, interviews were conducted with a total of 30 farmers at six sites (Muungoni (n = 2, 6°19'11.7"S 39°24'43.2"E), Jambiani (n = 11, 6°19'15.1"S 39°32'54.9"E), Uzi (n = 4, 6°20'23.5"S 39°23'03.4"E), Unguja Ukuu (n = 6, 6°19'01.3"S 39°22'16.4"E), Nyamanzi (n = 2, 6°16'03.5"S 39°14'55.9"E), and Fukuchani (n = 5, 5°50'09.5"S 39°17'01.7"E)) between October and December 2022, with the support of a local translator in Swahili. To understand the socio-economic profile of the farmers, constraints of different culture systems, seasonal influences, weather, and market situations, the coastal farmers were interviewed at all six-sites, using a combination of focus groups and individual interviews. All interviews were semi-structured and open-ended questionnaires, where the interviewers not only asked questions, but encouraged farmers to provide opinions and recommendations. In order to include both sexes and a range of age groups, the interviewees were chosen at random from among the farmers who were willing and able to participate (Charisiadou *et al.*, 2022). To preserve uniformity among respondents, the survey's interviews with each respondent lasted between 20 and 30 minutes (Fröcklin *et al.*, 2012). The focus of discussion topics included problems and conflicts related to mariculture practices. Data were processed with MS Excel and frequency statistics were analysed using IMB SPSS (version 29.0.1.0).

Results

Water quality

Water temperatures ranged from 24.1 °C to 27.5 °C in the RAS system, and from 27.9 °C to 30.1 °C in the IMTA systems, with lower temperatures in the beginning, progressively getting warmer. Average salinity was 30.2 ± 1.1 (± SD) ppt and 31.5 ± 1.1 ppt and average pH 8.2 ± 0.2 and 7.9 ± 0.4 for the IMTA and RAS

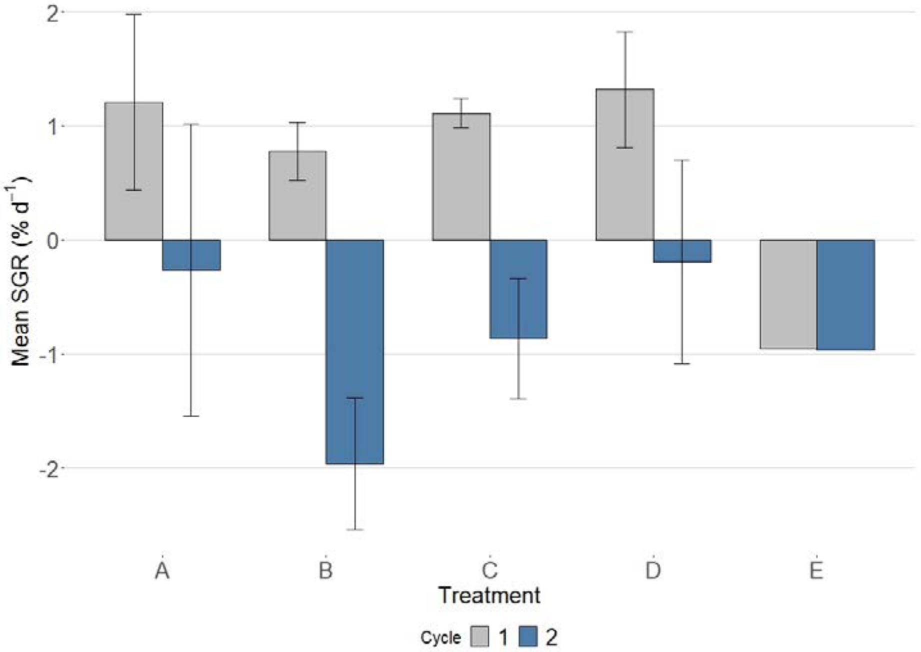

Figure 3. Seaweed specific growth rate (SGR, % d^{-1}) in two cycle periods (45 days for treatment A-D; 35 days for treatment E). Treatments A-D represent ratios 1:0:0, 1:1:1, 2:1:1, and 1:2:2, respectively of seaweed, sea cucumbers and pearly oyster. Treatment E represents seaweed, sea cucumbers and milkfish. Grey indicates the first growth period, blue the second one. Data are in mean values ± standard deviation, n = 4 for treatments A-D, treatment E is only one data point per cycle.

designs, respectively. Dissolved oxygen decreased in all tanks over time, ranging from 4.6 ± 0.05 mg L^{-1} to 3.7 ± 0.07 mg L^{-1} in the IMTA and 4.6 ± 0.72 mg L^{-1} to 4.2 ± 0.34 mg $^{-1}$ in the RAS, respectively for the first and second half, but never below critical values.

Growth and survival

Seaweed performance varied greatly between the first and second culture cycle, as seen in Figure 3. SGR in the first cycle was positive for treatments A to D and above 1 for A (SGR 1.21 ± 0.77 % d^{-1}), C (SGR 1.11 ± 0.13 % d^{-1}) and D (SGR 1.32 ± 0.51 % d^{-1}). In the second growth cycle, all treatments experienced severe seaweed loss, due to the sudden occurrence of ice-ice disease (Ward *et al.*, 2022). In treatment E ice-ice also occurred in the first growth cycle, resulting in SGRs of -0.95 % d^{-1} and -0.97 % d^{-1}, respectively.

Sea cucumber survival was 94.9 % in treatments A to D, and 100 % in treatment E. During the first cycle, treatment D showed the greatest weight loss (SGR -0.3 ± 0.22 % d^{-1}), while treatment C the least weight loss (SGR -0.03 ± 0.2 % d^{-1}). Specific growth rates in treatments B and D were also negative during the second cycle, with B experiencing even higher losses (SGR -0.89 ± 0.47 % d^{-1}). Treatment C showed positive growth in the second cycle (SGR of 0.35 ± 0.06 % d^{-1}). SGR of sea cucumbers in treatment E were 0.94 % d^{-1} and 0.01 % d^{-1} for the first and second half, respectively (Fig. 4).

The survival rate of the **sea oysters** in the IMTA was 97.22 %. Dead sea oysters were only found in treatment D, which also showed the highest SGR (0.11 ± 0.04 % d^{-1}). Survival rate of the first batch of **milkfish** was 9 %, because of this they were restocked on day 42 of the RAS experiment. The second batch had a survival rate of 100 % and an SGR of 0.42 ± 0.2 % d^{-1} over the 28 days left until the end of the experiment.

Nitrogen uptake and biofiltration by seaweed

Thallus nitrogen content in seaweed showed no significant difference in different stocking densities and on different days among the treatment tanks ($p > 0.05$), but there was a significant difference between cycles one and two ($p < 0.05$) (Fig. 5A, B). Assimilation rate was the highest in Treatment D in both culturing cycles.

In the RAS systems, there were no significant differences in the NH_4^+, NO_3^-, NO_2^- and PO_4^{3-} concentrations between the seaweed and control system. NH_4^+ in the seaweed system peaked at around 1.0 mg L^{-1}, in the control at 0.7 mg L^{-1} at day 7, after which it decreased in both systems to around 0.2 mg L^{-1} on day 20 (Fig. 5C). A second and third peak was only observable in the

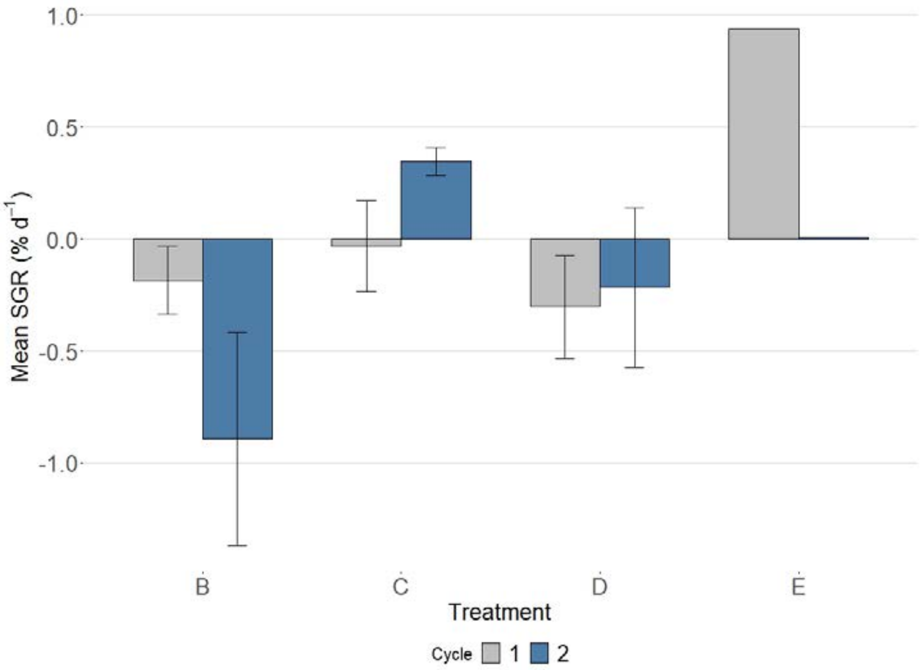

Figure 4. Sea cucumber specific growth rate (SGR, % d^{-1}) in two cycle periods (45 days for treatments A-D; 35 days for treatment E). Treatments B-D represent ratios 1:1:1, 2:1:1, and 1:2:2, respectively of seaweed, sea cucumbers and pearl oyster. Treatment E represents seaweed, sea cucumbers and milkfish. Grey indicates the first growth period, blue the second one. Data are in mean values ± standard deviation, n = 4 for treatments B-D, treatment E is only one data point per cycle.

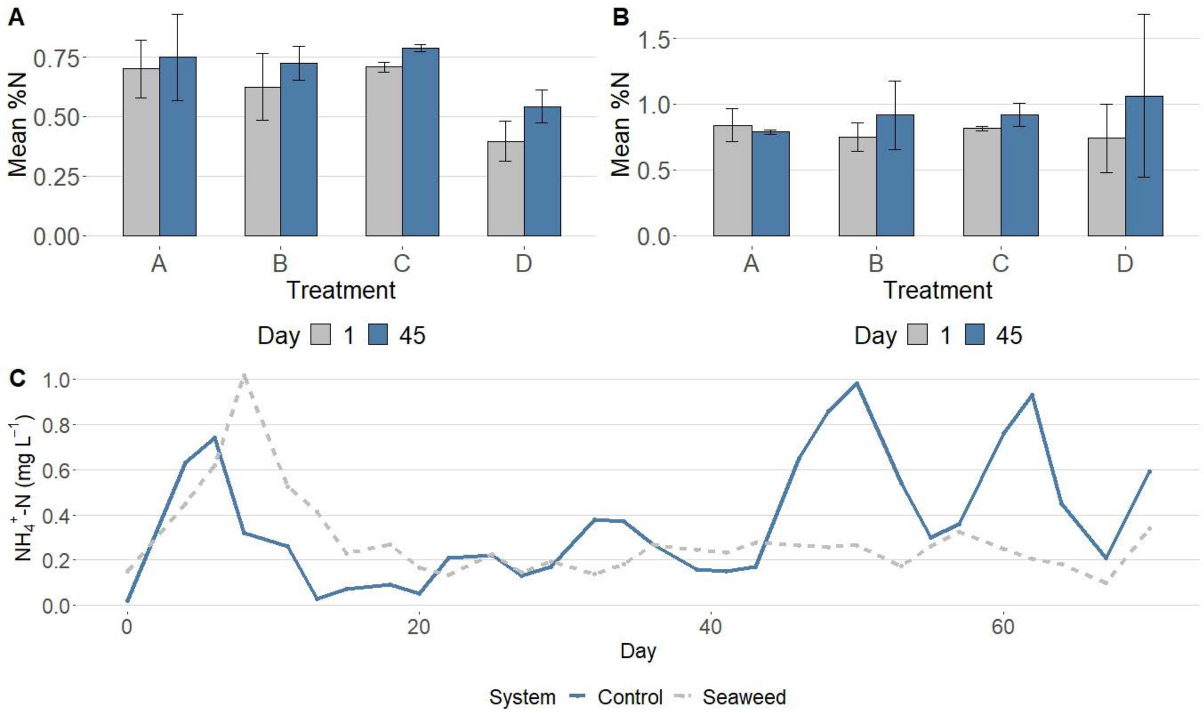

Figure 5. Nitrogen content in the seaweed thallus on day 0 and 45 in cycle one (**A**) and two (**B**). Treatments A-D represent ratios 1:0:0, 1:1:1, 2:1:1, and 1:2:2, respectively of seaweed, sea cucumbers and pearl oyster. Data are in mean values ± standard deviation, n = 2 for each treatment. (**C**) Average NH_4^+-N concentration (mg L^{-1}) in all three systems over the 70 days. The blue line is from water samples of the control system, the grey line from the seaweed system, n = 30 for control and seaweed system, respectively.

control on day 50 and 62, not in the seaweed system. Nitrite concentration peaked in the seaweed system shortly after the ammonium peak and stayed low afterwards, while the control experienced a strong increase in the last 10 days of the experiment. Nitrate levels fluctuated around 0.6 and 3 mg L^{-1} in both systems, and after day 50 it increased to 4.5 mg L^{-1} in the seaweed system. Phosphate concentrations stayed relatively low for the first 45 days of the experiment, not exceeding 0.2 mg L^{-1}. After day 50 it started to increase in both systems, with maximums reaching 0.5 mg L^{-1} in both on day 70.

Economic viability

In the IMTA systems, treatment D had the highest total yield for all species (635.36 g m^{-2}) and highest gross return (US$ 6.96 per m^2), while treatment B had the lowest yield (299 g m^{-2}) and gross return (US$ 3.15 per m^2). Treatment A demonstrated especially poor economic performance. After analysing cost, return, and BCR values on an annual basis, it was discovered that treatment C and D were economically viable with treatment D being slightly more viable (Table 1). In the RAS, economic feasibility was not satisfactory due to the loss of milkfish in the first batch (survival rate 9 %). This also makes an annualization difficult to perform, as the possible growth over the entire experiment duration is unknown.

Questionnaires for farmers' perception

In total, 30 participants in discrete age groups were interviewed, of which 60 % were females (n = 18) and 40 % males (n = 12), with all females actively engaging in seaweed culture. Interviewed people had diversified occupations (small scale business 27.8 %, fishing 26.4 %, mariculture 22.2 %, tourism 15.3 %, the rest were involved in crop and animal husbandry). This study tried to find out the principal constraints in specific aquaculture systems (Table 2). Seaweed farmers mentioned their main problems were low market prices, die-offs, and high cost of materials. Farmers of sea cucumbers suffered because of theft, the limited availability of fingerlings, and high cost of materials. Almost two-thirds of farmers agreed to accept new technologies such as IMTA, but highlighted the main obstacles in implementing such systems, including the lack of funding, security, and knowledge. The majority of participants (n=20, 66.67 %) stated concerns about the situation of the market and frequently mentioned that they are unable to directly connect with large consumers since primarily local traders purchased their products, and did not agree to pay reasonable prices. They frequently voiced specific requirements towards the government and NGOs who are involved in coastal aquaculture development such as financial support (small

Table 1. Estimation of yield, return and economic viability from all treatments per square meter cultivation area, and an annualization of these.

Culture type	Treatment	Cost (US\$/ m^2)	Total Yield (g/m^2)	Gross return (US\$/m^2)	BCR	Annualization		
						Total Yield (g/m^2)	Gross return (US\$/m^2)	BCR value
Seaweed mono-culture	A	6.04	60	0.05	0.01	360	0.31	0.05
IMTA	B	13.93	300	3.15	0.23	1270	12.65	0.91
	C	13.39	430	4.06	0.30	1930	16.44	1.23
	D	17.34	640	6.96	0.40	2670	27.95	1.61
	E (RAS)	36.19	1350	3.85	0.11	6900	19.37	0.54

Note: 1 US$ = 2322 TZS (25 September 2022)
Total yield of seaweed wet weight (1 wet kg = 0.12 dry kg)
Kappaphycus alvarezii market price= 0.87 US$/ dry kg
Total yield of sea cucumber is given in live weight
(1 kg of live = 0.54 kg of gutted)

Holothuria scabra market price = 15.07 US$/ gutted kg
Pinctada margaritifera market price = 10.77 US$/ kg
Chanos chanos market price = 2.15 US$/ kg

scale credits), instrument and training facilities, security for reducing poaching, establishing a hatchery to produce fingerlings, introducing new technique (e.g., integrated aquaculture), subsidies or allowances (during rainy season aquaculture production is hampered seriously), collaboration with foreign buyers, availability of a health facility, interaction and monitoring.

Discussion

Biomass production under different culturing conditions

Seaweed cultured in the RAS got infected by the ice-ice disease shortly after the stocking and the absolute biomass therefore decreased in both 35-day intervals. The ice-ice disease causes rotting of the stems and

Table 2. Constraints regarding three farming methods mentioned by the individual farmers and their frequencies (frequency equals 100 % means that a reply appeared in all the members in a specific group).

Frequency (%)	Constraints
Seaweed farmers (n=12)	
100	Low market price of dry seaweed
75	Seaweed die-offs for ice-ice disease
66.7	High price of equipment, after covid-19, market price suddenly increased
50	Extreme weather, especially December to May, with average temperature 30-36^{0}C
41.7	Serious health problems, skin diseases, pain, allergies, injured by sea urchin and other hard-shell species
41.7	Lack of support from government and non-government stakeholders
Sea cucumber farmers (n=10)	
100	Poaching is the main problem in open coastal aquaculture
70	Scarcity of fingerlings
50	Escaping, most of the farmers use fish net which have not enough capability to protect fingerling
40	Extreme weather, climate change has a great impact on this sector
40	Limited buyers, mainly China is the major importer and their purchase depends on the best quality of the products
IMTA farmers (n=8)	
100	Insufficient capital to facilitate the multi-trophic aquaculture
87.5	Theft, security maintenance more difficult in open area
62.5	Poor knowledge about multiculture, authority had less/no involvement with researchers
50	Lack of goverment support, mainly the funding and monitoring
37.5	High material cost

a significant loss of tissue. It is usually caused by environmental factors such as the warming of sea water, a decrease in salinity, low light levels (<125 µmol photon m^{-2} s^{-1}) or slow water movements (Ward *et al.*, 2022). Largo *et al.*(1995b) showed that lower temperatures (~25 °C) and salinities between 25 and 35 ‰ increased growth rates and inhibited the formation of ice-ice when culturing *K. alvarezii* under laboratory conditions. As temperatures were as high as 28 °C and the tanks were under a roof, this further facilitated the formation of ice-ice. Furthermore, the low fish feed input of 4 % bodyweight d^{-1}, as the main source of phosphate in aquaculture (Nora'aini *et al.* 2005), was most likely not sufficient. Only with the higher input of fish feed after stocking the second batch of milkfish, was the phosphate availability sufficient. With a 100 % survival of the first batch and a consistently high input of feed, phosphate levels probably would have been higher compared to what was found, which could have facilitated a better growth, as it has been shown that P can be a growth limiting factor in seaweed culture (Lapointe, 1987; Pedersen *et al.*, 2010).

As for the seaweed cultured in the IMTA design, SGRs in the first cycle were all positive, with treatment D (high sea cucumber and sea oyster stocking density) showing the best performance. However, the observed SGRs, with max. 2.3 % d^{-1}, were well below the recommended 3.5 % d^{-1} for commercial eucheumatoid farming (Doty, 1987; Wakibia *et al.*, 2006). Several factors could have limited the seaweeds growth, such as low light availability, low water movement or too little nutrient input. Additionally, because of the type of tank, the seaweed was only suspended around 20 cm from the surface, well below the recommended 0.5 to 1 m for *Kappaphycus* culture (Sahoo and Yarish, 2005; Zuldin *et al.*, 2016). Looking at the individual treatments, it is observable that seaweed growth was facilitated by the presence of sea cucumbers and sea oysters. This positive effect of sea cucumbers was also reported by Uthicke (2001) and Wolkenhauer *et al.* (2010), showing that they boost primary producers' productivity through recycling of nutrients. This is in contrast to Davis *et al.* (2011), who discovered that the survival of seaweed appeared to be little/not influenced by the presence of sea cucumbers.

In contrast, the performance in the second cycle of the present study strongly declined, with all treatments showing a decrease in absolute biomass. The more optimal water parameters in the first batch facilitated the growth, compared to the higher water temperatures in the second cycle. This led to higher environmental stress and more opportunities for pathogenic bacteria (Glenn and Doty, 1990; Ward *et al.*, 2020; Faisan *et al.*, 2021). The ice-ice disease and unwanted algae growth were noticed more severely in the second cycle. Seaweed growth and carrageenan yields are frequently hampered by the growth of epiphytes (Ask and Azanza, 2002), and infestation by the algae *Neosiphonia spp*. on grown *Kappaphycus spp.* has already been observed for seaweed farms in Tanzania (Msuya and Kyewalyanga, 2006; Vairappan *et al.*, 2008).

As temperatures in the two designs (RAS and IMTA) were similar and both situated underneath a roof, the occurrence of ice-ice in the first half of the RAS experiment cannot only be linked to high water temperatures and low light setting. While the water in the IMTA systems was changed regularly, the water in the RAS was only changed once during the entire 70 days, which was necessary to establish the microbial community to drive nitrification and denitrification (Keuter *et al.*, 2015). This, however, could also have enabled the bacteria responsible for the ice-ice disease to accumulate (Largo *et al.*, 1995a). As Ward *et al.* (2022) showed, the presence of ice-ice does not originate from only one stressor, but most likely a 'complex pathobiotic syndrome'. This means that both abiotic and biotic factors combined are responsible for the ice-ice disease.

Although overall water quality remained within tolerable limits in both experimental designs, parameters such as temperature, salinity, pH and DO need to be observed more tightly and adjusted if necessary. Specifically, water temperatures rose in the second half of the experiments, as the months of December and January are the hottest in Zanzibar (Muhando, 2002). As water temperature is one of the most important parameters in seaweed culture (Breeman, 1988; Wiencke and Bischof, 2012), it is necessary to adjust the temperature to the seasonal variations, to increase the possible yield.

Sea cucumber performance varied strongly between the treatments; while in the RAS they showed a high growth rate in the first 35 days, it dropped to almost zero for the second half. For the other treatments, only treatment C showed a positive growth rate in the second half. The IMTA systems had much higher stocking densities and less space available for the sea cucumbers, which could have resulted in the negative growth, as these factors have a significant impact on sea cucumber growth rates (Slater and Carton, 2007; Davis *et al.*, 2011; Namukose *et al.*, 2016). This phenomenon has been called 'aestivation', and links the limited space with low metabolic rates and a stop of feeding (Li *et al.*, 2013). Furthermore, studies showed, that the growth of sea cucumber individuals can greatly vary and is dependent on multiple environmental factors and individual genetics (Qiu *et al.*, 2014; Dumalan *et al.*, 2019). Also, depending on the type of tank and sediment, sea cucumber SGRs were found to be negative, meaning a weight loss over the time of rearing further showing that sea cucumber growth is unpredictable in tank culture (Robinson *et al.*, 2013).

Nitrogen uptake by seaweed

Even though the thallus nitrogen content did not show any significant difference between days 1 and 45 of the respective cycles, a clear trend was apparent that the seaweed took up inorganic nitrogen over time. It is well reported that *Kappaphycus* assimilates dissolved inorganic nutrients and uses them as a source of N for assimilation (Rosenberg and Ramus, 1984; Smith *et al.*, 1999; Dy and Yap, 2001; Granbom *et al.*, 2004). Looking at the treatments individually, it shows that seaweed in treatment D had the highest mean uptake of nitrogen after 45 days, originating from a higher nitrogen availability. With higher sea cucumber and oyster stocking densities the quantity of excretory products and therefore of inorganic nutrients was higher compared to the other treatments (Taylor and Rees, 1998).

Especially ammonium, excreted by sea cucumbers, but also milkfish (Mook *et al.* 2012), plays an important role in intensive aquaculture and was therefore, together with nitrite and nitrate, measured three times a week in the RAS. The build-up of ammonium right after the first stocking, observed in both the seaweed and control system, is normal in recirculating aquaculture. Removal of ammonium by nitrification takes up to 14 days, as the responsible bacterial communities first have to establish themselves in the system (Keuter *et al.*, 2015). The observed ammonium levels decreased even earlier than that, most likely due to the filtration properties of the seaweed (Neori *et al.*, 2003; Quintã *et al.*, 2015). In the first step of nitrification, ammonium is oxidized into nitrite by aerobic chemoautotrophic bacteria (Sharma and Ahlert, 1977; Camargo *et al.*, 2005). This is observable in the measured nitrite concentration, as about 5 to 10 days after the ammonium peaked, the nitrite also showed peak concentrations. Nitrite concentrations measured in the two systems are quite similar to the ones reported by Senff *et al.* (2020), and highly likely not lethal. The observed concentrations of nitrate (NO_3^--N < 5 mg L^{-1}) were well below critical limits of 10 to 20 mg L^{-1} (Spotte, 1979; Ward *et al.*, 2005). The regular water changes in the control system probably kept the nitrate levels low and stopped a built-up of nitrate, while in the seaweed system the seaweed could filter out the nitrate. Although, the nitrogen content in the seaweed thallus and the inorganic nutrient measurements in the water come from two different systems, the assimilation of ammonium and nitrate by the seaweed can be seen in both. The seaweed kept the nitrogen levels in the water low, while incorporating it in its thallus.

Economic viability and farmers' perception

IMTA can enhance production sustainability, mitigate the negative effects of intensive aquaculture operations, and generate financial gains through diverse products and faster production cycles (Knowler *et al.*, 2020). In this study, gross return and BCR were calculated and converted to annual figures to understand the experimental viability of the designs used. In comparison, seaweed monoculture had a low return, while having the same construction costs as the other IMTA systems. In the IMTA systems, the high-density treatment showed the highest return of US$ 6.96 per m^2. Whereas the RAS was found to have a comparatively low return of US$ 3.85 per m^2, while having the highest cost of US$ 36.00 per m^2 due to high initial construction and material costs. By annualizing the production of seaweed monoculture, it was shown that it is impossible to generate revenue over the long-term, while the IMTA system with high stocks of seaweed, sea cucumber, and sea oyster, generates an income of 27.95 US$ m^{-2} per year with initial costs being approximately 17.34 US$ m^{-2}, achieving a BCR of 1.61 over one year. The RAS had initial costs of 36.19 US$ m^{-2}, however, when calculating the yearly income, it was determined to be 19.37 US$ m^{-2} with a BCR of 0.54. This indicates that the system needs to continue for almost two years in order to recover its losses. A 100 % survival of milkfish could achieve a BCR above 1, although this would require further research and investment.

By conducting questionnaires on the perception of farmers, this study found that seaweed farming is largely female dominated, while men were more actively engaged in fishing, following the observations of Msuya *et al.* (2007). The fishing industry, not only in Zanzibar, but in Tanzania in general, is dominated by men (Shao *et al.*, 2003). During the survey, seaweed farmers claimed to have the lowest market price, though Msuya (2020) reported that the seaweed (dry) price has increased to approximately 1800 TZS per kg, most likely due the establishment of a processing plant in Pemba Island. In contrast, Makame *et al.* (2021) found that the seaweed price is fixed by buyers and 76.4 % of farmers are not satisfied with it. Additionally, the production of *Kappaphycus* has been significantly impaired by diseases, such as the ice-ice disease, as well as epiphyte outbreaks that are now more severe through rising sea surface temperatures associated with climate change (Msuya and Porter, 2014; Largo *et al.*, 2020).

To alleviate the above mentioned problems in the local seaweed industry, it is vital that different stakeholders should come forward in proposing solutions. One of which could be the regulation of prices of unprocessed seaweed by the local government on the basis of current instrument cost, as Jong Cleyndert *et al.* (2021) reported that weak bargaining power of farmers is the reason why they receive a low sale price. To improve the seaweed market's attractiveness, Msuya (2021) suggested value adding initiatives, such as training to process seaweed into more valuable products such as soap, shampoo, cookies, and juice. To further overcome the challenges induced by climate change and the associated rise in sea surface temperature, the SeaPoWer project, proposed a new technology for seaweed farming in deeper waters (>8m) using tubular nets (Brugere *et al.*, 2020).

Another obstacle for farmers in Zanzibar is the regular poaching of sea cucumbers due to their high market value. In this study, sea cucumber farmers frequently mentioned their main constraints being poaching and fingerling scarcity, high pressure on natural sources reducing fingerling availability and the lack of a recognized hatchery. Kunzmann *et al.* (2018) reported that fingerling production was initiated by the FAO hatchery supported by the Korean International Cooperation Agency (KOICA), but during this study they stopped the sea cucumber fingerling production (personal observation).

The pearl oyster is mainly cultured for producing half-pearl, which was initiated in Zanzibar in 2005 (Mmochi, 2015). There were two farmers found during this study, who are mainly fishermen, but they also cultured oysters to produce half pearl and make jewellery, which are sold to the local market. Farmers mentioned that the main constraints to producing half pearls are poaching, inadequate spat (Ishengoma *et al.*, 2011), an irregular and unreliable market, and lack of expertise (Charisiadou *et al.*, 2022). In Zanzibar there is a great potential for producing half pearl, as in the south pacific countries, where oyster culture is popular for forming valuable pearls due to less labour input, capital, and training requirements (Johnston *et al.*, 2020). To get a broader view of the local communities' perception of the introduction of new aquaculture practices, a more detailed survey would be necessary, as the current questionnaire only reflects the local farmers perception.

Improvements to the systems

Some improvements to the IMTA and RAS systems have already been mentioned, but this section aims to highlight them in detail and give recommendations for future systems. The biggest constraint found in this study was the appearance of the ice-ice disease and the subsequently fouling of seaweed. This reduced the economic viability of all treatments. There are already several suggestions how to overcome this disease, including a stricter control and adjustment of water parameters, such as temperature and salinity as well as light intensity (Largo, 2002; Tahiluddin and Terzi, 2021). Another potential method is to manually clean the seaweed from macroalgae epiphytes and filamentous epi-endophytes, which can cause the ice-ice disease (Largo *et al.*, 2020; Kambey *et al.*, 2021). The setup of tanks also seems to play an important role; seaweed should be able to be suspended at least 50 cm into the water column (Sahoo and Yarish, 2005; Zuldin *et al.*, 2016) and steady water movement should be maintained (Ward *et al.*, 2022). Furthermore, there are suggestions that *E. denticulatum* can withstand adverse environmental factors better, and would therefore be better suited for this type of aquaculture (Tisera and Naguit, 2009; Pang *et al.*, 2015). Kambey *et al.* (2021) showed that seaweed farms integrating various biosecurity measurements, not only achieve higher growth rates and reduced infection rates, but also increase the quality of the carrageenan yield and therefore the products value. Apart from biosecurity measurements, it would be beneficial to increase the tank size and change the tank shape, not only for seaweed, but also for the sea cucumbers (Slater and Carton, 2007; Davis *et al.*, 2011; Li *et al.*, 2013) and milkfish (Oca *et al.*, 2004; Duarte *et al.*, 2011; McLean, 2021). Large circular tanks/ponds or raceway systems would be possible solutions. In particular for the RAS, a possible solution could be the use of just one big tank, which can be divided into compartments for each species, ensuring a better dispersion of nutrients and diminish technical failures due to power failures and broken equipment, which is especially important in developing countries lacking infrastructure. Furthermore, it seemed that the sea cucumbers in the IMTA were lacking a source of nutrients, which could be overcome by artificially feeding them, either with algal extracts or sludge from fish cultures. Because of the type of tank used for the fish, the removal of sludge was not possible. This is, therefore, another possible improvement to boost the sea cucumbers growth. Overall, more testing is necessary for both types of systems to overcome the ice-ice issue and to boost growth rates. However, as this study demonstrated, sustainable tank culture of seaweed in IMTA can be economically feasible and provide farmers with a secure income. Seaweed farming has been a profitable business for local farmers over the last decades and will likely stay that way (Msuya and Kyewalyanga, 2006) and farmers are willing to adapt to new farming techniques (Lumenyela *et al.*, 2023). Improved versions of the systems used could be viable options for farmers not only on Zanzibar but in many developing tropical countries. The initial investments would need to be covered by microcredit schemes from local government or NGOs.

Conclusions

In conclusion, the study highlights the importance of developing sustainable aquaculture systems to meet the increasing demand for food, especially in developing countries, as well as the complexities and challenges that are associated with this. Both experimental designs, IMTA and RAS, show promising results to be used as low-tech and sustainable alternatives to already existing practices. However, for both designs, there are still a lot of improvements necessary, especially regarding the seaweed culturing. The prevalence of the ice-ice disease, driven by high water temperatures and inadequate nutrient input, significantly hampered seaweed growth. Only the IMTA system in the first growth cycle showed more robust growth, most likely attributed to the beneficial effects of nutrient recycling from sea cucumbers and oysters. The results of the economic analysis revealed that the IMTA system, particularly with high-density seaweed, sea cucumber, and pearl oyster stocks, was more profitable compared to RAS and monoculture systems, thus IMTA is a more practical system for Zanzibar and Tanzania in general. Equally, IMTA can be a better choice for Zanzibar given that the IMTA system achieved a higher BCR, indicating better financial viability over time. Socially, seaweed farming remains a crucial livelihood for many, particularly women, though market challenges such as low prices and disease outbreaks persist. With appropriate support and investments, these novel aquaculture practices can mitigate the impacts of environmental stressors, enhance production, and ensure long-term viability.

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References

Ahmed N, Glaser M (2016) Can Integrated Multi-Trophic Aquaculture (IMTA)" adapt to climate change in coastal Bangladesh? Ocean & Coastal Management 132: 120-131 [doi: 10.1016/j.ocecoaman.2016.08.017]

Ahmed N, Thompson S, Glaser M (2019) Global aquaculture productivity, environmental sustainability, and climate change adaptability. Environmental Management 63 (2): 159-172 [doi: 10.1007/s00267-018-1117-3]

Ahmed N, Turchini GM (2021) Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. Journal of Cleaner Production 297: 126604 [doi: 10.1016/j.jclepro.2021.126604]

Ask EI, Azanza RV (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. Aquaculture 206 (3-4): 257-277 [doi: 10.1016/S0044-8486(01)00724-4]

Badiola M, Mendiola D, Bostock J (2012) Recirculating aquaculture systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering 51: 26-35 [doi: 10.1016/j.aquaeng.2012.07.004]

Badiola M, Basurko OC, Piedrahita R, Hundley P, Mendiola D (2018) Energy use in recirculating aquaculture systems (RAS): A review. Aquacultural Engineering 81: 57-70 [doi: 10.1016/j.aquaeng.2018.03.003]

Breeman AM (1988) Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: Experimental and phenological evidence. Helgolander Meeresunters 42 (2): 199-241 [doi: 10.1007/BF02366043]

Brugere C, Msuya FE, Jiddawi N, Nyonje B, Maly R (2020) Can innovation empower? Reflections on introducing tubular nets to women seaweed farmers in Zanzibar. Gender, Technology and Development 24 (1): 89-109 [doi: 10.1080/09718524.2019.1695307]

Buschmann AH, Camus C, Infante J, Neori A, Israel Á, Hernández-González MC, Pereda SV, Gomez-Pinchetti JL, Golberg A, Tadmor-Shalev N, Critchley AT (2017) Seaweed production: overview of the global state of exploitation, farming and emerging research activity. European Journal of Phycology 52 (4): 391-406 [doi: 10.1080/09670262.2017.1365175]

Camargo JA, Alonso A, Salamanca A (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 58 (9): 1255-1267 [doi: 10.1016/j.chemosphere.2004.10.044]

Charisiadou S, Halling C, Jiddawi N, Schreeb K von, Gullström M, Larsson T, Nordlund LM (2022) Coastal aquaculture in Zanzibar, Tanzania. Aquaculture 546: 737331 [doi: 10.1016/j.aquaculture.2021.737331]

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. Journal of Phycology 37 (6): 975-986 [doi: 10.1046/j.1529-8817.2001.01137.x]

Davis BM, Stead SM, Jiddawi NS, Abdulaziz M, Slater MJ (2011) A novel polyculture system for sea cucumber *Holothuria scabra* and seaweeds (*Eucheuma denticulatum* and *Kappaphycus alvarezii*) in Tanzanian lagoons. MSc Aquaculture Enterprise and Technology Thesis, Newcastle University, United Kingdom

Dawes CJ, Trono GC, Lluisma AO (1993) Clonal propagation of *Eucheuma denticulatum* and *Kappaphycus alvarezii* for Philippine seaweed farms. Hydrobiologia 260-261 (1): 379–383 [doi: 10.1007/BF00049044]

Doty MS (1987) The production and use of *Eucheuma*. FAO Fisheries Technical Paper 281. Rome. pp 123-164

Duarte S, Reig L, Masaló I, Blanco M, Oca J (2011) Influence of tank geometry and flow pattern in fish distribution. Aquacultural Engineering 44 (2): 48-54 [doi: 10.1016/j.aquaeng.2010.12.002]

Dumalan RJP, Bondoc KGV, Juinio-Meñez MA (2019) Grow-out culture trial of sandfish *Holothuria scabra* in pens near a mariculture-impacted area. Aquaculture 507: 481-492 [doi: 10.1016/j.aquaculture.2019.04.045]

Dy DT, Yap HT (2001) Surge ammonium uptake of the cultured seaweed, *Kappaphycus alvarezii* (Doty) Doty (Rhodophyta: Gigartinales). Journal of Experimental Marine Biology and Ecology 265 (1): 89-100 [doi: 10.1016/S0022-0981(01)00325-2]

Engle CR, Kumar G, Senten J (2020) Cost drivers and profitability of US pond, raceway, and RAS aquaculture. Journal of the World Aquaculture Society 51 (4): 847-873 [doi: 10.1111/jwas.12706]

Eriksson H, Robinson G, Slater MJ, Troell M (2012) Sea cucumber aquaculture in the Western Indian Ocean: challenges for sustainable livelihood and stock improvement. Ambio 41 (2): 109-121 [doi: 10.1007/s13280-011-0195-8]

Ertör I, Ortega-Cerdà M (2015) Political lessons from early warnings: Marine finfish aquaculture conflicts in Europe. Marine Policy 51: 202-210 [doi: 10.1016/j.marpol.2014.07.018]

Fabiani G, Namukose M, Katikiro RE, Yussuf YS, Steinmann NM, Msuya FE (2023) Tanzania case study. In: Wolff M, Ferse SC, Govan H (eds) Challenges in Tropical Coastal Zone Management. Springer International Publishing, Cham. pp 133-148

Faisan JP, Luhan MRJ, Sibonga RC, Mateo JP, Ferriols VMEN, Brakel J, Ward GM, Ross S, Bass D, Stentiford GD, Brodie J, Hurtado AQ (2021) Preliminary survey of pests and diseases of eucheumatoid seaweed farms in the Philippines. Journal of Applied Phycology 33 (4): 2391-2405 [doi: 10.1007/s10811-021-02481-5]

FAO (2020) Sustainability in action. The state of world fisheries and aquaculture 2020. Food & Agriculture Organisation, Rome. 206 pp

FAO (2022) Towards blue transformation. The state of world fisheries and aquaculture 2022. Food & Agriculture Organisation, Rome. 236 pp

Felaco L, Olvera-Novoa MA, Robledo D (2020) Multitrophic integration of the tropical red seaweed *Solieria filiformis* with sea cucumbers and fish. Aquaculture 527: 735475 [doi: 10.1016/j.aquaculture.2020.735475]

Franchini AC, Costa GA, Pereira SA, Valenti WC, Moraes-Valenti P (2020) Improving production and diet assimilation in fish-prawn integrated aquaculture, using iliophagus species. Aquaculture 521: 735048 [doi: 10.1016/j.aquaculture.2020.735048]

Fröcklin S, La Torre-Castro M de, Lindström L, Jiddawi NS, Msuya FE (2012) Seaweed mariculture as a development project in Zanzibar, East Africa: A price too high to pay? Aquaculture 356-357: 30–39 [doi: 10.1016/j.aquaculture.2012.05.039]

García-Poza S, Leandro A, Cotas C, Cotas J, Marques JC, Pereira L, Gonçalves AMM (2020) The evolution road of seaweed aquaculture: Cultivation technologies and the industry 4.0. International journal of environmental research and public health 17 (18): 6528 [doi: 10.3390/ijerph17186528]

Glenn EP, Doty MS (1990) Growth of the seaweeds *Kappaphycus alvarezii*, *K. striatum* and *Eucheuma denticulatum* as affected by environment in Hawaii. Aquaculture 84 (3-4): 245–255 [doi: 10.1016/0044-8486(90)90090-A]

Granbom M, Chow F, Lopes PF, Oliveira MC de, Colepicolo P, Paula EJ de, Pedersén M (2004) Characterisation of nitrate reductase in the marine macroalga

Kappaphycus alvarezii (Rhodophyta). Aquatic Botany 78 (4): 295–305 [doi: 10.1016/j.aquabot.2003.11.001]

Hall SJ, Delaporte A, Phillips MJ, Beveridge M, O´Keefe M (2011) Blue frontiers: managing the environmental costs of aquaculture. The WorldFish Center, Penang, Malaysia. 92 pp [ISBN: 978-983-2346-78-4]

Ishengoma EB, Jiddawi NS, Tamatamah RA, Mmochi AJ (2011) Wild black-lip pearl oyster (*Pinctada margaritifera*) spat collection in Tanzania. Western Indian Ocean Journal of Marine Sciience 10: 49-57

Johnston B, Kishore P, Vuibeqa GB, Hine D, Southgate PC (2020) Economic assessment of community-based pearl oyster spat collection and mabé pearl production in the western Pacific. Aquaculture 514: 734505 [doi: 10.1016/j.aquaculture.2019.734505]

Jong Cleyndert G de, Newman R, Brugere C, Cuni-Sanchez A, Marchant R (2021) Adaptation of seaweed farmers in Zanzibar to the impacts of climate change. In: Oguge N, Ayal D, Adeleke L, Da Silva I (eds) African Handbook of Climate Change Adaptation. Springer International Publishing, Cham. pp 3-28

Kambey CSB, Campbell I, Cottier-Cook EJ, Nor ARM, Kassim A, Sade A, Lim PE (2021) Seaweed aquaculture: a preliminary assessment of biosecurity measures for controlling the ice-ice syndrome and pest outbreaks of a *Kappaphycus* farm. Journal of Applied Phycology 33 (5): 3179-3197 [doi: 10.1007/s10811-021-02530-z]

Kennedy P, Kennedy H, Papadimitriou S (2005) The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. Rapid communications in mass spectrometry: RCM 19 (8): 1063-1068 [doi: 10.1002/rcm.1889]

Keuter S, Beth S, Quantz G, Schulz C, Eva S (2015) Longterm monitoring of nitrification and nitrifying communities during biofilter activation of two marine recirculation aquaculture systems (RAS). International Journal of Aquaculture and Fishery Sciences: 51-61 [doi: 10.17352/2455-8400.000029]

Knowler D, Chopin T, Martínez-Espiñeira R, Neori A, Nobre A, Noce A, Reid G (2020) The economics of Integrated Multi-Trophic Aquaculture: where are we now and where do we need to go? Reviews in Aquaculture 12 (3): 1579-1594 [doi: 10.1111/raq.12399]

Kunzmann A, Beltran-Gutierrez M, Fabiani G, Namukose M, Msuya FE (2018) Integrated seaweed – sea cucumber farming in Tanzania. Western Indian Ocean Journal of Marine Science 17 (2): 35 [doi: 10.4314/wiojms.v17i2.4]

Kunzmann A, Todinanahary G, Msuya FE, Alfiansah Y (2023) Comparative environmental impacts and development benefits of coastal aquaculture in three tropical countries: Madagascar, Tanzania and Indonesia. Tropical Life Sciences Research 34 (3): 279-302 [doi: 10.21315/tlsr2023.34.3.15]

Lapointe BE (1987) Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (*Rhodophyceae*) in the Florida Keys: an experimental field study. Marine Biology 93 (4): 561-568 [doi: 10.1007/BF00392794]

Largo DB, Fukami K, Nishijima T (1995a) Occasional pathogenic bacteria promoting ice-ice disease in the carrageenan-producing red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). Journal of Applied Phycology 7 (6): 545-554 [doi: 10.1007/BF00003941]

Largo DB, Fukami K, Nishijima T, Ohno M (1995b) Laboratory-induced development of the ice-ice disease of the farmed red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). Journal of Applied Phycology 7 (6): 539-543 [doi: 10.1007/BF00003940]

Largo DB (2002) Recent developments in seaweed diseases. In: Proceedings of the National Seaweed Planning Workshop held on August 2-3 2001. SEAFDEC Aquaculture Department, Tigbauan, Iloilo. pp 35-42

Largo DB, Diola AG, Marababol MS (2016) Development of an integrated multi-trophic aquaculture (IMTA) system for tropical marine species in southern Cebu, Central Philippines. Aquaculture Reports 3: 67-76 [doi: 10.1016/j.aqrep.2015.12.006]

Largo DB, Msuya FE, Menezes A (2020) Understanding diseases and control in seaweed farming in Zanzibar. FAO Fisheries and Aquaculture Technical Paper 662: 0_1,1-3,5-39,41,43,45,47,49,I-VII,IX-X [doi: Food]

Li J, Dong S, Gao Q, Wang F, Tian X, Zhang S (2013) Total organic carbon budget of integrated aquaculture system of sea cucumber *Apostichopus japonicus* , jellyfish *Rhopilema esculenta* and shrimp *Fenneropenaeus chinensis*. Aquaculture Research 45: 1825-1831 [doi: 10.1111/are.12131]

Logan M (2011) Biostatistical design and analysis using R: A practical guide. EBL-Schweitzer. Wiley, Somerset. 546 pp

Lumenyela RA, Magasha O, Dimosso P (2023) Adoption of seaweed improved farming techniques among farmers in Zanzibar: An application of Adoption and Diffusion Outcome Prediction Tool. Social Sciences & Humanities Open 7 (1): 100472 [doi: 10.1016/j.ssaho.2023.100472]

Magondu EW, Fulanda BM, Munguti JM, Mlewa CM (2022) Toward integration of sea cucumber and cockles with culture of shrimps in earthen ponds in Kenya. Journal of the World Aquaculture Society 53 (5): 948-962 [doi: 10.1111/jwas.12861]

Makame MO, Hamad AR, Said MS, Mushi A, Sharif K (2021) Moving seaweed farms from shallow to deep seawater to cope with warming and diseases in Zanzibar. Current socio-economic and cultural barriers. Journal of Sustainable Development 14 (5): 29 [doi: 10.5539/jsd.v14n5p29]

McLean E (2021) Fish tank color: An overview. Aquaculture 530: 735750 [doi: 10.1016/j.aquaculture.2020.735750]

Mmochi AJ (2015) Chapter 22, Mariculture, in Part V: Assessment of food security from marine resources. In: UNEP-NAIROBI Convention (ed) The Regional State of the Coast Report: Western Indian Ocean. UNEP and WIOMSA, Nairobi, Kenya. pp 289–304

Mook WT, Chakrabarti MH, Aroua MK, Khan G, Ali BS, Islam MS, Abu Hassan MA (2012) Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination 285: 1–13 [doi: 10.1016/j.desal.2011.09.029]

Msuya FE, Kyewalyanga MS (2006) Quality and quantity of the phycocolloid carrageenan in the seaweeds *Kappaphycus alvarezii* and *Eucheuma denticulatum* as affected by grow out period, seasonality, and nutrient concentration in Zanzibar, Tanzania. Report submitted to Degussa Texturant Systems/Cargill Texturizing Solutions, France. 46 pp

Msuya FE, Shalli MS, Sullivan K, Crawford B, Tobey J, Mmochi AJ (2007) A comparative economic analysis of two seaweed farming methods in Tanzania. The Sustainable Coastal Communities and Ecosystems Program. 27 pp

Msuya FE, Porter M (2014) Impact of environmental changes on farmed seaweed and farmers: the case of Songo Songo Island, Tanzania. Journal of Applied Phycology 26 (5): 2135-2141 [doi: 10.1007/s10811-014-0243-4]

Msuya FE, Muumin H, Hamed S (2016) Status of aquaculture in the Zanzibar Islands, Tanzania. World Aquaculture 47 (3): 25-29

Msuya FE, Hurtado AQ (2017) The role of women in seaweed aquaculture in the Western Indian Ocean and South-East Asia. European Journal of Phycology 52 (4): 482–494 [doi: 10.1080/09670262.2017.1357084]

Msuya FE (2020) Seaweed resources of Tanzania: status, potential species, challenges and development potentials. Botanica Marina 63 (4): 371-380 [doi: 10.1515/bot-2019-0056]

Msuya FE (2021) The Zanzibar Seaweed Cluster Initiative: Fostering seaweed farming and value addition innovation to cope with impact of climate change in Tanzania. In: Dash JW (ed) World Scientific Encyclopedia of Climate Change: Case Studies of Climate Risk, Action, and Opportunity 2. World Scientific. pp 185-192

Muhando CA (2002) Seawater Temperature on shallow reefs off Zanzibar Town, Tanzania. In: Programme CrditIO (ed) Coral reef degradation in the Indian Ocean: Status report 2002. CORDIO, Kalmar. pp 40-46

Namukose M, Msuya FE, Ferse SC, Slater MJ, Kunzmann A (2016) Growth performance of the sea cucumber *Holothuria scabra* and the seaweed *Eucheuma denticulatum*: integrated mariculture and effects on sediment organic characteristics. Aquaculture Environment Interactions 8: 179–189 [doi: 10.3354/aei00172]

Neori A, Msuya FE, Shauli L, Schuenhoff A, Kopel F, Shpigel M (2003) A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. Journal of Applied Phycology 15 (6): 543–553 [doi: 10.1023/B:JAPH.0000004382.89142.2d]

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231 (1-4): 361–391 [doi: 10.1016/j.aquaculture.2003.11.015]

Nora'aini A, Wahab Mohammad A, Jusoh A, Hasan MR, Ghazali N, Kamaruzaman K (2005) Treatment of aquaculture wastewater using ultra-low pressure asymmetric polyethersulfone (PES) membrane. Desalination 185 (1-3): 317-326 [doi: 10.1016/j.desal.2005.03.084]

Oca J, Masaló I, Reig L (2004) Comparative analysis of flow patterns in aquaculture rectangular tanks with different water inlet characteristics. Aquacultural Engineering 31 (3-4): 221-236 [doi: 10.1016/j.aquaeng.2004.04.002]

Pang T, Liu J, Liu Q, Li H, Li J (2015) Observations on pests and diseases affecting a eucheumatoid farm in China. Journal of Applied Phycology 27 (5): 1975-1984 [doi: 10.1007/s10811-014-0507-z]

Pedersen MF, Borum J, Leck Fotel F (2010) Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. Marine Ecology Progress Series 399: 103-115 [doi: 10.3354/meps08350]

Putro SP, Widowati S, Muhammad F (2015) The application of Integrated Multi Trophic Aquaculture (IMTA) using Stratified Double Net Rounded Cage (SDFNC) for aquaculture sustainability. International Journal of Science and Engineering 9 (2): 85-89

Putro SP, Sharani J, Widowati, Adhy S, Suryono (2020) Biomonitoring of the application of monoculture and Integrated Multi-Trophic Aquaculture (IMTA) using macrobenthic structures at Tembelas Island, Kepulauan Riau Province, Indonesia. Journal of Marine Science and Engineering 8 (11): 942 [doi: 10.3390/jmse8110942]

Qiu T, Zhang L, Zhang T, Bai Y, Yang H (2014) Effect of culture methods on individual variation in the growth of sea cucumber *Apostichopus japonicus* within a cohort and family. Chinese Journal of Oceanology and Limnology 32 (4): 737-742 [doi: 10.1007/s00343-014-3131-5]

Quintã R, Santos R, Thomas DN, Le Vay L (2015) Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120: 414-421 [doi: 10.1016/j.chemosphere.2014.08.017]

Rahman H, Mirza JA, Hossain A, Asif AA, Haq E, Chwakravorty P, Rahman M (2017) Economics of fish production in paddy fields in Bangladesh. Asian Journal of Medical and Biological Research 3 (3): 379-390 [doi: 10.3329/ajmbr.v3i3.34528]

Ren JS, Stenton-Dozey J, Plew DR, Fang J, Gall M (2012) An ecosystem model for optimising production in integrated multitrophic aquaculture systems. Ecological Modelling 246: 34-46 [doi: 10.1016/j.ecolmodel.2012.07.020]

Robinson G, Slater MJ, Jones CL, Stead SM (2013) Role of sand as substrate and dietary component for juvenile sea cucumber *Holothuria scabra*. Aquaculture 392-395: 23-25 [doi: 10.1016/j.aquaculture.2013.01.036]

Rosenberg G, Ramus J (1984) Uptake of inorganic nitrogen and seaweed surface area: Volume ratios. Aquatic Botany 19 (1-2): 65-72 [doi: 10.1016/0304-3770(84)90008-1]

Sahoo D, Yarish C (2005) Mariculture of seaweeds. In: Andersen RA (ed) Physiological methods: Algal culturing techniques. Academic Press, New York. pp 219-237

Senff P, Blanc PP, Slater M, Kunzmann A (2020) Low-technology recirculating aquaculture system integrating milkfish *Chanos chanos*, sea cucumber *Holothuria scabra* and sea purslane *Sesuvium portulacastrum*. Aquaculture Environment Interactions 12: 471-484 [doi: 10.3354/aei00377]

Shao F, Mlay E, Mushi V (2003) Review of marine fisheries for Tanzania. Annex 1.1 to understanding associated livelihoods and the constraints to their development in Kenya and Tanzania: (No. DFID Fisheries Management Science Progshaoramme Project No. R8196), London: Marine Resources Assessment Group Ltd. 78 pp

Sharma B, Ahlert RC (1977) Nitrification and nitrogen removal. Water Research 11 (10): 897-925 [doi: 10.1016/0043-1354(77)90078-1]

Shpigel M, Shauli L, Odintsov V, Ashkenazi N, Ben-Ezra D (2018) *Ulva lactuca* biofilter from a land-based integrated multi trophic aquaculture (IMTA) system as a sole food source for the tropical sea urchin *Tripneustes gratilla elatensis*. Aquaculture 496: 221-231 [doi: 10.1016/j.aquaculture.2018.06.038]

Slater MJ, Carton AG (2007) Survivorship and growth of the sea cucumber *Australostichopus (Stichopus) mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture 272 (1-4): 389-398 [doi: 10.1016/j.aquaculture.2007.07.230]

Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution (Barking, Essex : 1987) 100 (1-3): 179-196 [doi: 10.1016/S0269-7491(99)00091-3]

Soto D (2009) Integrated mariculture: A global review. FAO Fisheries and Aquaculture Technical Paper 529. Food & Agriculture Org, Rome 183 pp

Southgate PC, Rubens J, Kipanga M, Msumi M (2006) Pearls from Africa. SPC Pearl Oyster Information Bulletin 17: 16-17

Spotte SH (1979) Fish and invertebrate culture: Water management in closed systems. Wiley, New York. 179 pp

Strickland J, Parsons TR (1972) A Practical handbook of seawater analysis, 2nd edition. Fisheries Research Board Canada, Ottawa. 310 pp

Tahiluddin A, Terzi E (2021) Ice-Ice disease in commercially cultivated seaweeds *Kappaphycus spp*. and *Eucheuma spp*.: A review on the causes, occurrence, and control measures. Marine Science and Technology Bulletin 10 (3): 234-243 [doi: 10.33714/masteb.917788]

Taylor R, Rees TAV (1998) Excretory products of mobile epifauna as a nitrogen source for seaweeds. Limnology & Oceanography 43 (4): 600-606 [doi: 10.4319/lo.1998.43.4.0600]

Tisera WL, Naguit MRA (2009) Ice-ice disease occurrence in seaweed farms in Bais Bay, Negros Oriental and Zamboanga del Norte. The Treshold 4: 1-16

Troell M, Halling C, Neori A, Chopin T, Buschmann A, Kautsky N, Yarish C (2003) Integrated mariculture: asking the right questions. Aquaculture 226 (1-4): 69-90 [doi: 10.1016/S0044-8486(03)00469-1]

Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang J-G (2009) Ecological engineering in aquaculture — Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297 (1-4): 1-9 [doi: 10.1016/j.aquaculture.2009.09.010]

United Nations (2019) World population prospects 2019: highlights. UN Department of Economic and Social Affairs Population Division. 39 pp

Uthicke S (2001) Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology 265 (2): 153-170 [doi: 10.1016/S0022-0981(01)00329-X]

Vairappan CS, Chung CS, Hurtado AQ, Soya FE, Lhonneur GB, Critchley A (2008) Distribution and symptoms of epiphyte infection in major carrageenophyte-producing farms. Journal of Applied Phycology 20 (5): 477-483 [doi: 10.1007/s10811-007-9299-8]

van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquacultural Engineering 53: 49-56 [doi: 10.1016/j.aquaeng.2012.11.010]

Wakibia JG, Bolton JJ, Keats DW, Raitt LM (2006) Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. Journal of Applied Phycology 18 (3-5): 565-573 [doi: 10.1007/s10811-006-9058-2]

Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, VanDerslice J (2005) Workgroup report: Drinking-water nitrate and health—recent findings and research needs. Environmental Health Perspectives 113 (11): 1607-1614 [doi: 10.1289/ehp.8043]

Ward GM, Faisan JP, Cottier-Cook EJ, Gachon C, Hurtado AQ, Lim PE, Matoju I, Msuya FE, Bass D, Brodie J (2020) A review of reported seaweed diseases and pests in aquaculture in Asia. Journal of the World Aquaculture Society 51 (4): 815-828 [doi: 10.1111/jwas.12649]

Ward GM, Kambey CSB, Faisan JP, Tan P-L, Daumich CC, Matoju I, Stentiford GD, Bass D, Lim P-E, Brodie J, Poong S-W (2022) Ice-Ice disease: An environmentally and microbiologically driven syndrome in tropical seaweed aquaculture. Reviews in Aquaculture 14 (1): 414-439 [doi: 10.1111/raq.12606]

Wiencke C, Bischof K (eds) (2012) Seaweed biology. Ecological studies. Springer Berlin Heidelberg, Berlin, Heidelberg. 514 pp

Wolkenhauer S-M, Uthicke S, Burridge C, Skewes T, Pitcher R (2010) The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. Journal of the Marine Biological Association of the United Kingdom 90 (2): 215-223 [doi: 10.1017/S0025315409990518]

Zuldin WH, Yassir S, Shapawi R (2016) Growth and biochemical composition of *Kappaphycus* (Rhodophyta) in customized tank culture system. Journal of Applied Phycology 28 (4): 2453-2458 [doi: 10.1007/s10811-016-0792-9]

Original Article

Uncovering the relationship between light intensity and *Tripneustes gratilla* (collector sea urchin): implications for aquaculture

Shamira Payet[1], Bas de Vos[1,2]*, Maria Rose[1], Danilla Adonis[1], Aubrey Lesperance[1], Thomas Hecht[3,4]

[1] Seychelles Fishing Authority, Aquaculture Department, Seychelles

[2] GOPA, Bad Homburg, Germany

[3] Department of Ichthyology & Fisheries Science, Rhodes University, Makhanda (Grahamstown), South Africa.

[4] Advance Africa Management Services, 2 Beach Road, Kleinemonde, South Africa

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*** Corresponding author:**
bascdevos@gmail.com

Abstract

The sea urchin species *Tripneustes gratilla* has high aquaculture potential. It is known for using debris to cover itself, likely to avoid light, which suggests that high light levels may impact its production. This study aimed to validate these concerns through four experiments. The first experiment assessed if *T. gratilla* preferred opaque or transparent covering materials, finding no evidence that shading ability of the material influenced their choice ($F_{(2,\ 33)} = 0.27$, $p = 0.765$). The second experiment investigated the impact of shade on the number of covering materials collected, showing that higher solar radiation correlated with increased material collection per urchin ($H_{(3)} = 7.844$, $p = 0.049$). The third experiment evaluated righting response time as a fitness trial, finding that high light intensity reduced fitness to urchins acclimated to low light ($H_{(2)} = 5.615$, $p = 0.020$). The fourth long-term experiment demonstrated that *T. gratilla* can acclimatize to higher light intensities, with no significant differences in fitness, mortality, disease susceptibility, growth, and gonadosomatic index ($p > 0.128$). While this suggests that shading may not be a strict requirement for *T. gratilla* aquaculture, it is recommended for consideration. These findings underscore the importance of acclimatization when altering light conditions for *T. gratilla*.

Keywords: aquaculture, sea urchin, *Tripneustes gratilla*, echinoderm aquaculture

Introduction

The gonads (uni) of sea urchins such as *Hemicentrotus pulcherrimus, Heliocidaris crassispinam*, *Paracentrotus lividus*, *Strongylocentrotus droebachiensis* and *Tripneustes gratilla* are in high demand and greatly valued as a culinary delicacy (Tsukiji Market, 2020). *Tripneustes gratilla* is a circumglobal, tropical and sub-tropical sea urchin species that holds significant commercial and ecological importance (Toha *et al.*, 2017). This species is considered a promising candidate for full-lifecycle aquaculture due to their high value, fast growth rates, palatability of both sexes and large marketable gonads (Cyrus *et al.*, 2013; Shpigel *et al.*, 2018). However, commercial production of *T. gratilla* uni is not yet at scale, partly due to knowledge gaps regarding optimal conditions to promote high survival and growth and therefore increasing yield. A factor potentially influencing the production of *T. gratilla* may be the intensity of solar radiation upon the urchins (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al.*, 2021).

The urchin's behavioural tendency to shade itself by actively utilizing materials from the substratum to cover its aboral surface suggests that there may be a negative correlation between light intensity and *T. gratilla* production. This process involves the use of

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its podia (tube feet) and spines to move material actively onto its aboral surface (Ziegenhorn, 2016). This covering behaviour has been observed, to a lesser extent, in other urchin species where it has been demonstrated to correlate with temperature, solar radiation, feeding, availability of cover material, predation, surge and suspended particles (Adams, 2001; Sigg *et al*., 2007; Dix, 1970; Dennis and Gerald , 1972; Sharp and Gray, 1962; James, 2000; Agatsuma, 2001, Kehas *et al*., 2005).

There is some evidence for *T. gratilla* that the covering behaviour serves to assist urchins against the effects of currents and waves, preventing them from becoming detached from the substrate (Park and Cruz, 1994), but the most substantiated reason is that the urchin will cover itself to avoid light (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al*., 2021). This is physiologically feasible due to the urchins' ability to sense light using photo-receptors located on the podia (Millott *et al*., 1975). Ziegenhorn (2016) demonstrated that *T. gratilla* prefers opaque over translucent materials to cover itself, which implies this is either a photo-defensive behaviour (protecting itself from solar radiation) or a cryptic behaviour (avoiding predation). Li *et al*. (2021) investigated the influence of different spectrums of light on urchin fitness, which the current study defines as the overall health, adaptability, and performance of *T. gratilla.* The metric used for fitness in Li *et al*. (2021) is righting response which is the time sea urchins take to return to their normal posture after being inverted (Brothers and McClintock, 2015). Li *et al*. (2021) found that *T. gratilla* exposed to short-wavelength irradiation (blue light) had significantly reduced fitness compared to higher wavelength irradiation (red and full spectrum light). There is further evidence suggesting that without sufficient shading *Tripneustes ventricosus* will use their feed (e.g., macroalgae) to cover their bodies, presenting a trade-off between feeding and ingesting, negatively impacting growth and gonad quality (Kehas *et al*., 2005). These studies (Park and Cruz, 1994; Kehas *et al*. 2005; Ziegenhorn, 2016; Li *et al.*, 2021) provide evidence that *T. gratilla* are influenced by light conditions. However, it is unclear if these relationships between light, covering behaviour, and fitness are relevant to *T. gratilla* aquaculture or what specific actions aquaculture operators should take to maximize *T. gratilla* production, such as shading culture facilities.

The main goal of this study was to determine if and how solar radiation affects *T. gratilla* in Seychelles and to provide subsequent management advice to *T. gratilla* production facilities. It is important to note that while *T. gratilla* covers itself to avoid light in other regions (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al*., 2021), this has not been proven for the *T. gratilla* population from Mahe, Seychelles, used in this study. Due to significant geographic differences in *T. gratilla* behaviour, such as spawning time and feed preferences (Toha *et al*., 2017, personal observation, 2023), it was necessary to confirm if the *T. gratilla* in Seychelles covers itself to provide shading and not for another reason.

Methods

Experimental design and system

A total of four experiments were conducted in this study, the first three were short-term experiments focussing on behavioural responses to light, whereas the fourth experiment assessed the long-term influence of light on *T. gratilla* production. All experiments were conducted in fiberglass raceways with dimensions 6.2 m x 2 m x 0.8 m filled with seawater to a working depth of 60 cm fiberglass raceways. The exchange rate during all experiments was approximately 0.25 full tank exchanges per hour (1860 $l.hr^{-1}$). Fresh seawater was passed through a sand filter prior to entering the tanks. There was constant aeration distributed evenly across the raceway. For all experiments the urchins were held in plastic crates that measured 50 x 32 x 26.5 cm, with a different number of urchins in each crates depending on the experiment.

Once a week the tank was cleaned by draining the raceway completely and scrubbing it. Prior to draining the raceway all baskets with sea urchins were transferred to a clean raceway already filled with filtered seawater and aerated. The temperature and dissolved oxygen levels were measured daily and on average (±standard deviation) were 28.97 (± 2.89) °C and 5.2 (± 0.772) $mg.L^{-1}$ respectively. Light intensity was measured in LUX with a Lutron LX-1108 and UV was measured with a Lutron UV-340A. The measurements for LUX and UV were taken daily by positioning the meter beneath each crate shading materials (Table 1), during the different experiments.

Experimental animals

T. gratilla were collected from the wild typically at water depths ranging from 1 to 2 meters. They were then transported to the sea urchin research facility situated at the Seychelles Maritime Academy in Providence, Mahe, Seychelles. During transport, the urchins were distributed into separate containers to avoid overcrowding and covered with *Sargassum spp*.

Table 1. The shading treatments, and the materials used to achieve the shading levels and the observed mean LUX and UV values beneath the materials.

Treatment (% shaded)	Shading material	Mean LUX (lm/m²)	Min LUX (lm/m²)	Max LUX (lm/m²)	Average UV
0	None	116300	126,500	112900	4939
32	Mesh	36675	78500	102000	2738
41	Green corrugated roofing	47241	28200	62000	120
77	Shade cloth	89708	28300	41600	515

to shield them from direct sunlight exposure. Upon arrival, they were transferred to plastic crates with 41 % shading and placed in a raceway where they were allowed to acclimate for a period of 14 days.

Experiment 1: Covering material opacity preference trial

To examine the correlation between light and covering of *T. gratilla* from Seychelles, an experiment was conducted to determine the preference for selecting opaque or transparent covering materials by *T. gratilla*. On the day of this experiment, 12 *T. gratilla* were removed to a separate raceway and were placed individually in uncovered crates. These urchins had an average (± SD) wet weight, test diameter and test height of 353.9 ± 69.87 g, 87.1 ± 7.51 mm and 61.8 ± 7.0 mm respectively. The height and test diameter were determined using vernier callipers. An average LUX of 117,450 ± 937.54 lm/m² was recorded during the study. Each crate was randomly scattered with three different types of covering materials of various opacities, with 15 pieces of each type. The covering materials used were corrugated plastic roofing sheets cut into 2.5 x 2.5 cm pieces of either blue (opaque), green (partially transparent) or clear (fully transparent). After 3 hours, the number and type of covering materials collected by each *T. gratilla* in each crate were recorded.

Experiment 2: Influence of solar radiation on quantity of covering materials collected by *T. gratilla*

This experiment was conducted to determine if solar radiation intensity would influence the quantity of covering material collected by *T. gratilla*. Four treatment levels of solar radiation intensity where achieved by placing different types of shading materials (mesh, green corrugated roofing, shade cloth), or lack therefore, on top of the crates and above the water surface. The light intensity beneath the shading material within each crate was measured daily and the average value calculated (Table 1)..

Twelve *T. gratilla* were measured for wet weight, test diameter and test height, with mean ± SD values of 368.8 ±60.1 g, 89.4 ± 7.8 mm, and 65.3 ±7.0 mm respectively before each was placed individually into a crate. Following this, 45 pieces of 2.5 x 2.5 cm opaque (not transparent) plastic sheet covering materials were added inside the crates amongst the urchins. Besides these green plastic sheets, no other covering materials were provided. Note that this covering material differs from the shading material (Table 1) which is above the water surface and out of the urchins' reach. After 30 minutes, the number of covering materials collected by each *T. gratilla* in each crate was recorded. Thirty minutes was required for this trial (as opposed to the three hours of the previous trial), as the urchins did not need time to select different materials.

Experiment 3: Short-term effects of solar radiation on *T. gratilla* fitness

In this experiments *T. gratilla* were placed in crates that had three different light regimes: full light (control), 37 % light reduction and 100 % light reduction. The amount of light was controlled using different covering materials (none, corrugated roofing and black plastic sheeting respectively). A total of 60 *T. gratilla* were included in the study, conducted over five experimental runs, with four *T. gratilla* randomly allocated to each crate per run. The experiment was only undertaken on days with no cloud cover (between 10:00 hrs and 14:00 hrs). *T. gratilla* in this experiment had an average ± SD of wet weight of 399.42±71.35 g, diameter of 77.51±23.33 mm and, height of 55.43±8.79 mm.

At the start of the experiment, LUX and UV radiation intensity were recorded, using the same methods previously described, at the water surface in all the crates. After three hours they were removed to be tested for righting response time. To do so, *T. gratilla* was gently placed in individual plastic containers of 50 cm x 40 cm x 30 cm with their aboral side down. The urchins were positioned in the centre of the container so they could not access the sidewalls to assist with righting themselves. The containers were filled with seawater. Aeration and water flow were not used to avoid potential impacts on the righting behaviour. The time taken

Table 2. The shading treatments, and the materials used to achieve the shading levels and the observed LUX and UV values beneath the materials.

Treatment (% shaded)	Shading material	Mean LUX (lm/m²)	Min LUX (lm/m²)	Max LUX (lm/m²)	Average UV
0	None	188298	7982	351700	6824
37	Corrugated roofing	69128	1608	272400	238
100	Black plastic sheeting	0	0	0	0

for the sea urchins to right themselves, with their aboral side facing upwards was measured and recorded.

Experiment 4: The long-term influence of light on *T. gratilla* production

A long-term experiment was conducted to assess the influence of light on *T. gratilla* production over 67 days. *T. gratilla* (n=90) was collected from the wild and acclimated for 14 days in crates covered with corrugated roofing. To begin the experiment, nine crates were placed in the raceway with 10 *T. gratilla* in each with an average ± SD wet weight, test diameter and test height of 336 ±82.3 g, 71.4 ±6.6 mm, 43.8 ±15.2 mm, respectively. Three of the crates were covered with corrugated roofing material, three with black plastic sheeting and three crates had no shading. The solar radiation intensities under each level of shading are shown in Table 2. LUX and UV were measured using the same instrument and method mentioned above. Mortalities and incidence of disease (bald spot) during the experiment were recorded. The appearance of bald spot disease (Becker *et al.*, 2007) was considered a sign of stress in *T. gratilla*. At the end of the experiment, three urchins were randomly chosen from each basket to test their righting response time. The same method as described above was used. Also, urchins from each replicate were measured for height, diameter and mass. Subsequently, the specific growth rate (SGR) was determined for each replicate (Equation 1).

$$SGR = 100x\ln (L_T/L_0)/t \qquad (1)$$

Where:
L_0=initial length/mass
L_T=final length/mass
t = days of culture

The urchins were then dissected to determine the gonadosomatic index (GSI, Equation 2).

$$GSI = 100x(GW/WW) \qquad (2)$$

Where:
GW = Gonad weight
WW = Wet weight

Statistical analysis

The statistical programming environment R (R Development Core Team, 2017) was employed in all the analytical procedures. Excel was utilized for data organization and presentation. It was ensured that the assumptions regarding independence and non-selectivity were satisfied, as elucidated within the context of the experimental designs. Significance was attributed to p-values below the threshold of 0.05.

All the data was tested for homogeneity of variance and normality of distribution using Levene's test and the Shapiro-Wilk test respectively. The data stemming from the experiments on cover preference and long-term specific SGR, GSI and fitness was found to be homogenous ($p > 0.29$) and parametric ($p < 0.056$) and therefore one-way ANOVAs were applied. When treatments were found to have a significant influence on the mean, pairwise Tukey tests were applied for multiple comparisons. The data from the experiments quantifying the covering response to light, short-term fitness, long-term survival and disease was found to be homogenous ($p > 0.091$) but not parametric ($p < 0.015$), subsequently, a Kruskal-Wallis test was applied. When the tests were found to be significant, Dunn post-hoc analyses were conducted.

Results

Experiment 1: Covering material opacity preference trial

At the end of the three-hour trial, all urchins had collected covering materials of various transparencies to cover themselves with a mean ±standard deviation (SD) of 1.72 ±1.92 pieces per urchin (Fig. 1). While the average number of opaque pieces (2.33 ±1.92) collected by the sea urchins was greater than that of the fully (1.83 ±1.89) and partially transparent (1 ±1.94) pieces (Fig. 1), there is no conclusive evidence that *T. gratilla* preferred covering material due to their ability to provide shade. There were instances where the individual urchins collected only transparent pieces, but no opaque pieces and vice versa. There was no significant influence of material transparency on the number of pieces collected by *T. gratilla* ($F_{(2, 33)} = 0.27$; $p = 0.765$).

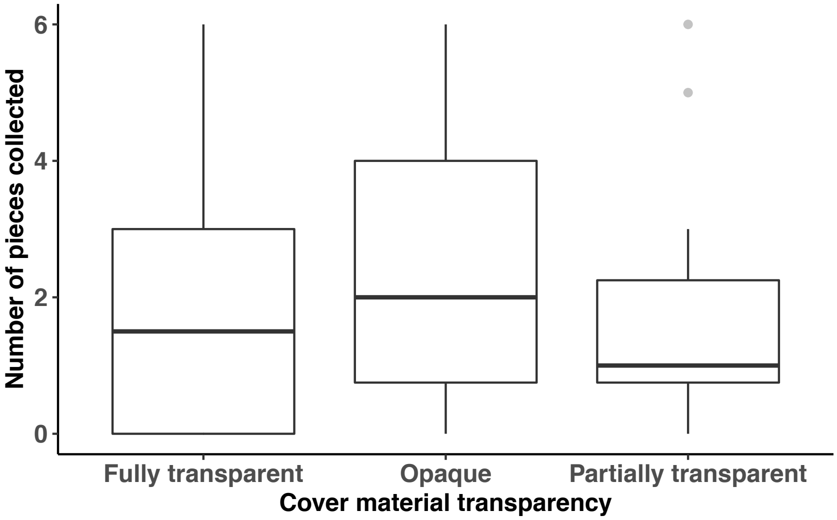

Figure 1. The number of covering material pieces of various transparencies collected by *T. gratilla* after three hours. The covering material pieces (n=45) provided to each urchin (n=12) were small sheets of hard plastic, either fully transparent (clear), partially transparent (green) or opaque (blue), with equal quantities of each type.

Experiment 2: Influence of solar radiation on covering materials collected.

All urchins collected some covering material (7.71 ±5.38 pieces per urchin) regardless of shading, except for one individual in an unshaded crate which did not collect any pieces. Overall, the amount of shading over the crate containing the urchins had a significant influence on the number of pieces collected ($H_{(3)} = 7.844$, $p = 0.049$). The average number of materials collected by urchins in the unshaded crates (11 ±6.85 pieces) was greater than that of the treatments with various levels of shade (6.42 ±4.15 pieces; Fig. 2). However, the urchins in the unshaded crates collected significantly more covering pieces than those in the maximally (77 % shade) shaded crates ($p = 0.039$), but not significantly more than in the other treatments ($p > 0.098$).

Experiment 3: Short-term effects of solar radiation on *T. gratilla* fitness

After three hours of being exposed to three different light intensities, the righting response of *T. gratilla* was recorded. The urchin's ability to right themselves took the longest in the crates without shade

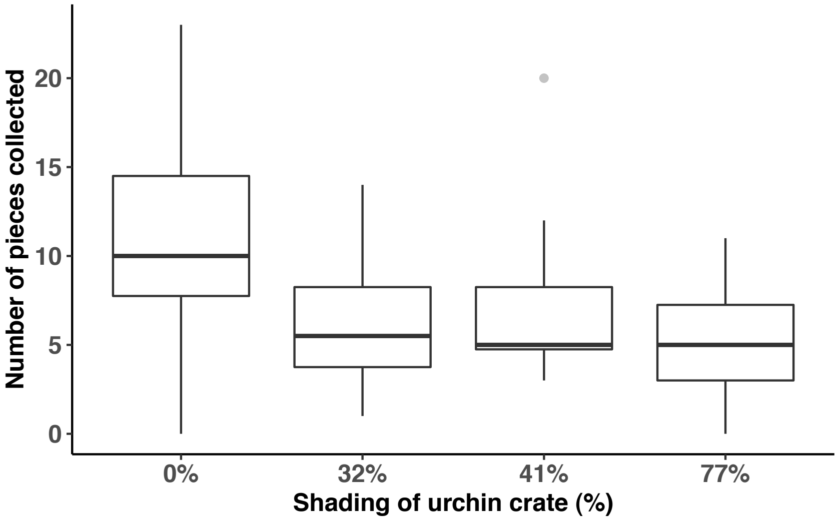

Figure 2. The number of covering materials (plastic sheets) collected by *T. gratilla* in crates with varying levels of shading. The 0 % shaded treatment had no shading, the 32 % shaded treatment had the crates covered by a fine mesh, the 41 % used an opaque plastic sheet, and the 77 % shade treatment was achieved by covering with a shade cloth.

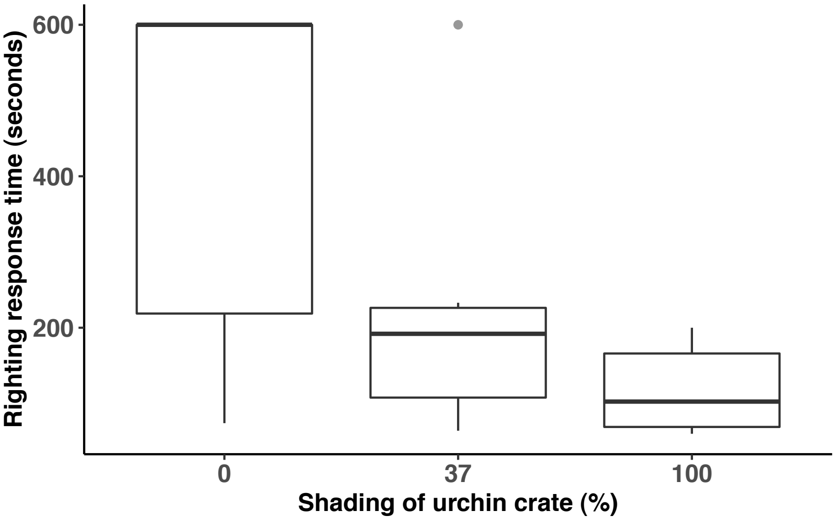

Figure 3. The righting response time (seconds) of *T. gratilla* after three hours exposed to three different light intensities. A total of 20 sea urchins exposed to each of the different light intensity either black plastic sheeting (0 % light intensity), corrugated roofing (37 % light intensity) and none (100 % light intensity).

(282 ±221 seconds), followed by those in 37 % shade under corrugated roofing material (241.4 ±197.12 seconds; Fig. 3). The urchins in 100 % shade under black plastic sheeting took the least time (143 ±113.97 seconds) to right themselves. The results showed that light intensity had a significant influence on righting response in *T. gratilla* ($H_{(2)}$=7.182, p = 0.027). A significant difference was observed between treatments 0 % and 100 % shading (p = 0.032).

Experiment 4: The long-term influence of light on *T. gratilla* production

At the end of the long-term trial, during which *T. gratilla* was exposed to three different light intensities for 67 days, the urchins' righting response was assessed to quantify their degree of fitness. The righting response of urchins exposed to a lower light intensity (100 % shade) was faster (128 ±92.79 seconds) than those under 37 % shade (285 ±205.17 seconds) and 0 %

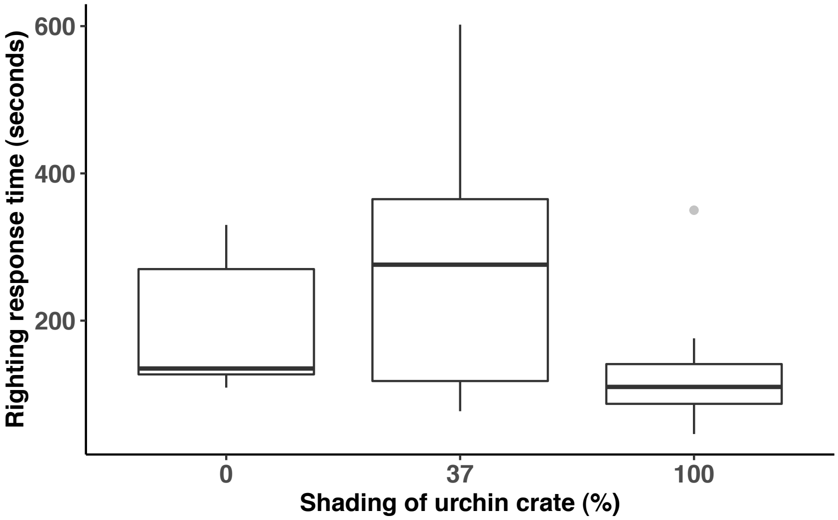

Figure 4. The fitness of *T. gratilla* (n=27) after long-term exposer to three different light intensities; 0 %, 37 % and 100 % shade. Fitness was assessed by the time taken for to right themselves from being inverted.

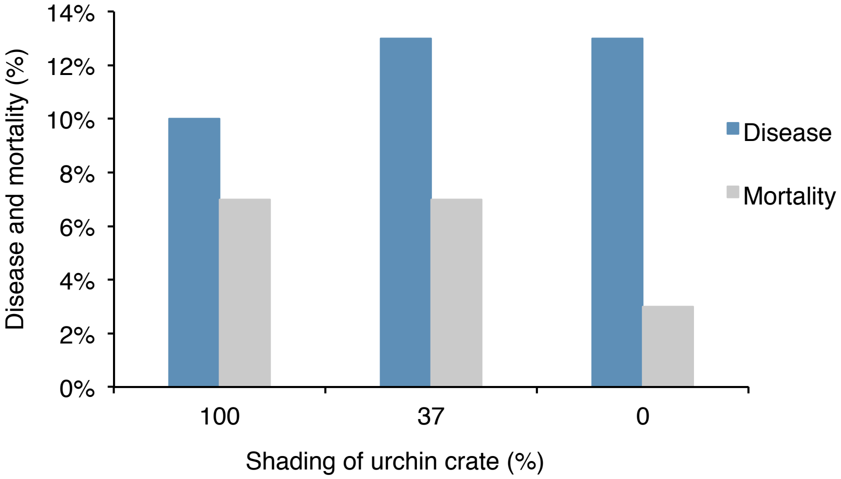

Figure 5. The disease and mortality rate (%) of *T. gratilla* after 67 days of exposer to three different light intensities; 0 %, 37 %, and 100 % shading.

shade (184 ±83.04; Fig. 4). However, there is no statistical evidence that light intensities influenced the righting abilities of *T. gratilla* ($H_{(2)}$ = 4.108 p = 0.128).

Across all treatments, the average specific growth rates (SGR) were low over the long-term trial. The mean ±SD SGR of the test diameter, height and wet mass was 0.04 ±0.06 mm, 0.04 ±0.07 mm, 0.07 ±0.15 g respectively. There were no significant differences between treatments for the SGR of height ($F_{(2, 6)}$ = 0.782; p = 0.499), diameter ($F_{(2, 6)}$ = 0.279; p = 0.766) or mass ($F_{(2, 6)}$ = 0.829; p = 0.481).

During the 67-day trial, the lowest light intensity treatment (100 % shade) had the lowest mortality rate (3.5 %), while the two treatments at higher light intensities had an equally greater mortality (7 %; Fig. 5). However, there was no evidence of a significant influence of light intensity on mortality ($F_{(2, 12)}$ = 0.09, p = 0.913). Similarly, even though fewer urchins with symptoms of the disease were recorded in fully shaded crates (Fig. 5), there were no significant differences between the number of diseased individuals and light intensity ($F_{(2, 12)}$ = 0.09, p = 0.913).

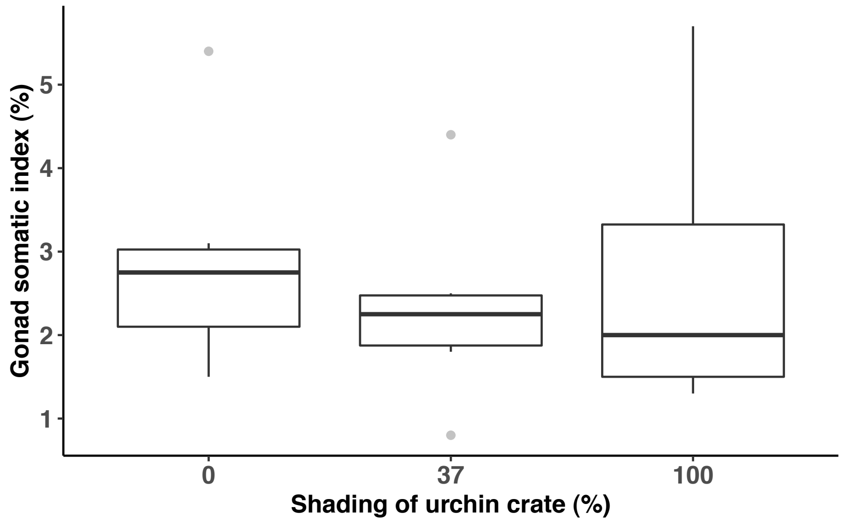

Figure 6. Gonad somatic index (%) of *T. gratilla* after being exposed in three different light intensity for 67 days. The covering materials were black plastic (0 % light intensity), corrugated roofing (37 % light intensity) and none (100 % light intensity).

The average gonad somatic index (GSI) was not significantly different under the three different light intensities ($H_{(2)} = 0.877$ $p = 0.644$). The mean ±SD GSI values for 100 %, 37 % and 0 % shaded treatments were low (Fig. 6), 2.6 ±1.73 %, 2.3 ±1.20 % and 2.9 ±1.36 % respectively.

Discussion

The observations from this study confirmed that *T. gratilla* covering behaviour is stimulated by greater light intensity (Experiment 2, Fig. 2), aligning with prior studies (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al*., 2021). Yet, evidence could not be provided that *T. gratilla* prefers opaque materials for covering (Experiment 1, Fig. 1), unlike Ziegenhorn (2016). Therefore, it cannot be concluded that *T. gratilla* in the Seychelles selected material for their ability to provide shade. Thus, the purpose of this covering behaviour remains unclear. While it may protect from UV radiation or have a cryptic function, they may also collect objects as a physical barrier against predators or to stabilize the urchins during surges. The contrasting finding of cover material opacity preference between this study and that of Ziegenhorn (2016) may stem from regional behavioural differences among *T. gratilla* populations (Toha *et al*., 2017; personal observation, 2023). This underscores the imperative to validate proposed aquaculture practices, findings, or technologies within the region of application, prior to upscaling for commercial implementation in general. More specifically, given the spatial variations in *T. gratilla* behaviour, it is essential to conduct similar investigations in different regions to obtain a comprehensive understanding of their responses to light.

The results of the short-term fitness trial (Experiment 3) complemented those of Li *et al*. (2021), where greater light exposure significantly reduces the fitness of urchins (Fig. 3). However, it should be noted that urchins in this study were acclimatised to lower light levels prior to this experiment and Li *et al*. (2021) did not specify what lighting the urchins were acclimatised to before experimentation. This is relevant as this fitness and light relationship was not observed when urchins were acclimatised to specific lighting conditions over a longer-term experiment (Fig. 4). This indicates that *T. gratilla* fitness will only be affected adversely by light when shocked by it, but they can acclimatise to higher light intensities. This emphasizes the importance of gradual acclimatization when altering light conditions for *T. gratilla*, where care should be taken by aquaculture operators to not rapidly change environmental conditions. Additionally, this finding does not provide conclusive evidence that *T. gratilla* production facilities require shading. This is further supported by the lack of evidence that light intensity impacted urchin mortality, disease susceptibility (Fig. 5), growth or gonad somatic indices (Fig. 6), suggesting that *T. gratilla* can adapt to higher light levels over an extended period, a novel finding that carries important implications for aquaculture practices.

While there were no significant differences in disease and mortality between the different light treatments, which suggests that *T. gratilla* can adjust to higher light intensity, it is important to note some limitations regarding urchin growth and GSI results. The GSI results might have been affected by spawning events that occurred before the dissections. Even though no spawning was observed during the trial, the very low GSI values (average of 2.84 %) suggest that spawning might have happened. Previous trials at this facility under similar conditions and feed showed much higher GSI values, around 10 % (personal observation, 2023). The lack of significant difference in growth rates is likely due to the urchins starting at a size where they no longer experience high growth rates (Dafni, 1992; Shpigel *et al*., 2018). Additionally, the measurement methods used may not have been precise enough (de Vos *et al*., 2023), making it unlikely to detect a significant difference. Future research should use smaller urchins that can continue to grow during the experiments and ideally control for spawning events.

Although these experiments did not establish a substantial influence of light intensity on *T. gratilla* production, the authors are not necessarily advising that shading should not be implemented in land-based urchin production facilities. There are likely alternative advantages of shading such as preventing salinity fluctuations due to rainwater, slowing the growth of epiphytes, and reducing temperature fluctuations in tanks.

In conclusion, this research contributes to a deeper comprehension of the complex relationship between light exposure, covering behaviour, and the production of *T. gratilla*. While the motives behind *T. gratilla's* covering behaviour remain somewhat enigmatic and are likely dynamic, the findings from this study underline the importance of gradual acclimatization to changing light conditions and regional variation in *T. gratilla* behaviour. This study does not provide definitive evidence that light directly hampers urchin production thus implying that shading of urchin production facilities may not be an absolute requirement for production. This research contributes another piece of the puzzle to advancing *T. gratilla* aquaculture by offering valuable insights into optimizing production practices while ensuring the welfare of this economically important species.

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References

Adams NL (2001) UV radiation evokes negative phototaxis and covering behavior in the sea urchin *Strongylocentrotus droebachiensis*. Marine Ecology Progress Series 213: 87-95 [doi: 10.3354/meps213087]

Agatsuma Y (2001) Effect of the covering behavior of the juvenile sea urchin *Strongylocentrotus intermedius* on predation by the spider crab *Pugettia quadridens*. Fisheries Science 67 (6): 1181-1183 [doi: 10.1046/j.1444-2906.2001.00379.x]

Becker P, Gillan DC, Eeckhaut I (2007) Microbiological study of the body wall lesions of the echinoid *Tripneustes gratilla*. Diseases of Aquatic Organisms 77 (1): 73-82 [doi: 10.3354/dao01821]

Brothers CJ, McClintock JB (2015) The effects of climate-induced elevated seawater temperature on the covering behavior, righting response, and Aristotle's lantern reflex of the sea urchin *Lytechinus variegatus*. Journal of Experimental Marine Biology and Ecology 467: 33-38 [doi:10.1016/j.jembe.2015.02.019]

Cyrus MD, Macey BM, Bolton JJ (2013) The development of a formulated feed containing *Ulva* (Chlorophyta) to promote rapid growth and enhanced production of high quality roe in the sea urchin *Tripneustes gratilla* (Linnaeus). Aquaculture Research 45 (1): 159-176 [doi: 10.1111/j.1365-2109.2012.03219.x]

Dafni J (1992) Growth rate of the sea urchin *Tripneustes gratilla elatensis*. Israel Journal of Zoology 38 (1): 25-33 [doi: 10.1080/00212210.1992.10688663]

Dennis CL, Gerald AC (1972) The covering response to surge, sunlight, and ultraviolet light in *Lytechinus anamesus*. Ecology 53 (6): 1127-1133 [doi:10.2307/1935425]

De Vos BC, Cyrus MD, Macey BM, Batik T, Bolton JJ (2023) Combining computer vision and standardised protocols for improved measurement of live sea urchins for research and industry. Aquaculture, Fish and Fisheries 3: 507-517 [doi: 10.1002/aff2.137]

Dix T (1970) Covering response of the echinoid *Evechinus chloroticus*. Pacific Science 24 (2):187-194 [doi: 10125/4041].

James DW (2000) Diet, movement, and covering behavior of the sea urchin *Toxopneustes roseus* in rhodolith beds in the Gulf of California, México. Marine Biology 137 (5-6): 913-923 [doi: 10.1007/s002270000423]

Kehas AJ, Theoharides KA, Gilbert JJ (2005) Effect of sunlight intensity and albinism on the covering response of the Caribbean sea urchin *Tripneustes ventricosus.* Marine Biology 146 (6): 1111-1117 [doi: 10.1007/s00227-004-1514-4]

Li YY, Pang SJ, Liu F, Qian PY, Zhang Y, Mao YZ, Zhou L, Sun J, Bao WY, Li X (2021) Embryo development and behavior in sea urchin (*Tripneustes gratilla*) under different light-emitting diodes condition. *Frontiers in Marine Science* 8 [doi: 10.3389/fmars.2021.684330].

Millott N, Station B, Station B (1975) The photosensitivity of echinoids. Advances in Marine Biology. Academic Press, New York. pp 1-52

Park I, Cruz C (1994) Masking behavior and distribution of the tropical sea urchin *Tripneustes gratilla*. Biological Geomorphology in Tropical Islands 4: 22-47

Sharp DT, Gray IE (1962) Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. Ecology 43 (2): 309-313 [doi: 10.2307/1931986]

Shpigel M, Shauli L, Odintsov V, Ashkenazi N, Ben-Ezra D. (2018) *Ulva lactuca* biofilter from a land-based integrated multitrophic aquaculture (IMTA) system as a sole food source for the tropical sea urchin *Tripneustes gratilla elatensis*. Aquaculture 496: 221-231 [doi: 10.1016/j.aquaculture.2018.06.038]

Sigg JE, Lloyd-Knight KM, Boal JG (2007) UV radiation influences covering behaviour in the urchin *Lytechinus variegatus*. Journal of the Marine Biological Association of the United Kingdom 87 (5): 1257-1261 [doi: 10.1017/S0025315407055865]

Toha AHA, Putranto TH, Bengen DG, Zamani NP (2017) Review: Biology of the commercially used sea urchin *Tripneustes gratilla* (Linnaeus, 1758) (Echinoidea: Echinodermata). Ocean Life 1 (1): 1-10. [doi: 10.13057/oceanlife/o010101]

Tsukiji-Market (2016) Tokyo Metropolitan Central Wholesale Market [https://www.shijou.metro.tokyo.lg.jp/]

Ziegenhorn MA (2016) Best Dressed Test: A study of the covering behavior of the collector urchin *Tripneustes gratilla*. PLoS ONE 11 (4) [doi: 10.1371/journal.pone.0153581]

Original Article

Unlocking the therapeutic treasures of seagrasses: Antioxidant and antimicrobial activities of *Halophila stipulacea*, *Halodule uninervis*, and *Thalassodendron ciliatum*

Bhuvaneshwaree Surroop[1*], Nadeem Nazurally[1, 2], Deena Ramful-Baboolall[1], Arvind Ruggoo[3]

[1] Department of Agricultural and Food science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius, 80837

[2] Faculty of Fisheries, Hokkaido University, Japan, 060-0817

[3] Department of Agricultural Production and Systems, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius, 80837

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*** Corresponding author:**
bsurroop28@gmail.com

Abstract

Seagrasses are essential to marine ecosystems and have been shown to possess pharmaceutical properties. This study evaluated the antioxidant and antimicrobial activities of the seagrass species, *Halophila stipulacea, Halodule uninervis*, and *Thalassodendron ciliatum* from a Mauritian lagoon. Two essential steps in the extraction process were investigated: drying method (oven-drying and freeze-drying) and maceration solvent (methanol and acetone), using a factorial design. The highest total phenolic content (60.1 mg GAE/g) was observed in oven-dried acetonic *T. ciliatum* extracts. Antioxidant activity was assessed through DPPH and ABTS assays, where all extracts demonstrated significant activities ($p < 0.05$). Oven drying and acetonic extractions resulted in greater activities (highest DPPH activity of 69.3 % with *T. ciliatum*). Significant interactions ($p < 0.05$) were found between species, drying methods, and solvents. *Escherichia coli* and *Bacillus cereus* were resistant to all extracts, while *Staphylococcus aureus* showed limited inhibition. *Pseudomonas aeruginosa* was more susceptible to freeze-dried extracts (greatest MIC of >1.25 mg/ml for acetonic *T. ciliatum*). *Candida albicans* was most susceptible to freeze dried acetonic *T. ciliatum* extracts (24.7 ± 4.06 mm). Consequently, this study encourages further exploration and use of seagrasses, especially, *T. ciliatum* in the pharmaceutical industry.

Keywords: Seagrass, Total phenolic content, Antioxidant, Antibacterial, Antifungal

Introduction

Nestled within the Indian Ocean's southern tropical belt, Mauritius is an island adorned with a diverse marine ecosystem, where seagrasses, crucial aquatic angiosperms, play a pivotal role. Montaggioni and Faure (1980) reported the presence of seven seagrass species in Mauritian lagoons, namely, *Syringodium isoetifolium, Thalassodendron ciliatum, Halophila ovalis, Halophila stipulacea, Halophila decipiens, Halodule uninervis* and *Cymodocea serrulata*. Seagrasses are vital marine species, providing nursery habitats for juvenile fish and supporting 20 % of the world's major fisheries (Madi Moussa *et al.*, 2020). They serve as key food sources for herbivores such as *Dugong dugon* and *Chelonia mydas* (Lin *et al.*, 2021). Additionally, they act as efficient carbon sinks, sequestering 27–44 Tg of organic carbon annually in sediments (Bijak *et al.*, 2023; Bedulli *et al.* 2020). Their ecological significance extends to the tourism industry in Mauritius through sediment filtration in water columns and enhanced coastline protection from erosion by resilient rhizomes (Tandrayen-Ragoobur *et al.*, 2022; Amone-Mabuto *et al.*, 2023).

Seagrasses have demonstrated noteworthy pharmacological significance (Gono *et al.*, 2022; Lahay and Amiin, 2023; Punginelli *et al.*, 2023). In fact, the presence of phenolic compounds in seagrasses has shed light on their potential as sources of therapeutic agents (Astudillo-Pascual *et al.*, 2021; Gono *et al.*, 2022). Antioxidants counter oxidative stress from reactive oxygen species (ROS), which can contribute to cardiovascular diseases, inflammation, and cancer (Halliwell, 2024) and seagrasses have been determined to possess antioxidant potential (Sansone *et al.*, 2021; Divyashri *et al.*, 2021). However, the antioxidant potential of Mauritian seagrasses has been only been investigated by Ramah *et al.*(2014).

The scourge of infectious diseases has claimed countless lives over decades and antimicrobial resistance is further exposing mankind to risks (Manso *et al.*, 2021). In pursuit of solutions, researchers have explored natural reservoirs of antimicrobials, with seagrasses emerging as a subject of interest (Punginelli *et al.*, 2023; Ozbil *et al.*, 2024). For example, Hamisi *et al.*(2023) discovered the antimicrobial effect of seven seagrasses against a causative agent of typhoid fever, *Salmonella typhi*. Despite such studies, it remains salient that no drugs derived from seagrasses have obtained Food and Drug Administration (FDA) approval (Marine Pharmacology, 2023). Furthermore, the antimicrobial potential of seagrasses in Mauritian lagoons remain largely untapped. In the Western Indian Ocean (WIO) region itself, studies on the antimicrobial potential of seagrasses are quite limited. Hence, comprehensive studies are necessary to firmly establish seagrasses' antimicrobial potential and secure their recognition in the realm of antimicrobial drugs.

The evaluation of seagrass bioactivity requires phytochemical extraction, influenced by factors like drying methods, extraction techniques, and maceration solvent (Astudillo-Pascual *et al.*, 2021; Benjamin *et al.*, 2022). Although freeze-drying and oven-drying of seagrasses are commonly reported separately, limited research has investigated the combined effects of different drying methods and solvents, potentially leaving room for inaccuracies in the findings (Bharathi *et al.*, 2019; Susilo *et al.*, 2023).

Therefore, the primary aim of this paper is to highlight the pharmacological significance of seagrasses in Mauritius. This study delves into assessing the phenolic content, antioxidant and antimicrobial potential of three distinct seagrasses species found in the lagoons of Mauritius, namely, *Halophila stipulacea, Halodule uninervis*, and *Thalassodendron ciliatum*. Additionally, this study seeks to elucidate the influence of different drying methods (freeze-drying and oven drying) and various maceration solvents (acetone and methanol) on these identified properties.

Materials and methods

Study area and material collection and preservation

Pointe-aux-Feuilles, situated on the east coast of Mauritius at geographical coordinates 20° 18' 21" South, 57° 46' 24" East, was the collection site for fresh samples of *Halophila stipulacea, Halodule uninervis, and Thalassodendron ciliatum*. Snorkeling and diving techniques were employed to collect samples from a depth of 2-3 meters at the same location (Fig. 1). The species were identified based on their morphological characteristics, following the descriptions provided in the available literature for the WIO (Richmond, 2011). The hand-pulling technique with the aid of a small shovel was used, involving the gathering of leaves, shoots, and roots with minimum damage to the surrounding seagrass root systems. The samples were washed in seawater to remove sand particles, then placed in ice-cold conditions for transportation. Upon arrival at the University of Mauritius Zoology lab, samples underwent additional washing with tap water, then distilled water (Yuvaraj *et al.*, 2012).

Phytochemicals extraction

Fresh seagrass (70 g) was oven-dried and freeze-dried separately and ground in a grinder (PACIFIC PM600) to a fine powder. Five grams of the dried powder was soaked in 50 ml of 70 % acetone and 70 % methanol, separately, macerated for 48 hours in a shaker, followed by pump filtration and rotary evaporation. Both the dried extracts, obtained from acetone and methanol maceration, were resuspended in methanol. The percentage extract yield was then obtained using the following formula:

Percentage Yield= (W1×100)/W2
W1: Weight of extract after removing the solvent; W2-Dry weight of the sample

Quantitative estimation of Total Phenolic Content (TPC)

The procedure outlined by Nopi *et al.* (2018) was employed, using gallic acid as a standard. A mixture of

1 ml 20 % Folin Ciocalteau reagent and 200 µl seagrass extracts or gallic acid was vortexed and rested for 4 minutes. Then, 750 µl of 7 % Sodium Carbonate solution was added, vortexed, and kept in the dark for two hours. Absorbance was measured at 750 nm.

Antioxidant activity

DPPH Radical-Scavenging Assay

The procedure based on Kavitha *et al.*(2022) was followed. A 2 ml solution of methyl alcohol with DPPH (25ug/ml) was prepared. This mixture was combined with 0.5 ml of extracts or ascorbic acid, stirred, and incubated at 30 °-35 °C for 30 minutes in darkness. Absorbance was measured at 517 nm, using ascorbic acid as a standard. The percentage radical scavenging activities were then determined:

Percentage radical scavenging activity = 100 - ((Ac - As) / Ac) × 100
AC: absorbance of the control solution
AS: absorbance of the seagrass extracts.

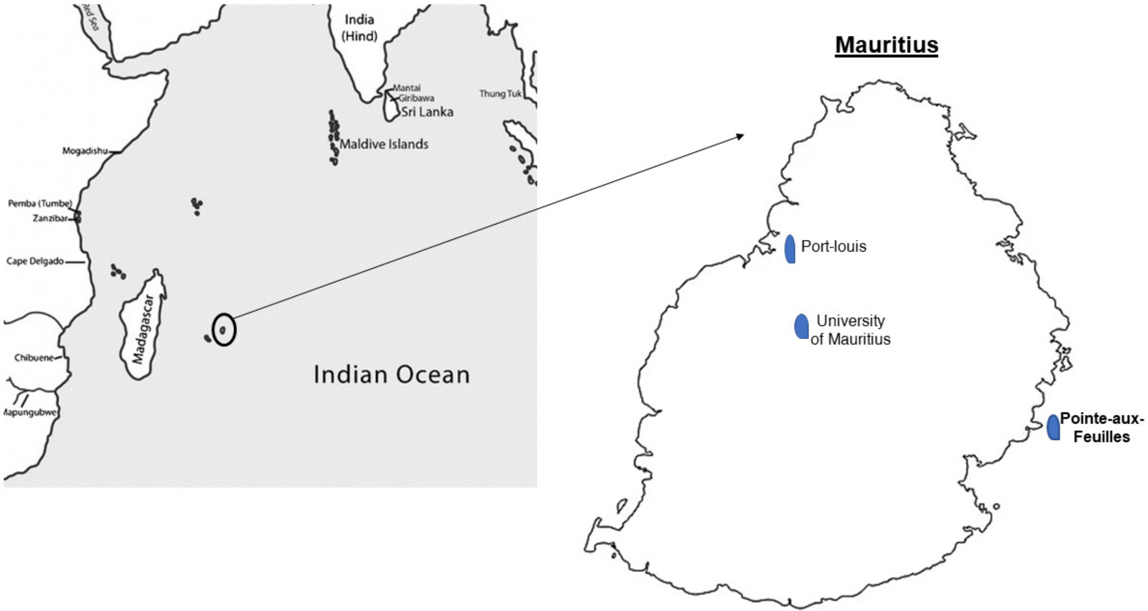

Figure 1. The location of Mauritius and sample collection site.

ABTS Assay

The ABTS assay, based on Re *et al.* (1999) with modifications, was used, with ascorbic acid as a standard. A 7 mM ABTS stock solution was prepared by mixing ABTS with potassium persulfate, and stored in the dark for 12-16 hours. The stock solution was standardized to an absorbance of 0.700 at 734 nm. For the assay, 20 µl of crude extract or ascorbic acid was mixed with 2 ml of diluted ABTS solution, incubated for 7 minutes in the dark, and absorbance was measured at 734 nm. The absorbance values obtained were used to compute the percentage radical scavenging activities.

Antimicrobial susceptibility testing (ast)

The antimicrobial assay followed the standard Clinical and Laboratory Standards Institute (CLSI) protocol (CLSI, 2012). Pure cultures of *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 were used.

Disk diffusion assay

The Mueller-Hinton agar (MHA) disk diffusion technique was employed. Sterile 6 mm filter paper discs (Whatman #1) impregnated with 10µL of 10 mg/ml extracts were air-dried and placed on the microbial-inoculated MHA. Methanol was included as the negative control as it was previously used as the solvent for resuspending the dried extracts, while ampicillin was included as the positive control. The plates were incubated at 37 °C overnight, and the inhibition zones around the discs were measured using a vernier caliper.

Broth microdilution assay

The Broth microdilution was performed in a 96-well plate, utilizing a two-fold dilution technique, according to the method described by Eloff (1998). Methanol and nutrient broth were included as negative control, and Chloramphenicol as positive control.

Statistical design and analysis

All the tests were carried out in three replicates and the results displayed as means for the antioxidant, antimicrobial and Total Phenolic Content tests. The disk diffusion assay was displayed as mean and standard deviation. A completely randomized design with a 3x2x2 factorial treatment structure was used for the antioxidant and TPC tests. The 12 treatments were a combination of the three seagrasses, two drying methods and two maceration solvents and each were replicated thrice. The resulting data were subjected to analyses of variance (ANOVA) and the treatment sums of squares were split up into the main and interaction effects. All statistical analyses were carried out using the software package JAMOVI 2.5 (Jamovi, 2024). A significance level of 5 % was used for all the statistical tests.

Results

Percentage yield

The mass of the dry extracts was measured, and yield percentages were calculated (Fig. 2). Higher yields were obtained when the samples were oven dried and methanol-macerated (highest for *Halophila stipulacea* at 24.7 %). The lowest yield (0.610 %) was obtained with freeze-dried acetonic extract of *Halodule uninervis.*

Total phenolic content (tpc)

Total phenolic content (TPC) was measured in milligrams of gallic acid equivalent (GAE) per gram of extract, ranging from 0.447 to 60.1 mg GAE/g (*Thalassodendron ciliatum* highest). Overall, oven drying resulted in higher TPC than freeze drying for all species. Acetonic extracts consistently showed over 30 % higher TPC compared to methanolic extracts (Fig. 3).

Antioxidant activity

DPPH radical scavenging activity

Percentage inhibition of DPPH was analyzed through ANOVA. The percentage inhibition ranged from 18.0 % to 69.3 % (freeze dried *Thalassodendron ciliatum* methanolic extract and oven dried *T.ciliatum* acetonic extract, respectively). All extracts demonstrated significant

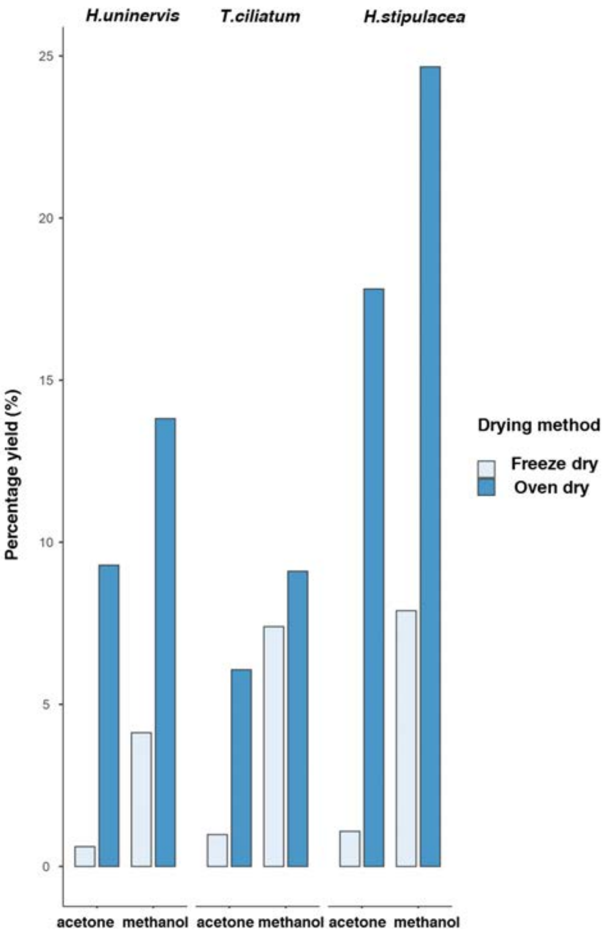

Figure 2. Dry extract percentage yield.

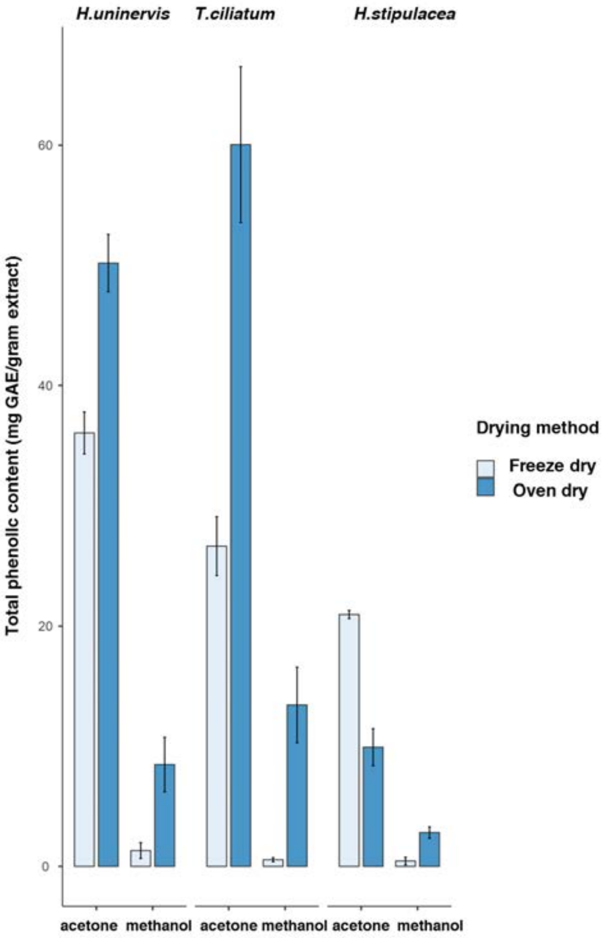

Figure 3. Total Phenolic Content of seagrass extract.

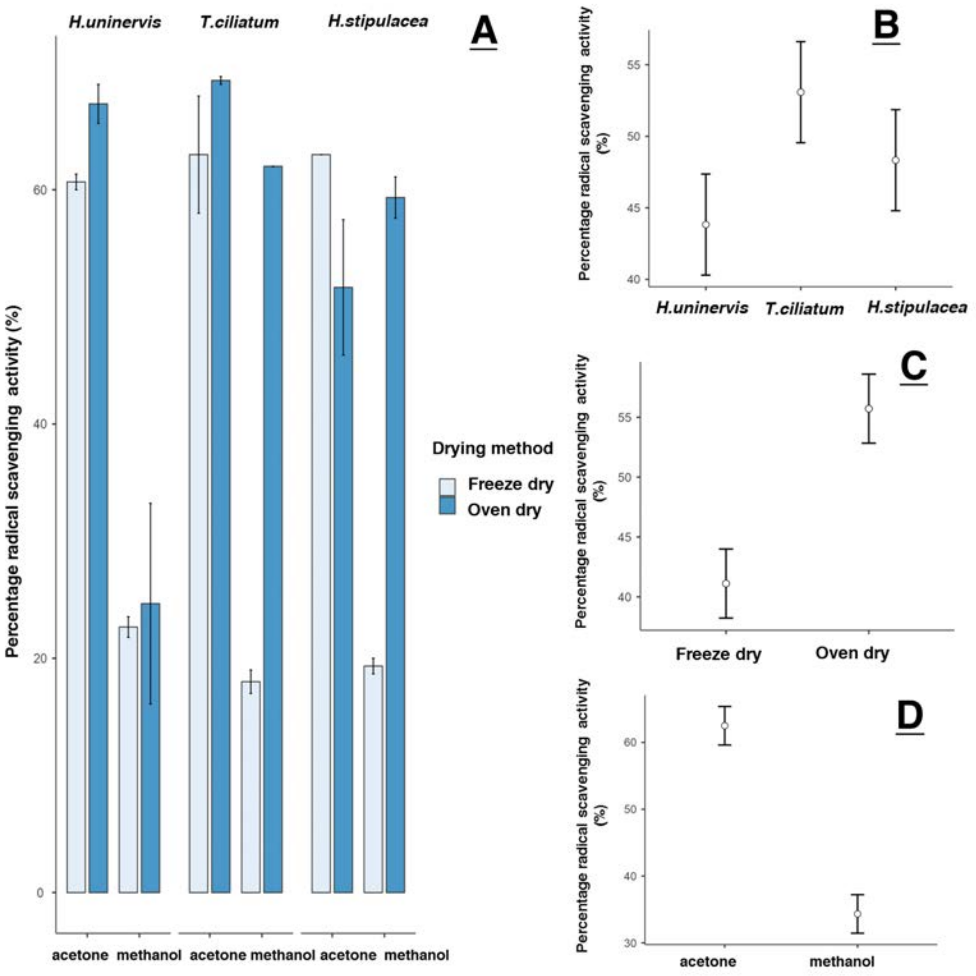

Figure 4. DPPH assay result (i). A: DPPH radical scavenging activity; B: Estimated marginal means of species; C: Estimated marginal means of drying method; D: Estimated marginal means for extraction solvent.

DPPH radical scavenging ($p < 0.05$). Main effects of the factors revealed significant distinct trends: *T.ciliatum* > *H.stipulacea* > *H.uninervis* for species, oven-drying > freeze-drying for drying method, and acetonic extracts > methanolic extracts for solvents.

The results showed clear evidence of significant interactions between the main factors ($p < 0.05$) (Fig. 4 and 5):

- **Species and drying method:** *T.ciliatum* had the highest marginal mean when oven-dried (around 65 %) and the lowest when freeze-dried (approx. 40 %). Notably, a reverse pattern emerged with freeze-drying.
- **Species and solvent:** *H.stipulacea* in methanol showed a 40 % significantly higher marginal mean inhibition than *H.uninervis*. Conversely, with acetone, *H.uninervis* exhibited higher inhibition (approx. 63 %).
- **Solvent and drying method:** In methanol, significantly higher marginal mean inhibition was seen in both freeze-drying (40 %) and oven-drying (30 %).
- **Solvent, drying method, and species:** Percentage marginal mean inhibition was relatively similar between drying methods when macerated in acetone, varying by approx. 2 %. But were significantly different when macerated in methanol.

ABTS radical scavenging activity

In the ABTS assay, *T.ciliatum* (acetone, oven-dried) exhibited the highest inhibitory activity at 29.33 %. All extracts displayed statistically significant ABTS radical scavenging activity ($p < 0.05$). Marginal plots revealed the main effects of the factors resulting in the following trends in percentage inhibition:

- Species: *T.ciliatum* > *H.stipulacea* > *H.uninervis*

- Drying Method: Freeze-drying > oven-drying

- Solvent: Acetonic extracts > methanolic extracts.

The ANOVA revealed significant interactions between the various main factors ($p < 0.05$) (Fig. 6 and 7):

- **Solvent and species**: The highest mean % inhibition was observed with *T.ciliatum* in both solvents, with a lower value in methanol (approx. 10 %) compared to acetone (approx. 25 %).

- **Drying method and species**: All species exhibited a higher mean % inhibition (approx. 2 %) when freeze-dried compared to oven-dried, except for *T.ciliatum*.

- **Solvent, drying method, and species**: When freeze-dried, *T.ciliatum* showed the highest mean activity (approx. 12 %) when macerated in methanol. Conversely, when oven-dried, its acetonic extract exhibited the highest activity (29 %).

Antimicrobial assay

The antimicrobial activity of the seagrass extracts was assessed in terms of zones of inhibition, as outlined in Table I. Notably, the extracts displayed pronounced effectiveness against *Pseudomonas aeruginosa*, particularly the freeze-dried samples, with larger inhibition zones (21.27 ± 3.12 mm and 27.3 ± 11mm for Acetonic Freeze-dried *T.ciliatum* and *H.stipulacea* respectively). In the case of *Staphylococcus aureus*, the highest zone of inhibition observed was 8.33 ± 7.89 mm. However, no activity was detected against *Escherichia coli* and *Bacillus cereus*. Concerning fungal strains, *T.ciliatum* extracts exhibited notable inhibition zones against *Candida albicans* (24.7 ± 4.06 mm).

A 2-fold broth microdilution assay was carried out for the freeze dried extracts to determine their MIC against *P.aeruginosa*. A minimum concentration of 1.25 mg/ml acetonic *T.ciliatum* extracts was needed for inhibition, while for acetonic *H.stipulacea*, a 5 mg/ml extract was required.(Table II)

The zones of inhibition produced by some of the seagrass extracts were subsequently compared with those of other plants, including terrestrial plants, as depicted in Table III.

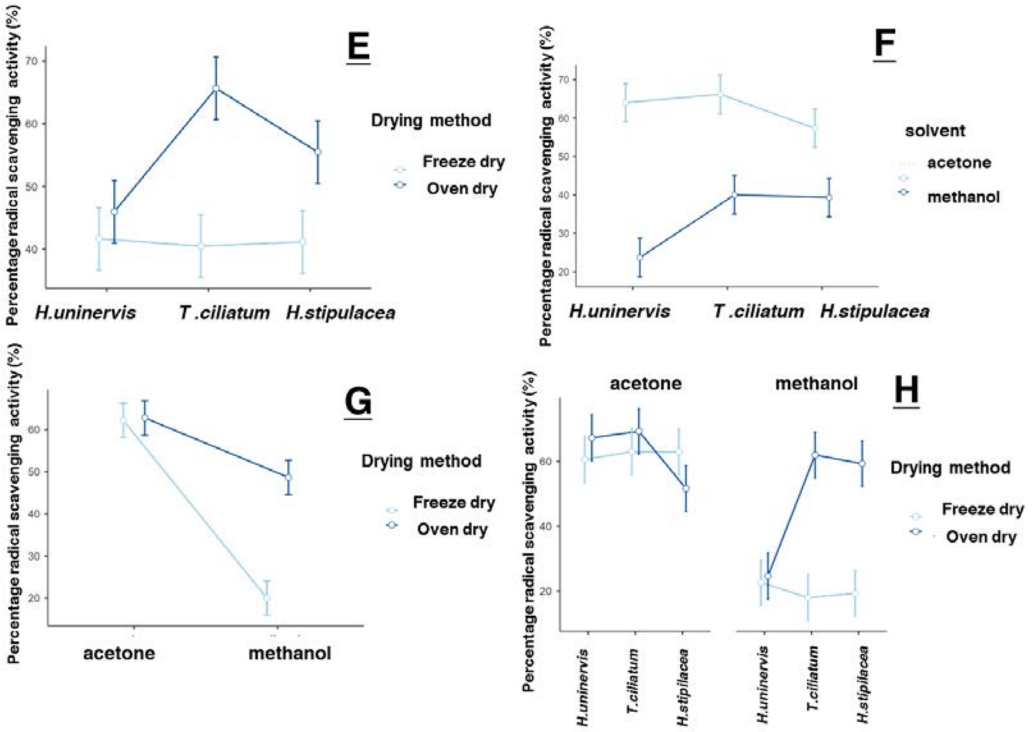

Figure 5. DPPH assay result (ii). E: Estimated marginal means Species ∗ drying method; F: Estimated Marginal Means species ∗ extraction solvent; G: Estimated Marginal Means extraction solvent ∗ Drying Method; H: Estimated Marginal Means species ∗ drying method.

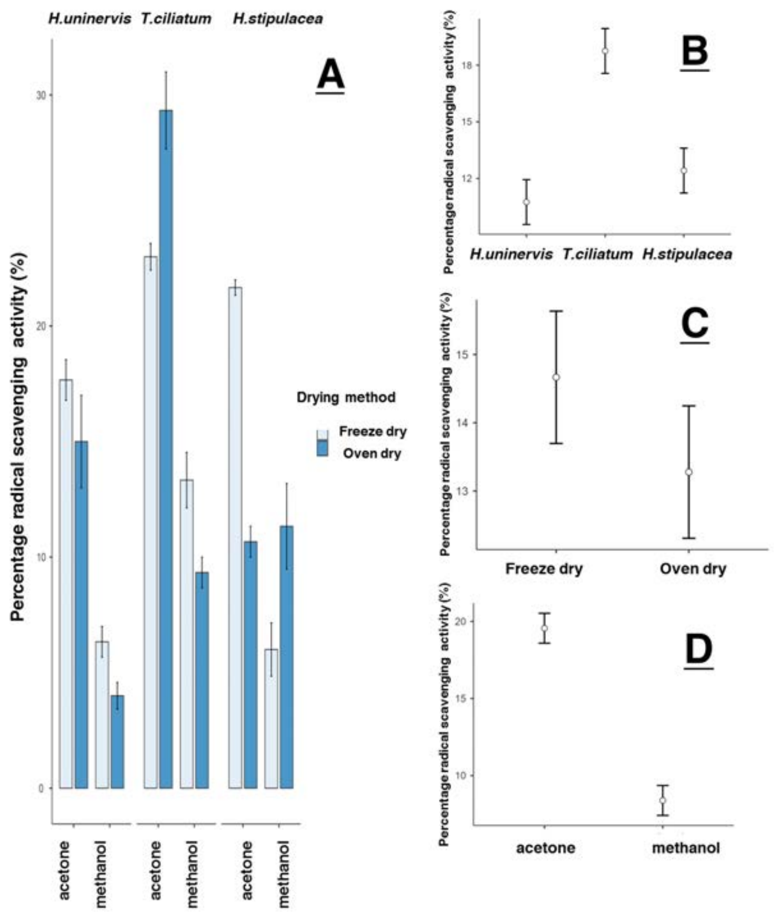

Figure 6. ABTS ASSAY RESULT (i). A: ABTS radical scavenging activity recorded(i). B: Estimated marginal means of species; C: Estimated marginal means of Drying method; D: Estimated marginal means of Extraction solvent.

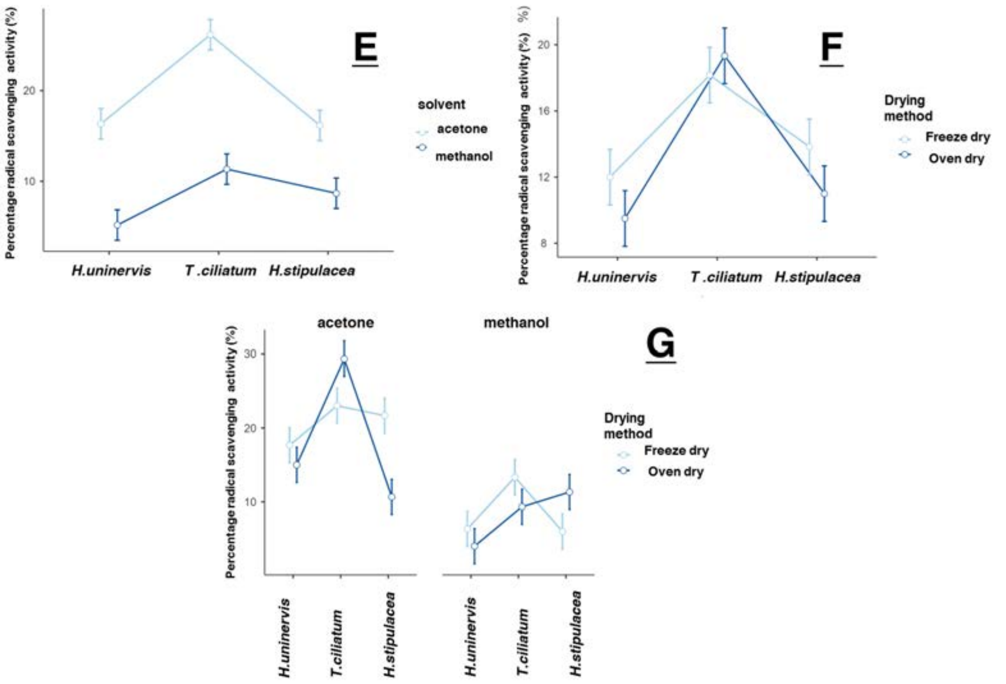

Figure 7. ABTS ASSAY RESULT (ii). E: Estimated Marginal Means species ∗ extraction solvent; F: Estimated Marginal Means species ∗ drying method; G: Estimated Marginal Means species ∗ extraction solvent ∗ drying method.

Table 1. Inhibition zones for disk diffusion assay.

Extracts			**Zone of inhibition(mm)**				
			Bacteria				**Fungus**
Species	**Drying method**	**extraction solvent**	***P. aeruginosa***	***E.coli***	***S. aureus***	***B. cereus***	***C. albicans***
H. stipulacea	**Oven dry**	**methanol**	-	-	5.17 ± 8.95	-	-
H. stipulacea	**Oven dry**	**acetone**	-	-	-	-	-
H. stipulacea	**Freeze dry**	**methanol**	10.77 ± 0.85	-	-	-	-
H. stipulacea	**Freeze dry**	**acetone**	27.3 ± 11.0	-	-	-	-
H. uninervis	**Oven dry**	**methanol**	11.63 ± 0.40	-	-	-	6.67
H. uninervis	**Oven dry**	**acetone**	8.03 ± 2.10	-	2.4 ± 4.16	-	7 ± 5.86
H. uninervis	**Freeze dry**	**methanol**	13.63 ± 2.18	-	4.37 ± 7.56	-	3.57± 6.18
H. uninervis	**Freeze dry**	**acetone**	16.6 ± 0.6	-	-	-	-
T. ciliatum	**Oven dry**	**methanol**	-	-	-	-	-
T. ciliatum	**Oven dry**	**acetone**	11.87 ± 3.88	-	8.33 ± 7.89	-	-
T. ciliatum	**Freeze dry**	**methanol**	12.13 ± 2.00	-	-	-	-
T. ciliatum	**Freeze dry**	**acetone**	21.27 ± 3.12	-	4.6 ± 7.97	-	24.7 ± 4.06
Positive control (Ampicillin for bacteria and Nystatin for fungus)			-	29.03 ± 1.33	18.1 ± 2.25	31.17 ± 9.76	-

Table 2. Minimum Inhibitory Concentration of freeze dried seagrass samples against *P. aeruginosa*.

species	**extraction solvent**	**MIC(mg/ml)**
H. stipulacea	methanol	>2.5
H. stipulacea	acetone	>5
H. uninervis	methanol	>2.5
H. uninervis	acetone	>2.5
T ciliatum	methanol	>2.5
T ciliatum	acetone	> 1.25
Chloramphenicol (positive control)	None	>0.938
Methanol		-
Nutrient broth		-

Table 3. Comparison of the antimicrobial properties of seagrass extracts with some terrestrial plants (ND: No data).

Plant		**Extract**	**Microbe (Zone of inhibition in mm)**				**References**
			S. aureus	**P. aeruginosa**	**E. coli**	**B. cereus**	
Seagrass	***T. ciliatum***	Acetonic (freeze-dried)	4.6 ± 7.97	21.27 ± 3.12	-	-	
	H. stipulacea	Acetonic (freeze-dried)	-	27.3 ± 11.0	-	-	
	H. uninervis	Acetonic (freeze-dried)	-	16.6 ± 0.6	-	-	
Mangrove	***Suaeda nudiflora***	Acetonic	5.76 ± 0.25	3 ± 0.2	ND	ND	(Eswaraiah *et al.*, 2020)
	Lumnitzera racemosa	Acetonic	7.3 ± 0.2	5.33 ± 0.15	ND	ND	
	Ipomoea tuba	Acetonic	5.83 ± 0.15	4.8 ± 0.2	ND	ND	
	Avicennia alba	Acetonic	6.83 ± 0.15	5.83 ± 0.15	ND	ND	
Aloe vera		Acetonic	12 ± 0.45	19 ± 0.57	14 ± 0.38	ND	(Nejatzadeh-Barandozi, 2013)
Licorice	***Glycyrrhiza glabra***	Methanolic	10.04 ±1.34	-	6 ±1.22	7 ±1	(Jafari-Sales and Bolouri, 2018)

Discussion

The ability of seagrass to produce secondary metabolites as a defense mechanism has drawn attention to their phytochemical properties and pharmacological potential (Kalaivani *et al.*, 2019). While numerous studies (Wisespongpand *et al.,* 2022; Lahay and Amiin, 2023; Punginelli *et al.*, 2023) on seagrass bioactivity have been conducted globally, research on Mauritian seagrasses remains limited. Only one study (Ramah *et al.*, 2014) has investigated their antioxidant potential, and none has explored their antimicrobial properties. This study, therefore, examines the phytochemical characteristics of three Mauritian seagrass species: *T. ciliatum*, *H. stipulacea*, and *H. uninervis*.

An essential aspect of this study involved optimizing the extraction process to obtain maximum phytochemical yield and bioactivity. In line with this, two drying methods (freeze-drying and oven- drying) and two solvents (Acetone and Methanol) were tested for phytochemical extraction. Oven drying yielded higher extract yield compared to freeze drying which was consistent with the results obtained by Lee *et al.*(2022) when they compared several drying methods. This difference may be ascribed to the greater porosity of the freeze-dried samples, potentially causing rapid moisture reabsorption (Benjamin *et al.*, 2022). Methanol extraction yielded higher phytochemical levels compared to acetone. The polarity of the solvent plays a crucial role in this difference, whereby methanol is more polar than acetone. In this case, it is suggested that the seagrasses possessed more polar compounds than non-polar. Ozbil *et al.* (2024), in a similar context, compared the effect of different polar and non-polar solvents including methanol and acetone, on seagrass *Posidonia oceanica* and methanol resulted in greater yield as compared to acetone in both the leaves and roots.

Phenolic compounds are renowned for their capacity to scavenge free radicals and reactive species that pose potential harm to cellular structures (Pratyusha, 2022). Throughout this study, the three seagrass species possessed different levels of phenolic compounds within their respective samples. Notably, *T. ciliatum* demonstrated the highest total phenolic content (TPC) among the three seagrasses. This discovery aligns with the study of Ramah *et al.* (2014), who also identified *T. ciliatum* as having the highest TPC among five seagrass species in Mauritius. Nevertheless, the TPC values obtained in this study were lower than those reported by Ramah *et al.*, and this difference could stem from their direct use of fresh seagrass samples, which contrasts with the dried seagrass utilized in this study. Remarkably, higher phenolic content resulted from oven drying, rather than freeze drying, deviating from some studies (Ningsih *et al.*, 2022; Wan *et al.*, 2021). Although, methanol is a commonly utilized solvent for phenolic extraction as phenolic compounds are more soluble in more polar solvents (Bharathi *et al.*, 2019), the current study revealed that acetonic extracts displayed a higher TPC than methanolic extracts..

DPPH and ABTS assays are two commonly used antioxidant assays. All the seagrass extracts in this study demonstrated significant DPPH and ABTS radical scavenging activities. Species-specific variations ($p=0.003$ for DPPH and $p<0.001$ for ABTS) were observed, with *T. ciliatum* showing the highest activity, agreeing again with the DPPH results of Ramah *et al.* (2014) in Mauritius. The obtained DPPH value for *T. ciliatum* was approximately similar with the results obtained by Hamdy *et al.* (2012) in Egypt. Hamdy *et al.* attributed this efficacy of *T. ciliatum* to the presence of flavonoids such as quercetin 3-O-β-d-xylopyranoside, asebotin, 3-hydroxyasebotin, rutin, and racemic catechin. Additionally, marked dissimilarity was observed between the ABTS and DPPH assay results, with oven drying yielding better results with the DPPH assay and freeze-drying yielding better results with the ABTS assay. The heightened antioxidant capacity observed with the DPPH assay may stem from its capacity to react with weaker antioxidants, as noted by Christodoulou *et al.* (2022). Moreover, as mentioned above, antioxidant activity arises due to the phytochemical components present. Therefore, the impact of the drying method on the targeted phytochemical compounds and their solubility is another aspect to consider (Sun *et al.*, 2015). The highest total phenolic content was observed in the oven-dried, acetone-macerated extracts, which also exhibited the strongest DPPH activity. This demonstrates that the phenolic compounds contribute significantly to antioxidant activity. This relationship of the antioxidant capacity with the TPC clarifies the significantly varying marginal means among species, drying methods, and solvents ($p<0.05$).

Heightened consumer concern about synthetic compounds has spurred research on natural antimicrobial compounds. This study explored the antimicrobial potential of three Mauritian seagrasses. Many studies (Bharathi *et al.*, 2019; Kavitha *et al.*, 2022; Amiin and Lahay, 2023) on seagrasses have shown that they possess this ability due to their phytochemical components, including phenols, flavonoids, and alkaloids (Amiin and Lahay, 2023). However, in this study, none of the seagrass extracts showed antimicrobial activity against the gram-negative *Escherichia coli*. *H. stipulacea* and other *Halophila* spp. showed effective antimicrobial activity against *E. coli* in other studies (Gumgumjee *et al.*, 2018; Yuvaraj *et al.*, 2012). Discrepancies in geography could account for this observation. Terrestrial plants like *Aloe vera* and *Glycyrrhiza glabra* could exhibit potential activity against *E. coli*, highlighting a distinction in bioactive components between marine and terrestrial plant species (Jafari-Sales and Bolouri, 2018; Nejatzadeh-Barandozi, 2013; Table III).

As for the other gram negative bacteria used in this study, varying positive results were observed against *Pseudomonas aeruginosa.* The freeze-dried acetonic extracts of *T. ciliatum* and *H. stipulacea* displayed large inhibition zones. Yet, in another study in Egypt, the same seagrass species were unable to inhibit *P. aeruginosa* (Ahmed *et al.*, 2023*)*. Conversely, oven-dried and methanolic extracts exhibited little to no activity against the bacteria, indicating a potential loss of antimicrobial components attributed to high-temperature drying and solubility of phytochemicals on maceration solvent. The MIC determination of the extracts against *P. aeruginosa* provided a more detailed insight. Species-wise, *T. ciliatum* exhibits the highest antimicrobial potential, which could be linked to *T. ciliatum*'s elevated phenolic content observed in this study. Interestingly, *H. uninervis* demonstrated a higher activity than *T. ciliatum* against the gram-negative *Salmonella typhi* in Tanzania (Hamisi *et al*., 2023). Significantly, the seagrass extracts were more effective against *P. aeruginosa* than several terrestrial plants, including the medicinal plant *Aloe vera.* (Table III).

Regarding gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus* were non-susceptible to the extracts. In contrary, in the WIO region, Mabrouk *et al.*, (2024) reported *H. stipulacea* as more effective against gram-positive bacteria (including *S. aureus*) than against gram-negative ones (including *P. aeruginosa*). On a similar note, *S. aureus* and *B. cereus* could also resist several seagrass leaf extracts of *Cymodocea rotundata* and *Cymodocea serrulata* (Wisespongpand *et al.,* 2022). Gram-positive bacteria have thicker cell walls containing teichoic acids, which are absent in gram-negative bacteria, which may explain this non-susceptibility (Jubeh *et al.,* 2020). Conversely, studies on mangroves and other terrestrial plants demonstrated significant inhibition of *S. aureus* growth, and *Glycyrrhiza glabra* extract effectively inhibited *B. cereus* (Table III). As for fungus, *Candida albicans* was most susceptible to the freeze-dried acetonic extract of *T. ciliatum,* while the other species exhibited minimal to no activity. Although anti-candidal studies of these species is absent in the WIO region, outside the WIO, *H. stipulacea* and other seagrasses were found to act against *C. albicans* (Mabrouk *et al.*, 2024; Punginelli *et al.*, 2023) .

Conclusions

In conclusion, to the knowledge of the authors, this is the first study in the WIO region assessing the combined effect of drying method and maceration solvent on the bioactivity of the three seagrasses, *H. stipulacea, H. uninervis, and T. ciliatum.* This study allowed identification of the optimum maceration solvent, drying method and species to use to obtain a high antimicrobial and antioxidant activity. Specifically, oven-drying and acetone proved more effective in extracting phenolic compounds. Notably, among the examined species, *T. ciliatum* demonstrated the highest antioxidant potential when subjected to oven drying and macerated in acetone, while also possessing the highest phenolic compounds. This study has also, for the first time, determined the antimicrobial activity of seagrasses in Mauritius. *T. ciliatum* displayed remarkable antimicrobial efficacy against *P. aeruginosa* and *Candida albicans*, particularly when freeze-dried and macerated in acetone. It is therefore recommended that more studies are carried out on the use of seagrass extracts, especially, *T. ciliatum*, in the pharmaceutical industry.

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References

Ahmed F, Mahmoud A-B, EL-Swaify Z, Salah El-Din R (2023) A comparative evaluation of phytochemical and antimicrobial properties of selected aquatic and terrestrial halophyte plants growing in Egypt. International Journal of Theoretical and Applied Research 2: 169-182 [doi:10.21608/ijtar.2023.220991.1070]

Amiin MK, Lahay AF (2023) Anti-bacterial effectiveness of *Cymodocea rotundata* extract and assay for primary bioactive composition. Journal of Aquatropica Asia 8: 6-12 [doi: 10.33019/joaa.v8i1.3920]

Amone-Mabuto M, Mubai M, Bandeira S, Shalli MS, Adams JB, Lugendo BR, Hollander J (2023) Coastal community's perceptions on the role of seagrass ecosystems for coastal protection and implications for management. Ocean & Coastal Management 244: 106811 [doi: 10.1016/j.ocecoaman.2023.106811]

Astudillo-Pascual M, Domínguez I, Aguilera PA, Garrido Frenich A (2021) New phenolic compounds in *Posidonia oceanica* seagrass: a comprehensive array using high resolution mass spectrometry. Plants 10: 864 [doi: 10.3390/plants10050864]

Bedulli C, Lavery PS, Harvey M, Duarte CM, Serrano O (2020) Contribution of seagrass blue carbon toward carbon neutral policies in a touristic and environmentally-friendly island. Frontiers in Marine Science 7: 1 [doi: 10.3389/fmars.2020.00001]

Benjamin MAZ, Ng SY, Saikim FH, Rusdi NA (2022) The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules 27 (19): 6458 [doi: 10.3390/molecules27196458]

Bharathi NP, Jayalakshmi M, Amudha P, Vanitha V (2019) Phytochemical screening and in vitro antioxidant activity of the seagrass *Cymodocea serrulata*. Indian Journal of Geo Marine Sciences 48 (08): 1216-1221

Bijak AL, Reynolds LK, Smyth AR (2023) Seagrass meadow stability and composition influence carbon storage. Landscape Ecology: 4419-4437 [doi: 10.1007/s10980-023-01700-3]

Christodoulou MC, Orellana Palacios JC, Hesami G, Jafarzadeh S, Lorenzo JM, Domínguez R, Moreno A, Hadidi M (2022) Spectrophotometric methods for measurement of antioxidant Activity in food and pharmaceuticals. Antioxidants 11: 2213 [doi:10.3390/antiox11112213]

CLSI (2012) Performance standards for antimicrobial disk susceptibility tests; Approved s tandard—Eleventh Edition. CLSI document M02-A11. Wayne, PA, Clinical and Laboratory Standards Institute, Vol. 32. 76 pp

Divyashri S, Arivarasu L, Sivaperumal P, Thangavelu L (2021) Antioxidant activity from *Cymodocea serrulata* seagrass crude extract. Journal of Pharmaceutical Research International 33 (61B): 309-317 [doi:10.9734/jpri/2021/v33i61B35603]

Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64 (8): 711-713 [doi:10.1055/s-2006-957563]

Eswaraiah G, Peele KA, Krupanidhi S, Kumar RB, Venkateswarulu TC (2020) Studies on phytochemical, antioxidant, antimicrobial analysis and separation of bioactive leads of leaf extract from the selected mangroves. Journal of King Saud University - Science 32 (1): 842-847 [doi:10.1016/j.jksus.2019.03.002]

Gono CMP, Ahmadi P, Hertiani T, Septiana E, Putra MY, Chianese G (2022) A comprehensive update on the bioactive compounds from seagrasses. Marine Drugs 20 (7): 406 [doi:10.3390/md20070406]

Gumgumjee NM, Bukhari DA, Alshehri WA, Hajar AS (2018) Antibacterial activity of *Halodule uninervis* leaves extracts against some bacterial pathogens strains. Pharmacophore 9 (2): 52-59

Halliwell B (2024) Understanding mechanisms of antioxidant action in health and disease. Nature Reviews Molecular Cell Biology 25: 13-33 [doi: 10.1038/s41580-023-00645-4]

Hamdy AH, Mettwally WSA, Fotouh MAE, Rodriguez B, El-Dewany AI, El-Toumy SAA, Hussein AA (2012) Bioactive phenolic compounds from the Egyptian Red Sea seagrass *Thalassodendron ciliatum*. Zeitschrift für Naturforschung C 67 (5-6): 291-296 [doi:10.1515/znc-2012-5-608]

Hamisi MI, Mbusi LD, Lyimo TJ (2023) Antibacterial activity against *Salmonella typhi* and phytochemical screening of seven seagrass species from the coast of Tanzania. Western Indian Ocean Journal of Marine Science 22: 83-93

Jafari-Sales A, Bolouri P (2018) Evaluation of the antimicrobial effects of *Glycyrrhiza glabra* on some gram positive and gram negative pathogenic bacteria in laboratory conditions. Jorjani Biomedicine Journal 6: 78-84 [doi:10.29252/jorjanibiomedj.6.4.78]

Jubeh B, Breijyeh Z, Karaman R (2020) Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. Molecules 25: 2888 [doi:10.3390/molecules25122888]

Kalaivani P, Kavitha D, Vanitha V (2019) A review on phytochemical and pharmacological activities of *Syringodium isoetifolium*. International Journal of Research in Pharmaceutical Sciences 11: 207-214 [doi: 10.26452/ijrps.v11i1.1808]

Kavitha D, Padmini R, Chandravadivelu G, Magharla DD (2022) Phytoconstituents screening and antioxidant activity of *Syringodium isoetifolium* leaf extracts. Indian Journal of Pharmaceutical Sciences 84(5): 1309-1322 [doi:10.36468/pharmaceutical-sciences.1028]

Lahay AF, Amiin MK (2023) Antibacterial potential of seagrass *Cymodocea rotundata* (Alismatales: *Cymodoceaceae*) extract on the pathogenic bacteria *Staphylococcus aureus*. Jurnal Biologi Tropis 23: 355-360 [doi:10.29303/jbt.v23i2.4884]

Lee SY, Ferdinand V, Siow LF (2022) Effect of drying methods on yield, physicochemical properties, and total polyphenol content of chamomile extract powder. Frontiers in Pharmacology. 13: 1003209 [doi: 10.3389/fphar.2022.1003209]

Lin J, Liu X, Lai T, He B, Du J, Zheng X (2021) Trophic importance of the seagrass *Halophila ovalis* in the food web of a Hepu seagrass bed and adjacent waters, Beihai, China. Ecological Indicators 125: 107607 [doi:10.1016/j.ecolind.2021.107607]

Mabrouk SB, Grami B, Ayache SB, Kacem A (2024) Antioxidant, antimicrobial and analgesic activities of the invasive seagrass *Halophila stipulacea* leaf and stem extracts. Authorea Preprints [doi:10.22541/au.171231882.23748315/v1]

Madi Moussa R, Bertucci F, Jorissen H, Gache C, Waqalevu VP, Parravicini V, Lecchini D, Galzin R (2020) Importance of intertidal seagrass beds as nursery area for coral reef fish juveniles (Mayotte, Indian Ocean). Regional Studies in Marine Science 33: 100965 [doi:10.1016/j.rsma.2019.100965]

Manso T, Lores M, de Miguel T (2021) Antimicrobial activity of polyphenols and natural polyphenolic extracts on clinical isolates. Antibiotics 11: 46 [doi:10.3390/antibiotics11010046]

Marine Pharmacology (2023) Marine Pharmacology. Mysite. [https://www.marinepharmacology.org]

Montaggioni L, Faure G (1980) Les Récifs coralliens des Mascareignes (Océan Indien). Université française de l'Océan indien, Centre universitaire de la Réunion. 151 pp

Nejatzadeh-Barandozi F (2013) Antibacterial activities and antioxidant capacity of *Aloe vera*. Organic and Medicinal Chemistry Letters 3 (1): 1-8 [doi:10.1186/2191-2858-3-5]

Ningsih AW, Syahrani A, Wahyuni KI (2022) Study of drying methods and extraction methods on phenolic content. In: International Conference on Green Energy and Materials Technology, Vol.1(1), Bandung City

Nopi NS, Anwar E, Nurhayati T (2018) Optimization of extraction condition to obtain antioxidant activity and total phenolic content of seagrass *Thalassia hemprichii* (Ehrenb.) Asch from Indonesia. Pharmacognosy Journal 10 (5): 958-962 [doi:10.5530/pj.2018.5.162]

Ozbil E, Ilktac M, Ogmen S, Isbilen O, Ramirez JMD, Gomez J, Walker JN, Volkan E (2024) In vitro antibacterial, antibiofilm activities, and phytochemical properties of *Posidonia oceanica* (L.) Delile: An endemic Mediterranean seagrass. Heliyon 10: e35592 [doi: 10.1016/j.heliyon.2024.e35592]

Pratyusha S (2022) Phenolic compounds in the plant development and defense: an overview. In: Plant stress physiology - perspectives in agriculture. IntechOpen 11: 125-140 [doi:10.5772/intechopen.102873]

Punginelli D, Catania V, Abruscato G, Luparello C, Vazzana M, Mauro M, Cunsolo V, Saletti R, Di Francesco A, Arizza V, Schillaci D (2023) New bioactive peptides from the Mediterranean seagrass *Posidonia oceanica* (L.) Delile and their impact on antimicrobial activity and apoptosis of human cancer cells. International Journal of Molecular Sciences 24: 5650 [doi:10.3390/ijms24065650]

Ramah S, Etwarysing L, Auckloo N, Gopeechund A, Bhagooli R, Bahorun T (2014) Prophylactic antioxidants and phenolics of seagrass and seaweed species: a seasonal variation study in a southern Indian Ocean island, Mauritius. Internet Journal of Medical Update-EJOURNAL 9 (1): 27-37

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237 [doi:10.1016/S0891-5849(98)00315-3]

Richmond M (2011) A field guide to the seashores of Eastern Africa and the Western Indian Ocean Islands. 3rd edition, Swedish International Development Agency (SIDA), Stockholm, WIOMSA. 464 pp

Sansone C, Galasso C, Lo Martire M, Fernández TV, Musco L, Dell'Anno A, Bruno A, Noonan DM, Albini A, Brunet C (2021) In vitro evaluation of antioxidant potential of the invasive seagrass *Halophila stipulacea*. Marine Drugs 19 (1): 37 [doi:10.3390/md19010037]

Sun Y, Shen Y, Liu D, Ye X (2015) Effects of drying methods on phytochemical compounds and antioxidant activity of physiologically dropped un-matured citrus fruits. LWT - Food Science and Technology 60: 1269–1275 [doi:10.1016/j.lwt.2014.09.001]

Susilo B, Setyawan HY, Prianti DD, Handayani MLW, Rohim A (2023) Extraction of bioactive components on Indonesian seagrass (*Syringodium isoetifolium*) using green emerging technology. Food Science and Technology 43: e086722 [doi:10.1590/fst.086722]

Tandrayen-Ragoobur V, Tengur ND, Fauzel S (2022) COVID-19 and Mauritius' tourism industry: an island perspective. Journal of Policy Research in Tourism, Leisure and Events: 1-17 [doi:10.1080/19407963.2022.2028159]

Jamovi (2024) The Jamovi Project (Version 2.5) [Computer Software]. [https://www.jamovi.org]

Wan Nasir WNH, Ibrahim NNA, Woon KH, Abu Bakar Sajak A, Sofian-Seng N-S, Wan Mustapha WA, Abdul Rahman H (2021) Effects of different drying methods and solvents on biological activities of *Curcuma aeruginosa* leaves extract. Sains Malaysiana 50 (8): 2207-2218

Wisespongpand P, Khantavong A, Phothong P, Wanghom W (2022) Antimicrobial, antioxidant, and antifouling activity from extracts of aboveground and below-ground parts of seagrasses *Cymodocea rotundata* and *Cymodocea serrulata*. Letters in Drug Design & Discovery 46: 123-129

Yuvaraj N, Kanmani P, Satishkumar R, Paari A, Pattukumar V, Arul V (2012) Seagrass as a potential source of natural antioxidant and anti-inflammatory agents. Pharmaceutical Biology. 50 (4) 458-467 [doi:10.3109/13880209.2011.611948]

Original Article

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Metal pollution in mangrove ecosystems in Dar es Salaam, Tanzania

Prisca Mziray[1]*, Ismael A. Kimirei[1]

[1]Tanzania Fisheries Research Institute (TAFIRI),
PO Box 9750, Dar es Salaam, Tanzania and
TAFIRI - Kigoma, PO Box 90, Kigoma,
Tanzania

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*** Corresponding author:**
priscamziray@gmail.com

Abstract

Environmental pollution through accumulation of metals in estuarine, coastal, and marine waters is a worldwide issue. Most contaminants originate inland and pass through these environments before dispersing into the ocean. The objectives of this study were to determine metal concentration levels of Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn in sediments, water, invertebrates and mangrove leaves collected from mangrove ecosystems along the coast of Dar es Salaam and Mbegani. Evidence of metal bioaccumulation of non-essential metals including Al, Cu, Fe, Mn and Zn was mostly found at sites located close to Dar es Salaam City centre, which decreased with increasing distance from the centre. *Avicennia marina* (mangrove leaves), *Uca spp*. and *Terebralia palustris* (invertebrates) were identified as suitable local species for monitoring metal concentrations in the region.

Keywords: Biomonitoring, invertebrates, mangrove leaves, sediments, water

Introduction

Environmental pollution due to metals in estuarine and coastal environments is a worldwide problem, since most contaminants from urban centres end up in these areas before dispersing into the ocean (Islam and Tanaka, 2004; Pan and Wang, 2012; Tam and Wong, 2000). Once introduced into these environments, the pollutants are distributed into different compartments that include sediment, water, plants, and animals, and eventually pose a risk to the ecosystem and humans. Concentrations of trace metals in water, sediments, and biota are usually the three measures used in marine habitats (Rainbow, 1995). Occurrence of metal pollution induced by anthropogenic inputs on coastal environments and estuaries is not uncommon, even in countries with low levels of industrialization in Africa (Biney *et al*., 1994; Otchere, 2019).

Species with the capacity of accumulating heavy metals in its tissues, which may be analyzed as a measure of metal bioavailability in the ambient habitat can be termed as a metal bio-monitor. Bio-monitors of metal pollution respond differently to different sources of bio-available metals (Rainbow, 1995). The levels of metal accumulation in marine organisms are not only attributed by water quality but also to different factors such as seasonality, temperature, salinity, diet, spawning and individual variation, among others. The levels of metals accumulated in some marine organisms may be higher than background concentrations and thus certain species and/or tissues of certain organisms can be suitable to use as bio-indicators of heavy metal pollution (Chan, 1989).

Mangrove ecosystems are intertidal estuarine wetlands thriving in relatively sheltered locations such as lagoons, bays and estuaries in tropical and subtropical regions and tend to face serious anthropogenic contamination due to their proximity to areas of dense human population, urban and industrial development and concentrated pressures from human activities (MacFarlane and Burchett, 2002; Sharma *et al*.,

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2021; Tam and Wong, 2000). Sediments in mangrove ecosystems are mostly anaerobic, reduced, and rich in sulphide and organic matter; thus have a high metal retaining capacity (Harbison, 1986; Lacerda *et al.*, 1993; Machado *et al.*, 2008; Silva *et al.*, 1990; Tam and Wong, 1996). Mangrove wetlands along the coast of Tanzania are mostly located in sheltered bays, estuaries and river mouths and receive most of their freshwater inflow from rivers, streams and runoffs (Kruitwagen *et al.*, 2008). Based on their location and ability to trap chemicals, the amount of contaminants in mangrove wetlands closely reflects the general level of pollution in coastal areas and the hinterlands (Kruitwagen *et al.*, 2008). The most common pollution sources along the coast include sewage discharge, municipal, industrial and agricultural wastes, transportation activities, coastal area urbanization and erosion.

Tanzania is experiencing increasing pressure from urbanization and industrialization which has resulted in an increase in environmental degradation (De Wolf and Rashid, 2008; Machiwa, 1992; Semesi, 1992; Yhdego, 2021). This has eventually led to a severe decrease of water and sediment quality since most of the industrial, agricultural and residential wastes are disposed-off directly into natural drainage systems that end up in coastal ecosystems (Machiwa, 1992; Mremi and Machiwa, 2003). For example, Msimbazi River, which is located in Dar es Salam city was reported to receive average and peak untreated effluent rates of 256 m^3/h and 606 m^3/h of wastes from industrial and other anthropogenic sources (Ak'habuhaya and Lodenius, 1988; De Wolf *et al.*, 2001).

Despite efforts undertaken to evaluate the potential environmental status of metal pollution along the coast of Dar es Salaam (De Wolf and Rashid, 2008; De Wolf *et al.*, 2001; Kruitwagen *et al.*, 2008; Machiwa, 1992; Mremi and Machiwa, 2003; Mtanga and Machiwa, 2008; Mtanga and Machiwa, 2007; Muzuka, 2008; Rumisha *et al.*, 2012), there is paucity of information regarding contaminant accumulation and their respective effects on local marine flora and fauna in Tanzania. The objectives of the present study were: (i) to evaluate the extent of metal contamination in the local coastal environment (water and sediments) and in the resident organisms (invertebrates and mangroves) in mangrove ecosystems of Dar es Salaam and the Coastal Region of Tanzania; and (ii) to identify the most suitable metal pollution bio-indicators from the selected flora and fauna species (mangrove leaves and invertebrates).

Materials and methods

Study sites

The study was conducted in mangrove ecosystems in Dar es Salaam (Msimbazi, Mtoni Kijichi, Kunduchi, Mbweni and Mbutu) region and in Mbegani (Coastal region) in Tanzania (Fig. 1). Sites were selected based on distance from the Dar es Salaam city centre. Msimbazi mangrove ecosystem (39.273262 longitude and -6.79798 latitude) is in the city centre and receives wastewater discharges from the city. Mtoni Kijichi mangrove ecosystem (39.280047 longitude and -6.878884 latitude) is located along the Dar es Salaam harbour channel, approximately 2.5 km south of the city centre and receives waste from intensive traffic of fishing boats and ferries, municipal outflows, and adjacent residential and agricultural areas. These sites are considered to be highly polluted. Kunduchi mangrove ecosystem (39.211938 longitude and -6.657801 latitude) is located within a tourist hotel area and Kunduchi fishing village, approximately 20 km north of the Dar es Salaam city centre. Mbweni mangrove ecosystem (39.139344 longitude and -6.578716 latitude) is located at the Mpigi River mouth. The last two sites, Mbutu (39.479969 longitude and -6.880632 latitude) and Mbegani (38.87321 longitude and -6.394379 latitude) mangrove ecosystems, are situated south and north of the city centre, respectively, and are considered to be less polluted.

Sampling

Sampling was conducted in the months of August and September 2009, at low tide in the upper part of the intertidal. At each site, three replicate sediment samples (top 10 cm from the surface) and water samples were collected and transferred to clean zip lock polyethylene bags and into clean 50- mL polypropylene tubes, respectively. Leaves of the mangroves *Avicennia marina*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Rhizophora mucronata*, and *Sonneratia alba*, and invertebrates (*Uca spp.*, *Volema pyrum*, *Turbo* spp., *Terebralia palustris*, *Cerithidea decollata*, *Cerithium caeruleum*, *Diala lauta* and *Zeuxius olivaceus*) were also collected, washed with Milli-Q water to remove any adhering sediment particles and stored in clean polyethylene bags per species and site. Some species were not present at all the sites. The samples were transported on ice to the Laboratory of Ecophysiology, Biochemistry, and Toxicology, University of Antwerp, Belgium for analysis.

Laboratory analysis

Grain size distribution in sediments

Sediment samples from each site were mixed, oven dried at 60 °C for 72 hours, and ground to obtain a homogeneous sample. The particle size (0.05 µm to 900 µm diameter) distribution was then analyzed using a laser diffraction particle size analyzer (Master sizer S, Malvern Instrument Ltd, Worcestershire, UK). During analysis, 10 g aliquots of each sample were repeatedly analysed over five minutes to obtain an average size distribution profile.

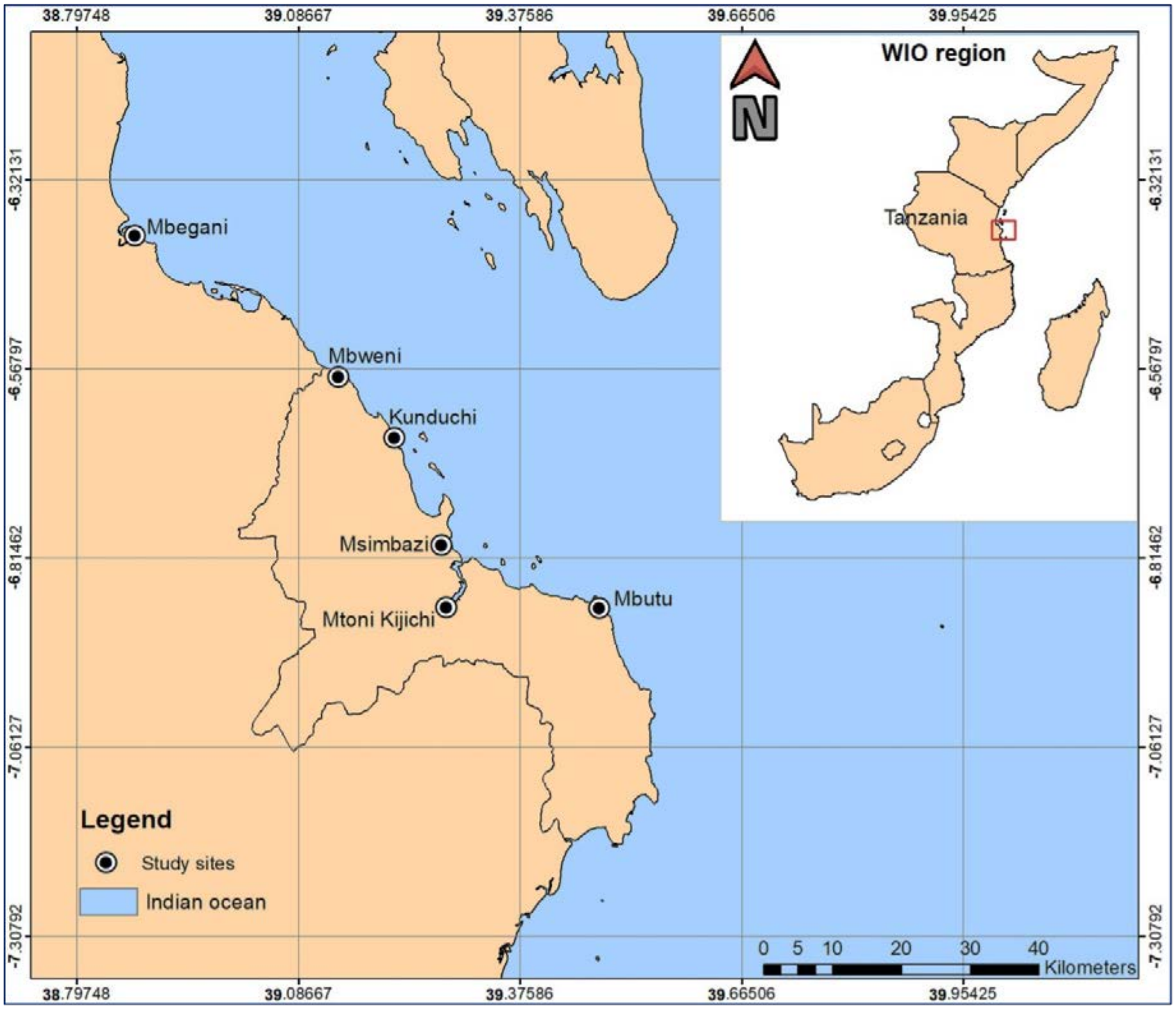

Figure 1. Map showing sampling sites.

Organic carbon content in sediments

Total Organic Carbon (TOC) in 1 g aliquots of dried sediment was determined using a Shimadzu TOC-VCPN analyzer equipped with a PC controller and a solid sample combustion unit (SSM-5000A).

Metal analysis of sediment

Sediment samples from each site were mixed, oven dried at 60 °C for 72 hours, and ground to obtain homogeneous samples, from which a 0.3 g aliquot was placed in a Teflon digestion vessel. To each sample, 1 mL of concentrated nitric acid (HNO_3) (69 %) and 3 mL of hydrochloric acid HCl (37 %) were added. Samples were then extracted in closed vessels using a microwave (ETHOS 900, Milestone, Shelton, CT, USA). Samples were digested in four sequential steps (90, 200, 350, and 500 watts) for 5, 3, 5, and 5 min respectively. The Certified Reference Material (CRM) BCR 144R (sewage sludge of domestic origin) and laboratory blanks were analysed with batches of samples during the preparation stage and through the analytical measurements and data calculations for quality control (Table 1). After microwave digestion, samples were diluted with Milli-Q water to make the volume 50 mL and then stored at -20 °C until analysis. The

Table 1. The recovery rates (expressed as percentage of certified values) of the reference material BCR 144R (sewage sludge of domestic origin) used in this study.

			Measured and certified concentrations (µg/g) of metals in the reference material BCR 144R												
Reference material	**Type of samples used for**		**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
		Measured value	8.68	22.25	151.4	1.82	14.34	97.39	291.1	4915	196.2	46.2	103.8	886	1038
BCR 144R	**Sediments**	**RSD (%)**	0.6	0.31	14.5	0.09	0.95	3.3	9.42	280.7	3	3.5	1.8	42	24
Sewage sludge of domestic origin		**Certified value**				1.82	15	104.3	308		207.9	47.7	106	932	
		Recovery (%)				**100.2**	**95.6**	**93.4**	**94.5**		**94.4**	**96.8**	**97.9**	**95.1**	

concentrations of metals (silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn)) were analysed using a High Resolution Inductively Coupled Plasma Mass Spectrometer (HR-ICP-MS; ELEMENT XR, Bremen Germany). Metal concentrations were corrected for recoveries in laboratory blanks.

Metal analysis of water

Water samples were acidified to 1 % with highly purified concentrated nitric acid and then diluted 5 and 20-fold for metal analysis. Arsenic (As), iron (Fe), and selenium (Se) were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; iCAP 4000, Thermo Scientific, Bremen Germany), while other metals (Ag, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) were analyzed using HR-ICP-MS. Certified reference material SRM 1643e (reconstituted water) and laboratory blanks were analysed with batches of samples throughout the preparation stage, analytical measurements and data calculations (Table 2). Metal concentrations were corrected for recoveries in laboratory blanks.

Metal analysis of mangrove leaves

For each species and site, mangrove leaf samples were placed in pre-weighed 50 mL polypropylene tubes and dried at 60 °C to constant weight, to determine the moisture content. To each sample, 5 mL of highly purified nitric acid (HNO_3) (69 %) was added and allowed to digest at room temperature for 48 h. Samples were further digested on a hot block at 105 °C (±2 °C) for 30 min. An aliquot of 0.5 mL of hydrogen peroxide (27 % H_2O_2) was then added and the samples were left to digest for a further 25-30 min. The digested samples were allowed to cool to room temperature. 50 mL of Milli-Q water was then added to each sample. The samples were held at -20 °C until analysis. Concentrations of metals (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn) were analysed using HR-ICP-MS. Analytical quality was evaluated

Table 2. The recovery rates (expressed as percentage of certified values) of the reference material SRM 1643e (reconstituted water) used in this study.

			Measured and certified concentrations (µg/g) of metals in the reference material SRM 1643e												
Reference material	**Type of samples used for**		**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
		Measured value	1.09	141.87	60.32	6.51	26.61	21.15	23.03	97.06	39.54	62.92	20.11	11.69	76.26
SRM 1643e	**Water samples**	**RSD (%)**	0.02	0.92	1.33	0.21	0.87	0.84	0.84	0.18	1.04	1.27	0.43	0.13	1.02
Reconstituted water		**Certified value**	1.062	141.8	60.45	6.568	27.06	20.4	22.76	98.1	38.97	62.41	19.63	11.97	78.5
		Recovery (%)	**103**	**100.1**	**99.8**	**99.1**	**98.4**	**103.7**	**101.2**	**98.9**	**101.5**	**100.8**	**102.4**	**97.6**	**97.2**

Table 3. The recovery rates (expressed as percentage of certified values) of the reference material BCR 279 ((*Ulva lactuca*)) used in this study.

			Measured and certified concentrations (µg/g) of metals in the reference material BCR 279												
Reference material	**Type of samples used for**		**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
		Measured value	0.07	586.85	2.92	0.26	2.1	10.51	13.06	2308	1989.34	12.76	12.82	0.61	48.77
BCR 279	**Mangrove leaves**	RSD (%)	0.01	22.42	0.12	0.02	0.08	0.8	0.84	83.18	52.87	0.45	0.68	0.1	5.8
Sea Lettuce (*Ulva lactuca*)		Certified value			3.09	0.274		10.7	13.14	2400	2090		13.5	0.593	51.3
		Recovery (%)			**94.6**	**96.6**		**98.2**	**99.4**	**96.2**	**95.2**		**94.9**	**103.3**	**95.1**

using certified reference material *Ulva lactuca* (BCR 279) and laboratory blanks (Table 3). Metal concentrations were corrected for recoveries in laboratory blanks.

Metal analysis of invertebrates

For each species and sampling site, the soft tissues of invertebrates were extracted from the shells and oven dried at 60°C to constant weight, and then cooled. Other laboratory procedures for metal analysis were the same as outlined for mangrove leaves. However, in this case, the certified reference material used was VMK 102 (mussel tissue) (Table 4). Blanks were also included to check for analytical quality. Metal concentrations were corrected for recoveries in laboratory blanks.

Statistical analysis

The data were first tested for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests respectively. Most of the data were not normally distributed even after log transformation, and thus non-parametric equivalents were used. Metal concentrations in sediment and water from sampling sites were compared using a Kruskal-Wallis test (non-parametric one-way ANOVA). Comparisons of metal concentrations between sites and species of biota (mangrove leaves and invertebrates) were tested by the same approach for comparisons of more than two groups, and a Mann-Whitney U test for comparisons of two groups. Multiple comparisons between groups and/or species were performed using a Dunn's test. Correlation between metal concentrations in species of biota and sediments (environment) was done using a non-parametric Spearman rank correlation analysis. All the statistical tests were performed using GraphPad Prism 5 (GraphPad Software Inc., USA). The graphical presentation of data was done using GraphPad Software Inc., USA and R Core Team version 4.4.1 of 2024.

Table 4. The recovery rates (expressed as percentage of certified values) of the reference material VMK 102 (mussel tissue) used in this study.

			Measured and certified concentrations (µg/g) of metals in the reference material VMK 102												
Reference material	**Type of samples used for**		**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Se**	**Zn**
		Measured value	3.14	36.41	15	2.81	2.28	1.62	10	188.8	4.91	6.89	6.46	6.05	121
VMK 102		RSD (%)	0.19	1.91	0.6	0.22	0.24	0.18	0.39	13.12	0.33	0.51	0.11	0.65	2.28
Mussel tissue	**Invertebrates**	Certified value				2.9			10.1	192	4.7				114
(In-house reference material)		Recovery (%)				**96.8**			**98.7**	**98.3**	**104.4**				**106.2**

Results

Grain size distribution and total organic carbon content (TOC) in sediment

The grain size analysis indicates a wide range of grain sizes for most sampling sites (Fig. 2). The TOC content at the Mbegani site (3.74±0.12 %) was higher than at other sites (Fig. 3), followed by Msimbazi (2.53±0.13 %), Mbutu (2.33±0.08 %), Mbweni (1.92±0.03 %), Mtoni Kijichi (1.26±0.13 %), and Kunduchi (0.64±0.04 %).

Metal concentrations in sediments and water

Metal concentrations in sediments from all the studied sites ranged between 2.60-0.03 for Ag, 152.39-65.96 for As, 1.28-0.01 for Cd, 11.29-0.92 for Co, 50.19-7.35 for Cr, 57.77-1.81 for Cu, 5843.14-2243.21 for Fe, 344.24-21.49 for Mn, 17.64-2.67 for Ni, 60.90-1.56 for Pb, 102.19-42.59 for Se and 371.59-17.27 for Zn (µg/g) (Fig. 4). Metal concentrations in sediments were significantly different between sites (Kruskal-Wallis, $H \geq 11.16$, $p < 0.05$, Fig. 4) except for Fe ($H = 6.555$, $p = 0.256$). In general, high metal concentrations were recorded at Msimbazi and Mtoni Kijichi, while low concentrations were recorded at Mbutu and Mbegani (Figure 4). Metal concentrations observed in water samples depicted a different pattern where, for some of the studied metals (e.g. Zn, Pb, Mn, Cr, Cd, and Ag), there was a decrease in concentration from polluted (Msimbazi and Kijichi) to pristine (Mbutu and Mbegani) sites. For some other metals (Ni and Cu), the opposite pattern emerged (Fig. 5). Metal concentrations in water from all the studied sites ranged between 0.05-0.03 for Ag, 20.18-9.29 for As, 0.05-0.04 for Cd, 0.00-0.00 for Co, 0.38-0.14 for Cr, 1.38-1.03 for Cu, 7.32-3.93 for Fe, 0.80-0.50 for Mn, 2.72-1.28 for Ni, 0.05-0.05 for Pb, 69.22-31.57 for Se and 1.59-1.14 for Zn (µg/g) (Fig. 5).

Metal concentrations in biota

Mangrove leaves

Metal concentrations in mangrove leaves from all the studied sites and species ranged between 0.13-0.00 for Ag, 511.01-6.07 for Al, 2.16-0.02 for As, 0.53-0.00 for Cd, 0.45-0.01 for Co, 1.17-0.02 for Cr, 14.73-0.55 for Cu, 541.27-15.83 for Fe, 187.48-10.86 for Mn, 2.61-0.02 for Ni, 5.05-0.01 for Pb, 3.23-0.06 for Se and 330.78-3.93 for Zn (µg/g). Metal concentrations in mangrove leaves differed significantly amongst sites and species (Kruskal-Wallis, $H \geq 22.65$, $p < 0.05$; Mann-Whitney, $U \geq 0.00$, $p < 0.05$) apart from Mtoni Kijichi and Mbweni, where there was no significant difference for some metals i.e. Al, As, Cd, Cr, Fe, Ni, and Pb for *B. gymnorrhiza* (Mann-Whitney, $U \geq 60.00$, $p > 0.05$). Metal concentrations were significantly different amongst all mangrove species (Kruskal-Wallis, $H \geq 10.84$, $p < 0.05$

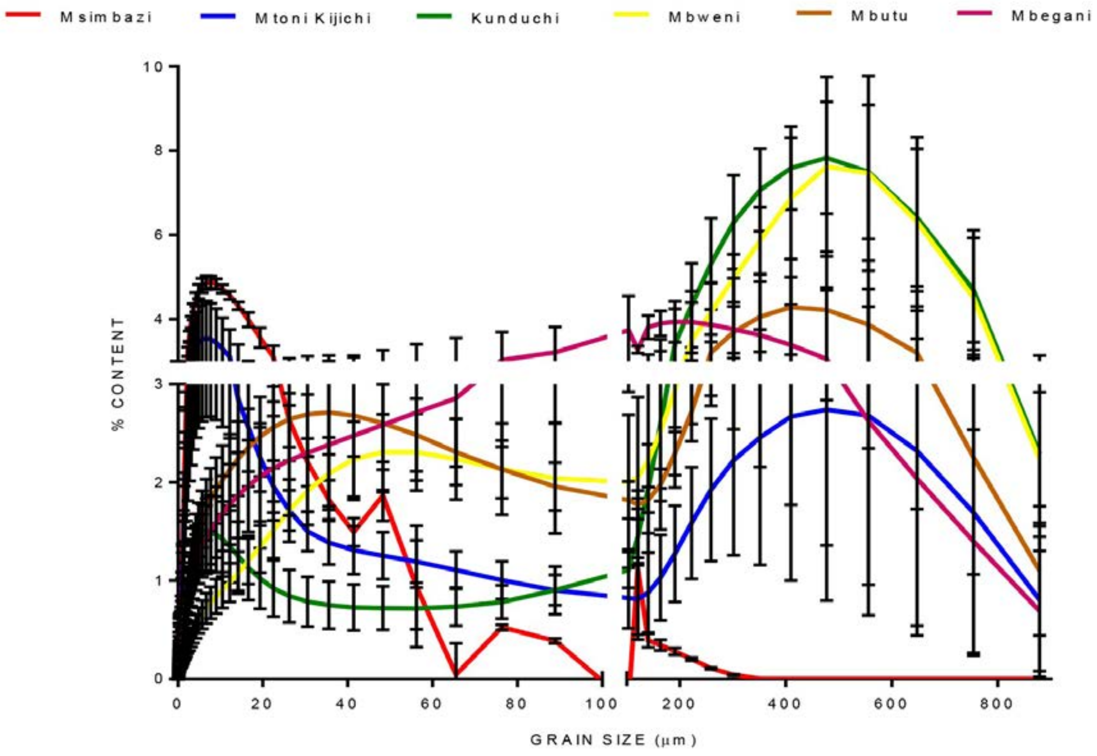

Figure 2. Particle size distribution of sediments (mean±SEM in µm) from the six sampling sites.

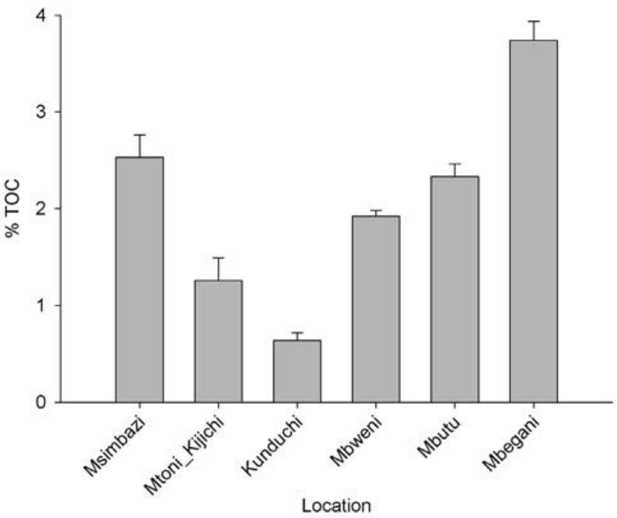

Figure 3. The total organic carbon (TOC) content in sediment.

or Mann-Whitney, $U \geq 0.00$, $p < 0.05$). Higher concentrations of metal were generally observed at Msimbazi and Mtoni Kijichi and mostly decreased with increasing distance from the city centre. Lower concentrations were mostly observed at Mbutu and Mbegani (Fig. 6).

Invertebrates

Metal concentrations in invertebrates from all the studied sites and species ranged between 7.68-0.18 for Ag, 1259.03-10.49 for Al, 85.99-3.64 for As, 7.19-0.10 for Cd, 29.94-1.67 for Co, 23.64-1.35 for Cr, 295.67-27.41 for Cu, 1803.45-178.49 for Fe, 1004.53-7.34 for Mn, 69.09-3.86 for Ni, 16.67-1.17 for Pb, 12.24-0.99 for Se and 710.68-28.60 for Zn (µg/g). Metal concentrations in invertebrates were significantly different amongst sites (Kruskal-Wallis, $H \geq 10.10$, $p < 0.05$; Mann-Whitney, $U \geq 0.00$, $p < 0.05$) apart from Kunduchi, Mbutu, and Mbegani, which showed no significant difference for a few metals (Al and Cd for *T. palustris*) (Kruskal-Wallis, $H \geq 3.89$, $p > 0.05$). The concentrations of metals showed a highly significant difference amongst invertebrate species (Kruskal-Wallis, $H \geq 8.83$, $p < 0.05$; Mann-Whitney, $U \geq 0.00$, $p < 0.05$) except for a few, which were not significantly different among species (Kruskal-Wallis, $H \geq 0.71$, $p > 0.05$; Mann-Whitney, $U \geq 23.00$, $p > 0.05$). Higher concentrations of metals were generally observed at Msimbazi and Mtoni Kijichi while lower metal concentrations were observed at Mbutu and Mbegani (Fig. 7).

Discussion

Sediments and water

In coastal areas, sediment characteristics, particularly the type and quantity of organic matter, grain size, cation exchange capacity, and mineral constituents, are known to have an important influence on metal binding and retention in sediments (Vertacnik *et al.*,

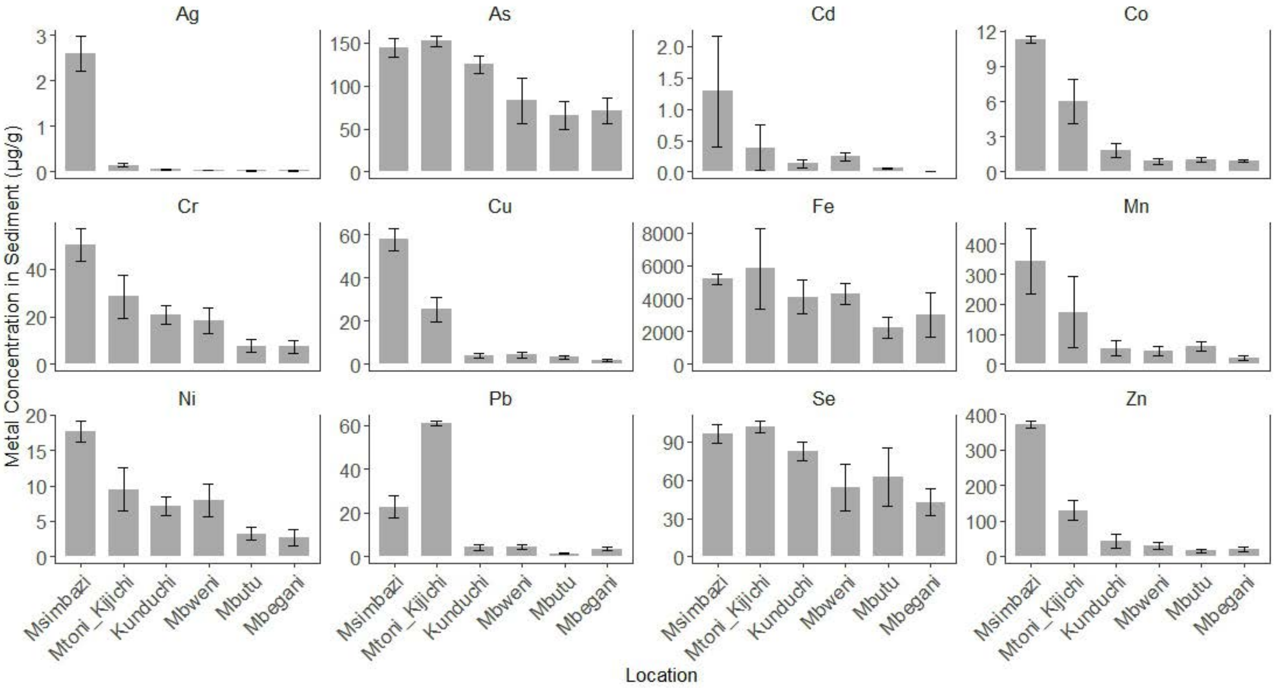

Figure 4. Metal concentration (mean ± SD) in sediments (µg/g dry weight).

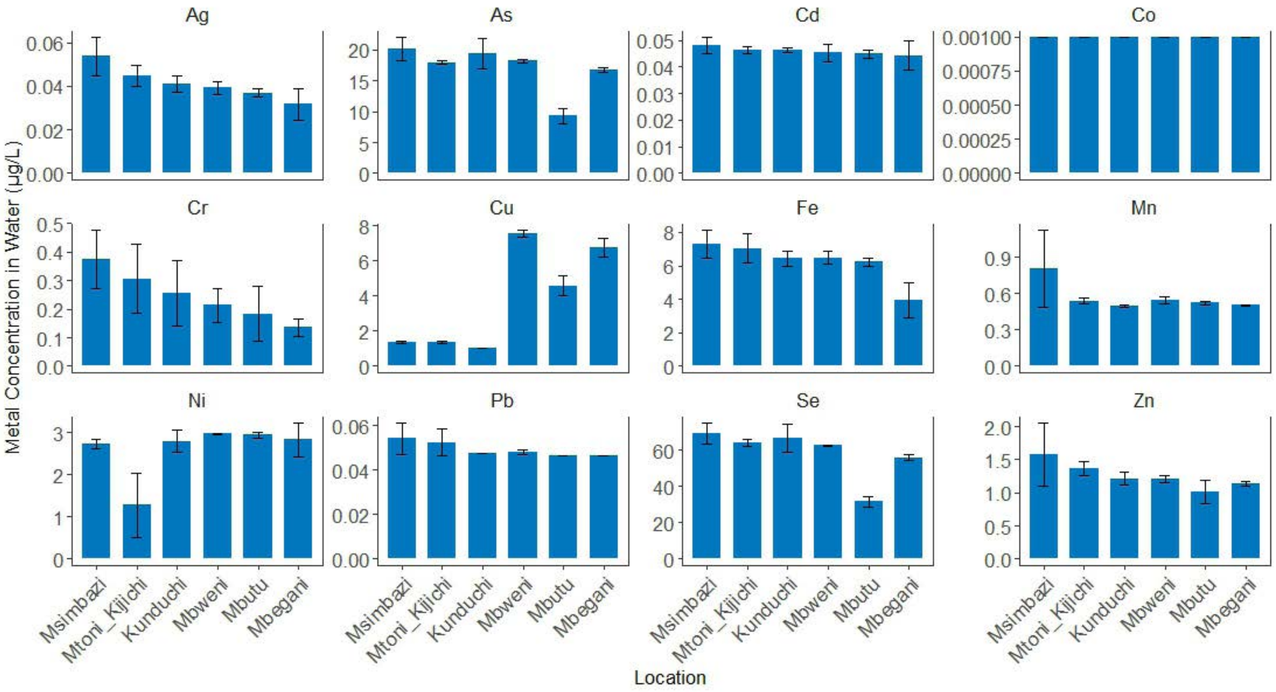

Figure 5. Metal concentration (mean ± SD) in water (µg/L).

1995). The fine-grained fraction (< 63 µm) of the sediment has a high surface area-to-volume ratio, and humic substances also provide a large surface area for metal adsorption (Cheriyan *et al*., 2015; Moore *et al*., 1989; Zhang *et al*., 2014). Sediments with high silt-clay and organic matter content have a high capacity to hold metals. In this study, Msimbazi and Mtoni Kijichi showed the highest silt-clay content, while Kunduchi, Mbweni, Mbutu, and Mbegani showed high sand contents (Fig. 2). This reveals that, metal concentrations observed in organisms in the more polluted sites may also have been in part due to enhanced bioavailability as a result of high silt-clay content and /or total organic carbon concentrations in sediments and vice versa for sites with sandy to coarse contents. The sediment from most sites, however, show a wide range of grain size. This may be due to the fact that sediment in especially vegetated locations like mangrove ecosystems, do not tend to be homogeneous. Non-homogeneity of sediments can be a result of various factors including the presence of decomposing organic matter. Another important point to note regarding sediment composition is that for some filter feeding and sediment-dwelling organisms, organic particles constitute an important direct source of metal intake. Therefore, locations with organically rich sediments will significantly increase metal bioavailability for these organisms.

Certainly, high TOC values alone do not imply high metal bioaccumulation. For example, in this study, Mbegani and Mbutu showed high TOC content, but other factors such as grain size distribution and distance from sources of pollution have contributed to low levels of metal contamination at these sites.

Differences in metal concentrations in sediments can be partly attributed to differences in site distance from the city of Dar es Salaam, which is considered a major pollution source. In general, sites in Dar es Salaam (Msimbazi and Mtoni Kijichi) recorded higher levels of metal concentration, while sites located far away from the city centre (Mbutu and Mbegani) had comparatively low metal pollution which indicates a decrease in metal pollution with increasing distance from the city centre. Studies by Kruitwagen *et al*. (2008), Mremi and Machiwa (2003), and Mtanga and Machiwa (2007) have reported similar results showing decreasing metal pollution with distance from Dar es Salaam city. The Msimbazi River was reported by Ak'habuhaya and Lodenius (1988), De Wolf *et al*. (2001), Mihale (2013), and Mihale (2021) as being heavily polluted. Kruitwagen *et al*. (2008), Machiwa (1992), and Mwevura *et al*. (2002) reported that the harbour of Dar es Salaam, which is located within the Mtoni Estuary, contains high levels of pollutants. Higher levels of metal concentration recorded at

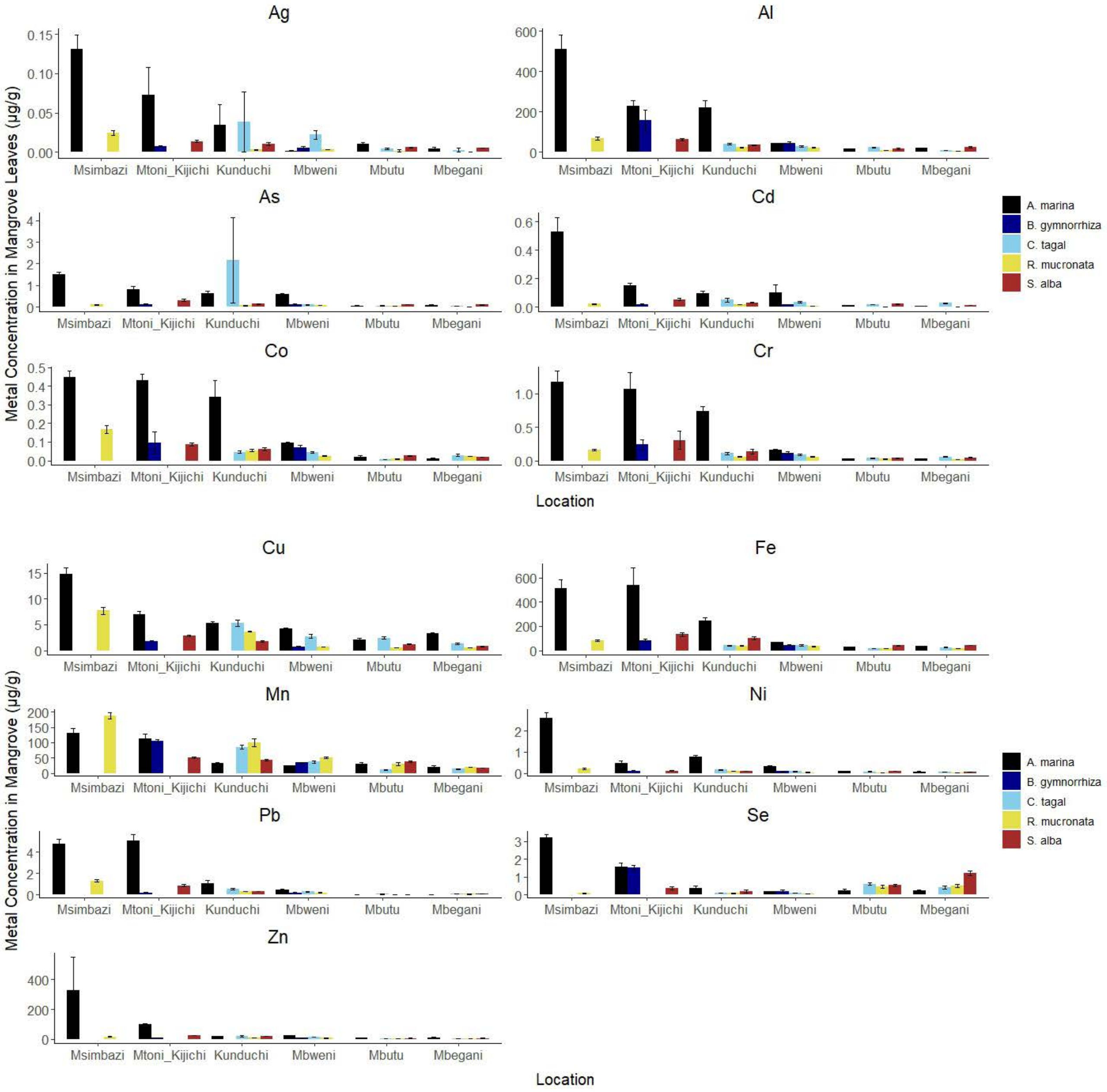

Figure 6. Measured values of different metal concentrations (mean±SD in µg/g dry weight) in mangrove leaves.

Msimbazi and Mtoni Kijichi may also be attributed to sediment grain size characteristics (high silt-clay and organic matter content) which have an influence on sediments capacity to hold metals.

Some metal concentrations in water did not conform to a general pollution decrease at sampling sites away from the city centre. Factors such as the presence of some localized sources (besides Dar es Salaam) at some distant sites may have contributed to higher than expected concentrations of these metals at these sites. Overall, water metal concentrations alone may generally not be a very good indicator of pollution unless at a much larger spatial scale.

Mangrove leaves

One of the main challenges studying (especially for monitoring purposes) chemical concentrations in large plants (trees) is to find suitable parts that represent the whole tree and that are easy to work with, preferably non-destructively, and thus allow repeated sampling. Leaves seem to be good candidates, but some challenges remain, mainly on how to standardize the age of the leaves. However, this is often overcome by

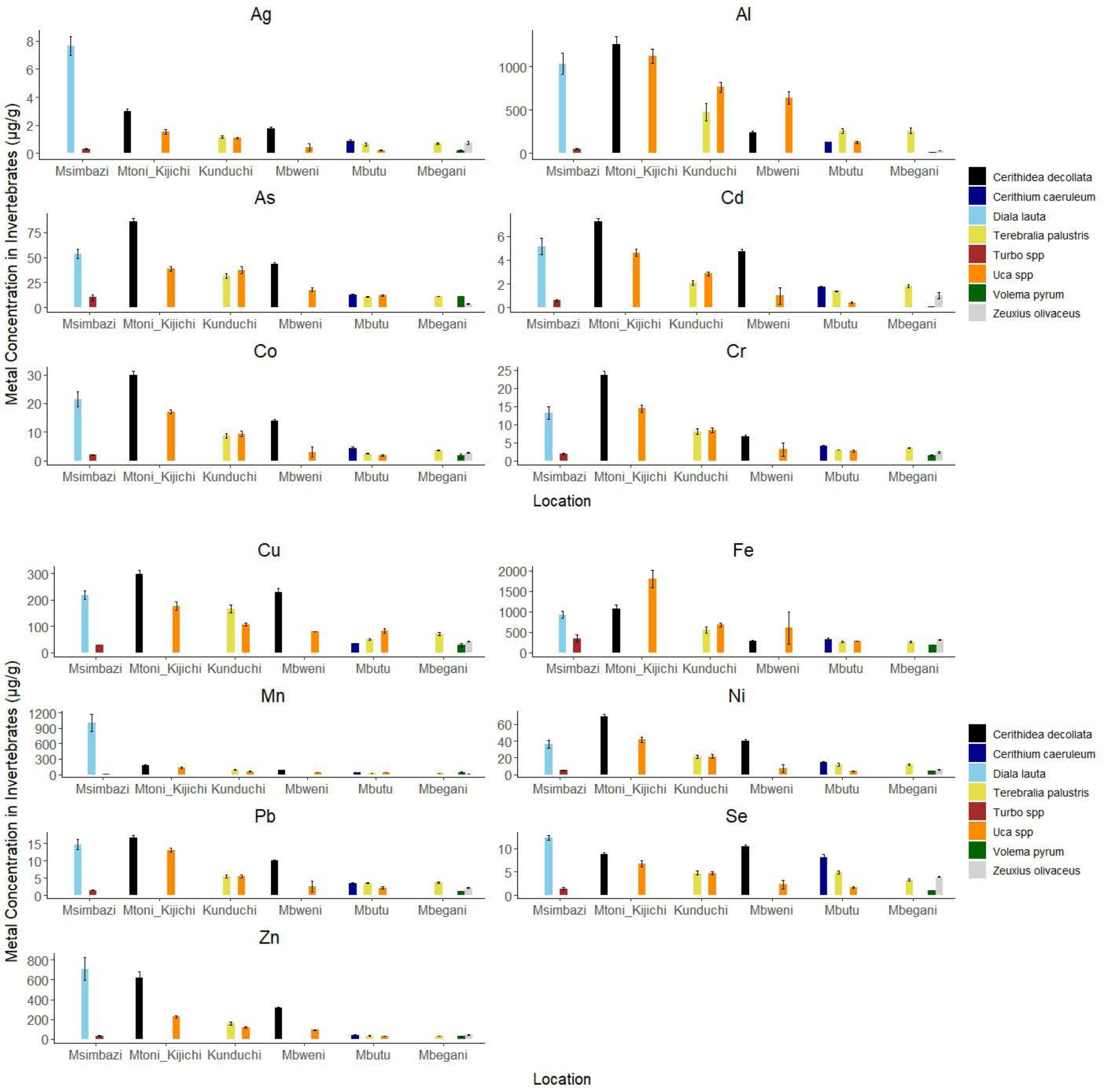

Figure 7. Metal concentrations (mean±SD in µg/g dry weight) in invertebrates.

always sampling either fresh leaves or those that have just fallen. Furthermore, leaves are easy to collect, store and transport. Metal determination procedures are fairly easy in leaves compared to other plant materials. Mangrove leaves have been used in several studies and revealed reasonable levels of metal concentrations (Al Hagibi *et al*., 2018; Almahasheer *et al*., 2018; Caregnato *et al*., 2008; Defew *et al*., 2005; MacFarlane, 2002; MacFarlane and Burchett, 2001; MacFarlane *et al*., 2007; Mremi and Machiwa, 2003; Parvaresh *et al*., 2011).

The differences in metal concentrations among mangrove species, and among sites, may be attributed fundamentally to differences in the levels of metal pollution among the study sites. The closest sites to Dar es Salaam city, such as Msimbazi and Mtoni Kijichi, are generally more polluted than those far away sites (Mbutu and Mbegani). Although there are only a few published studies on the status of metal pollution and their accumulation in mangrove trees, a study by Mremi and Machiwa (2003) showed similar results where metal concentrations in leaves of *A. marina* at Msimbazi were higher than those at Mbweni, which is located far from Dar es Salaam city. Mangroves are highly tolerant of metals despite their potential exposure to metal contaminated sediments (Yan *et al*., 2017). Their tolerance is partly attributed by their ability to exclude metals or regulate the uptake of metals at the root level and limit translocation to the shoot (MacFarlane and Burchett, 2002; Rahman *et al*., 2024). It was suggested by a number of laboratory-based toxicity trials that metal concentrations required to cause a significant negative effect in mangroves may be higher than in comparable aquatic and terrestrial plants (MacFarlane *et al*., 2007). For example, MacFarlane and Burchett (2002) found Pb (0–800 μg g^{-1}) to have little negative effect in the seedlings of *Avicennia marina*. In *A. marina*, Cu and Zn showed low relative toxicities in terms of emergence and biomass, with LC_{50}'s of 566 μg g^{-1} for emergence for Cu and 580 μg g^{-1} for Zn. The reduction in growth/biomass parameters had LOEC values of 400 μg g^{-1} for Cu and 500 μg g^{-1} for Zn. An inhibition of leaf and root development in *Kandelia candel* (L.) Druce seedlings was observed only at the highest applied metal concentrations (400 μg g^{-1} Cu and Zn) (Chiu *et al*., 1995). Based on the above studies, it is clear that the levels of metal contamination in mangrove plants reported in this study do not seem to pose a serious impact on these ecosystems along the coast of Tanzania. However, continuous input of metal contaminants in the studied area may lead to harmful effects in the future.

A. marina showed generally higher metal concentrations overall. These differences may be attributed to differences in metal uptake, tolerance, and metal accumulation capacities amongst mangrove species. The differences may be brought by their distinct morphological specializations for dealing with periodic inundation, anoxic sediments and maintenance of osmotic balance in (hyper) saline environments. For example, members of the families Avicennaceae and Sonneratiaceae possess aerial roots termed 'pneumatophores', while Rhizophoraceae possess aerial stilts to enable gaseous exchange and oxygenation for respiration (MacFarlane *et al*., 2007). Their nutritive root anatomy varies widely among taxa and even within families. Most mangrove species possess roots with two barriers to transport Na+ and other ions to the vasculature, with layers of epidermis and endodermis. The mechanism to deal with the regulation and /or exclusion of ion transport differs depending on whether a species is a salt secretor or a non-secretor. A greater ion mobility and translocation is generally shown by secretors (Lawton *et al*., 1981).

In some mangrove genera, for example *Avicennia* and *Aegiceras*, excessive Na+ and K+ are excreted through specialized glands or glandular trichomes on adaxial and abaxial leaf surfaces, a mechanism which is absent in non-secretors such as *Rhizophora* and *Sonneratia* (MacFarlane and Burchett, 1999). Mangroves with glandular tissues tend to excrete heavy metals concurrently with other solutes (MacFarlane and Burchett, 2000). Variations in mangroves glandular tissue to deal with the challenges of excess cations in saline environments and morphology/function of nutritive root tissue may thus have significant implications for metal accumulation, transport, partitioning and excretion amongst mangrove species (MacFarlane *et al*., 2007).

In order to explain the link between the observed levels of metals in mangroves and those found in the surrounding environment (particularly sediments), correlation analysis was performed between metal concentrations in mangrove species and those observed in sediments. As expected, the results showed in many cases a clear positive relationship between metal concentrations in mangrove leaves and those in the sediments (Table 5). However, in principle, this relationship is not always expected to be perfect. As shown in this study, some cases (metals and/or species) showed relatively weak or no significant correlations. This is because accumulation of metals in any organism is not only dependent on the total concentration of the metal in the environment or distance from a pollution source, but it also depend on other factors such as sediment characteristics (organic matter content, grain size, etc.). In this study, the sites showed differences in both grain size profiles and organic carbon content of sediment. Such differences play a big part in the observed differences in metal accumulation in mangrove trees.

According to results presented on Figure 6, *A. marina* generally accumulated higher concentrations than other mangrove species for most metals, especially at sites located closest to Dar es Salaam city. This elucidates its wider tolerance and accumulation capacity of metals. This species is also widely distributed throughout the study sites and many other mangrove sites along the coast of Tanzania. A*. marina* can thus be used as a bio-monitor of metals along the coast of Tanzania since it fits more of the criteria for metal bio-monitoring than other mangrove species. *A. marina* was also used in a number of previous studies of metal contamination as far as mangrove ecosystems are concerned (Alhassan and Aljahdali, 2021; Aljahdali and Alhassan, 2020; Caregnato *et al*., 2008; MacFarlane, 2002; MacFarlane and Burchett, 1999,

Table 5. Correlation of sediment metal concentrations (µg/g dry weight) and mangroves metal concentrations (µg/g dry weight). *p* values in bold indicate significance level at ≤0.05.

Metal	*A. marina*		*C. tagal*		*R. mucronata*		*S. alba*	
	r	*p*	r	*p*	r	*p*	r	*p*
Ag	0.61	<**0.001**	0.26	**0.047**	0.88	<**0.001**	0.70	<**0.001**
As	0.71	<**0.001**	0.72	**0.000**	0.54	<**0.001**	0.63	<**0.001**
Cd	0.85	<**0.001**	0.41	**0.001**	0.77	<**0.001**	0.80	<**0.001**
Co	0.70	<**0.001**	-0.11	0.418	**0.62**	<**0.001**	0.87	<**0.001**
Cr	0.86	<**0.001**	0.47	<**0.001**	0.85	<**0.001**	0.69	<**0.001**
Cu	0.82	<**0.001**	0.44	<**0.001**	0.75	<**0.001**	0.84	<**0.001**
Fe	0.76	<**0.001**	0.73	<**0.001**	0.88	<**0.001**	0.80	<**0.001**
Mn	0.79	<**0.001**	-0.06	0.638	**0.56**	<**0.001**	0.66	<0.001
Ni	0.75	<**0.001**	0.36	**0.006**	0.80	<**0.001**	0.52	<**0.001**
Pb	0.86	<**0.001**	0.77	**0.000**	0.90	<**0.001**	0.95	<**0.001**
Se	0.55	<**0.001**	-0.21	0.118	-0.36	<**0.001**	-0.57	<**0.001**
Zn	0.81	<**0.001**	0.75	**0.000**	0.84	<**0.001**	0.83	<0.001

2000, 2001, 2002; MacFarlane *et al.*, 2003; MacFarlane *et al.*, 2007; Maurya and Kumari, 2021; Usman *et al.*, 2013).

Invertebrates

Sites closest to Dar es Salaam city (Msimbazi and Mtoni Kijichi) were more contaminated with metals than more distant sites (Mbutu and Mbegani). This was also reported by other studies (Kruitwagen *et al.*, 2008; Machiwa, 1992; Kazimoto *et al.*, 2018; Mtanga and Machiwa, 2007. It is important to note here that besides the level of pollution and bioavailability, many other factors can result in differences in metal concentrations observed in marine organisms. Biological differences and tolerance levels among species, is one of the main secondary factors that can influence metal concentrations in organisms. Mubiana *et al.* (2006) showed the importance of other biological factors, like body size and physiological condition, as well as some environmental variables such as tidal effect.

Invertebrate species (*Uca spp.* and *T. palustris*) found at more than two sites were correlated with sediment metal concentrations (Table 6). This was performed to get a general picture of the relationship between the environment and organisms. The results generally show a clear significant correlation between what is in the environment to what these species of invertebrates

Table 6. Correlation of sediment metal concentrations (µg/g dry weight) and invertebrate metal concentrations (*Uca spp*. and *Terebralia palustris*, µg/g dry weight). *p* values in bold indicate significance level at ≤0.05.

Metal	*Uca* spp.		*Terebralia palustris*	
	r	*p*	r	*p*
Ag	0.88	<**0.001**	0.62	**0.001**
As	0.84	<**0.001**	0.80	<**0.001**
Cd	0.83	<**0.001**	0.22	0.285
Co	0.87	<**0.001**	0.52	**0.006**
Cr	0.89	<**0.001**	0.63	**0.001**
Cu	0.62	<**0.001**	0.54	**0.004**
Fe	0.86	<**0.001**	0.64	**0.001**
Mn	0.32	**0.039**	0.67	<**0.001**
Ni	0.83	<**0.001**	0.63	**0.001**
Pb	0.75	<**0.001**	0.64	**0.001**
Se	0.84	<**0.001**	0.56	**0.002**
Zn	0.94	<**0.001**	0.71	<**0.001**

have accumulated. *Uca spp.* and *T. palustris* are widely distributed among study sites. *Uca spp.* and *T. palustris* are both good bio-indicators of metal contamination and can be used as a bio-monitor in a metal- and species-specific bio-monitoring programme.

With respect to previous studies, Mtanga and Machiwa (2007) observed higher metal concentrations in the oyster *Saccostrea cucullata* collected from Mzinga creek (Mtoni Kijichi) compared to Ras Dege (which is close to Mbutu). In their study, higher concentrations were found for some metals such as Zn and Cu. Generally, Mtanga and Machiwa (2007) observed a decreasing level of metal concentrations in organisms away from the centre of Dar es Salaam. Mremi and Machiwa (2003) also observed the same trend of metal contamination in the crab *Neosarmatium meinerti* except for Co, Ni and Zn, for which crabs at Mbweni had higher and or equal concentrations compered to Msimbazi and Mtoni Kijichi.

Conclusions

The study provides evidence of metal accumulation in the environment (sediments and water) and biota (mangrove leaves and invertebrates) for especially Al, Fe, Mn, Zn, Cu, Se and As, which can be linked to (or explain) metal contamination prevailing at each site. For the purpose of choosing a suitable local mangrove species to be used as a bio-monitor or bio-indicator, *Avicennia marina* is recommended. Among the invertebrates studied, *Uca spp.* and *Terebralia palustris* have the best qualities as bio-monitors or bio-indicators of metal contamination in the region. Metal concentrations in the environment and biota were generally high at sites located close to Dar es Salaam city centre and decreased with increasing distance from the centre, indicating a need for establishment of a multi-species (representing both plants and animals) monitoring program for assessing metal pollution along the coast of Tanzania. According to the observed differences in environmental variables (TOC, grain size distribution) amongst sites, it is recommended that future studies include assessing the relative bioavailability of metals to explain cases where the expected relationship between metal concentrations in the environment and those found in the organisms are either weak or masked.

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References

Ak'habuhaya J, Lodenius M (1988) Metal pollution of river Msimbazi, Tanzania. Environment International 14: 511-514

Al Hagibi H, Nagi H, Al-Selwi K, Al-Shwafi N (2018) Assessment of heavy metals concentration in mangroves leaves of the Red Sea coast of Yemen. Journal of Ecology & Natural Resources 2: 120

Alhassan AB, Aljahdali MO (2021) Sediment metal contamination, bioavailability, and oxidative stress response in mangrove *Avicennia marina* in central Red Sea. Frontiers in Environmental Science 9: 691257

Aljahdali MO, Alhassan AB (2020) Ecological risk assessment of heavy metal contamination in mangrove habitats, using biochemical markers and pollution indices: A case study of *Avicennia marina* L. in the Rabigh lagoon, Red Sea. Saudi Journal of Biological Sciences 27: 1174-1184

Almahasheer H, Serrano O, Duarte, CM, Irigoien X (2018) Remobilization of heavy metals by mangrove leaves. Frontiers in Marine Science 5: 484

Biney C, Amuzu A, Calamari D, Kaba N, Mbome I, Naeve H, Ochumba P, Osibanjo O, Radegonde V, Saad M (1994) Review of heavy metals in the African aquatic environment. Ecotoxicology and Environmental Safety 28: 134-159

Caregnato FF, Koller CE, MacFarlane GR, Moreira JC (2008) The glutathione antioxidant system as a biomarker suite for the assessment of heavy metal exposure and effect in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin 56: 1119-1127

Chan H (1989) Temporal and spatial fluctuations in trace metal concentrations in transplanted mussels in Hong Kong. Marine Pollution Bulletin 20: 82-86

Cheriyan E, Sreekanth A, Mrudulrag S, Sujatha C (2015) Evaluation of metal enrichment and trophic status on the basis of biogeochemical analysis of shelf sediments of the southeastern Arabian Sea, India. Continental Shelf Research 108: 1-11

Chiu C-Y, Hsiu F-S, Chen S-S, Chou C-H (1995) Reduced toxicity of Cu and Zn to mangrove seedlings (*Kandelia candel* (L.) Druce.) in saline environments. Botanical Bulletin of Academia Sinica 36: 19-24

De Wolf H, Ulomi S, Backeljau T, Pratap HB, Blust R (2001) Heavy metal levels in the sediments of four Dar es Salaam mangroves: Accumulation in, and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda). Environment International 26: 243-249

De Wolf H, Rashid R (2008) Heavy metal accumulation in *Littoraria scabra* along polluted and pristine mangrove areas of Tanzania. Environmental Pollution 152: 636-643

Defew LH, Mair JM, Guzman HM (2005) An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Marine Pollution Bulletin 50: 547-552

Harbison P (1986) Mangrove muds—a sink and a source for trace metals. Marine Pollution Bulletin 17: 246-250

Islam MS, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Marine Pollution Bulletin 48: 624-649

Kazimoto EO, Messo C, Magidanga F, Bundala E (2018) The use of portable X-ray spectrometer in monitoring anthropogenic toxic metals pollution in soils and sediments of urban environment of Dar es Salaam Tanzania. Journal of Geochemical Exploration 186: 100-113

Kruitwagen G, Pratap HB, Covaci A, Wendelaar Bonga S (2008) Status of pollution in mangrove ecosystems along the coast of Tanzania. Marine Pollution Bulletin 56: 1022-1030

Lacerda LD, Carvalho CEV, Tanizaki K, Ovalle ARC, Rezende CED (1993) The biogeochemistry and trace metals distribution of mangrove rhizospheres. Biotropica: 252-257

Lawton JR, Todd A, Naidoo D (1981) Preliminary investigations into the structure of the roots of the mangroves, *Avicennia marina* and *Bruguiera gymnorrhiza*, in relation to ion uptake. New Phytologist 88: 713-722

MacFarlane G, Burchett M (1999) Zinc distribution and excretion in the leaves of the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Environmental and Experimental Botany 41: 167-175

MacFarlane G, Burchett M (2000) Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. Aquatic Botany 68: 45-59

MacFarlane G, Burchett M (2001) Photosynthetic pigments and peroxidase activity as indicators of heavy metal stress in the Grey mangrove, *Avicennia marina* (Forsk.) Vierh. Marine Pollution Bulletin 42: 233-240

MacFarlane G (2002) Leaf biochemical parameters in *Avicennia marina* (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. Marine Pollution Bulletin 44: 244-256

MacFarlane G, Burchett M (2002) Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove *Avicennia marina* (Forsk.) Vierh. Marine Environmental Research 54: 65-84

MacFarlane G, Pulkownik A, Burchett M (2003) Accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina* (Forsk.) Vierh.: biological indication potential. Environmental Pollution 123: 139-151

MacFarlane GR, Koller CE, Blomberg SP (2007) Accumulation and partitioning of heavy metals in mangroves: A synthesis of field-based studies. Chemosphere 69: 1454-1464

Machado W, Santelli R, Carvalho M, Molisani MM, Barreto R, Lacerda LD (2008) Relation of reactive sulfides with organic carbon, iron, and manganese in anaerobic mangrove sediments: implications for sediment suitability to trap trace metals. Journal of Coastal Research: 25-32

Machiwa JF (1992) Heavy metal content in coastal sediments off Dar es Salaam, Tanzania. Environment International 18: 409-415

Maurya P, Kumari R (2021) Toxic metals distribution, seasonal variations and environmental risk assessment in surficial sediment and mangrove plants (*A. marina*), Gulf of Kachchh (India). Journal of Hazardous Materials 413: 125345

Mihale MJ (2013) Biochemistry of micro-polutants in sediments of Mtoni estuary, Tanzania. Doctoral dissertation, Vrije Universiteite Brussels

Mihale MJ (2021) Metal contamination in sediments of coastal rivers around Dar es Salaam, Tanzania. Huria: Journal of the Open University of Tanzania 27: 2

Moore JN, Brook EJ, Johns C (1989) Grain size partitioning of metals in contaminated, coarse- grained river floodplain sediment: Clark Fork River, Montana, USA. Environmental Geology and Water Sciences 14: 107-115

Mremi S, Machiwa JF (2003) Heavy metal contamination of mangrove sediments and the associated biota in Dar es Salaam, Tanzania. Tanzania Journal of Science 29: 61-76

Mtanga A, Machiwa JF (2007) Heavy metal pollution levels in water and oysters, *Saccostrea cucullata*, from Mzinga Creek and Ras Dege mangrove ecosystems, Tanzania. African Journal of Aquatic Science 32: 235-244

Mtanga A, Machiwa J (2008) Assessment of heavy metal pollution in sediment and polychaete worms from the Mzinga Creek and Ras Dege mangrove ecosystems, Dar es Salaam, Tanzania. Western Indian Ocean Journal of Marine Science 6: 2

Mubiana VK, Vercauteren K, Blust R (2006) The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. Environmental Pollution 144: 272-279

Muzuka A (2008) Distribution of heavy metals in the coastal marine surficial sediments in the Msasani Bay-Dar es Salaam harbour area. Western Indian Ocean Journal of Marine Science 6: 1

Mwevura H, Othman OC, Mhehe GL (2002) Organochlorine pesticide residues in sediments and biota from the coastal area of Dar es Salaam city, Tanzania. Marine Pollution Bulletin 45: 262-267

Otchere FA (2019) A 50-year review on heavy metal pollution in the environment: Bivalves as bio-monitors. African Journal of Environmental Science and Technology 13: 220-227

Pan K, Wang W-X (2012) Trace metal contamination in estuarine and coastal environments in China. Science of the Total Environment 421: 3-16

Parvaresh H, Abedi Z, Farshchi P, Karami M, Khorasani N, Karbassi A (2011) Bioavailability and concentration of heavy metals in the sediments and leaves of grey mangrove, *Avicennia marina* (Forsk.) Vierh, in Sirik Azini Creek, Iran. Biological Trace Element Research 143: 1121-1130

Rahman SU, Han J-C, Zhou Y, Ahmad M, Li B, Wang Y, Huang Y, Yasin G, Ansari MJ, Saeed M (2024). Adaptation and remediation strategies of mangroves against heavy metal contamination in global coastal ecosystems: A review. Journal of Cleaner Production: 140868

Rainbow PS (1995) Biomonitoring of heavy metal availability in the marine environment. Marine Pollution Bulletin 31: 183-192

Rumisha C, Elskens M, Leermakers M, Kochzius M (2012) Trace metal pollution and its influence on the community structure of soft bottom molluscs in intertidal areas of the Dar es Salaam coast, Tanzania. Marine Pollution Bulletin 64: 521-531

Semesi AK (1992) Developing management plans for the mangrove forest reserves of mainland Tanzania, The Ecology of Mangrove and Related Ecosystems: Proceedings of the International Symposium held at Mombasa, Kenya, 24-30 September 1990. Springer. pp 1-10

Sharma D, Rao K, Ramanathan A, 2021 A systematic review on the impact of urbanization and industrialization on Indian coastal mangrove ecosystem. Coastal Ecosystems: Environmental importance, current challenges and conservation measures. pp 175-199

Silva CAR, Lacerda LD, Rezende CE (1990) Metals reservoir in a red mangrove forest. Biotropica: 339-345

Tam N, Wong Y (1996) Retention and distribution of heavy metals in mangrove soils receiving wastewater. Environmental Pollution 94: 283-291

Tam N, Wong Y (2000) Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. Environmental Pollution 110: 195-205

Usman AR, Alkredaa RS, Al-Wabel M (2013) Heavy metal contamination in sediments and mangroves from the coast of Red Sea: *Avicennia marina* as potential metal bioaccumulator. Ecotoxicology and Environmental Safety 97: 263-270

Vertacnik A, Prohić E, Kozar S, Juračić M (1995) Behaviour of some trace elements in alluvial sediments, Zagreb water-well field area, Croatia. Water Research 29: 237-246

Yan Z, Sun X, Xu Y, Zhang Q, Li X (2017) Accumulation and tolerance of mangroves to heavy metals: a review. Current Pollution Reports 3: 302-317

Yhdego M (2021) Urbanization and solid waste in Tanzania: Environmental degradation and health hazards. ERC Research Paper 11: 1-37

Zhang C, Yu Z, Zeng G, Jiang M, Yang Z, Cui F, Zhu M, Shen L, Hu L (2014) Effects of sediment geochemical properties on heavy metal bioavailability. Environment International 73: 270-281

Short Communication

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*** Corresponding author:**
ronald.fricke@smns-bw.de

New record of the pygmy pipehorse *Cylix* sp. from La Réunion, southwestern Indian Ocean (Teleostei: Syngnathidae)

Ronald Fricke[1]*, Christophe Cadet[2]

[1] Staatliches Museum für Naturkunde Stuttgart,
Gewann Rosenstein 1, 70191 Stuttgart,
Germany

[2] 08 Impasse Bel Air le Haut, 01390
Saint-André-de-Corcy, France

Abstract

The pygmy pipehorse *Cylix* sp. is recorded for the first time from La Réunion, southwestern Indian Ocean, based on a specimen photographed off Sainte-Rose in August 2024, at a depth of 12-18 m. The specimen is described, and compared with other species of the genus. The live colouration is described for the first time.

Keywords: biodiversity, seahorses, new record, Mascarenes, distribution

Introduction

The pygmy pipehorses of the genus *Cylix* Short and Trnski 2021 are currently known only from the western Indian Ocean and New Zealand. They inhabit benthic habitats, often attached to seagrass or algae with their tail. The genus includes a total of two valid species in the western Indian Ocean and New Zealand (Fricke *et al.*, 2024); however, "*Hippocampus tyro"* Randall and Lourie 2009 will be reclassified into genus *Cylix* in an upcoming paper (Graham Short, personal communication, Nov. 2024) (Table 1). The genus is similar to *Hippocampus* Linnaeus 1758, *Acentronura* Kaup, 1853 and *Idiotropiscis* Whitley, 1947, but characterized by its cup-like crest present anterodorsally on the supraoccipital; and large conspicuous midventral conical spines on the cleithral symphysis and first trunk ring between the pectoral-fin bases (Short and Trnski 2021).

Hippocampus tyro Randall and Lourie 2009 was described by Randall and Lourie (2009) based on a single specimen from the Amirantes Islands (Seychelles), dredged at 43-48 m depth. Another individual was recently observed in Mauritius, western Mascarenes (Anonymous, 2023), on a dive cruise near Rivière du Rempart.

A specimen of *Cylix* sp. was photographed on a sand bottom with macroalgae, at 5 m depth, off Sainte-Rose, La Réunion (France), western Indian Ocean. This new record is reported and discussed in the present paper.

Materials and methods

On 24 August 2024, a *ca.* 30 mm TL, female specimen of *Cylix* sp. was photographed by David Pleuret and Miguel Ramírez on a sand bottom with macroalgae, off Sainte-Rose, La Réunion (France), at ca. 21°07'29.20''S, 55°47'12.19''E, at 12-18 m depth of water. The specimen was photographed when diving. The size of the specimen was estimated by the diver, according to his experience.

Counts and measurements followed Hubbs and Lagler (1947); the total length is abbreviated TL. The genus and species classification follows Fricke *et al.* (2024), the family classification follows Van der Laan *et al.* (2014), the head spine terminology follows Randall and Lourie (2009). Collections are abbreviated according to Fricke and Eschmeyer (2024).

Specimens of seahorses used as comparative material: *H. borboniensis*: NHMI uncat. (1), Mauritius. -- *H. jayakari*:

Table 1. Species of the genus *Cylix* Short & Trnski, 2021, and their distribution.

Species	Primary types	Distribution
Cylix nkosi Short, Smith, Harastio & Claasens, 2024	SAM F041935	South Africa (KwaZulu-Natal)
Cylix tupareomanaia Short, Trnski & Ngātiwai in Short & Trnski, 2021	AIM MA122274	New Zealand (Northland)
Cylix sp.	--	Western Mascarenes (La Réunion, Mauritius)
"Hippocampus" tyro Randall & Lourie, 2009	BPBM 35555	Seychelles

MNHN 1073 (1), La Réunion; NHMI uncat. (1), Mauritius; SMNS 3476 (2), Red Sea; SMNS 9921 (1), Red Sea. -- *H. tyro*: BPBM 35555 (holotype), Amirantes.

Results

Description

Proportions (Table 2) are part of this description.

Dorsal-fin rays 15; pectoral-fin rays ca. 15 (left side). Trunk rings 14. Subdorsal rings 3; superior trunk ridge ending under dorsal fin with 2 enlarged subdorsal spines that angle posterolaterally; lateral trunk ridge continuous with inferior tail ridge; spines of trunk and tail ridges moderate in size and blunt; first 7 dorsal trunk spines as double spines; head at right angle to trunk (ca. 85°); one suborbital, two supraorbital, and two nose spines; coronet oval and cuplike, with a rugose spine on the side; two supraorbital spines; one suborbital spine; two very small, median dorsal nose spines. Head length 16.1 % of TL; snout length 7.3 % of TL; eye diameter 1.7 % of TL; maximum trunk depth 8.5 % of TL; trunk length 31.2 % of TL. Total length *ca.* 30 mm.

Live colouration (Fig. 1): Snout carmine read, head yellow, covered with whitish dots, posteriorly bright orange, ventrally olive green; eye whitish rose; trunk orange, with three wide brown bars, all covered with tiny white dots, ventrally olive green; dorsal fin translucent, with a submarginal red band, tips of fin rays whitish.

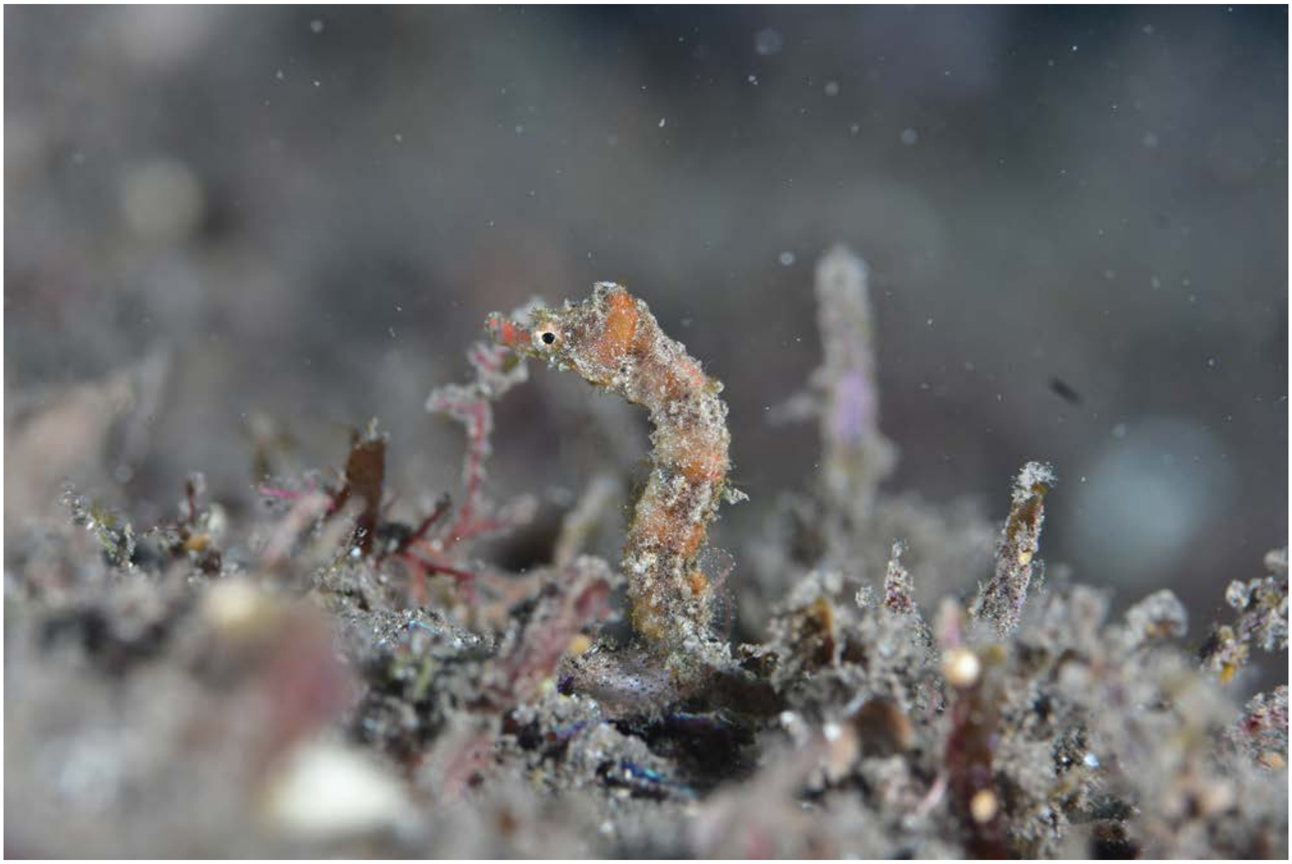

Figure 1. *Cylix* sp., specimen photographed off Sainte-Rose, La Réunion, at 5 m depth, August 2024. Photograph: David Pleuret and Miguel Ramírez.

Table 2. Proportions of the La Réunion specimen of *Cylix sp* (*ca.* 30 mm TL), compared with values of other species of the genus.

	***Cylix* sp. (La Réunion) (present specimen)**	***Cylix nkosi*, Short *et al*. (2024)**	***Cylix tupareomanaia*, Short *et al*. (2021)**	**"*Hippocampus*" *tyro*, Randall & Lourie (2009)**
Head length [in TL]	6.2	5.1-6.2	5.1-6.7	6.2
Maximum trunk depth [in TL]	11.8	13.0-13.8	11.2-13.6	11.2
Trunk length [in TL]	3.2	2.9-3.5	3.2-3.7	3.2
Eye diameter [in head length]	9.8	4.0-6.1	5.1-6.3	6.25
Head depth [in head length]	1.45	1.46-1.68	1.48-1.68	1.65
Snout length [in head length]	3.1	2.7-3.1	2.4-3.1	2.2
Maximum snout depth [in head length]	5.7	5.9-7.3	5.3-5.8	4.5
Coronet height [in head length]	7.0	1.2-2.0	1.9-2.1	9.4
Longest dorsal-fin ray [in head length]	4.2	--	--	3.1
Longest pectoral-fin ray [in head length]	4.3	3.2-3.6	4.8-6.5	4.2

Discussion

Cylix nkosi was described by Short *et al*. (2024) from two specimens, a male (46.6 mm TL) and a female (45.9 mm TL), collected at 22-50 m depth off KwaZulu-Natal (South Africa). *"Hippocampus" tyro* was originally described by Randall and Lourie (2009) from a single, female specimen of 61 mm TL, dredged at 43-48 m depth off the Amirantes (Seychelles). The present paper reports a female specimen, which documents a new record of the genus *Cylix* from La Réunion, differing in the shape of the coronet from all other described species (G. Short, pers. comm., Nov. 2024). Its specific identity remains uncertain as it differs from *Cylix nkosi* and *"Hippocampus" tyro*, but likely represents an undescribed species within the genus. A slightly indistinct photograph of a second specimen

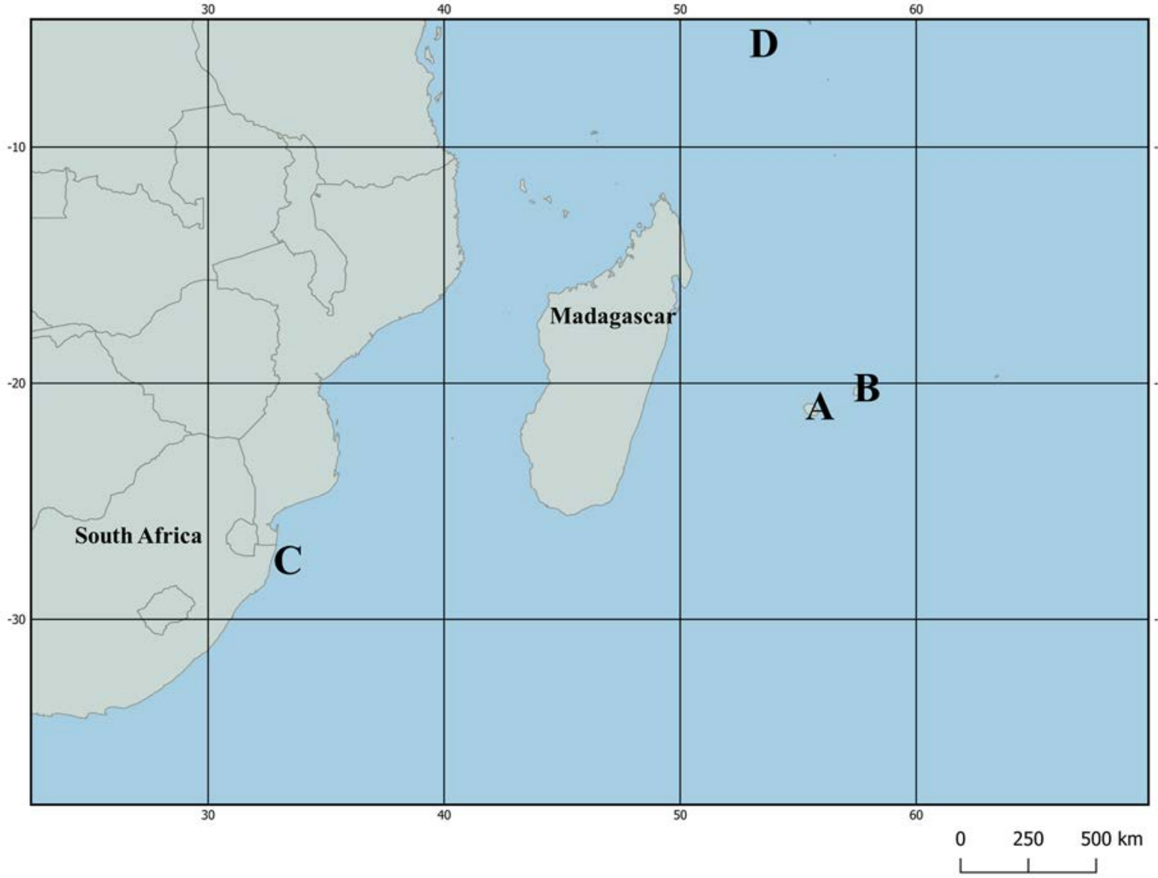

Figure 2. Geographical distribution of genus *Cylix* in the western Indian Ocean. A - New record of *Cylix* sp. from La Réunion; B - record of *Cylix* sp. by Anonymous (2023) from Mauritius (as *Hippocampus tyro*); C - *Cylix nkosi*; D - *"Hippocampus" tyro.*

from Mauritius was published by Anonymous (2023); the sex is indistinguishable, and the depth of occurrence is unknown, but it was photographed during a regular dive session in the lagoon. The known distribution range of *Cylix* sp. in the western Indian Ocean is illustrated in Figure 2.

During an exploration of the fish fauna, a specimen of *Cylix* sp. was photographed off Sainte-Rose, La Réunion. This represents a range extension from the type locality (Amirantes Islands) of ca. 1,710 km to the south, and a confirmed record of this species from the western Mascarenes. From another recent, unconfirmed record from Mauritius, this is an extension of 235 km to the southwest. Although the tail of the specimen is not visible on the photographs, it is clearly identifiable as a species of *Cylix*, and distinguished from other species by several features, including its unusual head shape with a very high crown and characteristic spination, and 14 trunk rings. The characters of the specimen in the photograph agree well with those of *H. tyro*; the proportions visible in the photograph are compared with those provided by Randall and Lourie (2009) in Table 2.

Randall and Lourie (2009: Fig. 1) illustrated a freshly dead specimen of *H. tyro* from the Amirantes Islands, which was overall orange brown with white spots, and the belly whitish; three dark bands were vaguely distinguishable, mainly by dark spots on the back. The present photograph (Fig. 1) is the first detailed documentation of the live colouration of *Cylix* sp. from La Réunion. Here, the snout carmine read, the head yellow, covered with whitish dots, posteriorly bright orange, ventrally olive green; the trunk orange, with three wide brown bars, all covered with tiny white dots, ventrally olive green; and the dorsal fin translucent, with a submarginal red band, tips of fin rays whitish.

The present specimen of *Cylix* sp. was photographed at a depth of 12-18 m (Fig. 3). The habitat is characterized by sand bottom with macroalgae of the species *Amansia detrichiana* Grunow, 1874 (family Rhodomelaceae Horaninow 1847); next to it was a rocky reef of about 15 x 5 m. This reef, extending from 18-28 m depth, was highly diverse, with anemones, nudibranchs, a few sponges, and numerous fish species.

Figure 3. Habitat of *Hippocampus tyro* Randall & Lourie 2009, off Saint-Rose, La Réunion, southwestern Indian Ocean. Photograph: David Pleuret and Miguel Ramírez.

Cylix sp. was photographed in relatively shallow water at 12-18 m depth, while the holotype of "*Hippocampus tyro*" was reported from 43-48 m (Randall and Lourie 2009), and *Cylix nkosi* is known from 22-50 m and *C. tupareomanaia* from 12-17 m. The preferred depth may be related to the water temperature, or, alternatively, the age of the specimen, or the availability of suitable habitats may play a role. Additional research on this rare and elusive species is necessary to gain more information about its biology, and depth distribution.

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References

Anonymous (2023) Faune et flore marines: présence de spécimens du très rare *Hippocampus tyro*. Le Mauricien 2023: 3 pp.

Fricke R, Eschmeyer WN (2024) A guide to fish collections in Eschmeyer's Catalog of Fishes. Online version of 4 Sept. 2024 [https://researcharchive.calacademy.org/research/ichthyology/catalog/collections.asp]

Fricke R, Eschmeyer WN, van der Laan R (2022) Eschmeyer's Catalog of Fishes: Genera, species, references. Online version of 4 Sept. 2024 [https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp]

Hubbs CL, Lagler KF (1947) Fishes of the Great Lakes Region. Bulletin Cranbrook Institute of Science (Bloomfield Hills, Michigan) 26: i-vi + 1-186

Randal, JE, Lourie SA (2009) *Hippocampus tyro*, a new seahorse (Gasterosteiformes: Syngnathidae) from the Seychelles. Smithiana, Publications in Aquatic Biodiversity, Bulletin 10: 19-21

Short GA, Trnski T (2021) A new genus and species of Pygmy Pipehorse from Taitokerau Northland, Aotearoa New Zealand, with a redescription of *Acentronura* Kaup, 1853 and *Idiotropiscis* Whitley, 1947 (Teleostei, Syngnathidae). Ichthyology and Herpetology 109 (3): 806-835

Short G, Smith R, Harasti D, Claassens L (2024) A new record and species of Pygmy Pipehorse of the genus *Cylix* (Teleostei, Syngnathidae) from South Africa and the African Continent. Ichthyology and Herpetology 112 (3): 315-327

Van der Laan R, Eschmeyer WN, Fricke R (2014) Family-group names of Recent fishes. Zootaxa 3882 (2): 1-230

Original Article

Participation of women in the blue economy value chain at Kilifi, Kenya

Solomon Njenga[1]*

[1] Institute of Climate Change and
Adaptation, University of Nairobi,
PO Box, 19132-00501-Nairobi, Kenya

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*** Corresponding author:**
solomonnjenga@gmail.com

Abstract

Participation of women in the blue economy value chain in Kilifi County in Kenya was assessed based on household surveys and key informant interviews. A sample of 96 households from the coastal Malindi sub-county was selected based on cluster sampling. The most senior female in each household was interviewed, and an additional 10 women leaders from 10 self-help groups were interviewed as key informants. Data from the household survey were analysed for mean, percentages and frequency while content analysis was used to analyse qualitative data. Based on the interview data, 88.5 % of women worked in fish processing, 63.2 % in trading of fish products, 62 % in fish eateries, 18.4 % in fishponds, 23 % in solid waste management, and 20.7 % in mining. Consequently, individual women participated in more than one activity, with a mean of 2.75 activities per woman. Work-related, cultural and economic challenges inhibited women's participation with mean responses of >3 on a 4-point Likert scale. Low levels of participation were observed in economic decision-making, access to information, ownership of productive resources, leadership positions and women's living conditions. Participation of women in the blue economy value chain should be prioritized in strategic decisions by government and stakeholders.

Keywords: women, participation, blue economy, value chain, seascape

Introduction

The economic paradigms currently dominating the world are not sustainable. The threats from climate change, exploitation-based approaches to commerce, and the excess acquisition of resources loom large (Lendoye, 2022). Maintaining a balance between development and ecosystems, aspirations for growth, and the need for sustainability is a prescient challenge (Vasseur, 2017). UN Sustainable Development Goals (SDGs) recognise that sustainable macroeconomic growth cannot be achieved without the full participation of women in economic opportunities (Katila, 2019). SDG 5 specifically speaks to achieving gender equality and empowering all women and girls (Hirsu, 2019). To achieve this goal, there is a need to ensure women's full and effective participation and equal opportunities for leadership at all levels of decision-making in political, economic, and public life (Lütz, 2023). Further, there is the need to undertake reforms to give women equal rights to economic resources, as well as access to ownership and control over land and other forms of property, financial services, inheritance, and natural resources, by national laws (Barbier and Burgess, 2019).

The blue economy refers to the sustainable use of ocean resources for economic growth, improved livelihoods, and jobs while preserving the health of the ocean ecosystem (Choudhary, 2021). The Blue Economy concept aspires to increase human well-being and social fairness while drastically lowering environmental dangers and ecological scarcity (Choudhary, 2021). This covers conventional ocean sectors like fishing, tourism, and maritime transportation, as well as emergent activities like offshore renewable energy, aquaculture, and marine biotechnology. The blue economy value chain is a series of actions that starts with manufacturing and concludes with markets. It involves

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both upstream and downstream entities, including producers and market participants. The micro level includes value chain operators and operational service providers, whereas the meso level includes specialised commercial or governmental players who offer support services. In the context of women, a blue economy value chain is a development process that strives to employ ocean resources sustainably for economic growth and improved livelihoods while maintaining the ocean ecosystem from production to sale and consumption. It is frequently described as three interwoven pillars: mentality creation, socioeconomic distribution, price, demand, and consumption. The blue economy value chain aims to use these pillars to provide a long-term and sustainable business model for the blue value chain (Choudhary, 2021).

Indeed, women's economic participation is one source of macroeconomic growth (Mose, 2024). Women's participation in economic opportunities is thus central to their economic participation. This research adopts the definition of women's participation suggested by Rimmer (2017) which held that women's participation has two elements. The first is 'Women Economic Advancement', meaning economic success and gain for individual women and groups of women based on the skills and resources necessary to compete in markets, plus fair and equal access to economic institutions (Nodirovna, 2024). The second is 'Women Power and Agency', meaning the ability of women to take and act on decisions and control their resources and profits (Bryan, 2024). In addition, Women's Participation is the process of change that gives women access to and control over resources and markets; increased agency and choice (Chacko, 2017). There is an increasing affirmation from scholars and policymakers involved in the development field worldwide that the Blue Economy Value Chain is a potent instrument for advancing economic participation, reducing poverty, generating employment, and fostering sustainable development, among other outcomes (Williams, 2023). The Blue Economy Value Chain around the ecosystem has immense economic opportunities for the general population and women (Rachmawati, 2024). The Blue Economy Value Chain has recently emerged as a novel development paradigm aimed at fostering growth and dynamism in coastal countries (Das, 2023). This paradigm emphasizes the importance of adhering to established norms related to environmental and ecological sustainability (Rachmawati, 2024). The Blue Economy Value Chain encompasses a wide range of activities, including fisheries, port and shipping operations, deep sea mining, ocean energy, coastal tourism, ecosystem services, and marine services (Haimbala, 2019). These activities hold significant potential for economic expansion (Haimbala, 2019). As countries increasingly vie for limited land resources, it is expected that competition will shift to the domain of the ocean sector (Mohanty and Dash, 2020). Furthermore, maritime transport remains a crucial means of navigation and trade (Haralambides, 2023). Many coastal nations in Asia and Africa boast extensive coastlines and large coastal communities, which can serve as catalysts for economic development (Haralambides, 2023). Given the importance of investment, employment, growth, and non-traditional security concerns, it is essential to have a deep understanding of the potential of various sectors within the Blue Economy Value Chain (Haimbala, 2019). Therefore, recognizing the pivotal role played by the Blue Economy Value Chain is of utmost importance (Haimbala, 2019). The fishery sector, for example, is intricately linked with food security, nutritional security, and livelihood security, making it a key component of the Blue Economy Value Chain. Eastern African countries such as Kenya, Madagascar, Mozambique, Comoros, and others, are strategically located in the Indian Ocean seascapes with rich marine heritage. (Lendoye, 2022). This advantageous position affords them significant opportunities to tap into the potential of marine resources for business expansion, entrepreneurship promotion, export growth, job creation, and participation of coastal communities (Haimbala, 2019). With the increasing awareness of the Blue Economy Value Chain and advancements in technology, there exists tremendous potential for investment and the stimulation of growth (Riddick, 2021). Despite their extensive coastlines and abundant ocean resources, the Eastern African countries have yet to fully harness the potential of the Blue Economy Value Chain for economic growth and other developmental objectives (Karani, 2022).

Indeed, women's involvement in onshore fisheries, aquaculture, marine product processing, waste recycling, eco-tourism, conservation, and disaster-risk reduction initiatives, has been identified as a powerful tool for economic participation and sustainable development (Rimmer, 2017). Further, Mukhopadhyay *et al.* (2020) noted that blue economy value chain enterprise offers opportunities around food security, energy, climate change, trade and investments, maritime connectivity, tourism, poverty alleviation, and socioeconomic growth. Doherty (2018) asserts that women's blue entrepreneurship is an innovative strategy for economic participation in the context of developing countries. It elucidates that women entrepreneurs have long been utilizing the abundant oceanic resources in coastal nations sustainably to enhance the future prosperity of the planet, promote welfare, and address the growing gender disparities. The study by Rimmer (2017) explores the prospects for the Women's Participation region from a feminist global governance perspective. The study noted that Trade policy should enable women to become key actors in sectors that benefit from trade, provide development pathways into technologically advanced sectors, and address the gender wage gap and women's time poverty. The study proposes a framework for achieving improvements in gender equality and women's participation, specifically targeting Sustainable Development Goal 5 (Doherty, 2018). The study notes that fair trade is critical to achieving women's participation. Fairtrade includes economic, social, and political dimensions, with market access serving as a tool to achieve social goals.

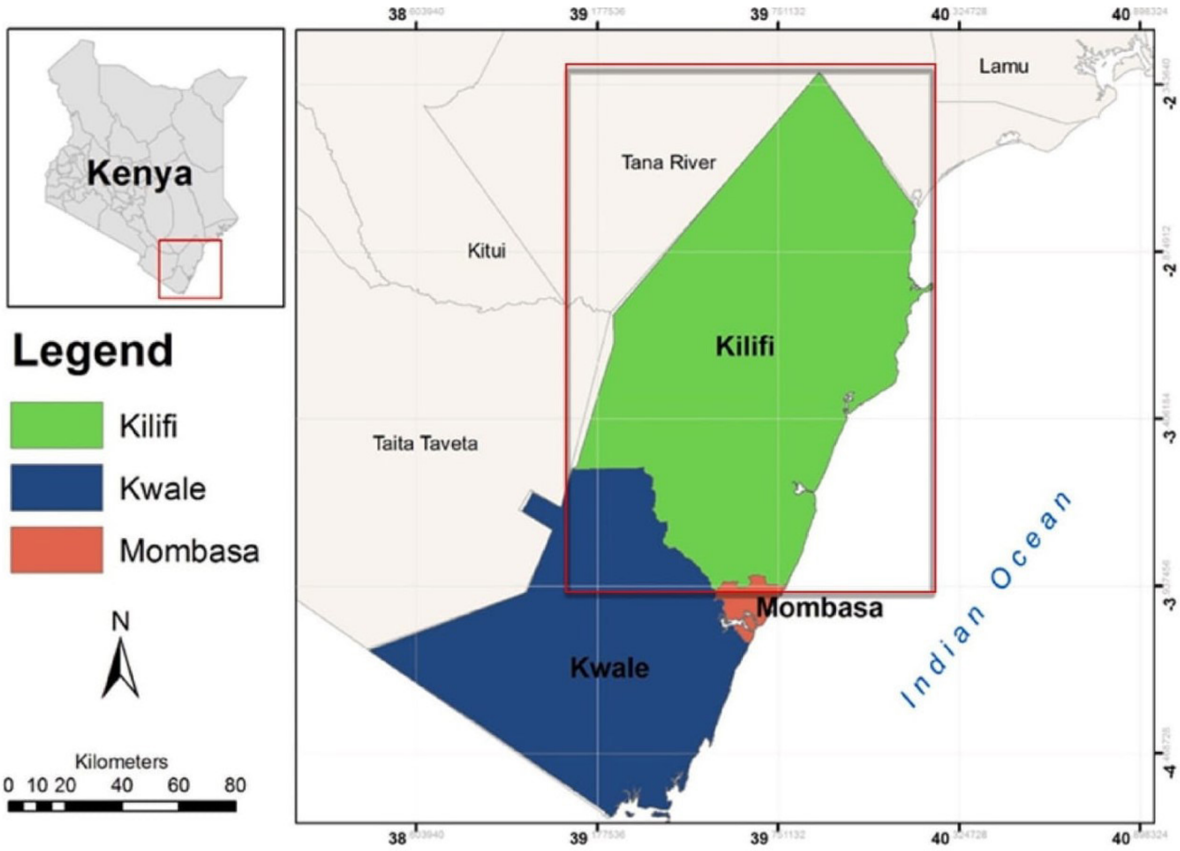

Figure 1. Map of Kilifi County.

Fairtrade International is the largest certification scheme for cocoa, paying both a minimum floor price and a social premium payment (Parra-Paitan and Verburg, 2023). Many chocolate confectionery companies are now promoting women's participation as part of their cocoa farming support programs. Chacko (2017) asserted that women's participation in blue economy value chain can be achieved through gender equality in work, which involves providing women with access to resources, markets, increased agency, and choice. Even with the critical role of women in sustainable development, the participation of women is still lagging behind that of men (Chacko, 2017). Empowering women in the economy and closing gender gaps at work are thus central to the 2030 Agenda for Sustainable Development (Carpentier, 2020). The UN estimates that at the current rate of change, it will take over 100 years for women and girls to have the same rights as men (Doherty, 2018). Achieving gender equality and the participation of all women and girls is Goal 5 of the sustainable development goals (Doherty, 2018). The participation of women in economic opportunities in general and the Blue Economy Value Chain has been constrained by several challenges: culture, religion, economic factors, and gender roles among others (Murunga, 2021). Murunga (2021) provides insight into how gendered power dynamics constrain and enable choices and opportunities for addressing gender inequality in small-scale fisheries. The authors note that the gendered-power dynamic is crucial for renegotiating gender equality with social norms and politics, including challenging simplistic views on poverty, vulnerability, and subordination of women. It's evident that women play a key role, in post-harvest activities relating to processing, marketing, and trade but their role remains undervalued (Biswas, 2018). Women are engaged in small-scale fishing activities across all regions of the globe. It is estimated that 11 % of participants involved in small-scale fishing activities are women (Galappaththi, 2022). These women collectively catch around 2.9 million tonnes of marine fish and invertebrates annually (Galappaththi, 2022). The monetary value of the catch made by women is estimated to be about USD 5.6 billion (Galappaththi, 2022). Most of these catches are obtained along the shoreline, either on foot or from small, non-motorized vessels (Manyang, 2019). The catches made by women are primarily intended for personal consumption and are therefore considered part of the subsistence sub-sector (Biswas, 2018). Kilifi County has a massive Blue Economy Value Chain investment potential arising from its 265 km long coastline and the 200 nautical mile Exclusive Economic Zone on its eastern side (Omukoto, 2024). This presents various economic opportunities in Kilifi County in areas such as fishing and fisheries, tourism, logistics and transport, ash crop growing, staple food growing, mining, and animal rearing (Molla, 2021). However, the participation level of women in such economic activities has been questioned. This study therefore sought to assess the status, trends and barriers to Women's Participation in the Blue Economy Value Chain among women in households in Kilifi County, Kenya.

Materials and methods

Study Area

Kilifi County (see Fig. 1) is located on Kenya's coast, 56 kilometres (35 miles) northeast of Mombasa, and is one of six counties in Kenya's coast region. The county has an area of 12,370.8 km² (Omollo, 2022). The county lies on Kilifi Creek and sits on the estuary of the Goshi River. Kilifi town is the capital of Kilifi County. Kilifi County is popular for its sandy beaches and the ruins of Mnarani, including mosques and tombs, dating from the 14th to the 17th century (Cheruiyot, 2022). There are seven sub-counties in Kilifi including Ganze, Kaloleni, Kilifi North, Kilifi South, Magarini, Malindi and Rabai (Tsofa, 2017). Fishing is the main economic activity in Kilifi due to its proximity to the Indian Ocean (Sheriff, 2022); the tourism industry plays a major role also due to the presence of wildlife sanctuaries, sandy beaches, and historical sites (Sheriff, 2022). Agriculture is also practiced and the county is known for growing sisal and cashew nuts. Kilifi County has appreciable quantities of mineral resources (titanium, iron ore and vast salt deposits) which are exploited to support development (Omollo, 2022).

Population and sampling

The study's key population included women in Kilifi County, Kenya. The population census undertaken in 2019 revealed that of the 1,453,787 people living in Kilifi County, 749,673 were women spread across 298,472 households (Kenya National Bureau of Statistics, 2019). The researcher adopted formulae suggested by Kothari (2015) to pick the sample size. Given the target population of 298,472 households, the sample size was arrived at as follows:

$$n = \frac{298{,}472 * 1.96 * 1.96 * 0.5 * 0.5}{0.1 * 0.1 * (298{,}472 - 1) + (0.5 * 0.5 * 1.96 * 1.96)} = \frac{286{,}652.5088}{2984.71 + 0.9604} = \frac{286652.5088}{2985.6704} = 96.00$$

The study adopted a cluster sampling method where the target population was segregated into seven sub-counties (Berndt, 2020). One of the major reasons why cluster sampling was chosen was its low cost. Money on travel and logistics was saved by focusing on select clusters rather than the total population. Then one sub-county was selected randomly with Malindi sub-county being selected to participate in the study. The sample size of 96 households was thereafter randomly drawn from the Malindi sub-county with the oldest female from each household being selected to participate in the household survey based on purposive sampling. In most cases, the oldest female was either the wife of the head of the household, the head of the household, or the oldest daughter in the household. The household survey was undertaken in homesteads with the assistance of research assistants from the Malindi sub-county. The survey questionnaire was structured into three parts. Part A collected demographic information such as marital status, education, and age. Part B of the questionnaire collected information on the participation of women in different Blue Economy Value Chain activities including fish processing, fish rearing in ponds, artisanal fishing, guiding tourists, selling artefacts and palm wine to tourists, trade in fish, eateries for fish and sea foods, solid waste collection and recycling, sand harvesting and mining, planting of mangrove forest and working in hotels and resorts. The participation in each activity was based on a Yes or No response question. Part C of the survey questionnaire sourced information on barriers to women's economic participation in the Blue Economy Value Chain (economic challenges, work-related challenges, cultural and religious barriers). The study adopted a 4-point Likert scale (where 1 was not at all, 2 was to a small extent, 3 was to a moderate extent and 4 was to a great extent) to examine the extent to which each barrier inhibited women's participation in economic opportunities. Part D of the survey instrument examined women's participation. The study used questions that collected information on five indicators (input in economic decisions, access to information, ownership of productive resources, participation in leadership positions and women's living conditions) of women's participation. The questions measuring women's participation were adapted from de Hoop *et al*. (2020). The household survey was undertaken in October 2023. The study also undertook a key informant interview (KII) with 10 women leaders from 10 self-help groups (SHGs) operating in Malindi based on purposive sampling. The KII guide was administered by the chief investigator in November 2023. Quantitative data from the household survey questionnaire was analyzed based on descriptive statistics tools such as frequency of distribution, mean, and percentages with the aid of Statistical Package for Social Sciences (SPSS) version 25 (Abu-Bader, 2021). SPSS Statistics V25 was chosen because it is a user-friendly statistical programme that has a wide variety of capabilities for new and sophisticated statistics for data analysis, data visualisation, data mining, and other applications. Further, content analysis was used to analyze qualitative information collected through KII. Major themes were identified around study objectives before they were explained.

Conceptual framework

The study adopted a conceptual framework (see Fig. 2) that outlines the key concepts, variables, relationships, and assumptions underlying the study. The framework provides a roadmap for understanding the phenomena under investigation. In the conceptual framework, women's participation in Blue

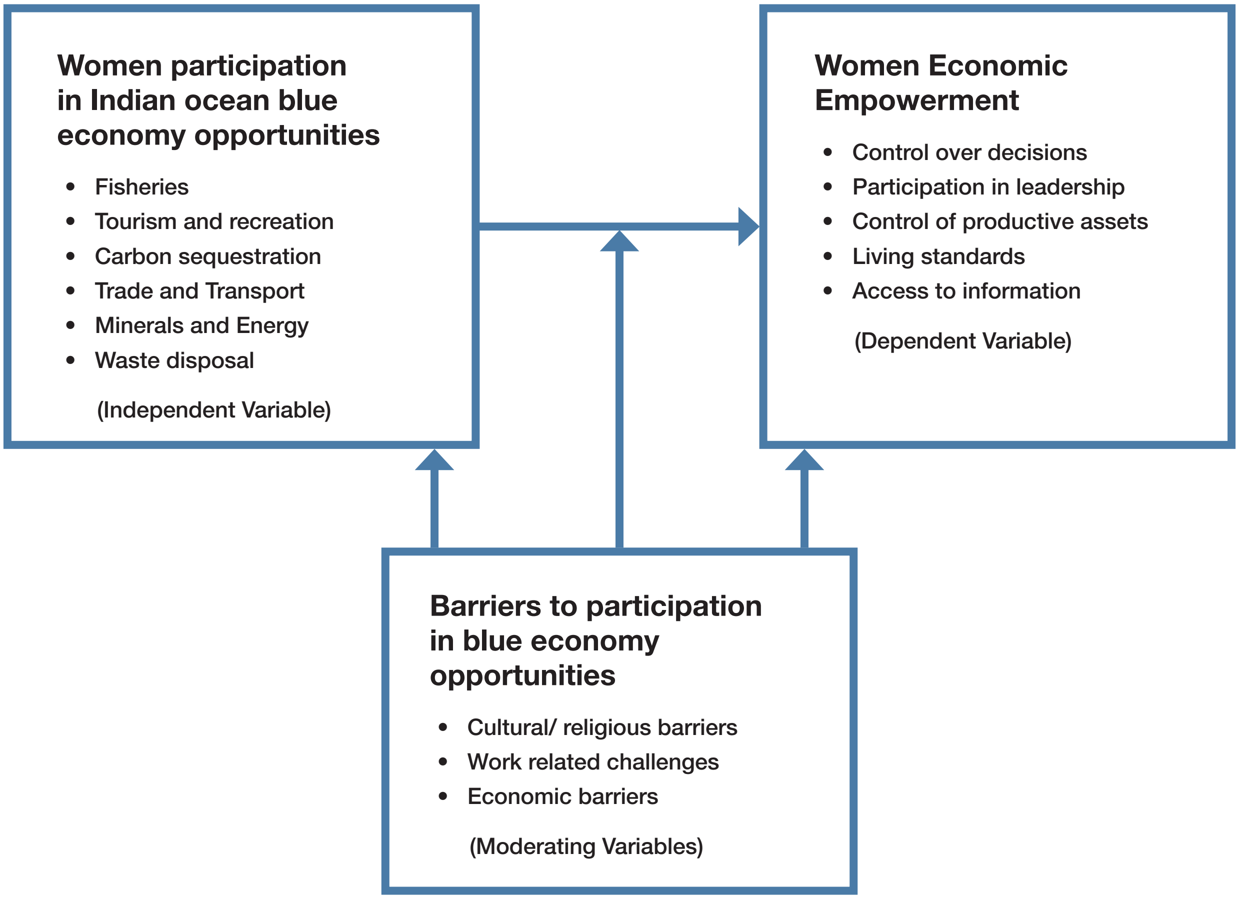

Figure 2. Conceptual Framework.

Economy Value Chain opportunities is the independent variable. Aspects of the Blue Economy Value Chain opportunities considered included fisheries, tourism and recreation, carbon sequestration, trade and transport, minerals and energy, and waste disposal. The dependent variable was Women's Participation captured with aspects such as control over decisions, participation in leadership, control of productive assets, living standards, and access to information. The moderating variable was barriers to women's participation in the Blue Economy Value Chain opportunities such as cultural/ religious barriers, work-related challenges, and economic barriers. The study validated the study's premise that women's engagement in Blue Economy Value Chain opportunities should lead to economic participation. Furthermore, the obstacles to participation should lead to a decrease in the opportunities offered by the Blue Economy Value Chain, hence reducing their economic involvement. The study adopted a positivist research paradigm that asserts that reality is objective and can be studied through objective measurement of a phenomenon of concern to the researcher. The philosophy further asserts that since variables can be operationalized in quantitative terms, it is possible to examine the causal effect relationship between them (Goddard and Melville, 2004). The study thus sought to establish the effect of women's economic participation in the Blue Economy Value Chain opportunities on their economic participation in Kilifi County. Such examination was based on objective data collection through a survey questionnaire tool. Further, the study was based on quantitative research methods. Quantitative research is applied when a phenomenon can be expressed in numerical or quantitative terms hence allowing the generation of relevant data in numerical form which can be subjected to rigorous quantitative analysis (Kothari, 2004).

Results and Discussion

Demographic information

The study was undertaken among 96 households with 87 households giving adequate information for analysis hence the response rate was 90.6 % which was considered adequate. The study examined demographic variables of the women from the Malindi area of Kilifi County in terms of marital status, education, and age. The study revealed that most women (75.9 %) who participated in the study were aged between 20 and 39 with those aged below 20 being 11.5 % and those aged 40 and above 12.6 %. As regards age, most women were youthful. Further, most (78.1 %) women possessed either primary or secondary education with the rest having no formal or college education. This finding implies that most women did not have specialized formal skills gained from college and university education. Finally, most women were married (59.8 %), followed by single and widowed at 16,1 % each, and finally separated at 8 %. The finding implies that most women were in marriage as expected from their strong cultural and religious doctrines that encouraged marriage.

Participation of women in Blue Economy Value Chain opportunities

The study team asked women if they had engaged in any economic activities within the previous year. The household survey findings in Table 1 revealed that women in Kilifi participated in multiple blue economic activities at the same time. The study revealed that 88.5 % of women participated in small-scale fish

Table 1. Participation of women in Blue Economy Blue Value Chain opportunities.

Question	Economic Opportunities	Responses			
		Yes		No	
		Freq	%	Freq	%
Did you participate in any of the economic activity in the past 12 months year?	Fishpond and fish rearing	16	18.4	71	86.6
	Small-scale fish processing	77	88.5	10	11.5
	Artisanal fish in the ocean	21	24.1	66	75.9
	Guiding Tourists on sites	27	31.0	60	69.0
	Selling palm wine and artefacts to tourists	27	31.0	60	69.0
	Trade in fish and other seafood	55	63.2	32	36.8
	Eatery for fish, sea foods and Swahili dishes	54	62.0	33	37.9
	Solid waste collection and recycling	20	23.0	67	77.0
	Sand harvesting and Mining of gypsum, limestone	18	20.7	69	79.3
	Planting of mangrove forest	32	36.8	55	63.2
	Working in hotels and resorts	46	52.9	41	47.1

processing, 63.2 % in trading in fish and seafood and 62 % in eateries for fish, seafood and Swahili dishes among other Blue Economy Value Chain opportunities. However, only 18.4 % participated in fish rearing in fishponds, 24.1 % in artisanal fishing in the ocean, 23 % in solid waste collection and recycling and 20.7 % in sand harvesting and mining of gypsum and limestone. These economic activities have tended to be preserved for men. In a KII with the woman leader of Machina Self-help Group, the respondent noted:

"Most women shy away from actual fishing because that is a preserve for the men. Woman's work is to receive fish at the harbour for clearing and drying before it is sold or prepared for eating at home."

The finding implies that women participated in the fishing industry doing small-scale fish processing and trading in fish and fish products. However, they participated less in mining, artisanal fishing, and solid waste collection. The study concluded that women were confined to fish processing and trade in fish products among the available Blue Economy Value Chain opportunities in Kilifi County. Empirical investigations corroborated the results that women participate in just a small proportion of available Blue Economy Value Chain opportunities. Vipinkumar *et al.* (2017) highlight the gender bias and inequalities in the marine fisheries sector. This emphasizes the importance of self-help groups in promoting gender mainstreaming. Meetei *et al.* (2016) who worked on women's active participation in various fisheries and aquaculture activities in Manipur note that participation in fisheries activities significantly contributes to the participation of rural women.

Rewards for the participation of women in Blue Economy Value Chain opportunities

The study also investigated the rewards received by women in Kilifi from their participation in Blue Economy Value Chain opportunities. The rewards were categorized as either profit, salary/wages or subsistence. The survey findings in Table 2 showed that of those women who participated in small-scale fish processing, 63.6 % earned a profit while the rest earned a wage or for subsistence. Further, of those who participated in trade in fish and seafood, 70.9 % of them did it for a profit with the rest earning wages for their services. Further, of those women who participated in sand harvesting and mining, solid waste collection, planting of mangrove forests and working in hotels, 77.3 %, 85.0 %, 87.5 %, and 78.3 % respectively earned wages with the rest earning a profit or for subsistence. In a KII with a woman group leader from Malindi town who operates an eatery in Malindi town she said:

> *"I operate this hotel to make a profit for taking care of my family. Here, I sell different kinds of food with fish being the popular dish among my customers."*

According to the findings, women who participated in more Indian Blue Economy Value Chain activities tended to make a profit from their work and therefore had control over their earnings, whereas women who participated in fewer activities tended to receive a wage or did so for subsistence. In these situations, the employer set their pay. Empirical studies have also noted that women do two more hours of unpaid work per day than men hence implementing policies to reduce and redistribute unpaid work could increase GDP by up to four percent. (Alonso *et al.*, 2019).

Table 2. Rewards for the participation in the Blue Economy Value Chain.

Question	Economic activity	Responses					
		Profits		Salary/Wage		Subsistence	
		Freq	%	Freq	%	Freq	%
Is your participation in the economic activities for a salary, profit or family subsistence?	Fishpond and fish rearing	10	62.5	5	31.3	1	6.2
	Small-scale fish processing	49	63.6	22	28.5	6	7.8
	Artisanal fishing in the ocean	16	76.2	0	0.0	5	23.8
	Guiding Tourists on sites	16	59.2	9	33.3	2	7.4
	Selling palm wine and artefacts	25	92.6	2	7.4	0	0.0
	Trade in fish and other seafood	39	70.9	16	29.1	0	0.0
	Eatery for fish, seafood	24	44.4	30	55.6	0	0.0
	Solid waste collection and recycling	3	15.0	17	85.0	0	0.0
	Sand harvesting, Mining of gypsum, limestone etc.	4	22.2	14	77.3	0	0.0
	Planting of mangrove forest	4	12.5	28	87.5	0	0.0
	Working in hotels and resorts	10	21.7	36	78.3	0	0.0

Barriers to women's economic participation in the Blue Economy Value Chain

Cultural and religious Barriers

Table 3 presents the mean response score for statements on cultural and religious challenges facing women in their participation in Blue Economy Value Chain opportunities. The most prominent cultural challenge was forced/early marriages with a mean response of 3.6 on the 4-point Likert scale; hence tending towards a great extent. The finding implies that women in Kilifi County were hindered in their participation in Blue Economy Value Chain opportunities by early or forced marriage. The least-faced cultural challenge was gender-based violence at a mean response score of 3.1 on the 4-point Likert scale coinciding with a moderate extent and implying that gender-based violence was also a cultural hindrance to women's participation in Blue Economy Value Chain opportunities. All mean responses on cultural and religious barriers to women's participation in Blue Economy Value Chain opportunities were 3 and above on the 4-point Likert scale implying that the women in Kilifi County were hindered by cultural barriers in their participation in Blue Economy Value Chain opportunities. Further, the KII with the Kivangaraa Women group leader revealed:

> *"...I am a woman and every day before I go out to sell fish, I have to feed the goats and do household chores. These activities eat into my time for selling fish..."*.

The findings imply that culture and religion bestow on women additional activities and roles that end up limiting their participation in Blue Economy Value Chain opportunities. These findings are in agreement with empirical studies (Muli, 2020; Azizi and Moradi, 2022; Alemu *et al.*, 2022). Muli (2020) noted that cultural practices, property ownership, and early marriages influenced women's involvement in economic projects while Female Genital Mutiation (FGM) and widow inheritance had minimal influence. Azizi and Moradi (2022) observed that barriers to women's presence in economic, social, and cultural dimensions and providing solutions for removing those barriers is needed to obtain practical results in the field of employment policy. Alemu *et al*. (2022) noted that factors inhibiting women's participation in income-generating activities included husbands not allowing participation, local customs, family size, land size, and livestock holdings, among others.

Work-related challenges

Table 3 presents the mean response score for statements on work-related challenges facing women in their participation in Blue Economy Value Chain opportunities. The most prominent work-related challenge women in Kilifi faced was unequal pay for women as evidenced by a mean response score of 3.3 on the 4-point Likert scale, tending towards a great extent. Women in Kilifi therefore received relatively lower pay compared to men in their participation in Blue Economy Value Chain

Table 3. Barriers to women's economic participation.

Cultural and Religious Barriers	Mean
Early/ forced marriages	3.6092
Participation in-home care and subsistence work	3.4713
Childbearing and rearing	3.3678
Denial of education opportunity	3.3218
Female genital mutilation	3.2414
Gender-based violence	3.1609
Work-Related Barriers	**Mean**
Unequal pay for women	3.3333
Assigning inferior tasks to women	3.2874
Assigning tasks based on sex	3.2874
Stereotypes of what women can do	3.2644
Sexual harassment	3.1839
Economic barriers	**Mean**
Inadequate skills training and education	3.3103
Financial illiteracy	3.2874
Low levels of income	3.1724
Low access to credit facilities	3.1724
Low access and ownership of productive resources	3.0345

opportunities. The least faced work-related challenge was sexual harassment at a mean score of 3.1 on the 4-point Likert scale, coinciding with a moderate extent. This finding means that even though sexual harassment was the least faced work-related challenge, it still had an impactful effect on the participation of Kilifi women in Blue Economy Value Chain opportunities. Further, all the mean response scores on work-related challenges were above 3 on the 4-point Likert scale. This implies that work-related challenges inhibited Kilifi women's participation in various Blue Economy Value Chain opportunities to a great extent. In KII with the woman leader of the Wachuma Women Group, the respondent stated:

> *"I am a woman who works as a fish seller, our culture does not allow women to go fishing. The activity is reserved for men. All we can do is clean the fish and sell it. I am not even allowed to own a boat as no male fisher will even dare work with it…"*

The findings mean that Kilifi women face a number of work-related challenges that hinder their participation in Blue Economy Value Chain opportunities. Critical work-related challenges include unequal pay in favour of men, inferior tasks for women, assigning tasks based on sex, and sexual harassment among others. Women's economic participation is influenced by structural biases, such as the sexual division of labour, the glass wall and glass ceiling effects, and the feminization of poverty (Masreka, 2020). Neyer and Stempel (2019) noted that in Nigeria discrimination leads to inefficient working time allocation between women and men—discrimination results in lower wages, output, and welfare for women labourers. Women's contributions to agriculture and economic development are underrepresented. Gender biases hinder women's participation in productive agricultural activities (Uche, 2019).

Economic barriers

Table 3 presents the mean response score for statements on economic barriers facing women in their participation in Blue Economy Value Chain opportunities in Kilifi. The most faced economic challenge was skills training level as evidenced by a mean response score of 3.3 on the 4-point Likert scale. The finding means that the most inhibiting economic challenge facing them in their participation in Blue Economy Value Chain opportunities in Kilifi County was the lack of skill sets needed. The least faced economic challenge was ownership of productive resources as evidenced by a mean response score of 3.0 on the 4-point-Likert scale. The finding implies that even though ownership of productive resources was the least faced economic challenge, it was still a major inhibitor to the participation of Kilifi women in Blue Economy Value Chain opportunities. Nevertheless, all the economic challenges were ranked above 3 on the 4-point Likert scale implying that economic barriers were a significant inhibitor to the women's participation. Further, data collected through KII also paints a picture of economic barriers limiting women's participation in Blue Economy Value Chain activities. A woman leader from the Malembe women group noted:

> *"We women do not have easy access to credit facilities to improve our livelihoods. Our ability to take loans is limited to the social capital that the group can offer each of us. Banks want collateral before they can advance loans to us. The land we own is ancestral and is in the name of my husband so we are not even allowed to talk about it let alone use it as collateral to get a loan to improve my business…"*

These findings imply that women face various economic challenges that inhibit their ability to participate effectively in Blue Economy Value Chain opportunities. The major economic challenges are access to credit, lack of skills, and financial literacy, among others. The findings have a basis in the empirical study by Ojwala (2012) who noted that the financial knowledge and education of women help in determining success in business enterprise and management while enhancing women's confidence and bargaining power. Further, training creates awareness of the issues affecting women and prepares or equips them on how to tackle and sustainably maintain a balancing act that meets their needs. Khalid and Raza (2022) noted that access to finance, legal constraints, and entrepreneurial skills also play a significant role in limiting women's participation in business activities. Finally, Jahan and Khan (2016) in a paper discussing the significance of women's role in agriculture development and the lack of technical advice and skills training they receive, suggest that this affects their participation in economic activities.

Women's participation in Kilifi County

The study examined the level of women's participation in Kilifi County as far as their participation in Blue Economy Value Chain opportunities were concerned, and challenges inhibiting such participation as presented in Table 4. Women's participation was examined in terms of women's input in economic decisions, their access to information needed to

Table 4. Women's participation level.

Statements	Responses							
	Little to no input		Input into some decisions		Input to all or most decisions			
	Freq	%	Free	%	Free	%		
Input in decisions on participation level in economic activity	29	33.3	34	39.1	24	27.6		
Input in decisions on how much output generated is sold or consumed	32	36.8	40	46.0	15	17.2		
Input in decisions on how income generated is spent	27	31.0	54	62.1	6	6.9		
	Not at all		Small extent		Moderate extent		Great extent	
	Freq	%	Freq	%	Freq	%	Freq	%
Access to information for decisions	33	37.9	28	32.2	23	26.4	3	3.4
Change in living conditions of self and family	28	32.2	27	31.0	19	21.8	13	14.9
	No		Yes					
	Freq	%	Freq	%				
Ownership of productive assets	68	78.2	19	21.8				
Participation in community leadership	53	60.9	34	39.1				

make economic decisions, their living conditions and that of the household, their ownership of productive assets and their participation in leadership positions in the community. Input in economic decisions was based on a 3-point scale (where 1 was little to no input, 2 was input in some decisions and 3 was input in all or most decisions). 72.4 % of the women in the household survey reported that they either had little to no input or some input in decision-making on the economic activity they participated in. The finding implies that the input in most decisions regarding the participation rate of Kilifi women in Blue Economy Value Chain opportunities was made by someone else other than them. Further, 82.8 % of the women in the household survey revealed that they either had little to no input or some input in decisions about how much output is sold or consumed in the family. The findings mean that the input into decisions on use of outputs was mainly made by someone else other than them. Finally, 93.1 % of the women in the household survey had either little to no input or some input into decisions regarding how income earned from participation in Blue Economy Value Chain opportunities is spent implying the use of income tended to be decided by someone else and not women themselves.

The study also examined the level of access to information needed to make economic decisions based on the 4-point Likert scale (where 1 is not at all, 2 is to a small extent, 3 is to a moderate extent and 4 is to a great extent. The finding showed that 70.1 % of the women in the household survey reported either not at all or to a small extent implying that in most cases Kilifi women did not have access to the right information for decision-making as regards participation in Blue Economy Value Chain opportunities. Further, the study evaluated the extent to which the living standards of the Kilifi women and that of their household had changed since they began participation in Blue Economy Value Chain opportunities with 63.2 % reporting either not at all or to a small extent. This finding implies that the living standard of the Kilifi women and that of their household had not changed much even after years of their participation in various Blue Economy Value Chain opportunities. Finally, on whether the economic activity women participated in had enabled them to own productive assets in their name or jointly with their spouse, 78.2 % of women in the household survey responded with a 'no' meaning that most of them did not own productive assets either in their name or jointly with their spouses. On whether their participation in Blue Economy Value Chain activities had enabled them to participate in leadership positions in their community, 60.9 % responded with 'no' implying that most women in Kilifi County were not in leadership positions in the community. The findings revealed that Kilifi women had a low level of economic participation on all indicators adopted to measure women's participation. In this case, low levels of economic involvement meant that women's participation in the blue economy value chain was minimal, and that their future access to economic resources was therefore limited.

With regard to the first indicator 'input in economic decisions', the Kilifi women had either little to no input or some input. This means that women in Kilifi County had little input in decisions around participation in spending output and rewards from the Blue Economy Value Chain opportunities. Concerning the second indicator 'access to information', Kilifi women did not have access to the right information for decision-making as regards participation in Blue Economy Value Chain opportunities. The third indicator 'living standards', showed that the living standard of the Kilifi women and that of their household had not changed much even after years of participation in various Blue Economy Value Chain opportunities. The fourth and fifth indicators, 'ownership of productive assets' and 'participation in leadership positions' revealed that most women did not own productive assets and were not in leadership positions in the community. Therefore, Kilifi women's participation level was low; a phenomenon that is attributed to inadequate participation in all Blue Economy Value Chain opportunities. The low participation was due to several barriers including work-related, economic, cultural and religious barriers. The empirical studies point towards a nexus between participation in economic opportunities and women's participation level. Empirical studies have shown that when women have increased participation in value chains, their relative bargaining power within the household improves, leading to a greater ability to contribute to household welfare through decision-making processes related to food, nutrition, branded food items, and child education (Pandey *et al*., 2021). Further, patriarchal norms can limit women's financial decision-making within households, resulting in lower participation in financial markets, limited equity holdings, and reduced asset diversification (Luigi, 2023). Elapata and De Silva, (2018) observed that decision-making power in the fisheries sector is concentrated among males and that women's contribution towards investment decisions in the sector is poor.

Conclusion

This study examined the levels of women's participation in the Blue Economy Value Chain in Kilifi Seascape, Kilifi County, Kenya. The study established that women in the Malindi area of Kilifi participated more in Blue Economy Value Chain activities such as fish processing, trading in fish and seafood and eateries of fish and seafood. However, few women participated in fish rearing in fishponds, artisanal fishing, guiding tourists and solid waste collection. These economic activities are reserved for the men. The findings also showed that cultural barriers (i.e., early/ forced marriages, participation in-home care, patriarchal society, polygamy, childbearing and rearing, denial of education), economic barriers (i.e., skills training level, financial literacy and access to use of productive resources) and work-related challenges (i.e., unequal pay for women, assigning inferior tasks to women and assigning tasks based on sex) inhibited Kilifi women participation in Blue Economy Value Chain opportunities. As regards women's participation, the findings showed most women had minimal input in decisions regarding the economic activity they participate in, how much output generated is sold or consumed in the family and how income generated from the economic activity is used. Further, the majority of women did not have access to the right information for decision-making. Moreover, their living standards and that of their families had not improved from their participation in the economic activity. Finally, most women did not own productive assets and were not in leadership positions in the community. Therefore, the economic participation level of women in the Malindi area of Kilifi County was low given their low participation in most activities and barriers inhibiting their participation in Blue Economy Value Chain opportunities.

Implications for policy and theory

The study's findings have practical and theoretical implications for women's involvement in the Blue Economy Value Chain potential in Kilifi and beyond. The study's conclusions are important for policymakers at both the subnational (Kilifi County) and national levels. Subnational and national governments, through their ministries and agencies, should encourage women to participate in Blue Economy Value Chain activities by identifying and removing impediments. The government and stakeholders should therefore prioritise skill development, access to financial resources, gender awareness sensitization, women's rights to equitable participation, preventing retrogressive cultural and religious traditions, and preserving women's employment rights, among other things. Gender mainstreaming should be implemented in sub-national and national development policy frameworks to enable women's participation in all aspects of the economy, including Blue Economy Value Chain opportunities. The study is also important for theory, particularly in terms of women's participation in economic activities and participation. Specifically, it informs on women's participation levels and factors (cultural, religious, economic, and work-related) influencing women's participation in economic opportunities presented by the Blue Economy around the world. This research is one of the few that measure women's engagement in Kenya as a direct result of their participation in the Blue Economy. The study findings should be evaluated within the context of Kilifi County, and any application outside of Kilifi should be done with caution.

References

Abu-Bader H (2021) Using statistical methods in social science research: With a complete SPSS guide. Oxford University Press, USA. pp 305-307

Alemu A, Woltamo T, Abuto A (2022) Determinants of women participation in income generating activities: evidence from Ethiopia. Journal of Innovation and Entrepreneurship 5 (2): 11-12

Alonso C, Brussevich M, (2019) Reducing and redistributing unpaid work: Stronger policies to support gender equality. IMF Working Paper 19 (225)

Azizi F, Moradi F (2022) An analysis of barriers to women's economic participation in Iran. The International Journal of Humanities 5: 99-107

Barbier EB, Burgess JC (2019) Sustainable development goal indicators: Analyzing trade-offs and complementarities. World Development 122: 95-105

Berndt AE (2020) Sampling methods. Journal of Human Lactation 36 (2): 224-226

Biswas N (2018) Towards gender-equitable small-scale fisheries governance and development: a handbook. UN -iLibrary. pp 67-69

Bryan E, Alvi M, Huyer S, Ringler C (2024) Addressing gender inequalities and strengthening women's agency for climate-resilient and sustainable food systems. CGIAR GENDER Impact Platform Working Paper (13). Nairobi, Kenya. pp 40-49

Carpentier L, Braun H (2020) Agenda 2030 for Sustainable Development: A powerful global framework. Journal of the International Council for Small Business 1 (1): 1-10

Chacko P (2017) Women's economic participation in the region through gender equality in work: building a common agenda: Journal of the Indian Ocean Region 13 (1): 1-8

Choudhary P (2021) Empowering blue economy: From underrated ecosystem to sustainable industry. Journal of Environmental Managemen 291: 91-92

Cheruiyot J, Kimanthi M, Shabani S, Nyamu F, Gathu C, Agoi F, De Meijer (2022) Climate change poses a threat to nutrition and food security in Kilifi County, Kenya. African Journal of Primary Health Care & Family Medicine 14: 14-19

Das J (2023) Blue economy, blue growth, social equity and small-scale fisheries: a global and national level review. Studies in Social Science Research 4 (1): 38-82

de Hoop T (2020) Protocol for a scoping review of the evidence base and gaps related to women's groups in Uganda. The Evidence Consortium on Women's Groups Press 1: 56-58

Doherty B (2018) Gender equality and women's participation through Fair Trade social enterprise: Case of Divine Chocolate and Kuapa Kokoo 8: 156-163

Elapata MS, De Silva A (2018) Women's position in the blue economy. Sabaragamuwa University Press, Sri Lanka. pp 68-69

Galappaththi M, Armitage D, Collins, AM (2022) Women's experiences in influencing and shaping small-scale fisheries governance. Fish and Fisheries 23 (5): 99-100

Goddard W, Melville S (2004) Research methodology: An introduction. Juta and Company Ltd, Manchester. pp 134-135

Haimbala T (2019) Sustainable growth through value chain development in the blue economy: a case study of the port of Walvis Bay. World Maritime University Press, Sweden. pp 35-39

Haralambides H (2023) The state-of-play in maritime economics and logistics research (2017–2023). Maritime Economics & Logistics 25: 429-451

Hirsu L, Hashemi L, Quezada-Reyes Z (2019). SDG 5: Achieve gender equality and empower all women and girls. Jean Monnet Sustainable Development Goals. Policy Brief Series. RMIT University. 32 pp

Jahan N, Khan N (2016) To study the participation of farm women in various agriculture and allied activities. GPR Publication, USA 2 (2): 263-265

Karani P, Failler P (2022) Africa's blue economy strategies are integrated in planning to achieve sustainable development in national and regional economic communities (RECs). Journal of Sustainability Research 4 (3): 22-25

Katila P, Colfer J (2019) Sustainable development goals. Cambridge University Press. pp 45-49

Kenya National Bureau of Statistics (2019) Kenya population and housing census press I. pp 20-23

Khalid R, Raza M (2022) The challenging factors affecting women's entrepreneurial activities. Journal of Liberty and International Affairs 8 (1): 51- 66

Kimanjara TM (2013) Influence of micro-finance on economic participation of women: A case of Kenya Women Finance Trust, Nakuru town, Kenya. Nairobi: Unpublished Masters Thesis, University of Nairobi. 178 pp

Kothari CR (2004) Research Methodology: Methods and Techniques. 2nd Edition, New Age International Publishers, New Delhi. pp 35-42

Lendoye M (2022) The blue economy and the sustainable development goal,s and corporate social responsibility. GASP Publication 3 (3): 240-262

Luigi G (2023) From patriarchy to partnership: Gender equality and household finance. Journal of Financial Economics 147 (3): 573-595

Lütz F (2023) Gender equality and artificial intelligence: SDG 5 and the role of the UN in fighting stereotypes, biases, and gender discrimination. Springer International Publishing 9 (2): 153-180

Masreka K (2020) Gender-based economic inequalities: A review of selected concepts. Journal of Economy Culture and Society 61: 371-382

Meetei WT, Saha B (2016) Participation of women in fisheries: a study on gender issues in Manipur, India. International Journal of Bio-resource and Stress Management 7 (4): 906-914

Mohanty S, Dash P (2020) Economic opportunities in blue economy in Africa. Asia-Africa Growth Corridor: Development and Cooperation in Indo-Pacific 3 (4): 89-110

Molla S (2021). Effects of fish post harvest handling practices on socio economics of fishermen and locals: A case study of Kukuwit Landing Site, Kilifi County, Kenya. Journal of Fisheries 2: 1-28

Mose N (2024) Economic Growth and Female Participation in the Labour Market: Gender Disaggregated Data: Business and Economic Research 14 (2): 93-110

Mukhopadhyay R, Loveson VJ, Sudarsan PK (2020) Blue Economy of the Indian Ocean: Resource economics, Strategic Vision, and Ethical Governance (1st ed). CRC Press. pp 23-26

Muli SM (2020) Factors influencing women's participation in economic development pojects: a case of women enterprise fund in Kilome Constituency, Makueni County, Kenya. Unpublished Masters Thesis, University of Nairobi. 205 pp

Murunga M (2021) Towards a better understanding of gendered power in small-scale fisheries of the Western Indian Ocean. Global Environmental Change 67: 69-78

Ojwala RA (2023) Gender equality in ocean science for sustainable development: Analysis of ocean science institutions in Kenya. pp 19-22

Neyer U, Stempel D (2019) Macroeconomic effects of gender discrimination. Research Papers in Economics: Journal of Düsseldorf Institute for Competition Economics (DICE) 13 (1): 10-12

Nodirovna S, Nazirjono'g'li D (2024) The role of women in business activity and measures taken in this regard. Journal of Economy, Tourism and Service 3 (3): 66-69

Omollo O (2022). Budgeting as a tool for implementing physical development plans: An insight from Kenya 3 (7): 45-46

Omukoto O, Graham A, Hicks C (2024) Fish markets facilitate nutrition security in coastal Kenya: Empirical evidence for policy leveraging. Marine Policy (164): 106-179

Pandey V, Nagarajan HK, Kumar D (2021) Impact of gendered participation in market-linked value chains on economic outcomes: Evidence from India. Food Policy 104: 42-52

Parra-Paitan C, Verburg P (2023) Large gaps in voluntary sustainability commitments covering the global cocoa trade. Global Environmental Change 8: 102-109

Rachmawati T (2023) Economic empowerment strategy model for coastal women based on blue economy and local potential in Surabaya. International Journal of Artificial Intelligence Research 8 (2): 11-24

Riddick S (2021) Innovation in the Blue Economy. Doctoral dissertation, Duke University. pp 97-100

Rimmer SH (2017) The future of women's economic participation in the region: governance challenges and opportunities. Journal of the Indian Ocean Region 13 (1): 4-24

Sheriff M, Mash R (2022) Climate change and primary health care in Chakama, Kilifi County, Kenya. African Journal of Primary Health Care & Family Medicine 14 (1): 11-23

Tsofa B, Goodman C, Gilson L, Molyneux S (2017) Devolution and its effects on health workforce and commodities management–early implementation experiences in Kilifi County, Kenya. International Journal for Equity in Health 4 (6): 1-13

Uche C (2019). Gender issues on economic diversification through agriculture in Nigeria: A thematic discuss. American Journal of Basic and Applied Sciences 2 (80): 19-20

Vasseur L, Horning D, Thornbush M (2017) Complex problems and unchallenged solutions: Bringing ecosystem governance to the forefront of the UN sustainable development goals. Ambio 46: 731–742

Vipinkumar, P, Narayanakumar R (2017) Gender mainstreaming and impact of self-help groups in marine fisheries sector. ICAR-Central Marine Fisheries Research Institute (Indian Council of Agricultural Research) 12: 276 – 287

Williams M (2023) Financing the blue economy: impacts and implications for gender equality and women's empowerment in the global south. Journal of Community Mobilization and Sustainable Development 17 (3): 763-769

Original Article

Spatio-temporal variation of macroalgal assemblages in southwestern Madagascar

Claudia Rodine[1]*, Henitsoa Jaonalison[4], Jean M. Kira[1], André Rakotoarimanana[2], Lantoasinoro N. Ranivoarivelo[1], Cicelin Rakotomahazo[1], Gildas B. G. Todinanahary[1], Gaëtan Tsiresy[3], Aina Le Don Nomenisoa[1], Fidèle Rakotonjanahary[1], Igor Eeckhaut[5], Mara E. Remanevy[1], Spencer Jamie[6], Richard Rasolofonirina[1], Thierry Lavitra[1]

[1] Institut Halieutique et des Sciences Marines (IH.SM), University of Toliara, 48B042 Rue Dr Rabesandratana HD, PO Box 141 - Toliara 601, Madagascar

[2] University of Toamasina, CUR Barikadimy, Building B, 4th, Toamasina 501, Madagascar

[3] University of Toamasina, ISTRCE, Box 60, Fénérive-EST 509, Madagascar.

[4] Laboratory of Evolutionary Ecology, FOCUS, University of Liège, B-4000 Liège, Belgium.

[5] University of Mons, 20 Place du Parc, 7000 Mons, Belgium.

[6] Feed-Back Madagascar NGO, Lot II M 98 i Antsakaviro, Antananarivo 101, Madagascar.

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*** Corresponding author:**
rodineclaudia@gmail.com

Abstract

The spatial and temporal distribution of macroalgal assemblages at 10 sites in the Baie de Ranobe and Baie de Toliara in southwestern Madagascar were investigated during warm (February to March) and cool (July to August) seasons. Algal species were identified and coverage estimated at six habitats between the shore and 15 m depth, based on surveys with 0.5 m x 0.5 m quadrats along transects of 30 m long by 5 m broad. Ninety eight taxa (53 red, 24 green, 21 brown) were identified of which 42 were edible. Species dominance varied by bay and season, with *Sargassum latifolium* dominating during the warm season, and *Hypnea musciformis* and *Ulva lactuca* prevalent during the cool season. Algal cover did not differ significantly between bays, but cover differed significantly between habitats. *S. latifolium*, *U. lactuca*, and *H. musciformis* were prominent on the algal shelf, *Ulva reticulata* on the inner shelf, and *Amansia rhodantha* on the outer shelf. Edible seaweed proportions increased during the cool season, particularly at algal and inner-shelf habitats. Generalized Linear Model analysis confirmed significant differences in edible algal cover across habitats and seasons. The potential of sustainably using macroalgae for aquaculture and human consumption in southwestern Madagascar is highlighted.

Keywords: species richness, species cover, edible seaweed, season, Ranobe Bay, Toliara Bay

Introduction

Madagascar, an island nation renowned for its extraordinary biodiversity and unique ecosystems, is situated in the Southwestern Indian Ocean off the coast of Africa. With an approximate land area of 587,041 Km^2, Madagascar boasts a coastline stretching over 5,603 km (Sanbar, 2015). Following its geographic isolation from Africa and India millions of years ago, Madagascar has evolved diverse flora and fauna species. Over 13,780 plant species and 2,108 animal species have been recorded, of which many are endemic to the island (CBD, 2024). Furthermore, the marine diversity surrounding Madagascar encompasses over 5,000 species, reflecting the island's ecological richness (MESUPRES, 2018).

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However, despite its natural ecosystem richness, Madagascar faces significant socioeconomic challenges. The country is consistently ranked among the world's poorest, with high levels of hunger and malnutrition. According to the Global Hunger Index (GHI) ranking in 2022, Madagascar was placed as the 119th of 121 countries in the world, highlighting the severity of the nation's socioeconomic issues (Grebmer *et al.*, 2022). Malnutrition, particularly prevalent among children, remains a major challenge, with nearly half of children under the age of five suffering from stunting (Rakotomanana *et al.*, 2016). In addition, the majority of Madagascar's population lives in extreme poverty, earning less than 2 USD per day (Razakamanana *et al.*, 2023). This disastrous situation is underscored by a low Multidimensional Poverty Index (valued at 0.4), which places Madagascar among the bottom five countries in Sub-Saharan Africa (GMPI, 2023) and highlights the need for urgent intervention (UNDP, 2022).

The southern region of Madagascar, comprised of the administrative regions of Atsimo-Andrefana, Androy, and Anosy, known as the 'Grand Sud' or 'Deep South,' is particularly vulnerable to these socioeconomic issues. With a population of approximately 2.74 million (11 % of the nation's total population), the Deep South is one of the areas with the highest rates of poverty and food insecurity and most limited access to essential services (Harrington *et al.*, 2022). Ninety percent of the population lives below the poverty line (Harrington *et al.*, 2022). Key indicators of living conditions in the region, such as poverty rates and undernourishment, are significantly worse than the national average, exacerbating the challenges faced by its inhabitants (Healy, 2018; Gondard *et al.*, 2023). Recurrent droughts due to the arid climate further compound this situation, leading to crop failures and food crises (Ralambomanantsoa *et al.*, 2023). Recently, a severe food crisis has struck the region, causing tens of thousands to face famine-like conditions (Harrington *et al.*, 2022).

To help address these challenges, the 'Institut Halieutique et des Sciences Marine' of the university of Toliara (IH.SM), in collaboration with Feedback Madagascar and Mara Seaweed Company, has launched the Global Seaweed STAR project. This initiative aims to address food insecurity in Madagascar by harnessing the nutritional potential of seaweed.

Seaweeds, also known as macroalgae, are multicellular photoautotrophic organisms predominantly found in coastal and marine ecosystems. Classified into three major groups according to the nomenclature of Algae Base, Rhodophyta (red algae), Chlorophyta (green algae), and Heterokontophyta (brown algae, class Phaeophyceae) seaweeds offer rich nutritional benefits and serve as essential resources for various industries (Mohiuddin *et al.*, 2023). With approximately 12,000 species identified so far, including nearly 500 species collected from natural sources and used locally and about 33 genera commercially farmed, seaweeds represent a promising alternative food source rich in micro- and macro-nutrients, vitamins, and other essential compounds (Akrong *et al.*, 2021).

Today, seaweed cultivation has emerged as one of the world's fastest-growing industries, with nearly 130 countries engaging in farming or harvesting seaweeds on industrial or experimental scales (FAO, 2021). The total output of seaweed production has risen significantly over the years, highlighting the economic and nutritional importance of seaweed (FAO, 2021).

Despite Madagascar's rich biodiversity, documentation of its marine flora remains inadequate, with current estimates likely underestimating the true algal diversity (Vieira *et al.*, 2021). Consequently, the aim of this study was to assess the spatial and temporal variation of seaweed assemblages in the southwest of Madagascar. By evaluating the potential of seaweed as a sustainable food source to combat food insecurity and contribute to the valorization of Malagasy seaweed, this research seeks to address pressing socioeconomic and environmental challenges facing Madagascar's coastal communities.

Materials and methods

Study area

The study was conducted in Baie de Ranobe (BR) and Baie de Toliara (BT) (Fig. 1). BR is located between 23°3'0" S and 43°33'0" E, and is limited by the Manombo river in the north and by the Fiherenana river in the south. The BT, situated between 23°25'0" S and 43°42'0" E, is a small bay adjacent to BR. It is located between the Fiherenana river in the north and the Onilahy river in the south. These rivers play a crucial role in transporting substantial terrigenous inputs from their respective watersheds, making them the primary contributors to lagoon sedimentation. Moreover, they have a significant influence on seawater turbidity, particularly during the rainy season.

The coral reefs present in BR and BT are classified as continental outer barrier reefs. The choice of these two bays as study areas was based on the fact that their reefs exhibit distinct characteristics compared to the reefs located farther north and south of these bays, which are classified as complex coastal barrier reef complexes (Mahafina, 2011). Additionally, these areas have received considerable attention in research and tourism due to their biodiverse marine (coral reefs, seagrasses, and seaweeds) and coastal (mangrove) ecosystems.

Five monitoring sites were established in each bay. In BR, these sites were referred to as BR1, BR2, BR3, BR4, and BR5, while the sites in BT were labeled as BT1, BT2, BT3, BT4, and BT5 (Fig. 1).

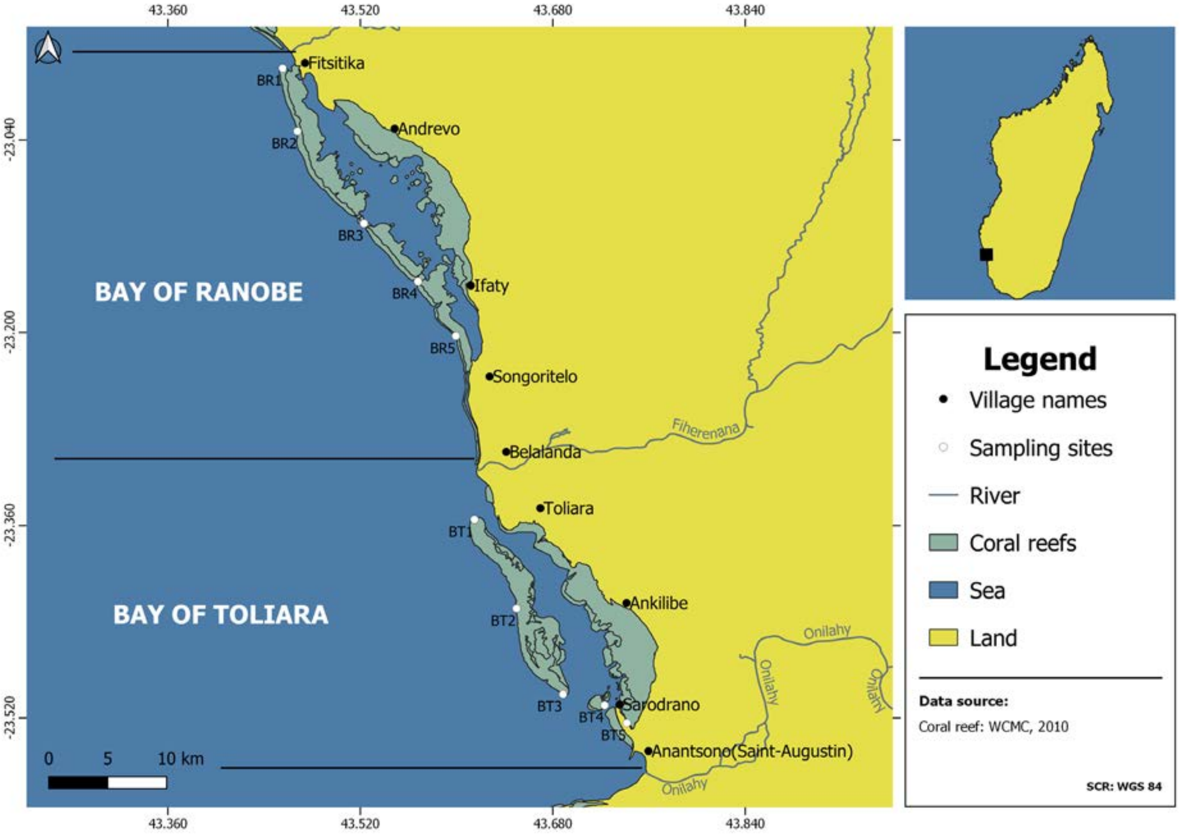

Figure 1. Location of the Baie de Ranobe and the Baie de Toliara and the studied sites.

Sample and data collection

The research was carried out during the warm season (February-March 2022) and the cool season (July-August 2022). Within each site, six habitats - littoral, inner-shelf, algal shelf and outer-shelf (0-5 m, 5-10 m and 10-15 m) - were surveyed. At each habitat, four transect lines (30 m), were haphazardly placed perpendicular to the waterline to inventory the macroalgae and to identify their distribution, with a minimum distance of 10 m between transects. Within each transect, the observation width extended to 2.5 m on both sides of the median line, giving an area of 150 m^2. Systematic identification of all encountered algae species within each transect was conducted. However, to estimate the percentage cover of algae species, six quadrats of 0.25 m^2 (0.5 m x 0.5 m) each were systematically positioned at 5 m intervals, altering between the left and right sides of the median line of the transect, from start to finish of the 30 m transect. Visual estimation and on-site scoring of the cover for each identified algal species within a quadrat were conducted, with cover recorded as rounded percentages based on visual observations. Additionally, each quadrat was photographed, serving as an observational and analytical reference for verification and further analysis. On-site identification of algae was performed macroscopically, primarily based on morphological characteristics. Uncommon specimens, not easily identifiable by divers, were initially photographed, carefully collected, and securely packaged for subsequent detailed examination in the laboratory. This approach allowed for more precise observations and analysis of these specimens. The algal identification process involved utilizing taxonomic keys (Richmond, 1997; Jha *et al.*, 2009; Pereira, 2016) and local checklists (Mollion, 2019; Vieira *et al.*, 2021) to determine the algae's taxonomic classification at the most specific level possible. The names of the identified species, along with their classifications, were cross-validated using AlgaeBase (www.algaebase.org), a trusted online resource for algae taxonomy and nomenclature.

The classification of species as edible was based on Pereira (2016), which provides insight into the functional uses of various algae.

At each site, in situ measurements of various parameters, including temperature, salinity, light, and pH, were conducted to characterize the environmental conditions. The mean salinity was 37.21 ± 1.61 psu, and the mean pH was 7.48 ± 0.07. Water temperature varied between 24.5 ± 1.25 °C (cool season) and 28.5 ± 0.51 °C (warm season), while luminosity ranged from 4,902 ± 6,181 lux (outer-shelf, 10-15 m) to 20,396 ± 36,690 lux (algal-shelf).

Data analysis

For marine macroalgae in general and edible species in particular, non-metric multidimensional scaling (nMDS) was used to visualize the spatial and temporal distribution of seaweed assemblages, i.e., between seasons (Cool and Warm), bays (BT and BR) and among habitats (Littoral, Inner-shelf, Algal-shelf, Outer-shelf 0-5 m, 5-10 m and 10-15 m). The eventual differences in macroalgal assemblages were then tested using the Analysis of Similarity (ANOSIM). When ANOSIM exhibited a significant difference, Similarity Percentage test (SIMPER) was performed to determine the taxa having the greatest contribution to the dissimilarity. Finally, Generalized Linear Model (GLM) was also used to examine differences in proportion of edible algae species among habitats. All the statistical analyses were performed with R software (version 4.2.1, R Core Team, 2021).

Results

Species richness

A total of 98 marine macroalgae taxa (53 red, 24 green and 21 brown) were observed in the coastal habitats of

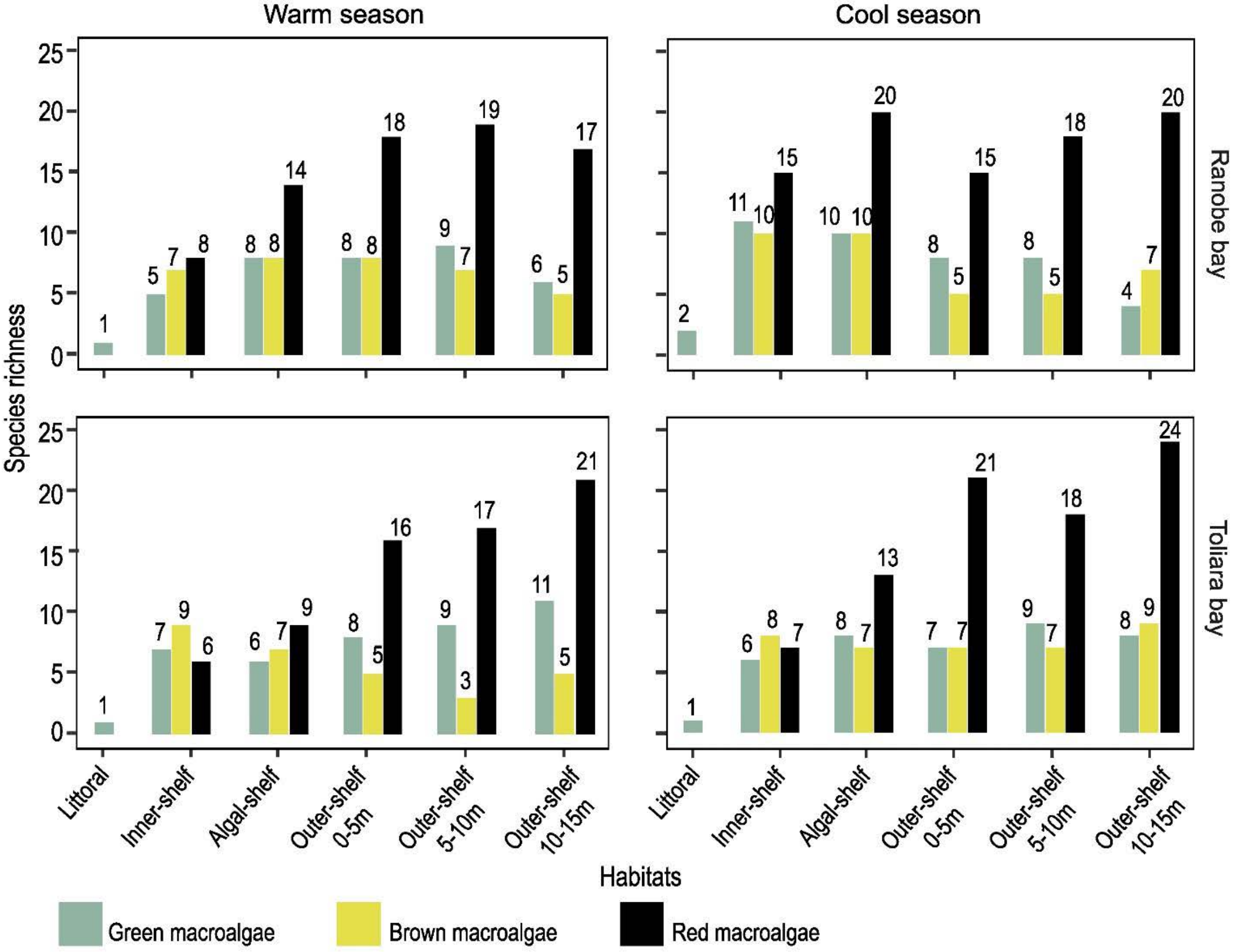

Figure 2. Spatial and seasonal distribution of algae species richness along the coastal habitats from the littoral to the reef outer-shelf at 15m depth.

Southwestern Madagascar, of which 42 are edible (16 red, 12 green and 14 brown) and 56 are not (37 red, 12 green and 7 brown, Table 1).

The species richness in terms of algae is higher in the Baie de Ranobe (89 taxa) than in the Baie de Toliara (80 taxa). The seaweed richness was spatially and seasonally dominated by the red algae (Fig. 2). Additionally, the cool season was richer in red algae species than the warm season. The highest species richness of red seaweed occurred in the algal shelf and outer-shelf, especially in the deepest outer-shelf (Fig. 2).

Table 1. Presence-Absence of algae species among habitats and seasons split by their use (edible or not) and the algae type (green, brown and red).

Color	Genus species	Seasons			Habitats					
		Edible	Cool	Warm	Littoral	inner-shelf	algal-shelf	Outer-shelf 0-5m	Outer-shelf 5-10m	Outer-shelf 10-15m
Red	*Acantophora spicifera*	Yes	1	1	0	1	1	0	0	0
	Amansia glomerata	Yes	1	0	0	1	1	1	1	1
	Amphiroa fragilissima	Yes	1	1	0	1	1	1	1	1
	Asparagopsis taxiformis	Yes	1	1	0	1	1	0	0	1
	Digenea simplex	Yes	1	1	0	1	1	0	1	1
	Euchema denticulatum	Yes	0	1	0	0	0	1	0	0
	Galaxaura rugosa	Yes	1	1	0	0	1	1	1	1
	Gelidiella acerosa	Yes	1	0	0	0	1	0	0	0
	Gracilaria corticata	Yes	1	1	0	1	1	1	1	1
	Gracilaria salicornia	Yes	1	1	0	1	1	1	1	1
	Halymenia durvillei	Yes	1	1	0	1	1	1	1	1
	Hypnea cervicornis	Yes	1	0	0	1	1	1	0	0
	Hypnea musciformis	Yes	1	1	0	1	1	1	1	1
	Hypnea pannosa	Yes	1	1	0	0	0	0	1	1
	Kappaphycus striatus	Yes	0	1	0	0	0	1	0	0
	Portieria hornemannii	Yes	1	1	0	0	0	1	1	0
Green	*Boodlea composita*	Yes	1	0	0	1	1	0	0	0
	Caulerpa laetevirens	Yes	1	0	0	0	1	0	0	0
	Caulerpa racemosa	Yes	1	1	0	1	1	1	1	1
	Codium arabicum	Yes	0	1	0	0	0	0	1	1
	Codium bursa	Yes	1	1	0	1	1	1	1	1
	Codium fragile	Yes	0	1	0	0	0	0	0	1
	Codium geppiorum	Yes	1	1	0	0	0	1	1	1
	Halimeda opuntia	Yes	1	1	0	1	1	1	1	1
	Ulva compressa	Yes	1	0	1	0	0	0	0	0
	Ulva intestinalis	Yes	1	1	1	1	1	0	0	0
	Ulva lactuca	Yes	1	1	0	1	1	1	1	1
	Ulva reticulata	Yes	1	1	0	1	1	1	1	1
Brown	*Colpomenia sinuosa*	Yes	1	0	0	1	1	0	1	1
	Dictyota dichotoma	Yes	1	1	0	1	1	1	1	1
	Dictyota friabilis	Yes	0	1	0	0	0	1	0	0
	Hormophysa cuneiformis	Yes	0	1	0	1	1	0	0	0
	Hydroclathrus clathratus	Yes	1	1	0	1	1	0	0	1
	Lobophora variegata	Yes	1	1	0	0	0	1	1	0
	Padina boryana	Yes	1	0	0	1	1	0	1	1
	Padina gymnospora	Yes	1	1	0	1	1	0	0	0
	Padina pavonica	Yes	1	1	0	1	1	1	1	1
	Sargassum crassifolium	Yes	1	1	0	0	0	1	1	1
	Sargassum ilicifolium	Yes	1	1	0	1	1	0	0	1
	Turbinaria conoides	Yes	1	1	0	1	0	1	0	0
	Turbinaria decurrens	Yes	1	1	0	1	1	1	1	1
	Turbinaria ornata	Yes	1	1	0	1	1	1	1	1

Color	Genus species		Seasons		Habitats					
		Edible	Cool	Warm	Littoral	inner-shelf	algal-shelf	Outer-shelf 0-5m	Outer-shelf 5-10m	Outer-shelf 10-15m
Red	*Acantophora muscoides*	No	1	0	0	0	1	0	0	0
	Actrinostrichia fragilis	No	1	0	0	1	1	0	0	0
	Amansia rhodantha	No	1	1	0	0	1	1	1	1
	Amphiroa anceps	No	1	1	0	1	1	1	1	1
	Amphiroa rigida	No	1	1	0	1	1	1	1	1
	Amphiroa sp1	No	1	0	0	0	0	0	0	1
	Amphiroa sp2	No	1	1	0	0	0	1	1	1
	Callithamnion stuposum	No	0	1	0	0	0	0	0	1
	Carpopeltis maillardii	No	0	1	0	0	0	1	0	1
	Cryptonemia natalensis	No	0	1	0	0	0	0	0	1
	Dasia stanleyi	No	1	1	0	0	0	1	1	1
	Dictyomenia sp	No	0	1	0	0	0	0	1	0
	Eucheuma platicladum	No	1	0	0	1	1	1	1	1
	Eucheuma sp	No	1	0	0	1	1	1	1	1
	Galaxaura obtusata	No	1	1	0	0	0	1	1	1
	Galaxaura tenera	No	1	1	0	1	1	1	1	1
	Galaxaura verprecula	No	1	0	0	1	1	0	0	0
	Gelidium pteridifolium	No	1	1	0	0	1	1	1	1
	Gibsmithia hawaiensis	No	0	1	0	0	0	0	0	1
	Gracilaria millardeti	No	0	1	0	0	0	0	1	0
	Hypnea sp	No	0	1	0	0	0	0	0	1
	Jania adhaerens	No	1	1	0	1	1	0	1	1
	Kappaphycus sp	No	1	1	0	1	1	1	1	1
	Laurencia complanata	No	0	1	0	0	0	1	1	1
	Laurencia filiformis	No	1	1	0	0	1	1	1	1
	Laurencia sp	No	1	0	0	0	1	0	0	0
	Liagora ceranoides	No	1	0	0	0	0	0	0	1
	Liagora divaricata	No	0	1	0	0	1	0	0	0
	Lithophyllum tortuosum	No	0	1	0	0	0	1	0	0
	Peysonnelia capensis	No	1	1	0	0	0	1	1	1
	Peysonnelia simulans	No	1	1	0	0	0	1	1	1
	Phacelocarpus neurymenioides	No	1	1	0	0	0	1	1	1
	Phacelocarpus tristichus	No	1	1	0	0	0	1	1	1
	Platysiphonia delicata	No	0	1	0	0	0	1	0	1
	Ptilophora sp	No	1	0	0	0	0	0	0	1
	Spyridia hypnoides	No	0	1	0	0	0	0	1	0
	Tricleocarpa fragilis	No	1	0	0	0	1	1	1	1
Green	*Boengersenia forbessi*	No	1	1	0	1	1	1	1	0
	Bryopsis myosuroides	No	0	1	0	0	0	0	0	1
	Chlorodesmis fastigiata	No	1	1	0	1	1	1	1	1
	Cladophora sp	No	1	0	0	1	1	0	0	0
	Dictyosphaeria cavernosa	No	1	1	0	1	1	1	1	1
	Dictyosphaeria versluysii	No	1	1	0	1	1	0	0	0
	Halimeda macroloba	No	1	1	0	1	1	1	1	1
	Halimeda tuna	No	1	0	0	0	0	1	1	1
	Monostroma obscurum	No	0	1	0	0	0	0	0	0
	Udotea sp	No	1	0	0	0	0	1	1	1
	Valonia fastigiata	No	1	1	0	0	1	1	1	1
	Ventricaria ventricosa	No	1	1	0	0	0	1	1	0
Brown	*Cystoseira myrica*	No	0	1	0	1	1	0	0	0
	Desmia sp	No	1	0	0	1	0	0	0	0
	Dictyota humifusa	No	1	1	0	0	0	1	1	1
	Dictyota liturata	No	0	1	0	0	0	0	1	0
	Sargassum densifolium	No	0	1	0	0	1	0	0	0
	Sargassum latifolium	No	1	1	0	1	1	1	1	1
	Stypopodium multipartitum	No	1	1	0	0	1	0	0	1

Species cover

The species with a cover of more than 6 % in both sites are presented in Figure 3 for each season. During the warm season, the species *Sargassum illicifolium*, *Sargassum latifolium* and *Hormohpysa cuneiformis* were dominant in BR (with cover more than 20 %) while *S. latifolium*, *Sargassum densifolium* and *Amansia rhodantha* were dominant in BT. The dominant species during the cool season in BR were *Ampiroa sp2*, *A. rhodantha* and *Ulva lactuca,* while those in BT were *Lobophora variegata*, *U. lactuca* and *Hypnea musciformis*. *S. latifolium* was dominant in both bays during the warm season while *U. lactuca* was dominant in both bays during the cool season.

Spatial and seasonal variation of seaweed coverage

The nMDS ordination showed that seaweed cover differs between the seasons (Fig. 4a). An ANOSIM test revealed a significant dissimilarity between seasons (R: 0.12; p-value = 0.001). A SIMPER test demonstrated that this significant seasonal distribution was due to the high cover of *S. latifolium* (40 %) during the warm season and *H. musciformis* (20 %) and *U. lactuca* (22 %) during the cool season (Table 2). The observed separation of the two groups of samples in the warm season may reflect site-specific environmental variations between the two bays, such as differences in local hydrodynamic conditions and nutrient availability, which could influence seaweed coverage. Although the nMDS showed a slight difference in algae cover between the two bays (Fig. 4b), no significant difference was observed using ANOSIM statistics ($p > 0.05$).

Seaweed cover differs significantly between habitats, particularly the algal shelf and inner-shelf habitats that were clearly separated from the others (Fig. 5) with an ANOSIM statistical R of 0.38 and a p-value of 0.001. The SIMPER test revealed that these differences were linked to the high cover of *S. latifolium*, *U. lactuca* and *H. musciformis* in the algal-shelf, the *Ulva reticulata* in the inner-shelf and the *A. rhodantha* in the outer-shelf from 0 - 15m (Table 3).

Proportion and cover distribution of edible algae species

Among the 42 edible algae species observed during this study, the most dominant (>5 % cover) 32 species

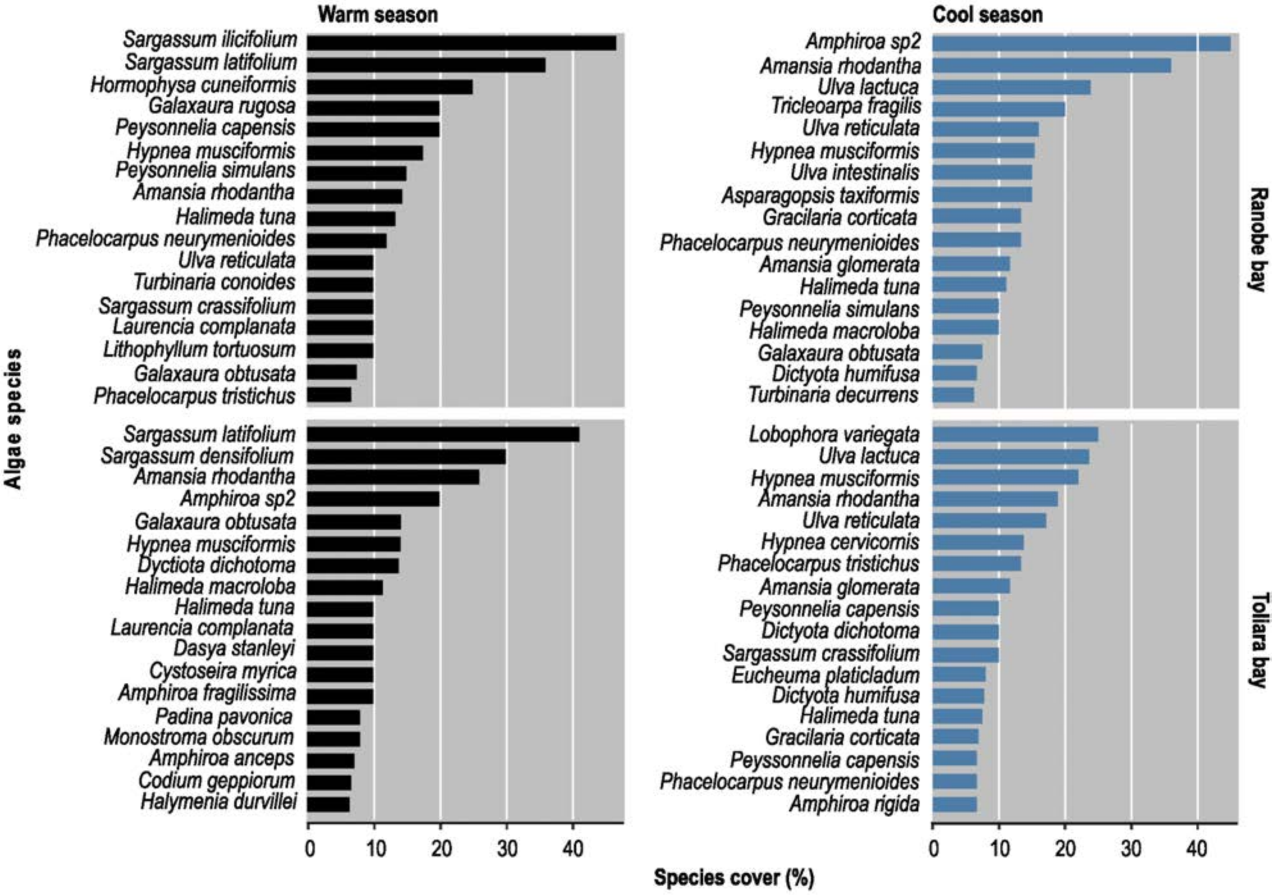

Figure 3. Species with a cover of more than 6 % in the two bays during warm and cool seasons.

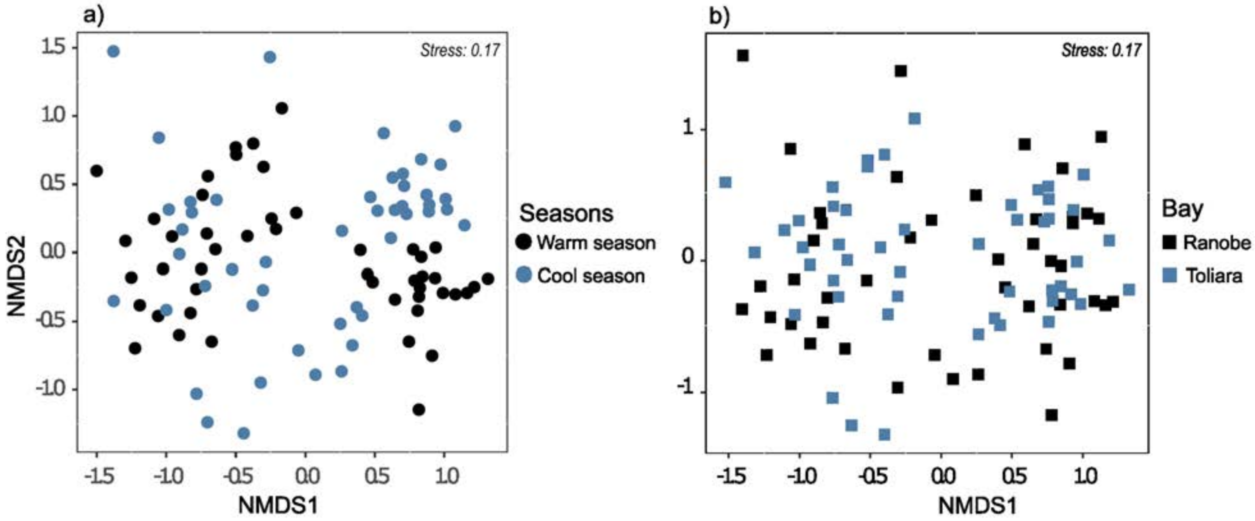

Figure 4. Seasonal distribution of seaweed cover through nMDS ordination; a) between seasons and b) between bays.

in BR and BT during the warm and cool seasons are presented in Figure 6. Dominance of edible species varies between the two bays and seasons. During the warm season, three species including *S. illicifolium, H. cuneiformis and Galaxaura rugosa* were most dominant in BR (>20 % cover) while *H. musciformis, Dictyota dichotoma*, and *Amphiroa fragilissima* are dominant in BT (>10 % cover). During the cool season, *U. lcatuca, U. reticulata and H. musciformis* were observed to be dominant in BR (>15 %) while *L. variegata*, *U. lactuca and H. musciformis* dominate in BT (>20 %). Seven species including *H. musciformis*, *U. reticulata*, *Halimeda opuntia*, *Turbinaria decurrens*, *Padina pavonica*, *Gracilaria corticata*, and *U. lactuca* were observed during the two seasons in both bays (Fig. 6).

The findings exhibit that the proportion of edible seaweed species was extremely important during the cool season. The highest proportion values during the cool season were found in the algal-shelf and inner-shelf (Fig. 7), in which more than 75 % of the recorded marine algae were edible species. Such a pattern has been observed in both the BR and BT and for all the studied sites in each bay. The Generalized-Linear Model (GLM) showed that the proportion cover of edible algae in the algal-shelf and inner-shelf differs significantly from other habitats with respective p-values of 0.049 and 0.02. On the outer-shelf, the marine algae comprised around 50 % of edible species.

The cover of edible marine algae also differed significantly between the warm and cool season (ANOSIM statistic R = 0.10, p-value = 0.001). SIMPER revealed that this difference is due to the high cover of *H. musciformis* and *U. lactuca* occurring during the cool season (Table 4). The cover of edible marine algae also significantly differed between the two bays and between different habitats, with p-values of 0.02 and 0.001 respectively. However, the ANOSIM statistic R was very low for the difference between bays (R=0.05)

Table 2. Top 10 discriminating species for each season (i.e., characteristic of each season).

Rank	Species	Warm season	Cool season	Cumulated contributions
1	*Sargassum latifolium*	0.15	0.01	8.9
2	*Hypnea musciformis*	0.10	0.14	17.6
3	*Amansia rhodantha*	0.10	0.08	25.6
4	*Ulva lactuca*	0.01	0.12	32.8
5	*Ulva reticulata*	0.02	0.06	37.0
6	*Dictyota dichotoma*	0.04	0.05	41.0
7	*Halimeda macroloba*	0.05	0.02	44.2
8	*Halimeda tuna*	0.02	0.04	47.1
9	*Galaxaura obtusata*	0.04	0.01	49.9
10	*Gracilaria corticata*	0.02	0.04	52.7

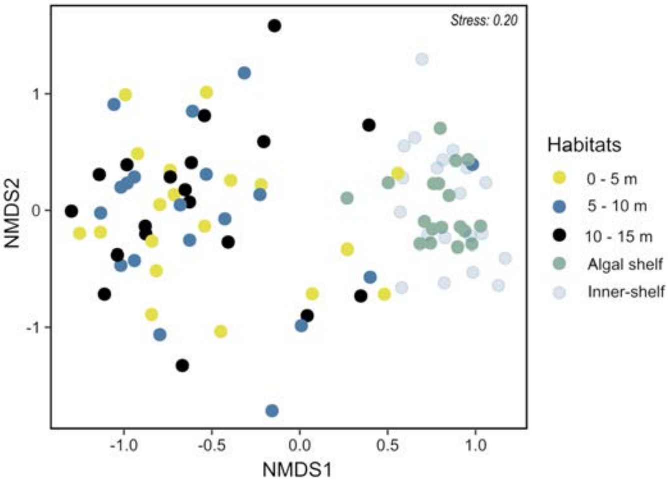

Figure 5. Ordination of the cover distribution of seaweed according to different habitats using nMDS.

while it was high among habitats (R=0.28). Based on SIMPER results, the difference in edible algae cover between habitats was mostly due to the high coverage of *H. musciformis* and *U. lactuca* on the algal shelf, *U. reticulata* on the inner-shelf, and *Dictyota dichotoma* on the outer-shelf between 5 – 15 m (Table 5).

Discussion

Seaweed species richness: Contrasting Southwest Madagascar with national diversity

In this study, 98 marine algae taxa were identified in the Southwestern region of Madagascar, consisting of 53 red, 24 green, and 21 brown species. In contrast, Mollion (2019) documented 69 species within the same Southwestern region, which included 35 red, 18 green and 16 brown species. The present study discovered additional 29 species comprising 18 red, 6 green and 5 brown likely due to a more comprehensive sampling approach. Mollion's study focused only on the fringing and barrier reefs without specifying the sampling period, while the current study covered a broader range of morphological areas and depths. These included the survey of the littoral, inner-shelf, algal shelf, and outer-shelf (0-5 m, 5-10 m, and 10-15 m) areas across the two bays, covering two distinct seasons. Furthermore, while Mollion identified a total of 92 species across multiple regions of Madagascar—including the Southwestern, Southern, Southeastern, and Northeastern regions, such as Saint Marie —the findings reported on here specifically reflect the diversity present in the Southwestern region alone. In contrast, Vieira *et al*. (2021) reported a total of 442 algae species in Madagascar, with 241 red, 116 green, and 85 brown algae. This national dataset highlights significant variations in seaweed distribution across different regions of Madagascar, underscoring the complexities of marine biodiversity that cannot be fully captured in localized studies alone. As an example, Mollion (2019) recorded 16 species in the Southern and Southeastern regions of Madagascar that this study did not identify in the Southwestern region. These include three species of green algae (*Bryopsis sp*, *Caulerpa taxifolia* and *Codium duthiae*), two species of brown algae (*Ecklonia sp* and *Stypopodium sp*) and 11 species of red algae (*Botryocladia madagascarensis*, *Cryptonemia. sp*, *Gelidium madagascariense*, *Gracilaria mamillaris*, *Martensia elegans*, *Plocamium sp*, *Porphyra sp*, *Sarconema filiforme*, *Solieria sp*, *Solieria robusta* and *Yoganugia ligulatus*). Moreover, the current study revealed 27 species that were not mentioned in the study by Vieira *et al.* (2021), comprising

Table 3. The characteristic species contributing to the difference between habitats from a SIMPER test.

Rank	Species	Algal-shelf	Inner-shelf	Outer-shelf		
				0 – 5m	5 – 10m	10 – 15m
1	*Sargassum latifolium*	0.17	0.16	0.02	0.03	0.01
2	*Ulva lactuca*	0.19	0.13	0.01	0.01	0.01
3	*Hypnea musciformis*	0.26	0.20	0.06	0.06	0.04
4	*Ulva reticulata*	0.06	0.12	0.01	0.01	0.01
5	*Amansia rhodantha*	0.01	0.00	0.16	0.13	0.15

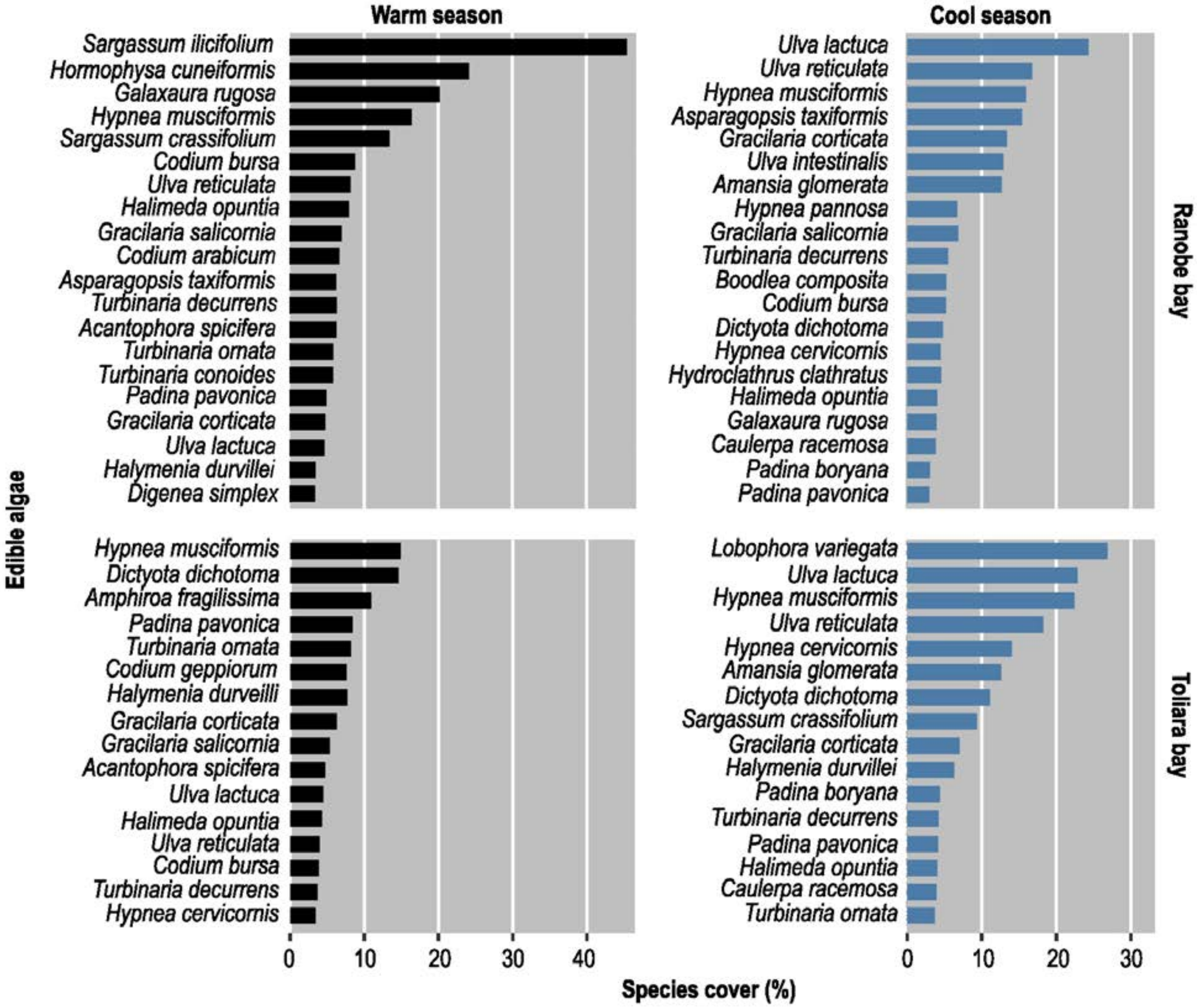

Figure 6. Dominant edible algae species (>5 %) in the BR and BT during the warm and cool seasons.

18 red, 6 green, and 3 brown algae. This divergence highlights the importance of localized studies in capturing region-specific biodiversity, as well as the need for continued research to fully catalog and understand the marine flora of Madagascar. It is noted that among the 27 species that were not reported by Vieira *et al*., 2021, 16 were already reported in the Indian Ocean Islands and 10 were observed in African countries.

Comparison of seaweed species richness: Madagascar and continental East Africa

In this section, the seaweed species richness of Madagascar with that of continental East Africa is compared, highlighting the significant biodiversity present in these regions. The 98 species identified in the present study contribute to the overall biodiversity of Madagascar's marine flora. Vieira *et al*. (2021) reported a total of 442 algae species in Madagascar, comprising 241 red, 116 green, and 85 brown algae. This species richness highlights an important aspect of Madagascar's unique marine ecosystems, although true biodiversity also encompasses factors such as species distribution and genetic variation.

According to AlgaeBase, Madagascar has a total of 606 recorded seaweed species. In comparison, Kenya has 875 species, Tanzania has 583 species, and Mozambique has 652 species (Guiry and Guiry, 2024). These figures illustrate the considerable diversity present along the African coast, which encompass distinct biogeographical regions characterized by unique seaweed communities (Bolton *et al*., 2003).

Overall, Africa hosts a total of 8,886 recorded seaweed species, with the Indian Ocean Islands accounting for 1,583 species. These comparisons emphasize the need for localized studies to understand the ecological dynamics and species distributions in Madagascar's marine environments.

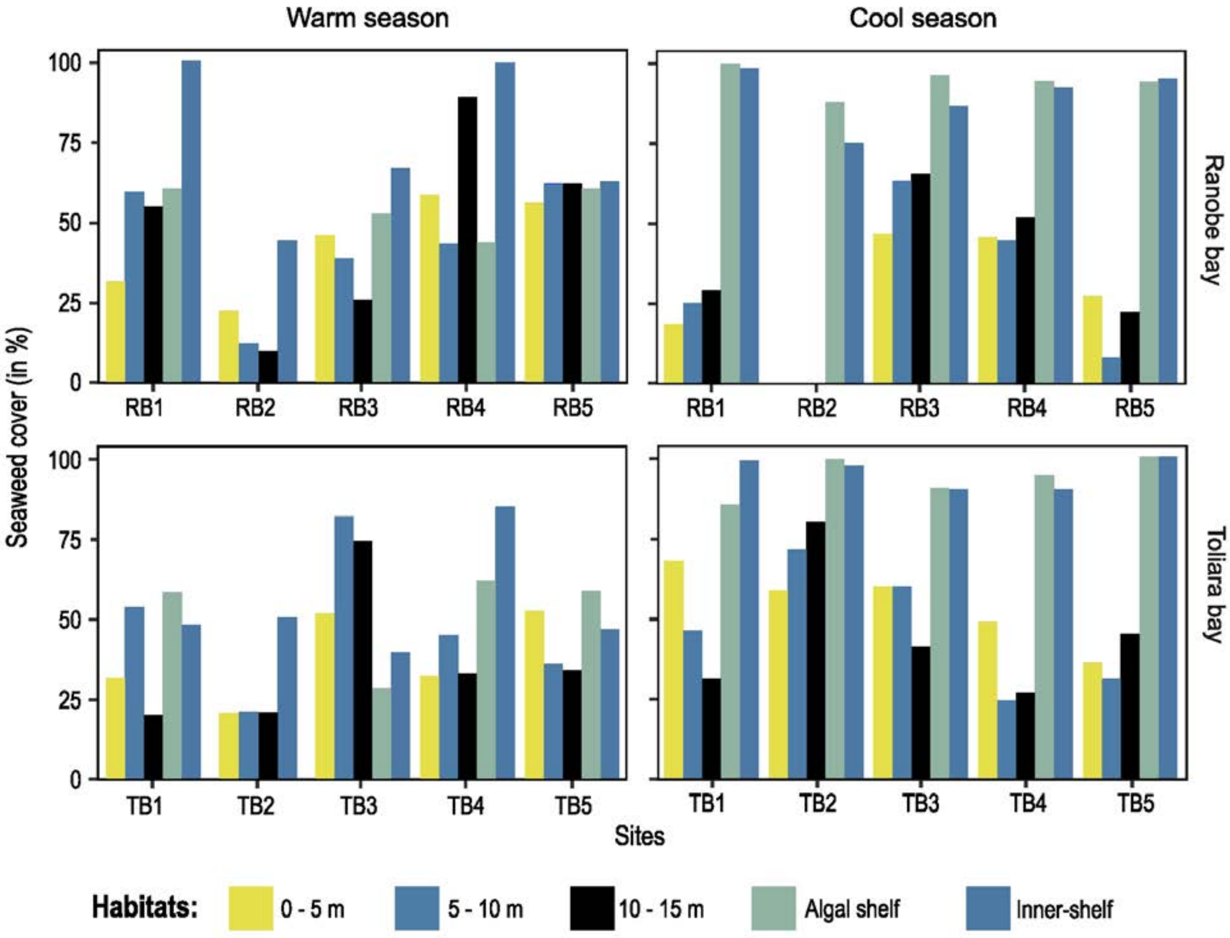

Figure 7. Spatial and seasonal variation in the proportion of edible marine algae.

Species richness among the three groups (red, green and brown)

This study highlighted that red algae are significantly richer in species compared to green and brown algae. This finding aligns with the species richness observed at the national level (Vieira *et al.*, 2021) and is consistent with results from various studies, except for that of Mushlilah *et al.*, 2021. Rhodophytes (red algae) are characteristically diverse and abundant in both tropical and temperate regions (Littler and Littler, 2003) exhibiting a wider ecological amplitude compared to the other two seaweed groups (Romdoni *et al.*, 2018). Typically, species richness of red algae is followed by the green algae and then brown algae, as seen in the results of this study. A similar trend has been reported by authors in different regions including South Africa (Bolton *et al.*, 2003), Ghana (Akrong *et al.*, 2021), NE and SE Brazil (Cavalcanti *et al.*, 2022), Atol das Rocas, Brazil (Villaça, 2010), the eastern coasts of Qeshm Island, Persian Gulf, Iran (Kobabi *et al.*, 2016), Vietnam (Nguyen *et al.*, 2013), Myanmar (Soe-Htun, 2010) and The Pari Island Reef Cluster, Jakarta, Indonesia (Zulpikar *et al.*, 2020). However, species richness of green algae is sometimes less than that of brown algae,

Table 4. Edible species contributing to the difference between seasons using the SIMPER test.

Rank	Species	Warm season	Cool season	Cumulated contributions
1	*Hypnea musciformis*	0.10	0.14	16.1
2	*Ulva lactuca*	0.01	0.12	28.0
3	*Dictyota dichotoma*	0.04	0.05	36.8
4	*Ulva reticulata*	0.02	0.06	43.8
5	*Padina pavonica*	0.05	0.01	49.3

Table 5. Edible species contributing to the difference between habitats using the SIMPER test.

Rank	Genus species	Algal-shelf	Inner-shelf	Outer-shelf		
				0 – 5m	5 – 10m	10 – 15m
1	*Hypnea musciformis*	0.26	0.20	0.06	0.06	0.04
2	*Ulva lactuca*	0.19	0.13	0.01	0.01	0.01
3	*Ulva reticulata*	0.06	0.12	0.01	0.01	0.01
4	*Dictyota dichotoma*	0.02	0.01	0.04	0.08	0.07

as reported in other studies like in Udo, Jeju Island in Korea (Kang *et al.*, 2011), Sao Miguel, in the Azores archipelago, Portugal (Neto, 2001) and the Persian Gulf (Niamaindi *et al.*, 2017).

To explore these patterns further, the Cheney ratio (R+G)/B was calculated, where R represents the number of red algae species, G the number of green algae species, and B the number of brown algae species. This study found a Cheney ratio of 3.66 in Southeastern Madagascar, which is slightly lower than the 4.2 reported by Vieira *et al.* (2021) for Madagascar as a whole. In comparison, Phu Yen Province in Vietnam reported a Cheney ratio of 3 (Hang *et al.*, 2020), while a notably high ratio of 23.6 was documented in Campeche, Mexico (Hernández-Casas *et al.*, 2024). The high Cheney index value observed in Campeche indicates a tropical affinity of its flora, as values greater than six signify tropical characteristics.

Biogeographic affinity can also be inferred from the Cheney index values. For instance, estuaries and coastal flora in Campeche exhibit Cheney indices between 6.5 and 13, further supporting their classification as tropical flora. In contrast, lower values found in the Northwest Atlantic—where ratios vary from 1.0 in Churchill, Hudson Bay (Saunders and McDevit, 2013) to 5.9 in tropical waters of Florida (Dawes and Mathieson, 2008)—indicate a transition to temperate affinities.

These comparisons illustrate the considerable diversity present along African coastlines and underscore the varying ecological dynamics and species distributions across different marine environments. Understanding these patterns provides valuable insights into the ecological dynamics of seaweed communities, particularly regarding biogeographic affinities and the importance of localized studies in characterizing marine biodiversity.

Spatial and seasonal variation of species richness

This research has revealed that the dominance of seaweed varies according to the season, bay, site, and habitat. This finding aligns with other ecological studies (Raffo *et al.*, 2014; Kobabi *et al.*, 2016; Melsasail *et al.*, 2018), which have shown that the structure and composition of macroalgal assemblages fluctuate both temporally and spatially due to seasonal variations in rainfall, salinity, nutrients, and light intensity (Kobabi *et al.*, 2016). According to Mushlilah *et al.* (2021), the distribution of macroalgae is influenced by various environmental factors, ranging from anthropogenic pressures such as the activities of local communities and tourists to the environmental parameters of the waters. Environmental parameters include wave action, substrate, and nutrients (Thakur *et al.*, 2008; Kang *et al.*, 2011; Petsut *et al.*, 2012). Dıez *et al.* (2003) have also noted that abiotic factors such as substratum, nutrients, water motion, sedimentation and pollution affect the structure and distribution of algal communities at a local scale. However, seawater temperature is considered one of the important physical factors determining seaweed distribution (Zhuang and Zhang, 2001).

The succession of seaweed assemblages over time may be attributed to the seasonal fluctuation of nutrient supplies in seawater in the study area (Kobabi *et al.*, 2016). In the present study, the dominance of Ulva species (*U. lactuca* and *U. reticulata*) was observed during the cool season and the dominance of Sargassum species during the warm season. Phillips and Hurd (2003) have reported that Ulva species are among the fast-growing algae and exhibit temporal differences, being abundant in winter and early spring but diminishing in summer (Kobabi *et al.*, 2016). However, the strategies of annual seaweeds that develop during late spring to summer in periods of low nutrient supplies, such as Sargassum species, are poorly investigated (Vaz-Pinto *et al.*, 2014). It is possible that these Sargassum species exhibit slow-growing perennial characteristics, similar to other perennial seaweeds, with low nutrient uptake rates. According to Kobabi *et al.* (2016), slow-growing perennials accumulate large nutrient pools in winter, which support their growth in spring/summer when light levels increase. While specific studies on Sargassum nutrient uptake strategies are limited, this general pattern may explain their dominance during the warm season in the current study.

Potential of seaweed for aquaculture and human consumption

During this study, seven dominant seaweed species with significant potential for aquaculture and consumption were consistently observed across the two bays (BR and BT) during the two seasons: *H. musciformis*, *U. reticulata*, *H. opuntia*, *T. decurrens*, *P. pavonica*, *Gracilaria corticata*, and *U. lactuca*. These species hold valuable nutritional properties that could address key food challenges in the southwestern part of Madagascar, a region known for its issues with food insecurity and malnutrition.

Among the species identified, *H. opuntia*, *T. decurrens*, and *P. pavonica* have been relatively underexplored in terms of large-scale farming. However, these species are rich in bioactive compounds and nutrients that are particularly beneficial in addressing dietary deficiencies. For example, *H. opuntia* has shown potential for use in enhancing human health, particularly due to its antioxidant properties, which can help protect cells from damage. Additionally, its antibacterial and antimicrobial qualities suggest its utility in promoting both human and aquaculture health (Nazarudina *et al*., 2022; Darfia *et al*., 2021; Radhika *et al*., 2022).

T. decurrens and *P. pavonica* are similarly promising. *T. decurrens* contains compounds with anticoagulant and anticancer properties, which could offer medicinal benefits alongside its nutritional potential (Shanthi *et al*., 2021; Sami and Nur, 2022). Moreover, it has shown efficacy in agricultural applications, such as controlling bacterial brown rot disease (Abd-El-Aziz, 2020), making it a candidate for addressing both health and agricultural productivity issues. *P. pavonica* has been studied extensively as a dietary supplement in fish aquaculture, with positive effects on growth and health, particularly for rabbitfish and Nile tilapia (Monier *et al*., 2022; Maghawri *et al*., 2023). Its consumption is considered safe due to its non-genotoxic and antioxidant properties (Güner, 2021), and its mineral content makes it a valuable nutritional addition to local diets.

U. reticulata, although less studied in terms of aquaculture trials, stands out for its medicinal and nutritional properties. It is rich in proteins, minerals, and iodine, making it a valuable food source for combating malnutrition, especially in regions where iodine deficiency is prevalent (Ratana-arporn and Chirapart, 2006). Its potential for managing diabetes, thanks to its ability to inhibit carbohydrate-metabolizing enzymes and promote insulin secretion, further highlights its value in addressing non-communicable diseases that may affect the population (Unnikrishnan *et al*., 2022).

The more widely cultivated species, such as *U. lactuca*, *H. musciformis*, and *G. corticata*, also demonstrate considerable nutritional potential. *U. lactuca* is rich in Ulvan, a polysaccharide known for its health benefits, including its ability to support immune function and reduce inflammation (Pappou *et al*., 2022; Dominguez and Loret, 2019). This species, already grown in various countries, could play a pivotal role in enhancing local diets by providing essential vitamins and minerals.

Similarly, *G. corticata* is recognized as a potential functional food due to its nutrient content, including essential fatty acids and proteins (Rosemary *et al*., 2019). The development of its cultivation could directly contribute to addressing protein shortages in the local population. *H. musciformis*, though lower in protein and carbohydrate content, is rich in essential minerals such as calcium, magnesium, sodium, and potassium (Balamurughan *et al*., 2013), which are vital for maintaining proper bodily functions and could help alleviate common mineral deficiencies in southern Madagascar.

In conclusion, the findings underscore the substantial nutritional potential of the identified seaweed species in the southwestern region of Madagascar. By promoting the cultivation and consumption of these species, local food challenges, including malnutrition and nutrient deficiencies, can be addressed. Further research and targeted cultivation efforts will be crucial for integrating these species into sustainable aquaculture systems and enhancing food security in the region.

Acknowledgements

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References

Abd-El-Aziz R (2020) Usage of brown algae *Padina pavonica* and *Turbinaria decurrens* to control bacterial brown rot disease. IJSRSD 3 (1): 1-18

Akrong MO, Anning AK, Addico GND, De Graft-Johnson KAA, Adu-Gyamfi A, Ale M, Meyer AS (2021). Spatio-temporal variations in seaweed diversity and abundance of selected coastal areas in Ghana. Regional Studies in Marine Science 44 (2021): 101719 [https://doi.org/10.1016/j.rsma.2021.101719]

Balamurughan M, Selvam GG, Thinakaran T, Sivakumar K (2013). Biochemical study and GC-MS analysis of *Hypnea musciformis* (Wulf.) Lamouroux. Am-Euras. Journal of Scientific Research 8 (3): 117-123 [doi: 10.5829/idosi.aejsr.2013.8.3.12071]

Bolton JJ, De Clerck O, John DM (2003) Seaweed diversity patterns in Sub-Saharan Africa. In: Decker C, Griffith C (eds) Proceedings of the Marine Biodiversity in Sub-Saharan Africa: The Known and the Unknown. Cape Town, South Africa. pp 229-241 [http://hdl.handle.net/1834/973]

Cavalcanti MILG, Sanchez PMG, Fujii MT (2022) Comparison of the diversity and biomass of beach-cast seaweeds from NE and SE Brazil, European Journal of Phycology 57 (3): 367-376 [doi: 10.1080/09670262.2021.2003867]

CBD (Convention on Biological Diversity) (2024) Country profile: Madagascar [https:// www. cbd. int/ countries/ profile/? country= mg]

Darfia, Kasmiati, Latama G (2021) Antibacterial activity and identification of active compounds of seaweed extract *Sargassum sp*., *Halimeda opuntia* and *Halymenia sp*. from Lae-Lae Island of South Sulawesi. International Journal of Environment, Agriculture and Biotechnology 6 (6): 187-196 [https://dx.doi.org/10.22161/ijeab.66.23]

Díez I, Santolaria A, Gorostiaga J (2003). The relationship of environmental factors to the structure and distribution of subtidal seaweed vegetation of the western Basque coast (N Spain). Estuarine, Coastal and Shelf Science 56 (5-6): 1041-1054 [doi:10.1016/s0272-7714(02)00301-3]

Dawes CJ, Mathieson AC (2008) The seaweeds of Florida. University Press of Florida, Gainesville, FL. 591 pp

Dominguez H, Loret EP (2019) *Ulva lactuca*, a source of troubles and potential riches. Marine Drugs 17: 357 [doi:10.3390/md17060357]

FAO (2021) The global status of seaweed production, trade and utilization. Globefish Research Programme 124. Rome. 120 pp

Filiezadeh Y (2002) Cultivation of Gracilaria (*Gracilaria corticata*) in the Qeshm Island. AquaDocs [http://hdl.handle.net/1834/12695]

Ganesan M, Thiruppathi S, Jha B (2006) Mariculture of *Hypnea musciformis* (Wulfen) Lamouroux in South east coast of India. Aquaculture 256: 201-211 [doi:10.1016/j.aquaculture.2006.01.039]

GMPI (Global Multidimensional Poverty index) (2023) Unstacking global poverty: Data for high impact action. United Nations Development Program and Oxford Poverty and Human Development Initiative. 26 pp

Gondard C, Tantely, Andrianantoandro VT, Cachau D, Delpy L, Droy I, Lallau B, Ralibera N, Ramiaramanana M, Rasolofo P, Razafindraibe M (2023) Le développement dans le Grand Sud malgache, Quelques enseignements de 30 ans de projets de développement: Étude de capitalisation bibliographique. Bordeaux Sciences Economiques, Université de Bordeaux, UMI Source, IRD [hal-04218183v2ff]

Grebmer KV, Bernstein, JB, Wiemers, MW, Reiner LR, Bachmeier MB, Hanano AH, Towey OT, Ní Chéilleachair RNC, Foley CF, Gitter SG, Larocque GL, Fritschel HF (2022) Global Hunger Index. Food systems transformation and local governance. Welt Hunger Hilfe & Concern Worldwide, Bonn/Dublin

Guiry MD, Guiry GM (2024) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway [https://www. algaebase.org]

Güner A (2021) In vitro risk assessment of *Padina pavonica* (Linnaeus) (Brown algae). Food and Health 7 (1): 31-38 [https://doi.org/10.3153/FH21004]

Hang NTT, Tu NV, Phu VV, Lam NN (2020) Species composition and distribution of seaweeds in Phu Yen province. Vietnam Journal of Marine Science and Technology 20 (4A): 69-80 [doi: https://doi.org/10.15625/1859-3097/15614]

Harrington LJ, Wolski P, Pinto I, Ramarosandratana AM, Barimalala R, Vautard R, Philip S, Kew S, Singh R, Heinrich D, Arrighi J, Raju E, Thalheimer L, Razanakoto T, Van Aalst M, Li S, Bonnet R, Yang W, Otto FEL, Van Oldenborgh GJ (2022) Limited role of climate change in extreme low rainfall associated with southern Madagascar food insecurity, 2019–21. Environmental Research: Climate 1: 021003 [https://doi.org/10.1088/2752-5295/aca695]

Healy T (2018) The deep south. Constraints and opportunities for the population of southern Madagascar towards a sustainable policy of effective responses to recurring droughts/emergencies: Socio-economic, historic, cultural, political, anthropological and environmental analysis of Madagascar's southern Region. The World Bank. IBRD-IDA, World Bank Group. 48 pp

Hernández-Casas CM, Mendoza-González AC, García-López DY, Mateo-Cid LE (2024) Spatio-temporal structure of two seaweeds communities in Campeche, Mexico. Diversity16: 344 [https://doi.org/10.3390/d16060344]

Jha B, Reddy, CRK, Thakur, MC, Rao, MU (2009) Seaweeds of India: The diversity and distribution of seaweeds of

Gujarat Coast 3. Springer Science & Business Media. Springer, Dordrecht, Heidelberg, London, New York. 215 pp [doi 10.1007/978-90-481-2488-6]

Kang JC, Choi HG, Kim MS (2011) Macroalgal species composition and seasonal variation in biomass on Udo, Jeju Island, Korea. Algae 26 (4): 333-342 [http://dx.doi.org/10.4490/algae.2011.26.4.333]

Kobabi M, Yousefzadi M, Razaghi M, Feghhi MA (2016) Zonation patterns, composition and diversity of macroalgal communities in the eastern coasts of Qeshm Island, Persian Gulf, Iran. Marine Biodiversity Records 9: 96. [doi 10.1186/s41200-016-0096-4]

Littler DS, Littler MM (2003) South Pacific reef plants. Off-shore Graphics, Inc., Washington, DC. 331 pp

Maghawri A, Marzouk SS, El-Din HME, Nashaat M (2023) Effect of brown algae *Padina pavonica* as a dietary supplement on growth performance and health status of cultured *Oreochromis niloticus.* Egyptian Journal of Aquatic Research 49: 379-385 [https://doi.org/10.1016/j.ejar.2023.03.001]

Mahafina J (2011) Perception et comportement des pêcheurs pour une gestion durable de la biodiversité et de la pêcherie récifale : application au niveau des réserves temporaires du Sud-Ouest de Madagascar. Thèse de Doctorat. Université de La Réunion et Université de Toliara 185 pp [http://hdl.handle.net/1834/9447]

Melsasail K, Awan A, Papilaya PM, Rumahlatu D (2018) The ecological structure of macroalgae community (seagrass) on various zones in the coastal watersof Nusalaut Island, Central Maluku District, Indonesia. AACL Bioflux 11(4): 957-966 [http://www.bioflux.com.ro/aacl]

MESUPRES (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique) (2018) Plan directeur de recherche en sciences marines. 74 pp

Mohiuddin M, Banik U, Iqbal MZ, Chamily FA, Rahman MM, Nahiduzzaman M, Wahabc MA Rahman MA, Asaduzzaman M (2023) Influence of cultivation systems and associated environmental factors on the growth performance of *Hypnea musciformis* seaweed at the south-east coast of the Bay of Bengal, Bangladesh. Aquaculture Reports 32: 101718 [https://doi.org/10.1016/j.aqrep.2023.101718]

Mollion J (2020) The seaweed resources of Madagascar. Botanica Marina 63 (4): 97-104 [https://doi.org/10.1515/bot-2018-0040]

Monier MN, Abdelrhman AM, Marzouk SS, Nashaat M,Eissa HA, El-Din HME, Goda AMAS (2022) The potential role of marine macroalgae *Padina pavonica* on growth performance, histological status, and resistance of the rabbitfish *Siganus rivulatus* to *Pseudomonas anguilliseptica* bacteria. Egyptian Journal of Aquatic Biology & Fisheries 26 (5): 797-819

Mushlilah H, Amri K, Faizal A (2021) Diversity and distribution of macroalgae to environmental conditions of Makassar City. Jurnal Ilmu Kelautan. SPERMONDE (2021) 7(1): 16-26. P-ISSN: 2460-0156. E-ISSN: 2614-5049

Nazarudina MF, Yasin ISM, Mazli NAIN, Saadi AR, Azizee MHS, Nooraini MA, Saad N, Ferdous UT, Fakhrulddin IM (2022) Preliminary screening of antioxidant and cytotoxic potential of green seaweed, *Halimeda opuntia* (Linnaeus) Lamouroux. Saudi Journal of Biological Sciences 29: 2698-2705 [https://doi.org/10.1016/j.sjbs.2021.12.066]

Neto AI (2021) Macroalgal species diversity and biomass of subtidal communities of São Miguel (Azores). Helgol Mar Res (2001) 55:101–111. [DOI 10.1007/s101520100074]

Nguyen TV, Le NH, Lin SM, Steen F, De Clerck O (2013) Checklist of the marine macroalgae of Vietnam. Botanica Marina 56 (3): 207-227 [doi10.1515/bot-2013-0010]

Niamaindi N, Bahmyari Z, Sheykhsagha N, Kouhgardi E, Vaghei RG (2017) Species diversity and biomass of macroalgae in different seasons in the northern part of the Persian Gulf. Regional Studies in Marine Science 15: 26-30 [http://dx.doi.org/10.1016/j.rsma.2017.07.001]

Pappou S, Dardavila MM, Savvidou MG, Louli V, Magoulas K, Voutsas E (2022) Extraction of bioactive compounds from *Ulva lactuca*. Applied Science 12: 2117 [https://doi.org/10.3390/app12042117]

Peng J, Min S, Qing P, Yang M (2021) The impacts of urbanization and dietary knowledge on seaweed consumption in China. Foods 10: 1373 [https://doi.org/10.3390/foods10061373]

Pereira L (2016) Edible seaweeds of the world. International Standard Book Number 13: 978-1-4987-3050-1 (eBook - PDF). 453 pp

Petsut N, Chirapart A, Keawnern M (2012) A stability assessment on seasonal variation of seaweed beds in the Trat peninsula of Thailand. Biodiversity Journal 3 (3): 229-236 [ISSN: 20390408]

Phillips JC, Hurd CL (2003) Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilisation in relation to shore position and season. Marine Ecology Progress Series 264: 31-48

R Core Team. 2021. R: a language and environment for statistical computing (version 4.2.1).Vienna, Austria: R Foundation for Statistical Computing.

Radhika RSR, Gayathri S, Gobalakrishnan M (2022) Marine biomolecule mediated synthesis of selenium nanoparticles and their antimicrobial efficiency against fish and crustacean pathogens. Research Square. 14 pp [https:/ /doi.org/10.21203/rs.3.rs-1594624/v1]

Raffo MP, Lo RV, Schwindt E (2014) Introduced and native species on rocky shore macroalgal assemblages:

zonation patterns, composition and diversity. Aquatic Botany 112: 57-65

Rakotomanana H, Gates GR, Hildebrand D, Stoecker BJ (2016) Determinants of stunting in children under 5 years in Madagascar. Maternal Child Nutrition: e1 2409. 10 [doi 10.1111/mcn.12409]

Ralambomanantsoa TF, Ramahatanarivo ME, Donati G, Eppley TM, Ganzhorn JU, Glos J, Kübler D, Ratovonamana YR, Rakotondranary JS (2023) Towards new agricultural practices to mitigate food insecurity in southern Madagascar. In: Dormann CF, Batáry P, Grass I, Klein AM, Loos J, Scherber C, Stean-Dewenter I, Wanger TC (eds) Defining agroecology – A festschrift for teja tscharntke. Hamburg. pp 205-222

Ratana-arporn P, Chirapart A (2006) Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata.* Kasetsart Journal (Natural Science) 40 (Suppl.): 75-83

Razakamanana MV, Andriannatoandro VT, Ramiandrisoa TO (2023) Do public health expenditures affect maternal and child health in Madagascar? Health Economics Review 13: 47 [https://doi.org/10.1186/s13561-023-00462-7]

Richmond MD (1997) A guide to the seashores of Eastern Africa and the Western Indian Ocean Islands. SIDA/Department for Research Cooperation, SAREC, Stockholm. pp 448

Romdoni TA, Ristiani A, Meinita MDN, Marhaeni B (2018) Seaweed species composition, abundance and diversity in Drini and Kondang Merak Beach, Java. E3S Web of Conferences 47. EDP Sciences: 03006

Rosemary T, Arulkumar A, Paramasivam S, Mondragon-Portocarrero A, Miranda JM (2019) Biochemical, micronutrient and physicochemical properties of the dried red seaweeds *Gracilaria edulis* and *Gracilaria corticata.* Molecules 24: 2225 [doi:10.3390/molecules24122225]

Sami FJ, Nur S (2022) Isolation, identification and biochemical studies of gallic acid from *Turbinaria decurrens* Bory. Current Research on Biosciences and Biotechnology 4 (1) 238-241 [doi: 10.5614/crbb.2022.4.1/X7DD09RY]

Sanbar S (2015) Environmental law in Madagascar: The Nagoya Protocol on genetic resource use, access and benefit sharing. Independent Study Project (ISP) Collection: 2176 [https://digitalcollections.sit.edu/isp_collection/2176UNDP, 2020]

Saunders, GW, McDevit, DC (2013) DNA barcoding unmasks overlooked diversity improving knowledge on the composition and origins of the Churchill algal flora. BioMed Central Ecology 13: 9

Setyati WA, Susanto AB, Pamungkas DBP, Makrima DB, Senduk JL (2023) Isolation and identification of seaweed-associated bacteria and their antibacterial activity against skin disease agents. Trends in Sciences 20 (6): 6517 [https://doi.org/10.48048/tis.2023.6517]

Shanthi N, Arumugam P, Murugan M, Sudhakar MP, Arunkumar K (2021) Extraction of fucoidan from *Turbinaria decurrens* and the synthesis of fucoidan-coated AgNPs for anticoagulant application. ACS Omega 6: 30998-31008 [https://doi.org/10.1021/acsomega.1c03776]

Soe-Htun U (2010) The seaweed resources of Myanmar. Outstanding Research Paper. 32 pp

Thakur MC, Reddy CRK, Jha B (2008) Seasonal variation in biomass and species composition of seaweeds stranded along port Okha, northwest coast of India. Journal of Earth System Science 117 (3): 211-218 [http://dx.doi.org/10.1007/s12040-008-0025-y]

UNDP (United Nation Development Program) (2022) Human development report 2021/2022 overview. Uncertain times, unsettled lives: Shaping our future in a transforming world. 1 UN Plaza, New York, NY 10017 USA. 44 pp

Unnikrishnan PS, Animish A, Madhumitha G, Suthindhiran K, Jayasri MA (2022) Bioactivity guided study for the isolation and identification of antidiabetic compounds from edible seaweed—*Ulva reticulata*. Molecules 27: 8827 [https://doi.org/10.3390/molecules2724882]

Vaz-Pinto F, Martínez B, Olabarria C, Arenas F (2014) Neighbourhood competition in coexisting species: the native *Cystoseira humilis vs* the invasive *Sargassum muticum*. Journal of Experimental Marine Biology and Ecology 454: 32-41

Vieira C, N'Yeurt ADR, Rasoamanendrika FA, D'Hondt S, Tran LAT, Den Spiegel DV, Kawai H, Clerck OD (2021) Marine macroalgal biodiversity of northern Madagascar: morpho-genetic systematics and implications of anthropic impacts for conservation. Biodiversity and Conservation 30: 1501-1546 [https://doi.org/10.1007/s10531-021-02156-0]

Villaça R, Fonseca AC, Jensen VK, Knoppers B (2010) Species composition and distribution of macroalgae on Atol das Rocas, Brazil, SW Atlantic. Botanica Marina 53: 113-122

Zhuang SH, Zhang M (2001) Biodiversity investigation. II. The biodiversity in intertidals of Yantai littoral regions. Shandong Map Publisher, Jinan. 155 pp

Zulpikar F, Handayani T, Renyaan J, Rifai H, Perisha B (2020) Species composition and distribution of tropical marine macroalgae in the Pari Island Reef Cluster, Jakarta. Omni-Akuatika 16 (2): 141-150 [http://dx.doi.org/10.20884/1.oa.2020.16.2.819]

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Original Article

Effects of environmental change on phytoplankton in Kuwait Bay, Arabian Gulf: Emerging Critical Issues

Subba V. Durvasula[1]*, Nageswara Rao Venkata Chava[2],
Ashlesha Saxena[3]

[1]Department of Biology, University of New Mexico, Albuquerque, NM, U.S.A, 8713

[2] NRI Institute of Technology, Pothavarappadu, Krishna Dt., A.P., India, 521212

[3] National Center for Polar and Ocean Research, Vasco-da-Gama, Goa, 403 804, India

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*** Corresponding author:**
seshu35@gmail.com

Abstract

Declining fluvial discharge, dust storms, salinity increases to ~44 PSU and anthropogenic activities impact the phytoplankton of Kuwait Bay, located in the hyper-arid desert climate of the north-western Arabian Gulf. Historical trends in phytoplankton dynamics in the bay are reviewed, which include decadal changes in phytoplankton communities and episodic algal blooms. The number of identified dinoflagellate species has increased from 45 to 213 since 1931, with increases in six categories of potentially toxigenic species, of which *Gymnodinium catenatum*, *Karenia papilionacea* and *Pyrodinium bahamense* pose the highest risk. A decline in chlorophyll *a* between 2002 and 2020, despite available nutrient sources, likely contributed to the decline in Arabian Gulf fisheries. Apart from declining pelagic fish catches, several mass fish mortality events have been reported for mullets, sobaity seabreams and sea cucumbers. Experimental manipulation of offshore Kuwait surface waters (~42 PSU) to salinities of 32, 37, 42 and 50 PSU resulted in phytoplankton bloom proportions in four days at 32 and 37 PSU. The measured trends in key ecosystem variables together with the decline in diatoms to dinoflagellates, and mass mortalities of fish, suggest a rapidly changing structure and functioning of phytoplankton communities in Kuwait Bay. Restoration measures are suggested to improve the ecological condition of the bay and surrounding Arabian Gulf, including greater regional collaboration to reduce the flow of brine and waste water nutrients into the Gulf.

Keywords: Environmental perturbations, desalination, eutrophication, remediation, oligotrophication, Arabian Gulf

Introduction

The Arabian Gulf (AKA. Persian Gulf) is a unique, subtropical, nearly enclosed, hyper-saline inland sea in a semi-arid region (Fig.1). The Gulf is approximately 1000 km long, 200-300 km wide, 2.39 x 10^5 km^2 in area, with an average depth of 35 m and 8630 km^3 volume (Al-Yamani, 2008). To quote Brewer and Dyrssen (1985), the Gulf experiences 'extraordinary neglect and tanker traffic'. Besides the hot climate, natural perturbations affecting the Gulf include the discharge from Shatt Al-Arab River - the only freshwater source, and dust storms. Shatt Al-Arab River- the confluence of the rivers Tigris, Euphrates, and Karun contributes 35-133 km^3 y^{-1} freshwater and 62.4 x 10^6 tons y^{-1} sediment (Reynolds, 1993). Several publications (Al-Yamani *et al.*, 2007, 2008, 2021; Jones *et al.*, 2008; Ben-Hasan *et al.*, 2018) addressed the importance of the Shatt Al-Arab River flow on the Gulf marine environment. Additionally, the dust storms contribute 60-200 x 10^6 tons y^{-} aeolian dust (Al-Dousari *et al.*, 2021).

Kuwait Bay (Bay) in the north-western Arabian Gulf, is a mesotidal hyper saline semi enclosed Bay with 130 km coastline, 720 km^2 area and ~3.76 km^3 volume.

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The average depth of the Bay is 5.2 m, and its maximum is 30m. The Bay experiences natural and anthropogenic perturbations similar to those in the six Gulf Co-operation Council (GCC) countries.

The Gulf region is a harsh environment, and the high temperatures compound the stress caused by multiple perturbations that operate at multiple time scales. The present Gulf population of 43.2 million people is growing rapidly by 4 % y^{-1} and is expected to reach 59.8 million by 2050 (Le Quesne *et al.*, 2021). With an average <100 mm y^{-1} rainfall, decreasing flow from Shatt Al-Arab, Gulf Co-operation Council (GCC) countries receive much of their freshwater requirements from 1483 desalination plants that produce 9×10^9 hm^3 y^{-1}, and this figure is expected to grow substantially (Qureshi, 2020). Within the GCC, approximately 40 coastal towns with 300 wastewater treatment plants discharge 3455 hm^3 y^{-1} of which 73 % is treated and 39 % of this is reused, with the rest is discharged into the sea (Qureshi, 2020) . The socio-economic conditions in many regions of the Gulf are similar and accelerate the environmental stresses that would increase in future as a direct consequence. Common to the GCC countries, whose solid waste management practices are similar (Al-Hasawi, 1999), are the incessant anthropogenic environmental perturbations that have been on the rise and unabated for decades (Hosseini *et al.*, 2021). Intensive dredging, reclamation and infilling impact coastal habitats. Additionally, the Gulf acts as a receptacle for unintended spillage from oil tankers, discharges of ballast waters from shipping, sanitary wastewater, industrial wastes, effluents from slaughterhouses, dairy plants and mariculture operations which perturb the ecosystem (Table 1).

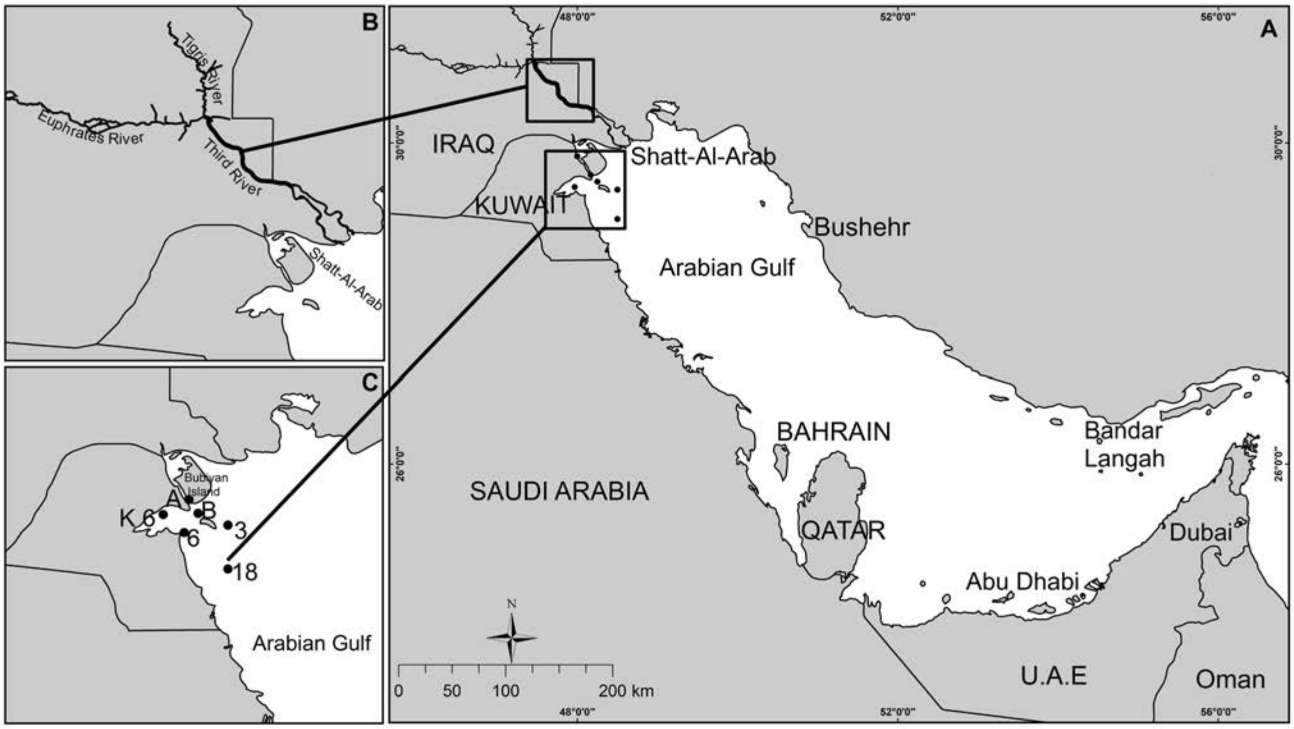

Figure 1. Location of Gulf countries, Euphrates and Tigris, Third River, marshlands of Mesopotamia (top left) and station locations in Kuwait Bay (bottom left).

To protect water quality and further environmental deterioration and habitat loss in the Arabian Gulf, GCC countries established a Regional Organization for the Protection of the Marine Environment (ROPME) in 1979. ROPME is mandated to co-ordinate efforts of the eight member countries to protect the marine and coastal environment and ecosystems. However, much of the data generated from the Gulf are disparate and disorganized spatially or temporally, compared to data from the western countries. Of all the GCC countries, Kuwait has a more organized environmental database that incorporates environmental data from its monitoring program which began in 1967. In this paper these long-term series datasets are focused on, as a representative study indicative of the environmental issues in the Gulf.

Table 1. Natural and anthropogenic perturbation impacting the Gulf ecosystem.

Category	Perturbation	Contribution y^{-1}	Reference
Natural	Shatt Al-Arab out flow	35-133 km^3 freshwater and 62.4 x 10^6 tons sediment)	Reynolds, 1993
	Evaporation	~350-800 km^3	Swift and Bower, 2003
	Incursion of Indian Ocean Surface water Sverdrup	0.10 - 0.20 0.28 Sv	Swift and Bower, 2003 Johns *et al.*, 2003
	Aeolian dust due to Dust storms	60-200 x10^6 tons	Safar, 1985
Anthropogenic	Third River (Khor Al-Zubir -Khor Al-Sabiyah	6.57 km^3 Drainage from Salt encrusted fields	Richardson *et al.*, 2005
	Brine from 1483 Desalination plants km^3	1.264	Qureshi, 2020
	Wastewater x $10^4 km^3$	2.39 average	Qureshi, 2020; Chalit, 2009; GCC wastewater Report, 2020
	Waste water discharged into sea km^3	1.09	Al-Anzi *et al.*, 2012
	Sewage km^3	1.84	Aleisa and Al-Zubari, 2017b
	Discharge from slaughterhouses, diary plants	9125 tons	Bustillo-LeCompte and Mehrvar, 2017
	Spillage of oil	160 x 10^6 t	Jacob and Al-Muzaini, 1995
	Dredging, coastline encroachments, reclamation	Details not available	

Dorgham and Moftah (1989) published the first account on the plankton of the northwest Gulf based on data assembled by the Kuwait Institute. Subsequent studies discussed the environmental conditions and phytoplankton ecology (Subba Rao and Al-Yamani, 1998, 2000), and climatic factors regulating phytoplankton (Nezlin *et al.*, 2010). Sheppard *et al.* (2010) reviewed the large-scale activities impacting this shallow Gulf, and concluded that environmental conditions within this young sea were on the decline. Dorgham (2013) identified the achievements and limitations in plankton research in the Gulf. Essential gaps exist due to irregular collection of samples, sporadic studies in localized areas, the lack of integrated studies between the GCC countries, and the absence of comprehensive regular monitoring studies in the whole Gulf. After the Sheppard *et al.* (2010) study, new information on the Gulf has been published on water quality from 1983 to 2016, (Devlin *et al.*, 2015, 2019), diatoms, dinoflagellates, flagellates, and cyanobacteria (Al-Kandari *et al.*, 2009; Al-Yamani and Saburova, 2019 a and b), winter phytoplankton (Polikarpov *et al.*, 2016), hydrography (Al- Yamani *et al.*, 2017), and algal blooms (Al-Yamani *et al.*, 2012; Al-Yamani and Saburova, 2019 a; Polikarpov *et al.*, 2020) which are included in this review.

Despite the uncertainties in the data (Dorgham, 2013), reorganization of the metadata provides a valuable means to assess the environmental perturbations and their impact on the Gulf phytoplankton ecology. Based on a meta-analysis of data collected since 2010, this review was conducted to understand the combined impact of natural and anthropogenic perturbations, and increasing salinity on phytoplankton ecology, a crucial ecosystem component. It is hypothesized that the current high temperature and high salinity regime in the Arabian Gulf, the heavy loading of nutrients and chemical elements, and the longer residence times of bay waters are causing shifts in phytoplankton biomass to the detriment of the ecosystem. It is contended that the resultant breakdown of any seasonal phytoplankton cycle and the consequential loss of pelagic fish catch is an alarming sign of the shifting structure and functioning of this unique environment. This study is used to reiterate the need for more systematic time series data collections among GCC countries and for initiation of laboratory algal culture studies aimed at *understanding* the structure and physiological functioning of phytoplankton.

Sources of data

The physical, chemical and biological data presented here are based on various publications but were not balanced with regard to sampling. Temperature and salinity plots for 2004-2014 (Al-Yamani et al., 2014) were used to show their temporal changes. A Sea Bird electronic 25 profiler was also used to obtain profiles of temperature and salinity. Nutrients were analyzed using standard methods with an automated Auto-analyzer- Skalar SANplus Segmented Flow Analyzer Model. The precision obtained was greater than 0.2 % for nitrate, 1.1 % for phosphate and 0. 2 % for silicate. Phytoplankton biomass expressed as chlorophyll *a* (chl. *a*) was determined on duplicate samples using the fluorometric method utilizing a Turner Design Fluorometer Model 10-AU. Extraction of pigments was in 15 ml 90 % acetone + 5ml DMSU, in dark at 0 °C for 24 h (Barnes *et al.*, 1992). Water samples preserved with acidified Lugol solution were used for enumeration of phytoplankton by the Utermöhl method. These are described by Al-Yamani *et al.* (2006, 2012, 2017), Al-Yamani and Saburova (2019 a and b), Al-Said *et al.* (2017), Al-Kandari *et al.* (2009), and Polikarpov *et al.* (2016). Taxonomic nomenclature is based on WoRMS Editorial Board (2023).

Additionally, remotely sensed ocean color data from January 2002 to December 2002 were used for chlorophyll *a* estimations. Ocean color data was downloaded from Ocean color https://www.oceancolour.org site. The Ocean Color data sets consist of a time-series of merged and bias- corrected MERIS, MODIS Aqua, and SeaWiFS data at 4 km-by 4 km resolution (https://oceancolor.gsfc.nasa.gov.). Chlorophyll *a* data was processed using QGIS and ArcGIS Pro software for Geometric correction, and calibration. Data are downloaded in NetCDF format; the NetCDF file was converted to GeoTIFF format using QGIS. Final processing and analysis are performed in ArcGIS Pro for the chl. *a* distribution and final cartographic products are generated for 2020.

Results and Discussion

Natural perturbations

a) Fluvial discharge from Shatt Al-Arab

Strong inter annual variations in nutrient loading can be seen due to variations in riverine flow. The Shatt Al-Arab fluvial outflow contributes to the nutrient distribution (µ mol) in the Gulf (Table 2); nutrients were high and ranged from 1.82-7.07 µ mol PO_4-P, 365.9 – 733.8 µ mol NO_3-N, and 135.6 -306.9 SiO_2 -Si (Talling, 1980; Al-Ansari *et al.*, 2019; Al- Said *et al.*, 2017; Saad, 1985). An earlier study showed that in the Kuwait Bay, nutrients were not exhausted during 1985-89 when there was good river discharge; of the 28 instances, only on two occasions in Kuwait Bay waters and three instances in the offshore, SiO_2-Si was <11.32 **µ**m and chl. *a* ranged between 5.4 to 9.9 mg chl. *a* m^2 (Subba Rao and Al-Yamani, 1999). When freshwater inflow was moderate the SiO_2-Si: salinity (SiO_2:PSU) is usually about 1 and can be used as a tracer of freshwater. During 1997 the mean SiO_2: PSU in the Bay (sampling station K6) was 2.92 (Al-Yamani *et al.*, 2006) and the mean for 2006-2023 at station Z04 (located~ 48 ^{0} E and ~29 ^{0}.50N in the vicinity of K6) was 0.68 (Devlin *et al.*, 2015) suggesting a decrease in the flow of freshwater.

Nutrients in the Bay varied between 0-5.91 PO_4-P, 0 - 111.27 NO_3 N and 0.05-68.96 SiO_2-Si and are higher than those reported for 1983-2013 (Devlin *et al.*, 2015) (Table 2). A compilation of nutrient levels in the Gulf (Shatt Al-Arab, Basrah Estuary, Kuwait, Iran-Busheir, North of Iran, South of Iran, Qatar, UAE, Hormuz Strait, Gulf of Oman) shows existence of a large variation in the nutrient levels; i.e., 0-6.01 PO_4-P, 0-103.5 NO_3-N and 0-306.9 SiO_2-Si (Subba Rao and Al-Yamani, 2000). Compared to the northern Kuwait waters, nutrient levels in the south, near Hormuz and the Sea of Oman are low (Ismail and Al Shehhi, 2022; Emara, 2010) possibly due to reduced freshwater admixture. Besides the contribution by Shat Al-Arab, in these tidally well mixed shallow Gulf waters, the multiple sources of anthropogenic enrichments (Table 1) obliterate any seasonal trends, and account for large variations in nutrients.

Various sources contribute to elemental loadings to the Bay (Table 3). Kuwait surface water samples yielded high levels (µM) of Fe 670-28160, Cu 10260-23250, Ni 15.63- 23.25, Co 50-1340, Zn 5140-25330 and Mn 0.91-1.09, generally higher than in the offshore (Al-Said *et al.*, 2018 a). Shatt Al-Arab sediments are rich in Fe, Cu, Ni, Co and Zn and their ranges and mean (µ M) correspond to 0.00067-0.028 ($\bar{x}$ 0.0144 Fe), 0.0039-0.023 ($\bar{x}$ 0.014 Cu), 0.0078-0.0248 ($\bar{x}$ 0.0163 Ni), 0.00051-0.00134 ($\bar{x}$ 0.000925Co) and 0.005-0.025 ($\bar{x}$ 0.015Zn) (Al-Said *et al.*, 2018 b). Of interest is the existence of a gradient of Fe, Cu, Ni, Co, Zn Cr, Mn, V levels in the sediments, similar to the macronutrients, with high values in the north decreasing to the south (Basaham and Al-Lihaibi, 1993).

Although there was a reduction in the Shat al-Arab flow, the high level of nutrients is attributed to increased loading associated with industrial discharges and sewage outfalls. Devlin's analysis suggests several hypotheses related to the structure and functioning of phytoplankton in the Bay but data from perturbation experiments utilizing natural or cultured algae are required. Results of an experimental study in the Northern Arabian Gulf showed high denitrification 404±78 g NO_3-N ha day (Al Ghadban *et*

Table 2. Nutrient levels (µM) in the Arabian Gulf.

Region	PO$_4$ - P	NO$_3$ - N	SiO$_2$ - Si	Reference
Shatt Al-Arab	1.82-7.07	365.9 - 733.8	NA	Talling, 1980
	1.15-6.46	41.13- 114.52	135.6-306.9	Al-Ansari *et al*., 2019. Saad, 1985
Kuwait waters	0.07-0.59	0.29- 4.60	0.10-11.32	Al-Said *et al*., 2017
(mean 2000-2012)	Traces –5.91	Traces – 111.27	0.05-68.96	Al-Said *et al*., 2017
(monthly 1997-98)	0-5.16	0-14.12	1.19-52.48	Al-Yamani *et al*., 2006
(mean 1983-2013)	0.4-0.7	2.1-3.5	25.8-33.5	Devlin *et al*., 2015
Nov-Dec 2018	~3.1	~9	30.0	Ahmed *et al*., 2022
Qatar	0.03-1.23	0.12-0.90	0.66-5.12	Dorgham and Mofta, 1989
UAE	0.07-0.84	0.17-0.54	2.14-6.26	Emara, 2010
Strait of Hormuz	0.23-0.49	0.15-0.23	0.39-0.99	Dorgham and Mofta, 1989
Gulf of Oman	0.19-0.79	0.12-0.59	1.62-5.48	Dorgham and Mofta, 1989
Sea of Oman	0.08-1.65	0.08-3.44	2.49-22.47	Emara, 2010
Hormuzgan Sea	0.82-2.15	5.2-27.3		Esmaeili *et al*., 2021

al., 2012) corroborated by Al-Yamani and Naqvi (2019) who reported high nitrite levels in the euphotic zone of the Gulf. Hypoxia occurs in the Arabian Gulf due to oxidation of dissolved organic material. Similar to denitrification, there are no estimates of the sources of organic matter. The only work using clean sampling and analytical techniques is that of Al-Said *et al*., 2018 b, who suggested that anthropogenic inputs are important for Total Organic Carbon.

b) Dust Storms

The Gulf countries experience 15-20 dust storms per year that contribute 60-200 x10^6 tons of dust km^{-2} y^{-1}. This enormous quantity of dust is deposited over the land and sea (Prakash *et al*., 2015; Al-Dousari *et al*., 2021). The annul amount of dust received varies regionally; the Kuwait region experiences on the average 373 tons (Al-Dousari *et al*., 2017). The most abundant elements delivered by dust storms (Al-Awadhi, 2005) are Fe (19.62 g·kg^{-1}) and Al (9.672 g·kg^{-1}) followed by Cr (0.4318 g·kg^{-1}) and Mn (0.3941 g·kg^{-1}) besides large quantities of Si, Zn, P, Cu and Ni. The total dust fallout into the Kuwait Bay from August 2010 to July 2011 was estimated as 94,282.0 t that contributed 4569 t iron, 12,743 t clay, 99,818 t quartz, 14,177 t sulfate, and 169.167 t of ash (Neelamani and Al-Dousari, 2016).

Shatt Al-Arab sediments act as store houses of Fe, Cu, Ni, Co and Zn, and their ranges and mean (µM) are presented in Table 3. Dredging is an ongoing coastal activity for expansion of berths, deepening the approach channel, construction of marinas, and millions of tons of dredged spoil are redistributed. The Aeolian dust swept over the bays sinks to the bottom and is buried in the sediment. The surface one-meter layer of the Gulf sediments is rich in total organic carbon (TOC), Fe, Mn, Zn, V, Cr, Cu, Ni and Pb (Al-Ghadban *et al*., 1994, Al-Sarawi *et al*., 2002).

Dredging churns up the sediments and releases stored nutrients. Of interest is the existence of a gradient of Fe, Cu, Ni, Co, Zn Cr, Mn, and V levels in the sediments with high values in the north and decreasing to the south (Basaham and Al-Lihaibi, 1993). The surface one-meter layer of the Gulf sediments is rich in TOC carbon and heavy metals (Al-Ghadban *et al*., 1994). In Kuwait Bay during summer, TOC ranged between 101 -318.4 µM with a mean 161.2 µM (Al-Said *et al*., 2018 b) and is attributed to anthropogenic input. Sediments in Sulaibikhat Bay, an offshoot of Kuwait Bay, contained large quantities of Fe, Mn, Zn, V, Cr, Cu, Ni and Pb (Al-Sarawi *et al*., 2002), and are mostly derived from sewage. Large depositions of trace elements could be toxic to phytoplankton (Paytan *et al*., 2009). The dust, rich in several trace elements, stimulated phytoplankton growth when added to surface seawater in certain quantities (Subba Rao *et al*., 1999 b).

c) Other multiple sources of enrichment

In the Gulf Countries sewage disposal is a major form of coastal pollution that increases nutrient levels. Also,

Table 3. Elements (µM) in Kuwait Bay together with associated perturbations.

			Kuwait Bay			Seawater
			Anthropogenic			
			Brine (Kuwait Bay) [1,2]	Sanitary water [3,4]	Kuwait Bay [5,6,7]	[8]
	Quantity y^{-1} → **Element ▾**		1.264 km^3	2.39 x 10^{-4} km^3	3.76 km^3	
1	Nitrogen	Range	124.3-848.9	3142.9-7142.9	0-5.63	1107.14
		Mean	486.6	5142.9	1.53	
2	Phosphorus	Range	NA	451.6-2064.5	0-15.1	2.84
		Mean	NA	1258.1	0.355	
3	Silicon	Range	1.8-21749.4	NA	NA	103.25
		Mean	10875	NA	NA	
4	Iron	Range	0.5-799.8	-	71.95-182.6	
		Mean	400.2	1.3 x 10^{-3}	145.2	6.0 x 10^{-2}
5	Manganese	Range	0.9-76.9	-	2.2x10^{-2}-2.7x10^{-2}	
		Mean	38.9	4.0 x 10^{-5}	2.5 x 10^{-2}	7.3 x 10^{-3}
6	Copper	Range	0.8-635.5	-	8.7 x 10^{-2} - 0.57	
		Mean	318.1	11016	0.29	1.4 x 10^{-2}
7	Zinc	Range	0.8-91.5	-	0.28-3.44	
		Mean	46.15	30621	1.22	7.65 x 10^{-2}
8	Cobalt	Range	NA	NA	0.29-0.42	
		Mean	NA	NA	0.38	6.6 x 10^{-3}
9	Nickel	Range	0-0.17	-	0.72-2.13	
		Mean	8.0 x 10^{-3}	1891.2	1.6	0.112
10	Molybdenum	Range	NA	NA	NA	
		Mean	NA	NA	NA	0.411
11	Cadmium	Range			4.7 x 10^{-2} -5.0 x 10^{-2}	
		Mean		186.8	4.7 x 10^{-2}	9.8 xc 10^{-4}
12	Chromium	Range	0.38-675.9		0.37-0.78	
		Mean	338.13	1538.6	0.56	3.8 x 10^{-3}
13	Lithium	Range	NA	NA	1.44-1.96	
		Mean	NA	NA	1.73	24.50
14	Vanadium	Range	NA	NA	0.55-0.64	
		Mean	NA	NA	0.59	3.7 x 10^{-2}
15	Mercury	Range	NA	-	NA	
		Mean	NA	289.15	NA	7.5 x 10^{-4}
16	Lead	Range	NA	-	NA	
		Mean	NA	1626.4	NA	1.5x 10^{-4}
17	Aluminum	Range	NA	-	NA	
		Mean	NA	1.4 x 10^{-3}	NA	3.7 x 10^{-2}
18	Strontium	Range	56.6-498.2	NA	NA	
		Mean	277.4	NA	NA	92.45
19	Barium	Range	NA	NA	NA	
		Mean	NA	NA	NA	0.153

[1] Ahmed *et al*., 2001; [2] Ahmed *et al*., 2004; [3] Enezi *et al*., 2004; [4] Aleisa and Al-Shayii, 2017a; [5] El-Anbaawy *et al*., 2018; [6] Al-Mutairi *et al*., 2014; [7] Al Said *et al*, 2018 b; [8] Anthoni, 2006. NA: Not Available

sludge from dredging the channels, discharge of ships' ballast water, slaughterhouse wastewater and effluents from mariculture operations enrich the Gulf. The annual wastewater collection in the Gulf is about 4.0 km^3 and 73 % is treated in 300 wastewater treatment plants (Qureshi, 2020). Many of the treatment facilities are either outdated or are exceeding their design capacity. Despite extreme water poverty, only 39 % of the treated wastewater is reused, and the remainder is discharged into the sea (Qureshi, 2020). Human influence enriches the Kuwait Bay with N, P, Zn, Ni, Al, Fe, and Mn (Aleisa and Al-Shayii, 2017a). Mean concentrations of NO_3 and PO_4 in the Bay were 14.9 and 52.5 **µg** $^{-1}$ (Al-Mutairi *et al.*, 2014). Estimated slaughterhouse wastewater (SWW) from the GCC countries is 9125 tons y $^{-1}$ (Qatar, 2015). Analyses of SWW (Bhunia *et al.*, 2022) showed these are rich in surfactants, volatile biosolids, chloride anions, carbon, nitrogen, heavy metals, and TOC impacts on the environment when discharged into the Gulf.

Effluents from mariculture are another potential source of nutrient enrichment. A BLOOM ECO model developed (Bhunia *et al.*, 2022) for 73 cages for Seabream fish in Kuwait Bay yielded dissolved nutrient inputs (tons y^{-1}) of 9.27 inorganic nitrogen and 0.89 inorganic phosphate and particulates as well. However, the expansion of marine cage aquaculture operations in Kuwait were halted in 2008 because of several mass fish kills and this has been stagnant with production of around 412 tons y^{-1} (Almutawa and Alfraih, 2023). Under the New Kuwait 2035 Plan, several projects are being considered to provide technical help for growing fish and shrimp by 2029. In this GCC region aquaculture is on the increase and there are 80 mariculture farms (Feidi, 2009). Cage mariculture produces organic matter that could also increase the nutrient loads up to six-fold by 2050 (Islam, 2005) which may promote an increase in harmful algal blooms (Bouwman *et al.*, 2013). There are plans to establish 200,000 tons of cage fish production in the Arabian Gulf. Based on a nutrient model Risk *et al.* (2021) concluded that the resulting nutrient footprint could affect the entire Gulf raising ecological concerns. In the Kuwait Bay with a 5.2 m average depth and a 30 m maximum depth and a tidal range 3.5 to 4.0 m (Abu-Seedo *et al.*, 1990), the water column would be well mixed facilitating nutrient distribution. This could have devastating impacts on the AG ecosystem and it is emphasized that Environmental Impact Assessment (EIA) studies are important before embarking on this ambitious goal.

Thus, multiple perturbations enrich the Bay with several elements (Table 3); their range and mean levels are very high compared to the mean (µM) 0.0056 (Cd), 0.515 (Cu), 0.146 (Fe) 0.0073 (Mn), 0.019 (Ni) and 0.512 (Zn) reported in seawater of the Bahrain environment (Juma and Al-Madany, 2008). Some of these elements could be toxic to phytoplankton and all other forms of marine life depending on their speciation, i.e., oxidation state when they are more stable (e.g., Pb 0 +2 and +4, Mo +2 to +6, Cr +3 and +6, V +2, +3, +4 and +5, Mn +2, +4, and +7, Hg+1 and+2, As +3 and +4, Cu +1, +2). As estimates of these elements are not available for Kuwait wastewaters, values given by Baawain *et al.* (2014) in Oman waters were used; overall average levels of Cd, Cr, Cu, Pb, Mo, Ni and Zn in activated sludge (RAS) corresponded to 38,139,99,39,8,34 and 2800 mg/kg, and slightly lower in recycled activated sludge. Suffice is to say that natural and anthropogenic perturbations also impact waters in other regions of the Gulf and contribute N, P, Si, Fe, Mn, Cu, Zn, Co, Ni, Mo, Cd, Cr, Li, Va, Hg, Pb, Al and Sr.

d) Salinity

Decrease in the annual discharge of the Tigris and Euphrates Rivers affected the salinity in Kuwait Bay and the Gulf as well. The flow of these rivers decreased significantly (Al-Ansari *et al.*, 2019). Analysis of flow model data indicates that the average annual flow volume (10^9 m^3) is 80 for the period 1965–1973, and decreased to 55 by 1973–1989, to 50 by 1989–1998 (Jones *et al.*, 2008) and to 20 by 2010 (Abdullah, 2016). This decline is due to stream regulation in the riparian countries (Al-Yamani *et al.*, 2017) and will have a significant effect on the closely coupled phytoplankton ecology in Kuwait Bay and the Gulf. Gulf countries depend on intense activity of water desalination and the most relevant impacts are related to discharge of the brine. Discharges amount to 7.31 km^3 y^{-1} and may increase to 29.2 km^3 y^{-1} by 2050 (Le Quense *et al.*, 2021). Kuwait discharges 1.26 km^3 y^{-1} brine (Bashitialshaaer *et al.*, 2011). Multi-stage flash (MSF) desalination plants account for 86.7 % of the desalting capacity, while reverse osmosis accounts for only 10.7 % (Qureshi, 2020). Desalination results in an increase of salt concentration to 1.5 to >2 times than that of the seawater. Brine also contains 17 elements (µM), with chloride as the highest (1101749) followed by Na (354304), Mg (57188), Ca (15350), K (12349), and carbonates (7344) (Ahmed *et al.*, 2004). Nitrate (3804), Si (7.7), Fe (3.76), Cu (4.33), Zn (0.92) and Mn (1.09) were also present in concentrations higher than in normal coastal waters (Anthoni, 2006; Duxbury *et al.* 2020) and are known to impact micro algal growth (Ahmed *et al.*, 2004). During 2002 to 2020, salinity off Kuwait increased from about 37 to 44 PSU, which affected the phytoplankton community (Al-Said *et al.*, 2017). Surface temperature also increased during 2002-2020.

Kuwait Bay is impacted by the “Third River”, a 565 km outfall drainage (Fig. 1) installed by Iraq to remedy the chronic salinity problem in the >7,750 km^2 farmland between Tigris and Euphrates Rivers (Al-Handal and

Hu, 2015). It collects wastewater and drainage from 1.5 x 10^6 km^2 salt-encrusted fields (Fig. 1) and discharges 0.018 km^3d^{-1} into the Arabian Gulf via Khor Al-Zubair and Khor Al-Sabbiya (Pearce, 1993). The international 'Basra Water Crisis" workshop 15-16 February 2024 Save the Tigris (coordinator @ savethetigris.org) discussed increase in salinity levels of Shatt al-Arab and the possible solutions to this salinity crisis. Details about the flow are not available.

Phytoplankton

There are two seasons in the Gulf: a 'cooler' December to February, and a hot April-October season. There are no distinct winter, spring, summer, and autumn seasons and therefore the local conditions influence the phytoplankton of the Gulf. In near shore Kuwait waters during 1985 to 1990, chl. *a* ranged between 1.6 to 8.1 µg chl. *a* l^{-1} (Fig. 2); biomass integrated in the column ranged from 3.8- 113.4 mg chl. *a* m^2 with the high values in the near shore (Subba Rao and Al- Yamani, 1999 a). Remotely sensed chl *a*. in the littoral Kuwait waters during 2020 ranged between 6.2 and ~8.1 µg *a* l^{-1} (Fig. 2). Albanai (2021) observed chl. *a* level (7 mg m^{-3}) during winter in Kuwait Bay that were higher than in the open Gulf (< 2.5) and <0.5 in the central Gulf. However, off Kuwait, their monthly averages for 2002 to 2020 ranged between 2.85 and 3.64 µg chl. *a* l^{-1} during June and September (Fig. 3). There was no pronounced seasonal progression; increases in chl. *a* were small and were during March-May, and August-October-December. During 2002 to 2020 as the Kuwait Bay warmed up and the salinity increased, chl. *a* decreased. These findings are consistent with earlier findings (Nezlin *et al.*, 2010); the seasonal maxima were during late summer-autumn on the western coast. Al-Naimi *et al.* (2017) pointed out that satellite data do not recognize the optical depth and the satellite sensors systematically over estimated chlorophyll; they suggested a need for a regionally calibrated algorithm to estimate chlorophyll in the Gulf.

Silica is related to river discharge and would impact diatom populations. In Kuwait Bay there have been qualitative and quantitative shifts in the dominant phytoplankton groups, particularly the halophytes (Al-Said *et al.*, 2017). In particular, perturbations in the silicon cycle caused by the decrease in river flow has impacted the growth of diatoms, and the increase in the amount of organic loading is now promoting blooms of dinoflagellates. Changes in salinity

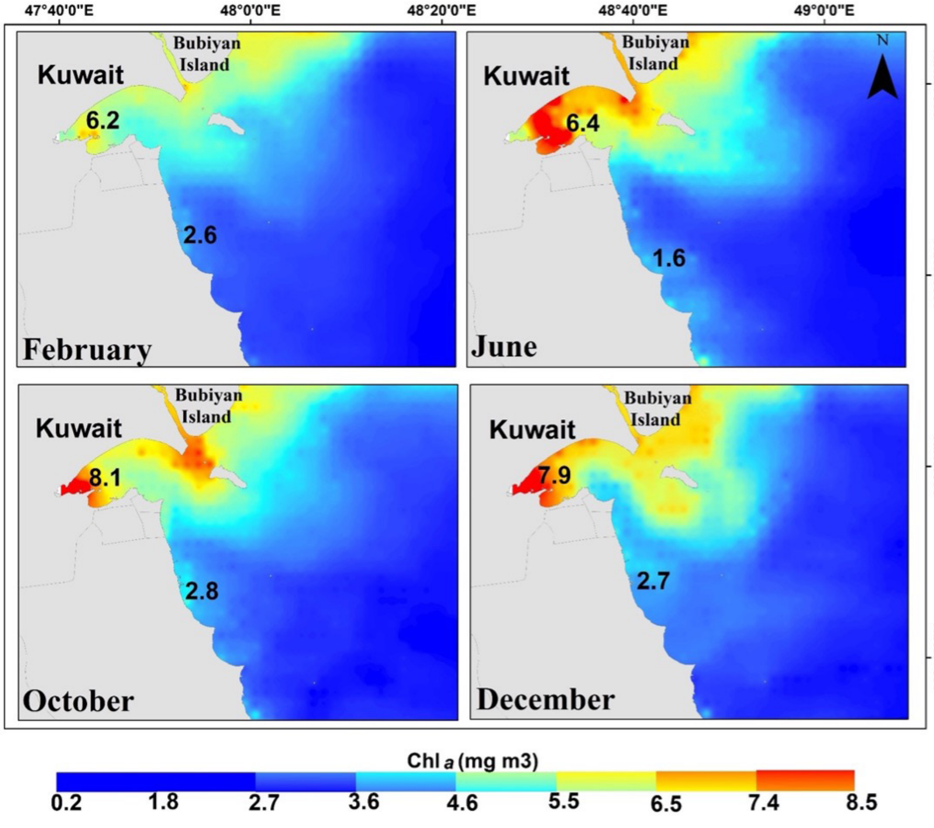

Figure 2. Distribution of average chlorophyll *a* (mg m^{-3}) in Kuwait waters during 2002 -2020. Based on *https://www.oceancolour.org site, https://oceancolor.gsfc.nasa.gov.*).

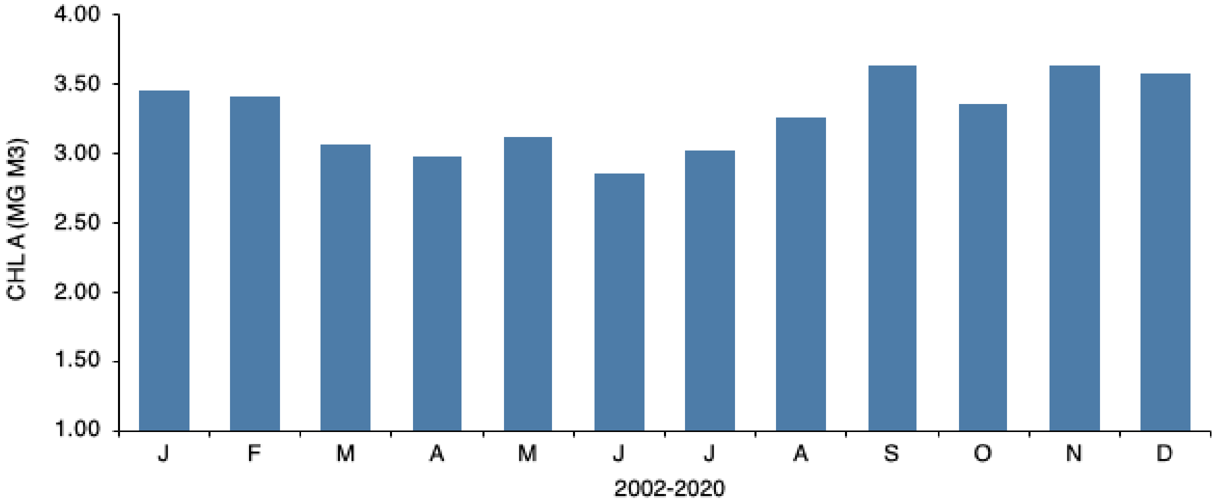

Figure 3. Distribution of average chlorophyll *a* in Kuwait waters during 2002 -2020 based on Ocean Color.

and anthropogenic perturbations seemingly impact the phytoplankton growth and species diversity in the inshore Kuwait waters. During 2000-2007 when the salinities were ~40 PSU, there were 243 diatom and 103 dinoflagellate species (Al-Yamani and Saburova, 2019 a) but by 2008 as the salinity increased to ~44 PSU their diversity decreased to 74 diatoms and 46 dinoflagellates (Al-Said *et al.*, 2017). In addition, in the offshore waters during 2005-2006 there were 108 diatom and 48 dinoflagellate species, but by 2012-2013 diatom diversity decreased to 83 species and dinoflagellates increased to 67 species. Members of Cryptophyceae and Prymnesiophyceae also increased. Such a replacement may be due to a physiological response of algae to salinity stress in these semi-arid, hyper saline, and warm Gulf waters. Brine salinity typically ranges from 1.6 to 2 times the salinity of seawater (Lee *et al.*, 2024) and part of the increase in salinity may be explained by the increased discharge of brine from desalination plants in the Gulf. Also, from thermal-based technologies brine could increase temperature to 1.37 to 1.82 times that of ambient seawater temperature (Lee *et al.*, 2024). Increased salinity and temperature resulted in a 32-60 % reduction in chlorophyll levels and total algal cell numbers in the AG waters (Omerspahic *et al.*, 2022). This corroborates with the results on osmotic and ionic effects due to NaCl resulting in the loss of activity of photosystems (Ps) I and II and loss of oxygen-evolving activity in *Synechococcus* sp. (Allakhverdiev *et al.*, 2000). Culture studies utilizing native microalgae in a salinity gradient would be necessary to establish that salinity is a crucial abiotic factor.

There is indirect evidence from Kuwait Bay to show that lowering of salinity is a highly effective stress reliever. Results of earlier microcosm experiments showed natural assemblages of phytoplankton positively responded to changes in salinity in real time. Incubations of offshore Kuwait surface water in a gradient of salinities 32, 37, 42 and 50 PSU, enriched with f/2 nutrients (0.2ml l^{-1}), showed a positive response only in samples with 32 and 37 PSU and not at salinities >37 (Madhusoodhanan *et al.*, 2018). Centric diatoms *Leptocylindrus, Lauderia, Streptotheca, Chaetoceros, Eucampia* and pennate diatoms *Thalassionema, Thalassiothrix, Nitzschia* and *Bacillaria* grew rapidly leading to a bloom in four days. At high salinities (42 and 50 PSU), algal growth was poor, indicative of their sensitivity to salinity stress. Also, results on the physiology of the chlorophyte *Clamydomonas plethora* and the naviculoid diatom *Nitzschia frustula* isolated from the coastal waters off Kuwait with 40 PSU but cultured in Atlantic Ocean water (34 PSU) medium f /2 are of interest. Results showed high growth rates μ max) of 2.5 for *C. plethora* and 3.4 for *N. frustule* (Subba Rao *et al.*, 2005). Their photosynthetic functioning measured as assimilation numbers (P^B_m: μg C [μg Chl *a*] $^{-1}$ h^{-1}): 22.8 for *C. plethora* and 18.1 for *N. frustula* and initial slopes (α^B: ng C [μg Chl *a*] $^{-1}$ $h^{-1)}$ [μmol m^{-2} s^{-1}] $^{-1}$ was also the highest observed so far, with 79.5 for *C. plethora* and 39.6 for *N. frustula* confirming their potential when cultured at lower salinity (Subba Rao *et al.*, 2005). Flash floods with a discharge 1400 of m^3 s^{-1}, exceeding the earlier records of 80 m^3 s^{-1}, lowered the salinity and promoted algal growth (4 -7 chl. *a* mg m^3) along the Kuwait coast (Alosairi *et al.*, 2019). Floodwater contributes silica and facilitates uptake of biogenic silica. Floods due to heavy rains in the winter of 1991-1992, lowered the salinity of the hyper saline waters of the Dead Sea to 70 % of their formal salinity, and a bloom of *Dunaliella parva* (15 x10^6 cells l^{-1}) developed (Oren *et al*., 1995).

Aperiodic biomass

Unlike in the temperate seas that experience clear seasonal events such as the winter overturning, upwelling and monsoons that drive phytoplankton blooms, the shallow, semi enclosed, heavily nutrient enriched waters of the Arabian Gulf are not characterized by well-defined seasonal blooms. In the Gulf, multiple processes, prevailing high temperatures, salinities, nutrients, trace elements, aeolian dust, pollutants, and ballast water introductions operate at multiple time scales. Their interplay influences their constituents, when and where ephemeral phytoplankton pulses or blooms occur, and their duration and magnitude. Results of Winder and Cloern (2010) on the diversity of constituent species and their ephemeral nature in temperate and subtropical zones are relevant here. Of their 125-time series of phytoplankton biomass, about a third of them had a series of irregular pulses of biomass but no seasonal cycle. Anthropogenic perturbations, aperiodic weather events and strong coupling between phytoplankton and herbivores in Kuwait Bay may result in a high noise to signal ratio and probably masks any seasonal cycle.

There were 31 dinoflagellates reported from the Arabian Gulf and the Gulf of Oman (Bohm, 1931) and their numbers have increased since (Table 4). Using plankton nets with 55 **µM** pore size Dorgham and Moftah (1989) reported 88 diatom and 37 dinoflagellate taxa, 135 diatom and 9 dinoflagellate taxa in Kuwait waters during 1989, compared to 175 diatoms and 124 dinoflagellate taxa in the U.A.E and Qatar waters. A two-year study in the Gulf of Oman (Al-Azri *et al*., 2010) showed a chlorophyll maximum of 3 mg^3, and the phytoplankton was dominated by diatoms and the dinoflagellate *Noctiluca*. From the coastal waters of Oman, Al-Hashmi *et al*. (2015, 2019) reported a high diversity of 130 diatom and 80 dinoflagellate species and their abundance related to the Northeast and Southwest monsoons. Maximum diatom abundance was 1832704 cells per m^3, four-fold less compared to the previous decade but dinoflagellates were dominated by *Noctiluca* (3x 10^6 m^3). There were 24 potentially harmful algal species identified, including 11 species of dinoflagellates and eight species of diatoms.

Long-term phytoplankton surveys in Kuwait waters (Al-Kandari *et al*., 2009; Al-Yamani and Saburova, 2019 a) are more instructive than information from a few samples (Dorgham *et al*., 1987). Diatoms were dominant in the unfractionated Kuwait water samples (Table 4). Recently, the ratio of diatoms to dinoflagellates was suggested to be a new environmental

Table 4. Phytoplankton diversity in Kuwait waters, ROPME Sea Area and the Arabian Gulf.

	Kuwait waters[1]	Kuwait waters[2]	Kuwait waters[3]	Kuwait waters[4]	Kuwait waters[5]	Central Gulf[6]	ROPME*[7]	Arabian Gulf[8]
Total species ▸	327	200	250		138	223	376	1220
Taxonomic group ▾								
Bacillariophyceae	202	134		250	125	134	171	888
Dinoflagellata	108	56	213		13	86	194	211
Cryptophytes	1	1	5				1	1
Cyanobacteria	2	2	2			2	2	
Dictiochales	2	2	3			1	2	15
Haptyophytes			3				1	15
Chlorophytes	6	3	6				4	90
Raphidophceae	1		4					
Chromista	5		1					
Ebriophytes		1	3				1	
Prasinophytes		1	4					
Prymnesiophyta			2					
Litostomaetea *(Myrionecta rubra)*			1					

[1] Al-Kandari *et al*., 2009; [2] Polikarpov *et al*.,2009, [3] Al-Yamani and Saburova, 2019 a; [4] Al-Yamani and Saburova, 2019 b; [5] Dorgham *et al*., 1987; [6] Dorgham *et al*.,1987; [7] Polikarpov *et al*., 2016; [8] Jacob Al-Muzzaini, 1995

indicator; in the Baltic Sea due to eutrophication a shift from diatom to dinoflagellates is affected (Spilling et al., 2018). In Kuwait waters the diatom:dinoflagellate numbers corresponded to 202:108 (Al-Kandari *et al*., 2009), 134:56 (Polikarpov *et al*., 2009) 92:38 (Al-Yamani and Saburova, 2019a). Samples from ROPME areas (across the Arabian Gulf, the Strait of Hormuz, and the Sea of Oman) revealed 131 diatom and 194 dinoflagellate species (Polikarpov *et al*., 2016).

Besides the usual diatoms, dinoflagellates and other groups of microalgae, picoplankton is an integral component of phytoplankton biomass and responds to environmental perturbations. The relative contribution of picoplankton to the phytoplankton in Kuwait waters is not known but may be important.

Additionally, the results from this study on the impact of enrichment with aeolian dust in stimulating diatom growth are of interest (Subba Rao *et al*., 1999 b). Surface water 51.7 km off Kuwait with low phytoplankton collected during summer was enriched with aeolian dust comparable to the fall out levels 0-100 g m^2 sea surface (Gharib *et al*., 1986). Initial micronutrients (µM) were 1.4 - 4 NO_3, 6.6 -8.4 PO_4, and 18.3-28.1 SiO_2. Data from the present study showed the doubling rate of algal biomass increased from 0.56 to 1.42; in 5 days, 29 species of diatoms, mostly 6x3 µM naviculoids, grew rapidly and attained a maximum biomass (527 µg l^{-1} chl. *a*) (Subba Rao *et al*., 1999 b). Dust storms are a source of enrichment of Fe, Al, Cr, Mn, Si, Zn, P, Cu, and Ni (Al-Awadhi, 2005) some of which could stimulate phytoplankton growth. Satellite observations have shown that aeolian dust fertilizes the Gulf waters (Nezlin *et al*., 2010). The impact of these trace elements on marine phytoplankton is rather complex and could be either positive or inhibitory depending on the algal species, salinity of the medium, frequency of enrichment, stoichiometry, solubility state, oxidative reductive states, availability of chelators like organic ligands, siderophores, and on microbial feedbacks.

Episodic blooms

In these hyper saline Gulf waters, algal blooms are ephemeral, episodic, few and far between. They are atypical in respect of their non-seasonality, non-recurrence, and multi species in contrast to seasonal HAB blooms of *Dinophysis* sps., *Prorocentrum* sps. *Karenia brevis*, *Gamberidiscus* sps., *Pyrodiniumbahamense, Karlodinium venefiucum* and *Alexandrium tamarense* from other regions (Subba Rao and Durvasula, 2020). In Kuwait Bay there were significant short-term elusive pulses of red tides during 1987- 2000; both the constituent species and their abundance varied. Based on phytoplankton monitoring data for 2004-2017, Al-Yamani and Saburova (2019 a) recorded incidences of 50 algal blooms. About 29 species attained > 3 x 10^6 cells l^{-1} or 10 µg l^{-1}, within the blooms. Most blooms contained more than one species, that included *Chrysochromulina* sp., *Gymnodinium* spp, *Heterosigma akashiwo, Karenia papilionacea, Karenia selliformis, Karlodinium* sp, *Leptocylindrus* sp., *Prorocentrum* spp., *Nanoflagellates, Nitzschia cf. laevis, Nitzschia longissima, Nitzschia* sp., *Nitzschia* spp., *Phaeocystis globosa, , Leptocylindrus* sp., *Prorocentrum* spp., *Prorocentrum balticum, Pseudo-nitzschia seriata, Rhodomonas* sp., *Skeletonema grevillei, Takayama* spp., and *Thalassiosira* spp. Highly exceptional blooms contained 1008.9 x 10^6 l^{-1} cells and 4525.5 µg l^{-1} chl. *a* (Subba Rao *et al*., 2003). Monospecific blooms observed include *Chaetoceros socialis*, *Dunaliella salina*, *Myrionecta rubra*, *Noctiluca scintillans*, *Oltmannsiella lineata*, *Phaeocystis globosa*, and *Trichodesmium erythraeum*.

In September 2001, the globally invasive *Gymnodinium catenatum* and *Gyrodinium impudicum* were the main bloom-forming species (>10^6 cells/l) that suggests their ability to tolerate the prevailing high salinity (Glibert *et al*., 2002). Analyses of historical records of algal blooms in the Indian Peninsula (1908-2017) similarly showed a high variability; 24 genera and 30 species including Cyanophyceae, Bacillariophyceae, Dinophyceae, Raphidophyceae, Prymnesiophyceae, Trebouxiophyceae and Ciliates in the 154 events (Oyeku and Mandal, 2021). The non-recurrence of taxa and their biomass varied depending on the sea surface salinity, eutrophication and high nutrient conditions.

Due to continued perturbations in the Gulf, probably a few resistant species grew better. Based on a mechanistic competition theory, Flöder and Sommer (2015) showed that factors other than macro-nutrients affect the structure of macroalgal communities. Rengefors (2020) discussed adaptive C, S, R strategies (Competition, stress tolerance and disturbance tolerance) as a response to the environment, favoring distinct species. In the Bay that experiences incessant perturbations, competition for resources among phytoplankton species and groups can determine which species become dominant. Mesocosm experiments of 90 days duration with sequential pulse perturbations, showed the resistance and recovery index of algae, finally resulting in a failure of the community to recover (Stelzer *et al*., 2022). In addition to the salinity gradient studies mentioned earlier, experiments along the lines of Folt (1999) may be taken up by the GCC and utilize cultures of microalgae native to the Gulf to understand their response to synergism (increased stress) and antagonism (decreased stress). Not a single nutrient but an array of complex factors including optimal temperature and light and a mix or multiples of the macro, micro and trace elements regulate phytoplankton growth. If their levels are higher than the threshold, the nutrients become toxic to phytoplankton. The Fe, Cu, Co, Zn and Mn levels in Kuwait Bay (Table 5) are much higher than those in f/2 algal culture medium (Guillard, 1975). However, culture medium has much higher P, N, Si concentrations than in Kuwait Bay (Al-Said *et al*., 2018a).

Elevated levels of PCBs, PBDEs and dioxins were reported near crude oil industries along the Kuwait Bay coastline. Alshemmari (2021) in a review of persistent organic pollutants in Kuwait sediments (g/ DW) reported ranges of TPH 40-240 µg, and PCBs 0.40 -81.7 ng. Further, elevated levels remain relatively high (426-459 µg) in industrial areas and near oil loading terminals. Their longevity, high hydrophobicity and resistance to degradation are a matter of concern. In the highly saline Kuwait waters these may be toxic for phytoplankton growth and may prevent progression of phytoplankton into blooms Studies such as that of Gallo *et al*. (2020) indicating that PAHs from sediment elutriate inhibit growth of *Phaeodactylum tricornutum*, *Skeletonema costatum* and the halophyte green alga *Dunaliella tertiolecta* are required for the AG region. In addition, precise physiological ecology studies utilizing cultures of native algae are required to understand their response and recovery to perturbations and to answer the question as to why the algal blooms in the Gulf are sporadic and not regular.

Ballast waters: microalgae

More than 17 million barrels of ballast water from various geographical regions are transported to the ROPME area annually and may have inadvertently introduced exotic microalgae (Al-Yamani *et al*., 2015) and heavy metals as well (Dobaradaran *et al*., 2017). Comparison of a list of dinoflagellate records from Kuwait waters shows a steady increase in dinoflagellate species from 39 (Dorgham *et al*., 1987), to > 108 (Al-Kandari *et al*., 2009; Al-Yamani and Saburova, 2019 a) and included several first-time records. In the whole Gulf the increase has been from <45 species in 1931 (Bohm, 1931) to 509 species (Subba Rao and Durvasula, 2020). It is plausible that ballast water from 50,000 ships visiting the Gulf annually from various geographic regions may have introduced species new to the Gulf. Of interest are the changes in the constituent species; 19 species from Dorgham *et al*. (1987) were absent in observations of Al-Kandari *et al*. (2009) while 155 species additional were reported by Al-Yamani and Saburova (2019 a). Only 33 species from the first study are common to those of the second. Assuming 60 % of these, particularly the athecate, were "poorly preserved" the remaining were thecate and well preserved. While such an increase in dinoflagellate diversity could be partly due to an underestimation of native taxa, it is plausible that at least some of the 40 % new of thecate dinoflagellates constitute the "suspect" transient species brought in by ballast waters. However, it appears that their physiological conditions and low abundance precluded their development into a bloom.

It has been observed previously that a few of the opportunistic species when "inoculated" in sufficient numbers in enriched coastal waters can result in aperiodic, episodic non-recurrent blooms as in *Karenia*

Table 5. Enrichment levels (µM) in f/2 algal culture medium compared to their range in Kuwait waters.

Enrichment	f/2 medium (Guillard, 1975)	Range in Kuwait waters (Al-Said *et al*., 2018)
Phosphorus	36.3	0.07-0.45
Nitrogen	883	0.08-2.85
Silica	107	0.26-9.72
Iron	10	670-28160
Copper	0.04	10260-23250
Cobalt	0.05	50-1340
Zinc	0.08	5140-25330
Manganese	0.9	-1.09

Additionally, *f*/2 contains 3 x 10^{-8} Molybdenum, Vitamin B_{12} (3.69 x 10^{-10} M), biotin 2.05 x 10^{-2}M. and 2.96 x 10^{-7} thiamine.

(*Gymnodinium*) *mikimotoi, Gonyaulax polygramma, Noctiluca scintillans*, *Pfiesteria piscicida*, *Prorocentrum cordatum* (Burkholder, 1998). *Heterocapsa circularisquama,* a species of hardy alien opportunistic and toxic dinoflagellate from the Western Pacific developed into blooms in Kuwait Bay during June-July 2020 (Saburova *et al*., 2022). During the blooms, chl. concentrations were 20 µg l^{-1} and cell concentrations were 267.9 x 10^6 cells l^{-1} along with the native blooming diatoms *Thalassiosira delicatula, T. exigua*, and *Minutocellus polymorphus* (Saburova *et al*., 2022). Their geographical distribution suggests introduction from Malaysia, Brunei, Philippines, New Guinea, Japanese waters, Australia, Belize, Russia, and South African seas, like the occurrence of Indo-Pacific dinoflagellates in the Mediterranean (Gómez, 2006). It is desirable to establish the viability of such suspect introduced alien species using culture methods (Subba Rao *et al*., 1994). In the enclosed Black and Caspian Seas some of the novel transient hardy species *Lingulodinium machaerophorum* and *Impagidinium caspienense* introduced by shipping activities bloomed (Sala-Perez *et al*., 2020; Lattuada *et al*., 2020). Clarke *et al*. (2020) reported the possible introduction of a wide range of 136 invasive non-native species of which 56 potentially invasive species from four microalgae to fish in the Arabian Gulf and Sea of Oman. Such bio-invasions could affect at local, regional and global scales.

Harmful algae

Species of algae reported harmful elsewhere causing Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Amnesic Shellfish Poisoning (ASP), Ichthyotoxin poisoning, Yesso toxin poisoning, are present in Kuwait waters (Devlin *et al*., 2019; Al-Yamani and Saburova, 2019 a; Saburova, *et al*., 2022). Out of a total of 62 harmful algal species, 43 are potentially toxic to humans and marine biota (Al-Yamani *et al*., 2012). These species are: PSP -, *Alexandrium acatenella, A. leei, A. ostenfeldii, A. pacificum, A. tamiyavanichii, Gymnodinium catenatum,* DSP - *Phalacroma rapa, P. rotundatum,* ASP -, *Pseudo-nitzschia seriata,* and *Halamphora coffeaeformis,* Neurotoxin - *Karenia brevis, Prorocentrum cordatum, Heterosigma akashiwo, Karenia papilionacea,* and Icthyotoxin–*Margalefidinium polykrikoides, Karenia mikimotoi* and Yessotoxin producer *Protoceratium reticulatum. Karenia selliformis* produces a compound gymnodimine in culture that may accumulate in shellfish with no effect proven for human health. Analysis of 214 species from a data set (2007-2016) listed 39 potential Harmful Algal Bloom (HAB) species (Devlin *et al*., 2019) in Kuwait waters. Species that produced phycotoxins are *Alexandrium minutum, A. tamarense, Gymnodinium catenatum, Pyrodinium bahamense* (PSP), *Dinophysis acuminata, D. acuta, D. caudata, D. fortii, D. miles, D. norvegica, D. tripos, Prorocentrum lima*, *P. mexicanum (*syn *P.rhathymum), P. concavum,* (DSP*), Gonyaulax spinifera (*Yessotoxin*), Karenia selliformis* (NSP), *Heterosigma akashiwo, Karenia brevis (*syn *K. papilionacea), Prorocentrum minimum,* (Neurotoxin), *P. reticulatum* (Yessotoxin), *Karenia mikimotoi* (Icthyotoxin), *Pseudo-nitzschia pungens*, and *Pseudo-nitzschia seriata*,(ASP). Additionally, Devlin *et al.* (2019) reported the occurrence of *Akashiwo sanguinea* a surfactant species, *Chaetoceros socialis, C. curvisetus, Chaetoceros* spp, *Cyclotella* sp., *Cylindrotheca closterium, Eucampia zodiacus, Guinardia flaccida, Leptocylindrus* sp., *Nitzschia laevis, Noctiluca scintillans Peridinium quinquecorne, Prorocentrum micans, Pseudo-nitzschia americana, Tripos furca, T. fusus, Dictyocha fibula, Dictyocha speculum* and *Trichodesmium erythraeum*. Cell densities (l^{-1}) of these were for the non-toxigenic diatoms *Eucampia zodiacus (*929,333), the toxigenic *Karenia brevis* (116,333) and *Pyrodinium bahamense* (110,667) and were probably sufficient to be toxigenic. Al-Yamani and Saburova (2019 a) listed 57 blooms in Kuwait waters during 1987-2017, and 22 dinoflagellate species were the constituents. Cell densities (l^{-1}) ranged between 0.4 x 10^6 and 1x 10^9 and chl. *a (*µg l^{-1}) between 4.1 to 4256 (Table 1 in Al-Yamani and Saburova, 2019 a). Of these only 14 had >10 chl. µg l^{-1} (Al-Yamani and Saburova, 2019 a). Biosynthesis and bioaccumulation of phycotoxins in *Pseudo-Nitzschia multiseries* (Pan *et al*., 1996) and *Prorocentrum lima* (Pan *et al*., 1999) is dependent on sufficient cell biomass and their growth phase. *Alexandrium pseudogonyaulax* that produces a mucous trap and *Scripsiella trochoidea* were also recorded in Kuwait waters. The bloom of *S. trochoidea* was nontoxic; however, their high blooms can negatively impact coastal ecosystems and cause mortality in marine biota (Hallegraeff, 1993; Hold *et al*., 2001). As discussed earlier it is possible that prevailing high salinity in the Gulf, inhibited growth of these toxic species preventing their growth into bloom proportions that are needed for elevating toxin levels in the water. It is recommended that culture studies are conducted on isolates from Kuwaiti waters and investigations are carried out at various phases of their growth for phycotoxin production in this harsh environment.

Mass mortalities

Marine mortalities in the Gulf include 25-30 tons of mullets *(Planiliza macrolepis)*, approximately 80,000 tons of farmed Sobaity (*Sparidentex hasta)* in floating cages in Kuwait Bay during 1999, Gilthead Seabream (*Sparus aurata)* in aquaculture cages, Fringescale (*Sardinella fimbriata)* in marinas in 2005, and Catfish (*Netuma thalassina* and *Policofollis tenuspinus)* in 2006, 2015, 2017 and 2019 (Al-Yamani *et al*., 2020). The most well-known fish kill of *Planiliza macrolepis* in Kuwait occurred in 1999, as a result of a bloom of ichthyotoxic *Karenia selliformis* (Heil *et al*., 2001, Al-Yamani *et al*., 2020). A large-scale fish kill event worthy of mention was associated with intensive heterotrophic bacterial activity (*Streptococcus agalactiae*) on dead algae in Kuwait Bay during August-September 2001 (Glibert *et al*., 2002). This study also reported various harmful algae including *Gymnodinium catenatum, Gyrodinium impudicum*, and *Pyrodinium bahamense* var. *compressum*. Cell numbers of *G. catenatum* and *G. impudicum* exceeded 10^6 l^{-1}, probably sufficient to deliver phycotoxins. But these algae were neither cultured nor phycotoxin production determined. Data are needed to conclude whether the fish mortality may have been caused either by a phycotoxin or due to hypoxic conditions that followed the bloom.

The Gulf acts as a topographical shelter and concentrates organic carbon in the water column ranging from 101.0 – 318.4 µM (Al-Yamani and Naqvi, 2019). Oxidation of this organic matter and bacterial heterotrophic activity on dead algae also exacerbate deoxygenation leading to formation of dead zones with low oxygen. The prevailing high temperatures, excessive pollutants, and bacterial action of *Streptococcus agalactiae* may have resulted in hypoxia caused by the die off from phytoplankton, leading to the mortalities. A three-dimensional numerical model (Alosairi and Al-Sulaiman, 2020) showed depletion of dissolved oxygen is local and near the pollution out falls limited to Kuwait Bay. A bloom of toxic *Heterocapsa circularisquama* in Kuwait's waters during June–July 2020 (Saburova *et al*., 2022) was associated with shellfish mortality.

In Kuwait's southern shore mass mortalities of pearl oyster *Pinctada radiata* and scallop *Chlamys livida* in 2013 was associated dredging activities. The mass mortalities of Sea Cucumbers, *Holothuria arenicola* in 2018 on Kuwait's southern shore are believed to be due to osmotic shock resulting from a drastic decrease in salinity from 45 PSU to 5 PSU (Al-Yamani *et al*., 2020). This change in salinity was caused by rainwater discharge and coincided with dominance of the nano flagellate *Plagioselmis prolonga* (Al-Yamani *et al*., 2020*)*. Nevertheless, to establish the causative factors it is stressed that it is necessary to conduct culture experiments. Richlen *et al*. (2010) established cultures of *Margalefdinium polykrikoides* isolated from coastal waters near Ras Al-Khaimah, located in the northern United Arab Emirates. In one study, Al-Muftah *et al*. (2016) isolated cultures of the ichthyotoxic dinoflagellate *Vulcanodinium rugosum* from the coastal areas of Doha, Qatar, and these strains contained between 603 and 981 ng pinnatoxin (PnTx) H per mg dry weight in addition to being positive for portimine. It is suggested that going forward, molecular analysis of organism-level gene regulation and transcriptiomics under hypoxic conditions would be illuminating.

Fisheries

The natural and anthropogenic perturbations presented above resulted in concomitant changes in the hydrographical conditions during 2000-2013 that caused shifts in functional plankton groups. Phytoplankton biomass (chl. *a*) in Kuwait waters decreased from ~6 to 2 µg l^{-1} with a long term mean of 3.64 µg l^{-1} (Al-Said *et al*., 2017) and 1.01- 2.27 (Devlin *et al*., 2015). This in turn may have altered the pelagic trophodynamics evident from a drastic decline in the fish stocks. Fish and shrimp production declined (Alqattan and Gray, 2021). Off Kuwait, the landings (tons) of the pelagic feeders decreased during 2000 and 2012 (Al-Husaini *et al*., 2015); i.e., *Tenulosa ilisha* (Suboor) 642 to 72, *Carangoides* sp. (Hamam) 117 to 76, *Otolithes ruber* (Nowaiby) 728 to 535, *Parastromateus niger* (Halwaya) 50 to 42, and *Epinephelus coioides* (Hamoor) 262 to120. Correlation between the flow of the Tigris-Euphrates rivers and estimated finfish recruitment trends are positive (Ben–Hasan *et al*., 2018). Declining catches in the Northwestern Arabian Gulf indicate possible over-fishing. Ben-Hasan *et al*. (2018) on the other hand, allude to such declines as being the result of recruitment changes caused by reduced freshwater flow in the Tigris-Euphrates Rivers. In the Aral Sea where surface salinity increased from 10 PSU in 1960 to 92 PSU by 2004 (Zavialov, 2005), the commercial fish catch was decimated from 43,430 tons in 1960 to zero in 1980. It is to be noted that damming the Nile in 1965 reduced freshwater flow to the Mediterranean by 90 %, resulting in concomitant changes in the nature of the productive ecosystem and a collapse of the fishery within 15 years (Nixon, 2003). Changes in nutrient load caused by damming the Danube River similarly affected the food web structure in the Black Sea surface waters (Humborg *et al*., 1997).

Conclusions

Environmental protection practices in the Gulf countries are similar, and it is assumed that natural and anthropogenic perturbations like those in Kuwait would prevail in other Gulf countries. High sea surface temperature together with reductions in river flow, and enrichment due to natural and anthropogenic perturbations result in stressful environmental conditions for marine biota in the Gulf that have far-reaching consequences that need to be addressed. In the GC, 61 % of treated wastewater is not utilized but discharged into the sea (Qureshi, 2020). This study calls for a concerted effort by GCC for a systematic reduction in the release of treated wastewater in the AG for ecosystem restoration as was demonstrated in the highly eutrophicated Seto Inland Sea, Japan (Imai *et al*., 2006). Long-term reduction of nutrient inputs has been considered as the most effective pathway to mitigate eutrophication to improve the structure and functioning of the ecosystem (Imai *et al*., 2006). Prior to the reduction in wastewater outflow, about 299 harmful algal blooms developed and damaged a fishery worth US$ 60 million per annum. The causative microalgae included *Chattonella antiqua*, *C. marina*, *C. ovata* and *Heterosigma akashiwo* (Raphidophyceae), and *Karenia mikimotoi* and *Margalefidinium polykrikoides* (Dinophyceae) and in 1988 a novel red-tide of dinoflagellate species *Heterocapsa circularisquama*. The "Law Concerning Special Measures for Conservation of the Environment of the Seto Inland Sea" was legislated in 1973 and nutrient loading was systematically reduced to half the level of 1972. The incidence of harmful algal blooms also decreased to 100. A similar success story is that of Thau Lagoon, France, where a reduction of phosphorus loading as a means of 'de-eutrophication' resulted in a decrease in dinoflagellate abundance (Gowen *et al*., 2015).

The Arabian Gulf is experiencing "extraordinary neglect compounded in large part by excessive tanker traffic (Brewer and Dyrssen, 1985) and unabated discharge of pollutants from land. As a result of the warming trend and the prolific increase in the number of desalination plants all around its coast, the Arabian Gulf is emerging as one of the most thermohaline stressed environments. Compounding thermohaline stress, are both natural and anthropogenic perturbation levels of inorganic nutrients, nutrient stoichiometries, trace elements, heavy metals, and the PAHs, PCBs and PBDEs concentrations to the detriment of the environment. When several exogenous cumulative stresses exist beyond a threshold for biological tolerance, the threats to the ecosystem increase dramaticaaly. In order to maintain the integrity of the Gulf ecosystem, it is suggested that brine should not be discharged into the AG but pumped further inland on to the desert flat. Although this would decrease sea level, it would be compensated by incursion of relatively less saline (~35.4 PSU) Arabian Sea Water into the Gulf.

It is emphasized that it is time for all the GCC countries to come together to strengthen cooperation to implement of strategic management programs that reduce environmental stressors and build resilience of the Arabian Gulf ecosystem. This study calls for implementation of GCC-wide, sustained long term series data acquisition programs. Furthermore, research studies that include cultures of microalgae native to the Gulf and from ballast waters, improved screening of suspect toxigenic algal cultures for phycotoxins, and assessment of the impact of 'oligotrophication' to reduce nutrient loading and its impact on phytoplankton, are called for.

In addition a regional ecosystem-based nutrient management strategy plan is called for, like the HELCOM Baltic Sea Action Plan (HELCOM BSAP, 1974) in the Baltic Marine environment which has demonstrated the effectiveness of long-term and large-scale reduction of nutrient inputs to improve the Baltic Marine environment (OSPAR, 2020; Murray *et al*., 2019). Duarte *et al*. (2009) cautioned that coastal eutrophication results in shifting baselines and reduced chlorophyll, but prediction of trajectories of 'oligotrophication' of individual ecosystems is still a challenge and should be based on more long-term systematic data.

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References

Abdullah AD (2016) Modeling approaches to understand salinity variations in a highly dynamic tidal river: The case of the Shatt Al-Arab River. PhD thesis, Delft University of Technology and of the Academic Board of the UNESCO-IHE Institute for Water Education. 207 pp

Abu-Seedo F (1990) Tidal and turbidity effects on the shallow-water fish assemblage of Kuwait Bay. Marine Ecology Progress Series 65: 213-223

Ahmed M (2001) Brine disposal from reverse osmosis desalination plants in Oman and United Arab Emirates. Desalination 133: 135-147 [https://doi.org/10.1016/S0011-9164(01)80004-7]

Ahmed M, Hoey D, Shayya W, Goosen MFA (2004) Brine disposal from inland desalination plants: Current status, problems and opportunities. In: Zannetti P (ed) Environmental Sciences and Environmental Computing Vol. II. The Environmental Computing Institute [http://www.envirocomp.org]

Ahmed A, Al-Said T, Madhusoodhanan R, Wajih S, Naqvi A, Sarkar A, Fernandes L, Thuslim F, Al-Zakri W, Al-Yamani F (2022) Environmental impact of a series of flash flood events on a hypersaline subtropical system in the Northwestern Arabian Gulf. Marine Pollution Bulletin 175 (2): 113394 [doi:10.1016/j.marpolbul.2022.113394]

Al-Ansari N, Jawad S, Adamo N, Sassakian K (2019) Water quality and its environmental implications within Tigris and Euphrates Rivers. Journal of Earth Sciences and Geotechnical Engineering 9: 57-108 [http://www.diva-portal.org/smash/get/diva2:1199708/fulltext01.pdf]

Al-Anzi B, Abusam A, Shahalam A (2012) Assessment of wastewater reuse in Kuwait and its impact on amounts of pollutants discharged into the Sea. Journal of Environmental Protection 3: 935-939 [http://dx.doi.org/10.4236/jep.2012.328108]

Al-Azri AR, Piontkovski SA, Al-Hashmi K, Goes JI, Gomes HR (2010) Chlorophyll a as a measure of seasonal coupling between phytoplankton and monsoon periods in the Gulf of Oman. Aquatic Ecology 44: 449-456 [doi 10.1007/s10452-009-9303-2]

Al-Awadhi J (2005) Dust fallout characteristics in Kuwait: A case study. Kuwait Journal of Science & Engineering 32 (2): 135-152

Albanai JA (2021) Seasonal spatial and temporal distribution of Chlorophyll-*a* concentration over Kuwait and the Arabian Gulf using satellite and in-situ data. Preprints. 2021070232 [doi: 10.20944/preprints202107. 0232.v1.]

Al-Dousari A, Doronzo D, Ahmed M (2017) Types, indications and impact evaluation of sand and dust storms trajectories in the Arabian Gulf. Sustainability 9: 1526 [doi:10.3390/su9091526]

Al-Dousari A (ed.), (2021) Atlas of fallen dust in Kuwait. Springer International Publishing. ISBN: 978-030-6676-8 [doi: 10.1007/978-3-030-66977-5]

Aleisa E, Al-Shayii K (2017 a) Analysis on reclamation and reuse of wastewater in Kuwait. Kuwait Journal of Science and Engineering 7: 1-27

Aleisa E, Al-Zubari W (2017 b) Wastewater reuse in the countries of the Gulf Cooperation Council (GCC): the lost opportunity. Environmental Monitoring and Assessment 89: 553 [https://doi.org/10.1007/s10661-017-6269-8]

Al-Hashmi KA, Smith Sl, Claereboudt M, Piontkovski SA, Al-Azri A (2015) Dynamics of potentially harmful phytoplankton in a semi-enclosed bay in the Sea of Oman. Bulletin of Marine Science 91 (2): 141-166 [http://dx.doi.org/10.5343/bms.2014.1041]

Al-Hashmi K, Piontkovski S, Bruss G, Hamza W, Bryantseva YV, Popova E (2019) Seasonal variations of plankton communities in coastal waters of Oman. International Journal of Oceans and Oceanography 13 (2): 395-426 [https://www.ripublication.com/ijoo19/ijoov13n2_13.pdf]

Al-Ghadban AN, Jacob PG, Abdalli F (1994) Total organic carbon in the sediments of the Arabian Gulf and need for biological productivity investigations. Marine Pollution Bulletin 28: 356-362 [https://doi.org/10.1016/0025-326X(94)90272-0]

Al-Ghadban AN, Uddin S, Maltby E, Al Khabbaz A, Al Mutairi A (2012) Denitrification potential of the Northern Arabian Gulf- n experimental study. Environmental Monitoring and Assessment1 84 (12): 7103-12 [doi 10.1007/s10661-011-2483-y]

Al-Handal A, Hu C (2015) MODIS observations of human-induced changes in the Mesopotamian marshes in Iraq. Wetlands 35: 31-40 [http://dx.doi.org/10.1007/s13157-014-0590-6]

Al-Hasawi H (1999) Investigation of municipal solid waste management in GCC states. Thesis submitted for PhD. University of Sterling, U.K. 318 pp

Al-Husaini M, Bishop JM, Al-Foudari AF, Al-Baz AF (2015) A review of the status and development of Kuwait's fisheries. Marine Pollution Bulletin 100: 597-606 [doi: 10.1016/j.marpolbul.2015.07.053]

Al-Said T, Al-Ghunaim A, Subba Rao DV, Al-Yamani F, Al-Rifaie K, Al-Baz A (2017) Salinity-driven decadal changes in phytoplankton community in the NW Arabian Gulf of Kuwait. Environmental Monitoring and Assessment 189: 268 [http://dx.doi.org/10. 1007/s10661-017-5969-4]

Al-Said T, Madhusoodhanan R, Pokavanich T, Al-Yamani F, Kedila R, Al-Ghunaim A, Al-Hashem A (2018 a) Environmental characterization of a semiarid hypersaline system based on dissolved trace metal-macronutrient synergy: A multivariate spatio-temporal approach. Marine Pollution Bulletin 129: 846-858 [https://doi.org/10.1016/j.marpolbul.2017.10.009]

Al-Said T, Naqvi SWA, Al-Yamani F, Goncharov A, Fernandes L (2018 b) High total organic carbon in surface waters of the northern Arabian Gulf: Implications for the oxygen minimum zone of the Arabian Sea. Marine Pollution Bulletin 129: 35-42

Al-Sarawi MA, Massoud, MS, Khader SR (2002) Recent trace metal pollution in bottom sediments of Sulaibikhat Bay. Kuwait Technology 8: 38-50

Alshemmari H (2021) An overview of persistent organic pollutants along the coastal environment of Kuwait. Open Chemistry 19: 149-156

Al-Yamani F (2008) Importance of the freshwater influx from the Shatt-Al-Arab River on the Gulf marine environment. In: Abuzinada AH, Barth HJ, Krupp F, Böer B, Al Abdessalaam TZ (eds) Protecting the Gulf's marine ecosystems from pollution. Birkhäuser, Basel. pp 207-222 [https://doi.org/10.1007/978-3-7643-7947-6_11]

Al-Yamani F, Subba Rao DV, Mharzi A, Ismail W, Al-Rifaie K (2006) Primary production off Kuwait, an arid zone environment, Arabian Gulf. International Journal of Oceans and Oceanography 1: 67-85

Al-Yamani FY, Bishop JM, Al-Rifaiae K, Ismail W (2007) The effects of the river diversion, Mesopotamian Marsh drainage and restoration, and river damming on the marine environment of the northwestern Arabian Gulf. Aquatic Ecosystem Health and Management 10: 277-289 [doi: 10.1080/14634980701512384]

Al-Yamani F, Saburova M, Polikarpov I (2012) A preliminary assessment of harmful algal blooms in Kuwait's marine environment. Aquatic Ecosystem Health and Management 15 (1): 64-72

Al-Yamani F, Stokozov N, Polikarpov I (2014) Oceanography Annual Report Card, OARC. KISR

Al-Yamani F, Skryabin V, Subba Rao DV (2015) Suspected ballast water introductions in the Arabian Gulf. Marine invasive species: Management of ballast water and other vectors. Journal of Aquatic Ecosystem Health and Management18 (3): 280-289 [https://doi.org/10.1080/14634988.2015.1027135]

Al-Yamani F, Yamamoto T, Al-Said T, Alghunainm A (2017) Dynamic hydrographic variations in northwestern Arabian Gulf over the past three decades: Temporal shifts and trends derived from long-term monitoring data. Marine Pollution Bulletin 122: 488-499

Al-Yamani F, Naqvi SWA (2019) Chemical oceanography of the Arabian Gulf. Deep-Sea Research. Part II: Tropical Studies in Oceanography 161: 72-80 [https://doi.org/10.1016/j.dsr2.2018.10.003]

Al-Yamani F, Saburova MA (2019 a) Marine phytoplankton of Kuwait's waters. Vol 1. Cyanobacteria, Dinoflagellates, Flagellates. Kuwait Institute for Scientific Research. 469 pp

Al-Yamani FY, Saburova MA (2019 b) Marine phytoplankton of Kuwait's waters. Vol II. Diatoms. Kuwait Institute for Scientific Research. 336 pp

Al-Yamani FY, Polikarpov IG, Saburova M (2020) Marine life mortalities and Harmful Algal Blooms in the Northern Arabian Gulf. Aquatic Ecosystem Health and Management 23 (2): 196-209 [doi: 10.1080/14634988.2020.1798157]

Al-Yamani F, Polikarpov IG, Saburova M (2021) Northern Gulf marine biodiversity in relevance to the river discharge. In: Jawad LA (ed) Southern Iraq's marshes. Coastal Research Library 37. Springer Nature Switzerland AG. pp 379-438

Baawain MS, Al-Omairi A, Choudri BS (2014) Characterization of domestic wastewater treatment in Oman from three different regions and current implications of treated effluents. Environmental Monitoring and Assessment 186 (5): 2701-2716 [doi:10.1007/s10661-013-3572-x]

Barnes IJD, Balaguer L, Mnrique E, Elvira S, Davison AW (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. Environmental and Experimental Botany 22: 85-100 [https://doi.org/10.1016/0098-8472(92)90034-Y]

Basaham AS, Al-Lihabi SS (1993) Trace elements in sediments of the Western Gulf. Marine Pollution Bulletin 27: 103-107

Bashitialshaaer R, Persson KM, Aljaradin M (2011) Estimated future salinity in the Arabian Gulf, the Mediterranean Sea and the Red Sea. Consequences of brine discharge from desalination. International Journal of Academic Research 3 (1): 133-140

Ben-Hasan A, Walters C, Christensen V, Al-Husaini M, H. Al-Foudari H (2018) Is reduced freshwater flow in Tigris-Euphrates rivers driving fish recruitment changes in the Northwestern Arabian Gulf? Marine Pollution Bulletin 129 (1): 1-7 [https://doi.org/10.1016/j.marpolbul.2018.02.012]

Bhunia S, Bhowmik A, Mukherjee J (2022) Waste management of rural slaughterhouses in developing countries. Advanced Organic Waste Management: 425-449 [https://doi.org/10.1016/B978-0-323-85792-5.00019-8]

Bohm A (1931) Peridineen aus dem Persischen Golf und dem Golf von Oman. Archiv für Protestenkunde 74: 188-197

Bouwman L, Beusen A, Glibert PM, Overbeek C, Pawlowski M, Herrera J, Mulsow S, Yu R, Zhou M (2013) Mariculture: significant and expanding cause of coastal nutrient enrichment. Environmental Research Letters 8: 044026 [doi:10.1088/1748-9326/8/4/044026]

Brewer PG, Dyrssen D (1985) Chemical oceanography of the Persian Gulf. Progress in Oceanography 14: 41-55

Burkholder JM (1998) Implications of harmful microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. Ecological Applications 8 (1): Supplement: Ecosystem Management for Sustainable Marine Fisheries (Feb. 1998): S37-S62

Bustillo-LeCompte, Mehrvar M (2017) Slaughterhouse wastewater: treatment, management, and resource recovery. In: Robert Farooq, Zaki Ahmad (eds) Physico Chemical Wastewater Treatment and Resource Recovery. pp 153-174 [doi: 10.5772/65499]

Chalit G (2009) FAO Report [Qatar.http://www.fao.org/nr/water/aquastat/countries_regions/qat/ QAT- CP_ara.pdf]

Clarke SA, Vilizzi L, Lee L, Wood LE, Cowie WJ, Burt JA, Mamiit RJE, Ali H, Davison PI, Fenwick GV, Harmer R, Skóra ME, Kozic S, Aislabie LR, Kennerley A, Le Quesne WJF, Copp GH, Stebbing PD (2020) Identifying potentially invasive non-native marine and brackish water species for the Arabian Gulf and Sea of Oman. Globe Change Biology 26: 2081-2092 [https://doi.org/10.1111/gcb.14964]

Devlin MJ, MS Massoud MS, Hamid SA, Al-Zaidan A, Al-Sarawi H, Al-Enezi M, Al-Ghofran L, Smith AJ, Barry J, Stentiford GD, Morris S, da Silva ET, Lyons BP (2015) Changes in the water quality conditions of Kuwait's marine waters: long term impacts of nutrient enrichment. Marine Pollution Bulletin 100 (2): 607-620 [https://doi.org/10.1016/j.marpolbul.2015.10.022]

Devlin MJ, Breckels M, Graves CA, Barry J, Capuzzo E, Huerta FP, Al Ajmi F, Al-Hussain MM, Lequesne WJF, Lyons BP (2019) Seasonal and temporal drivers influencing phytoplankton community in Kuwait marine waters: Documenting a changing landscape in the Gulf. Frontiers in Marine Science 6: 141 [doi: 10.3389/fmars.2019.00141]

Dobaradaran S, Soleimani F, Nabipour I, Saeedi R, Mohammadi MJ (2017) Heavy metal levels of ballast waters in commercial ships entering Bushehr port along the Persian Gulf. Marine Pollution Bulletin 126: 74-76 [https://doi.org/10.1016/j.marpolbul.2017.10.094]

Dorgham MM, Muftah A, El-Deeb KZ (1987) Plankton studies in the Arabian Gulf II. The autumn phytoplankton in the Northwestern Area. Arab Gulf Journal of Scientific Research B5: 215-235

Dorgham MM, Moftah A (1989) Environmental conditions and phytoplankton distribution in the Gulf and Gulf of Oman. Journal of the Marine Biological Association of India 31: 36-53

Dorgham MM (2013) Plankton research in the ROPME Sea Area, Achievements and Gaps. International Journal of Environmental Research 7 (3): 767-778

Duarte C M, Conley DJ, Carstensen J, Sanchez-Camacho M (2009) Return to neverland: Shifting baselines affect eutrophication restoration targets. Estuaries and Coasts 32: 29–36 [doi10.1007/s12237-008-9111-2]

Duxbury AC, Byrne RH, Mackenzie FT (2024) Seawater. Encyclopedia Britannica 5 Nov. 2020 [https://www.britannica.com/science/seawater]

El-Anbaawy E, Abdelhalim A, Al Ajmi D, Al Sarawi M (2018) Environmental assessment and evaluation of anthropogenic impacts on Sulaibikhat Bay tidal flat area, Kuwait. Journal of Health and Environmental Research 4 (1): 21-34 [http://www.sciencepublishinggroup.com/j/jher] [doi: 10.11648/j.jher.20180401.14]

Emara HI (2010) Nutrient salts, inorganic and organic carbon contents in the waters of the Persian Gulf and the Gulf of Oman. Journal of the Persian Gulf (Marine Science) 1: 33-44

Enezi G, Hamoda MF, Fawzi N (2004) Heavy metals content of municipal wastewater and sludges in Kuwait. Journal of Environmental Science and Health Part A-Environmental Science and Engineering and Toxic and Hazardous Substance Control A39 (2): 397-407 [doi: 10.1081/ESE-120027531]

Esmaeili FM, Mortazavi MS, Banadaki AD, Saraji F, Nozar SLM (2021) Algal blooms historical outbreaks in the northern coastal waters of the Persian Gulf and Oman Sea (1980–2015). Environmental Monitoring and Assessment 193: 648 [https://doi.org/10.1007/s10661-021-09413-3]

Feidi IH (2009) Aquaculture industry in the Arab World. Project report: FAO Aquaculture Industry in the Arab World. FAO, Rome. pp 12

Flöder S, Sommer U (2015) Competition of aquatic microalgae in variable environments: the disturbance effect. Chapter 15 In: Subba Rao DV (ed) Algal cultures, analogues of blooms and applications. Science Publishers, Enfield, USA. pp 1-42

Folt CL, Chen CY, M. V. Moore MV, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnology and Oceanography 44: 864-877

GCC Wastewater Report (2020) Water management in the GCC- statistics and facts. Statista Research Department.

Gallo A, M. Guida M, Armiento G, Siciliano A, Mormile N, Carraturo F, Pellegrini D, Morroni L, Tosti E, Ferrante MI, Montresor M, Molisso F, Sacchi M, Danovaro R, Lofrano G, Libralato G (2020) Species-specific sensitivity of three microalgae to sediment elutriates. Marine Environmental Research 156: 104901 [https://doi.org/10.1016/j. marenvres. 2020.104901]

Gharib I, Al-Hashash, Anwar M (1986) Dust fallout in the northern part of the ROMPE Sea Area. I. Physical characteristics of the dust fallout in the northern part of ROPME Sea Area. ROMPE/GC 5/0 EES 66 KISR Technical report no 2058 p 193-195

Glibert PM, Landsberg JH, Evans JJ, Al-Sarawi MA, Faraj M, Al-Jarallah MA, Haywood A, Ibrahem S, Klesius P, Powell C, Shoemaker C (2002) A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: the roles of bacterial disease, harmful algae, and eutrophication. Harmful Algae 1: 215-231

Gómez F (2006) Endemic and Indo-Pacific plankton in the Mediterranean Sea: a study based on dinoflagellate records. Journal of Biogeography 33: 261-270

Gowen RJ, Y. Collos Y, Tett P, Scherer C, Bec B, Abadie E, Allen M, O'Brien T (2015) Response of diatom and dinoflagellate lifeforms to reduced phosphorus loading: A case study in the Thau lagoon, France. Estuarine, Coastal and Shelf Science 162: 45-52 [https://doi.org/10.1016/j.ecss.2015.03.033]

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of Marine Invertebrate Animals. Plenum Press, New York, USA p 29-60

Hallegraeff G M (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32: 79-99 [https://doi.org/10.2216/i0031-8884-32-2-79.1]

Heil CA, Glibert PM, Al-Sarawi MA, Faraj M, Behbehani M, Husain M, (2001) First record of a fish killing Gymnodinium sp. bloom in Kuwait Bay, Arabian Sea: Chronology and potential causes. Marine Ecology Progress Series 214: 15-23

HELCOM BASP (1974) Baltic marine environment protection commission [http://www.helcom.fi/1974 https://doi.org/10.1016/j.rsma.2020.101472]

Hold Gl, Smith EA, Rappé MS, Maas EW, Moore ERB, Stroempl C, Stephen JR, Prosser JI, Birkbeck TH, Gallacher S (2001) Characterization of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea.* FEMS Microbiology Ecology 37 (2): 161-173 [doi:10.1016/S0168-6496(01)00157-X]

Hosseini H, Saadaoui I, Moheimani N, Al Saidi ,M, Al Jamali F, Al Jabri H, Ben Hamadou R (2021) Marine health of the Arabian Gulf: Drivers of pollution and assessment approaches focusing on desalination activities. Marine Pollution Bulletin 164: 112085 [doi: 10.1016/j.marpolbul.2021.112085]

https://www.oceancolour.org site

https://oceancolor.gsfc.nasa.gov

Humborg C, Ittekkot V, Cociasu A, Bodungen B (1997). Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. Nature 386: 385-388

Imai I, Yamaguchi M, Hori Y (2006) Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. Plankton Benthos Research 1 (2): 71-84

Islam MS (2005) Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: Review and analysis towards model development. Marine Pollution Bulletin 50: 48-61 [doi: 10.1016/j.marpolbul.2004.08.008]

Ismail KA, Al Shehhi MR (2022) Upwelling and nutrient dynamics in the Arabian Gulf and sea of Oman. PLOS ONE 17 (10): e0276260 [https://doi.org/10.1371/journal.pone.0276260]

Jacob PG, Al-Muzaini S (1995) Marine plants of the Arabian Gulf and effect of oil pollution. Mahasagar 28: 83-101

Johns WE, Yao F, Olson DB, Josey S, Grist JP, Smeed D (2003) Observations of seasonal exchange through the Straits of Hormuz and the inferred heat and freshwater budgets of the Persian Gulf. Journal of Geophysical Research 108 (C12): 3391

Jones C, Sultan M, Yan E, Milewski A, Hussein M, Al-Dousari A, Al-Kaisy S, Becker R (2008) Hydrologic impacts of engineering projects on Tigris-Euphrates system and its marshlands. Journal of Hydrology 353: 59-75

Juma HA, Al-Madany IS (2008) Concentration of heavy metals in the territorial sea water of Kingdom of Bahrain, Arabian Gulf. Arab Gulf Journal of Scientific Research 26: 19-32

Lattuada M, Wilke T, Raes N (2020) Caspian Sea environmental variables: an extension of the Bio-Oracle

Ocean data set. Ecology 101 (9): e03076 [https://doi.org/10.1002/ecy.3076]

Lee CH, Ho H-J, Chen W-S, Iizuka A (2024) Total resource circulation of desalination brine: A review. Advanced Sustainable Systems 8: 2300460 [https://doi.org/10.1002/adsu.202300460]

Le Quesne WLF, Fernand L, Ali TS, Andres O, Antonpoulou M, Burt JA, Dougherty WW, Edson PJ, El Kharraz J, Glavan J, Mamiit RJ, Reid KD, Sajwani A, Sheahan D (2021) Is the development of desalination compatible with sustainable development of the Arabian Gulf? Marine Pollution Bulletin 173: 112940 [doi: 10.10D16/j.marpolbul.2021.112940]

Madhusoodhanan R, Al-Yamani F, Subba Rao DV, Ahmed A, Fernandes L, Al-Said T, Madhavapeddi S (2018) Salinity and microzooplankton grazing as forcing variables affecting phytoplankton growth and primary productivity in the NW Arabian Gulf. Gulf International Conference, 5-7, November 2018 Al Aion, UAE

Murray CJ, Muller-Karulis B, Carstensen J, Conley DJ, Gustafsson BG, Andersen JH (2019) Past, present and future eutrophication status of the Baltic Sea. Frontiers in Marine Science 6: 2 [https://doi.org/10.3389/fmars.2019.00002]

Neelamani S, Al-Dousari A (2016) A study on the annual fallout of the dust and the associated elements into the Kuwait Bay, Kuwait. Arabian Journal of Geosciences 9: 210 [doi.10.1007/s/12517-2236-2]

Nezlin N P, Polikarpov IG, Al-Yamani FY, Subba Rao DV, Ignatov AM (2010) Satellite monitoring of climatic factors regulating phytoplankton variability in the Arabian (Persian) Gulf. Journal of Marine Systems 82 (1-2): 47-60

Nixon S (2003) Replacing the Nile: Are anthropogenic nutrients providing fertility once brought to the Mediterranean by a Great River? AMBIO: A journal of the human environment 32: 30-39

Omerspahic M, Al-Jabri H, Siddqui SA, Saadaoui I (2022) Characteristics of desalination brine and its impacts on marine chemistry and health, with emphasis on the Persian/Arabian Gulf: A review. Frontiers in Marine Science 9: 845113 [https://doi.org/10.3389/fmars.2022.845113]

Oren A *et al*. (1995) A bloom of *Dunaliella parva* in the Dead Sea in 1992: biological and biogeochemical aspects. Hydrobiologia 297: 173-185 [https://doi.org/10.1007/BF00019283]

OSPAR Commission (2020) Atmospheric deposition of Nitrogen to the OSPAR maritime area in the period 1995-2017. Publication 758, London, UK. pp1-94 [ISBN 978-1-911458-97-5]

Oyeku O G, Mandal SK (2021) Historical occurrences of marine microalgal blooms in Indian Peninsula: Probable causes and implications. Oceanologia 63: 51-70

Pan Y (1996) Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-Nitzschia multiseries* under phosphate limitation. Journal of Phycology 32 (3): 371-381 [https://doi.org/10.1111/j.0022-3646.1996.00371.x]

Pan Y, Cembella AD, Quilliam MA (1999) Cell cycle and toxin production in the benthic dinoflagellate *Prorocentrum lima*. Marine Biology 134: 541-549 [doi:10.1007/s0022700505690xigenic]

Paytan A, Mackey KRM, Chen Y, Post AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. Thiemens MH (ed), University of California at San Diego, La Jolla, CA 106 (12) 4601-4605 [https://doi.org/10.1073/pnas.0811486106]

Pearce F (1993) Draining life from Iraq's marshes. New Scientist. 138: 11-12

Polikarpov I, Al-Yamani F, Saburova M (2009) Space-time variability of phytoplankton structure and diversity in the north-western part of the Arabian Gulf (Kuwait). BioRisk 3: 86-96 [https://doi.org/10.3897/biorisk.3.8]

Polikarpov I, Saburova, M, Al-Yamani F (2016) Diversity and distribution of winter phytoplankton in the Arabian Gulf and the Sea of Oman. Continental Shelf Research 119: 85-99

Polikarpov I, Saburova, M, Al-Yamani F (2020) Decadal changes in diversity and occurrence of microalgal blooms in the NW Arabian/Persian Gulf. Deep-Sea Research II 179: 104810 [https://doi.org/10.1016/j.dsr2.2020.104810]

Prakash PJ, Stenchikov G, Kalenderski S, Osipov S, Bangalath H (2015) The impact of dust storms on the Arabian Peninsula and the Red Sea. Atmospheric Chemistry and Physics 15: 199-222

Qatar (2015) [http://www.fao.org/nr/water/aquastat/countries_regions/qat/QAT-CP_ara.pdf]

Qureshi AS (2020) Wastewater to manage water scarcity crises in the Gulf Cooperation Council (GCC) Countries. Water 12: 1-16

Rengefors K (2020) Species assemblies and seasonal succession of dinoflagellates. Ch. 3 In: Subba Rao DV (ed) Dinoflagellates Classification, evolution, physiology, and ecological significance. Nova Science Publishers, Inc. New York. pp 63-84 [ISBN: 978-1-53617-888-3]

Reynolds RM (1993) Physical oceanography of the Gulf, Straits of Hormuz, and the Gulf of Oman: Results from the *Mt. Mitchell* Expedition. Marine Pollution Bulletin 27: 35-59

Richardson CJ, Reiss P, Hussain NA, Alwash AJ, Pool DJ (2005) The restoration potential of the Mesopotamian marshes of Iraq. Science 307: 1307-1311

Richlen ML, Morton SL, Jamali E, Anbiah R, Anderson DM (2010) The catastrophic 2008-2009 red tide in the Arabian Gulf region, with observations on the identification and phylogeny of the fish-killing dinoflagellate *Cochlodinium polykrikoides*. Harmful Algae 9: 163–172

Risk MJ, Haghshenas SA, Arab AR (2021) Cage aquaculture in the Persian Gulf: A cautionary tale for Iran and the world. Marine Pollution Bulletin 166: 112079 [https://doi.org/10.1016/j.marpolbul.2021.112079]

Saad MAH (1985) Distribution of nutrients in Shatt Al-Arab Estuary. Bulletin of the National Institute of Oceanography and Fisheries. Cairo Government 11: 24-72

Saburova M, Al-Kandari M, Polikarpov I, Akbar A, Hussain S, Rahmeh R, Al-Zakri W, Al-Yamani F (2022) Alien toxic dinoflagellate *Heterocapsa circularisquama* from the Western Pacific in Kuwait, NW Indian Ocean. Deep Sea Research Part II: Topical Studies in Oceanography 19: 6105027 [https://doi.org/10.1016/j.dsr2.2022.105027]

Safar MI (1985) Dust and dust storms in Kuwait. State of Kuwait Directorate General of civilization and Meteorological Department. 212 pp

Sheppard C, Al-Husiani M, Al-Jamali F, Al-Yamani F, Baldwin R, Bishop J, Benzoni F, Dutrieux E, Dulvy NK, Subba Rao DV, Jones DA, Loughland R, Medio D, Nithyanandan M, Pilling GM, Polikarpov I, Price ARG, Purkis S, Riegl B, Saburova M, Zainal K (2010) The Gulf: A young sea in decline. Marine Pollution Bulletin 60 (1): 13-38 [doi: 10.1016/j.marpolbul.2009.10.017]

Spilling K, Olli K, Lehtoranta J, Kremp A, Tedesco L, Tamelander T, Klais R, Peltonen H, Tamminen T (2018) Shifting diatom-dinoflagellate dominance during spring bloom in the Baltic Sea and its potential effects on biochemical cycling. Frontiers in Marine Science 5: 327 [https://doi.org/10.3389/fmars.2018.00327]

Stelzer JAA, Mesman JP, Gsell AS, de Senerpont Domis LN, Visser PM, Adrian R, Ibelings BW (2022) Phytoplankton responses to repeated pulse perturbations imposed on a trend of increasing eutrophication. Ecology and Evolution 12 (3): e8675 [https://doi.org/10.1002/ece3.8675]

Subba Rao DV, Sprules WG, Locke A, Carlton JT (1994) Exotic phytoplankton from ships' ballast waters: risk of potential spread to mariculture sites on Canada's east coast. Canadian data report of Fisheries and Aquatic Sciences 937: 51

Subba Rao DV, Al-Yamani F (1998) Phytoplankton ecology in the waters between Shatt-Al-Arab and Straits of Hormuz - the Arabian Gulf. Plankton Biology and Ecology 45: 101-116

Subba Rao DV, Al-Yamani F (1999) Analysis of the relationship between phytoplankton biomass and the euphotic layer off Kuwait, Arabian Gulf. Indian Journal of Maine Science 28: 416-423

Subba Rao DV, Al-Yamani F, Lennox A, Pan Y, Al-Said TFO (1999 a) Biomass and production characteristics of the first red tide noticed in Kuwait Bay, Arabian Gulf. Journal of Plankton Research UK 21: 805-810

Subba Rao DV, Al-Yamani F, Nageswara Rao CV (1999 b) Aeolian dust affects phytoplankton in the waters off Kuwait, the Arabian Gulf. Naturwissenschaften 86: 525-529

Subba Rao DV, Al-Yamani F (2000) Ch. 53, Arabian Gulf: In: Sheppard C (ed) Seas at the Millennium. Elsevier Science Ltd., UK. pp 1-16

Subba Rao DV, Al-Hassan JM, Al-Yamani F, Al-Rafiae K, Ismail W, Nageswara Rao CV, Al-Hassan M (2003) Elusive red tides in Kuwait Coastal waters. Harmful Algal News. IOC-UNESCO Newsletter 24: 10-13

Subba Rao DV, Pan Y, Al-Yamani F (2005) Growth and photosynthetic rates of *Chlamydomonas plethora* and *Nitzschia frustula* cultures isolated from Kuwait Bay, Arabian Gulf, and their potential as live algal food for tropical mariculture. Marine Ecology 26 (1): 63-71 [https://doi.org/10.1111/j.1439-0485.2005.00043.x]

Subba Rao DV, Durvasula R (2020) Ch. 15, Putting it all together: The Arabian Gulf dinoflagellates. In: Subba Rao DV (ed) Dinoflagellates, classification, evolution, physiology and ecological significance. Nova Science Publishers, New York. pp 527-569

Swift SA, Bower AS (2003) Formation and circulation of dense water in the Persian/Arabian Gulf. Journal of Geophysical Research 108: 3004 [doi:10.1029/2002JC001360]

Talling JF (1980) Water characteristics. In: Rzoska L (ed) Euphrates and Tigris: Mesopotamian ecology and destiny. Monographiae Bilogicae 38: 63-86

Winder M, Cloern JE (2010). The annual cycles of phytoplankton biomass. Philosophical Transactions of the Royal Society B 365: 3215-3226 [https://doi.org/10.1098/rstb.2010.0125]

WoRMS Editorial Board (2023) World Register of Marine Species [https://www.marinespecies.org at VLIZ; doi:10.14284/170]

Zavialov P (2005) Physical oceanography of the dying Aral Sea. Praxis Publishing Ltd., Chichester. pp 1-170

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    - figures (maps, graphs and others) in best possible resolution.

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[1] https://www.ajol.info/index.php/wiojms/login

[2] https://www.wiomsa.org/publications-2/wio-journal-of-marine-science/

- Original files of graphs and diagrams (such as Excel, Power-point, e-Primer, R script, etc.).
- Files of maps (pdf, tiff or jpeg) in high resolution.

In each published WIOJMS issue, the cover has a different colour photograph relevant for the themes of the respective articles. Should the author(s) have high-quality images for which they hold the copyright, they may propose its inclusion as cover for the issue.

The Manuscript

1. The manuscript is your own original work, and does not duplicate any other previously published work, including your own previously published work.

2. The manuscript has been submitted only to the Western Indian Ocean Journal of Marine Science; it is not under consideration or peer review or accepted for publication or in press or published elsewhere.

3. By submitting your manuscript to the Western Indian Ocean Journal of Marine Science, you are agreeing to any necessary originality checks your manuscript may undergo during the peer-review and production process.

4. Contributions must be written in English. Any consistent spelling and publication styles may be used. Please use single quotation marks, except where 'a quote is "within" a quotation'. Long quotations of 40 words or more should be indented without quotation marks. If English is not your first language we suggest that an English-speaker edits the text, before submission.

5. The journal recommends that authors have their manuscripts checked for language by an English native speaker before submission.

6. All persons who have a reasonable claim to authorship should be named in the manuscript as co-authors and should have been involved in the fundamental aspects of the manuscript; the corresponding author acts on their behalf in all matters pertaining to publication of the manuscript. The order of names should be agreed by all authors.

7. The manuscript must be typed in a normal type font (e.g. Times New Roman font size 12, or Arial font size 11) and 1.5 line spacing. Sequential line numbers should be inserted on the manuscript. The total number of pages should not exceed 20 manuscript pages (excluding figures and tables), both for Original Articles and Review Articles. Short Communications must not exceed 8 manuscript pages.

8. Species names must be in italics; the genus is written in full at the first mention in the Abstract, again in the main text, figure and table legends, and abbreviated thereafter.

9. Tables and Figures (maps, graphs and others): originals of all figures should follow the **Guidelines for illustrations** referred below. The lettering should be of a size readable after reduction for the final layout. Authors are requested to indicate the recommended position of figures and tables in the left-hand margin of the text. A separate sheet at the end of the document should be used for each table and figure, with the corresponding legend.

10. The international system of units (SI Units) must be used throughout; abbreviations and acronyms should be identified where they first appear; mathematical symbols and formulae should be used only when absolutely necessary and should be clearly defined in the text.

11.A complete **Original Article** manuscript must include the following: title page, abstract, keywords, introduction, materials and methods, results, discussion, acknowledgements, references, tables and figures (with figure legends) in that order.

a. Title Page: this should contain a concise title and the names of authors followed by affiliations and their complete postal addresses, phone numbers, and email addresses. The corresponding author and email address must be indicated.

b. Abstract: the abstract should not exceed 200 words and should be a single paragraph. It should briefly describe the main points of the manuscript.

c. Keywords: four to six key words are required for indexing purposes. Keywords must be written in lower case letters unless when referring names, separated by comas. Avoid using the same words as in the title.

d. Introduction: a brief survey of relevant literature and objectives of the work should be given in this section. Thus, the introduction should largely be limited to the scope, purpose and rationale of the study.

e. Materials and Methods: the methodology used should be clearly explained, including relevant references, such that another person can repeat the procedures. It should provide the framework to gain answers to the questions or problems identified. Sampling methods must be elaborated as well as analytical frameworks and model specifications.

f. Results: the text should be as objective and descriptive as possible. Only material pertinent to the subject should be included. Avoid presenting the same information in both graphical and tabular form.

g. Discussion: this section could be combined with the above to present "Results and Discussion". It should interpret the results in view of the problems identified in the introduction, as well as in relation to other published work. The final paragraph of this section could include concluding remarks and recommendations for future work.

h. Citations: authors should be cited using their surnames, followed by the year of publication. Two authors should be separated by 'and'. If there are more than two authors, only the first author, followed by "*et al.*", should be given. This and other Latin or foreign terms should be italicized.

i. Acknowledgement/s: this section should be brief. Authors are advised to limit acknowledgements to substantial contributions to the scientific and technical aspects of the paper, financial support or improvements in the quality of the manuscript.

j. References: the reference section must contain an alphabetical list of all references mentioned in the text of the manuscript, and the journal rules should be followed strictly. Limit punctuation and special fonts as indicated and give all journal names in full. Cross checking of references in the text to the cited literature and vice-versa is the responsibility of the author(s). Examples for citations from periodicals, books and composite works are given below:

- **Periodicals.** Here the following should be sequentially listed: author's name/s, initials, year of publication, full title of paper, periodical (in full), volume, first and last page numbers.

  Example: Richardson K, Beardall J, Raven J (1983) Adaptation of unicellular algae to irradiance: An analysis of strategies. The New Phytologist 93: 157-191

- **Books.** The following should be listed: author's or editor's name, initials, year of publication, full title, publisher, place of publication, total pages.

  Example: Kirk TJO (1983) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge. 401 pp

- **Composite works or serials.** The sequence should be as above, but also should include full title of paper followed by In: editor(s) if any, full title of publication, publisher, etc., and the first and last page numbers.

  Example: Sathyendranath S, Platt T (1993a) Remote sensing of water-column primary production. In: Li WKW, Maestrini SY (eds) Measurement of primary production from the molecular to the global Scale. ICES Marine Science Symposia, Vol. 97, Copenhagen. pp 236-243

- **Articles with a Digital Object Identifier (DOI).**

  Example: Gooseff MN, McKnight DM, Lyons HJ, Blum RJ (2002) Weathering reactions and hyporheic exchange controls on stream water chemistry in a glacial meltwater stream in the McMurdo Dry Valleys. Water Resources Bulletin 38 [doi: 10.1029/2001WR000834]

k. Tables and Figures: each table and figure should be numbered consecutively, accompanied by a complete caption, and must be cited in the text. Please follow the **Guidelines for illustrations** for details.

l. Supplementary material: in case it is found relevant, authors may submit appendices with relevant information of major interest for the interpretation of the manuscript results. This is not applicable for the raw data of normal research. The editors will decide its eventual inclusion as appendices.

12. A complete **Review Article** manuscript must include the following: title page, abstract, keywords, introduction, main body text (the central sections vary with specific divisions according to the theme), acknowledgements, references, tables and figures (with figure legends) in that order.
13. A complete **Short Communication** manuscript must include the same structure as an Original Article in a shorter format.

Failure to follow any point of these guideline may delay or compromise the editorial process. Unless there are communication problems, all communications and exchange of documents shall be made strictly via the AJOL editorial interface. Authors must comply with timing for processing manuscripts, either requested revisions or proof check, or otherwise inform the editors of any delays.

Guidelines for illustrations

Figures

To maintain a graphic consistency throughout all articles and issues of the journal, we advise the use of these guidelines.

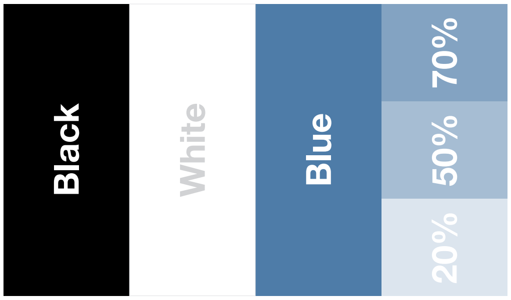
The original figures files should be sent separately from the text file to ensure the good quality of the figure reproduction.

Color palette

To maintain visual harmony in the illustrations, we recommend using mainly a monochromatic palette with the introduction of the Western Indian Ocean Journal's identity blue color.

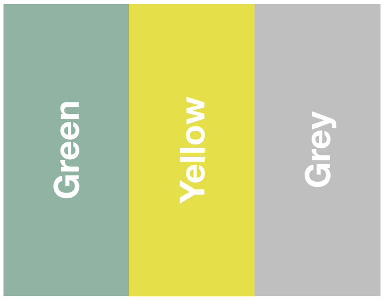
When necessary to allow greater contrast between patterns and colors, the use of the secondary palette is considered.

main



R 0	**R 255**	**R 77**	**Shades of blue**
G 0	**G 255**	**G 124**	
B 0	**B 255**	**B 168**	

secondary



R 144	**R 229**	**R 191**
G 179	**G 223**	**G 191**
B 162	**B 73**	**B 191**

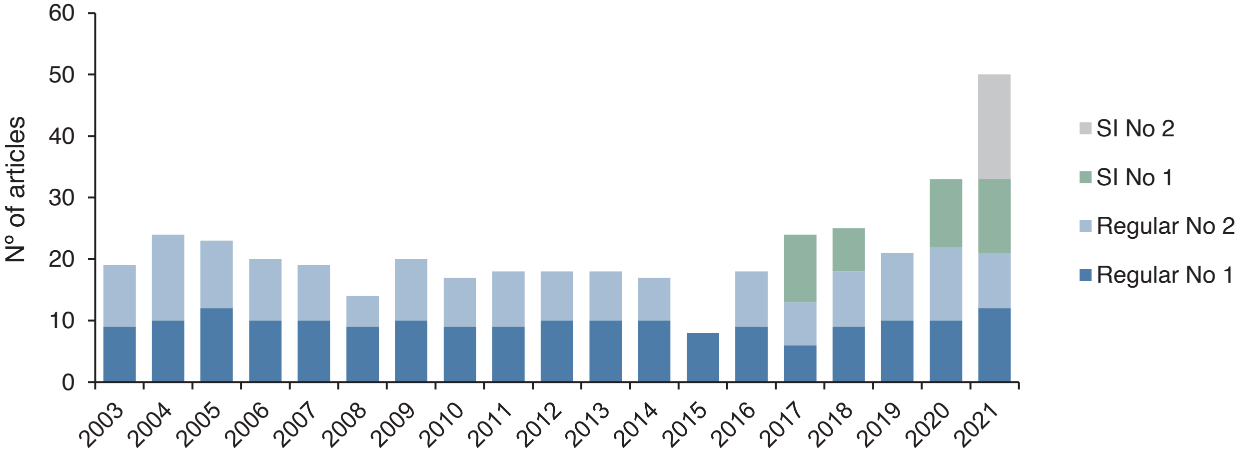
Graphs

Graphs should be clear and appealing figures.

The lettering should be of a size readable after reduction for the final layout.

We recommend using the Helvetica font in its various styles (Regular, Medium, Italic, Bold) for the axes and captions of graphs and other figures, as it is a classic, simple and easy reading font.

Preferably exported to pdf files to maintain viewing quality. They must be also sent separately in an editable format in the program in which they were made, for possible edits if necessary.



Example of formatted graph according to WIOJMS graphical guidelines

Published articles per year on WIOJMS.

Photographs

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

Example of low resolution

not enough for printing

Example of high resolution

good for printing

Maps

Maps must have good resolution for efficient viewing and good reading

Example of low resolution

not enough for printing

Example of high resolution

good for printing

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