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# Shelf life assessment of hot smoked African catfish stored under different storage conditions at Lake Kenyatta, north coast, Kenya

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## Abstract

Catfish (Family Clariidae) from Lake Kenyatta in coastal Kenya was smoked using an improved smoking (Chorkor) oven and subjected to storage under different packaging conditions. Biochemical, proximate composition and sensory parameters were used to determine the shelf life of the product for a period of 30 days. Peroxide values for samples stored under open, ambient air, and vacuum packaging increased significantly ($p < 0.05$) from 7.296 $megO_2$/kg to 24.890 $meqO_2$/kg, 28.940 $meqO_2$/kg and 18.729 $meqO_2$/kg, respectively. Thiobarbituric acid reactive substances increased from 0.459 mg/kg to 4.653 mg/kg, 1.473 mg/kg and 0.339 mg/kg during storage under open, ambient air, and vacuum packaging, respectively. Total volatile bases-nitrogen increased significantly with storage days, from 1.349 mgN/100g to 5.182 mg N/100g, 6.700 mgN/100g and 2.001 mgN/100g for open, ambient air, and vacuum packaging, respectively. During storage, proximate composition for the stored samples differed significantly between the open and ambient air package only, while sensory changes were observed on day 30 only. Texture remained the same to day 30 for all samples stored under difference storage conditions. Water activity ranged between 0.7 and 0.79 during the same period in the three packaging conditions. In general, the 30 days storage period did not compromise the acceptability of smoked products.

**Keywords:** storage, smoking, Chorkor, Lake Kenyatta, shelf life, packaging

## Introduction

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world (FAO, 2016). Recent reports have highlighted the tremendous potential of the oceans and inland waters as significant current and future contributors to food security and adequate nutrition for a global population expected to reach 9.7 billion by 2050 (FAO, 2016). However, fish is classified as a highly perishable food commodity whose shelf life depends on the initial quality as well as the subsequent storage conditions under which it is kept. Reports indicate that 46% of total direct human consumption of fish is in the live, fresh or chilled form (FAO, 2016). However, the rest of the edible production is in various processed forms, with about 12% (17 million tonnes) dried, salted, smoked or cured in other ways, while 13% (19 million tonnes) is in prepared and preserved forms, and 30% (about 44 million tonnes) is frozen (FAO, 2016).

Smoking forms one of the oldest methods used to process and preserve fish (Bilgin *et al.,* 2008). It can inhibit the formation of toxins in products and reduce growth of bacteria due to lower water activity (Rørvik, 2000). Smoking also gives special colour and flavour to food (Alcicek and Alar, 2010; Hattula *et al.,* 2001) and extends its shelf-life via the effect of dehydration, and the antimicrobial and antioxidant properties of the smoke compound (Goulas and Kontominas, 2005; Alcicek and Atar, 2010; Pagu *et al.*, 2013). Huss *et al.* (1995) stated that methods used to produce

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smoked fish varies among different producers within one country, and throughout world. This means that production parameters vary, and also the quality and shelf life of the product. It has been reported that since 1990, the consumption of smoked fish in the market has increased, and smoked salmon is the most consumed product followed by smoked trout and herring (Cardinal *et al.,* 2006).

Even though live, fresh or chilled fish is preferred in most markets, developing countries have challenges as far as infrastructure for this type of preservation is concerned. Poor roads and inadequate electricity supply in the fishing areas hamper the use of cold rooms, freezers and refrigerators. This leaves many developing countries with the option of practising mainly sun-drying and smoking methods of fish processing. In Kenya, fishermen face the same challenges, with most areas practising sun drying, deep frying and smoking as the major preservation methods. Processing procedures vary from one region to the other with no standardized processing methods or hygienic conditions.

At Lake Kenyatta in the Kenyan north coast region, smoking is carried out in traditionally built smoking kilns. Wood from various tree species is used for smoking with very little consideration of hygienic conditions and smoking temperature. Salting and duration of smoking is not standard. Reports indicate that the quality of smoked fish depends on the raw material (Cardinal *et al.,* 2001), condition of processing (Duffes, 1999), composition of smoke (Cardinal *et al.*, 2006) and storage conditions. This study was therefore designed to determine the quality and shelve life of smoked fish products using an improved smoking oven, and to determine the shelf life of the product under different storage conditions. Community participation was encouraged so as to empower the communities economically and to enhance food security.

## Materials and Methods

### Study area

This study was conducted at Mpeketoni fish landing site at Lake Kenyatta in Lamu County on the north coast Kenya (Fig. 1). Fishermen in this area are mainly artisanal and are engaged in both farming and fishing. Fish smoking is mainly done by women while fishing activities are dominated by men. Traditional smoking kilns of different sizes are used for smoking fish. Cichlidae (*Tilapia* sp.) and Clariidae (catfishes) are the main fish species smoked for marketing. Smoking is done in settlement areas with the majority preferring to smoke fish in their homes. The smoked products are kept in bags (gunny bags) awaiting customers who are mainly wholesalers. The smoked products are transported to nearby (Mombasa) and distant markets (Nairobi and Kisumu) for sale.

### Study design

Three smoking kilns were selected at random as replicates from the eight improved smoking ovens previously constructed by the Kenya Marine and Fisheries Research Institute (KMFRI) for this study. Pieces of catfish (150) of approximately equal sizes were bought from the fishermen and processed. In each kiln the fire was lit and left to char off for the production of smoke. Fifty pieces of catfish were placed on a wire mesh on top of each kiln, covered with ply wood to avoid contamination, and allowed to smoke dry. Smoking was done for a period of 30 hours.

### Fish handling and processing

Quality fish was selected and weighed using a top loading electronic weighing balance. The fish was then eviscerated, washed using 5% brine salt for 1 hour, and then left to drain for another 1 hour on a drying rack. During this period the fire was lit in each of the three ovens and a known amount of fuel introduced into each smoking oven for smoking. Acacia wood was used in all the three ovens for uniformity.

### Smoke-Drying

In each oven, three pieces of fish of equal sizes were marked for monitoring. Temperatures were monitored using a hand held thermometer with temperatures ranging from 70°C to 90°C. The three pieces of fish were weighed at an interval of 2 hours until no change in weight was detected. This marked the end of the drying period. The smoked fish were collected, placed in gunny bags and carried to the laboratory for quality determination and shelf life evaluation under different storage conditions.

### Laboratory treatment

Three replicates were chosen (one from each oven) and ground using a blender for analysis of biochemical, proximate and water activity parameters. The remaining samples were divided into three portions with each being stored under three different storage conditions. One portion was vacuum packed using vacuum packer (vacuum package). The second portion was place in polythene bags and sealed under ambient atmospheric conditions (ambient air


**Figure 1.** Map showing Lake Kenyatta study sites on the North coast of Kenya

package) using a sealing machine, while the third portion was placed in open containers (open package). All samples were stored in the laboratory at ambient air temperature (27°C ± 4°C) for a period of 30 days. Sampling from each was done every 10 days for biochemical, sensory and water activity parameters.

### Laboratory Analysis

Each sample was presented to 10 pre-trained panellists at the beginning of storage. The attributes tested were taste, texture, appearance, and general acceptability. The attributes were based on a 5-point scale for each attribute according to Haider *et al*. (2011). Water activity readings were obtained in replicates using a water activity meter. Total volatile basic-nitrogen (TVB-N) was determined according to the method adopted from Siang and Kim (1992) using Conway's Micro Diffusion Unit, while the extraction of crude fat was carried out according to the method of Bligh and Dyer (1959). Peroxide values (PV) were determined according to Kirk and Sawyer (1991).

Crude protein content was determined based on the Kjeldahl method (AOAC, 1990), whereas crude fat content was determined by the AOCS (1997) official method of analysis.

Dry matter was calculated by analysing moisture content according to the AOCS (1997) official method of analysis. Moisture content was then subtracted from 100% to get dry matter content (%). Ash content was determined according to the AOCS (1997) official method of analysis.

### Data Analysis

Data were analysed using MINITAB® 14 statistical software. All data were tested for normality using Shapiro Wilk (1965) before being subjected to analysis of variance (ANOVA). Where differences were noted, tests for significance differences in means was conducted using Turkey's pair-wise comparison analysis. All tests were considered significant at a confidence level of 95% ($\alpha$ = 0.05).

## Results

### Effect of storage on biochemical parameters

*Peroxide Values (PV)*

The results (Table 1) show an increase in peroxide values with storage period.

**Table 1.** Turkey's pairwise comparison on mean Peroxide values (PV) of fish during storage under different packaging conditions.

| Packaging conditions | Storage Period | | | Limit of acceptability |
|---|---|---|---|---|
| | Day 0 | Day 15 | Day 30 | |
| Open Packaging | 7.296 ± 2.316[a] | 32.583 ± 3.458[b] | 24.890 ± 9.838[b] | 10-16 $meqO_2/kg$ |
| Ambient air packaging | 7.296 ± 2.316[a] | 19.324 ± 4.652[b] | 28.940 ± 4.905[c] | |
| Vacuum packaging | 7.296 ± 2.316[a] | 16.573 ± 3.458[b] | 18.729 ± 3.585[c] | Okpala *et al.* (2014) |

Different superscript letters in the same row indicate significant difference ($p < 0.05$). The values are expressed as Mean ± standard deviation. Units are expressed in meq O2/kg.

The lowest PV was observed on Day 0 and the highest on Day 30 (Table 1). All packaging conditions showed increased PV with storage time. However, ambient air packaging had the highest PV of 28.940 ± 4.905 $meqO_2/kg$ after 30 days storage, followed by the open packaging, while the lowest value during the same storage time was observed in the vacuum packaged samples at 18.729 ±3.585 $meqO_2/kg$. There was a significance difference ($p < 0.05$) in all PVs during the storage period, except for day 15 and 30 for open packaging. Both open and ambient air samples surpassed the PV limit of acceptability (10-16 meqO2/kg) by day 15 of storage, while the vacuum packaged samples were still within the acceptable range on day 15 of the storage period. PVs for all products under the three packaging conditions however surpassed the limit of acceptability after 30 days of storage.

*Thiobarbituric Acid Reactive Substances (TBARs)*

The results showed an increase in TBARs values with storage days in products stored under open packaging conditions (Table 2). The highest value was observed on day 30 of the storage period (4.653 ± 0.832 mg/kg).

Vacuum packaging showed the least changes in TBARs values during the storage period of 30 days. This was followed by ambient air packaging while the highest values were observed on samples under open packaging. There were significant differences ($P<0.05$) in TBARS values for products stored under open packaging and ambient air packaging during the storage period. Products stored under vacuum packaging did not show any significance differences for the whole storage period. Open and ambient air packaged products surpassed the limit of acceptability (1mg/kg) at day 15 and day 30 respectively, while vacuum air packaged products remained within the limit of acceptability during the same period.

*Total Volatile Bases-Nitrogen (TVB-N)*

Results showed that ambient air packaging had the highest value of TVB-N on day 30 (6.700 ± 0.284 mg N/100g), followed by open packaging (5.182 ± 0.284 mg N/100g), while the lowest value was vacuum packaging (2.001 ± 0.214 mg N/100g). All packaging conditions showed significance differences in TVB-N values over the 30 day storage period. All the TVB-N values for all packaging conditions remained within the acceptability limits (<25mgN/100g) during the rest of the storage period. In all biochemical parameters, the ambient air storage conditions show comparatively higher values than those stored under open and vacuum packaging conditions on day 15 and day 30, respectively.

**Table 2.** Turkey's pairwise comparison on mean Thiobarbituric acid reactive substances (TBARS) of fish during storage under different packaging conditions.

| Packaging condition | Storage period | | | Limit of acceptability |
|---|---|---|---|---|
| | Day 0 | Day 15 | Day 30 | |
| Open | 0.459 ± 0.059[a] | 1.941 ± 0.269[b] | 4.653 ± 0.832[c] | < 1 mg/kg |
| Ambient air | 0.459 ± 0.059[a] | 0.233 ± 0.012[a] | 1.473 ± 0.335[b] | (Kezban and Nuray, |
| Vacuum | 0.459 ± 0.059[a] | 0.473 ± 0.057[a] | 0.339 ± 0.091[a] | 2003) |

Different superscript letters in the same row indicate significant difference ($p < 0.05$). The values are expressed as Mean ± standard deviation. Values are expressed in mg/kg.

**Table 3.** Turkey's pairwise comparison on mean Total Volatile Bases-Nitrogen of fish during storage under different packaging conditions.

| Packaging conditions | Storage period | | | Limit of acceptability |
|---|---|---|---|---|
| | Day 0 | Day 15 | Day 30 | |
| Open Packaging | 1.349 ± 0.082[a] | 3.721 ± 0.426[b] | 5.182 ± 0.325[c] | < 25mgN/100g Bono & Badaluco, (2012) |
| Ambient air packaging | 1.349 ± 0.082[a] | 5.574 ± 0.741[b] | 6.700 ± 0.284[b] | |
| Vacuum packaging | 1.349 ± 0.082[a] | 1.535 ± 0.139[a] | 2.001 ± 0.214[b] | |

Different superscript letters in the same row indicate significant difference ($p < 0.05$). The values are expressed as Mean ± standard deviation. Values are expressed in mg N/100g.

### Water activity variations

The results (Fig. 2) on water activity of the samples ranged between 0.70 and 0.79 for the whole storage period, with the lowest value being observed in ambient air packaging on day 30. However, no significance difference was seen during the storage period in all storage conditions.

### Temperature Variations

There was minimal changes in temperatures during the storage period (Table 4) with the highest value being 25.423 ± 0.282° C and the minimum being 24.953 ± 0.071° C.

### Effect of storage on sensory attributes under different packaging conditions

Taste, texture, appearance, and overall acceptability was scored by the 10 panelists. As shown in Fig. 3, panelists detected no change in taste for the products stored for 15 days. However, day 30 resulted in a lower score in taste for all products stored under different packaging with a mean score of 3.0.

On the other hand, texture did not change in score rating for the whole storage period, having a mean score of 4.0. The scores on appearance also did not change for the whole storage period in all products, except for the product stored in the open, having a mean score of 3.0 on day 30. A lower score of 3.0 was observed on overall acceptability for open and ambient air packaged samples. However, vacuum packaged samples had no change in scores for the rest of the storage period.

### Effect of storage on proximate composition

The results indicated a continuous decrease in protein content over the storage period in fish samples stored under open and ambient air packaging conditions. On the contrary, the values for vacuum packaged samples increased. Open packaging products gave protein value of 60.499% while ambient air packaging had a value of 61.154%, respectively. Percentage fat composition did not show any significance difference for both packaging and storage period. This was also observed in the ash (%) composition, except for the

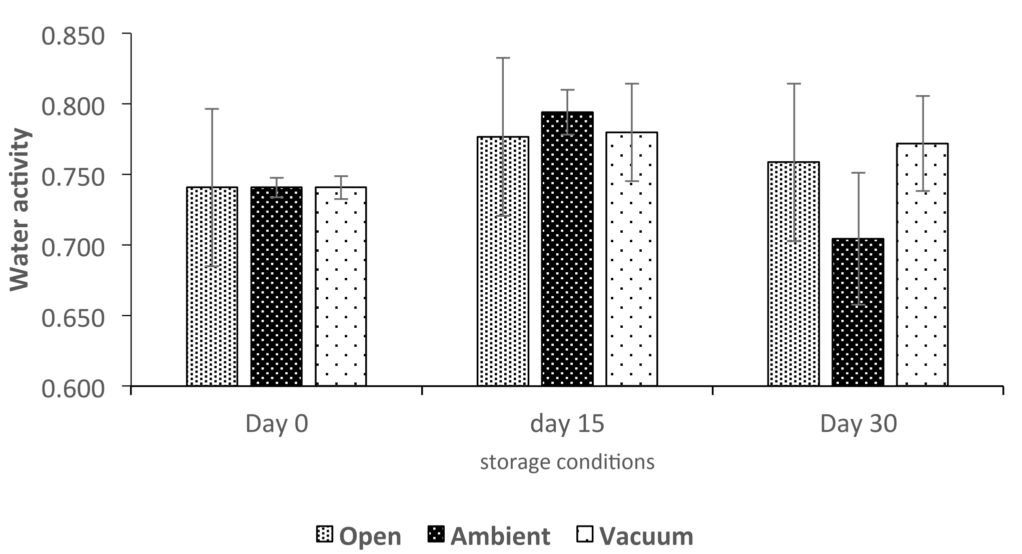


**Figure 2.** Changes in water activity during storage under open, ambient and vacuum packaging.

**Table 4.** Mean temperature variation during storage under different packaging conditions.

| Days of storage | Mean Temp.$^0$C ± Stdev |
|---|---|
| Day 0 | 25.423 ± 0.282 |
| Day15 | 25.193 ± **0.062** |
| Day 30 | 24.953 ± 0.071 |

open packaged samples, that had a significant difference on day 30 of storage. During the 30 days storage period, there was a significant change in percentage moisture content in fish products stored in the open and ambient air packaging (Table 5). However, the products stored under vacuum packaging did not show a significance difference in the moisture content over the 30 days storage period.

## Discussion

### *Peroxide Value*

The observed increase in PV in this study indicates an increase in rancidity of the oil leading to the "off" flavour of catfish samples. Similar observations were made by Nirmal and Benjakul (2009), and Chaijan (2011). Consumer acceptability of PV value in fish has been categorised as follows: 0-2 mmol/kg - very good; 2-5 mmol/kg – good; 5-8 mmol/kg – acceptable; and 8-10 mmol/kg - spoilt (Okpala *et al.*, 2014). Conversion of these values to meq $O_2$/kg gives values of 0-4 meq $O_2$/kg as very good, 4-10 meq $O_2$/kg as good, 10-16 meq $O_2$/kg as acceptable, and 16 - 20 meq $O_2$/kg as spoilt. Peroxide value results showed that quality of the smoked fish products stored under open and ambient air packaging deteriorated beyond acceptable level at day 15. However, vacuum packaged sampled were at the end limit level of acceptability (16.573±3.458 meq $O_2$/kg). High values observed in the open package could be associated with free contact to air. On the other hand, the values observed in the ambient air packaging could be due to availability of sufficient air (oxygen) in the package leading to elevated PV in comparison to vacuum packaging. The lowest PVs observed in the vacuum packaging was mainly attributed to low oxygen in the package hence restricting the oxidation process of oil in the fish products.

### *Thiobarbituric Reactive Substances (TBARs)*

TBARs is one of the most widely used assays for measuring lipid oxidation in the food industry. It is formed as a degradation product of fats (Malondialdehyde-MDA) present in a sample as well as

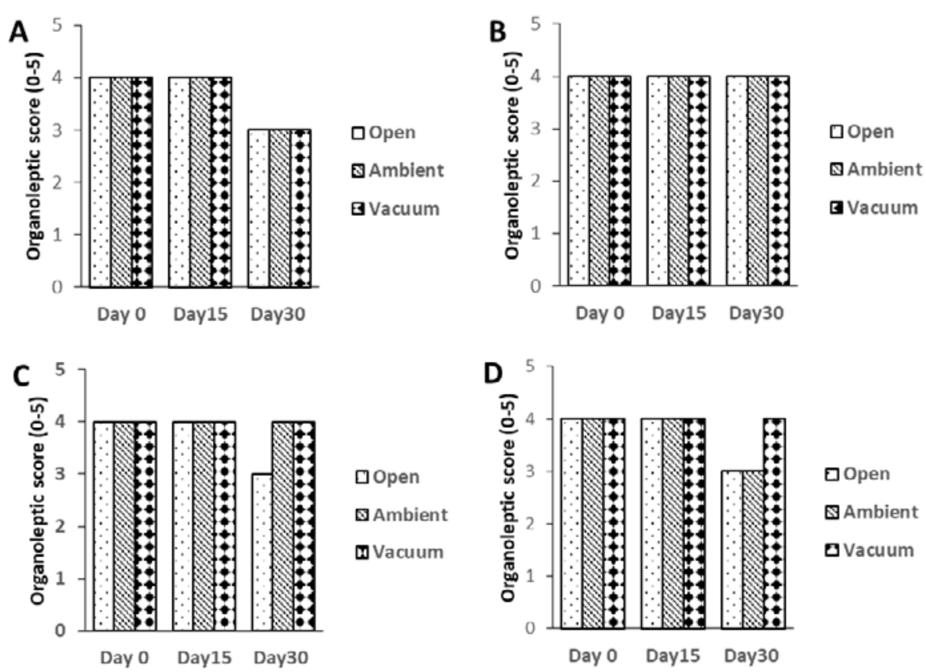


**Figure 3.** Changes in sensory scores and fish products for Taste (A), Texture (B), Appearance (C) and Overall acceptability (D) during storage

**Table 5.** Turkey's pairwise comparison on mean proximate composition of fish during storage under different packaging conditions.

| Parameter | Packaging | Storage Days | | |
|---|---|---|---|---|
| | | Day 0 | Day 15 | Day 30 |
| Moisture (%) | Open | 17.911 ± 0.964[a] | 18.597 ± 2.906[a] | 29.002 ± 6.693[b] |
| | Ambient air | 17.911 ± 0.964[a] | 20.943 ± 3.489[a] | 25.708 ± 4.342[b] |
| | Vacuum | 17.911 ± 0.964[a] | 19.381 ± 1.551[a] | 20.017 ± 1.123[a] |
| Protein (%) | Open | 77.318 ± 0.147[a] | 71.792 ± 1.457[b] | 60.449 ± 0.689[c] |
| | Ambient air | 77.318 ± 0.147[a] | 72.112 ± 1.636[a] | 61.154 ± 0.000[b] |
| | Vacuum | 77.318 ± 0.147[a] | 74.719 ± 1.295[a] | 82.044 ± 0.709[b] |
| Fat (%) | Open | 6.328± 0.933[a] | 5.222 ± 0.735[a] | 5.174 ± 0.720[a] |
| | Ambient air | 6.328 ± 0.933[a] | 3.818 ± 0.385[b] | 5.250 ± 1.059[a] |
| | Vacuum | 6.328 ± 0.933[a] | 6.673 ± 0.494[a] | 5.551 ± 1.391[a] |
| Ash (%) | Open | 3.369 ± 0.159[a] | 4.123 ± 0.213[a] | 4.047 ± 0.188[b] |
| | Ambient air | 3.369 ± 0.159[a] | 3.244 ± 1.565[a] | 3.603 ± 0.805[a] |
| | Vacuum | 3.369 ± 0.213[a] | 3.499 ± 1.175[a] | 4.440 ± 0.453[a] |

Different superscript letters in the same row indicate significant difference ($p < 0.05$). The values are expressed as Mean ± standard deviation (%) composition. Values are expressed in % composition.

malodialdehyde generated from lipid hydro peroxides. The significant increase in TBARs values from day 0 to day 30 in open and ambient air conditions during storage indicated continued degradation of fats on the smoked product. This could be attributed to unlimited air (oxygen) contact in both open and ambient air packages. On the contrary, the insignificant change in the vacuum packaged samples indicated non-degradation of fats for the 30 days period. This could be due to restricted air contact due to the vacuum packaging condition during storage,

### *Total Volatile Bases-Nitrogen (TVB-N)*

TVB-N serve as a quality indicator to estimate the level of freshness in fishery products. There was an increase in values of TVB-N with an increase in storage days in each packaging condition. This indicates a cumulative spoilage trend or quality loss with storage days. Similar observations were reported by Ali *et al.* (2013). Studies have reported scales of acceptability for TVB-N in shrimps to range as follows: <12 mg N/100 g for fresh raw shrimps; 12 – 20 mg N/100 g for edible but lightly decomposed shrimps; 20 – 25 mg N/100 g for borderline shrimps; and > 25 mg N/100 g for inedible and decomposed shrimps (Lannelongue *et al.*, 1982; Bono and Badaluco, 2012; Okpala *et al.*, 2014). TVB-N values in this study indicated that the fish samples did not surpass the limit of freshness for the whole storage period, despite the increase in value with time. Packaging conditions had a significant effect on the quality of the fish. However, the products in all the three packaging conditions remained fresh (<12 mg N/100g). Despite being within the limit of freshness in all packaging conditions, it was observed that vacuum packaging had the lowest values. It has been reported that vacuum packaging extends the shelf life in comparison to ambient air packaged products (Kumar & Ganguly, 2014). This was said to be attributed to the restricted quantity of air in the package leading to reduced bacterial activity (Kumar *et al.*, 2015). On the other hand, the availability of air in the open and ambient air packages allowed the bacterial activity to continue with little restrictions, leading to higher values.

### Water activity

Water activity of any given food system is an important index to consider, particularly because of the resultant chemical effects during food processing. Richardson and Finley (1986) stated that water activity is able to influence the oxidation of fresh foods, particularly during storage. Water activity of less than 0.70 and 0.62 is able to retard the growth of bacteria and fungi respectively (Sandulachi, 2012). The water activity levels of between 0.70 and 0.79 in this study were therefore likely to retard bacterial activity leading to prolonged shelf life, but fungal growth was not effectively retarded, and could still lead to spoilage. Therefore, more dehydration during drying to water activity level of < 0.62 is necessary.

### Effect of storage on Sensory attributes under different packaging conditions

A range in scores of between 4.0 and 5.0 on the fish samples was considered a good response on product acceptability. Between day 0 and 15, the overall acceptability scores remained at 4.0. This indicated a good panelist response on the product quality for the 15 days period. However, the overall acceptability score of 3.0 for open and ambient packaging samples on day 30 indicated reduced quality of the product under these treatments.

### Effect of storage on proximate composition

The highest value (29.002%) in moisture content for open packaging storage was noticed on day 30. During the same period vacuum packaging gave a value of 20.017% moisture content. This increase in moisture content could be attributed to absorption of air moisture, since the products were stored in open packages. Lack of air contact in the samples stored in vacuum packaging could be the contributor to insignificant change in moisture content over the storage period.

Protein content was significantly different in all packaging conditions during the 30 days storage, with the highest value being 82.044 for vacuum packaged samples. Both ash (%) and fat content showed no significant change during the entire storage period.

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# Post-bleaching mortality of a remote coral reef community in Seychelles, Western Indian Ocean

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## Abstract

The 2015–2016 global coral reef bleaching event was the most persistent and widespread in history. In its aftermath, efforts are required to understand the extent of the post-bleaching coral mortality and the ability of reefs to recover. This study used benthic photographic data to assess the post bleaching mortality of a coral reef community at D'Arros Island and St Joseph Atoll in the Republic of Seychelles, Western Indian Ocean. Results showed that April 2016 exhibited anomalously high sea temperatures that were above the regional coral bleaching threshold. In response, hard coral cover declined significantly from pre-bleaching levels of 28.5% in 2015 to 14.7% in 2017. Post-bleaching coral cover was significantly affected by site, with shallow reefs dominated by acroporids and pocilloporids exhibiting greater declines in hard coral than deeper sites. There were no changes to the macroalgal community but significant post-bleaching increases in coralline algae, which could facilitate reef recovery. This may be influenced by the reef's associated herbivorous fish community and lack of concurrent anthropogenic stressors. Continued monitoring is required to assess long-term impacts of the bleaching event, however, initial evidence suggests D'Arros Island and St Joseph Atoll provide a suitable environment for post-bleaching coral recovery.

**Keywords:** Coral reefs, Coral bleaching, Post bleaching mortality, 3rd Coral Reef Bleaching Event, Western Indian Ocean Coral Reef

## Introduction

Coral reefs are highly biodiverse ecosystems that provide important goods and services to an estimated 500 million people worldwide (Pratchett *et al.*, 2008; Burke *et al.*, 2011). Despite their economic and ecological value, coral reefs are threatened by a suite of human induced stressors (Wilkinson, 1999; Hughes and Connell, 1999; Hughes *et al.*, 2017a) of which sea temperature rise, resulting in coral bleaching, is arguably the most problematic (Baker *et al.*, 2008; Hoegh-Guldberg *et al.*, 2017; Hughes *et al.*, 2018). Mass regional coral bleaching and subsequent mortality was first noted in 1983 (Coffroth *et al.*, 1990; Glynn, 1993), with the first global bleaching event recognized in 1998 (Spalding and Brown, 2015). Further global bleaching events occurred in 2002, 2006, 2010 and 2015–2016, with the latter recorded as the longest and most widespread in history (Hoegh-Guldberg *et al.*, 2017; Hughes *et al.*, 2017b).

Research following past bleaching events suggests that reefs can recover from coral loss over decadal timescales if not affected again by mass bleaching or the presence of other anthropogenic stressors (Hoegh-Guldberg *et al.*, 2017). However, current trajectories of global warming make this scenario seem unlikely (Bellwood *et al.*, 2004; Zinke *et al.*, 2014; Perry and Morgan, 2017) and evidence from progressive bleaching events suggest that the recovery potential of reefs may be diminished with synergistic stress events

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(Hughes, 1994; Hughes *et al.*, 2017b). After the 1998 global bleaching event, coral cover in the Western Indian Ocean region declined substantially but reefs, particularly in Seychelles, showed signs of recovery following the event (Obura, 2005; Stobart *et al.*, 2005). However, after the 2015–2016 global bleaching event, an assessment of the risks to coral reefs suggested that coral reefs in Seychelles may be particularly susceptible to future decline (Beyer *et al.*, 2018). Thus, there remains a need to assess the post-bleaching status of coral reefs, particularly in Seychelles, and identify those that show signs of recovery in order to prioritize conservation efforts accordingly (Beyer *et al.*, 2018).

In this study, sea temperature data and benthic photographic data collected between 2011 and 2017 is assessed to determine the mortality of hard corals and the status of the post-bleaching benthic community at a remote coral reef in the Amirante Islands, Republic of Seychelles.

## Material and methods

### Study site

D'Arros Island and St Joseph Atoll are situated on the Amirantes Bank approximately 255 km southwest of Mahé in the Republic of Seychelles (Fig. 1) and together make up 3.03 km$^2$ of low lying land fringed by extensive reef flats and associated outer reef slopes (Stoddart *et al.*, 1979).

### Sea temperature

Sea temperature was recorded at 10 minute intervals from 2012 to 2017 at 5m and 12m depth at D'Arros Island (Fig. 1) using HOBO ProV2 water temperature loggers.

### Benthic surveys

Benthic surveys were conducted annually using a stratified random sampling protocol. Eleven representative sites were selected at D'Arros Island and St Joseph Atoll. Sites 1, 3, 5, 7 and 9 were located at a depth of 5m whilst sites 2, 4, 6, 8, 10 and 11 were located at a depth of 12m. Annually, 80 quadrats (1m$^2$) were positioned randomly within each selected site and photographs were taken of each quadrat by a diver positioned perpendicular to the substrate. Photographs were taken using a Nikon D7100 camera with a Nikon 10-22mm rectilinear lens and external strobes. Sampling was conducted in the months of November (2011, 2012, 2014, 2016), December (2015), January (2013), and July (2017).

### Benthic classification

Benthic photographs were analysed using Coral Point Count with Excel extensions (CPCe) software v.4 (Kohler and Gill, 2006) whereby each photo quadrat was calibrated and 30 randomly generated points per sample were overlaid and categorised, generating 26,400 points a year. Each point was categorized as either: abiotic, algae, soft coral (including gorgonians),

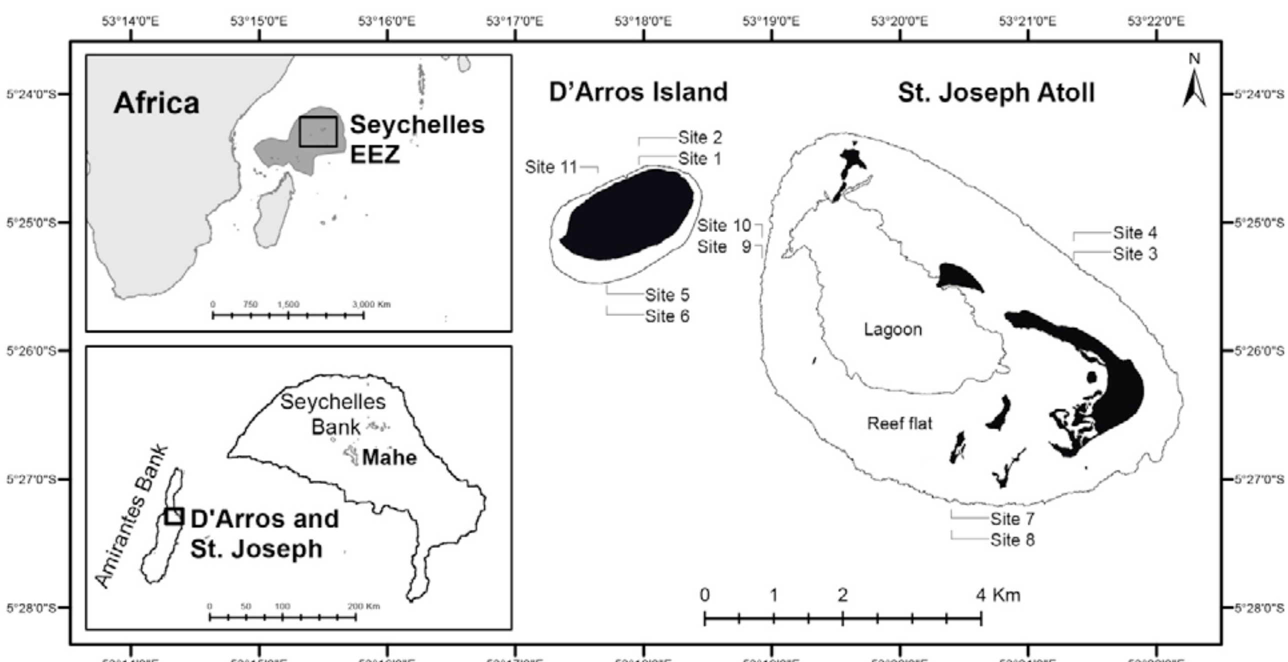


**Figure 1.** D'Arros Island and St. Joseph Atoll located in the Amirante Island Group within the Outer Islands of the Republic of Seychelles in the West Indian Ocean. Site numbers indicate survey locations (n=11).

hard coral or "other". Points that fell outside the quadrats were not included. Corals were classified as "healthy", "bleached", "fluorescing" and "recently dead". Additionally, abiotic classifications were categorized as bare rock, sand, or coral rubble (defined as "dead coral that had turned to rubble"), and algae was divided into either coralline algae or macroalgae. "Other" included mobile invertebrates, sessile invertebrates (sponges, giant clams) and fish.

### Data analysis

All statistical analyses were performed in the R statistical platform (3.3.1; http://cran.r-project.org). CPCe produced annual per site averages of cover for hard coral, soft coral, bare ground (abiotic), macroalgae, coralline algae, and "other" which were used as response variables in analyses. Transformations were applied using square root to relevant variables (√hard coral and √macroalgae) to reduce skew and improve linearity. Response variables were tested for intercorrelation to identify serious collinearity ($r>0.7$ and $VIF>2$). One-way ANOVAs were used to compare benthic cover trends and annual per site averages from 2011 to 2017. Transformed data was normally distributed and met the assumptions for a one-way ANOVA. Any significant changes in benthic coverage across years were compared using a post hoc Tukey test to identify year to year differences for $p<0.05$.

The pre-bleaching hard coral cover in 2015 was also compared separately with the hard coral cover during the bleaching event in 2016 and after it in 2017. The data analysed consisted of 880 points per year, as opposed to annual averages used for previous analyses. These data were not normally distributed and could not be transformed, therefore nonparametric methods were used. A Kruskal-Wallis test compared hard coral cover across the three years and a post hoc Dunn's test determined specific year differences for $p<0.05$. Site differences were also tested for significant changes in coral cover from 2015 to 2017. Since the data were paired and not normally distributed, a nonparametric Wilcoxon signed-rank test was used. All summary data were calculated as means and standard deviation.

## Results

### Sea temperature

Between 2012 and 2015, mean monthly sea temperature at 5m at D'Arros Island typically peaked at 29.65 °C in April (Fig. 2). In April 2016, this figure rose to 30.25 °C, which was 0.50 °C warmer than the next warmest month on record (Fig. 2). Maximum annual sea temperatures recorded at 5m at D'Arros Island ranged between 30.42°C in 2013 and 31.31°C in 2016 over the six-year study period (Fig. 3). Similarly, maximum annual temperatures recorded at 12m ranged between 30.17°C in 2013 and 31.05°C in 2016. The coral bleaching temperature threshold, considered to be 30.5°C in Seychelles (NOAA), recorded at 5m depth was reached on 6 days in 2012, on 11 days in 2014, on 4 days in 2015, and on 38 days in 2016 (Fig. 3). Temperatures of 30.5 °C or more were also recorded at 12m depth for 20 days between March and May in 2016.

### Hard coral cover

In 2011, mean hard coral cover was 18.8% (SD ± 15.9) across all sites and this increased steadily from 2013 until it reached 28.5% (SD ± 15.9) in 2015. Following bleaching in May 2016, hard coral cover decreased to 18.1% (SD ± 10.2), then went down further to 14.7%

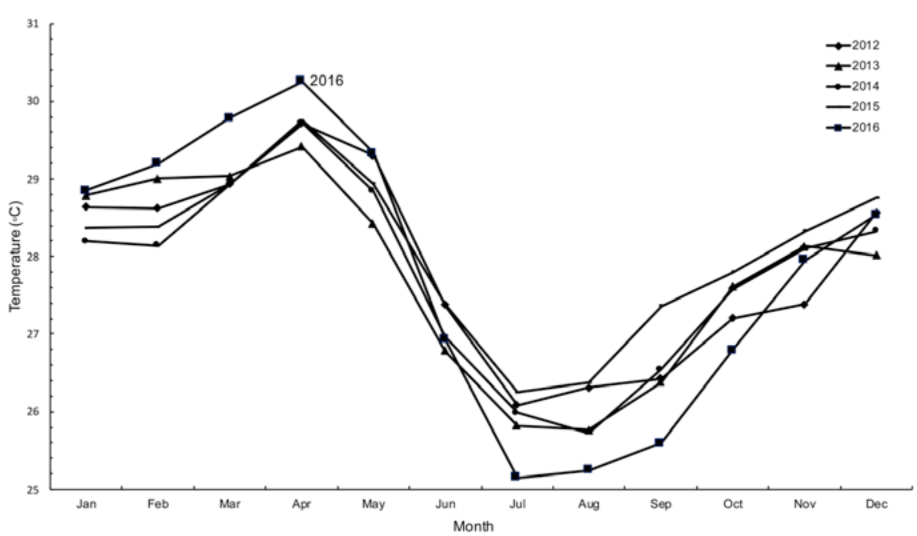


**Figure 2.** Monthly mean temperature recorded at 5m at D'Arros Island between 2012 and 2016.

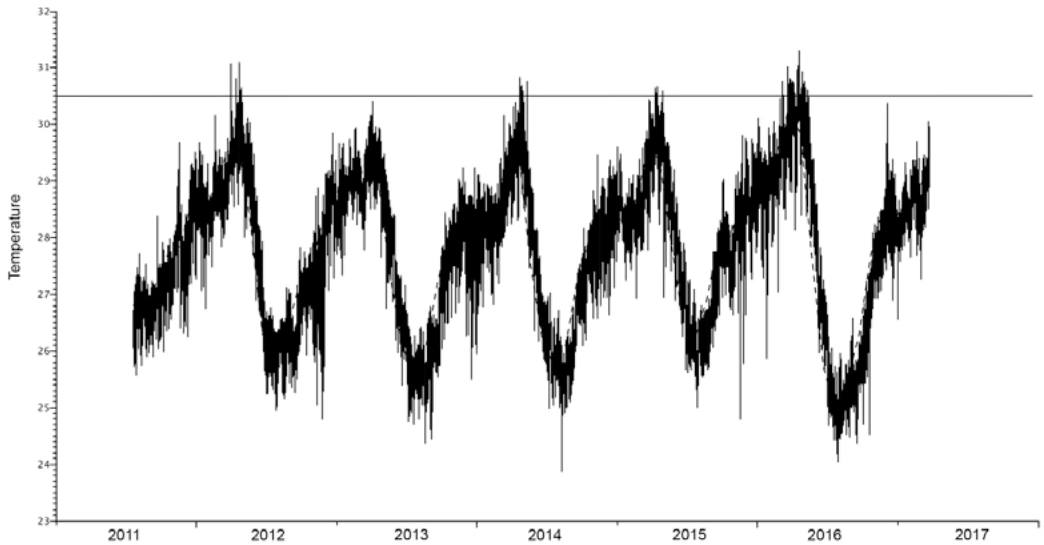


**Figure 3.** Daily sea temperature recorded at D'Arros Island at 5m between 2011 and 2017. Horizontal line represents the 30.5°C coral bleaching temperature threshold reported for the region.

(SD ± 7.9) in 2017 (Fig. 4). Between 2015 and 2017, the overall, mean hard coral cover reduced by almost half (48.3%), although the influence of year was not statistically significant when per site averages were compared (One-way ANOVA $F(6, 70)=1.26$, $p=0.28$). However, when change in average coral cover was compared between 2015 and 2017, a significant decline was apparent ($x^2(2)=197.3$, $p=0.001$, $n=880$) with 2015, 2016 and 2017 all exhibiting significant differences in post hoc testing ($p<0.001$).

## Hard coral cover between survey sites

Fig. 5 illustrates pre-bleaching (2015), bleaching (2016), and post-bleaching (2017) hard coral cover at the 11 study sites. Sites 1, 4, 5 and 9 showed high average loss, equating to declines of 21% to 36%. Site 1 exhibited the greatest decrease in hard coral cover, declining from yearly averages of 50.8% to 14.08%. At sites 2, 3, 7, 8 and 10, declines of hard coral cover were small at only 1.8 to 5.1% between 2015 and 2017. Site 6 lost 14.6% of its coral cover, while Site 11 increased by 2.2% from 2015 to 2017. A Wilcoxon signed-rank test showed that the amount of hard coral cover before and after the 2016 bleaching was significantly affected by site ($Z=-19.215$, $p=0.001$ (two tailed)).

## Benthic composition

Benthic composition differed across years, with a

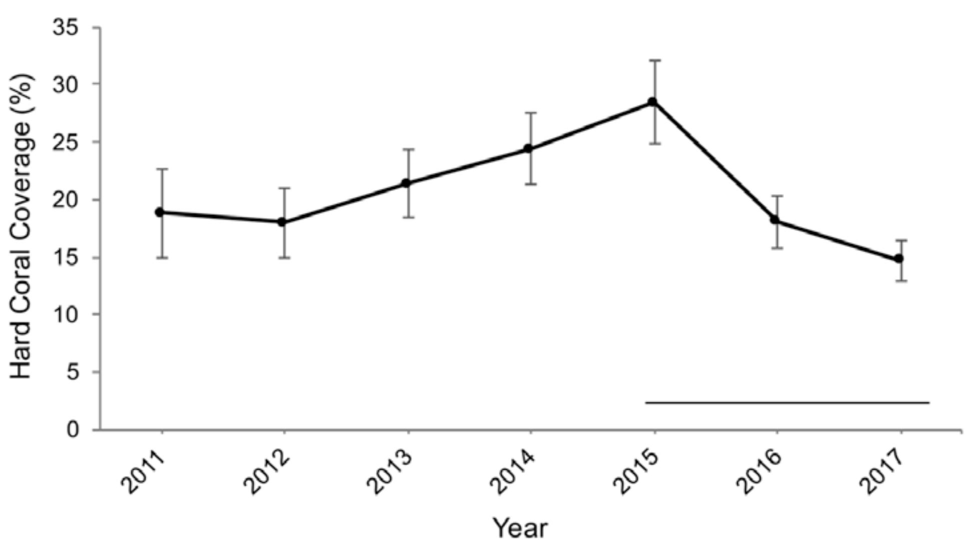


**Figure 4.** Mean hard coral cover across all sites surveyed between 2011 and 2017. Horizontal line represents significant difference between years. Error bars represent 95% confidence intervals.

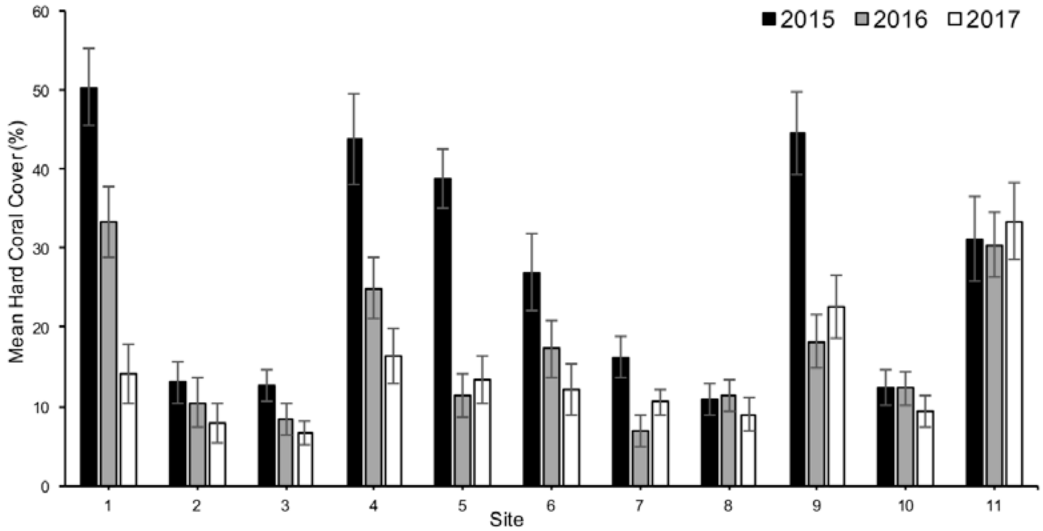


**Figure 5.** Mean hard coral cover for each survey site before the coral reef bleaching event in 2015, during it in 2016, and afterwards in 2017. Error bars represent 95% confidence intervals.

significant ($p<0.01$) increase in coralline algae cover for the two years following 2015 (Fig. 6). As hard coral cover decreased in 2016, coralline algae increased from 12.5% (SD ± 6.3) in 2015, to 22.7% (SD ± 10.9) in 2016. In 2017, coralline algae comprised 24.6% (SD ± 11.9) of the benthos which is close to the cover in 2011 of 25.3% (SD ± 15.7). Macroalgae remained between 2.35% and 2.37% from 2015 to 2017, comparable to coverage in 2011 of 2.4%. The amount of bare ground remained similar from 2011 to 2017, ranging from 51% to 55%. Soft corals and "other" did not exhibit significant differences across years.

## Discussion

This study confirmed that D'Arros Island and St Joseph Atoll experienced anomalously high sea temperatures in April 2016, consistent with regional sea temperature anomalies associated with the global coral reef bleaching event of 2016 (Obura *et al.*, 2017). Persistently elevated sea temperatures above the regional coral bleaching threshold were presumed to be the primary driver of the mass coral bleaching in 2016 and associated post-bleaching mortality, although other factors such as solar radiation may have also contributed to the coral bleaching event (Berkelmans, 2002; Obura

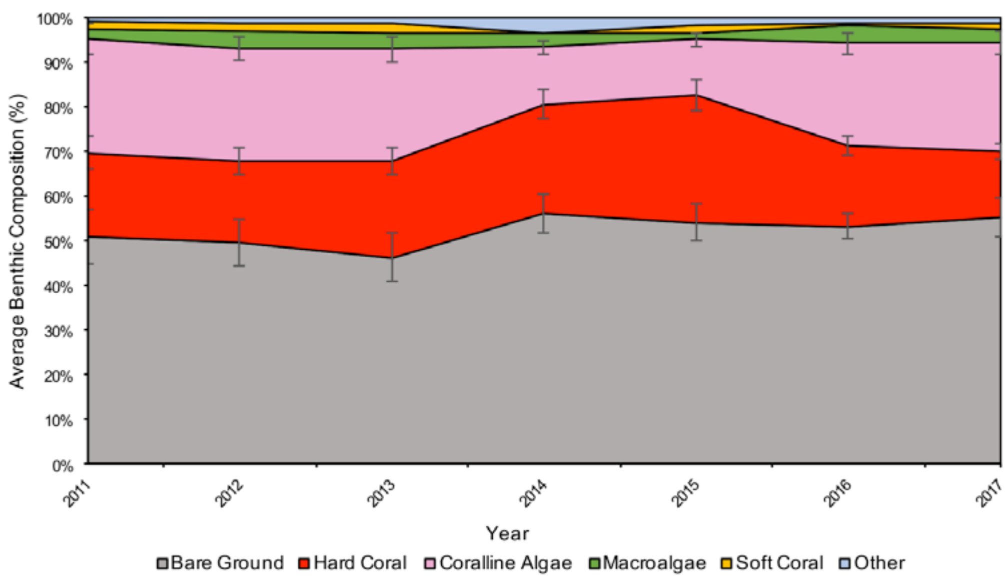


**Figure 6.** Benthic composition across all sites surveyed between 2011 and 2017. Error bars represent 95% confidence intervals.

*et al.*, 2017). Due to the remote and relatively pristine environment at D'Arros Island and St Joseph Atoll, it is unlikely that local human disturbance influenced coral bleaching or post-bleaching coral mortality.

Evidence of increasing hard coral cover at D'Arros Island and St Joseph Atoll between 2011 and 2015 was similar to broader trends recorded in Seychelles as coral recovered after the 1998 and 2010 mass coral bleaching events (Obura *et al.*, 2017; Smith *et al.*, 2017). The observed loss of approximately half (48.3%) of the hard coral coverage in 2017 was less than the inner islands that exhibited on average 60% loss, but more than the average outer island loss of approximately 17% (Smith *et al.*, 2017; Gudka *et al.*, 2018). Additionally, the hard coral coverage decline described in this study was more than many other reports from the broader region (Tanzania, Kenya, Madagascar) but less than areas in Maldives (Gudka *et al.*, 2018; Perry and Morgan, 2017). However, caution should be taken when comparing hard coral coverage loss between regions due to the difference in methods employed to assess coverage (Gudka *et al.*, 2018). Nonetheless, the relative hard coral loss described in this study is consistent with regional studies that relied on the same methods (Gudka *et al.*, 2018)

While D'Arros Island and St Joseph Atoll exhibited a significant overall loss in hard coral cover after the 2016 bleaching event, there was some variability between sites. Typically, those that exhibited the greatest post-bleaching mortality were shallow (i.e. at 5m) with relatively high hard coral cover before 2016 (i.e. sites 1, 5 and 9). The low post-bleaching mortality at Site 7 was an exception perhaps because the site had a relatively high percentage of poritid colonies which are typically resistant to coral bleaching and associated mortality (Bridge *et al.*, 2014). Other sites with low post-bleaching mortality were 2, 8 and 10, all of which were at 12m and had relatively low coral cover before 2016. Additionally, deeper sites experienced fewer days at which the recorded temperature reached the regional coral reef bleaching threshold. As a whole, sites which exhibited the greatest decrease in hard coral cover (i.e. sites 1 and 9) were dominated by acroporids and pocilloporids which are thought to be particularly susceptible to bleaching (Marshall and Baird, 2000). In general, amongst the study sites, those where coral communities appeared most resistant to post-bleaching mortality had strong currents and were in proximity to deeper water. Previous research has shown that such conditions, alongside exposure to frequent upwelling events, may contribute to the resistance of bleaching induced coral mortality (West and Salm, 2003; Goreau *et al.*, 2000).

Benthic cover after the bleaching event in 2016 was largely unchanged for macroalgae, soft coral and bare ground. However, there was an increase in cover of coralline algae and a decrease in hard coral cover, similar to post-bleaching trends in Seychelles waters after the 1998 bleaching event (Stobart *et al.*, 2005). Such slightly increased coralline algal cover may help to facilitate coral reef recovery (McCook *et al.*, 2001; Friedlander *et al.*, 2014), especially if the macroalgal community remains stable (West and Salm, 2003). Indeed, the unchanged macroalgal community at D'Arros Island and St Joseph Atoll may be facilitated by the diverse and healthy herbivorous fish community as well as the relatively pristine environment with minimal anthropogenic influence (Hughes *et al.*, 2007; Daly *et al.*, 2018). Thus, ensuring the continued conservation of marine resources and limiting anthropogenic disturbance will likely promote conditions favourable to recovery of the local coral reef community at D'Arros Island and St Joseph Atoll (Fung *et al.*, 2011).

In summary, this study found that after the 2016 bleaching event, hard coral cover at D'Arros Island and St Joseph Atoll declined from 28.5% in 2015 to 14.7% in 2017. Although this represented a substantial decline in hard coral cover, the benthic community in general did not appear to shift to a rubble or algal dominated community over the timeframe of the study. However, further monitoring is required to assess the status of the coral reef community over broader timescales. Additionally, some monitored sites exhibited minimal post-bleaching coral mortality. Specifically, some deeper sites probably exhibited less hard coral cover decline as they experienced fewer days of sea temperatures above the regional coral reef bleaching threshold and were not dominated by corals susceptible to bleaching (acroporids and pocilloporids) compared to the shallower sites. A 2017 post-bleaching hard coral cover of 14.7% suggests that local coral recruitment will contribute to the slow recovery of coral reefs in the region (Graham *et al.*, 2015). Furthermore, the low level of anthropogenic impact at D'Arros Island and St Joseph Atoll in terms of minimal pollution, fishing pressure and coastal development provide a suitable environment for post-bleaching recovery (Wilkinson *et al.*, 1999; Fung *et al.*, 2011; Hughes *et al.*, 2017a).

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# Improving bycatch mitigation measures for marine megafauna in Zanzibar, Tanzania

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## Abstract
This study was conducted to explore the governance processes and socio-economic factors relevant to the potential implementation of bycatch mitigation for various vulnerable marine megafauna (rays, sharks, marine mammals and turtles) in Zanzibar, Tanzania. Questionnaire-based interviews were conducted between February and April 2017 with fishers (n= 240) at eight landing sites. One focus group discussion was held in each site and eleven key informant interviews were carried out. The study showed that current measures to manage bycatch rates are not explicit; no rules govern ray and shark bycatch; and rules regarding marine mammal and sea turtle bycatch are poorly enforced. Binary logistic regression was used to determine the effects of five selected socio-economic factors (education, age, proportional fishing income, fishing experience, and the number of adults who bring income into the household) on the willingness of fishers to participate in potential future bycatch mitigation measures for marine megafauna. The results indicate that only one factor (the number of adults who bring income into the household) had any significant effect ($p=0.016$). These findings could benefit the future governance and management of marine megafauna in Zanzibar through a better understanding of what mitigation measures are more likely to be supported.

**Keywords:** fisheries, conservation, marine mammals, elasmobranchs, turtles

## Introduction
Marine megafauna play major roles in ecosystem structure and function (Bowen, 1997). Their status as apex and meso-predators and as mega-grazers mean they directly influence community structure, community dynamics and nutrient cycling (Preen, 1995; Aragones, *et al.,* 2006; Heithaus, *et al.*, 2008). Therefore, threats to the survival of these species have potentially wide-ranging consequences for marine ecosystems and those who rely upon them. In the past, loss of their natural habitats contributed to considerable mortality (Pusineri and Quillard, 2008). However, currently, fisheries are the greatest anthropogenic threat to these taxa at the global level (Lewison, *et al.,* 2004; Kiszka *et al.*, 2009; Riskas, *et al.*, 2016), where they may present as both targeted catch and bycatch. Persistent growth in human activities has increased interactions with megafauna, contributing to injuries, damage and finally death (Capietto *et al.*, 2014). Thus, in order to preserve these species, the ecosystems they affect, and the people who rely upon the marine environment, fisheries bycatch requires immediate action (Reeves *et al.*, 2013).

Bycatch in small-scale fisheries receives limited attention from either local or global fisheries authorities (Moore *et al.,* 2010). Although small-scale fishers generally use simplistic and smaller gears compared to their industrial counterparts, their gears and fishing strategies are generally less selective and their volume means they pose a serious bycatch threat to marine

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megafauna (Adimey *et al.,* 2014). Indeed, a growing number of researchers believe that marine megafauna bycatch in small-scale fisheries might be as extensive or even greater than in industrial fisheries (Alfaro-Shigueto *et al.*, 2011; López-Barrera *et al.*, 2012; Mancini *et al.*, 2012). In the South Western Indian Ocean, where small-scale fisheries employ at least 495,000 people, bycatch is widely reported (Temple *et al.,* 2017). Kiszka (2012) found that 31 species of marine megafauna were caught by small-scale fishers in Zanzibar; five species of sea turtles, five species of marine mammals, and 21 species of elasmobranchs.

Although many studies have focused on the by-catch problem in large-scale or industrial fisheries (Komoroske and Lewison, 2015), the bycatch problem in artisanal fisheries remains largely ignored (Curtis *et al.,* 2015). Attempts to manage and, where required, mitigate bycatch in small-scale fisheries are limited firstly by insufficient information on the scale and composition of the bycatch itself (Temple *et al.,* 2017). Moreover, implementation of mitigation strategies must consider the complex interactions between cultural, economic, social and environmental issues in order to achieve their goals (Read, 2008). This complexity is reflected in the growing recognition of the role of social and economic research approaches in facilitating the implementation of mitigation plans (Komoroske and Lewison, 2015). Social and economic factors can influence the effectiveness of bycatch mitigation measures, because fishers dependence on a fishery will influence how likely they will follow laws which may impact their social and economic well-being (Peterson and Stead, 2011; Teh *et al.*, 2015). Knowledge of socio-economic factors such as the numbers of people in certain areas, their beliefs, and their age can contribute to an understanding of how fishers can impact the sustainability of the megafauna populations (Stead *et al.*, 2006; Brewer *et al.*, 2012). Adequate understanding of social and economic features of fisher's communities are also essential requirements for good governance (Kittinger, 2013; Turner *et al.*, 2014).

Good governance and appropriate management are acutely relevant to the bycatch problem. Government intervention is needed to assist widespread bycatch reduction, whether through coercion or incentives, and so understanding fishers' perceptions of current governance processes and their effects on fisher behaviour is vital (Eriksson *et al.,* 2015; Turner *et al.*, 2017). The term 'governance' is a more comprehensive term than 'management', and it goes further than imposing controls or creating opportunities (Chuenpagdee and Sumaila, 2010). Good governance entails having accountability, participation, predictability, transparency, the rule of law and strong institutions (Lockwood *et al.*, 2010; Turner *et al.*, 2014). In order to reduce bycatch problems in the South Western Indian Ocean region all of these characteristics of good governance are required in the fisheries sector. Good governance can also help sustainable natural resource management by securing the availability of food, strengthening the rural economy, safeguarding the marine sustainable ecology, and promoting alternative livelihoods (Finkbeiner and Basurto, 2015).

The aim of this study is to identify governance processes within a socio-economic context that may hinder, or contribute to, the introduction and widespread use of bycatch mitigation methods. The outputs of the research are intended to include recommendations about how to mitigate bycatch through a better understanding of the human dimension of the fisheries.

## Materials and methods

The Zanzibar archipelago is part of the United Republic of Tanzania, consisting of many small islands and two large ones, Unguja and Pemba. Like many other African nations, Zanzibar is considered as (part of) a developing state. It has a GDP of \$ 675 million (Murphy *et al.*, 2016) and a total population of 1,303,569 (Population and Housing Census, 2013). This study was conducted in Unguja Island which is located at 6° 13'S and 39°13' E, situated approximately 40 miles off the coast of mainland Tanzania. Nearly 70% of Zanzibar's population is found on Unguja Island. In this study, data were collected from eight fisheries landing sites (See Fig. 1). These sites were chosen on the basis of geographic spread, fishing gear composition (with a bias toward sites with high numbers of long-lines, drift and bottom-set gillnets) and logistical constraints. Data collection took place between February and April 2017. A mixed-methods approach was used to obtain qualitative and quantitative information from different stakeholders. This approach was taken so as to reduce the weakness of mono-method research (Place and Kelle, 2008), and allow for triangulation of information (reinforcement of findings).

Face-to-face structured questionnaires were administered in a survey of 240 fishers (30 individual from each study site) to collect data on: (i) socio-economic factors comprising education (years spent in school), age, proportion of income from fishing to household, number of adults who bring income to the house, and fishing experience with gears; (ii) willingness of fishers to participate in potential future bycatch mitigation; (iii) perceptions of current management in relation to principles of good governance; and (iv) appropriate persons/organisations to involve when making decisions on marine megafauna bycatch management. Simple random sampling was used to select fishers, and survey questionnaires were administered at landing sites when fishers returned from fishing trips, repaired their fishing gears, relaxed at landing sites, or at their homes. Interviews were conducted face-to-face. Fishers were asked for their consent before the interview was conducted, anonymity was assured, fishers were free to choose not to answer any questions that they did not feel comfortable with, and could end the interview at any time.

One focus group discussion (FGD) was conducted in each study site where the moderator led different stakeholders such as leaders of the villages, fishers and members of Shehia fisheries committees. Each group contained six participants who were selected on the basis of their expert knowledge, their fisheries experience and the length of time they had lived in the area, thus taking account of historical context. Information obtained from the fishers included fishers' perceptions of catching marine megafauna, current laws regarding marine megafauna, their enforcement, and ways to conserve marine megafauna. The discussions were tape-recorded with the permission of the participants. Charlesworth and Rodwell (1997) suggested that FGDs should be comparatively small in size; not less than five and not more than eight participants, to give them more

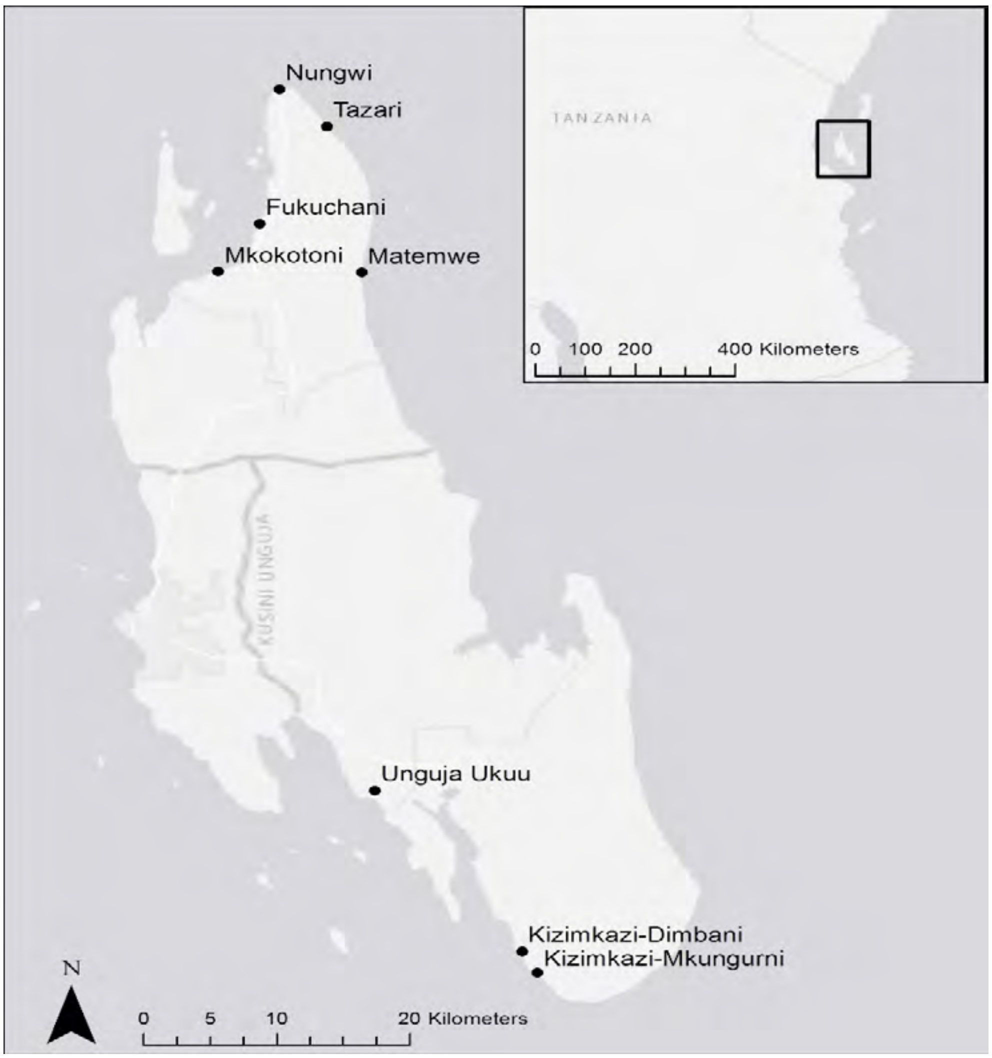


Figure 1. Map of Unguja Island highlighting the study areas.

time to discuss their views, experiences, and enable moderators to manage active discussions better than with larger groups.

Eleven key informant (KI) interviews were carried out, comprising one stakeholder from each study site and three from the fisheries department (a lawyer, a fisheries officer, and the Manager of Menai Bay conservation area). These participants were selected for their knowledge, role in the setting, and willingness and ability to provide useful information on the topic. These KI interviews were conducted to obtain a more synoptic perspective on the marine megafauna bycatch problem. The interviews were tape-recorded with the consent of the participants.

Quantitative data from the survey questionnaire returns were analysed by using statistical software SPSS version 20 wherein binary logistic regression was employed to assess the effect of socio-economic factors on the willingness of fishers to implement bycatch mitigation measures for marine megafauna. The socio-economic factors of the level of education, age, proportion of income from fishing to household, number of adults who bring income to the house, and fishing experience with the gears were taken as independent variables. A significance level (α) of 0.05 was used. Evidence of collinearity between variables used in the analysis and resultant variance inflation factors (VIF) on the binomial model was assessed. In the event of significant collinearity and high VIF (VIF >10) only one of the independent variable was submitted to the final model. Content analysis was employed to analyse qualitative data from focus group discussions and key informant interviews, where opinions recorded were listened to carefully, coded and interpreted to provide meaningful data which are presented below in the form of tables.

## Results

### Governance of marine megafauna bycatch

*Perceptions of fishers about governance principles:* From the survey questionnaire returns, the results showed that trust is the most important governance principle for effective decision-making on bycatch and fisheries issues since it was ranked number one by 29.1% of the 240 fishers surveyed, compared to 19.4% for accountability and 16.4% for effectiveness as shown in Fig. 2.

*Perceptions of fishers about appropriate persons/ organisations for making decisions on marine megafauna bycatch management:* In the survey, 38% of respondents perceived that Shehia fisheries committees are the most suitable organisations for making decisions on management of marine megafauna bycatch in Zanzibar, followed by the fisheries department and leaders of the

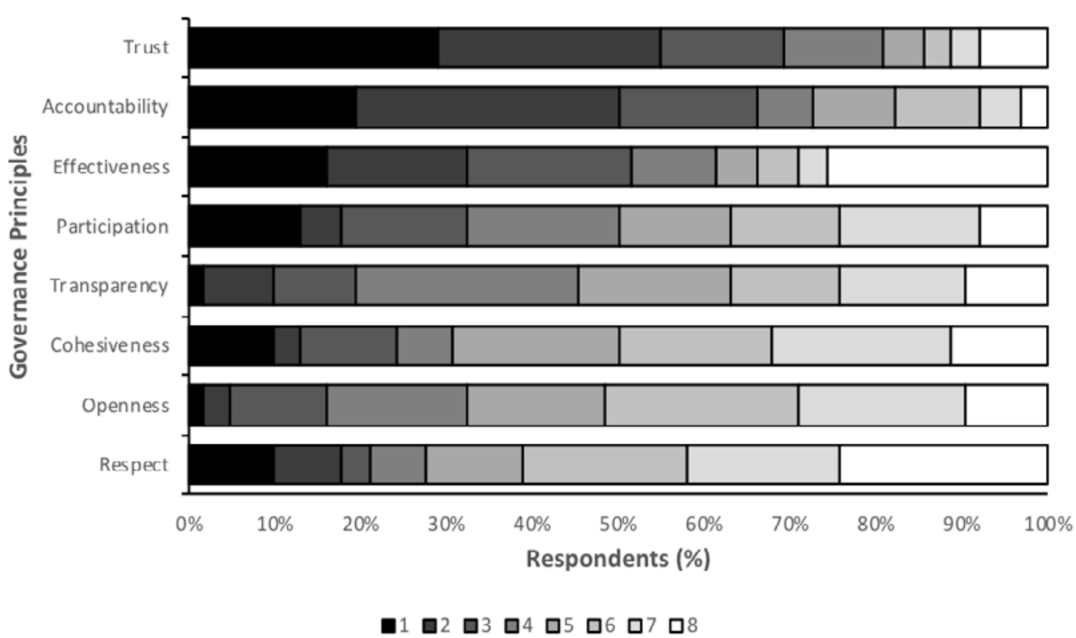


**Figure 2.** Governance principles ranked by stakeholders according to their perception of the importance of effective decision-making on bycatch issues, where number 1 (presented in black) is the most important principle, while number 8 (presented in white0 is least important.

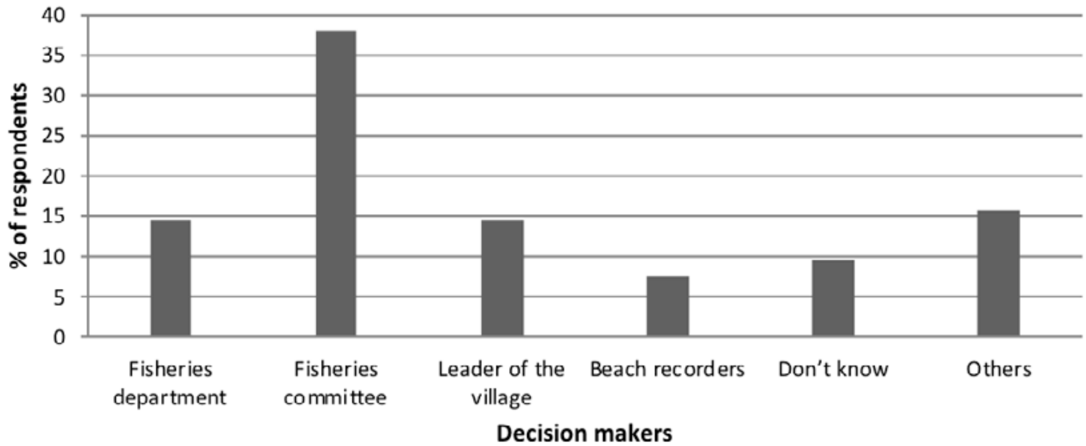


**Figure 3.** Perceptions of fishers about appropriate persons/organisations for making decisions on management of marine megafauna bycatch.

villages, both of which were rated as the most suitable by 14.6% of respondents (See Fig. 3). These Shehia fisheries committees are found in each village.

*Rules for catching marine megafauna in Zanzibar:* There are rules that forbid catching, landing or using products of some marine megafauna in Zanzibar such as sea turtles, whales and dolphins, and if these marine megafauna are caught accidentally in the gears they must be released. However, there are no such rules for elasmobranch species (rays and sharks) in Zanzibar. Currently, the rules are set by the fisheries department in collaboration with Shehia fisheries committees. Results from FGDs showed that awareness of fishers about these rules is high due to considerable efforts made by the government to educate fishers. However, the level of enforcement is considered very low (See Table 1).

**Table 1.** Reasons for low enforcement of rules, ways of improving conservation of marine megafauna and techniques used to educate fishers. The items in the columns are listed in order of the number of times they are mentioned by stakeholders, with the most mentioned items at the top.

| Reasons for low level of rules enforcement | Ways of improving conservation of marine megafauna | Techniques used to educate fishers about the rules |
|---|---|---|
| Inadequate resources for rule enforcement, for example, there are few patrol boats and insufficient fuel for them | More patrols and stricter enforcement of the rules | Outreach programs through fisheries officers to educate fishers in the villages |
| Corruption between rule enforcers and rule-breakers | Establish marine protected areas to reduce fishing in biodiversity hotspots | Awareness programs through mass media such as television, radio and newspapers |
| Rule enforcers often come from the same villages and even the same families as the rule-breakers | Accountability of managers and rule enforcers | Members Shehia fisheries committees host meetings with fishers to educate them about rules |
| There is a poor system for supervising and monitoring the work of the rule enforcers | Improve environmental awareness of the importance of marine megafauna | |
| Fishers are skilled at concealing their illegal activities by hiding when they catch dolphin and sea turtle. | Require fishers to use more selective fishing gears to avoid unwanted bycatch | |
| | Suggest fishers move out to deeper water where there is less marine megafauna | |
| | More cooperation between management and fishers, making better use of fishers' knowledge | |

### Socio-economics of marine megafauna

*Perception of fishers about marine megafauna and their mitigation measures:*

Results from FGDs revealed that most fishers believe that catching dolphins, whales and sea turtles is wrong because they are more valuable alive for use in tourism. Moreover, FGDs showed that most fishers consider them as bycatch because targeting them is illegal; however some did not consider them as bycatch and still actively target them for food and bait. Elasmobranchs are not considered as bycatch by most fishers and they believe it is not a bad thing to catch them since they provide marketable products such as fins, teeth, meat and livers for anti-fouling paint on boats, and also it is still legal to catch them.

The majority of fishers surveyed in face-to-face interviews (84%) perceived that implementing mitigation measures would not affect their livelihood and they were willing to implement those mitigation measures, while a small minority (16%) perceived that implementing mitigation measures would affect their livelihood by reducing their catch and therefore they were not willing to comply with them.

*Effects of socio-economic factors on the willingness of fishers to implement mitigation measures:*

Evidence of collinearity was found between age and experience (0.56, $p<0.001$) and also between number of adults bringing income to the household and proportion of household income from fishing (-0.43, $p<0.001$). However, VIFs in the model were small (VIF = 1.516073, 1.510253, 1.443569, 1.365072) suggesting that collinearity had no substantive effect on the outputs of the results, so all independent variables were retained in the model.

**Education:** From the survey 43.4% of all fishers interviewed had reached ordinary secondary school which is about 10 years of school, but only 0.4% had reached higher education level (university). Statistical results revealed that education levels had no significant effect on fishers' willingness to implement mitigation measures ($p>0.05$) (Table 2).

**Age:** 45.5% of all fishers surveyed were aged within the range of 41 to 63 years, while 8.7% were aged above 63 years. The statistical results showed that the age of the fishers had no significant effect on their willingness to implement mitigation measures ($p>0.05$) (Table 2).

**Proportion of household income from fishing:** Fishing activity was the main source of income in most households: 47.5% of all fishers surveyed said that fishing activities contributed 81-100% of household income; 35% of the fishers said fishing activity contributed between 61-80% of the household income; and 17.5% of fishers said fishing activities contributed 40-60% to their household income. Statistical results indicated that the proportion of household income from fishing had no significant effect on the willingness of fishers to implement mitigation measures ($p>0.05$).

**Experience of fishers with main fishing gear:** With regard to experience with the main fishing gears, 37.9% of fishers said that they had experience of between 1-10 years, while 0.8% of interviewed fishers said they had experience of greater than 60 years. This factor also had no significant effect on the willingness of fishers to implement mitigation measures ($p>0.05$).

**Table 2.** Binary logistic regression analysis on socio-economic factors effecting willingness of fishers to implement mitigation measures.

| Socio-economic factors | Coefficient (β) | SE | Exponent of (β) | p-value |
|---|---|---|---|---|
| Age | 0.011 | 0.014 | 1.011 | 0.438 |
| Education | -0.071 | 0.046 | 0.931 | 0.120 |
| Income proportion from fishing | 0.017 | 0.013 | 1.017 | 0.215 |
| Adults bring income to the household | 0.419 | 0.174 | **1.520** | **0.016** |
| Experience with fishing gear | 0.007 | 0.016 | 1.007 | 0.657 |

**Number of adults who bring income into the household:** The average number of adults bringing income into a household was 2, and the survey showed that most households (87.9%) had 1-3 adults who contributed to the income of the household, while 0.5% of respondents said they had more than 6 people who bring income into their households. The numbers of adults bringing income into the household had a positive statistically significant effect on fishers' willingness to implement mitigation measures ($p<0.05$).

## Discussion

### Governance of marine megafauna bycatch

This study shows that management actions to reduce bycatch of marine megafauna in Zanzibar are ineffective. There are no laws governing either catch or bycatch of elasmobranchs, and while laws do exist for marine mammals and sea turtles, they are poorly enforced. Fishers know about the rules that are in place, a result of substantial efforts by the government to educate fishers, though some still believe catching sea turtles and mammals is legal, and conversely, others believe that catching elasmobranchs is illegal. However, catching sea turtle species appears to be common despite their relatively low market value, reflecting fisher's observations of limited enforcement and thus limited risk of punishment for breaking these rules. On the lack of rules on elasmobranch species, the results found that there are no rules about them in small-scale fisheries, and fishers target them for their meat and fins. However, fishers said that the price they obtained for sharks fins had fallen dramatically since the shark fins trade (including exportation) was prohibited in Zanzibar. These findings support the observation of Temple *et al.* (2017) when they reported that the Government cancelled the shark fins export licence in Zanzibar.

On understanding why there is poor enforcement of the rules governing other marine megafauna, there were three main reasons given by those surveyed. First, there were insufficient resources for enforcing the current laws. Several studies show that lack of human resources, fewer patrol trips and less investment in equipment like boats, trigger rule-breaking events and undermine the effectiveness of conservation law enforcement (Ehler, 2003; Gilman, 2011; Peterson and Stead, 2011 Gilman, *et al*., 2014). Second, there was a lack of trust in the people who are responsible for governing and managing fisheries activities. For example, respondents claimed rule enforcers like fisheries officers who carried out patrols, took bribes from rule-breakers. This meant that fishers stopped reporting rule-breaking activities, and since when they did report, no action was seen to be taken. Smith and Walpole (2005) indicated that corruption can seriously reduce the efficiency of conservation measures and lead to over-exploitation of vulnerable species. Third, there was no system to make government officers accountable for their actions (or inactions). For example, there were inadequate mechanisms for supervising and monitoring the work of the rule enforcers. Lockwood *et al.* (2010) consider accountability to be the crucial governance principle for effective conservation of natural resources.

Results of the perceptions of fishers about appropriate persons or organisations for decision- making on fisheries management (as presented in Fig. 3) show that 38% of the fishers surveyed perceived that Shehia fisheries committees are the appropriate organisations for decision-making on fisheries issues, including mitigating megafauna bycatch. The reason behind this perception is that Shehia fisheries committees involve local stakeholders, including ten fishers from the village, the leader of the village, and the beach recorder who represents the fisheries department. Carlsson and Berkes (2005) found that the cooperative approach is the best governance approach in decision making of common pool resources since it reduces the marginalization of many stakeholders, empowers them, enables them to share their knowledge, and facilitates their sense of collective strength through unity. As for the 14.6% of fishers who perceived that the fisheries department was the most appropriate organisation to make decisions on fisheries management, their main reason was that the fisheries department is responsible for all fisheries activity in the country and they have resources for implementing their decisions.

### Socio-economic considerations of marine megafauna bycatch mitigation

In the past, fishers targeted dolphins and used them as bait for sharks. However, nowadays, most fishers perceived that dolphins, whales and turtles are less valuable to them as meat than kept alive as tourism attractions. Although 47.8% of interviewed fishers depend on fishing for 81-100% of their household income, most young fishers in coastal villages like Kizimkazi Mkunguni, Kizimkazi Dimbani and Nungwi are also involved in marine ecotourism which is a lucrative source of income and has led to a reduction in the number of fishers targeting these marine megafauna species in those villages. However, this kind of tourism activity itself has some negative impacts on marine megafauna because of disturbance from boats and swimmers, so it needs to be well managed as recommended by Stensland and Berggren (2007) and Christiansen *et al.* (2010).

The majority of fishers (84%) perceived that implementing mitigation measures will not affect their income, while only 16% perceived that implementing mitigation measures will have a negative impact on their income. The latter believed that such measures will cause catch reductions not only of marine megafauna species but also of other marine species, hence reducing their income. These results are in line with the findings of Bennett and Dearden (2014), who reported that some communities had negative perceptions about conservation measures since they believed such measures would harm their livelihoods, and therefore did not provide any support for them. Fishers who had a positive perception of mitigation measures on their livelihoods stated that these marine megafauna have less value to them when they catch them compared to other species, so mitigation measures will not reduce their income since they will catch other more valuable species. Fishers from Kizimkazi Dimbani, Kizimkazi Mkunguni and Unguja Ukuu stated that when they did pilot trials with 'pingers' to avoid catching marine mammals, they did not experience any reduction in the catches of their target species, so the results encouraged most fishers to be willing to implement the measures.

Results from binary logistic regression indicated that the willingness of fishers was significantly affected by the number of adults who bring income into the household. Households with a higher number of adults who bring income were more willing to implement mitigation measures. The magnitude of effect of this socio-economic factor was higher than that of other socio-economic factors studied (Table 2). For an additional one adult bringing income in the household, the odds of willingness rose by 1.5. The assumption is that households with more adults who bring in income have a higher income compared to those with fewer adults, and Liobikiene and Juknys (2016) found that income levels have a positive influence on environmental concern. In support of this finding, the 'social class hypothesis' proposed by Liere and Dunlap (1980) argues that the households with higher incomes are more concerned about the environment since they already satisfy their basic needs, unlike households with less income who will do whatever they can to get their basic needs satisfied, even through activities which destroy their environment.

Another finding from this study is that all the socio-economic factors studied had positive coefficients, except education which had a negative coefficient (Table 2); for every additional year fishers spent in school the odds of willingness to implement mitigation measures is lowered. This implies that fishers who had a low level of education were more willing to implement mitigation measures than those with higher education. This finding is contrary to the conclusion of Liobikiene and Juknys (2016) who found that concern for the environment increases with the number of years that people spent in school. In their hypothesis, education has a major contribution in making people understand environmental issues, and therefore increases their awareness and encourages a greater sense of environmental responsibility in them. However, this is not always the case (Kollmuss and Agyeman, 2002). In this study, fishers with low levels of education explained that employment opportunities outside fishing are lower for them compared to those with higher education, thus they are willing to implement mitigation measures to sustain their jobs.

In conclusion, marine megafauna are ecologically, socially and economically important for most coastal communities. However, populations of marine megafauna are at significant risk as a result of bycatch globally. In order to reduce or to eliminate this decline, rules and regulations for catching elasmobranch species should be established and those for other megafauna species should be strictly enforced. Furthermore, fishers must be encouraged to implement bycatch mitigation measures, and to achieve this encouragement there is need to understand socio-economic factors that influence fishers' willingness to collaborate with the authorities in introducing regulations and to then comply with those regulations. The present study can form a basis for understanding these socio-economic factors and the educational processes needed to encourage the willingness of fishers, but further studies are needed to understand the institutional governance of bycatch and to find alternative livelihoods in order to reduce pressure on the marine resources.

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# Hook size selectivity in the artisanal handline fishery of Shimoni fishing area, south coast, Kenya

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## Abstract

Selectivity of five handline fishing hook sizes was determined following Holt's 1963 model using data that was collected during January to June, 2016. A total of 966 fish specimens comprising of 65 species belonging to 23 families were sampled. Fish abundance was low for large sized hooks while catch rate was higher. Species diversity was higher during the northeast monsoon season and at the study sites of Mpunguti and Nyuli. However, species diversity decreased with increase in hook size. All hook sizes had a higher selection for mature *Lethrinus borbonicus* while hook size No. 8 selected immature *Lethrinus lentjan*. Hook sizes No. 9 and 10 selected mature *L. lentjan* and *Lethrinus rubrioperculatus*, hook size No. 15 selected immature *L. lentjan*, *L. rubrioperculatus* and *Aprion virescens*, while hook size No. 16 selected immature *A. virescens* and *L. rubrioperculatus.* Species similarity was higher for fish caught by hook sizes No. 16 and 15, and No. 8 and 9, while those captured by hook size No. 10 differed from those caught by other hook sizes. The larger hook size No. 8 is recommended for the sustainable exploitation of species in the artisanal handline fishery in Shimoni fishing area. Future work needs to consider the effects of bait type and size and the stock status of the fish under exploitation.

**Keywords:** Artisanal handline fishery; hook size; species selectivity; Shimoni fishing area

## Introduction

Globally, small-scale coastal and marine fisheries support the livelihoods of thousands of fisher folks providing food, fish protein and income to coastal communities. In Kenya, landings from the small-scale coastal marine fisheries average »9,134 Mt/year, valued at »KES 1.3 billion (Government of Kenya, 2013). The fishery directly supports about 13,000 fishers employing various fishing gear and vessel types (Government of Kenya, 2016). The number of handlines has increased over the years from about 4,100 in 2008 to over 6,000 lines in 2014, indicating a substantial increase in fishing effort in the fishery (Government of Kenya, 2012; 2014). However, there was a decrease in the number of handlines to 4, 364 in 2016 (Government of Kenya, 2016). At Shimoni, handlines contribute the highest effort by fishers (1,265 fisher days) compared to other gears. However, handline fishery catches are relatively low at 622kg per month, compared to other gears (Okemwa *et al*., 2015).

The handline fishery also plays an important role in the broader western Indian Ocean region, with Mozambique recording the highest number of about 12,683 handlines, comprising 23% of the total of 42,300 fishing gears in 2016. In Madagascar, 2,500 handlines were recorded and 356 in Mauritius, while the use of handlines was not recorded in Comoros during the same year (Jacquet and Zeller, 2007; WIOFish, 2017).

Overfishing and capture of juveniles of both target and non-target fish species is likely to threaten the sustainability of marine fisheries (Malleret-King *et al*., 2003; Mangi and Roberts, 2007). Furthermore, gear and species selectivity may also act as a key driver of fish population structure, species composition, trophic structure and the natural structure of the stock. Hook size selectivity, a measure of how hooks select fish of different fish sizes, is important in setting up size limits for particular fisheries, and helps guide fisheries management in designing policies and sustainable exploitation

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strategies for marine fish populations. Setting up size limits is important in conserving the older and bigger fish individuals whose fecundity levels are usually higher and their spawning periods are often extended compared to smaller individuals (Love *et al.*, 1990; Berkeley *et al.*, 2004; Arlinghaus *et al.*, 2010).

Handlines present some of the most selective fishing gears used by small-scale fishers and their use of handlines has been on the increase, especially in Kwale and Kilifi counties on the Kenyan coast, except in 2016 when there was a slight decrease in the use of handlines (Government of Kenya, 2014, 2016). Despite the increased use, many aspects of the handline fishery have not been studied comprehensively. In particular, data and information on the selectivity of handline hooks used in the small-scale coastal marine fisheries is lacking. This study provides baseline information for the sustainable management of the small-scale handline fishery along the Kenyan coast.

Numerous studies have been conducted on the small-scale fisheries of Kenya, from biological, ecological and socio-economic analyses (Stergiou and Erzini, 2002; Fulanda, 2003; Mangi, 2006; McClanahan *et al.*, 2008; Fulanda *et al.*, 2009, 2011; Munga *et al.*, 2011, 2012, 2013). However, studies on the different aspects of the handline fishery, including hook and line, longlines and related fishing gears are clearly lacking. Some studies have assessed hook selectivity in longline fisheries (Løkkeborg and Bjordal, 1992; Erzini *et al.*, 1996; Ekanayake, 1999; Peixer and Petrere, 2007) with little attention given to the handline fishery locally, regionally and globally. Therefore, there is need to assess the selectivity of different hook sizes in the coastal and marine artisanal handline fishery so as to establish suitable hook size limits for sustainable exploitation.

Hook size selectivity is useful in formulating species-specific management recommendations, hence the characterization of the selectivity of handline hooks for the small-scale fisheries of Kenya cannot be understated. The aim of this study was to assess hook size selectivity for the handline fishery in the Shimoni fishing area on the south coast of Kenya through sampling artisanal handline catches, determining the size frequency distribution of the fish species captured, and evaluation of the impact of handline hooks on the fish stocks.

## Materials and methods

### Study Area

This study was conducted in Shimoni fishing area straddling 04°38'49" S and 39°22'49" E (Fig. 1). The study area has distinct seasonality influenced by the movement of the Inter-tropical Convergence Zone (ITCZ) that creates two distinct seasons; the northeast monsoon (NEM), locally known as 'kas kazi' and the southeast monsoon (SEM), or 'kusi'. The SEM season prevails from April to October and is characterized by wet, windy and cooler weather accompanied by rough seas. The NEM season prevails from November to March and is characterized by warmer weather with calm seas and smaller wave heights (McClanahan, 1988).

The mean annual rainfall in Shimoni, south coast Kenya ranges from 1000–1600 mm and occurs during two distinct periods; the long rains last from March to May while the short rains are experienced during the months of October to December (Mutai and Ward, 2000; Camberlin and Phillipon, 2002). The sea surface temperature ranges between 24°C in August and 30°C in February, and the air temperature ranges from 24°C during July-August to 33°C in February-March, with a mean monthly evaporation rate of 1300-2200 mm (McClanahan, 1988; Swallow *et al.*, 1991; UNEP, 1998). Four oceanic currents influence the eastern Africa coastal waters; the East Africa Coastal Current (EACC), the Somali Current (SC), the Southern Equatorial Current (SEC) and the Equatorial Counter Current (ECC). The former two currents cause high productivity of the water (UNEP, 1998).

The artisanal fishery in the study area is dominated by the handline fishery compared to other areas of the Kenyan coast (Government of Kenya, 2012). The study was conducted at four selected sites within the Shimoni fishing area dominated by handline fishery namely; Mpunguti, Waga, Nyuli and Mundini fishing areas (Fig. 1).

### Field Sampling and Data Collection

Sampling was carried out from January to June, 2016, covering the late NEM (January to March) and early SEM (May to June) seasons using experimental fishing. Sampling was conducted for two days each month at each of the four selected sites using a 6 m fibre glass reinforced plastic (GRP) boat powered by a 40 hp outboard engine. Five hooks of different sizes (Youvella® brand round bend type; No. 16, 15, 10, 9 and 8 with the widths (Mean ± SD, mm) of 6.3 ± 0.1, 7.2 ± 0.1, 11.4 ± 0.1, 12.9 ± 0.1 and 15.0 ± 0.1mm, respectively, were used (Fig. 2). The mean widths of the hooks, which correspond to the gape size of fish, were determined by measuring and averaging the width of 20 hooks of each hook size. The numbering of hooks follows the order that the size decreases as the number increases (Bishop, 2019).

The hooks were attached to monofilament nylon lines of 0.30, 0.40, 0.50, 0.60 and 0.70mm thickness, respectively. The thickness of nylon lines was determined by the size of the hooks, thus large sized hooks were used with thicker lines, and vice *versa*. The experimental fishing was preferred to sampling the catches landed by the artisanal fishers in order to ensure full control over the use of the hooks and minimize bias in the method of fishing employed to collect the samples.

All the hooks were baited with equal-sized pieces of frozen squid. Depending on the water depth and current speeds, lead sinkers of varied weights were attached at the fore-tip of the fishing lines to ensure that the hooks sank but remained above the sea bed to allow the bait to attract the fish. Fishing was conducted in the morning between 08h00 and 12h00 and during the night between 23h00 and 05h00, although the latter was only conducted when weather and currents were too rough to allow for daytime fishing. The order in which the hooks were fished was alternated randomly on every fishing trip with each fisher using one specific size of hook on each fishing trip.

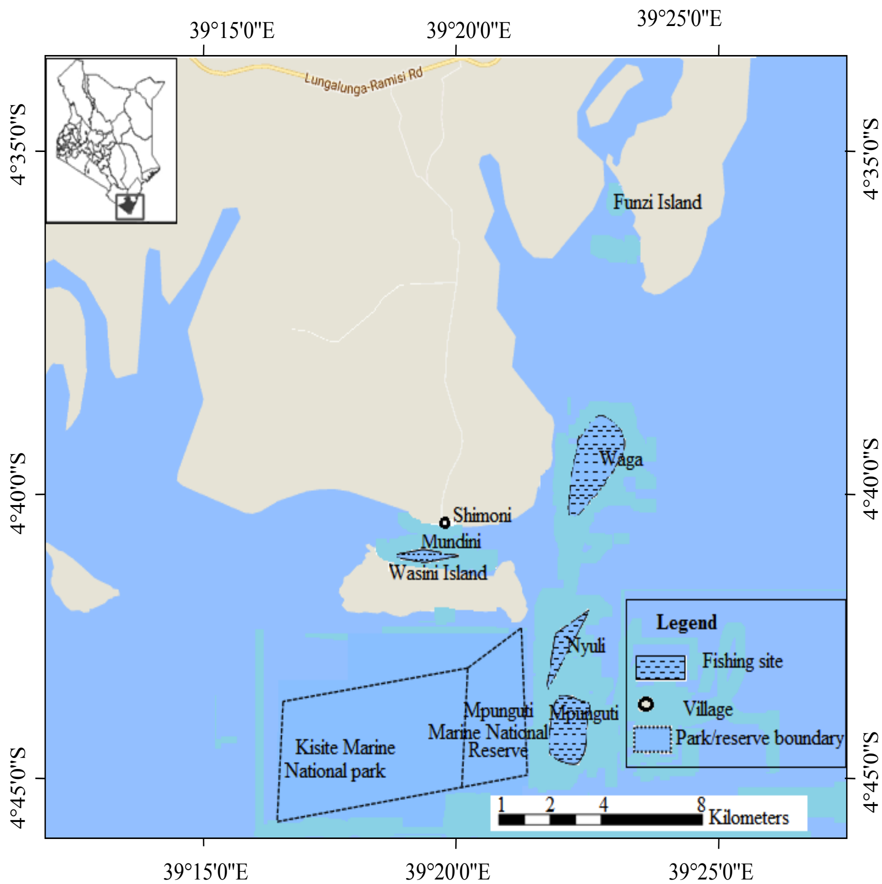


**Figure 1.** A map of Kenya (inset) showing the south coast and the location of the study sites.

At the fishing grounds, all fish caught were sorted according to hook sizes, placed in cooler boxes and transferred to Shimoni landing site for further sample categorization. All the specimens were sorted to species level at the landing site and identified using fish identification guides (Lieske and Myers, 2001; Anam and Mostarda, 2012). Fish that could not be identified at the landing site were photographed and later identified in the laboratory at Kenya Marine and Fisheries Research Institute (KMFRI) using additional fish identification guides including Fisher and Bianchi (1984) and Smith (2003). The total length (TL) of all the specimens

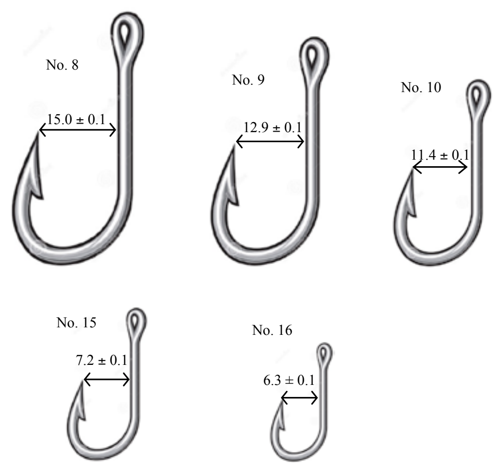


**Figure 2.** Width (mm) of hooks (No. 8, 9, 10, 15 and 16) used to fish during the experiment.

was measured from the tip of the snout to the tip of the caudal fin, with the tail fin pinched together, to the nearest 0.1cm using a standard fish-length measuring board. Body weight was measured to the nearest 0.01g using a hand-held portable electronic weighing balance (Weiheng, W40kg /10g, Japan).

## Data Analysis

Data was entered into an MS Excel spreadsheet and cleaned by confirming that species and family names were correctly written, and the fish measurements were entered in the respective columns. The number of fish caught for all species was determined for the various hook sizes to evaluate the species with representative data for selectivity analysis. The length data was grouped into 2cm size classes and data tallied into a table showing the length classes against the number of observations (specimens), or frequencies in each class for the different hooks used during the study. This was done for each species which had a frequency that could be tallied into the 2cm length classes and gave continuous catch proportions for at least one pair of corresponding hook sizes. Holt's (1963) model as explained by Pauly (1984) was used to determine the catch proportions for the various hook sizes that were plotted against the mid lengths of the length classes to obtain the selectivity curves for the different hook sizes. Holt's (1963) model was used, as the population size in the fishing areas was not known. Pauly (1984) explains Holt's (1963) model using a set of stepwise equations (equations i-vi) as illustrated below. First, the natural logarithms, *Ln*, of the catch ratios of the bigger hook to that of the smaller hook were determined using equation (1):

$$Ln = {}^{C_1}/_{C_2} \quad \text{……………………..……Equation (1)}$$

Where: $C_1$ are the catches from the larger hook and $C_2$ are catches from the smaller hook for each pair of hook sizes. The natural logarithms of the catch ratios (*Ln*) were regressed against the mid-point of the length class to obtain the intercept and slope, '*a*' and '*b*' respectively.

The selectivity factor (*SF*) was obtained using the '*a*' and '*b*' values

$$SF = \frac{-2a}{b(M_1 + M_2)} \quad \text{……………..……Equation (2)}$$

Where:
*SF* is the selectivity factor,
'*a*' is the intercept and '*b*' is the slope, both from the regression line,
$M_1$ is the gape size (mm) of the smaller sized hook, and
$M_2$ is the gape size (mm) of the larger sized hook for each pair of hooks.

Optimum catching lengths ($L_{opt}$) for the smaller sized hook ($L_{M1}$) and larger sized hook ($L_{M2}$) were calculated using equations (iii) and (iv), respectively. When two estimates of $L_{opt}$ were obtained for the same hook size due to comparison of two length-frequency distributions, their mean value was taken as the $L_{opt}$ corresponding to the particular hook size:

$$L_{M1} = SF \; x \; M_1 \text{……………………..Equation (3)}$$

$$L_{M2} = SF \; x \; M_2 \text{……………..………Equation (4)}$$

Where:
$L_{M1}$ is the optimum catching length for the smaller hook at every length class,
$L_{M2}$ is the optimum catching length for the larger hook at every length class,
$M_1$ is the gape size (mm) of the smaller hook, and
$M_2$ is the gape size (mm) of the larger hook for each pair of hooks.

The common standard deviations ($S^2$) of the two corresponding hooks were calculated using the following equation:

$$S^2 = SF \; x \; \frac{M_2 - M_1}{b} \quad \text{……………Equation (5)}$$

Where,
'*b*' is the slope
$S^2$ is the common standard deviation of the corresponding pair of hook sizes

$SF$ is the selectivity factor,
$M_1$ is the gape size (mm) of the smaller hook, and
$M_2$ is the gape size (mm) of the larger hook for each pair of corresponding hook sizes.
The common standard deviations of the hooks were then employed to determine the catch proportions, $SL$, for the corresponding hook sizes as shown in equation (6):

$$SL_{M1} = exp\ \{-\frac{(L - L_{M1})^2}{2\ x\ S^2}\} \quad \text{........Equation (6)}$$

Where:
$SL_{M1}$ is the catch proportion at each length class,
$L_{M1}$ is the optimum catching length for the smaller hook at every length class,
$L$ is the midpoint of each length class, and
$S^2$ is the common standard deviation for the two corresponding hook sizes.

The catch proportions were then plotted against the midpoints of the length class in Microsoft® Excel 2007 to generate selectivity curves for the individual hook sizes separately.

The selectivity ranges of the respective hook sizes were subsequently determined from the width of the selectivity curves, and the optimum length (selectivity) of fish caught by the different hook sizes was estimated from the highest point (mode) of the selectivity curves. The approach of Holt, 1963 model was not applied to all species caught during the study period because it calculates ratios of catches across pairs of hook sizes, and to avoid highly variable ratios, counts that were not sufficient were avoided (Holt, 1963). The length at maturity ($L_{mat}$) and the maximum length attained when the fish is fully grown (infinite length, $L\infty$) for the dominant species was compared with the optimal selection lengths of the different hook sizes to establish the impact of the hooks on the fish stocks according to Froese and Pauly (2017).

Catch rate by hook size was calculated based on daily catches (kg) for all the hooks of the same size, divided by the number of hooks for each size used to fish on a single day (kg/hook/day), both for each season and the entire period, as calculated below:

$$\text{Catch rate (hook size No. 8)} = \frac{\text{Total catch of all hooks of size No. 8 used in fishing (kg)}}{\text{Number of hooks of size No. 8 used in fishing (TN hooks)}}$$

### Statistical Analysis

The difference in mean seasonal catch rate was determined with the student's t-test using STATISTICA© (ver. 7.0.61.0) software (Hay, 1988). Species abundance and distribution across sites, season and hook sizes were assessed using $K$-dominance curves (Warwick *et al.*, 2008). The abundance of each fish species was cumulatively ranked against the log of the species rank using the method adopted from Jennings *et al.* (2001). The values of $K$-dominance against species rank were then plotted into a graph to produce the $K$-dominance curves for each species. Species diversity is reflected in the slope of the curve; a steep and more elevated curve represents a less diverse species assemblage, while small and more gentle gradients indicate high species diversity, and where the $K$-dominance curves cross, they indicate points of similarity in the species dominance (Rice, 2000). This analysis was executed in PRIMER-E (ver. 6.1.5) software (Clarke and Gorley, 2006).

The species abundance data for each hook size was square root transformed to a normal distribution curve, after which Bray-Curtis (1957) similarity analysis was used to evaluate the similarity of species caught by the different hook sizes during the study period. Two dimensional dendrograms were used to sequentially link the relative abundances of all fish species according to their similarity or dissimilarity using the method adopted from Clarke and Warwick, (2001) in PRIMER-E ver. (6.1.5) software. The vertical axis of the dendrogram indicates the percentage level of similarity for the different hook sizes in a cluster (Clarke and Gorley, 2006). Before analysis, the data was subjected to a normality test (of the total length distribution data) using the Shapiro-Wilk's W-test (Shapiro *et al.*, 1968). Thereafter, Analysis of Covariance (ANCOVA) was employed to determine the effect of hook size, season and sampling sites on the size of fish caught during the study period, using the method described by Yang and Juskiw, (2011). All tests were considered significant at the 95% confidence level ($\alpha = 0.05$).

## Results

### Catch Composition

A total of 966 specimens belonging to 65 species of 23 families were sampled during the study period. The numbers of specimens caught from each of the fishing grounds were: Nyuli (347), Mpunguti (337), Waga (166) and Mundini (116). The smaller hooks (No. 16) caught the highest number of fish (290 specimens) during

**Table 1.** Number of fish caught at each study site by the different hook sizes during the study period.

| Fishing ground /hook size | No. 8 | No. 9 | No. 10 | No. 15 | No. 16 | Total |
|---|---|---|---|---|---|---|
| Nyuli | 19 | 53 | 115 | 53 | 107 | 347 |
| Mpunguti | 44 | 35 | 135 | 87 | 36 | 337 |
| Waga | _ | _ | 1 | 93 | 72 | 166 |
| Mundini | _ | _ | _ | 41 | 75 | 116 |
| Grand Total | 63 | 88 | 251 | 274 | 290 | 966 |

the sampling period while hook size No. 8 caught the lowest number of fish (63 specimens) compared to hook sizes No. 15, 10 and 9, with 274, 251 and 88 specimens, respectively (Table 1). These results show that the abundance of fish capture decreased with increase in hook size. Hook sizes No. 8 and 9 did not catch any fish at Mundini and Waga fishing grounds while hook size No. 10 did not capture any fish at Mundini fishing ground (Table 1). During the experimental fishing eight hooks (four (4) of size No. 16, two (2) of size No. 8 and two (2) hooks of size No. 15) were lost and were not considered in the analyses.

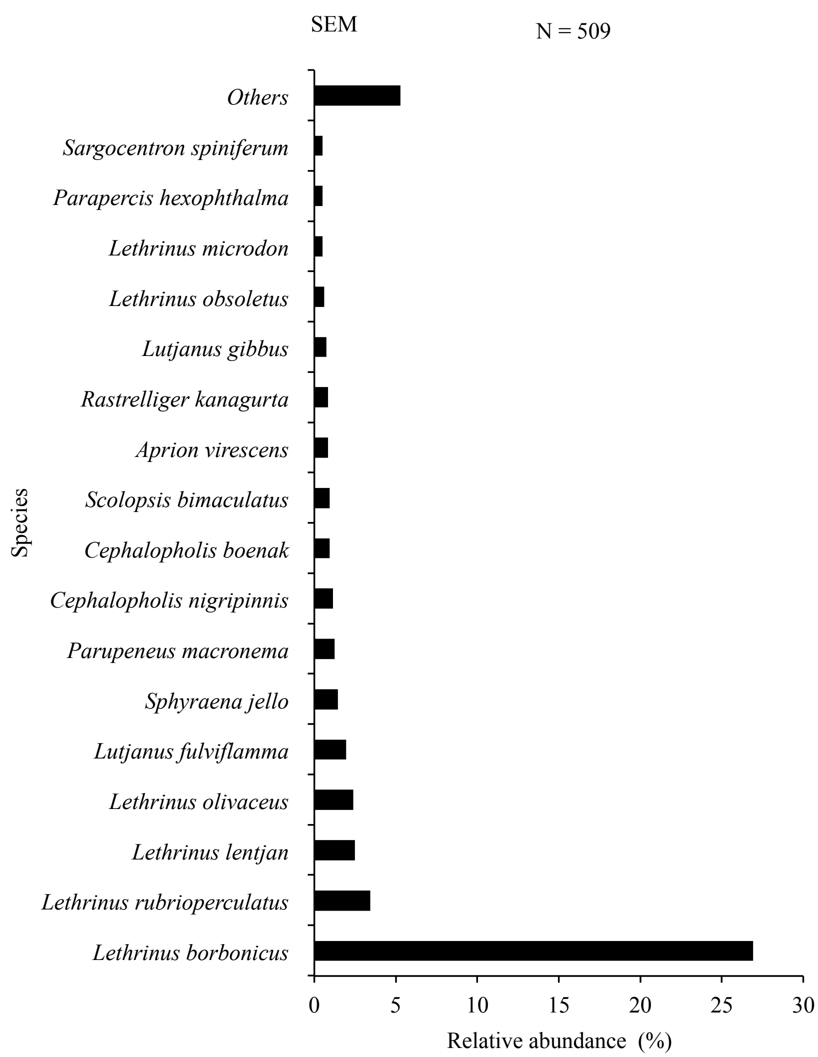


**Figure 3.** Relative abundance (%) of the fish species caught during the southeast monsoon (SEM) season.

## Seasonal Catch Variation

A total of 509 fish weighing 204.92 kg were caught during the SEM season with the Snubnose emperor, *Lethrinus borbonicus* Valenciennes, 1830 being the most abundant, representing 51.1% of the total catch in this study (Fig. 3). During the NEM season, a total of 457 fish weighing 165.87 kg were landed, dominated by Pink-ear emperor, *Lethrinus lentjan* Lacepède, 1802, representing 13.8% of the total catch (Fig. 4). Fish species with smaller proportions were grouped together as 'others' with this category being more abundant during the calmer NEM season than the rougher SEM season.

Hook size No. 8 had the highest mean catch rate during both the NEM and SEM seasons, at 1.29 ± 0.74 kg/hook/day during NEM, and 0.67 ± 0.28 kg/hook/day during SEM season. Hooks sizes No. 8, 9 and 10 gave the highest mean catch rates during the NEM season compared to the catches during the SEM season, while hook size No. 15 recorded similar mean catch rate for both seasons. On the contrary, the smallest hook size No. 16 recorded lower mean catch rate during the calmer NEM season compared to the rougher SEM season. However, the medium hook size No. 10 recorded the highest total catch during both the NEM and SEM seasons. Student's $t$–tests indicated that the mean catch rates for hook sizes No. 8 and 9 during the NEM and SEM differed significantly ($t$ = 1.36, $P$ = 0.25 for hook size 8, and $t$ = 1.08, $p$ = 0.31 for hook size 9, respectively). However, the catch rates for hook sizes No. 10, 15 and 16 were not significantly different between seasons (Table 2).

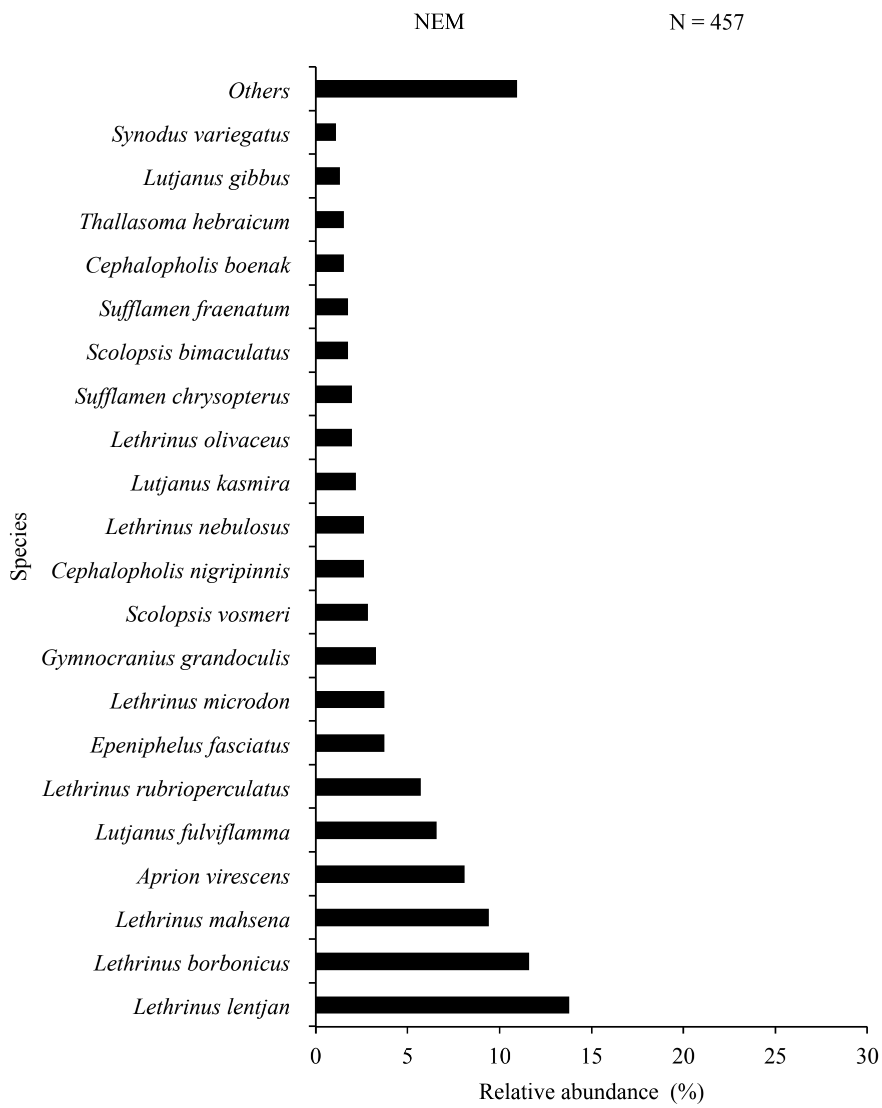


**Figure 4.** Relative abundance (%) of the fish species caught during the northeast monsoon (NEM) season.

**Table 2.** Seasonal mean catch rate (kg/hook/day) for the hook sizes used during the study period.

| | NEM | | SEM | |
|---|---|---|---|---|
| Hook size | Total weight (kg) | Mean catch rate ± SD | Total weight (kg) | Mean catch rate ± SD |
| No. 8 | 19.6 | 1.29 ± 0.74 | 12.4 | 0.67 ± 0.28 |
| No. 9 | 14.3 | 1.12 ± 2.88 | 6.8 | 0.15 ± 0.06 |
| No. 10 | 36.4 | 0.31 ±0.16 | 25.2 | 0.26 ± 0.10 |
| No. 15 | 22.8 | 0.13 ± 0.04 | 17.7 | 0.13 ± 0.10 |
| No. 16 | 12.8 | 0.12 ± 0.05 | 21.6 | 0.13 ± 0.08 |

### Species Dominance

The *K*-dominance analysis showed that the curve for the NEM season was lower than that for the SEM season suggesting that fish species dominance was lower during the NEM season; an indication of higher species diversity during this season. The curve for the SEM season showed that species dominance was higher, and hence a lower diversity of fish species during the SEM season (Fig. 5). A comparison of the *K*-dominance curves for the different hook sizes showed lower species dominance for hook sizes No. 15, 16 and 10 while for the other two hook sizes, No. 8 and 9, the curves showed higher dominance (Fig. 6). These results show that the diversity of fish species caught by hook sizes No. 15, 16 and 10 was higher than the diversity of fish species caught by hook sizes No. 8 and 9 during the study period.

A comparison of the *K*-dominance curves for the different study sites showed lower species dominance for Mpunguti and Nyuli fishing grounds, while for Mundini and Waga the curves showed higher species dominance (Fig. 7). These results show that the diversity of fish species caught at Mpunguti and Nyuli was higher than the diversity of fish species caught at Mundini and Waga during the study period.

### Effects of hook size, season and fishing ground interaction on the size of fish caught

ANCOVA showed that the size of hooks alone did not have a significant effect on the length of fish caught during the study period ($p$ = 0.12), but fishing ground had a significant effect on the size of fish captured ($p < 0.05$). The interaction of season and sampling site had a significant effect on the length of fish caught during the study period ($p < 0.05$). The interaction of season and hook size; fishing ground *versus* hook size, had no effect on the length of fish captured ($p$ = 0.884 and $p$ = 0.057), respectively. Similarly, the interaction of season, sampling site and hook size had no effect on the length of fish captured during the study period ($p$ = 0.195; Table 3).

### Selectivity

Selectivity of all the hook sizes used during the study period was determined for *L. borbonicus*, four

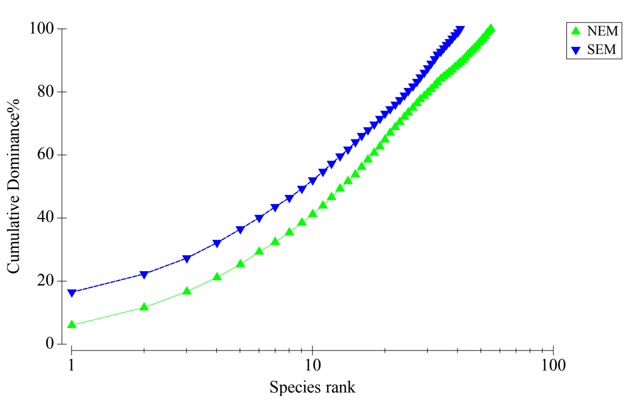


**Figure 5.** *K*-dominance curves for the fish species caught during NEM and SEM seasons.

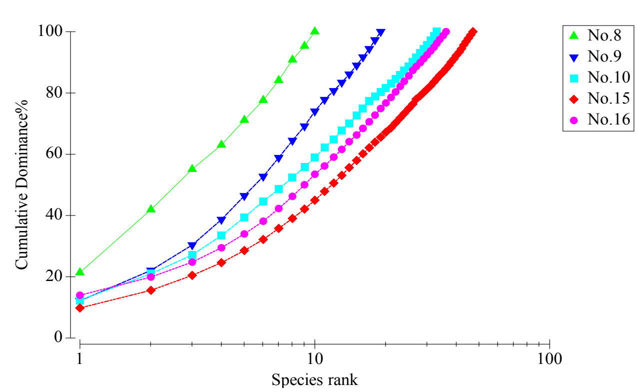


**Figure 6.** *K*-dominance curves for the fish species caught by the different hook sizes during the study period.

**Table 3.** P–values for the Analysis of Covariance (ANCOVA) on the effects of season, fishing site and hook size on the length of fish captured during the study period.

| Effect | SS | MS | F | P |
|---|---|---|---|---|
| Season | --- | --- | --- | --- |
| Fishing site | 563 | 563 | 10.06 | 0.002 |
| Hook size No. | 135.4 | 135.4 | 2.42 | 0.12 |
| Season*Fishing site | 262.3 | 262.3 | 4.69 | 0.031 |
| Season*Hook size No. | 1.2 | 1.2 | 0.02 | 0.884 |
| Fishing site*Hook size No. | 687.9 | 114.7 | 2.05 | 0.057 |
| Season*Fishing site*Hook size No. | 484.1 | 80.7 | 1.44 | 0.195 |

hook sizes for *L. lentjan* and *L. rubrioperculatus*, and two hook sizes for *A. virescens* and *L. fulviflamma*. The length at which *L. borbonicus* matures is 21.3cm and it grows to a maximum length of 40.0cm (Froese and Pauly, 2017) as indicated in Appendix 1. All the hook sizes used for the study period had optimal selection lengths above the length at which *L. borbonicus* matures, showing that all the hook sizes selected mature *L. borbonicus* individuals. Hook sizes No. 9 and 10 had optimal selection lengths above the maximum length for *L. borbonicus*, while hook sizes No. 8, 15 and 16 had optimal selection lengths below maximum length of this species. This implies that hook sizes No. 9 and 10 caught *L. borbonicus* individuals which had attained maximum growth size, while hook sizes No. 8, 15 and 16 caught *L. borbonicus* individuals which had not attained maximum growth size. Selection curves for all hook sizes used during the study period had wide selection ranges for *L. borbonicus*, except for hook size No. 8 which showed a narrow selection range (Fig. 8).

The length at which *L. lentjan* matures is 24.7 cm and the fish grows to a maximum length of 52.0cm (Froese and Pauly, 2017) as shown in Appendix 1. Hook sizes No. 15 and 8 had optimal selection lengths less than the length at which *L. lentjan* matures indicating that the hooks selected immature *L. lentjan* individuals. On the other hand, the optimal selection lengths of hook sizes No. 10 and 9 were above the length at which *L. lentjan* matures (Fig. 9). This indicates that hook sizes No. 10 and 9 selected mature *L. lentjan* during the study period. However, all the hooks caught *L. lentjan* individuals that had not attained maximum growth size. The length at first maturity for *L. rubrioperculatus* is 20.0 – 26.0 cm and it grows to a maximum length of 50.0cm (Froese and Pauly, 2017; Appendix 1). The optimal selection length of hook sizes No. 16 and 15 was less than the length at which *L. rubrioperculatus* matures while the optimal selection length of hook sizes No. 10 and 9 was above this length. This indicates that hook sizes No. 16 and 15 captured immature *L. rubrioperculatus* individuals and hook sizes No. 10

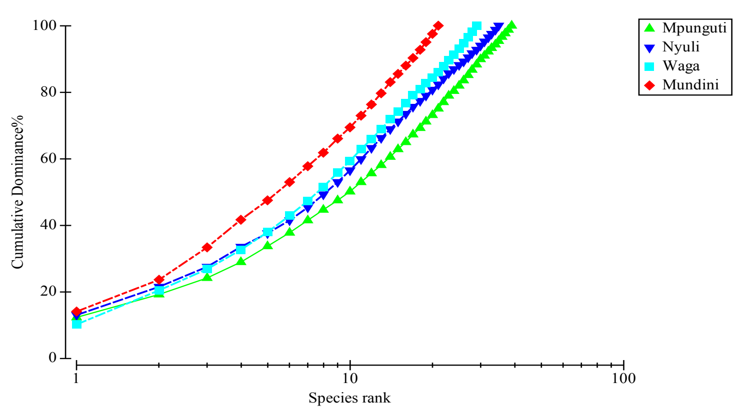


**Figure 7.** *K*-dominance curves for fish species caught at the study sites during the study period.

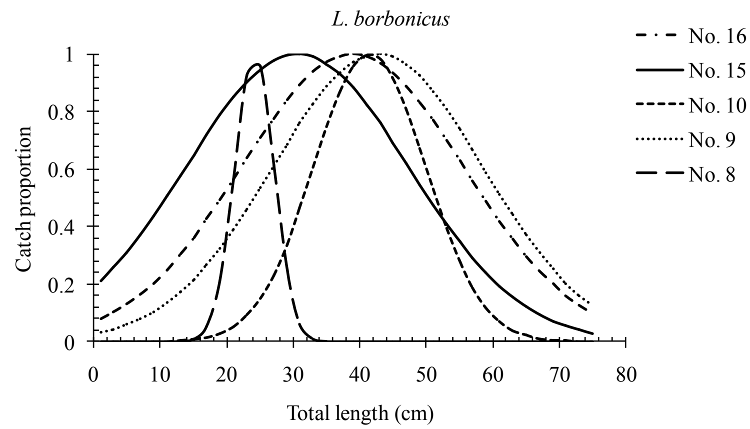


**Figure 8.** Selectivity curves for the various hook sizes used to capture *Lethrinus borbonicus* specimens during the study period.

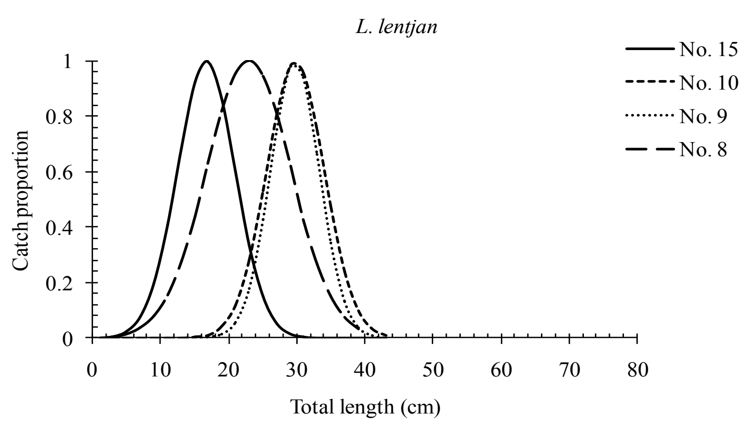


**Figure 9.** Selectivity curves for the various hook sizes used to capture *Lethrinus lentjan* specimens during the study period.

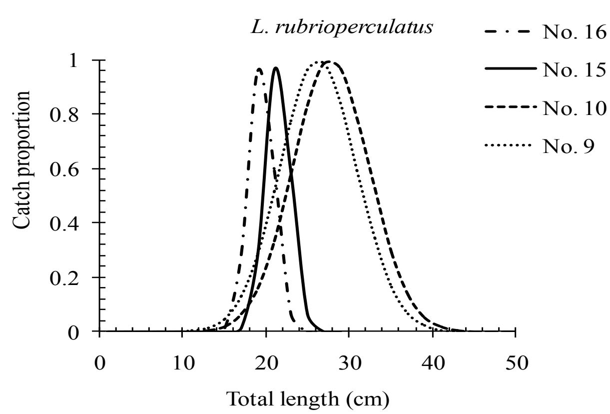


**Figure 10.** Selectivity curves for the various hook sizes used to capture *Lethrinus rubrioperculatus* specimens during the study period.

**Table 4.** Number of specimens per species, mean length (± SD, cm) and optimal selection length (cm) of the hooks used for the study.

| Species | N | Mean Length (cm) | Optimal selection length (cm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | No.16 | No.15 | No.10 | No.9 | No.8 |
| *L. fulviflamma* | 49 | 19.0 ± 3.3 | 19 | 21 | _ | _ | _ |
| *A. virescens* | 45 | 21.7 ± 15.9 | 7 | 9 | _ | _ | _ |
| *L. rubrioperculatus* | 59 | 20.4 ± 3.6 | 19 | 21 | 27 | 27 | _ |
| *L. lentjan* | 87 | 25.5 ± 5.3 | _ | 17 | 29 | 29 | 23 |
| *L. borbonicus* | 313 | 19.9 ± 4.2 | 39 | 31 | 41 | 43 | 25 |

and 9 captured mature *L. rubrioperculatus* individuals during the study period. The optimal selection length of hook sizes No. 16, 15, 10 and 9 were less than the maximum length attained by *L. rubrioperculatus* (Fig. 10). This indicates that the hooks captured *L. rubrioperculatus* individuals before they had attained their maximum growth size.

*Lutjanus fulviflamma* matures at a length of 17.1cm and grows to a maximum length of 35.0cm (Froese and Pauly, 2017; Appendix 1). The optimal selection lengths for hook sizes No. 15 and 16 (21.0cm and 19.0cm, respectively) were above the length at which *L. fulviflamma* matures indicating that hook sizes No. 15 and 16 selected mature *L. fulviflamma*. However, the

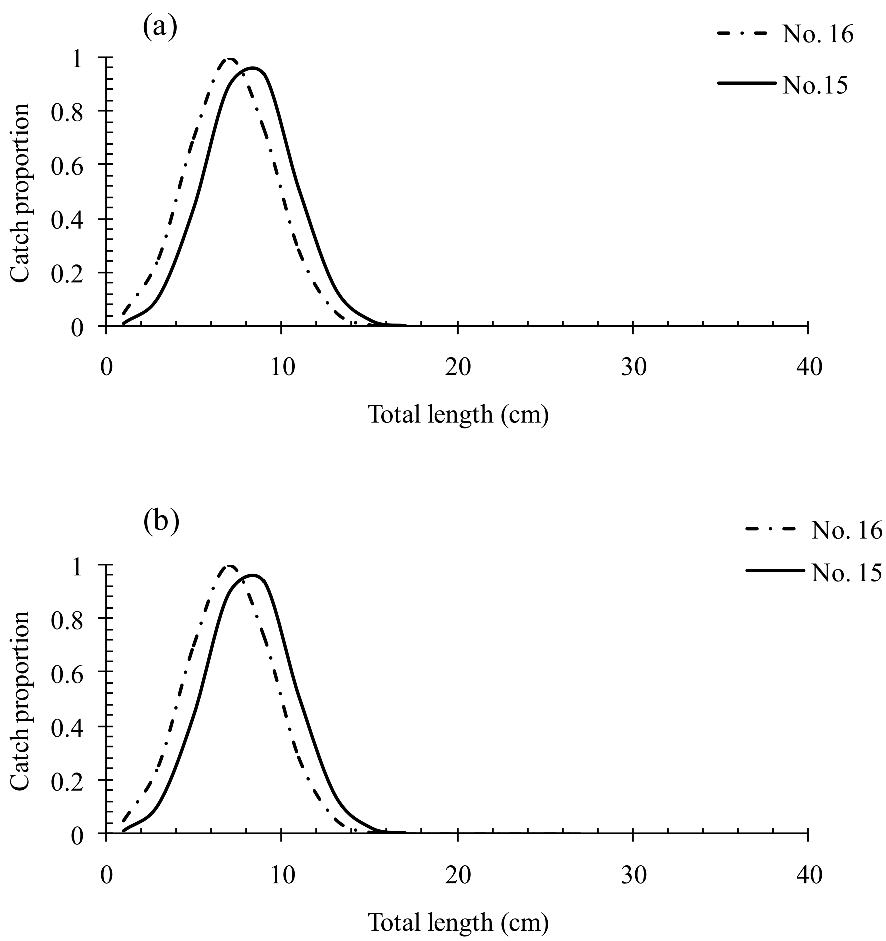


**Figure 11.** Selectivity curves for hook sizes No. 16 and 15 that captured *Lutjanus fulviflamma* (a) and *Aprion virescens* (b) specimens during the study period.

optimal selection lengths were less than the maximum length attained by *L. fulviflamma* indicating that hook sizes No. 15 and 16 captured *L. fulviflamma* individuals which had not attained maximum growth size.

*Aprion virescens* matures at a length of 44.7 cm and grows to a maximum length of 112.0cm (Froese and Pauly, 2017; Appendix 1). The optimal selection lengths for hook sizes No. 16 and 15 (7.0cm and 9.0cm, respectively) were lower than the length at which *A. virescens* matures and this indicated that both hook sizes No. 16 and 15 selected immature *A. virescens* during the study period. Also, hook sizes No. 16 and 15 captured *A. virescens* which had not attained maximum growth size (Fig. 11 a & b).

Hook size No. 16 had the same optimal selection length (19.0cm) for *L. fulviflamma* and *L. rubrioperculatus*, while hook sizes No. 10 and 9 had the same optimal selection length for this species (27.0cm). Similarly, hook sizes No.10 and 9 had the same optimal selection length (29.0cm) for *L. lentjan* during the study period (Table 4).
Similarity of species composition for the fish caught by the different hook types

Hierarchical cluster analysis was carried out to investigate the similarity of fish species composition for the different hook sizes used during the study period (Fig. 12). There was a high level of similarity in the species caught by hook sizes No. 16 and 15 (64.9%). Also, the fish species caught by hook size No. 8 were similar to those captured by hook size No. 9 (46.3%). This shows that the fish species caught by hook size No. 16 were comparable to those caught by hook size No. 15, while the species caught by hook size No. 8 were comparable to those caught by hook size No. 9. Hook size No. 10 can singled out, with fish species not similar to those caught by the other hook sizes used during the study period (Fig. 12).

## Discussion

Hook size has considerable effects on the size and composition of fish captured. This study assessed fish size selectivity of different hook sizes to ascertain whether the use of large sized hooks could reduce the capture of undersized individuals in the artisanal handline fishery of Shimoni on the south coast of Kenya. This was achieved by assessing the species composition of fish captured by five different hook sizes (Nos. 16, 15, 10, 9 and 8) and estimating the optimal selection lengths of the hooks for the most abundant species captured. The results indicated that there was a higher diversity of fish species caught during the calmer NEM season compared to the rougher SEM season. This could be due to reduced fishing effort as a result of rough sea conditions during the SEM, or migration of fish and reduced density due to a deeper thermocline and cooler waters in the SEM (McClanahan, 1988).

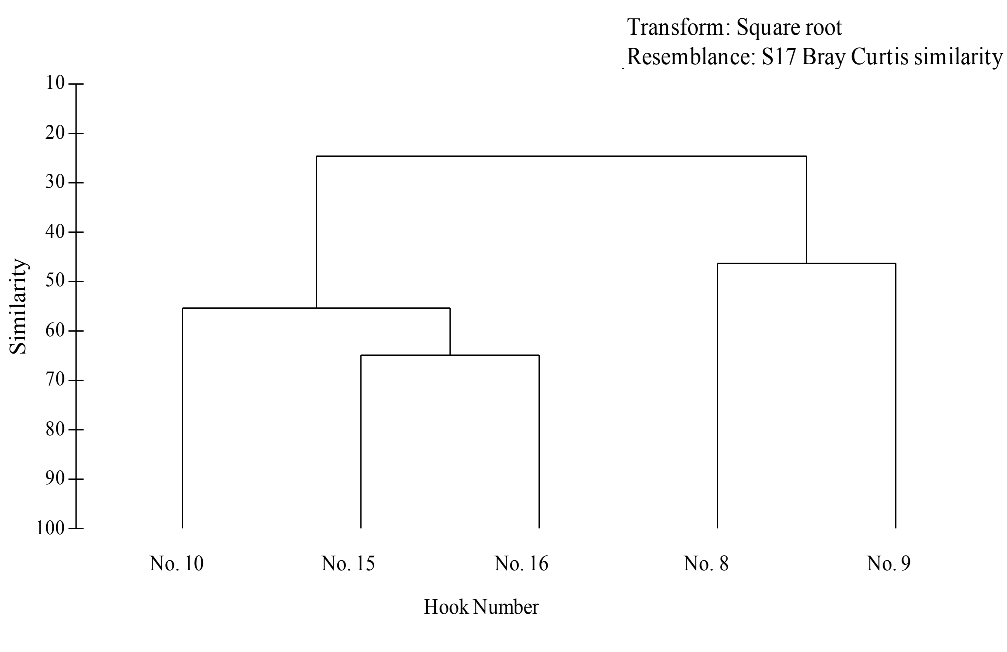


**Figure 12.** Cluster analysis dendrogram showing the similarity in species composition for various hook sizes.

Results from this study indicate that small sized hooks captured greater numbers of fish compared to large sized hooks which captured less and larger fish. These results are in agreement with the findings of Bjorndal and Løkkeborg (1996), where smaller hooks produced more fish than larger hooks. Similarly, the smaller hook size No. 12 captured small snappers while the larger hook size No. 8 captured large snappers (Ralston, 1990). In this study the larger hook size No. 8 was more effective in capturing and holding larger fish which gave higher catch rate, and showed lower species diversity compared to the smaller hook size Nos. 15 and 16. These results are in agreement with those of Patterson *et al.*, (2012) in which the diversity of fish caught decreased with an increase in hook size.

The decline of the number of smaller fish with increasing hook size and the abundance of fish could be due to gape limitations (Bacheler and Buckel, 2004) and small hooks being swallowed easily and becoming hooked deeply in the body, reducing the chances of fish escape (Alós *et al.*, 2008). Also, the decrease in the number of fish with an increase in hook size from this study concur with the findings of Mongeon *et al.* (2013) where the smaller hook size No. 10 caught more spotted rose snappers, *Lutjanus guttatus* than the large hook sizes No. 6 and 8. The results clearly indicate that there was an increase in the length of fish caught with increase in hook size and this could be as a result of large fish avoiding small hooks or the limitations of the mouth sizes of fish. These results concur with those obtained from a study conducted by Otway (1993) where an increase in absolute hook size led to a substantial increase in the mean size of snappers captured.

Results of this study showed that the sizes of fish caught at Mpunguti fishing ground did not differ from the sizes of fish caught at Nyuli. Similarly, the sizes of fish caught at Waga did not differ from the sizes of fish caught at Mundini fishing ground. However, there was higher species diversity at Mpunguti and lower species diversity at Mundini. This could be attributed to differences in fish size composition and species composition at the fishing grounds. The results indicated that the size of hooks alone did not have any effect on the size of fish captured, but different fishing grounds resulted in variations in the size of fish captured. This could be attributed to differences in the size composition of fish in the fishing grounds. A combination of both season and fishing grounds led to variations in the total length of fish caught, and could be an indication that the sizes of fish were influenced by season. However, when both season and hook size or fishing ground and hook size are changed, the length of fish caught did not change. Also, a simultaneous change of season, fishing ground and hook size did not change the total length of fish caught during this study.

The decrease in selection length with increase in hook size recorded for *L. borbonicus* agrees with the findings of Amarasinghe *et al.* (2014) in which the selection range of the giant trevally, *Caranx ignobilis*, and the naked breast trevallay, *Carangoides gynostethuse*, decreased with increase in hook size. The lower selection ranges for hook sizes No. 15 and 16 shown in *L. fulviflamma* and *A. virescens* selection curves could be as a result of large fish avoiding these hooks and the failure of these hooks in retaining large fish. The findings of this study show important differences in terms of the number of fish caught by different hook sizes and this could be due to the preference of the fish to the different hook sizes, the size of mouth gape or the size composition of the fish poulations. The smaller hook sizes No. 16, 15 and 10 caught large numbers of fish resulting in high species diversity compared to the larger hook sizes No. 8 and 9 which caught less numbers of fish, resulting in low species diversity.

In this study selectivity was determined for only five species (*L. borbonicus*, *L. lentjan*, *L. rubrioperculatus*, *L. fulviflamma* and *A. virescens*) and for those hooks which produced representative data. The lack of selectivity analysis for the other species caught by the handlines could be due to limited size ranges in the fishing areas (Erzini *et al.*, 1996), or an overlap in the length frequency distribution of fish and low variation in the sizes of fish captured, making curve adjustment difficult (Peixer and Petrere, 2007). The selection characteristics of *L. borbonicus*, *L. lentjan*, *L. rubrioperculatus*, *L. fulviflamma* and *A. virescens* indicated unimodal curves for the different hook sizes used during the study. This conforms to the principle of geometric similarity which states that all fish of the same species which are geometrically similar are caught by geometrically similar gears producing similar selection curves (Baranov, 1948; Hamley, 1975). These findings are similar to those recorded for masu salmon, *Oncorhynchus masou* (Shimizu *et al.*, 2000), yellowfin tuna, *Thunnus albacores* (Cortes-Zeragoza *et al.*, 1989), and for the giant trevally, *Caranx ignobilis*, together with those of the naked breast trevally, *Carangoides gynostethus* caught in the hook-and-line fishery off Nagombo, Sri Lanka (Amarasinghe *et al*., 2014) which reported unimodal selection curves for the respective species. However, Ralston (1982) and Peixer and Petrere (2007) found that hook selectivity can conform to a sigmoid selection curve which represents yield per recruit (Silvestre and Pauly, 1991).

The selectivity of all the hooks used in this study was above the length at which *L. borbonicus* matures (Table 6) indicating that the hooks did not capture immature individuals. However, the use of hook sizes No. 16, 15, 10 and 9 should be controlled, since they have wider selection ranges, to conserve the older and bigger fish that have high fecundities and longer spawning periods than smaller fish (Love *et al*., 1990; Berkeley *et al*., 2004). Hook sizes No. 16 and 15 captured mature *L. fulviflamma* and immature *A. virescens* specimens. These results are controversial when it comes to decision making on whether to avoid these sizes of hooks or not, since the fishery is multispecies. Also, hook sizes No. 15 and 8 captured mature *L. lentjan*, while hook sizes No.10 and 9 captured immature *L. lentjan*. The selectivity of hook sizes No. 15, 10 and 9 (Table 6) revealed that these hooks captured mature *L. rubrioperculatus* fish while hook size No. 16 captured immature *L. rubioperculatus* during the study period.

Generally, these results indicate an overlap in the selectivity of the hook sizes No. 16, 15, 10, 9 and 8 for *L. borbonicus*, *L. lentjan*, *L. rubrioperculatus*, *L. fulviflamma* and *A. virescens*. For certain species the hooks selected mature fish and for other species the specific hooks selected immature fish, making it difficult for decision making in the multispecies fishery. However, the larger hook size No. 8 proved to be the suitable hook for the handline fishery given that these hooks captured mature fish, gave narrow selectivity curves and yielded higher catch rates during the study period.

## Conclusion and recommendations

In conclusion, results from the present study indicate that varying hook sizes in the Shimoni artisanal handline fishery had significant effects: smaller hooks caught more fish with higher species diversity compared to the larger hooks that caught less fish with lower species diversity. However, the larger hooks had higher catch rates compared to the smaller hooks. From the results, it can be generally concluded that the selectivity of the different hooks used in this study vary with the fish species. However, the larger hook size No. 8 could be suitable for the Shimoni artisanal handline fishery as it resulted in higher catch rate and a selection curve with narrow selection ranges targeting fewer cohorts, and gave higher yields. This will result in reduced capture of immature individuals and conserve the more productive older fish in the population (Arlinghaus *et al*., 2010). However, if the current level of fishing is sustainable, other hooks with wider selection ranges could be used so that more length classes are harvested.

The use of large sized hook No. 8 is therefore recommended for the Shimoni artisanal handline fishery, which resulted in a higher catch rate and narrower selection ranges compared to the smaller sized hooks. This analysis was done without consideration of the hooks which got lost due to fish escapes, size of fish mouth, bait type and duration of soaking for specific hook sizes. Therefore, it is recommended that a study be conducted to address these aspects, and to assess the stock status of fish populations in the fishing area to allow for the application of other methods of determining selectivity such as "iterative estimates" (Regier and Robson, 1966)), and McCombie and Fry's (1960) methods to give absolute selectivity for the fishery, and for comparisons. Given the diversity of fish species caught by the handline fishery, a multispecies assessment approach would be required, or hook selectivity should be evaluated through single species assessment techniques.

## Acknowledgements

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**Appendix 1.** Family, species, number of fish (N), size range, length at $1^{st}$ maturity, $L_{mat}$ and infinite length, L¥ (Froese and Pauly, 2017) of the fish species caught during the study period.

| Family | Species | N | Size range (cm) | $L_{mat}$ (cm) | $L_\infty$ (cm) |
|---|---|---|---|---|---|
| Lethrinidae | *Lethrinus borbonicus* | 313 | 11.0–34.0 | 21.3 | 40.0 |
| Lethrinidae | *Lethrinus lentjan* | 87 | 14.9–20.0 | 24.7 | 52.0 |
| Lutjanidae | Lutjanus fulviflamma | 49 | 12.2–26.0 | 17.1 | 35.0 |
| Lethrinidae | *Lethrinus mahsena* | 46 | 23.6–41.0 | 19.0 | 65.0 |
| Lethrinidae | *Lethrinus rubrioperculatus* | 59 | 14.5–29.0 | 20.0-26.0 | 50.0 |
| Lutjanidae | *Aprion virescens* | 45 | 11.0–70.5 | 44.7 | 112.0 |
| Lethrinidae | *Lethrinus olivaceus* | 32 | 13.7–47.0 | 34.0 | 100.0 |
| Lethrinidae | *Lethrinus microdon* | 22 | 19.0–37.0 | 29.1 | 80.0 |
| Serranidae | *Epeniphelus fasciatus* | 19 | 12.0–26.0 | 17.5 | 40.0 |
| Nemipteridae | *Scolopsis bimaculatus* | 17 | 17.1–24.0 | _ | 31.0 |
| Lethrinidae | *Gymnocranius grandoculis* | 16 | 15.4–38.0 | _ | 80.0 |
| Serranidae | *Cephalopholis nigripinnis* | 23 | 10.5–22.5 | _ | 28.0 |
| Sphyraenidae | *Sphyraena jello* | 14 | 47.2–59.8 | _ | 150.0 |
| Nemipteridae | *Scolopsis vosmeri* | 13 | 11.9–16.6 | _ | 25.0 |
| Lethrinidae | *Lethrinus nebulosus* | 13 | 14.0–19.5 | 39.4 | 87.0 |
| Lutjanidae | *Lutjanus gibbus* | 13 | 14.4–44.0 | _ | 50.0 |
| Balistidae | *Sufflamen chrysopterus* | 12 | 14.0–20.6 | _ | 30.0 |
| Lutjanidae | *Lutjanus kasmira* | 12 | 14.5–26.5 | _ | 40.0 |
| Serranidae | *Cephalopholis boenak* | 16 | 10.0–75.0 | 12.2 | 30.0 |
| Mullidae | *Parupeneus macronema* | 16 | 14.9–20.0 | 12.3 | 40.0 |
| Balistidae | *Sufflamen fraenatum* | 11 | 17.9–32.6 | _ | 38.0 |
| | Others | 118 | | | |
| | Total | 966 | | | |

# Effects of urea and lipid removal from *Carcharhinus leucas* and *Galeocerdo cuvier* white muscle on carbon and nitrogen stable isotope ratios

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## Abstract

The analysis of stable isotope ratios of carbon and nitrogen is a tool commonly used in trophic ecology. However, the presence of nitrogen compounds and lipids in tissues of studied organisms can bias the ratio measurements. Treatments to eliminate problematic compounds have been highlighted in the literature. In this study the effects of two different treatments and their combination on the $\delta^{15}N$ and $\delta^{13}C$ ratio values of *Carcharhinus leucas* and *Galeocerdo cuvier* white muscle samples were tested. All sharks were caught along the west coast of Reunion Island (western Indian Ocean), within the framework of a shark-control programme. Deionized water rinsing proved to be the most effective treatment for nitrogen compound removal and the lipid extraction, using a 2:1 chloroform-methanol solution, the most effective treatment for lipid removal. The combination of both treatments was as effective as deionized water rinsing for nitrogen compound removal but produced an unexpected decrease of $\delta^{13}C$ ratio values. Deionized water rinsing caused a similar decrease on some $\delta^{13}C$ values in the bull shark. Some differences on the effects of the different treatments appeared when considering the sexes separately. Analytical normalization equations for the different treatments on the two stable isotope ratios are provided.

**Keywords:** $^{15}N$, $^{13}C$, TMAO, Lipid extraction, Shark, Reunion Island

## Introduction

Sharks, as apex or mesopredators, play major roles in the functioning of ecosystems in which they evolve, affecting the dynamics of their prey populations directly through consumption and indirectly through risk avoidance behavior (Heithaus *et al.*, 2008; Roff *et al.*, 2016). Apex predators are usually the largest species, (Ferretti *et al.*, 2010; Heupel *et al.*, 2014) they can undertake large-scale movements and therefore transport energy, nutrients and other materials through the oceans, over long distances (Estes *et al.*, 2016). Recently, anthropogenic pressures have caused the decline of several shark populations, raising concerns about their conservation and the effect of their removal on the functioning of their ecosystems (Ferretti *et al.*, 2010). Top-down effects have been highlighted in certain shark species (Heithaus *et al.*, 2007; Myers *et al.*, 2007) but data are still lacking on the trophic dynamic of many others (Ferretti *et al.*, 2010).

One method to study trophic ecology is the analysis of stable isotopes, and more specifically the $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) and $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) ratios (Fry, 2006). Their use is based on the fact that the isotopic composition of a consumer is dependent of its diet, presenting a mix of the isotopic proportions of its prey plus a small increase due to fractionation throughout the food web (Fry, 2006; Layman *et al.*, 2011). In the case of $\delta^{15}N$, the increase from the prey to the predator is typically estimated to 2-5 ‰ per trophic

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level, allowing the determination of trophic positions. The fractionation is more conservative in the case of $\delta^{13}C$, usually with 0-1 ‰ per trophic level, and is typically used to identify the production at the base of the food chain and foraging location (Post, 2002; Martínez del Rio *et al.*, 2009; Hussey *et al.*, 2012). Accurate ecological interpretation of stable isotope data relies on confidence in a number of underpinning assumptions, including accounting for biasing effects of polar compounds, namely lipids, urea and trimethylamine oxide (Shipley *et al.*, 2017).

Of concern when measuring $\delta^{13}C$ values, is the presence of lipids in the samples. Indeed, lipids are $^{13}C$-depleted compared to proteins and carbohydrates and introduce a bias in $\delta^{13}C$ values by lowering these (Newsome *et al.*, 2010). The presence of such a bias has been highlighted in certain studies of elasmobranches, but the low lipid proportion in some species suggests this bias is not systematic (Hussey *et al.*, 2010; Matich *et al.*, 2010; Kim and Koch, 2012; Li *et al.*, 2015). The C:N ratio is traditionally used to determine if a sample contains enough lipids to introduce a bias by assuming that ratios lower than 3.5 are mostly composed of proteins (Post, 2002; Pethybridge *et al.*, 2012). However, if this assumption is true in teleosts (Hoffman and Sutton, 2010), the use of nitrogenous compounds for osmoregulation in shark muscles imply that C:N ratios below 3.5 could still contain important lipid quantities (Shipley *et al*, 2017). Thus, it is recommended that lipids should be extracted from samples before stable isotope analysis to remove bias and standardize samples between species and across food webs (Hussey *et al.*, 2012; Shipley *et al.*, 2017).

The measurement of $\delta^{15}N$ ratios values can also be biased, especially in elasmobranches. Indeed, their tissues contain urea and trimethylamine oxide (TMAO) used to maintain osmotic balance. These nitrogenous compounds are $^{15}N$ depleted, which can lead to lowering $\delta^{15}N$ values when conducting stable isotope analyses. The removal of these compounds is necessary prior to analyses in elasmobranches (Kim and Koch, 2012; Hussey *et al.*, 2012). For lipids, although this bias is not systematic, it is recommended that elasmobranch samples are treated for urea to standardize samples.

Currently, lipids are commonly removed using a 2:1 chloroform methanol extraction following a modification of the Bligh and Dyer (1959) technique. Although nitrogenous compounds can be removed by the same technique in elasmobranch muscle, a deionized water rinsing has been shown to be the most effective technique to remove urea and TMAO from shark tissues. Combined lipid extraction and deionized water rinsing have also proven to be useful and even more effective than separated techniques in some instances (Li *et al.*, 2015).

This study is part of a long-term project that is investigating the trophic ecology of bull (*Carcharhinus leucas*) and tiger (*Galeocerdo cuvier*) sharks in coastal ecosystems of Reunion Island (western Indian Ocean). Samples were collected from specimens caught in the local shark-control programme implemented by the French government and local authorities after a series of shark attacks on surfers and bathers since 2011. The main aim of the programme is to better understand the place and role of the two species in the functioning of coastal ecosystems, and how the removal of individuals could affect these ecosystems. A first description of the diet and position of the species in food chains has been conducted by Trystram *et al.* (2016), highlighting differences in feeding habits and resource use between the two studied species. Although preliminary tests conducted by Trystram *et al.* (2016) on the effect of lipids and urea removal on stable isotope ratios of carbon and nitrogen did not reveal significant effects of these components on isotopic values, a more systematic investigation of the lipid extraction and urea rinsing seemed necessary. Indeed, several recent studies suggested significant effects of these treatments, especially for large shark species (Li *et al.*, 2015; Carlisle *et al.*, 2016; Shipley *et al.*, 2017).

This study followed the protocol described in Li *et al.* (2015) to investigate the effect of lipid and urea removal on isotopic values of bull and tiger shark white muscle. Treatment-related differences were investigated both at the scale of the species and for the sexes separately. When a significant difference was observed between the control (no treatment) and treated samples, an analytical normalization was proposed to adjust the isotopic values of non-treated samples in the future.

## Materials and methods

### Sample collection

Samples were collected from individuals caught along the west coast of Reunion Island in the framework of the Reunion Island shark control programme, using both horizontal bottom longlines and smart drumlines (Guyomard *et al.*, 2019). Dead individuals were stored at 4°C in a cold room shortly after their capture and dissected as soon as possible, and up to 36 h later. The total length (TL, cm) of each individual was measured to the nearest centimeter and the total weight (W, kg) of each individual was measured whenever possible, or otherwise derived from biometric equations (Pirog *et al.*, in press). A portion of white muscle was sampled from the back of each individual, from the front of the anterior dorsal fin, and frozen at -20°C shortly after sampling. Sixteen female and 15 male bull sharks and 14 male and 15 female tiger sharks, representative of the size range of the captures, were randomly selected for this study. All samples came from individuals caught in 2016 to limit possible effect of the year of catch on stable isotope values.

### Sample preparation and analysis

All frozen white muscle samples were freeze-dried at -50 °C for 48 h using a CRIOS Cryotec freeze dryer. Dry samples were reduced by milling for 3 minutes to a homogeneous powder using a Mixer Mill Retsch MM400 at 30 Hz. Each powdered sample was divided into four equivalent subsamples and four different treatments were applied to each: Urea extraction (DW), lipid extraction (LE), lipid and urea extraction (LE+DW) and no treatment (control, C), following the methods of Li *et al.* (2015). In summary, deionized water was used to remove urea from muscle tissues and a 2:1 chloroform-methanol mixture was used to extract lipids (see supplementary materials for the detailed protocol). After each treatment all samples were dried again in an oven at 50 °C for 24 h.

0.3 to 0.9 mg of dry powdered material was put into a tin capsule for each sample for stable isotope analyses after completion of the treatment. The exact mass was weighed using a precision balance to the nearest 0.1 mg. The capsules were then folded into small spheres, placed in a 96-sink plate and sent to the IRMS platform at the University of La Rochelle for $\delta^{13}C$ and $\delta^{15}N$ measurements. $\delta^{13}C$ and $\delta^{15}N$ values were determined for each sample using a Thermo Scientific Flash EA 1112 elemental analyzer coupled with a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer with a Conflo IV interface. The machines were calibrated using the working standards USGS-61 (Caffeine) and USGS-62 (Caffeine). All results are expressed in the standard notation relative to the international standards Pee-Dee Belemnite for carbon and atmospheric $N_2$ for nitrogen. Replicate measurements of internal laboratory standards provided measurement errors <0.10 ‰ for both $\delta^{15}N$ and $\delta^{13}C$ values.

### Statistical analysis

For each species, $\delta^{15}N$ and $\delta^{13}C$ values were statistically compared to test whether they differed between treatments. Parametric conditions were assessed using Bartlett's tests for homogeneity of variances and Shapiro's tests for normality. Pairwise paired t-tests with the Benjamini-Yekutieli p-value adjustment method were conducted when the data adhered to parametric assumptions. When this was not the case, a pairwise paired Wilcoxon rank sum test was conducted with the same p-value adjustment method, as the logarithmic and square-root data transformations did not allow parametric analyses. To assess for a sex-related response to treatments, the same statistical procedures were conducted for both sexes for each species. The differences between sexes for each treatment were determined using t-tests or Wilcoxon rank sum tests respectively, for parametric and non-parametric conditions.

When a significant effect of a treatment on stable isotope values was observed, an analytical normalization of non-treated samples was established with linear models. In order to test for species and sex-related differences in linear models, values observed and predicted by the models were statistically compared using either a t-test or Wilcoxon rank sum test, depending whether the dataset adhered to parametric assumptions.

Differences in C:N ratios between non-treated and treated samples were assessed for each species. As the data did not follow parametric assumptions, Kruskal-Wallis tests followed by Dunn post-hoc analyses with Bonferroni corrections were used.

All statistical analyses were performed using the software R version 3.4.3 with a significance level of 0.05.

## Results

The DW treatment resulted in significantly higher $\delta^{15}N$ values but did not modify $\delta^{13}C$ values when compared to the control, except for *C. leucas* when considering both sexes together. In this case the DW treatment resulted in a significantly lower $\delta^{13}C$ value compared to the control. The LE treatment resulted in higher $\delta^{15}N$ values than the control except for *C. leucas* males where the value was significantly lower. The LE $\delta^{13}C$ values were higher than the control except for *C. leucas* males

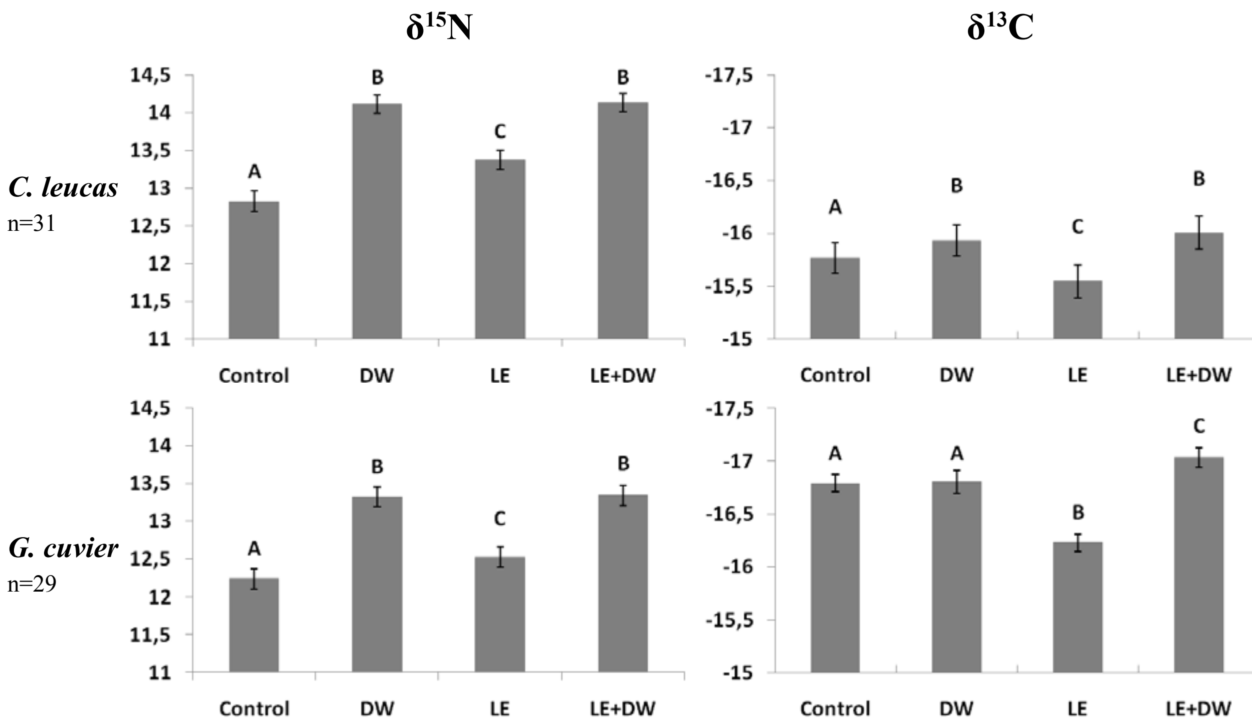


**Figure 1.** Histograms of the mean $\delta^{15}N$ and $\delta^{13}C$ values for *Carcharhinus leucas* and *Galeocerdo cuvier*. Significant results are indicated by different letters. Error bars are standard errors.

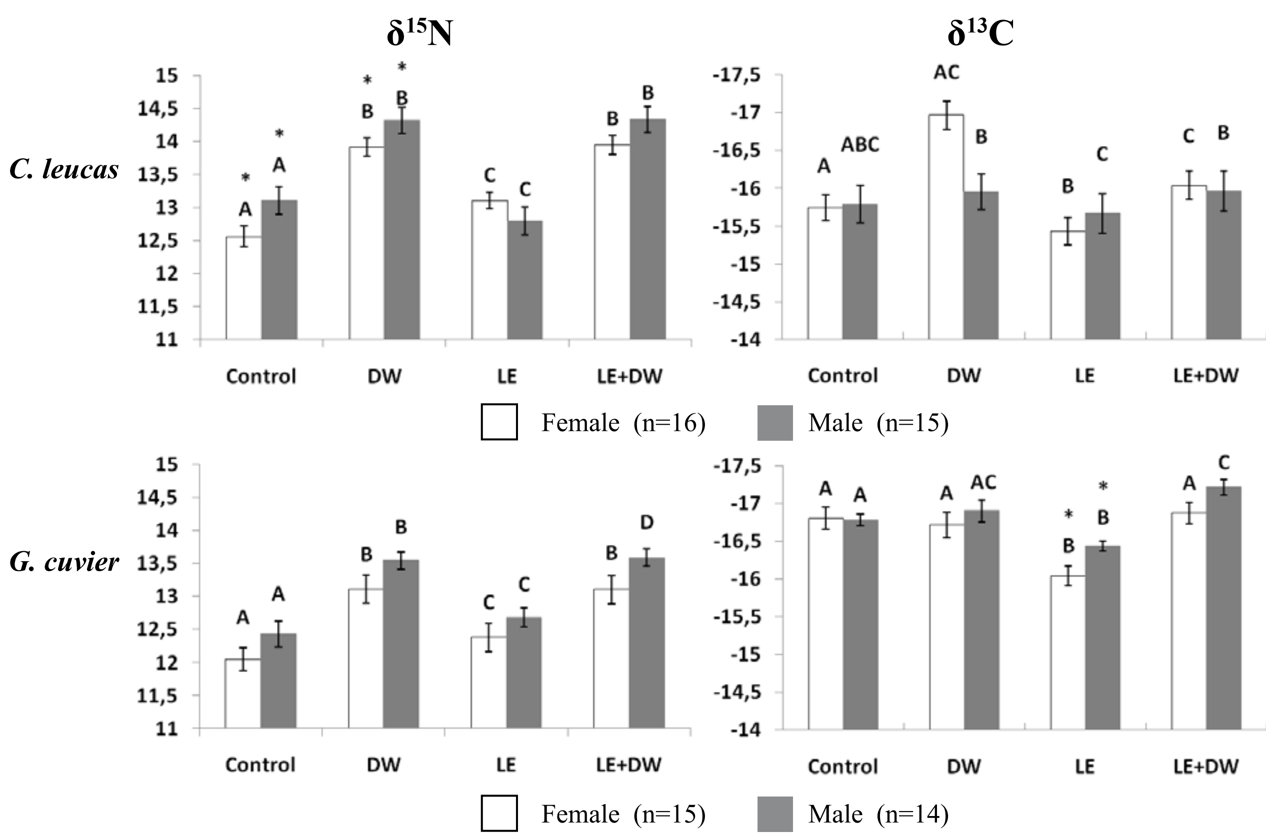


**Figure 2.** Histograms of the mean $\delta^{15}N$ and $\delta^{13}C$ values for male and female *Carcharhinus leucas* and *Galeocerdo cuvier*. Significant results within a sex are indicated by different letters. Asterisks indicate significant differences between two sexes for one treatment. Error bars are standard error.

where there was no significant difference. The LE+DW treatment resulted in an increase of $\delta^{15}N$ values and a decrease of $\delta^{13}C$ values compared to the control. The only exceptions were for the female *G. cuvier* and the male *C. leucas* $\delta^{13}C$ values, which were not significantly different between LE+DW treatment and control. LE samples always had significantly lower $\delta^{15}N$ values and higher $\delta^{13}C$ values than DW and LE+DW. These last two treatments generally did not significantly change $\delta^{15}N$ values, except for male *G. cuvier* for which the LE+DW treatment had a significantly higher value compared to the control. For the $\delta^{13}C$ values, the two treatments were significantly different for *G. cuvier* only, and LE+DW has the lowest value (Fig. 1). When comparing the means between sexes within a treatment, there were only significant differences for the control and DW treatment of $\delta^{15}N$ for *C. leucas* and the LE treatment of $\delta^{13}C$ for *G. cuvier* (Fig. 2).

**Table 1.** Regression equations displaying the relationship between the Control treatment and other treatments. The species column presents the species and the sexes. Sex equations are only presented when significantly different from the equations using both sexes. CL = *Carcharhinus leucas*. GC = *Galeocerdo cuvier*. The $R^2$ of the regression analyses are presented. All p-values <0.05. The equations recommended for the normalization values of non-treated samples (see discussion) are represented in grey.

| Species | | Parameter | Equation | $R^2$ |
|---|---|---|---|---|
| CL | | $\delta^{13}C$ | $\delta^{13}C_{LE} = 1.043*\delta^{13}C_{Control} + 0.903$ | 0.94 |
| | Female | $\delta^{13}C$ | $\delta^{13}C_{LE} = 1.075*\delta^{13}C_{Control} + 1,492$ | 0.93 |
| | Male | $\delta^{13}C$ | $\delta^{13}C_{LE} = 1.022*\delta^{13}C_{Control} + 0.469$ | *0.96* |
| CL | | $\delta^{13}C$ | $\delta^{13}C_{LE+DW} = 0.966*\delta^{13}C_{Control} - 0.779$ | 0.8 |
| | Female | $\delta^{13}C$ | $\delta^{13}C_{LE+DW} = 0.993*\delta^{13}C_{Control} - 0.408$ | 0.79 |
| | Male | $\delta^{13}C$ | $\delta^{13}C_{LE+DW} = 0.955*\delta^{13}C_{Control} - 0.886$ | *0.8* |
| CL | | $\delta^{13}C$ | $\delta^{13}C_{DW} = 0.939*\delta^{13}C_{Control} - 1.131$ | *0.86* |
| GC | | $\delta^{13}C$ | $\delta^{13}C_{LE} = 0.749*\delta^{13}C_{Control} - 3.66$ | 0.56 |
| | Female | $\delta^{13}C$ | $\delta^{13}C_{LE} = 0.826*\delta^{13}C_{Control} - 2.162$ | *0.88* |
| GC | | $\delta^{13}C$ | $\delta^{13}C_{LE+DW} = 0.772*\delta^{13}C_{Control} - 4.067$ | 0.44 |
| | Female | $\delta^{13}C$ | $\delta^{13}C_{LE+DW} = 0.784*\delta^{13}C_{Control} - 3.703$ | *0.62* |
| CL | | $\delta^{15}N$ | $\delta^{15}N_{DW} = 0.852*\delta^{15}N_{Control} + 3.192$ | 0.88 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{DW} = 0.823*\delta^{15}N_{Control} + 3.576$ | *0.85* |
| CL | | $\delta^{15}N$ | $\delta^{15}N_{LE+DW} = 0.834*\delta^{15}N_{Control} + 3.437$ | 0.82 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{LE+DW} = 0.824*\delta^{15}N_{Control} + 3.595$ | 0.73 |
| CL | | $\delta^{15}N$ | $\delta^{15}N_{LE} = 0.873*\delta^{15}N_{Control} + 2.176$ | 0.84 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{LE} = 0.762*\delta^{15}N_{Control} + 3.54$ | 0.82 |
| | Male | $\delta^{15}N$ | $\delta^{15}N_{LE} = 0.91*\delta^{15}N_{Control} + 1.735$ | *0.8* |
| GC | | $\delta^{15}N$ | $\delta^{15}N_{DW} = 0.813*\delta^{15}N_{Control} + 3.37$ | 0.66 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{DW} = 1.068*\delta^{15}N_{Control} + 0.235$ | 0.71 |
| | Male | $\delta^{15}N$ | $\delta^{15}N_{DW} = 0.544*\delta^{15}N_{Control} + 6.781$ | *0.66* |
| GC | | $\delta^{15}N$ | $\delta^{15}N_{LE+DW} = 0.832*\delta^{15}N_{Control} + 3.159$ | 0.67 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{LE+DW} = 1.074*\delta^{15}N_{Control} + 0.157$ | 0.72 |
| | Male | $\delta^{15}N$ | $\delta^{15}N_{LE+DW} = 0.559*\delta^{15}N_{Control} + 6.642$ | *0.66* |
| GC | | $\delta^{15}N$ | $\delta^{15}N_{LE} = 0.858*\delta^{15}N_{Control} + 2.031$ | 0.73 |
| | Female | $\delta^{15}N$ | $\delta^{15}N_{LE} = 1.099*\delta^{15}N_{Control} - 0.861$ | 0.74 |
| | Male | $\delta^{15}N$ | $\delta^{15}N_{LE} = 0.659*\delta^{15}N_{Control} + 4.495$ | 0.81 |

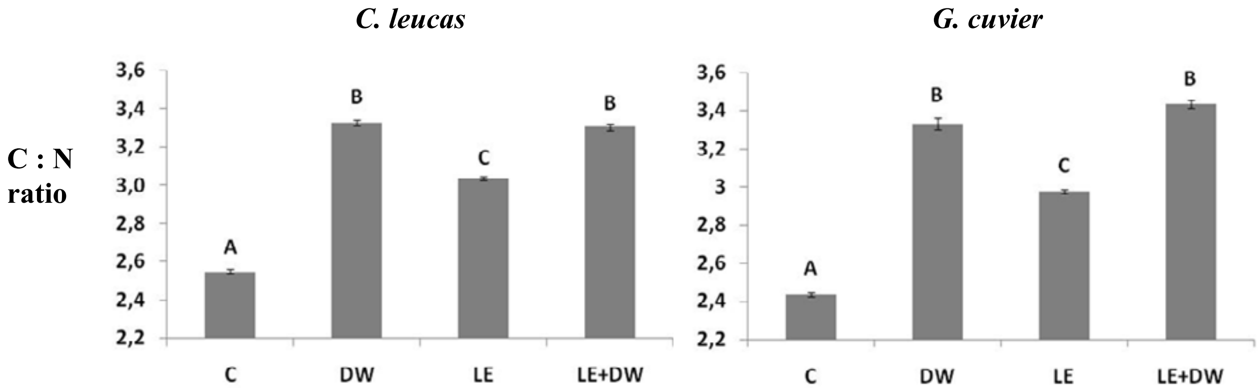


**Figure 3.** Histograms of the mean C:N ratios for *Carcharhinus leucas* and *Galeocerdo cuvier*. Significantly different results are indicated by different letters.

Equations of the linear models to normalize non-treated samples are shown in Table 1. When the general equation and the female and/or male equations produced significantly different datasets, all the equations are shown. The non-linear relations and the non-significant regressions (p-value >0.05) are not presented.

The C:N ratios increased between the control and the different treatments in both species (Fig. 3). The increase was more significant in DW and LE+DW treatments. The LE treatment also resulted in an increase, but this was less significant. The comparison of the results obtained and the results of Li *et al.* (2015) is shown in Table 2.

**Table 2.** Comparison of effects of the treatments on stable isotope values for the two studied species and other shark species (Li *et al.*, 2015). Different letters and colours indicate significant differences between control and treatments within each species. The comparison is shown for both the carbon and nitrogen analyses.

| | Species | C | DW | LE | LE + DW |
|---|---|---|---|---|---|
| | *Carcharhinus leucas* | A | B | C | B |
| | *Galeocerdo cuvier* | A | A | B | C |
| | *Carcharhinus falciformis* | A | B | C | D |
| | *Prionace glauca* | A | B | B | C |
| $\delta^{13}C$ | *Sphyrna zygaena* | A | B | B | C |
| | *Sphyrna lewini* | A | AB | B | B |
| | *Carcharhinus longimanus* | A | B | BC | C |
| | *Isurus oxyrinchus* | A | A | A | A |
| | *Alopias pelagicus* | A | A | B | B |
| | *Carcharhinus leucas* | A | B | C | B |
| | *Galeocerdo cuvier* | A | B | C | B |
| | *Carcharhinus falciformis* | A | B | C | B |
| | *Prionace glauca* | A | B | C | B |
| $\delta^{15}N$ | *Sphyrna zygaena* | A | B | C | BC |
| | *Sphyrna lewini* | A | B | C | B |
| | *Carcharhinus longimanus* | A | B | C | B |
| | *Isurus oxyrinchus* | A | A | A | A |
| | *Alopias pelagicus* | A | B | C | B |

## Discussion

It is critically important to obtain correct stable isotope values in order to accurately analyze food webs. In this context, sample preparation using a deionized water rinsing and lipid extraction was deemed necessary in sharks (Fisk *et al.*, 2002; Hussey *et al.*, 2012) and the results of the present study support this. Indeed, significant changes in isotopic values of bull and tiger shark white muscle and C:N ratios were observed when applying the different treatments to extract lipids and/or urea, compared to non-treated samples (control samples).

When considering $\delta^{15}N$ values, all treatments resulted in a significant increase of the values compared to control values, which could lead to an underestimation of the trophic positions of the individuals. Such a result was expected for DW treatment as it is known that the presence of urea and trimethylamine oxide (TMAO) in the muscles of sharks result in lowering the $\delta^{15}N$ value and corresponding trophic level (Fisk *et al.*, 2002; Hussey *et al.*, 2012). The LE treatment, initially designed to remove lipids, also resulted in increasing $\delta^{15}N$ values, though this increase was lower than for DW or LE+DW treatments. Such a result was recently observed for several species of deep-sea sharks by Shipley *et al.* (2017), who also recommended that an additional DW rinse be performed to remove any remaining urea from shark muscle tissue. Hussey *et al.* (2010) suggested that lipid extraction removes soluble urea, and this is likely why this small increase in $\delta^{15}N$ values was observed. However, the water rinsing had a greater impact, which confirms that this treatment is more effective than lipid extraction for urea and TMAO removal. Interestingly, the combined treatment LE+DW had the same effect as the DW treatment, suggesting that water rinsing is sufficient to remove all the urea and TMAO present in samples, and that no additional lipid extraction is needed to produce accurate $\delta^{15}N$ values. The only exception was for the male tiger sharks where the combined treatments increased the $\delta^{15}N$ value even more than water rinsing only. However, this additional increase was marginal with a maximum of 0.04‰, a value close to the internal laboratory measurement error, which suggests that either the difference is an artifact that could disappear with additional replicates, or the difference is real, but weak enough to keep the DW treatment only.

In the case of *C.leucas* and *G.cuvier* $\delta^{15}N$ analysis, the DW treatment alone seems to be adequate to remove urea and TMAO. Li *et al.* (2015) suggest that the LE+DW treatment is the most effective because it reduces urea concentration in pelagic shark muscles to a greater extent than the DW treatment alone. Similarly, Dale *et al.* (2011) suggested that water rinsing may not be enough to remove all the influence of urea on $\delta^{15}N$ values for a sting ray (*Dasyatis lata*). The same kind of effect could be observed for the tiger and bull sharks, but the urea concentration was not measured in the samples in this study. However, the maximal difference of 0.04 ‰ between DW and LE+DW mean values for each species and sex in these results suggests that the DW treatment is sufficient.

It is known that lipids are depleted in $^{13}C$ compared to carbohydrates and proteins, and that lipid-rich samples cause the $\delta^{13}C$ values to decrease (Newsome *et al.*, 2010; Hussey *et al.*, 2012). Thus, lipid extraction is necessary in cases of high lipid content in samples and the $\delta^{13}C$ value is expected to increase with it. Such a significant increase in $\delta^{13}C$ values was observed in this study for the LE treatment. This result confirms the need to extract lipids from both tiger and bull shark muscles to result in correct $\delta^{13}C$ values. In addition, C:N ratios were under 3.5 for all the controls. Thus, assuming that these samples contained mainly proteins is incorrect, as lipid extraction caused significant $^{13}C$ changes. Nitrogenous compound washing also affected the C:N ratio, confirming previous research showing that the presence of these compounds make this ratio an unreliable proxy for lipid presence estimation in sharks (Shipley *et al.*, 2017).

For both DW and LE+DW treatments, the $\delta^{13}C$ value decreased in both species, a result which was not expected. The hypothesis of repeated manipulation error is not relevant here because of the number of replicates, the success of the LE treatment and the consistency in the effect in both species and for each sex. Therefore, this could result from an unknown aspect of the tiger and bull shark physiology causing water rinsing to decrease $\delta^{13}C$ in powdered muscle samples; for example, by an unidentified compound washed by deionized water and enriched in $^{13}C$ that would decrease the $\delta^{13}C$ value. Further research is needed to elucidate this unexpected effect.

Lipid extraction alone seems to have had the expected effect and successfully increased the $\delta^{13}C$ value. The only exception was for the male bull sharks where no significant effect was observed. This could be explained by the low percentage of lipids in muscles of male bull sharks. Differences in lipid quantity have previously been observed between sexes of *Mustelus mustelus*, and the authors suggest that female fishes should have more lipids for maturation and embryo development (Bosch *et al.*, 2013). Furthermore, it has been highlighted that in some species of sharks, the quantity of lipids present in the muscles is very low and lipid extraction is not needed prior to SIA (Matich *et al.*, 2010; Trystram *et al.*, 2016). However, except for the male bull sharks, there was still an increase in $\delta^{13}C$ value, and this suggests that lipid extraction should still be undertaken in the two studied species.

Because of the confusing effect of water rinsing on $\delta^{13}C$ value, it is impossible to recommend one treatment for both $\delta^{13}C$ and $\delta^{15}N$ SIA for *C.leucas* and *G.cuvier*. Instead, water rinsing should be undertaken for $\delta^{15}N$ SIA and lipid extraction for $\delta^{13}C$ SIA on separated sub-samples. The combination of the two treatments, although usable for $\delta^{15}N$, is not recommended for $\delta^{13}C$. Such an effect of water rinsing on $\delta^{13}C$ values highlights the importance of assessing each species of shark separately when determining which sample treatment is necessary.

As these treatments are lengthy and costly, an alternative method used to result in correct values is the application of analytical normalization. For this purpose, a series of equations were produced which allowed the estimation of corrected isotopic values based on the values of non-treated samples. Considering the recommendation of treatments for carbon and nitrogen values in white muscle of tiger and bull sharks, the equations that should be used for the normalization of non-treated values are those highlighted in Table 1. When possible, the separated sex equations should be used. Interestingly, the models are less robust for tiger sharks compared to bull sharks (lower $R^2$ values). This suggests that tiger sharks display more variability in the lipid and urea contents in white muscles, and this could be linked to their life cycle.

When comparing the results from this study to those of Li *et al.* (2015), an interesting pattern appears for $\delta^{15}N$ values (Table 2). The effects of the different treatments are similar in each species except for *Isurus oxyrinchus* in which treatments had no significant effects. This indicates that the deionized water rinsing has the same outcome in various offshore pelagic species, as well as in the two coastal benthopelagic species studied, supporting the idea that this treatment is necessary at least in all large bodied shark species. The comparison of $\delta^{13}C$ results in more interspecific differences, underscoring once more the importance of species-specific tests in order to determine the most effective treatments. Again, *I. oxyrinchus* displays no difference between treatments. This species is believed to be the fastest and most active shark in the world (Ebert *et al.*, 2013) and could possess physiological attributes explaining the very low concentrations of both urea and lipids in its muscle. For the other species, the age of the individuals and their physiological and reproductive status could be factors explaining the differences in the results of treatments, as they might indicate different lipid contents in white mucles. Male bull sharks used in this study were, in particular, mostly caught outside of the reproductive period (pers. obs.), and this could explain the low lipid content of these individuals, which led to no significant effect of the LE treatment on $\delta^{13}C$ values. Further investigations could confirm or reject this hypothesis.

In conclusion, this research demonstrates the need to correct the stable isotope values of carbon and nitrogen in the white muscle of tiger and bull sharks, either by using a treatment or by analytical normalization. This conclusion is in accordance with previous studies conducted on other shark species (Li *et al.*, 2015; Carlisle *et al.*, 2016; Shipley *et al.*, 2017). A comparison of the results of treatments to extract lipids and urea in shark tissues from individuals from different locations could indicate whether analytical normalizations are specific to local individuals of a species, or to all specimens of the same species from any location.

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# Supplementary material
## Detailed protocol
### *Control*

For stable isotope analysis, 0.3 to 0.9 mg of powdered material was put in a tin capsule for each sample. The exact mass was weighed using a precision balance. The capsules were then folded into small spheres, placed in a 96-sink plate and sent from Reunion Island to the University of La Rochelle. There, the $\delta^{13}C$ and $\delta^{15}N$ values were determined for each sample using a Thermo Scientific Flash EA 1112 and a Thermo Scientific Delta V Advantage with a Conflo IV interface. The machines were calibrated using the working standards USGS-61 (Caffeine) and USGS-62 (Caffeine).

### *Urea extraction*
First, 1.8 ml of deionized water was added to each sample using a 2 ml scaled needle. The samples were then vortexed for 30 seconds. After that the closed tubes were left undisturbed at room temperature for 24 h. Following this, a Fugamix CM-50M centrifuge was used to sediment the material at 2000 rpm for 5 minutes. The water was then removed from the tube using a 1000 µL micropipette while being careful not to disturb the settled material. The described procedure was repeated 3 times in total. After that, the samples were placed in a dryer at 50 °C for 48 h. Finally, the samples were crushed in order to obtain a fine powder. Each sample then followed the steps described for the control.

### *Lipid extraction*
The lipid extraction was carried out under a fume hood and with proper protective equipment. First, a 2:1 solution of chloroform-methanol was prepared using a scaled beaker. 1.8 mL of this solution was added to the tube of each sample using a scaled needle. The samples were then vortexed for 10 seconds. The closed tubes were then placed in a 30 °C water bath for 24 h. After that, the tubes were centrifuged at 2000 rpm for 6 minutes using a Fugamix CM-50M centrifuge. The chloroform-methanol solution was then poured off the tubes by tilting. 1.8 mL of a fresh 2:1 chloroform-methanol solution was then added to each sample. The tubes were again vortexed for 10 seconds and immediately centrifuged. The chloroform-methanol was again poured off the sample tubes. After that the sample tubes were left open under the fume hood for 24 h. Finally, the samples were crushed in order to obtain a fine powder. Each sample then followed the steps described for the control.

### *Urea and lipid extraction*
For the urea and lipid extraction, the samples were subjected first to a lipid extraction and then urea extraction following the protocols described above. Each sample then followed the steps described for the control.

# Ecological classification of estuaries along the Tanzanian mainland: a tool for conservation and management

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## Abstract

Estuaries are unique and important coastal ecosystems providing significant and diverse services to ecosystems and human kind. Worldwide, estuaries are overwhelmed by human disturbance and over utilization of their resources which threatens their existence. This study aimed to develop a classification framework for Tanzanian mainland estuaries using abiotic variables (ecoregion, latitude and catchment size) and validate the resulting estuary types using biota (fish and prawns). Biota were sampled from five selected estuaries (Manyema, Lukuledi, Matandu, Rufiji and Ruvuma) using a seine net and identified to species level. Multivariate analyses including analysis of similarities, cluster analysis, Bray-Curtis, Pairwise and similarity percentage analysis were used to analyse biota data. Ecoregion, latitude and catchment size resulted in two (Pangani and Central East African), three (Lower (5°- 6°S), Middle (> 6°S- 8°S), Higher (> 8°S)) and five (Smaller (<1000km$^2$), Small (1000-10000km$^2$), Medium (10000-50000km$^2$), Large (50000-100000km$^2$), Larger (>100000km$^2$)) classes of estuary, respectively. Two classification options; latitude-catchment size and ecoregion-catchment size, have been proposed. The latitude-catchment size classification produced nine estuary types while the ecoregion-catchment size classification produced seven estuary types. The latitude-catchment size produced estuary types with higher significant differences (global R=0.926, $p$=0.01) than ecoregion-catchment size (global R=0.659, $p$=0.03).

**Keywords:** estuary classification, validation, estuary type, ecoregion, latitude, catchment size

## Introduction

Estuaries have significant ecological and socio-economic importance, and are a major focus for human activities (Saenger, 1995). Their importance has compromised the integrity of estuarine ecosystems resulting in large scale alterations of their natural communities (Graham *et al.,* 2000). Estuaries are influenced by human activities at a local scale (e.g. through mangrove harvesting, salt pans, industrial and urban waste disposal, dredging of shipping channels, and construction of port facilities) and at a broader scale in the upper catchment (e.g. through agriculture, livestock keeping, deforestation and water abstraction for hydroelectric power production and water supply). Local and large-scale stressors on estuaries create complexity for their conservation and management resulting in unsustainable resource utilization and ecosystem services provision.

The existence of an estuary depends on hydrological features such as freshwater inflow from inland areas and tidal inundations from the sea (Kennish, 1986). Hydrological and environmental variations in estuaries include variations in tidal range, freshwater availability, salinity, temperature, dissolved oxygen and turbidity, which together have an influence on the biota. Therefore, biodiversity and ecosystem integrity of estuaries are directly determined by the prevailing hydrological and environmental characteristics which vary among estuaries. Consequently, the occurrence and distribution of biota are expected to differ across estuaries. Ecosystem responses to various

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conservation and management interventions are also expected to vary across different estuaries. Conservation of estuaries also requires an understanding that different estuaries are subjected to particular types and levels of human impacts.

Estuary classification refers to the grouping of similar estuaries into estuary types. An estuary type is defined as 'a group of estuaries with similar abiotic and biotic characteristics which shows distinct characteristics from another estuary type'. Estuary classification can be used as a tool for efficient conservation and management of estuary ecosystems (Bucher and Saenger, 1991; Saenger, 1995). Classification of estuaries could also serve as a tool for identifying potential Estuary Protected Areas to serve as estuary conservation units. Estuary classification as a management tool has been globally applied, for example in Australia (Saenger, 1995, Graham *et al.,* 2000), New Zealand (Hume *et al.,* 2007; NIWA, 2013), South Korea (Jang and Hwang, 2013), UK (Davidson *et al.,* 1991) and South Africa (Colloty *et al.,* 2002; Harrison and Whitfield, 2006). Although the Tanzania National Water Policy (URT, 2002) requires classification of all water resources including estuaries, Tanzanian estuaries have not yet been classified. Therefore, this study aimed to develop a classification framework for Tanzanian estuaries using abiotic variables and to validate the developed classification framework in selected estuaries using biota (fish and prawns).

## Materials and Methods

### Study area

Classification of estuaries was carried out for the entire Tanzanian mainland coast, which extends for a length of 1424km from the border with Kenya in the north to the Ruvuma estuary in the south. The Tanzania coastline is intersected by numerous estuaries, which vary from large, permanently open systems to small systems that are only occasionally connected to the ocean (Kimirei *et al.,* 2016). Validation of the classified estuary types was done on the selected estuaries of Manyema creek, Lukuledi, Matandu, Rufiji and Ruvuma.

### Study sites (estuaries)

Manyema creek is a tidal inlet on the Msasani-Kunduchi shoreline in the Dar es Salaam seascape formed by the northward accretion of 3km of sandy shore. The creek is flushed by semi-diurnal tides which have a maximum spring tide range of about four metres and a neap tide range of about one metre. Lukuledi creek is located at the southern border of Lindi Urban District. The estuary is surrounded by a fringing mangrove forest. Matandu estuary is found in Lindi region at Kilwa Kivinje. It has a funnel-shaped river mouth and surrounded by a fringing mangrove forest. Rufiji estuary occurs in Rufiji District, Pwani Region. The estuary has a deltaic formation. The delta extends some 24km inland (tides influence the river for some 40km upstream) and has eight major branches. Ruvuma estuary is located in Mtwara on the border with Mozambique. This estuary has a deltaic formation made up of tidal creeks rather than river tributaries. It is the second largest estuary in Tanzania with a large area of mangroves, sand banks and mud flats, and many channels and tributaries.

### Classification of estuaries using abiotic characteristics

Estuaries along the Tanzanian mainland coast were identified on maps and reviewed in the literature. A desktop study was used to review information on physical features that could be potentially useful for estuary classification. Key reviewed information for each estuary included latitude, ecoregion and catchment size. Additional reviewed information included climatic data, rainfall and temperature, which were obtained from the Tanzania Meteorological Agency using weather stations near the estuaries. In this study, a two-level framework, which allows integration of climatic, hydrological and other catchment features, was used to classify Tanzanian estuaries. A two-level classification provides different levels of resolution and options for selection of the most appropriate level of resolution, as per different objectives (Frissel *et al.,* 1986). The two proposed levels in this study were 'latitude' and 'ecoregions' as the first level and 'catchment size' as the second level. Both levels of characteristics have previously been used in ecosystem classification and are considered as good reflectors of biotic communities (Chaves *et al.,* 2005; Dodkins *et al.,* 2005). Freshwater ecoregions which have been previously described for Africa by Thieme *et al.,* (2005) were adopted for this study to classify the Tanzania coastline at level one classification. To incorporate climatic characteristics as defined by latitudinal difference, a latitudinal zonation along the Tanzania coastline was also developed and used at level one. Furthermore, a catchment size classification was developed and used at level two. Catchment size further allows the classification to capture hydrological and ecological features which influence estuaries socio-ecological characteristics and their management.

### Validation of classified estuary types using biotic characteristics

A total of eleven sites from five estuaries; namely Manyema creek (3), Lukuledi estuary (3), Matandu estuary (1), Rufiji estuary (2) and Ruvuma estuary (2) were selected for biotic validation. These five estuaries are distributed among three and four estuary types for ecoregion-catchment size and latitude-catchment size classifications, respectively, as classified in this study. Fish and prawn species were sampled for use in the biotic validation of the classified estuary types.

### Fish and prawn sampling procedure

Fish and prawns were collected by dragging a 35m seine net with a 3m drop and 13mm mesh onto the shore. Samples at each site were recorded and counted to obtain abundance. Samples which could not be identified at the site were preserved and transported to the University of Dar es Salaam for further taxonomic identification.

### Data analysis: validation of estuary types

Estuary types were validated using combined biotic data for fish and prawn samples at each site. Analysis of similarity (ANOSIM) was used to test whether or not there were significant differences in biotic (fish and prawns) assemblages amongst classification classes of both ecoregion-catchment size and latitude-catchment size classification frameworks. The Pairwise analysis was then carried ou to ascertain strength in differences among estuary types. Non-metric multidimensional scaling (NMDS) was used to visualise biotic patterns using Bray–Curtis analysis. Cluster analysis was carried out to show group similarities among estuaries for both ecoregion-catchment size and latitude-catchment size classification frameworks. A similarity percentage (SIMPER) analysis was undertaken to show average similarity and dissimilarity within groups based on taxa.

## Results

### Classification of estuaries using abiotic characteristics

*Level I: Ecoregions and latitude*

Ecoregions

Ecoregion classification developed by Thieme *et al.* (2005) and latitudinal differences of the Tanzania freshwater ecosystems were used in the level I classification of Tanzanian estuaries. Ecoregion classification divides the Tanzanian mainland coastline into two ecoregions: the Pangani Ecoregion on the northern side, and the Central East Africa Ecoregion on the southern side. This resulted in two estuary classes being identified; the Pangani estuary type and Central East Africa estuary type. The Pangani estuary type includes the estuaries of Pangani, Msangazi, Mkulumuzi and Sigi. The Central East Africa estuary type includes the estuaries of Msimbazi, Mzinga, Mpiji, Tegeta, Manyema, Wami, Ruvu, Matandu, Rufiji, Mbwemkuru, Mavuji, Lukuledi and Ruvuma (Table 1).

Latitude

The latitudinal range of the Tanzanian mainland coast is from 5 °S in Tanga region to 10°S in Ruvuma region. Three latitudinal classes were proposed as the lower latitude estuary type (≤6°S), middle latitude estuary type (6°-8°S) and higher latitude estuary type (>8°S). The lower latitude estuary type includes estuaries occurring from 6°S northwards, namely Pangani, Msangazi, Mkulumuzi and Sigi estuaries. The middle latitude estuary type includes the Msimbazi, Mzinga, Mpiji, Tegeta, Manyema, Wami, Ruvu and Rufiji, while the lower latitude estuary type includes the Mavuji, Matandu, Lukuledi, Mbwemkuru and Ruvuma estuaries.

*Level II: Catchment Size*

Catchment size was used as a level II classification factor to further divide either ecoregions or latitude classes proposed in level I. Catchments draining the Tanzania mainland estuaries range in size from small (<50km$^2$), for example Manyema and Tegeta creeks, to large (about 183,79km$^2$) in the case of the Rufiji delta. Five size classes of catchments were suggested, which ranged from smallest (<1,000km$^2$), small (1,000 to 10,000km$^2$), medium (>10,000 to 50,000km$^2$), large (>50,000 to 10, 0000km$^2$), and largest (>10, 0000km$^2$) catchments.

*Estuary classification framework*

A two-level classification framework is proposed for defining estuary types on the Tanzanian mainland. Three abiotic attributes, *viz*: Ecoregion, latitude, and catchment size, were used to produce classification options as ecoregion–catchment size classes and latitude-catchment size classes (Fig. 1).

The advantage of the two classification options is that ecoregion-catchment size classification can be used as a broader class while latitude-catchment size classification can be used for a zoomed- in classification. The option for ecoregion-catchment size classification produces 7 estuary types, while the latitude-catchment size classification produces 9 estuary types (Table 1).

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**Table 1.** Estuary types following the Ecoregion-Catchment size and Latitude-Catchment size classifications.

| | | Estuary Type | Description | Estuaries |
|---|---|---|---|---|
| 1 | Ecoregion-Catchment size (7) | Estuary Type 1 | Pangani Ecoregion – smaller catchment size (<1000 Km²) | Mkulumuzi |
| 2 | | Estuary Type 2 | Pangani Ecoregion – small catchment size (1000-10000 Km²) | Sigi and Msangazi |
| 3 | | Estuary Type 3 | Pangani Ecoregion – large catchment size (>50000-100,000 Km²) | Pangani |
| 4 | | Estuary Type 4 | Central East African Ecoregion – smaller catchment size (<1000 Km²) | Mpiji, Msimbazi, Mzinga, Tegeta and Manyema |
| 5 | | Estuary Type 5 | Central East African Ecoregion – small catchment size (>1000-10000 Km²) | Mavuji |
| 6 | | Estuary Type 6 | Central East African Ecoregion – medium catchment size (>10000-50000 Km²) | Wami, Ruvu, Matandu, Mbwemkuru and Lukuledi |
| 7 | | Estuary Type 7 | Central East African Ecoregion – larger catchment size (>100000 Km²) | Rufiji and Ruvuma |
| 8 | Latitude-Catchment size (9) | Estuary Type 1 | Lower latitudes – very small catchment size (5°- 6°S - <1000 Km²) | Mkulumuzi |
| 9 | | Estuary Type 2 | Lower latitudes – small catchment size (5°- 6°S - >1000-10000 Km²) | Sigi and Msangazi |
| 10 | | Estuary Type 3 | Lower latitudes – large catchment size (5°- 6°S ->50000-100,000 Km²) | Pangani |
| 11 | | Estuary Type 4 | Middle Latitudes – smaller catchment size (>6-8° S - <1000 Km²) | Mpiji, Msimbazi, Mzinga, Tegeta and Manyema |
| 12 | | Estuary Type 5 | Middle Latitudes – medium catchment size (>6-8°S -10000-50000 Km²) | Wami and Ruvu |
| 13 | | Estuary Type 6 | Middle Latitudes – larger catchment size (>6-8°S - >100000 Km²) | Rufiji |
| 14 | | Estuary Type 7 | Higher latitude – small catchment size (>8°S - >1000-10,000 Km²) | Mavuji |
| 15 | | Estuary Type 8 | Higher latitude – medium catchment size (>8°S - 10000-50000 Km²) | Matandu, Mbwemkuru and Lukuledi |
| 16 | | Estuary Type 9 | Higher latitude – larger catchment size (>8° S - >100000 Km²) | Ruvuma |

## Validation of estuary types using biota (fish and prawns)

A total of 42 fish and 4 prawn species were identified from the five studied estuaries. Across the five estuaries, higher abundances of *Encrasicholina heteroloba* (516), *Sardinella gibbosa* (156), *Upeneus vittatus* (103), *Valamugil seheli* (48), *Upeneus sulphureus* (45), *Penaeus indicus* (41), *Penaeus monodon* (32) and *Gaza minuta* (28) were apparent. Highest catches in terms of abundance were recorded for *Encrasicholina heteroloba* (346) and *Upeneus sulphureus* (96) in Lukuledi estuary, and *Sardinella gibbosa* (120) in Ruvuma estuary. Prawn catches were highest in the Rufiji delta. Higher abundance was recorded in Lukuledi estuary (727), Rufiji estuary (333), Ruvuma Estuary (108), Matandu estuary (53) and Manyema estuary (25).

Analysis of similarity (ANOSIM) for ecoregion-catchment size estuary type showed a significant difference between the estuary types (global R = 0.659, $p$ = 0.03). Pairwise analysis showed strongest separation between Estuary Type 6 and Estuary Type 4 (R=1, $p=0.001$), Estuary Type 7 and Estuary Type 4 (R=0.927, p=0.001)

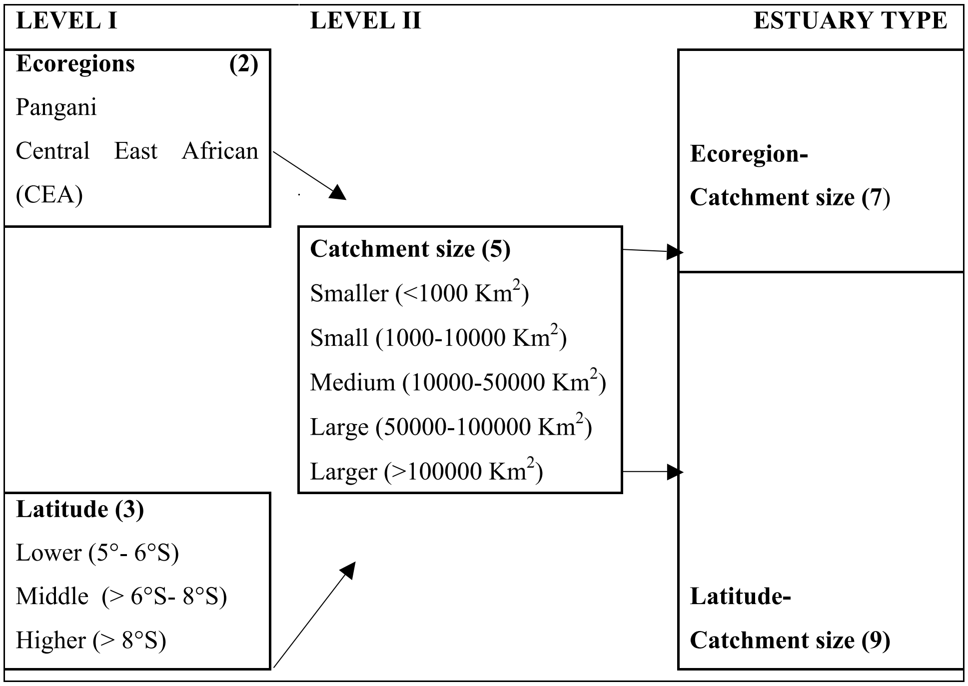


**Figure 1.** Classification framework for Tanzanian mainland estuaries.

and weakest separation between Estuary Type 6 and Estuary Type 7 (R=0.406, p=0.001) (Table 2). ANOSIM showed a strong significant variation of species among the nine latitude-catchment size groups (global R=0.926, $p$=0.01), based on latitude-catchment size classification. Strongest variations were obtained between all groups and were slightly less significant between Estuary Type 8 and Estuary Type 9 (R=0.607).

Cluster analysis for the two classification options (Fig. 2) shows the percentage levels at which samples are similar to form a group; that is, estuary type based on fish samples. In ecoregion-catchment size classifications, abiotic factors grouped the five estuaries into estuary types 4, 6 and 7 where estuary type 7 comprised of the Ruvuma and Rufiji estuaries. Biological validation, however, grouped the five estuaries into four groups separating Rufiji and Ruvuma estuaries. The Ruvuma and Rufiji estuaries together show a 20% similarity, while when separated, samples from Rufiji and Ruvuma showed a similarity of about 40% and 20%, respectively. Cluster analysis of the latitude-catchment size classification validated biotic differences between Ruvuma and Rufiji estuaries which belong to different estuary types.

Patterns of fish assemblages were visualized using a non-metric multidimensional scaling (MDS) for the latitude-catchment size classification. The MDS analysis showed a clearer separation of estuary types in latitude-catchment size than ecoregion-catchment size classification with a 2D stress value of 0.09. The MDS was overlaid with the cluster analysis to emphasize the biota grouping pattern (Fig. 3).

**Table 2.** Pairwise test for ANOSIM statistics of estuary groups based on Latitude-Catchment size classification.

| Estuary Types Groups | R significant, p=0.001 |
|---|---:|
| Estuary Type 8 and Estuary Type 9 | 0.607 |
| Estuary Type 8 and Estuary Type 4 | 1 |
| Estuary Type 8 and Estuary Type 6 | 0.907 |
| Estuary Type 9 and Estuary Type 4 | 1 |
| Estuary Type 9 and Estuary Type 6 | 1 |
| Estuary Type 4 and Estuary Type 6 | 1 |

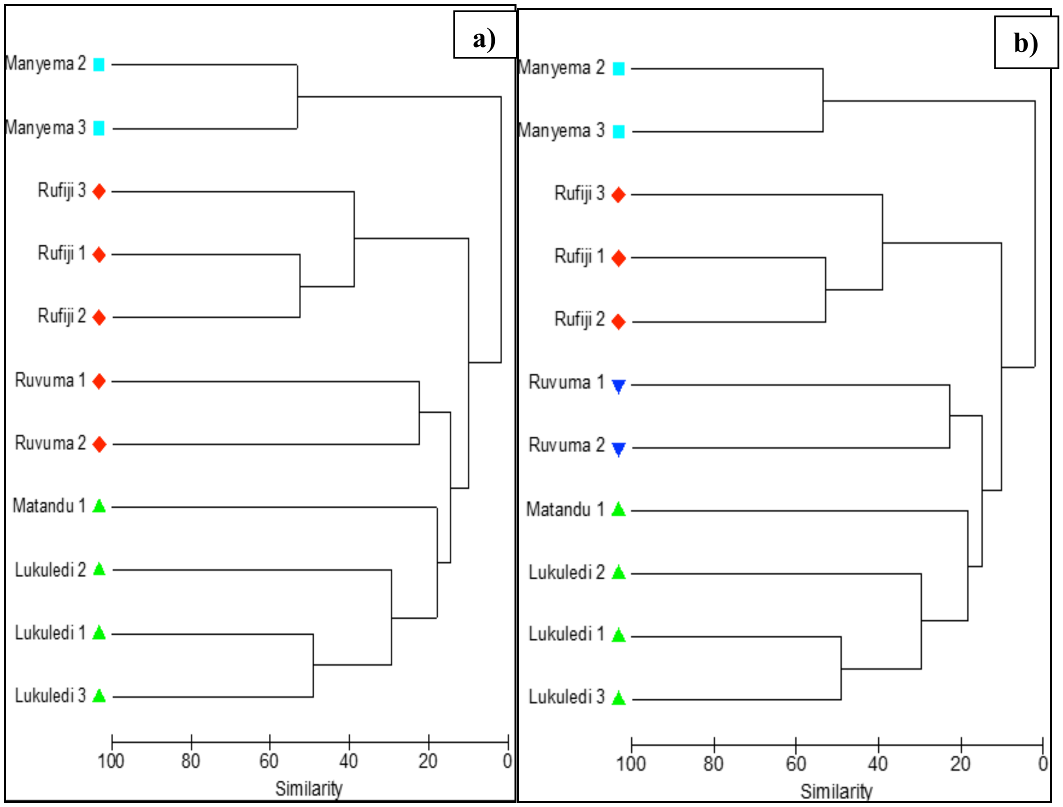


**Figure 2.** Cluster analysis of fish samples for a) ecoregion-catchment size and, b) latitude-catchment size classifications from estuaries along the Tanzania mainland, 2016.
(Ecoregion-catchment size: ■= Estuary Type 4; ▲= Estuary Type 6; ♦= Estuary Type 7 and latitude-catchment size; ■= Estuary Type 4; ▲= Estuary Type 8; ▼= Estuary Type 9; ♦ = Estuary Type 6).

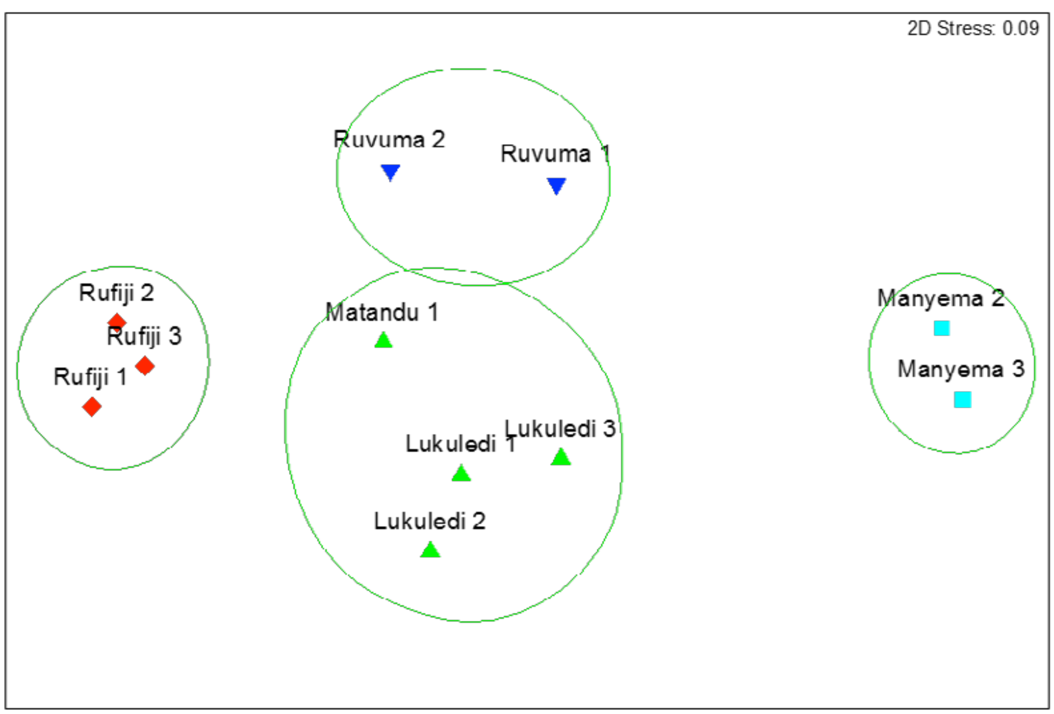


**Figure 3.** Non-metric multidimensional scaling plots showing grouping of estuaries based on biota assemblages overlaid on the cluster analysis for the latitude catchment size classification.

**Table 3.** Analysis of similarity percentage (SIMPER) of fish samples from four estuary types on the Tanzania mainland, 2016. Percentage of contribution by fish and prawn species for each estuary type are presented.

| Latitude-size Estuary Type | Sample | Average similarity (%) | Species | Contribution (%) | Cumulative contribution (%) |
|---|---|---|---|---|---|
| Estuary Type 8 | Lukuledi 1 | 27.11 | *Penaeus monodon* | 29.74 | 29.74 |
| | Lukuledi 2 | | *Encrasicholina heteroloba* | 21.87 | 51.60 |
| | Lukuledi 3 | | *Gerres acinaces* | 15.70 | 67.31 |
| | Matandu 1 | | *Alectis ciliaris* | 9.69 | 76.99 |
| Estuary Type 9 | Ruvuma 1 | 22.47 | *Sardinella gibbosa* | 84.77 | 84.77 |
| | Ruvuma 2 | | *Penaeus indicus* | 15.23 | 100.00 |
| Estuary Type 4 | Manyema 2 | 53.34 | *Leiognathus equlus* | 55.05 | 55.05 |
| | Manyema 3 | | *Etelis carbunculus* | 44.95 | 100.00 |
| Estuary Type 6 | Rufiji 1 | 43.51 | *Penaeus monodon* | 28.11 | 28.11 |
| | Rufiji 2 | | *Rastrelliger kanagurta* | 9.84 | 37.95 |
| | Rufiji 3 | | *Penaeus semisulcatus* | 8.32 | 46.27 |
| | | | *Johnius dussumieri* | 7.83 | 54.10 |
| | | | *Thryssa vitrirostris* | 7.80 | 61.90 |
| | | | *Macrobranchium rude* | 7.44 | 69.34 |
| | | | *Lutjanus argentimaculatus* | 7.00 | 76.34 |
| | | | *Valamugil seheli* | 6.75 | 83.09 |
| | | | *Arius africanus* | 6.44 | 89.53 |
| | | | *Penaeus indicus* | 5.51 | 95.05 |

A similarity percentage (SIMPER) analysis for the latitude-catchment size samples based on fish taxa showed that average similarity was 53.34%, 43.51%, 27.11%, 22.47% for Estuary Type 4, Estuary Type 6, Estuary Type 8 and Estuary Type 9, respectively. *Leiognathus equlus* contributed 55% of group similarity in Estuary type 4. In Estuary type 6, 28.11%, 9.84%, 8.32% and 7.83% of group similarity was contributed by *Penaeus monodon, Rastrelliger kenagurta, Penaeus semisulcatus* and *Johnieops sinain*, while in Estuary type 8, 29.74% and 21.87% was contributed by *Penaeus monodon* and *Encrasicholus heterolobus*, and in estuary type 9, 84.77% and 15.23% was contributed by *Sardinella gibbosa* and *Penaeus indicus* (Table 3).

## Discussion

Estuaries are coastal ecosystems which are among the most productive biomes globally, and support important and diverse life forms, including humans (Day *et al.*, 1989, Constanza *et al.*, 2014). Diverse provisioning and servicing by estuaries increasingly contributes to the disappearance and loss of some of the functional value and importance of these systems. Classification of estuaries is considered important for conservation and management purposes (Durr *et al.*, 2011; Ramos *et al.*, 2016; Mahoney and Bishop, 2018). Estuary classification may be useful in identifying groups of ecologically similar estuaries, for which common conservation strategies might be developed or adopted. Mahoney and Bishop (2018) summarised various schemes of estuary classification developed in different countries including Australia, Canada, Europe, New Zealand, United Kingdom, USA, South Korea and South Africa, where most schemes have used hydrological, geomorphological and physical-chemical classification variables (Mahoney and Bishop, 2018). Estuarine habitat mosaic and geomorphic classes can be influenced by the size of drainage basins, hydrology and climate through wave action and runoff. Climatic influence results in latitudinal zonation of estuaries following light, temperature and precipitation distribution patterns (Harris *et al.,* 2002).

The classification scheme developed in this study for Tanzania estuaries has used ecoregion, latitude and catchment size classification variables which pulls together the combined effect of climate, hydrology and drainage basin size. These classification variables were considered to have an influence on naturally partitioning of estuaries into estuary types. In this study, a classification option based on the combination of latitude and catchment size produced stronger differences between estuary types and stronger similarities among estuaries within the same estuary type than the ecoregion and catchment size classification. Latitudinal zonation is an important factor influencing the occurrence and distribution of living organisms along coasts (Engle and Summers, 1999). Fish occurrence and diversity in estuaries has specifically been described to be latitudinally influenced (Harrison and Whitfield, 2006). For example, in South Africa, estuarine fish diversity declines with decreasing latitude (from the east coast to the west coast) (Day *et al.,* 1981; Whitfield, 1992). In this study, fish composition separated Estuary type 6 and 9 of the latitude catchment size classification which occurs in the same Central East African ecoregion (Thieme *et al.,* 2005), but distinguished by latitude, Estuary type 9 (10°28'27.82"S) is further to the south than Estuary type 6 (7°49'28.77"S).

The size of the catchment draining into the estuaries contributes to the amount of freshwater discharge and sediment loads entering the estuary and has a significant impact on estuary productivity. Estuary type 8 and 9; and Estuary type 4 and 6 occur within similar latitudinal zones but showed significant differences between each other. This is attributed to the difference in catchment sizes forming the estuary types under comparison. This emphasizes the importance of the size of the draining catchment and its resulting abiotic characteristics. On the same note, the extent of human disturbance, ecosystem resilience and management options are influenced by catchment size. The upstream-downstream effect on estuaries is also influenced by the size of the catchment. Larger catchments are more susceptible to complex multi-sectoral impacts and conflicts than smaller catchments; however, they have higher potentials for economic importance and revenues than smaller catchments. Therefore, classifying similar types of estuaries allows for collective management of individual estuaries under a common entity (estuary type) (Mahoney and Bishop, 2018), thus facilitating extrapolation, adoption and comparison among estuaries of the same type.

Based on the validation and performance of classification options, it is suggested that the latitude-catchment size estuary types are used. Looking at the cluster analysis, the relationships between estuary types may be visualized. Estuary type 8 is more like Estuary type 9, while together they are similar to Estuary type 6, and the three groups are similar to Estuary type 4. This relationship may be related to their latitudinal locations.

## Conclusion

This study proposes a classification framework for Tanzania mainland estuaries using abiotic variables (ecoregion, latitude and catchment size). The framework gives options of using either an ecoregion-catchment size classification or latitude-catchment size classification.

The latitude-catchment size classification produced nine estuary types, while the ecoregion-catchment size classification produced seven estuary types along the Tanzania mainland coast.

The biotic validation of estuary types using biotic composition (fish and prawns) showed that latitude-catchment size classification was significantly stronger in partitioning estuary types than ecoregion-catchment size classification.

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# The status of Mtwapa Creek mangroves as perceived by the local communities

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## Abstract

Local coastal communities depend on mangrove ecosystems for valuable goods and services. As a result, mangroves have suffered degradation due to overexploitation to serve the ever-increasing demand for wood and wood products, as well as human activities along riparian areas which have equally had a significant impact on adjacent mangrove wetlands. Socioeconomic characteristics of five local communities living around Mtwapa Creek were examined to establish their perceptions on the status of the adjacent mangrove forest. The results show that although local communities distance themselves from responsibility on the status of the forest which they perceived as being poor, they appreciate mangroves as an integral component of their livelihood. Secondary data on mangrove harvesting within Kilifi County reflected a possible lack of alternative sources of energy as shown by the progressive increase in illegal fuelwood harvesting over the years from 1991. The local communities recognise the potential influence of both legal and illegal harvesting on the status of Mtwapa Creek mangroves, while only a small proportion perceive observed anthropogenic activities in riparian areas as a possible threat to mangroves. These findings have been obtained against a backdrop of mixed opinions amongst local coastal communities which is associated with gender, living standards, education level and knowledge about mangroves as a resource.

**Keywords:** mangrove status, local communities, perception, human activities

## Introduction

Mangroves provide products and services at both the local scale and beyond, but local communities may have the closest relationship to mangroves through their livelihoods and direct impacts. It therefore follows that the perception of both utilization and impact are intimately related. The basis of local livelihoods associated with mangroves may include timber and non-timber forest products (Dahdouh-Guebas *et al.*, 2000; Balmford *et al.*, 2002) as well as the associated ecosystem goods (Saenger, 2002; Crona and Rönnbäck, 2005; Lee *et al.*, 2014) which can be harvested by local communities within the mangroves or adjacent systems. Depending on the quality of the forest, mangroves may prevent coastal erosion and play a crucial role in mitigating disaster risk by acting as barriers that dissipate wave energy (Dahdouh-Guebas *et al.*, 2005; Latief and Sofwan, 2007; Lee *et al.*, 2014). The arguments by these authors align with those of Das and Vincent, (2009) who used data on several hundreds of villages to prove that mangroves would indeed protect lives in incidences of cyclones and tropical storm surges. Mangroves also help in sediment stabilization (Kimeli, 2013) and

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mitigation of climate change through their high carbon storage capacities (Donato *et al.*, 2011).

Owing to the multiple benefits that accrue from mangrove ecosystems, establishing a balance between the use and non-use values still remains a challenge (Millennium Ecosystem Assessment, 2005; Okello *et al.*, 2012). This is because the benefits attached are not always tuned to accrue at the same time scale and to the same people. In fact, while making important steps towards achieving the vision 2030, Kenya for instance is still encountering challenges in reversing environmental degradation (Government of Kenya, 2007). As a result, mangroves have faced continued cover loss in Kenya (Kirui *et al.*, 2012) as well as globally (Duke *et al.*, 2007; Spalding *et al.*, 2010). The progressive rise in population in coastal areas (McGranahan *et al.*, 2007; Samoilys *et al.*, 2015), and the consequent increasing demand for agricultural land, urban development as well as other forms of related anthropogenic disturbances have subjected mangroves to increased pressure and degradation (Bosire *et al.*, 2013). In fact, degradation due to development may require the longest time to restore mangrove functionality as opposed to other forms of degradation (Mukherjee *et al.,* 2014).

Several attempts have been made worldwide and in Kenya to restore degraded mangrove areas (Field, 1996; Kairo *et al.*, 2001; Okello *et al.*, 2012; Kodikara *et al.*, 2017) and to ensure effective management of these forests. It has however been noted that conservation and sustainable management is a superior strategy to restoration or reforestation (Vannucci, 2004). Since the declaration of mangroves as government reserve forests in 1932 (FAO, 2007), their management has been limited to the licensing of extraction of wood products, authorized by the Ministry of Environment and Natural Resources; where annual quotas for extraction are decided on an unspecified basis, and extraction operations are not always supervised (FAO, 2007). The Forest Conservation and Management Act, 2016 (No. 34 of 2016) however, provides for involvement of the private sector and local people in mangrove management through the formation of Community Forest Associations (CFAs) (Samoilys *et al.*, 2015), a system that is quickly picking up pace along the coast, and could offer a breakthrough (Frank, 2014). Further, the assumption that people always destroy mangroves has been put in question following self-initiated mangrove planting and management programmes by the local people (Walters *et al.*, 2008).

Socio-economic studies have been conducted among various communities living adjacent to mangrove forest patches in Kenya to analyze utilization pattern and establish possible cause-effect relationships between the people and these forests (Kairo, 1992; Dahdouh-Guebas *et al.*, 2000; Mohamed, 2009). However, since demographic characteristics of local human communities may vary significantly from one geographic locality to another (Government of Kenya, 2012), each mangrove area has to be treated as a separate entity for purposes of effective integration into national management plans. It is also important to incorporate local perceptions in order to ensure successful conservation ventures of natural resources (Nazarea *et al.*, 1998; Horowitz, 2001; Marcus, 2001; Frank, 2014). This study highlights the nature of activities of the local human community and the impacts they may exert on the bordering peri-urban mangrove ecosystems. Such understanding of how socioeconomic characteristic influence people's values of the environment can be an important tool in the development of an effective conservation strategy while solving the real causes of degradation of a resource (Cinner and Pollnac, 2004). The underlying hypothesis was that socioeconomic status (village, education, income, gender, house type) is associated with the nature of activities carried out by local communities and their perceptions on the status of adjacent mangrove ecosystems.

## Materials and Methods

### Study site

The study was conducted in five villages along Mtwapa Creek, Kenya (3° 57' 0 S, 39° 45' 0 E), bordering Mombasa and Kilifi counties to the south and north respectively (Fig. 1). The villages (Kashani, Kidutani, Mdengerekeni, Mtepeni and Mtomondoni) which border the creek on both shores were chosen purposively based on proximity to the mangrove forest (Fig. 1).

As per the Kenya electoral boundary commission, the surveyed villages fall under two sub-locations, Shimo la Tewa and Mtepeni (Government of Kenya, 2012), which have a population density of 21 and 65 persons per km$^2$, respectively (Government of Kenya, 2010). Data obtained from the village heads of Kashani, Kidutani, Mdengerekeni, Mtepeni and Mtomondoni villages indicate that they have 142, 258, 92, 284 and 308 households, respectively.

Current laws ban individuals from cutting mangroves, unlike previously when licenses were issued to

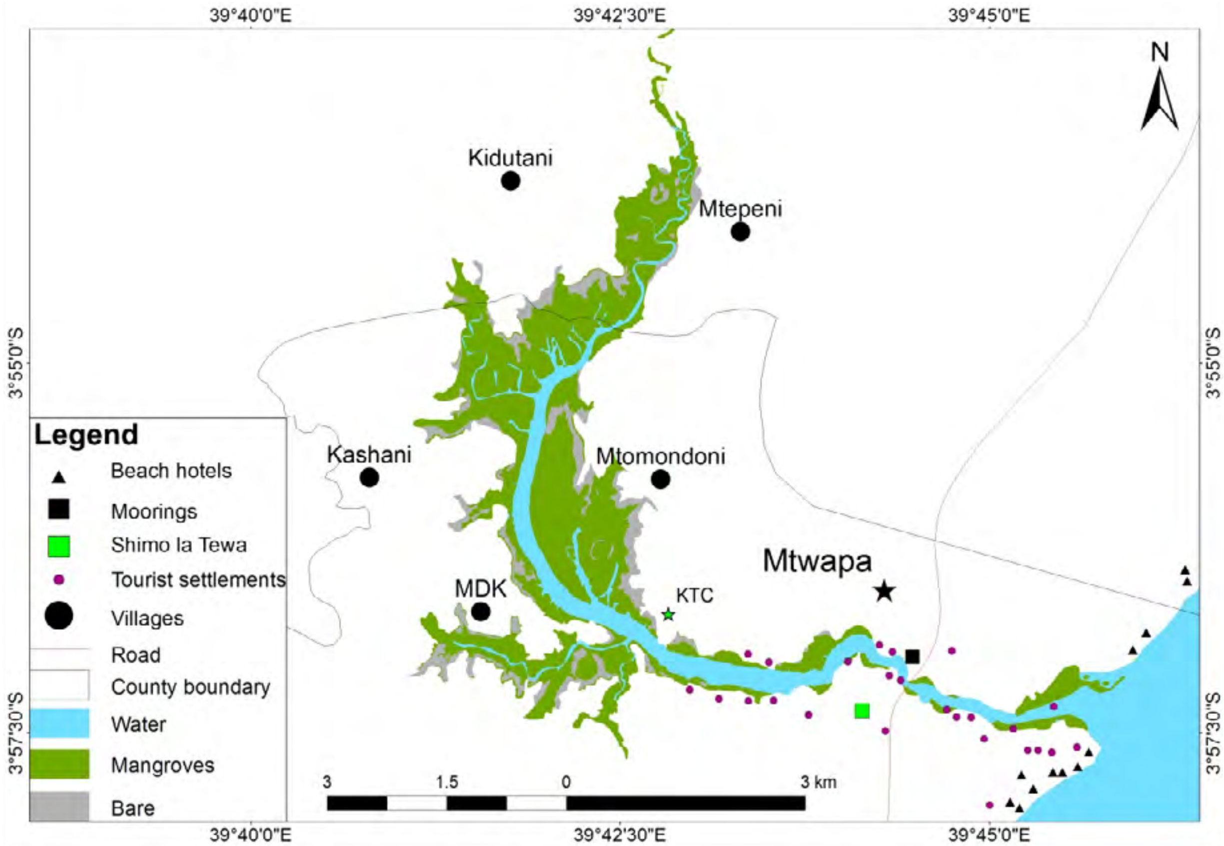


**Figure 1.** Map of Mtwapa Creek showing the location of the five villages, Mtomondoni, Mtepeni, Kashani and Mdengerekeni (MDK), within which the study was conducted. Inset is the map of the entire Kenyan coastline locating the creek. Kwetu Training Center (KTC) and several hotels and tourist settlements are also shown as well as the Shimo la Tewa prison. Source: CORDIO East Africa, Kenya

mangrove cutters, most of whom do not live around the study area. With the supervision of the Kenya Forest Service (KFS), such individuals were expected to cut within a given locality. In Mtwapa Creek, no such licenses have been issued since the placement of a presidential ban on local harvesting of mangroves in the year 2000 (Abuodha and Kairo, 2001), allowing the local community to only collect dead wood for use as firewood upon being issued with permits by KFS. Such permits costs about half a dollar per week and a bundle of firewood (*tita*) is sold for USD 1.19–1.78 (exchange rate USD 1 = KES 84, in 2011).

## Methods

Primary data was collected in April 2011 to gain insights on the socioeconomic characteristics of the local communities and their perceptions of the status of the mangrove forest of Mtwapa Creek. This was achieved through a combination of participant observation (captured in photographs and transect walks), semi-structured questionnaires, key informant interviews, and focus group discussions (Bunce *et al.*, 2000). Kiswahili was the general language used in communication and where necessary, the local language (of the indigenous inhabitants who were from the native Mijikenda community) was integrated into the conversations to enhance understanding. The questions were administered by Okello, Mwakha and the Kenya Marine and Fisheries Research Institute (KMFRI) socio-economic team (see acknowledgement). The team was assisted by one member of the local community identified by each village head and who could identify well with the people and speak the local language fluently. The following section details the data collection methods that were applied.

Participant observations – where the researchers got involved in the activities of study - these were useful to enhance understanding of activities and as a way to bond with the respondents, and obtain insight into what the activities meant to them. Transect walks and photographs were mainly employed to capture activities and features of specific interest, as well as to identify adversely impacted scenarios, both on land and in mangrove ecosystems.

Semi-structured interviews – were administered to systematically selected households from a full list of households in the five villages provided by the village heads. Selection of households was carried out by the team without influence from the village heads by picking every third home in a row. In cases where a selected household was absent during the survey, the next home in the row would be visited. Only one member per household (in most cases the household head) was interviewed with the exception of where another member contributed significantly to the family earnings. A total of 17, 31, 11, 34 and 37 persons, making up 12% of the total number households provided by the village heads, were interviewed in Kashani, Kidutani, Mdengerekeni, Mtepeni and Mtomondoni, respectively. These individuals were from 122 households in the villages (Table 1). Questionnaires containing both open- and closed- ended questions were applied. In this way, it was possible to probe answers, follow up on questions as they appeared in the questionnaires, and pursue new ideas. The questions explored their demographic and socio-economic characteristics; their perceptions on the state of mangroves and harvesting techniques of mangrove-related products for various uses; as well as land-based activities they were engaged in. Material style of life indicators which are regarded

**Table 1.** Description, mean and variation of the respondents living in the 5 villages surveyed around Mtwapa Creek. Only two of the respondents from Mdengerekeni were not natives of the area, while the rest were all Mijikenda.

| Indicator | Description | Villages | Range (mean ± standard deviation) |
|---|---|---|---|
| **Age** | Age of respondents | Kashani | 20–59(38.0±12.4) |
| | | Kidutani | 18–80(46.2±19.2) |
| | | Mdengerekeni | 23–66(45.5±13.4) |
| | | Mtepeni | 18–70(43.6±15.5) |
| | | Mtomondoni | 19–85(47.2±17.5) |
| | | Total | 18–85(44.7±16.6) |
| **Gender** | Percentage number of respondents of a given sex | Kashani | Male 76.5%; Female 23.5% |
| | | Kidutani | Male 61.3%; Female 38.7% |
| | | Mdengerekeni | Male 90.9%; Female 9.1% |
| | | Mtepeni | Male 41.2%; Female 58.8% |
| | | Mtomondoni | Male 48.6%; Female 51.4% |
| | | Total | Male41.2%–90.9%(63.7±20.4%) Female9%–59%(36.3±19.2%) |
| **Household size** | Number of individuals per household includingdependants both children (< 18 years old) & adults (>18 yearsold) | Kashani | 1–10(3±3) |
| | | Kidutani | 2–15(7±4) |
| | | Mdengerekeni | 2–11(6±3) |
| | | Mtepeni | 4–24(8±4) |
| | | Mtomondoni | 1–21(7±4) |
| | | | Total1–24(7±4) |
| **Income** | Percentage number of respondents with selected income ranges earned per week (1 US$ = KES 84) | US$0–5.95 | 10%–46%(31±13.2%) |
| | | US$5.96–11.90 | 11%–40%(26±11.1%) |
| | | US$11.92–17.86 | 9%–40%(18±12.4%) |
| | | US$17.87–35.71 | 8%–32%(19±10.7%) |
| | | US$>35.71 | 0%–16%(6±7%) |

as measures of the wealth of households were recorded in each case. These included mode of house construction, including house type (permanent, semi-permanent, temporary); roof type (coconut fronds-*Makuti*, other leaves, iron sheets or tiles); wall type (*Makuti*/ other leaves, poles and mud, stones/bricks, other); cooking fuel (fuelwood, charcoal, kerosene, other) and lighting fuel (kerosene, candle, electricity, other).

Key informant interviews – provided qualitative data that were used for triangulation of the results. The key informants were selected through prior communication with the village heads in order to gain confidence of the individual. This is because mangrove harvesting is considered a sensitive issue and local communities tend to shy away from discussing it. The village heads together with the key informant also helped identify participants for focus group discussions in each village. Willingness to be interviewed was the overriding factor for individuals to join the discussion group. Other factors such as gender balance and main economic activities of the respondents were used as secondary criteria. One focus group discussion was conducted in each of the five villages. Each focal group had 5-10 members with whom a series of open-ended questions were discussed.

Questions regarding knowledge were gauged as follows:

- Good working knowledge: Interviewee is able to explain what mangroves are, to identify at least three common species, and to identify at least three uses of mangroves
- Rough idea: Interviewee can associate mangroves with the intertidal area but does not know species. He/she knows the main use of mangroves in the area
- No idea: Interviewee does not know anything related to mangroves

Secondary data on mangrove utilization in Kilifi County was obtained from the draft national mangrove management plan (NMMP, under preparation). The data available was for between the year 1990 and 2012 and obtained from the NMMP working group. Additional information was provided by the Kenya Forest Service (KFS) and the municipal council of Mtwapa town.

## Data analysis

Data analysis was carried out using Ms Excel table sheets and SPSS 17.0 software. The analyses employed were mostly descriptive, which help to transform raw data into a form that summarizes a set of factors in a way that is easy to understand and interpret. Various quantitative variables in the study were also tested for relationships. The data sets by village did not meet the normality and homogeneity of variance requirement of parametric tests, even after being transformed. The Kruskal-Wallis test was therefore used, with no statistically significant differences in age of respondents between the villages visited being noted ($H(4) = 3.287$, $p = 0.511$). In subsequent statistical tests, the villages were therefore considered as one entity when dealing with age. Association among various variables was tested using the Pearson Chi-squared test. The 18 items used as material of life indicators were factor analyzed using principal component analysis techniques and varimax rotation resulting in two factors that explained the variance. The items that had the highest positive loadings have a stronger contribution on wealth than those with low or negative loadings (Cinner and Pollac, 2004).

## Results

### Socio-economic profile of the respondents around Mtwapa Creek

The overall male to female sex ratio of the respondents was 6:4. However, there were variations in other characteristics among the villages surveyed (Table 1). Only two respondents from Mdengerekeni were from the Kisii and Kikuyu tribes, while the rest of those interviewed belonged to the Mijikenda, which is the native tribal group in the area. The primary data collected showed an overall mean household size of $6.7 \pm 0.3$ members with Mtepeni village having significantly higher frequencies of large household sizes ($F=4.066$, $p<0.05$, $N=130$). Ninety-four percent of all the respondents were household heads while the rest were dependants who lived with their parents or guardians but contributed in one way or another to the household's income.

Education levels were quite low among the respondents. On average, most of the respondents had primary level education (48.8%), with the smallest proportion attaining secondary education (7.9%), while the remaining respondents had no formal education.

### Livelihoods of Mtwapa Creek local communities

Most respondents (31%) reported an average annual income of less than US$ 285.6 (Table 1). This value also included goods for direct consumption produced by each household.

Farming provided the major source of income in the

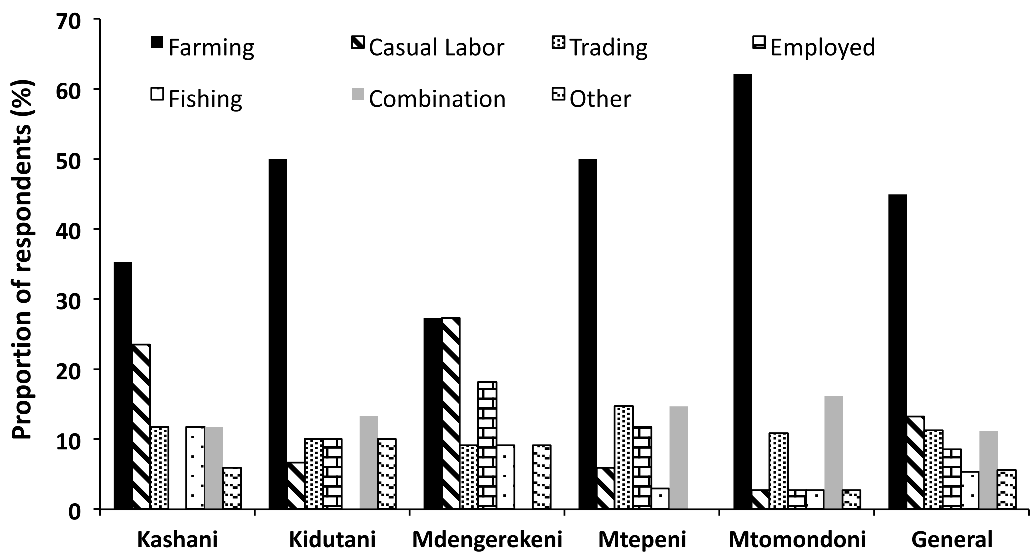


**Figure 2.** Economic activities of the people living in the five villages surveyed around Mtwapa Creek.

area contributing more than 60% of the total revenue. A total of 49.6% of the respondents practiced farming (Fig. 2) with 67% of these farmers engaged in farming as a full-time activity. Casual labour and trading in small-scale businesses involving fast moving household goods (mainly food stuffs) was generally considered the second and third most important source of livelihood respectively by local communities, but there were variations from village to village (Fig. 2). Those living in Kashani and Mdengerekeni had the least number of respondents depending on farming with relatively high proportions as casual labourers in building and construction industries and on farms. Mdengerekeni also had the highest relative proportion of those employed on either a permanent or contract basis working in the beach hotels, or as teachers in schools and in various industries in Mombasa city and Mtwapa town.

Other important income generating activities that the respondents engaged in included fishing and masonry. Although fishing was considered important, it only accounted for 3.9% of local community income sources as it was practiced by a small proportion of people living close to the creek, mainly in Kashani and the adjacent villages of Kidongo and Majaoni. The fishing was artisanal, undertaken for both subsistence and commercial needs. The fishermen used small traditional fishing boats and cast nets or employed hook and line techniques. Fish catch seldom reached the nearby Mtwapa town as it was often sold at the landing site directly to the local communities, or to local fish traders who supplied fish within the same villages.

From the focus group discussions, it emerged that both farming and fishing have encountered dwindling returns over the years. Fish catches were reported to have progressively declined, attributed to reduction in the depth of the creek. The reduction of depth was said to be as a result of sediment deposition in the creek waterways, although the local fishermen were not able to systematically ascertain the sediment source. Farming, on the other hand, had been affected by bad weather conditions and the escalating cost of farm inputs forcing men to seek employment as casual laborers in the fast-expanding town of Mtwapa and Mombasa city, while women engaged in small scale businesses such as the sale of food stuffs. The conspicuously low level of education (more than 40% having no formal education) greatly affected the level of engagement in formal employment, considering that more than 70% of the employed had some education. From the interviews, it was clear that the fluctuations in trends of engagement in various activities always followed opportunities and the need for better earnings.

### Material style of life indicators of the local communities adjacent to Mtwapa Creek

Most of the houses (68.8%) were temporary structures, with semi-permanent and permanent houses constituting only 25% and 6.2% of the total sample, respectively. A cross tabulation of mangrove usage against house type revealed a significant association between

**Table 2a.** Percent number of individuals associated with given material style of life items in the five villages surveyed in Mtwapa Creek. The percentages for each item are compared across the 5 villages.

| | Villages | | | | |
|---|---|---|---|---|---|
| **Items** | **Kashani** | **Kidutani** | **Mdengerekeni** | **Mtepeni** | **Mtomondoni** |
| Permanent house | 0 | 0 | 0 | 12 | 88 |
| **Semi-permanent house** | 16 | 22 | 6 | 31 | 25 |
| **Temporary house** | 12 | 27 | 10 | 26 | 25 |
| **Iron sheet roof** | 13 | 20 | 4 | 27 | 36 |
| **Makuti roof** | 13 | 26 | 9 | 26 | 26 |
| Other leaves as roof | 0 | 33 | 67 | 0 | 0 |
| Makuti wall | 0 | 0 | 100 | 0 | 0 |
| **Sticks-and-mud wall** | 13 | 28 | 8 | 27 | 24 |
| Stones/bricks wall | 17 | 0 | 0 | 18 | 65 |
| Other wall type | 0 | 0 | 0 | 100 | 0 |
| Charcoal cooking fuel | 0 | 0 | 0 | 0 | 100 |
| **Firewood cooking fuel** | 12 | 25 | 9 | 27 | 27 |
| Kerosene cooking fuel | 100 | 0 | 0 | 0 | 0 |
| Other cooking fuel types | 0 | 0 | 0 | 0 | 100 |
| Candle for lighting | 83 | 0 | 0 | 17 | 0 |
| Electricity lighting | 0 | 0 | 0 | 0 | 100 |
| **Kerosene lighting** | 9 | 25 | 10 | 29 | 27 |
| Other lighting sources | 20 | 0 | 0 | 0 | 80 |

Bold denotes common items across the villages; italicized are items present in/used by all households

the two ($\chi^2$ (3, N= 130) =8.74, p<0.05). Compared to the other villages in the study, Mtomondoni had the highest proportion of items perceived to be owned by the more privileged in the society, followed by Mtepeni (Table 2a). These included permanent houses, iron sheet roofing, stones/ brick walls and electrical lighting. Results from factor analysis of the 5 selected indicators showed that they all had high factor loading in the five villages, except for sticks-and-mud wall in Kidutani and Mdengerekeni (Table 2b). The extraction showed one component that explained more than 70% of the variance in each of the villages.

**Table 2b.** Principal component analysis of selected material style of life found in the villagessurveyed.

| | Villages | | | | |
|---|---|---|---|---|---|
| **Items** | **Kashani** | **Kidutani** | **Mdengerekeni** | **Mtepeni** | **Mtomondoni** |
| Semi-permanent house | 0.933 | 0.957 | 0.989 | -0.924 | -0.741 |
| Temporary house | 0.906 | -0.957 | -0.989 | 0.976 | 0.956 |
| Iron sheet roof | -0.971 | 0.953 | 0.989 | -0.97 | -0.954 |
| Makuti roof | 0.971 | -0.92 | -0.725 | 0.937 | 0.954 |
| Sticks-and-mud wall | 0.758 | 0.159 | 0.224 | 0.668 | 0.887 |
| % of variance explained | 83.027 | 72.198 | 70.16 | 81.398 | 81.422 |

Bold denotes high factor loading (> 0.4)

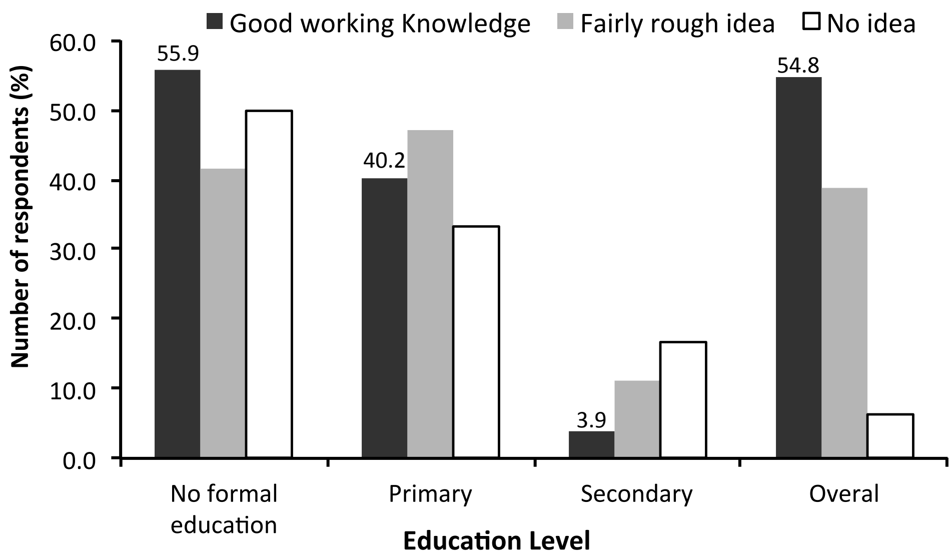


**Figure 3.** Knowledge of respondents on mangroves against their education level.

Households depended mainly on wood for cooking with more than 90% of the respondents using firewood in all the villages. Fuelwood collection was carried out by women, who did not wish to reveal the source of the wood. There were however no woodlots observed in the area during the survey. In addition, the results from the interviews revealed that villages which are much closer to the mangrove patches and where the terrain allowed ease of access (Kidutani, Mtomondoni and Mtepeni) had 100% dependency on firewood. These are villages within a range of 2km from the creek. Alternative sources of energy mentioned by the respondents were kerosene, palm fronds and gas.

## Knowledge of mangroves and the benefits

A large percentage of those interviewed had a good working knowledge (54.8%), while 35% had a rough idea and only 4.9% had no idea about the importance of mangroves. Most respondents, irrespective of the type of house they occupied, had a fairly rough idea or good working knowledge of mangrove importance. Similarly, no association was found between age category and mangrove knowledge level ($\chi^2$ (8, N=125) =3.6, $p>0.05$). However, there was a significant association between education level of respondents and knowledge on mangroves ($\chi^2$ (9, N=130) =48.96, $p<0.001$). Examination of frequencies showed that of the 54.8% of those interviewed with good working knowledge, 95% either had no education or only primary level (Fig. 3). Although the number of non-native individuals (not of coastal origin) encountered during the survey was too small to make a conclusive remark, they engaged in both trading and farming and had no idea about mangroves.

Among all the benefits of mangroves known by the local community, construction was the most frequently mentioned in all five villages (Fig. 4a). Specifically, male respondents considered construction as the most important mangrove use while fuelwood was favoured by women (Fig. 4b). Additionally, observations showed that more households in temporary houses made the most use of mangrove goods in each of the categories identified. Other benefits that were considered important were mangroves as fencing poles, charcoal, mariculture sites, traditional medicine (herbs) and ecotourism. Preference in usage of mangrove products did not only vary by gender but also by age, as was established from the key informant interviews. Children engaged in simple fishing activities where they caught crabs and small fish within the tidal inlets during low tide, while adult males mostly referred to mangrove forests as a source of building materials.

Eighty-six percent of the respondents admitted that mangroves were exploited in Kilifi County, but of these, only 41% said mangroves in Mtwapa Creek were harvested. Cutting of mangrove trees for construction of houses was mentioned in all the villages. Harvesting of standing mangrove trees for charcoal production in Mtepeni was also mentioned by respondents from Mtomondoni village. Most of the charcoal was not used locally but transported out of the area by road by both middlemen and producers for sale in the nearby Mtwapa town (2km) and Mombasa (15km).

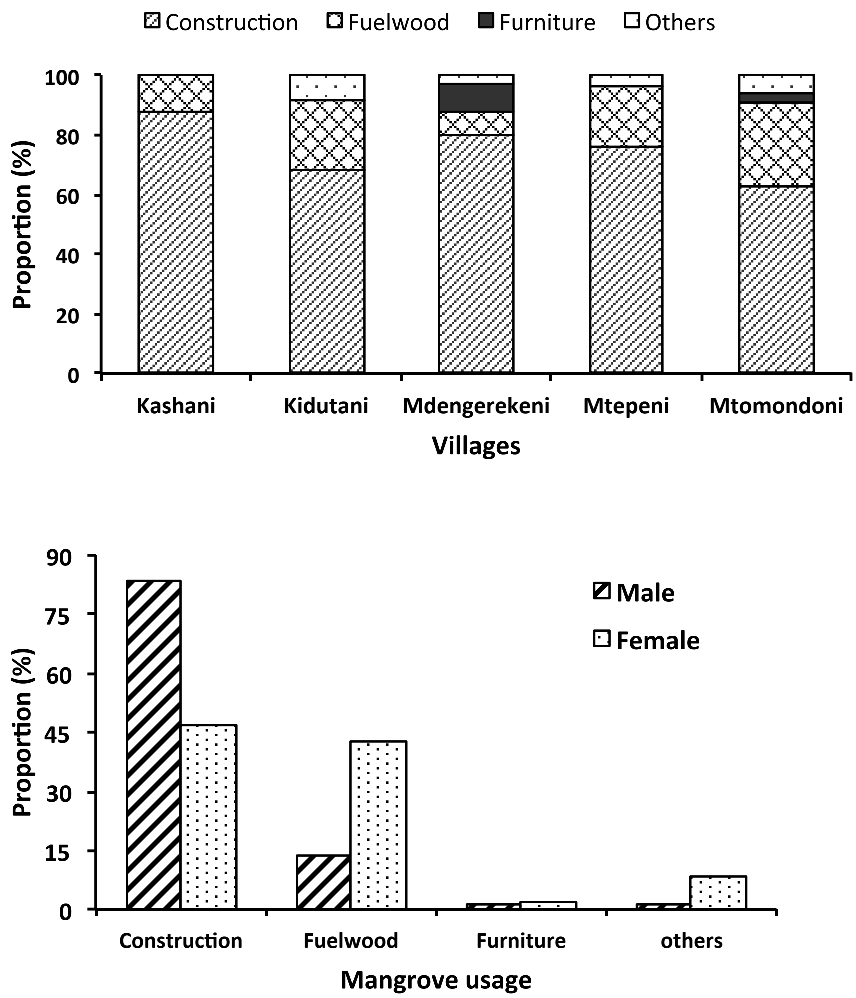


**Figure 4(a).** Mangrove usage patterns in the five villages surveyed around Mtwapa Creek.
**(b)** Cumulative preferred mangrove usage grouped by gender of the respondents.

Secondary data on mangrove harvesting revealed that illegal harvesting was a major threat for the mangroves in Kilifi County (Fig. 5). Losses due to illegal harvesting of construction poles progressively increased after the imposition of the total ban on harvesting of mangrove wood in the year 2000 (Fig. 5c), while illegal fuelwood extraction had been increasing since 1992 (Fig 5d). It should however be noted that the harvest data was for the entire Kilifi County and may only partly reflect the harvest in Mtwapa Creek. Local communities were well aware of the restrictions on access to the resource and as such, most of the harvesting in Mtwapa Creek occurred in the heart of the forest, limiting the sighting of trespassers by the forest guards. The trend was similar in the five villages with no significant association noted in the response obtained from either various age groups or education status. Local respondents blamed illegal harvesting on the high poverty levels in the area, laxity of KFS guards and corruption. Overall in Kilifi County, a complete ban was placed in the year 2000 to 2005, a period which saw a significant rise in estimated illegal extraction of mangrove poles from 348.5 to 650 scores annually, and firewood from 214.8 $m^3$ to 313.8 $m^3$ annually (Table S2 - data from KFS).

### Perception of local communities on current forest status as compared to the past

More than 50% of the respondents said that the forest was depleted of poles that could be used for construction (Table 3) and that the forest had degraded over the last 10 years. A total of 38% of the respondents, however, felt that the mangrove forest status was good or recovering while another 6% felt there had been no change and thus the mangroves were very healthy. There was a significant association between gender and response on forest status ($\chi^2$ (5, N= 100) =13.94, $p<0.05$), where most women either had no idea, or felt the forest was very healthy (Table 3).

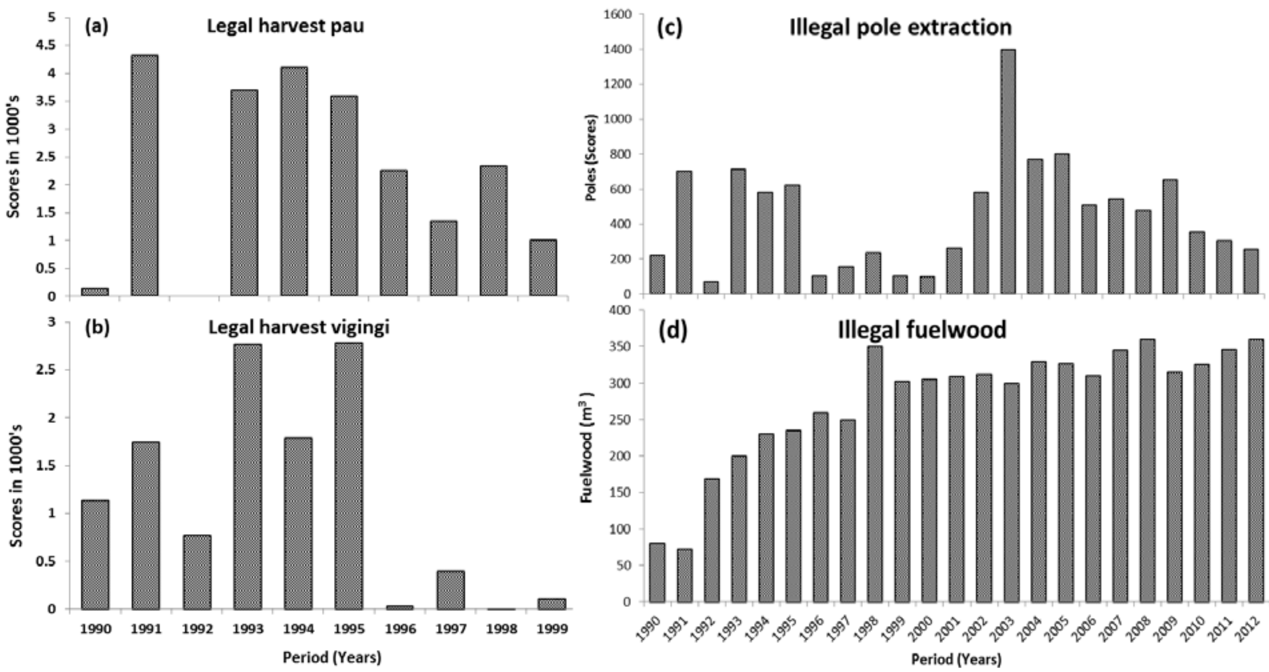


**Figure 5.** Legal (a, b) and illegal (c, d) mangrove wood extraction in Kilifi county. Only pau (butt diameter 4.0 – 7.4 cm) and vigingi (20 – 35 cm) were allowed to be harvested before the ban in the year 2000. Data obtained from KFS and the National Mangrove Management Plan (NMMP) working group.

Most respondents owning permanent houses felt the forest was in good condition while those with temporary houses mostly claimed that it was degraded, citing depletion of building poles (Table 3). This argument also emerged in all the focus group discussions as well as from four out of six of the key informant interviews. The focus group discussions also revealed an idea among the local communities that the *Rhizophora mucronata* (known locally as *mkoko*) in Mtwapa Creek are 'different' from those found in other areas of the coastline of Kenya, due to what they term as extremely thick bark and the crooked nature of the main stem. The Creek is occupied predominantly by *R. mucronata* with other species including *Xylocarpus granatum* (*mkomafi*) and *Avicennia marina* (*mchu*) being quite rare within the mangrove swamp.

## Perceived causes of mangrove degradation

Various causes of degradation of mangroves in Mtwapa Creek were identified, with cutting pressure being mentioned by most (78.9%) of the respondents. Out of this, 76.8% believed mangroves were getting destroyed solely because of cutting, while the rest attributed degradation to a combination of exploitation and other causes. Natural tree deaths and lack or excess of rain were the other factors mentioned by 10% of the respondents in each case. The reason for *A. marina* being rare in this mangrove swamp, was for

**Table 3.** Perception of the local community on the present status of Mtwapa Creek mangrove forest in comparison to the past 10 years- **by gender** (n = 101) **by house type (n = 101)**.

| Status | General (%) | By Gender (%) | | Perception by house type (%) | | |
|---|---|---|---|---|---|---|
| | | Male | Female | Temporary | Semi-permanent | Permanent |
| Degraded | 51.5 | 71.7 | 28.3 | 60.3 | 40.0 | 23.3 |
| Recovering | 13.4 | 46.2 | 53.8 | 8.8 | 24.0 | 11.7 |
| Good | 25.8 | 56.0 | 48.0 | 20.6 | 28.0 | 50.0 |
| No change/ Very healthy | 6.2 | 16.7 | 83.3 | 7.4 | 0 | 5.0 |
| No idea | 3.1 | 33.3 | 66.7 | 2.9 | 8.0 | 10.0 |

instance attributed to the death of the saplings of the species at an early stage, leaving the entire forest occupied predominantly by *R. mucronata*.

Only 1.4% of all the respondents mentioned the influence of land-based human activities, with farming, sewage and litter disposal being recognised in both Mtomondoni and Mdengerekeni during the focus group discussions. The terrain around the mangrove forest is generally characterized by steep slopes dotted with agricultural farms. The survey established that the local communities' farm close to the creek where they say the soil is more fertile. An analysis of the proximity of the farms showed that of the interviewed farmers, 50-70 % of the farmers had their farms within 10-100 m distance from the highest spring water mark.

## Discussion

### Socio-economic profile of the respondents around Mtwapa Creek

Most of the respondents in the current study were middle aged men. The variation in gender of the respondents was largely attributed to the cultural order in existence that conferred household headship (our target respondents) on the husband and not the wife. However, in certain instances the wife assumed headship in her partner's absence due to death, divorce or occupational engagements. As a result, possible gender bias effects on successive results cannot be ignored. The fact that all respondents except two belonged to the native tribal group of the area may also have a bearing on the results. This is because natives are considered to be more informed on mangrove resources because of their wide range of local traditional knowledge and experiences that are linked with their historical dependency and continuity in coastal and marine resource use and associated customary management practices (Drew, 2005). However, since the proportion of immigrants was so low, this cannot be proved from this study.

The mean household size values obtained in this survey were comparable to the county projections of 6.17 as per the 1999 population census, which is regarded as large (Kilifi District Planning Team, 2000). Considering the large household size and associated high number of dependants (Table 1), there is a greater financial burden being imposed on those who are working to support the other members (Cinner and Pollnac, 2004). Further, the income levels reported in the area were far less than those reported 10 years earlier for the villages bordering Mida Creek in a similar ecological setting further north along the Kenyan coast (Zorini *et al.*, 2004). This suggests a significant reliance of the local human community on natural resources for livelihoods due to their high poverty status (Cinner and Pollnac, 2004; Cinner *et al.*, 2009). This is further highlighted by the fact that of the 18 indicators of wealth used, house type showed the most obvious association with the responses obtained, with most houses being temporary structures, and their occupants making the most use mangrove goods.

Although there were multiple sources of livelihood identified in the Mtwapa Creek area, farming was marked as the major source of income, a case also seen among local communities around Mida Creek (Gang and Agatsiva, 1992). Like in other parts of the Kenyan coast, many households had diversified their sources of income (Cinner *et al.*, 2010), for instance farming households were also engaged in small-scale businesses. Such diversification of livelihoods is viewed as a way of increasing income to households (Cinner *et al.*, 2010). This is particularly important considering that Kenyan coastal areas have a greater percentage (62%) of the population living below the poverty line, with less than USD 1.25 per day (UNICEF, 2014), a situation replicated in Mtwapa Creek villages. It would thus be an important area of focus considering that Sustainable Development Goals (SDGs) 1 and 15 lay emphasis on poverty alleviation and environmental conservation.

High poverty levels could also be as a result of lack of diversification of earnings by the local communities which is tied to the low levels of education observed in the study, which in turn compromises engagement of individuals in formal jobs (Little *et al.*, 2009). Most respondents possess primary level as the highest level of education attained as is also the case for the Kenyan coastal region in general (Samoilys *et al.*, 2015). In fact primary school enrolment in the region increased from 63% to 84% upon the introduction of the free education system between 1999-2011 (UNICEF, 2015). Secondary education is however still wanting, as of the 60% enrolment, only 41% attend and the transition rate from primary school in 2006 was only 50% (Ngware *et al.*, 2006).

### Utilization of mangrove goods and services in Mtwapa Creek

The levels of knowledge of the local community about mangroves reported in this study is in agreement with findings by Naylor and Drew (1998), who noted that local communities living adjacent to a mangrove ecosystem have adequate working knowledge of mangroves attributed to their frequent interaction with the vegetation, almost on a daily basis for their subsistence needs. In Kenya, mangrove trees have numerous traditional uses for both subsistence and commercial users, which varies with species type (Dahdouh-Guebas *et al.*, 2000). The major uses highlighted in Mtwapa Creek (construction and fuelwood) show similarity in value attached to mangrove goods and services with other communities along the Kenyan coast (e.g. in South Coast of Kenya, Rönnbäck *et al.*, 2007; in Mida Creek, Dahdouh-Guebas *et al.*, 2000).

Lack of woodlots in the area together with secondary data obtained from the Kenya Forest Service (KFS) showing a progressive rise in illegal harvesting of mangrove wood for fuel over the years (Fig. 5d), may be an indicator of dependence on the adjacent mangrove forest for provision of cooking fuel. Generally in Kenya, fuelwood (charcoal and firewood) provides the main source of energy, contributing 70% of energy requirements nationally, and 90% of rural households use fuelwood (Githiomi and Oduor, 2012).

Local communities may however, rank these uses differently depending on site. Consequently, identification of mangrove goods and services, knowledge about mangroves and attitudes towards their conservation can vary significantly amongst user groups based on their gender, occupation and location (Rönnbäck *et al.*, 2007). Other than the role of mangrove in fisheries which was mentioned by a few respondents, under category 'others' (Fig. 5), none of the ecological roles considered as very important globally in an expert survey (Mukherjee *et al.*, 2014) were mentioned, suggesting a greater focus on the extractible benefits by the human community at local level. This also undermines the economic reasons for conserving nature as expressed by Balmford *et al.* (2002). Ecotourism was only mentioned by respondents who belonged to the conservation groups described in Okello *et al.* (2012), who are engaged in planting mangroves within the Creek.

### Perception of local communities on forest status

A number of factors have been mentioned that influence how people perceive resources, including migration, education and wealth (Cinner and Pollnac, 2004). In this study, gender and wealth status greatly influenced the locals' opinion of forest status. The respondents viewed degradation based on two criteria; cover loss, and pole size and quality. The largest proportion of respondents stated that changes in the mangroves was apparent by a decline in the desired sizes or overall tree density, similar to reports by Dahdouh-Guebas *et al.* (2000). Further, the fact that most of the respondents owning temporary houses claimed that the forest was depleted of poles suitable for construction could be attributed to their heavy dependence on the forest for building poles, compared to those who had permanent houses.

Local communities rate natural mangroves higher than plantations due to the multiple goods and services they provide, except for mangrove poles which are considered less durable than those from other natural forests (Rönnbäck *et al.*, 2007). This was however not the case in Mtwapa Creek where the dominant mangrove tree species (*Rhizophora mucronata* (mkoko)) is regarded by the locals as providing poles unsuitable for construction. The results from this study corroborate the findings from a structural survey conducted in 2010 by Okello *et al.* (2013). Additionally, the local respondents' argument regarding the scarcity of *X. granatum* (mkomafi) and *A. marina* (mchu), which is common in other areas along the Kenyan coast, is also in agreement with Okello *et al.* (2013).

Perceived causes and effects of mangrove degradation
The study identified various causes of mangrove degradation, with cutting pressure being singled out as the most important. Unsustainable exploitation and illegal extraction of mangrove trees, particularly for timber, building poles and firewood, has been cited as the major cause of historical decline in mangrove forests along the Kenyan coast (Dahdouh-Guebas *et al.*, 2000; Kairo *et al.*, 2001; Rönnbäck *et al.*, 2007; Mohamed *et al.*, 2009). This has seen a decline in mangrove forest cover, with the highest rate of loss being observed in the peri-urban areas (Mohamed *et al.*, 2009; Bosire *et al.*, 2013). However, cover change analysis between the year 2000 and 2010 suggested a 12% increase in mangrove cover (Okello, 2016), highlighting the idea of cryptic degradation, as also suggested by the local communities, which appears to be the major form of degradation in Mtwapa Creek. The fact that only *pau* and *vigingi* (Fig. 5a and b) were allowed to be harvested before the ban may have equally compromised the structural stability of the forest over time.

Apart from exploitation-related causes which are widely mentioned in the literature, the respondents attributed mangrove degradation to natural tree deaths, among other indirect causes. Such a combination of threats could lead to degradation of mangrove ecosystems and consequent loss of the ecosystem services they provide (Dahdouh-Guebas *et al*., 2005; Bosire *et al*., 2013). The local communities believe that the forest status may get worse or better depending on the line of action taken in terms of provision of alternatives such as conservation, including favorable policies and improved participatory forest management. Attempts by local respondents living around Mtwapa Creek to counteract illegal harvesting have been quite remarkable through the formation of environmental conservation groups (Okello *et al*., 2012). Some of the interviewees who were members of these groups, however, cite lack of support from the KFS and uncooperative non-members as factors thwarting their conservation efforts. While they live close to the mangrove area and carry out alternative livelihood activities within the forest, they do not have the power to arrest illegal harvesters who they frequently encounter. Under the new Forest Act, participatory forest management is upheld through formation of Community Forest Associations (CFAs) and has showed major successes in the involvement of local communities in conservation of mangroves in Mida Creek further north on the coast (Frank, 2014). This is however still at an infancy stage, with CFAs having only been formed in a few areas along the coast (Government of Kenya, 2017).

Land use practices, including poor farming practices in the riparian and catchment areas, damming of rivers, clearing of vegetated areas for development, and poor location of properties tend to increase instability of physical coastal formations, and hence increase soil erosion and consequent degradation of mangroves (UNEP, 2001).

## Conclusion

This study shows that the local communities perceive the status of mangroves differently depending on their gender and living standards as portrayed by house type. This implies that perspectives of all stakeholders, regardless of their gender, should be integrated in the implementation of management plans. Such perspectives demonstrate the importance of local knowledge in an area where poverty levels are high and degradation of mangrove ecosystems is ongoing due to stressors such as harvesting pressure, and support the implementation of a co-management approach to mangrove conservation.

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# A review of nudibranch (Mollusca: Euthyneura) diversity from the Republic of Mauritius: Status and Future Work

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## Abstract

Nudibranchs are considered one of the most diverse groups of opisthobranchs. Their history in Mauritius dates from 1832, with first records appeared in expedition reports and systematic works. Recent review of their biodiversity in Mauritius identified 23 species. The present study provides a list of nudibranch species using data from both systematic works and internet records as a means of maintaining an inventory from Mauritius. Some 105 species belonging to 20 families (excluding undescribed taxa and those recorded as cf.) have been identified in Mauritius. Most species have been collected in the north-west part of the island which is dominated by hotels and not subjected to easterlies which could be one reason explaining their abundance. Providing a list of nudibranchs species is important, to be able to design better ways of conserving them in the future, if the need arises. With a wide maritime zone and considered as a striking biodiversity hotspot, further species might be discovered from both Mauritius and Rodrigues.

**Keywords:** biodiversity, inventory, nudibranchs, opisthobranchs, Republic of Mauritius

## Introduction

Mauritius and Rodrigues islands form the Republic of Mauritius in the South Western Indian Ocean. Mauritius lies 20°S and 57°E and Rodrigues is located 19°S and 63°E, 574 km east of Mauritius (Thébaud *et al.,* 2009). The islands are both of volcanic origin, arising from an oceanic hotspot and known to be topographically distinct units (Louchart *et al.*, 2018; McDougall & Chamalaun, 1969). The study of McDougall & Chamalaun (1969) demonstrates Mauritius island as the oldest of the Mascarenes (7.8 million years old) and Rodrigues as the youngest and most isolated (1.8 million years old). Having always shared a close association with several of its islets but secluded from large land masses, the Republic of Mauritius is known to have a reservoir of intact communities. The Mascarenes have thus, been listed among the world's top biodiversity hotspot (Thébaud *et al.*, 2009). A total of 284 marine molluscs species including 175 marine gastropods and 109 bivalve species have been reported in Rodrigues by the Ministry of Agro Industry and Food Security (2015). The Republic of Mauritius has an overall of 13 marine protected areas (MPA) with Mauritius holding eight and Rodrigues owning five, of which the South East Marine Protected Area (SEMPA) is gazetted as the biggest MPA of the Republic of Mauritius (Pasnin *et al.*, 2016). Rodrigues Island is also known to have the best developed reef in the Mascarenes (Naim *et al.,* 2000; McDougall *et al.*, 1965), providing home for innumerable species, hence, a unique biodiversity of both marine fauna and flora (Beedessee *et al.*, 2015). The fifth national report under the convention on biological diversity for the Republic of Mauritius provided no information concerning the distribution and diversity of nudibranchs from these two islands (Ministry of Agro Industry and Food Security, 2015). However, report concerning the status of the marine reserves of Rodrigues indicated the presence of nudibranchs (Desiré *et al.*, 2011). Unfortunately, no elaboration pertaining to the different species contained in each of the four marine protected areas was

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given. Nudibranchs, poetically known as *butterflies of the sea*, constitute a diverse group of marine gastropod, representing roughly over 4700 known species (Dean & Prinsep, 2017; Anderson, 1995). Nudibranch (Mollusca: Euthyneura) is classified under the subclass Heterobranchia proposed by Haszprunar (1985) (Bouchet *et al.*, 2017). Formerly, they were known to belong to the infraclass Opisthobranchia. However, recent research by Wägele *et al*. (2014) denoted the peculiar infraclass as paraphyletic or even polyphyletic. Hence, Opisthobranchia was rejected as part of traditional taxa by Wägele *et al.* (2014) and considered as outdated by Schrödl *et al.* (2011) and Yonow (2015). Instead, Euthyneura has been recognised as the new infraclass with Nudipleura as its first offshoot (Bouchet *et al.*, 2017; Schrödl *et al.*, 2011). Nudibranchs have lost their shells through evolution which made them rely mostly on chemical defence to protect themselves from predators (Yonow, 2015). However, they are also known to sequester important metabolites from their prey and produce *de novo* defences (Dean & Prinsep, 2017). The sea slugs can be found in a wide range of habitat ranging from polar regions to the tropics and have been continuously assessed for their chemistry over the years (Dean & Prinsep, 2017; Chavanich *et al.*, 2013). In addition of being highly attractive, nudibranchs are also of high economic value, providing new leads to drug discovery (Dean & Prinsep, 2017; Jensen, 2013).

The current paper aims at giving an overview of nudibranch species collected in both Mauritius and Rodrigues (data obtained from both systematics works and internet records). Cataloguing a list of species is also an element of biodiversity. Biodiversity itself describes the number and variety of living organism and can be defined in terms of species, genes and ecosystems (Vitorino & Bessa, 2018; Magurran, 2004). The first component describes the methodology employed to construct the list of nudibranch species from the Republic of Mauritius. The second part confers to the results. The result section outlines the physical geography of the Republic of Mauritius, reports the history of nudibranchs in Mauritius and reviews the occurrence of nudibranchs. Finally, conclusion and further works are reported in the third constituent.

## Materials and methods

The list of species compiled is restricted to sea slug of the order Nudibranchia only. Data were screened from both regional checklist, systematic works as well as online data sources including photo-sharing website such as South-west Indian Ocean Seaslug site (http://seaslugs.free.fr/nudibranche/a_intro.htm). Systematic works includes, Tibiriçá *et al.* (2018), Tibiriçá *et al.* (2017), Yonow (2012), Yonow & Hayward (1991), Bergh (1888). Species were compiled with peculiar interest towards the site collection. Scientific names were confirmed using the World Register of Marine Species (WoRMS). Only taxa which could be identified following WoRMS were included in the species list. Undescribed taxa and those recorded as cf. on website or systematics were not included in the list.

## Results

In total, systematics works have identified 60 species. Together with internet records, the number of nudibranchs species found in the Republic of Mauritius would amount to 105 belonging to 20 families (Table 1).

### Physical geography of the Republic of Mauritius

Along with Mauritius and Rodrigues, the Republic of Mauritius also consists of many outer islands including St Brandon, Agalega, Tromelin and Chagos Archipelago including Diego Garcia. Mauritius is surrounded by a total of 49 offshore islets while 18 islets lie in the lagoon of Rodrigues. Mauritius Island has an Exclusive Economic Zone (EEZ) of over 2.3 million km$^2$, of which 99% is still unexplored (Ministry of Agro Industry and Food Security, 2015; Kauppaymuthoo, 2010). Further, Mauritius is made up of ten districts out of which seven are known as coastal, two as inland with Rodrigues making up the tenth districts. Mauritius is surrounded by 150 km of protective corals which are unfortunately being degraded. Around 50 to 60% of the coral cover which make up the reef of the Mauritian lagoon has already been lost. Such a loss in coral reefs habitats indicate serious threat to the biological diversity of the Republic of Mauritius (Ministry of Agro Industry and Food Security, 2015; Kauppaymuthoo, 2010). Mauritius covers a surface area of 1865 km$^2$, bordered by coral reefs of both fringing and barrier type which are interrupted by major river mouths, enclosing a lagoon area of 300 km$^2$ of varying widths (0 to 8 km) (Naim *et al.*, 2000; Fagoonee, 1990). Rodrigues is the smallest island with an area of 104 km$^2$, is 18.3 km long by 6.5 km wide with the entire coast bordered by fringing reef (90 km), covering an area of 200 km$^2$. The presence of patch reefs, atolls and reef flats are significant around Rodrigues (Ministry of Agro Industry and Food Security, 2015). Figure 1 shows the location of the Republic of Mauritius in the South Western Indian Ocean.

### History of nudibranchs in Mauritius

The history of nudibranchs in Mauritius dates back from 1832. The first records of opisthobranchs in Mauritius arose from expedition reports and systematic works (Yonow & Hayward, 1991; Claude, 1985; Bergh, 1888; Quoy & Gaimard, 1832). In 1888, Bergh first collected and described species from Mauritius Island belonging to both lineages; Cladobranchia and Anthobranchia (Bergh, 1888). Bergh introduced the genus *Baeolidia* in 1888, based on the description of a single specimen, *Baeolidia moebii* which eventually contained contradictory information and thus, led to morphological confusion (Carmona *et al.,* 2014a). *Limenandra fusiformis* (Baba, 1949) reported from Mauritius, has initially been reported as *Baeolidia* species. However, recent study validated the genus *Limenandra* and *Limenandra fusiformis* was attributed to this genus only (Carmona *et al.*, 2014b). Among the cladobranchs described included *Anteaeolidiella indica* (Bergh, 1888) which is based on the drawing and notes of Moebius. No additional materials were obtained to outline the morphological characteristic of the species (Carmona *et al.*, 2014c). Apart from expedition reports and systematic works, accounts of nudibranchs have been given in publications by Michel Claude. In 1985, 50 species of nudibranchs were recorded in Mauritius belonging to 9 families by Michel Claude (Claude, 1985). Yonow and Hayward reviewed the biodiversity of opisthobranchs in 1991. In October and November 1985 also in February and March 1990, Yonow and Hayward described thirty-five opisthobranchs species from the coral reefs habitats in Mauritius. Of the thirty-five species, twenty-three belonged to the order Nudibranchia (Yonow & Hayward, 1991). Recent review of the opisthobranchs from the western Indian Ocean localities which include Mauritius, described the occurrence of seventy opisthobranchs species in details (Yonow, 2012). Over the years, species described from Mauritius relied mostly on morphology and anatomical studies. Morphological description has frequently been challenged by modern identification techniques such as molecular data, in addition to the parallel evolution of numerous organ systems (Wägele *et al.*, 2014). Taxonomy is a dynamic field, experiencing regular revision in both nomenclature and classification. As a result, many of the names proposed by previous works appear as synonyms. New species are still being recorded in the South Western Indian Ocean (Tibiriçá *et al.*, 2019, 2018, 2017; Yonow, 2012). Inventories carried out by the Ministry of Agro Industry and

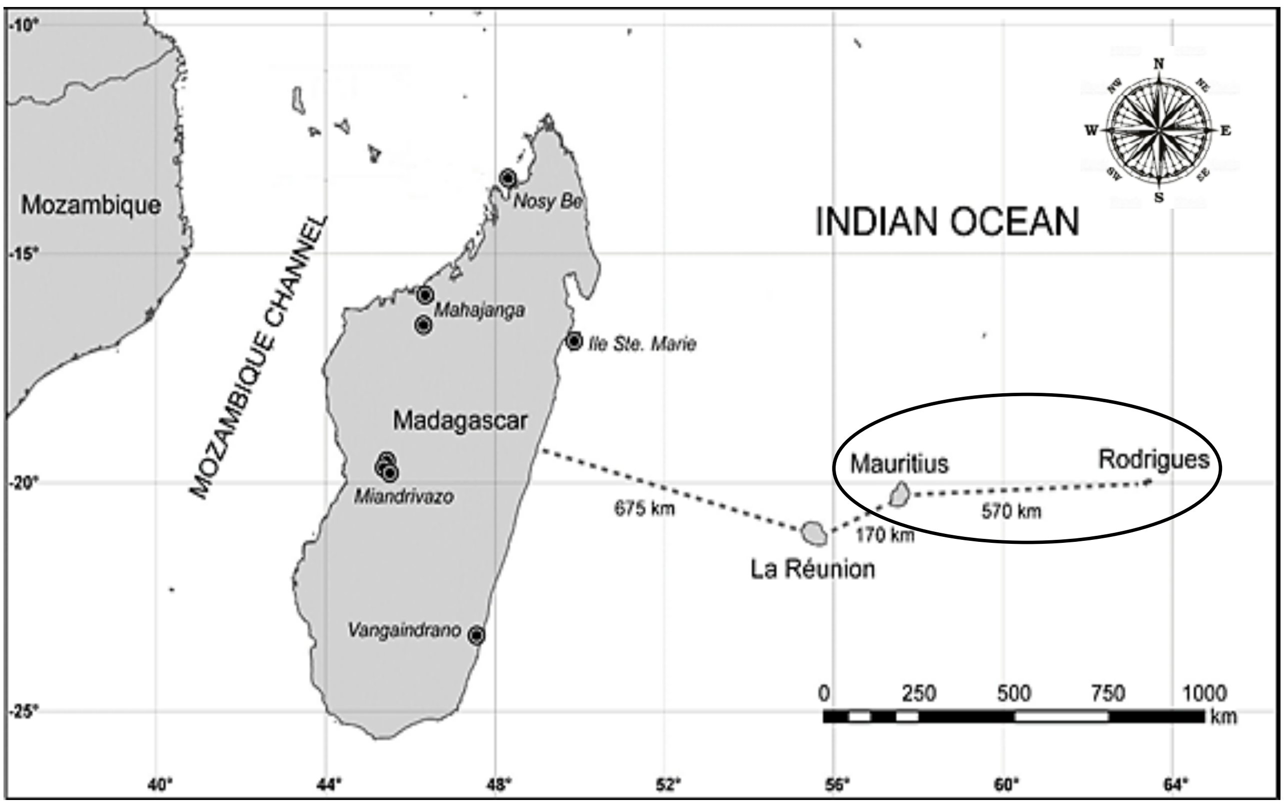


**Figure 1:** Map showing the Republic of Mauritius (black circle) in the South Western Indian Ocean, redrawn and adapted from (Chan *et al.*, 2011)

Food Security in 2015 reported incomplete information pertaining to the malacofauna status in Mauritius (Ministry of Agro Industry and Food Security, 2015). Nudibranchs are slow moving organisms, casting spectacular coloration making them highly photogenic among underwater photographers and scuba divers. Concerned about the environment, most divers collect only pictures of sea slugs however, it becomes difficult to identify species from pictures such that sometimes, it is suspected that the latter is an undescribed species (Jensen, 2013). Other than articles and books, databases such as the Sea Slug Forum and South-west Indian Ocean Seaslug site also provide considerable information on the proper morphological identification of nudibranchs, species distributions as well as a complete set of species list. The website South-west Indian Ocean Seaslug site provides a specific list of nudibranchs species recorded in Mauritius, Reunion Island, Mayotte, Madagascar and Seychelles. In addition to a specific list, the website also furnishes information about the specific location the picture was taken, date, name of the diver, maximum size of the organism, abundance, taxonomy information and pictures of the organism. To date, internet record contains more nudibranchs species than systematic works in Mauritius. The website South-west Indian Ocean Seaslug contains 410 nudibranchs species belonging to 35 families out of which 100 species belonged to Mauritius and 239 species belonged to Reunion Island. The website also contains undescribed species of the superfamily Doridoidea, one of which was observed on Mauritius Island (Summers, 2014), unassigned Cladobranchia, two species belonging to the family Janolidae (Pola *et al.*, 2019) were also found on Mauritius Island; *Janolus* sp. 1 (Arnim, 2010a) and *Janolus* sp. 2 (Arnim, 2010b). Additionally, the website hold species with uncertain identification (species with abbreviation cf.); three belonging to the Chromodorididae, two from Polyceridae, one from Tritoniidae, Fionidae and Facelinidae, a total of eight species. Nudibranch is known to exhibit notable polymorphism in their colour pattern which can mask diversity (Matsuda & Gosliner, 2018). The genus *Glossodoris* is recognised to contain multiple cryptic and pseudocryptic species. Among the species of uncertain identification on the South-west Indian Ocean Seaslug website include *Glossodoris* cf. *cincta* found in Mauritius which is highly similar to Bergh's description of *Glossodoris cincta* (type locality: Mauritius). As a result, to be able to resolve species complexity, further studies which include collection of *Glossodoris cincta* in Mauritius is required (Matsuda & Gosliner, 2018). Other databases include the Mauritius Oceanography Institute (MOI) which consists of four types of online databases. The first type provides both taxonomic and geographic information of marine organism of Mauritius. The second type is the genetic databank which furnishes morphometric as well as genetic data. Both of these databases provide limited information pertaining to the nudibranchs species in Mauritius. Other types include oceanographic data mapping and characterisation of aquaculture site in the Republic of Mauritius. The project started by the MOI in 2010 which consisted of assessing the marine living resources in the Mauritian waters using both traditional taxonomic and molecular identification techniques furnishes complete set of information only for fish and sea cucumbers (Mauritius Oceanography Institue, 2017). Even though limited ecological and biodiversity information relating to nudibranchs species in Mauritius were available, pharmaceutical research involving the latter had already begun. In 2015, while attempting to discover novel metabolites from Mauritian marine organisms, Beedessee *et al.* noticed the outstanding proportion of dorid nudibranchs among other mollusc species. The authors studied the cytotoxicity activities of 20 different nudibranchs collected around the island at both different location and depth. Promising cytotoxic activities were obtained for *Notodoris citrina* (Bergh, 1875) (Aegiridae) when tested on both epidermoid carcinoma and acute promyelocytic leukemia cells (100 ± 1% at 10 µg/ml) (Beedessee et al., 2015).

## Nudibranchs occurrence in the Republic of Mauritius

Based on their general morphology and digestive glands, nudibranchs can be classified into two distinct groups; the dorids and aeolids (Dean & Prinsep, 2017). Additionally, depending on their prey association, nudibranchs can be further divided into; sponge grazers, bryozoan grazers, hydroid grazers and a miscellaneous category. Nudibranchs belonging to either groups are best suited to their prey. Aeolids are less bulky and more buoyant to prey upon delicate and erect hydroids. In contrast, dorids are bulky, flattened and consist of an invariably broad radula with multiple rows of simple hook-shaped teeth to be able to graze encrusting sponges (Todd, 1983). It is usually believed that nudibranchs are a group of highly specialised predators (Megina *et al.*, 2002) feeding on few related prey species. Penney (2013) showed that diets for some species are broader than expected. The coastal habitats from east to west and from north to south of Mauritius are quite diverse (Fagoonee, 1990). Mauritius is known to contain 163 species of corals, of which 132 species are also found in Rodrigues (Moothien-Pillay *et al.*, 2002). The study conducted by Fenner *et al* in 2004 identified 130 named species of hard corals in Rodrigues, out of which eight were unidentified species. According to Fenner *et al.* (2004), thirty-seven species are new records for the southern Mascarene archipelago. Nudibranchs are also associated with corals for instance, the aeolid nudibranch *Phestilla lugubris* (Bergh, 1870) which is found in Mauritius (Summers, 2015). The latter is known to feed on the coral *Porites* (Rudman, 1999). However, the coral reef habitats around the Republic of Mauritius are being degraded (Ministry of Agro Industry and Food Security, 2015). Oceanographic survey report has been carried out both in Mauritius and Rodrigues which revealed that 40.26% of corals within peculiar marine park are heavily damaged (Kauppaymuthoo, 2010). On the contrary, marine protected areas (MPA) in Rodrigues are being strictly monitored. Of the four marine reserves in Rodrigues, nudibranchs have been spotted in three of them; Riviere Banane, Grand Bassin and Passe Demi marine reserves. However, the report pertaining to the status of marine reserves in Rodrigues provide no elaboration of the different species of nudibranchs spotted in the reserves (Desiré *et al.*, 2011). Opisthobranchs documented by Yonow & Hayward (1991) were taken from four coastal districts; Pamplemousses, Riviere du Rempart, Flacq and Black River. Out of the 23 nudibranchs species described by Yonow & Hayward (1991), most species came from the Chromodorididae (21.7%) and Phyllidiidae (30.4%) families. The Phyllidiidae are known to display themselves during daylight (Su *et al.*, 2009). In their study, Yonow and Hayward provided no mention of the time of collection (Yonow & Hayward, 1991). Nudibranchs are known to be nocturnal, cryptic (Su *et al.*, 2009), consist of flexible colour pattern and bathymetric range limits (Layton *et al.,* 2018). Recent study showed that external morphology can be unreliable in taxonomic identification of nudibranch, as a result of mimicry between species (Layton *et al.*, 2018). Hence, more nudibranchs species are yet to get discovered or identified as colour variant of the same species. On the other hand, internet record revealed nudibranch species from five districts particularly Pamplemousses, Riviere du Rempart, Flacq, Grand Port and Black River. Out of the 117 proclaimed beaches in Mauritius, only 22 have been investigated in the past years including both systematics (Yonow & Hayward, 1991) and internet record (South-west Indian Ocean Seaslug site). Nudibranchs from four islets have also been recorded; Ile aux Cerfs, Ile aux Benitiers, Ile aux Aigrettes and Ile Sancho. Most species (including both systematics and internet record) have been collected in the north-west part of the island (Trou aux Biches and Pereybere containing 20 species while Grand Baie contained 15 species) where waves are known to be less strong (Fagoonee, 1990) followed by Pointe d'Esny which is found in the southeast part of the island (19 species). The north part of the island is dominated by several hotels, surprisingly it contained the most species. Likely, the northern sides of the island are not subjected to easterlies (south east trade winds) which could be among the many reasons why most nudibranch species were found there (Fagoonee, 1990). A list of species found in the Republic of Mauritius is provided in Table 1 below.

**Table 1.** Species recorded from both Mauritius (MAU) and Rodrigues (*), a compilation of data obtained from website South-west Indian Ocean (SWIO) Seaslug site and systematics work with solid circle indicating proper classification of species and non-solid circle showing improper classification or species is still recognised by its synonymised name on website/systematics.

| Family | Species | Systematics | | | | | Website | Distribution |
|---|---|---|---|---|---|---|---|---|
| | | Bergh (1888) | Yonow & Hayward (1991) | Yonow (2012) | Tibiriçá *et al.* (2017) | Tibiriçá *et al.* (2018) | SWIO Seaslug Site | Mauritius (MAU) |
| Cadlinidae | *Aldisa fragaria* (Tibiriçá, Pola & Cervera, 2017) | | | | | | ● | MAU |
| Chromodorididae | *Ardeadoris angustolutea* (Rudman, 1990) | | | | ● | | ● | MAU |
| | *Cadlinella ornatissima* (Risbec, 1928) | | | | | | ● | MAU |
| | *Chromodoris aspersa* (Gould, 1852) | | ● | | | | ● | MAU |
| | *Chromodoris porcata* (Bergh, 1889) | ● | | | | | | MAU |
| | *Doriprismatica atromarginata* (Cuvier, 1804) | ○ | ○ | | | | ● | MAU |

| Family | Species | Systematics | | | | | Website | Distribution |
|---|---|---|---|---|---|---|---|---|
| | | Bergh (1888) | Yonow & Hayward (1991) | Yonow (2012) | Tibiriçá *et al.* (2017) | Tibiriçá *et al.* (2018) | SWIO Seaslug Site | Mauritius (MAU) |
| **Chromodorididae** (continuation) | *Goniobranchus albopunctatus* (Garrett, 1879) | | | | | | ● | MAU |
| | *Goniobranchus conchyliatus* (Yonow, 1984) | | | | | | ● | MAU |
| | *Goniobranchus fidelis* (Kelaart, 1858) | | | | | | ● | MAU |
| | *Goniobranchus geminus* (Rudman, 1987) | | ○ | | | | ● | MAU |
| | *Goniobranchus lekker* (Gosliner, 1994) | | | | | | ● | MAU |
| | *Goniobranchus tennentanus* (Kelaart, 1859) | | | | | | ● | MAU |
| | *Goniobranchus tinctorius* (Rüppell & Leuckart, 1830) | | | | | | ● | MAU |
| | *Glossodoris cincta* (Bergh, 1888) | ● | | | | | | MAU |
| | *Glossodoris hikuerensis* (Pruvot-Fol, 1954) | | | | | | ● | MAU |
| | *Glossodoris pallida* (Rüppell & Leuckart, 1830) | | | | | | ● | MAU |
| | *Hypselodoris bullockii* (Collingwood, 1881) | | | ○ | | | ● | MAU |
| | *Hypselodoris carnea* (Bergh, 1889) | | | | ● | | | MAU |
| | *Hypselodoris whitei* (A. Adams & Reeve, 1850) | | | | | | ○ | MAU |
| | *Hypselodoris maculosa* (Pease, 1871) | | | ● | ● | | ● | MAU |
| | *Hypselodoris maridadilus* (Rudman, 1977) | | ● | | | | ● | MAU |
| | *Hypselodoris nigrolineata* (Eliot, 1904) | | | | | | ● | MAU |
| | *Hypselodoris nigrostriata* (Eliot, 1904) | | | | | | ● | MAU |
| | *Hypselodoris pulchella* (Rüppell & Leuckart, 1830) | | | ○ | | | ● | MAU |
| | *Mexichromis katalexis* (Yonow, 2001) | | | | | | ● | MAU |
| | *Mexichromis lemniscata* (Quoy & Gaimard, 1832) | ○ | ○ | | | | ● | MAU |
| | *Verconia varians* (Pease, 1871) | | | | | | ● | MAU |
| **Discodorididae** | *Asteronotus cespitosus* (Van Hasselt, 1824) | ● | | | ● | | ● | MAU |
| | *Carminodoris grandiflora* (Pease, 1860) | ○ | | | | | | MAU |
| | *Carminodoris mauritiana* (Bergh, 1891) | ● | | | | | | MAU |
| | *Discodoris cebuensis* (Bergh, 1877) | | | | | | ● | MAU |
| | *Halgerda formosa* (Bergh, 1880) | ● | ● | ● | | ● | ● | MAU |
| | *Jorunna funebris* (Kelaart, 1859) | | | | ● | | ● | MAU |

| Family | Species | Systematics | | | | | Website | Distribution |
|---|---|---|---|---|---|---|---|---|
| | | Bergh (1888) | Yonow & Hayward (1991) | Yonow (2012) | Tibiriçá *et al.* (2017) | Tibiriçá *et al.* (2018) | SWIO Seaslug Site | Mauritius (MAU) |
| Discodorididae (continuation) | *Jorunna rubescens* (Bergh, 1876) | ○ | | | ● | | ● | MAU |
| | *Peltodoris murrea* (Abraham, 1877) | ○ | ○ | | | | ● | MAU |
| | *Platydoris scabra* (Cuvier, 1804) | ● | | | | | ● | MAU |
| | *Discodoris coerulescens* (Bergh, 1888) | ● | | | | | | MAU |
| | *Discodoris concinniformis* (Bergh, 1888) | ○ | | | | | | MAU |
| | *Sebadoris fragilis* (Alder & Hancock, 1864) | ○ | ○ | | | | ● | MAU |
| | *Sebadoris nubilosa* (Pease, 1871) | | | | | | ● | MAU |
| Dorididae | *Doriopsis granulosa* (Pease, 1860) | | | | | | ○ | MAU |
| | *Doris verrucosa* (Linnaeus, 1758) | | | | | | | MAU |
| | *Doris venosa* (Quoy & Gaimard, 1832) | ○ | | | | | | MAU |
| Dotidae | *Doto indica* (Bergh, 1888) | ● | | | | | | MAU |
| Goniodorididae | *Trapania naeva* (Gosliner & Fahey, 2008) | | | | ● | | ● | MAU, * |
| Dendrodorididae | *Dendrodoris carbunculosa* (Kelaart, 1858) | ● | | | | | ● | MAU |
| | *Dendrodoris denisoni* (Angas, 1864) | | | | | | ● | MAU |
| | *Dendrodoris fumata* (Rüppell & Leuckart, 1830) | ○ | ○ | ● | ● | | | MAU |
| | *Dendrodoris krusensternii* (Gray, 1850) | ○ | | | | | | MAU |
| | *Dendrodoris limbata* (Cuvier, 1804) | ● | | | | | | MAU |
| | *Dendrodoris nigra* (Stimpson, 1855) | ● | ● | ● | | | ● | MAU |
| | *Dendrodoris pustulosa* (Alder & Hancock, 1864) | ● | | | | | | MAU |
| | *Dendrodoris tuberculosa* (Quoy & Gaimard, 1832) | ● | | | | | | MAU |
| Phyllidiidae | *Phyllidia alyta* (Yonow, 1996) | | | ● | ● | | ● | MAU |
| | *Phyllidia coelestis* (Bergh, 1905) | | | | | | ● | MAU |
| | *Phyllidia ocellata* (Cuvier, 1804) | ○ | | | ● | | ● | MAU |
| | *Phyllidia marindica* (Yonow & Hayward, 1991) | | ○ | ● | ● | | ● | MAU |
| | *Phyllidia multituberculata* (C. R. Boettger, 1918) | | ● | ● | | | | MAU |
| | *Phyllidia varicosa* (Lamarck, 1801) | ○ | ○ | ● | | | ● | MAU,* |
| | *Phyllidia rueppelii* (Bergh, 1869) | ○ | | | | | | MAU |
| | *Phyllidiella meandrina* (Pruvot-Fol, 1957) | | ○ | ● | ● | | ● | MAU |

| Family | Species | Systematics | | | | | Website | Distribution |
|---|---|---|---|---|---|---|---|---|
| | | Bergh (1888) | Yonow & Hayward (1991) | Yonow (2012) | Tibiriçá *et al.* (2017) | Tibiriçá *et al.* (2018) | SWIO Seaslug Site | Mauritius (MAU) |
| **Phyllidiidae** (continuation) | *Phyllidiella pustulosa* (Cuvier, 1804) | ○ | ○ | ● | | | ● | MAU |
| | *Phyllidiella rosans* (Bergh, 1873) | | | ● | ● | | ● | MAU |
| | *Phyllidiella striata* (Bergh, 1889) | ○ | ○ | ● | | | | MAU |
| | *Phyllidiella zeylanica* (Kelaart, 1859) | | ○ | | ● | | | MAU |
| | *Phyllidiopsis cardinalis* (Bergh, 1876)) | | | | | | ● | MAU |
| | *Phyllidiopsis gemmata* (Pruvot-Fol, 1957) | | | | ● | | ● | MAU |
| | *Phyllidiopsis loricata* (Bergh, 1873) | | | | | | ● | MAU |
| | *Phyllidiopsis xishaensis* (Lin, 1983) | | | ● | | | ● | MAU |
| **Aegiridae** | *Notodoris citrina* (Bergh, 1875) | | | | | | ● | MAU |
| | *Notodoris minor* (Eliot, 1904) | | | ● | | | ● | MAU, * |
| | *Notodoris gardineri* (Eliot, 1906) | | ● | | | | | MAU |
| **Polyceridae** | *Gymnodoris striata* (Eliot, 1908) | | | | | | ● | MAU |
| | *Gymnodoris citrina* (Bergh, 1877) | | | | | | ● | MAU |
| | *Gymnodoris ceylonica* (Kelaart, 1858) | | | | | | ● | MAU |
| | *Gymnodoris crocea* (Bergh, 1889) | ● | | | | | | MAU |
| | *Martadoris limaciformis* (Eliot, 1908) | | | | | | ○ | MAU |
| | *Nembrotha cristata* (Bergh, 1877) | | | | | | ● | MAU |
| | *Nembrotha kubaryana* (Bergh, 1877) | | ● | ● | ● | | ● | MAU |
| | *Nembrotha lineolata* (Bergh, 1905) | | | | | | ● | MAU |
| | *Plocamopherus margaretae* (Vallès & Gosliner, 2006) | | | | ● | | ● | MAU |
| | *Roboastra gracilis* (Bergh, 1877) | | | | | | ● | MAU |
| | *Tambja affinis* (Eliot, 1904) | | | | | | ● | MAU |
| | *Tambja morosa* (Bergh, 1877) | | ○ | ● | ● | | ● | MAU |
| | *Tyrannodoris luteolineata* (Baba, 1936) | | | | | | ○ | MAU |
| **Hexabranchidae** | *Hexabranchus sanguineus* (Rüppell & Leuckart, 1830) | ○ | ● | ● | | | ● | MAU, * |
| **Aeolidiidae** | *Anteaeolidiella indica* (Bergh, 1888) | ○ | | | | | | |
| | *Baeolidia moebii* (Bergh, 1888) | ● | | | ● | | | MAU, * |
| | *Cerberilla affinis* (Bergh, 1888) | | | | | | ● | MAU |
| | *Limenandra fusiformis* (Baba, 1949) | | | | | | ● | MAU |

| Family | Species | Systematics | | | | | Website | Distribution |
|---|---|---|---|---|---|---|---|---|
| | | Bergh (1888) | Yonow & Hayward (1991) | Yonow (2012) | Tibiriçá *et al.* (2017) | Tibiriçá *et al.* (2018) | SWIO Seaslug Site | Mauritius (MAU) |
| Facelinidae | *Facalana pallida* (Bergh, 1888) | ○ | | | | | | MAU |
| | *Facelina rhodopos* (Yonow, 2000) | | | | | | ● | * |
| | *Favorinus mirabilis* (Baba, 1955) | | | | | | ● | MAU |
| | *Herviella mietta* (Er. Marcus & J. B. Burch, 1965) | | | | | | ● | MAU |
| | *Pteraeolidia semperi* (Bergh, 1870) | | | | | | ● | * |
| Glaucidae | *Glaucus atlanticus* (Forster, 1777) | ● | | | | | ● | MAU |
| Trinchesiidae | *Phestilla lugubris* (Bergh, 1870) | | | | | | ● | MAU |
| | *Phestilla melanobranchia* (Bergh, 1874) | | | | | | ○ | MAU |
| Samlidae | *Samla bicolor* (Kelaart, 1858) | | | | | | ● | MAU |
| Bornellidae | *Bornella anguilla* (S. Johnson, 1984) | | ● | | ● | | ● | MAU |
| Tethydidae | *Melibe engeli* (Risbec, 1937) | | | | | | ● | MAU |
| | *Melibe viridis* (Kelaart, 1858) | ○ | | | | | ● | MAU |
| Tritoniidae | *Tritoniopsis elegans* (Audouin, 1826) | | | | | | ● | MAU |
| | *Marionia levis* (Eliot, 1904) | | | | ● | | | MAU |
| Arminidae | *Dermatobranchus rubidus* (Gould, 1852) | | | | | | ● | MAU |

## Conclusion and Future Works

This review summarises the existing nudibranchs species from the Republic of Mauritius (Table 1). Previous researches concerned the description of existing species however, no information of the time of collection was provided. In contrast to species belonging to the Phyllidiidae, many nudibranchs are nocturnal hence, further inventories need to be carried out to assess their biodiversity and distribution. To date, 60 species have been identified by systematic work. Together with internet records, the number of nudibranchs species found in the Republic of Mauritius would amount to 105 belonging to 20 families (excluding undescribed taxa and those recorded as cf., table 1). Further studies pertaining to resolve the issue of species complexity and clarifying morphological characteristic of *Anteaeolidiella indica* are required. Additionally, further works concerning the abundance of nudibranchs found in the Republic of Mauritius should be carried out. Marine protected areas are designed for biodiversity conservation and detailed study on its biodiversity is essential. Rodrigues Island is strictly reinforcing the management of its marine reserves, comparison of the different species found in both marine reserves and non-marine reserves will bring out surplus information relating to the diversity of nudibranchs. With a wide maritime zone and considered as a striking biodiversity hotspot, further species might be discovered from both Mauritius and Rodrigues altogether with key molecules of medical importance.

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# A first account of the elasmobranch fishery of Balochistan, south-west Pakistan

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## Abstract
Pakistan was listed as eighth globally in its landings of sharks and other elasmobranchs during the 1990s. Balochistan occupies over three-quarters of the coast of Pakistan yet the nature of the elasmobranch fishery there remains undocumented. Landings of elasmobranchs at landing sites were surveyed; the main species recorded were blacktip shark (*Carcharhinus limbatus*), bull shark (*C. leucas*) and spot-tail shark (*C. sorrah*). Altogether 25 shark species were identified, of which nine are regionally vulnerable, eight endangered, and one (the sand tiger shark, *Carcharias taurus*) critically endangered. Of the thirteen other elasmobranchs recorded, five are regionally vulnerable, two are endangered and one (the sawfish, *Pristis pristis*) critically endangered. Local fishers and processors were interviewed about their industry. Sharks were caught using both long-lines and nets, largely in May – July. The fishers retained some meat (for consumption) or liver (for the oil used for waterproofing boats), but did not process the sharks themselves, instead selling them to agents of companies that exported fins and other elasmobranch products. Results showed that recorded landings in both Balochistan and the neighbouring Sindh Province have declined to a tenth or less of peak catch. Meanwhile, the numbers of registered fishermen continued to increase, a persistent threat to elasmobranchs stocks. It is recommended that a realistic national plan of action and widespread public awareness programme, with support to fishers and processors would help to alleviate this critical situation.

**Keywords:** economic value, elasmobranch overfishing, fishers, fisheries, population decline, processors

## Introduction
The Indian Ocean and western Pacific contain the greatest diversity of living elasmobranchs (Fowler *et al.*, 2005). These regions have also experienced widespread collapse in elasmobranch abundance (Dulvy *et al.*, 2017), principally due to intensive fishing (Jabado *et al.*, 2018) stimulated during recent decades by the far-eastern demand for shark fin (Davidson *et al.*, 2015). Countries in the Western Indian Ocean and Arabian Gulf regions that developed significant shark fishing industries during that period include Iran (Gerami and Dastbaz, 2013; Nergi, 2014; Jabado and Spaet, 2017), Oman, Kuwait, Qatar and United Arab Emirates (Henderson *et al.* 2007, 2008; Moore *et al.*, 2012), Yemen (Shaher, 2007; Jabado and Spaet, 2017) and India (Akhilesh *et al.*, 2011; Varghese *et al.*, 2017). Pakistan, along with neighbouring India and Iran, was among the top 20 countries for shark landings during the periods 2000 to 2008 (Lack and Sant, 2009) and 2009 to 2013 (Dulvy *et al.*, 2017). However, until now very little has been documented of the nature of this fishery over the greater part of the Pakistan coast, which falls within the province of Balochistan (Fig. 1).

Estimated elasmobranch landings for the whole country have been reported annually by Pakistan to

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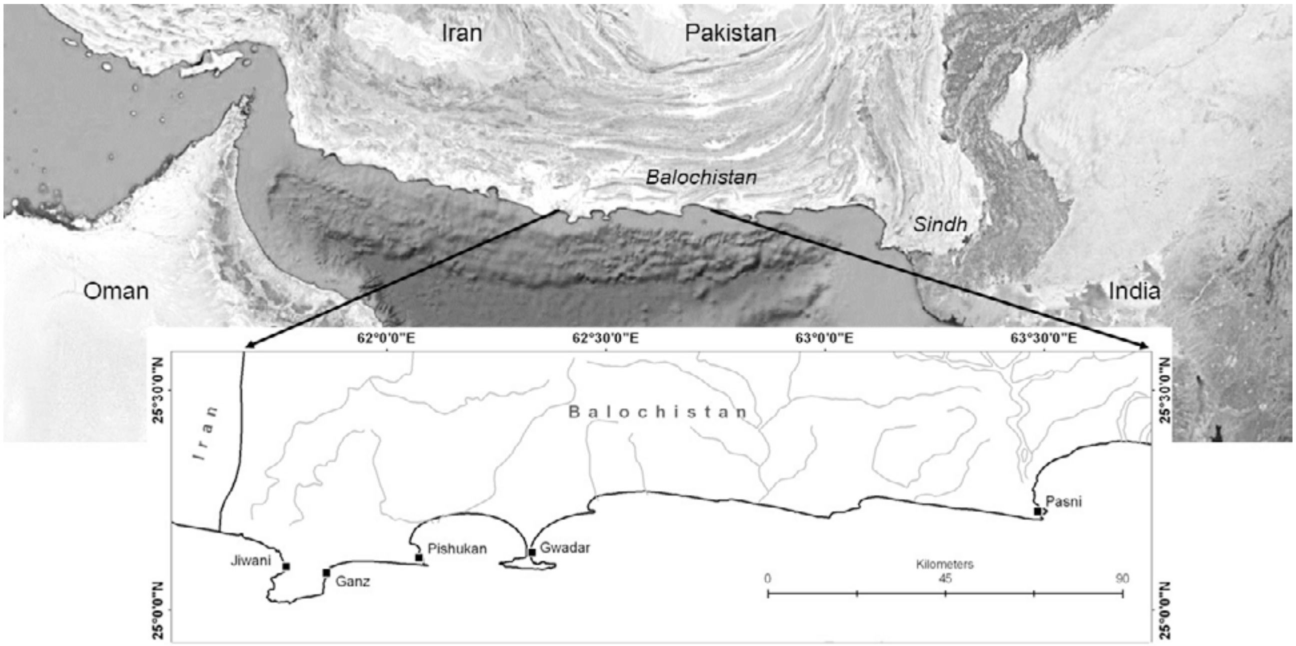


**Figure 1.** Upper map shows the Northern Indian Ocean with the two coastal provinces of Pakistan, Balochistan and Sindh, adjacent to Iran and India (Google Earth). Lower map gives details of main fishing towns along the Balochistan coast (WWF-Pakistan).

the United Nations Food and Agricultural Organisation (FAO). Between 1985 and 2000, gross landings increased by about 35%, but subsequently appeared to decline (Davidson *et al.*, 2015) and considerably more rapidly after 2007 (Fig. 13 in IOTC-2018-CoC15-RE). However, fisheries officers visiting landing places have normally only been able to make gross estimates of the combined weight of different classes, with neither the species fished nor the trade categories recorded by Pakistan's Marine Fisheries Department (2002, 2006, 2012). Until now, there has been minimal information on the species being caught in this region for commercial markets (see Clarke *et al.*, 2006; Fischer *et al.*, 2012). Without such basic data, stock assessments cannot be initiated, despite the impact of unsustainable fishing on elasmobranchs being of international concern (Stevens *et al.*, 2000). An opportunity arose during cetacean surveys that the authors undertook in Balochistan to detail the landings of elasmobranchs at a series of coastal ports and landing sites, and also to interview local fishers and fish processors about the details of the fishery.

## Materials and Methods

The Balochistan coast extends for 800 km between the border of Sindh Province and India in the east, and the border of Balochistan Province with Iran in the west. While the coast of Sindh is dominated by mangrove stands and mud flats around the Indus Delta, the coast of Balochistan consists mostly of alternating sandy and rocky shores, with sections of high cliffs. Below the shore, the seabed slopes to a shallow continental shelf, which is only 3km wide near Gwadar in the west but 73km wide near the Hub River in the east. Beyond the shelf, the seabed falls steeply to the Oman Abyssal Plain (Gore *et al.*, 2012) (Fig. 1).

Data on elasmobranch exploitation were collected from: a) landing sites; b) fishers; c) fish processors and their agents; d) fish export companies; and e) government sources. Between 16 April 2007 and 14 May 2010, all coastal settlements were visited as frequently as practicable and notes made of the species landed. On a total of 68 occasions, quantitative data were collected at 12 landing sites: 1) Afzal Bakar Naseer (both near Ganz), Ganz; 2) Adam Bakar, Bangali Para, Hussain Abdul, Kanpa, KD Bakar, Kinara and Murad Bakar (all in or near Jiwani); and 3) Pasni. For analysis, the sites were grouped into three sub-areas; Ganz, Jiwani, and Pasni, and analysed statistically using non-parametric statistics. On these occasions, most specimens were identified to species, their total length (nose tip to tail end) measured, and where possible the individual weight recorded. The prices (in local currency – Pakistani Rupees) being paid to fishers by processing company agents for the different species were also noted. On other occasions, either the visits to landing sites were brief, or the fishers or agents were not willing to allow time for quantitative data to be collected. On these occasions, attention was focused on noting any previously unrecorded species of shark that might be present and also on building a list of the species of other elasmobranchs that were also sometimes landed. A proportion of sharks could not, however, be identified with confidence; these have been recorded using the local terms pishik (small demersal sharks), pagas (medium bodied, coastal sharks), and warook (pelagic and large-bodied sharks).The length at maturity of species was referenced using Ebert *et al*. (2013).

Shark fishers, and processing plant managers and their agents, were interviewed using a standard list of questions covering their background, fishing method, catch statistics, prices paid to fishers, processing procedures, and prices paid to processors by exporting businesses. In addition, a workshop on shark fishing and conservation was held at WWF Jiwani, SW Balochistan, in November 2009. This was attended by 24 participants, including fishers, boat owners, processors' agents, fish processing company owners and exporters; the additional information gained was incorporated into the analyses. Government statistics on Pakistan's fishing industry were obtained from the Marine Fisheries Department in Karachi.

**Table 1.** Species of sharks and number recorded in 68 landings, separated into three sub-areas of Balochistan, between 16 April 2007 and 14 May 2010. Pishik is a local term for small bodied sharks including small demersal species, Pagas is the the term for medium sized coastal shark species, and Warook the term for large pelagic shark species.

| Scientific name | English name | Ganz | Jiwani | Pasni | Total |
|---|---|---|---|---|---|
| *Chiloscyllium griseum* | Grey bamboo | | 1 | | 1 |
| *Loxodon macrorhinus* | Sliteye | | 9 | | 9 |
| *Rhizoprionodon acutus* | Milk | 7 | | | 7 |
| *Rhizoprionodon oligolinx* | Grey sharpnose | | 1 | | 1 |
| *Scoliodon laticaudus* | Spadenose | | 1 | | 1 |
| Other Pishik | | | 43 | | 43 |
| *Carcharhinus limbatus* | Blacktip | 5 | 196 | | 201 |
| *Carcharhinus melanopterus* | Reef blacktip | | 1 | | 1 |
| *Carcharhinus sorrah* | Spot-tail | | 26 | | 26 |
| *Negaprion acutidens* | Sharptooth lemon | | 9 | | 9 |
| Other Pagas | | 1 | 62 | | 63 |
| *Alopias pelagicus* | Pelagic thresher | | | 1 | 1 |
| *Alopias superciliosus* | Bigeye thresher | | | 1 | 1 |
| *Carcharhinus leucas* | Bull | 39 | 5 | | 44 |
| *Carcharias taurus* | Sand tiger | 2 | 1 | | 3 |
| *Isurus oxyrinchus* | Shortfin mako | | 11 | | 11 |
| *Sphyrna lewini* | Scalloped hammerhead | | 2 | | 2 |
| *Sphyrna mokarran* | Great hammerhead | | 5 | | 5 |
| *Sphyrna zygaena* | Smooth hammerhead | | 3 | | 3 |
| Other Warook | | 3 | 9 | | 12 |
| Total of individual sharks identified to species | | 53 | 271 | 2 | 326 |

## Results

### Shark landings

Twenty species of shark were recorded among landings, of which the most frequent by number were blacktip shark, *Carcharhinus limbatus* (61.7%), bull shark, *C. leucas* (13.5%), and spot-tail shark, *C. sorrah* (8.0%) (Table 1). Pagas (medium-bodied coastal sharks) accounted for the greatest part of the catch (66.1%) compared to pishik (small coastal) and warook (large-bodied pelagic) sharks. There was a significant difference in the number of sharks landed in different sub-areas, with the greatest numbers of sharks landed in the Jiwani area and the least in the Pasni area (Friedman ANOVA $\chi^2$=16.1, N=20, df=2, p=0.0003).

The largest sharks landed were bull (*C. leucas*), shortfin mako (*Isurus oxyrinchus*) and sand tiger sharks (*Carcharias taurus*), the first of which varied considerably in size (Table 2). All individuals of the following species were mature: blacktip reef (*C. melanopterus*), grey bamboo (*Chiloscyllium griseum*), grey sharpnose (*Rhizoprionodon oligolinx*), spadenose (*Scoliodon laticaudus*), scalloped hammerhead (*Sphyrna lewini*), smooth hammerhead (*S. zygaena*) and spot-tail sharks, while all individuals of sharptooth lemon (*Negaprion acutidens*), milk (*R. acutus*) and great hammerhead (*S. mokarran*) were immature. In Jiwani, between April and May, both blacktip reef and spot-tail sharks were landed with 3-5 pups unborn, suggesting pupping occurred in that area.

In addition to the species recorded at landing sites, eight fishers from the Jiwani and Ganz sub-areas reported having in the past caught whale shark (*Rhincodon typus*). They stated that the species was seen regularly 20 to 25 years ago, when it was targeted for the liver, but that very few were seen currently and were only caught incidentally or as by-catch. Also, whitetip reef shark (*Triaenodon obesus*) were reported as having been caught by 36 of the fishers from the Jiwani and Ganz areas and Pishukan, but none were recorded during the landing site surveys.

**Table 2.** Lengths, mean weights and prices obtained by fishers for different species of sharks landed in Balochistan between 16/04/2007 and 14/05/2010. Max: maximum, Min: minimum, TL: total length, PKR: Pakistani rupees.

| Shark Species | Max TL (m) | Min TL (m) | Mean Weight (kg) | Max price PKR $kg^{-1}$ | Min price PKR kg-1 |
|---|---|---|---|---|---|
| *Chiloscyllium griseum* | 0.55 | 0.55 | | | |
| *Rhizoprionodon acutus* | 0.4 | 0.4 | | | |
| *Rhizoprionodon oligolinx* | 0.61 | 0.6 | | | |
| *Scoliodon laticaudus* | 0.5 | 0.46 | | | |
| Other Pishik | 0.46 | 0.3 | 1.31 | 50 | 50 |
| *Carcharhinus limbatus* | 1.52 | 0.6 | 10.59 | 145 | 70 |
| *Carcharhinus melanopterus* | 1.31 | 1.3 | | | |
| *Carcharhinus sorrah* | 1.52 | 1.2 | 27.5 | 140 | 40 |
| *Negaprion acutidens* | 1.04 | 1 | | 160 | 160 |
| Other Pagas | 1.86 | 1.2 | 41.6 | 100 | 45 |
| *Carcharhinus leucas* | 4.3 | 1.52 | 176 | 150 | 120 |
| *Carcharias taurus* | 3.7 | 3.05 | 212 | 150 | 150 |
| *Isurus oxyrinchus* | 3.96 | | | | |
| *Sphyrna lewini* | 3.1 | 2.74 | 175.5 | | |
| *Sphyrna mokarran* | 2.29 | 2 | 300 | 140 | 140 |
| *Sphyrna zygaena* | 2.62 | 2.6 | | | |
| Other Warook | 2.74 | 2.1 | 146.8 | 150 | 100 |

**Table 3.** List of scientific, English and corresponding Baluchi names of sharks and rays recorded during the study together with their regional (Arabian Seas Region) IUCN Red List status (Jabado *et al.*, 2017): CR = Critically Endangered, EN = Endangered, VU = Vulnerable and NT = Not Threatened. This list records elasmobranchs landed during dedicated surveys and opportunistic observations. *C. amboinensis* was observed landed in Sindh.

| Scientific name | English name | Balochi name | Regional Status |
|---|---|---|---|
| Sharks | | | |
| *Alopias pelagicus* | pelagic thresher | dumbi | EN |
| *Alopias superciliosus* | bigeye thresher | dumbi mushk | EN |
| *Carcharhinus amblyrhynchoides* | graceful | kanater | VU |
| *Carcharhinus amboinensis* | pigeye | | VU |
| *Carcharhinus brevipinna* | spinner | | VU |
| *Carcharhinus leucas* | bull | Loand, warook, balanwad | EN |
| *Carcharhinus limbatus* | blacktip | kanater, kalwani | VU |
| *Carcharhinus macloti* | hardnose | | NT |
| *Carcharhinus melanopterus* | blacktip reef | | VU |
| *Carcharhinus sorrah* | spot-tail | knaitar, mangra | VU |
| *Carcharias taurus* | sand tiger | Lohar, lunad | CR |
| *Chiloscyllium griseum* | grey bamboo | | NT |
| *Echinorhinus brucus* | bramble | | VU |
| *Galeocerdo cuvier* | tiger | narmani | VU |
| *Isurus oxyrinchus* | shortfin mako | nar manger | NT |
| *Loxodon macrorhinus* | sliteye | | NT |
| *Negaprion acutidens* | sharptooth lemon | balwand, jagri | EN |
| *Rhincodon typus* | whale | baren | EN |
| *Rhizoprionodon acutus* | milk | sorapi pishik | NT |
| *Rhizoprionodon oligolinx* | grey sharpnose | tailgo pishik | NT |
| *Scoliodon laticaudus* | spadenose | bhambol pishik | NT |
| *Sphyrna lewini* | scalloped hammerhead | mash bhuttar | EN |
| *Sphyrna mokarran* | great hammerhead | mahaish | EN |
| *Sphyrna zygaena* | smooth hammerhead | maish | EN |
| *Triaenodon obesus* | whitetip reef | lone | VU |
| Rays | | | |
| *Gymnura poecilura* | longtailed butterfly | | NT |
| *Himantura leoparda* | leopard whipray | | VU |
| *Himantura uarnak* | honeycomb stingray | | VU |
| *Pateobatus fai* | pink whiptail | | VU |
| *Taeniurops meyeni* | round ribbontail | | NT |
| Torpediniformes | | | |
| Narke dipterygia | spot-tail sleeper | | NT |
| *Torpedo sinuspersici* | Gulf torpedo | | DD |
| Rhinopristiformes | | | |
| *Rhina ancylostoma* | bowmouth | baradari | VU |
| *Glaucostegus granulatus* | sharpnose | zahro | EN |
| *Rhinobatos annandalei* | Annandale's | zahro | NT |
| *Rhynchobatus sp.* | wedgefish | khali | EN |
| *Glaucostegus halavi* | halavi | | VU |
| Sawfish | | | |
| *Pristis* | sawfish | bolundo | CR |

**Table 4.** List of Pakistani companies exporting shark and stingray products in 2010, showing nature of products: fresh, frozen or other value added products. "Bones" is the term used for cartilaginous skeleton). * = companies known to be still operating in 2018.

| Export Company | Fresh Products | Frozen Products | Other Value Added Products |
|---|---|---|---|
| Arham Group | fillets, fins | | |
| A2Z Enterprise* | | | fins |
| Badran Import / Export | | | fins (dried) |
| Fairbright Company | meat & fins, stingray | | fins, salted & unsalted "bones" |
| Forte | fins | fins | fins |
| Global Seafood | | | fins (dried) |
| Hansa | | | fins (dried) |
| Ocean Gold | | fins | |
| Pakfish International | fins | | |
| Sarah Brand* | fins | | fins, "bones", stingray skin |
| Sea Gold | fins | | |
| Zangi Fisheries* | fins, reef sharks | fins, reef sharks | fins, reef sharks |

The price paid to the fishers, reported by the fishers, agents, managers and owners of fish processing plants, was obtained for 45 landings and ranged between 40 and 160 PKR $kg^{-1}$ wet weight (Table 2).

### Fisher Interviews

Fifty four fishers were interviewed in their home villages on 16 separate occasions. All the fishers surveyed reported that they used both set nets and long-lines to fish for sharks. The long-line (mungar sungle) comprised of a heavy, multi-filament 12mm diameter nylon rope as the main line, up to 1km long, with 2.5m branch lines attached to the substrate every 10m, with a Mustad No. 2 or 3 hook attached by steel wire to the end of each branch line (see also Hussain and Amir, 2006). Long-lines with 100 to 200 hooks were deployed in deep water of 100m or more. The nets (arrseegh) had a mesh size of up to 23cm and were anchored at each end and left in place overnight.

All the fishers reported that the best period for shark fishing was during the hot season, largely June and July. They caught a variety of species, which were sold un-finned to agents from fish processing companies; fishers considered finning to be specialist work. All, except one of the fishers, occasionally retained sharks liver for caulking their boats. Fishers reported that shark was not a preferred fish, although 23 (43%) also retained shark meat on occasion for eating. The fishers from the Jiwani and Ganz areas all sold their shark catch to Jiwani (50% of all fishers). Those from Pasni, however, sold their shark catch to Gwadar and Karachi (44.4%), or only Karachi (37.0%), or Jiwani (14.8%), while a few sold the catch in Pasni (3.7%). Most fishers could identify sharks to the genus level and some to the species level and used Balochi names (Table 3). Some local names were unusual or of biological interest. For example, variations on maish and bhuttar ("beautiful doll" and "toy-like") were used for the hammerhead (*Sphyrna* spp.), and nar mangar ("dangerous") for shortfin mako (*Isurus oxyrinchus)*. Whale sharks were called baren ("innocent"). One of the landing sites was in the village of Pishukan, which translates as "pup of sharks", because sharks in pup were often landed there.

### Interviews with Fish Processors

Forty two visits were carried out to 15 fish processing plants; all of these plants bought sharks. Ten plants in Jiwani sent their products to Karachi and one also sent products to Gwadar. The four Ganz plants sent their products to Jiwani, and the Pishukan plant sent their products to Karachi. All plants appeared to process sharks of a wide range of sizes and species and mostly during June and July, with the product mainly being frozen prior to further use. The mean mass of shark a plant received per season (June – July) to process was 4408kg (range 200 – 25,000kg) and the price paid to agents by the processing plant ranged between 150 and 200 PKR$kg^{-1}$. Four of the plant owners/managers

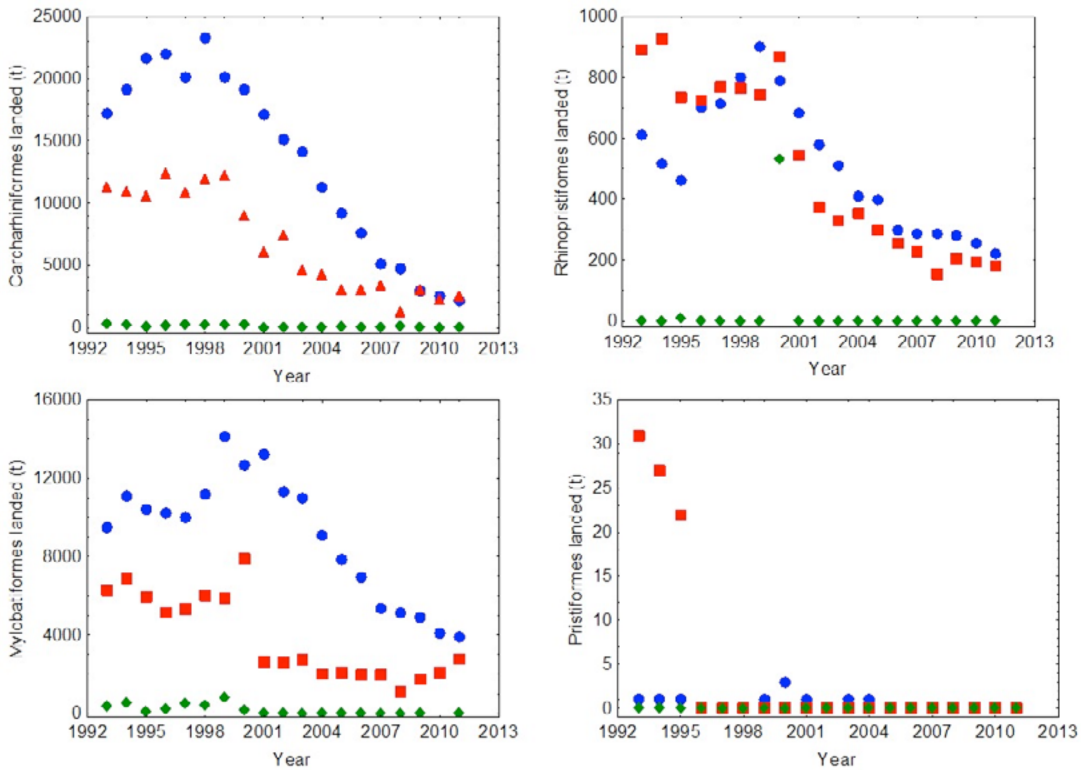


**Figure 2.** The estimated total wet weight landings (t) between 1993 and 2011 of each of four groups of elasmobranchs: sharks (Carcharhiniformes), guitarfish (Rhinopristiformes), rays (Mylobatiformes) and sawfish (Pristiformes) – separately for the two coastal provinces, Sindh (circles), Balochistan (triangles) and Pakistan's Exclusive Economic Zone (EEZ) (diamonds) collated from records of the Pakistan Government's Marine Fisheries Department.

reported also sourcing and selling their sharks on occasion from or to the port of Chabahar in Iran.

## Export of Shark Products

Up until September 2012, there were at least 12 businesses that exported shark products from Pakistan, either as fresh or frozen portions or as value added products, such as dried shark fin (Table 4). Two companies also sold stingrays. Shark fins were being exported to Asia (China, Hong Kong, South Korea and Japan), South Asia (Bangladesh, Sri Lanka, Myanmar, Singapore, Thailand and the Philippines), the Gulf region (Dubai) and Australia. Until at least 2000, shark fins were also being exported to the Czech Republic, France, Germany, Norway, Spain, Switzerland and the U.K. (Marine Fisheries Department, 2002, 2006, 2012). By July 2018, however, only three of these firms still had websites advertising shark products, including fins.

## Government Fisheries Data

Nineteen years of data on elasmobranch catches were provided by the Pakistan Marine Fisheries Department; these comprised 7–8% of total fish landings, the bulk of which were in Sindh. Elasmobranchs landed in Sindh and Balochistan and within Pakistan's Exclusive Economic Zone (EEZ outside of coastal waters) were recorded separately under four taxonomic groups: sharks (Carcharhiniformes); guitarfish (Rhinopristiformes); rays (Mylobatiformes); and sawfish (Pristiformes) (Marine Fisheries Department, 2002, 2006, 2012) (Fig. 2). The landings of sharks and rays in both provinces appeared to have increased slightly from

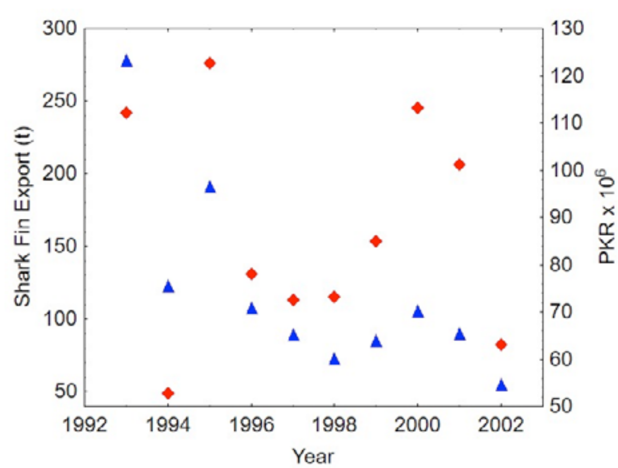


**Figure 3.** The weights of shark fin (t) (left axis, triangles) and its value (in Pakistani Rupees, PKR) (right axis, diamonds) between 1992 and 2002, from Pakistan government records.

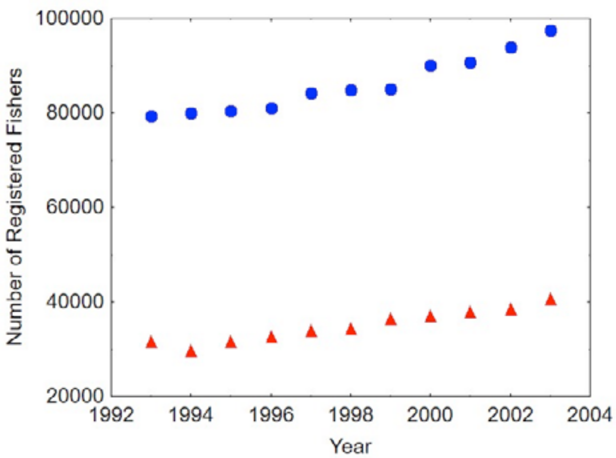


**Figure 4.** Numbers of registered fishers in the two Pakistan coastal provinces of Sindh (circles) and Balochistan (triangles) between 1993 and 2003.

1992 to about 1998 or 1999, and declined thereafter. Landings of guitarfish, on a much smaller scale (maximum < 1000t), varied irregularly until 2000, but then declined steadily. Landings of sawfish were even lower (maximum < 35t) and were confined almost entirely to Balochistan (where the stocks reportedly collapsed within three or four years during the early 1990s).

Shark fin exports peaked at over 250t in 1995 before declining until 1998. Stocks recovered somewhat in 2000 and 2001 and then declined further (Fig. 3). The monetary value of the shark fins exported appears to have increased in relation to its weight from 1999 to 2001, which may explain the temporary recovery of exports during this period.

The Marine Fisheries Department also registered and issued licenses to all fishing craft operating in Pakistan's territorial waters (Hussain and Amir, 2006). Data were available for the period 1992 to 2004. During that period the number of registered fishers in Balochistan steadily increased, while in Sindh there was an even steeper rise (Fig. 4).

## Discussion

In Pakistan, as in many other jurisdictions, elasmobranch landings have not been reported to species or even genus level by government fisheries officers, nor have individual shark weights and lengths generally been recorded. This lack of detail makes monitoring and management of individual stocks problematic, not least since the early decline of some species can be completely masked by increased exploitation of others. The present study provides a report of the shark species constituting the catch in Balochistan, the province accounting for the greater portion of the Pakistani coast.

Not only is species level information required for fisheries management purposes, but the status of many species is also a conservation issue. Of the 25 species of sharks encountered in the present study, nine are now regarded regionally as vulnerable, eight as endangered (including whale shark), and one (the sand tiger shark, *Carcharias taurus*) as critically endangered. Of the rays, guitarfishes and sawfishes, five are considered regionally as vulnerable, two as endangered and one (the sawfish, *Pristis pristis*) as critically endangered (IUCN Red List in Jabado *et al.*, 2017) (Table 3). Sawfish appear to have once been relatively abundant in Balochistan, judging by the extensive fencing made of their rostrums around houses in Ganz and neighbouring communities before 2004 (MG pers. obs.). A very steep decline in sawfish landings in Balochistan occurred over as little as three years in the early 1990s. Other scarce species may have been present, as it was not possible to confirm the identification of every individual in the time permitted by the fishers or the agents to whom they were being sold. A report of a rare bramble shark, *Echinorhinus brucus* (IUCN Red listed as Vulnerable: Jabado *et al.*, 2017), caught in Sindh's Swatch area, was featured in a leading local newspaper (http://dawn.com/ news/1048126/rare-bramble-shark-brought-to-fish-harbour); it was sold to fish meal manufacturers.

Given the Pakistan Marine Fisheries Department data and the accounts of fishers and fish processors, there is little doubt that there has been a general collapse in landings of all, or nearly all, elasmobranchs in both Balochistan and Sindh since about the turn of the century. By the time the present study was undertaken, total shark landings had returned to numbers similar to those being recorded in the 1950s (IOTC-2018-CoC15-RE), presumably before the demand for shark products led to their accelerating exploitation globally. However, catch rates did not necessarily increase monotonically since that time, as data reported by Pakistan to FAO indicated a sharp drop in the annual landings of both requiem sharks and batoids from about 70,000t to 20,000t in around 1983 (Fowler *et al.*, 2005). This finding suggests that these larger more vulnerable species began to be over-exploited from this earlier date. The more recent data reported here also shows temporary levelling, or even a drop, both in the landings of sharks (Carcharhiniformes), guitarfish

(Rhinopristiformes), and most clearly, rays (Mylobatiformes) (Fig. 2), and in the export of fins (Fig. 3) during the mid-1990s. These data suggested that sustained demand for and increased value of shark fin products probably encouraged fishers to extend their efforts to additional stocks and fishing areas. As a consequence, many species of shark landed did not exceed 1m in length, while the maximum length of even medium-bodied species rarely exceeded 1.5m (Table 2).

It was noticeable that almost all the blacktip, great hammerhead, sharptooth lemon and milk sharks landed were immature, suggesting that the areas being exploited were nursery grounds. Similarly, the blacktip reef and spot-tail sharks landed were typically gravid, giving birth to young on landing, with the pups being discarded as having no value. Clearly, the exploitation of nursery grounds represents a wasted resource, as these sharks would be better caught at a larger size. The landing of gravid females in particular represents a severe threat to stocks, as it also involves the loss of future breeding potential. Similarly, the discovery linked to the present study of two neonatal whale sharks that had been caught in fishing nets in 2000 off Ormara, Balochistan, (Rowat *et al*., 2007) suggested that there might be a pupping area for whale sharks in that region. However, fishers reported that for 20 or more years whale sharks were no longer frequently seen along the western Balochistan coast. This was despite whale sharks still appearing to be reported regularly in the Gulf of Oman and Arabian Gulf (Robinson *et al*., 2017).

Despite the declining stocks of elasmobranchs and also other fish, the number of fishing vessels and fishers in both Balochistan and Sindh continued to increase (Fig. 4), a trend also noted by Khan and Khan (2011). The fisher interviews showed that all the fishers in Balochistan operated on a full-time basis. These findings imply that pressure on stocks continued to increase during the period when there was a drastic decline in the numbers of sharks, guitarfish, and rays being landed (Fig. 2). Almost all fishers reported that since near shore areas were increasingly depleted of sharks and fish generally, they had to work in increasingly deeper waters. A similar shift from inshore to deep sea shark fishing in neighbouring India has also been ascribed to a reduction in coastal species (Akhilesh *et al*., 2011).

Lack and Sant (2009) have indicated that shark finning was not practiced in Pakistan, yet Vannuccini (1999) reported that Pakistan exported dried shark fins to Singapore and other Asian countries. Fowler *et al.* (2005) noted that Pakistan was responsible for 85% of the global dried or salted shark meat. The division of the industry in Balochistan (and similarly in Sindh) as described in the present study explains these apparent contradictions. As noted, fishers regarded shark finning as specialist work and sold elasmobranchs whole to agents, who in turn sold the catch on to processing plants. Thus, the fishers did not fin sharks (or rays). Further, while the processors interviewed all froze their sharks, exporters advertised fresh shark as well. However, the bulk of the shark body was of limited commercial value and it was shark fins that were the main interest for export companies. The price paid to Balochi fishers for whole sharks did not necessarily reflect the value of the fins on the export market, but it was noticeable that the price paid was greater for some species, ranging from the equivalent of US \$0.56–2.26kg$^{-1}$. Shortfin mako, *Isurus oxyrinchus*, and thresher shark, *Alopias spp*., are reported to be the most highly prized species in the wider shark fin market, presumably because of their proportionately much larger fins, but bull, spot-tail, great and scalloped hammerhead, and sharptooth lemon sharks are also preferred (Vanuccini, 1999) and found in the present study among the species being landed in Balochistan.

It is now widely appreciated that because of their low fecundities and slow growth rates, elasmobranchs generally are considerably more vulnerable to over-exploitation than other highly productive and heavily exploited stocks, such as anchoveta *(Cetengraulis mysticetus*) or shrimp *spp*. (CEA, 2012). CEA concluded that the main factor predicting stock decline was high susceptibility to fishing pressure, rather than high fishing pressure or low fishery productivity. This understanding, together with the realisation that threatened or endangered species of shark and ray are worth protecting for their own sake, has led to the introduction of a wide range of conservation measures by many countries. Size and catch limits have been enacted (e.g. South Africa - http://www.fishthesea.co.za/baglimits.htm) and bans on finning at sea (e.g. South Africa (1998), United Arab Emirates (1999), and India (2013) https://awionline.org/content/international-shark-finning-bans-and-policies), and a series of countries and territories including Egypt (2005), Palau (2009), the Maldives (2010) (https://awionline.org/content/international-shark-finning-bans-and-policies) and the Cayman Islands (Ormond *et al*., 2016) have established shark sanctuaries by giving full protection to sharks throughout their waters, and the most endangered species afforded global protection under the Convention on Trade in Endangered Species (CITES, www.cites.org) and the Convention on Migratory Species (CMS, www.cms.int). While the scope of such measures may seem limited, Ward-Paige and Worms (2017) found that banning commercial shark fishing and instituting laws that prohibit the possession, trade or sale of sharks and shark products led to less pronounced shark population declines. Thus, it was hoped that Pakistan would take steps to ensure the sustainability of its elasmobranch resources and of the associated benefits to fishers, processors and exporters. It was discouraging therefore to find that, according to Schmidt (2014), the Pakistan Marine Fisheries Department/FAO Fisheries Resource Appraisal Project have listed sharks as an extinct resource in Pakistan, except for coastal demersal species.

As a first stage in introducing effective management, the FAO encourages the development of both country (national) and (global) regional shark management plans (Polidoro *et al.*, 2008). Although a national plan of action for sharks (NPOA-sharks) was under discussion in late October 2004 (Cavanaugh *et al.*, 2009), Pakistan has still not introduced such a measure (Davidson *et al.*, 2015). Pakistan is a signatory to CITES, but it is not a signatory to the CMS Shark Memorandum of Understanding (https://www.cms.int/en/ legalinstrument/sharks-mou). Most recently there was a report that Pakistan had legislated (27 April 2018) a ban on shark finning (IOTC-2018-CoC15-RE). However, the Balochistan legislation bans catching, retention, marketing and trade of only five families of pelagic shark, together with pristids, mobulids, rhiinids, rhinobatids and rhynchobatids (Balochistan: No. 50 (Coord:) Fish/2-1/2013/3148-54 dated 08 September 2016). Further action to alleviate the situation is critical, beginning with a realistic national plan of action (NPOA-sharks). This will need buttressing by a widespread public awareness programme and targeted support for fishers and processors. Even partial success will be a worthwhile achievement given that much of Pakistan, including especially Balochistan, is much more ethnically diverse and more difficult to access than generally presumed.

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# The cavernicolous swimming crab *Atoportunus dolichopus* Takeda, 2003 (Crustacea, Decapoda, Portunidae) reported for the first time in the Western Indian Ocean during technical dives in the mesophotic zone

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## Abstract

The rare cavernicolous crab *Atoportunus dolichopus* Takeda, 2003, described from Kume-Jima Island, Ryukyu archipelago, is recorded for the first time since its description. Two specimens were observed in a marine cave off Mayotte Island, Western Indian Ocean, during technical dives in the mesophotic zone. The crabs were observed in total darkness at a depth of 75m, 120m from the entrance of the cave. No specimens were collected but morphological traits recognized on close-up photographs agree with those of *A. dolichopus*. This rare species is illustrated with comments on its remarkable disjunct geographical distribution and ecology.

**Keywords:** Cavernicolous crab, *Atoportunus*, Mayotte Island, Mesophotic zone

The marine mesophotic, or twilight zone, situated at depths of approximately 50-150m in the tropics, is still poorly known because it is beyond the usual depths of recreational dives. Exploring these depths necessitates technical dives with re-breather and trimix gas; techniques that are still mastered by only a few divers. The first two authors of this note are experienced technical divers. In 2018 they initiated a collaborative research programme to study the mesophotic zone around Mayotte (Barathieu, 2019). This programme brings together several experts on the marine fauna and flora around Mayotte and adds to another mesophotic research programme currently being conducted around Mayotte (MesoMay, funded by DEAL Mayotte).

During a dive by the first two authors the entrance of a cave was discovered at a depth of 50m southwest of Mayotte Island near 'Passe Bateau'. The entrance of the cave was very large, approximately 3-4m high by 15m long, opening into two separate galleries sinking gently into the basement of the island (Fig. 1). At the end of the longest gallery, about 120m from the entrance at a depth of 75m, in total darkness, a remarkable crab was observed during three successive dives with photographs taken on 28/11/2018 and 23/02/2019 showing two distinct specimens. A photograph of the first specimen was transmitted for determination to JP by Professor Bernard Thomassin of the collaborative research programme. Additional photographs of the second specimen, including close-up frontal views, were later examined (Fig. 2).

Based on these photographs the genus *Atoportunus* Ng and Takeda (2003) is recognized for the first time around Mayotte. This genus was established to accommodate two unusual swimming crabs living in marine caves, respectively *A. gustavi* Ng and Takeda, 2003 and *A. pluto* Ng and Takeda, 2003. These two species are superficially similar but differ in a series of subtle morphological characters. *Atoportunus pluto* is still unrecorded outside Hawaii where it is probably endemic. *Atoportunus gustavi* has a much wider distribution being present in the western Pacific (Guam, Marianas; and

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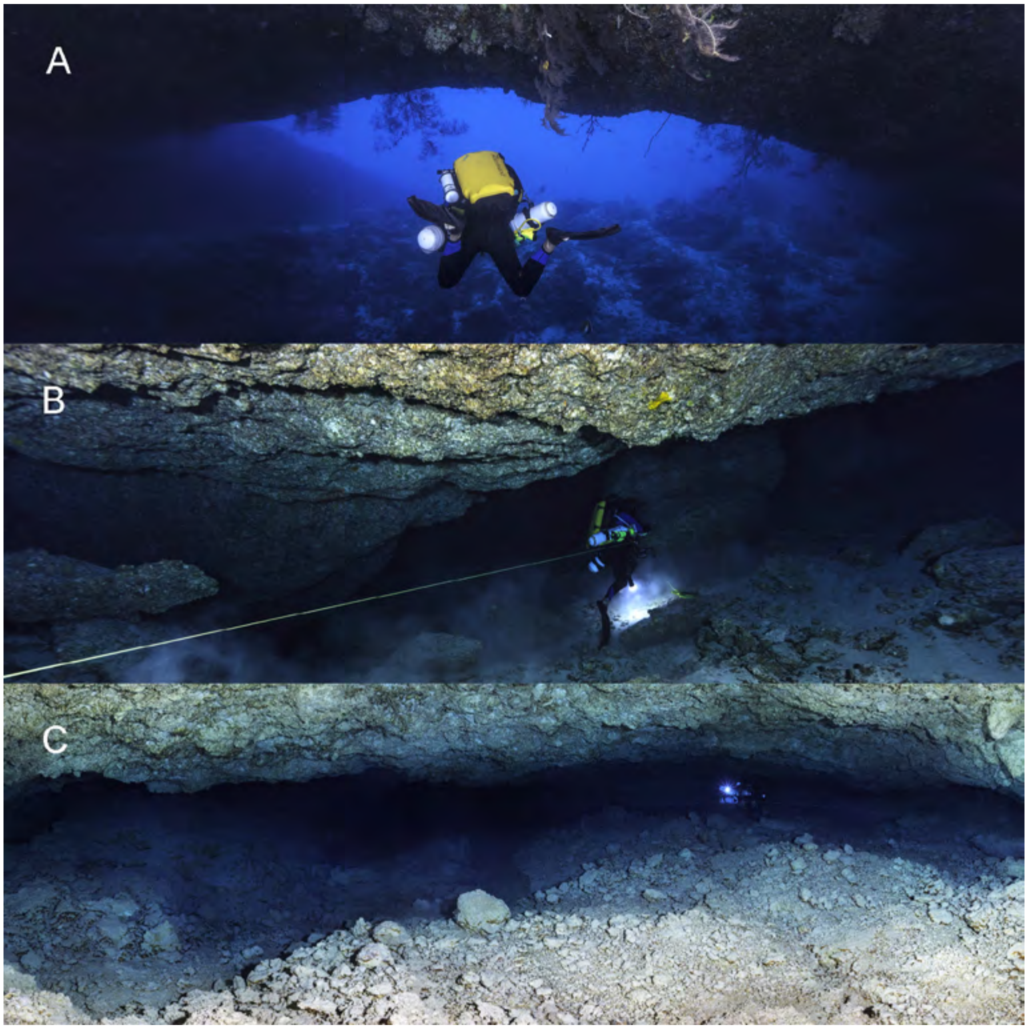


**Figure 1.** The *Atoportunus dolichopus* cave in Mayotte. A) Diver at the entrance of the cave, 50m deep; B) Exploring the 120m long tunnel with security line to avoid getting lost in the cave; C) End of the tunnel where the crab was observed with aspect of the bottom made up of rubble and calcareous muddy sand. Photographs G. Barathieu.

Yonaguni Island, Ryukyus) and in the eastern Indian Ocean (Christmas Island). It is probably common in marine caves as suggested by at least three more reports in the Ryukyus since its description in Shimojijima Island (Fujita *et al.*, 2013) and in Okinawa-jima Island and Ie-jima Island (Fujita and Mizuyama, 2016). *Atoportunus gustavi* and *A. pluto* have been reported in depths of 2-30m in coral rubble near or in caves, normally in dark places, hence the qualification of 'chalicophilous and cavernicolous' crabs by Ng and Takeda (2003). These authors have also indicated that *Atoportunus* is classified in the Portunidae Rafinesque, 1815, despite an unusual morphology and without appropriate comparison with other portunid genera. More recently Mantelatto *et al.* (2018, Fig. 1, Table 1) have sequenced a specimen of *Atoportunus gustavi*. It groups to *Carupa tenuipes* Dana, 1852, suggesting a potential affinity of *Atoportunus* with the Carupinae Paulson, 1875.

A third species of the genus, *Atoportunus dolichopus* Takeda, 2003, has been recognized in Japan based on two specimens collected off Kume-jima Island, Ryukyus. They were found in a cave in total darkness at a depth of 38m, approximately 60m from the entrance. The new species differed from the two previous *Atoportunus* species by at least six morphological characters: a) hemispheric carapace (vs. more flattened carapace); b) narrower carapace, carapace breadth CB (including lateral spine) on carapace length CL being 1.22-1.44 (vs. 1.67-1.83); c) last anterolateral tooth directed obliquely forward (vs. more laterally); d) longer legs and chelipeds, the cheliped being ca. 3 times CB (vs. 1.80-2.00); e) armature of the merus of cheliped with mesial margin having more than 10 spines on proximal half and 3 equidistant spines on distal half (vs. 6 spines distributed over the entire length); f) cutting edges of movable and immovable fingers of chela with respectively, 2 and 3 long spines of similar size directed obliquely (vs. 2 and 5 spines, of various sizes on immovable finger).

Morphological characters recognized on the photographs of the two *Atoportunus* specimens from Mayotte agree broadly with those of *A. dolichopus*: a) carapace hemispherical (Figs. 2B-C); b) last anterolateral spine directed obliquely forward (Figs. 2A, 3A); c) CB/CL ca. 1.45-1.55 (Fig. 3A); d) long legs with chelipeds more than 3 times CB (Fig. 2A); e) cutting edges of movable and immovable fingers of chela with respectively, 2 and 3 long spines of equal sizes directed obliquely (Figs. 2B, 3D). The armature of the mesial margin of the merus of the cheliped is intermediate between *A. dolichopus* and *A. gustavi/pluto* having 6-8 spines on the proximal half and 3-4 spines on the distal half disposed as illustrated in Fig. 3C. It seems, however, that this armature may display variation in *A. dolichopus* as illustrated in Takeda (2003) between the male holotype (Fig. 1A, 2E) and the female allotype (Fig. 1B-C). Despite this minor difference it seems reasonable, for the time being, to attribute the specimens from Mayotte to *A. dolichopus*. A new species closely affiliated to *A. dolichopus* cannot be totally excluded at this stage for Mayotte but more specimens and observations are necessary to confirm that hypothesis.

With this discovery, the geographical distribution of *A. dolichopus* appears remarkably disjunct with ca. 10 000km between Kume-jima and Mayotte Islands. Such a disjunct distribution has, however, already been observed for *A. gustavi* occurring in the Ryukyus, Marianas and Christmas Island, the latter being ca. 5 000km from the two former archipelagoes. *Atoportunus dolichopus* is probably widespread in the Indo-west Pacific (IWP) though rarely seen due to living in deep caves necessitating technical dives with complex and risky navigation in cave networks.

The eyes of *Atoportunus* crabs are reduced which is indicative of obligate cavernicolous species (Guinot, 1988; Ng and Takeda, 2003). In some cavernicolous crabs of the Potamidae, the reduction is so pronounced that the cornea is no longer visible (Guinot, 1988, Figs. 7-8). In the crabs examined from Mayotte, the cornea is still present but it is distinctly narrower than the ocular peduncle (Fig. 2C). Such a reduction is common in cavernicolous crabs. It has been documented recently by Wowor and Ng (2018) for three cavernicolous sesarmid of the genus *Karstarma*.

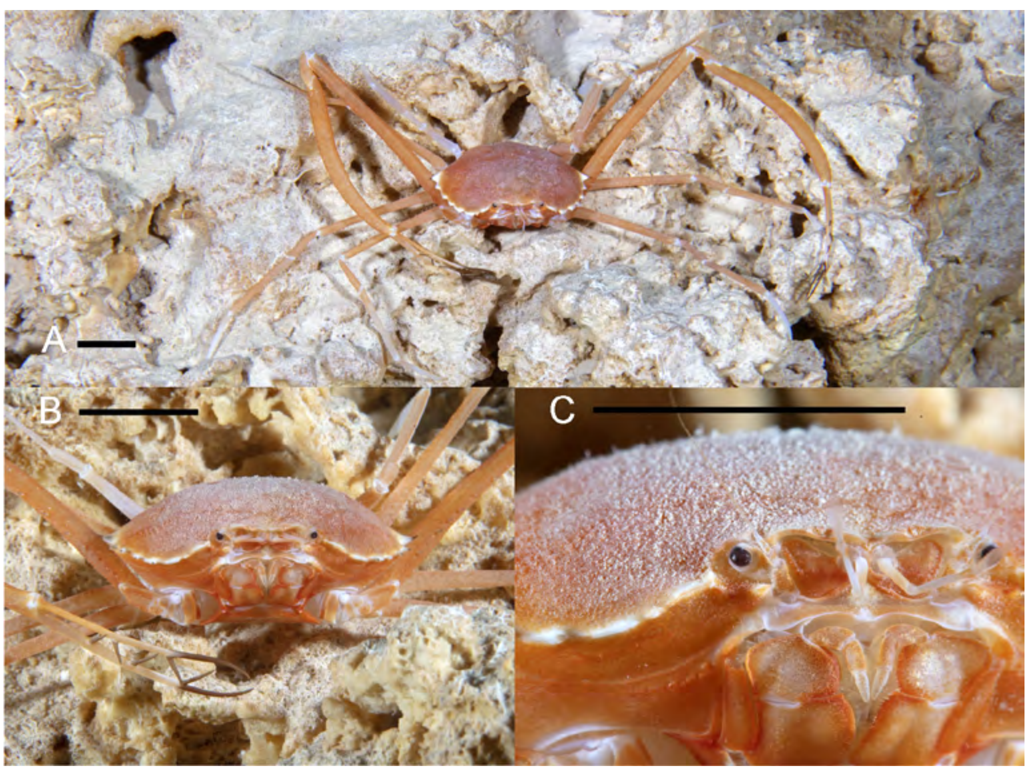


**Figure 2.** *Atoportunus dolichopus* in the cave at 75m depth at Mayotte Island, 23/02/2019. A) Defensive posture on hard substrate; B) Frontal view of carapace and aspect of right chela; C) Close-up frontal view showing orbits, epistome and buccal cavity. Estimated CB - 28mm. Scales bars - 10mm. Photographs G. Barathieu.

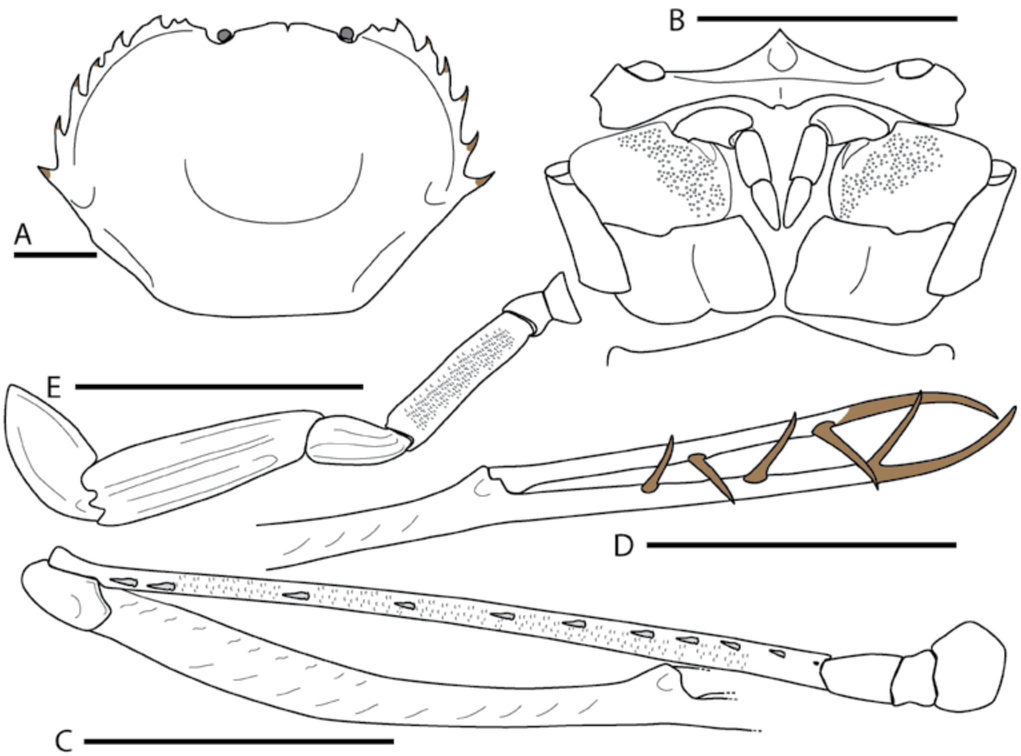


**Figure 3.** *Atoportunus dolichopus*, line drawings made from photographs. A, E, specimen photographed 28/11/2018; B, C-D, specimen photographed 23/02/2019; estimated CB 28mm for both specimens. A) Carapace, dorsal view with aspect of anterolateral armature; B) buccal cavity with MxP3 (setae omitted) and epistome (length of ischium is reduced because of oblique view); C) right cheliped showing mesial armature of merus; D) right chela, lateral view; E) right P5, dorsal view. Scale bars A-B, 5mm, C-E, 10mm.

Because of its hemispherical body (Fig. 2B-C), long legs (Fig. 2A) and reduced natatory P5 (Fig. 3E) this crab is probably not a good swimmer (Ng and Takeda, 2003; Takeda, 2003). The movement of the crabs in the cave was very slow and it could have been picked easily by hand which confirms Takeda's (2003) similar observation for Japanese specimens. The defensive posture of the crab (Fig. 2A) suggests that it probably hunts from a hide in total darkness. It must be able to quickly project its long chelipeds forward when it feels a prey within reach and harpoon it with the spear-like spines of its claws (Fig. 3D). No potential prey were observed during the dives in the immediate surroundings of crab but small shrimps and fishes were seen in the first tens of meters from the entrance where the crabs could possibly move for hunting. Three other macro-decapods were observed in the cave during the dives: a swimming crab, probably *Gonioinfradens paucidentatus* (A Milne-Edwards, 1861) hidden in a hole near the entrance; the hermit crab *Aniculus maximus* Edmonson, 1952 observed in total darkness, 60m from the entrance; and the shrimp *Parhippolyte misticia* (J Clark, 1989) with solitary individuals observed in several places in the tunnel - one in total darkness 100m from the entrance. *Parhippolyte misticia* is also a true cavernicolous shrimp originally described from a cave in Palau (Clark, 1989) and now reported from several IWP localities (Debelius, 2001). The two other species, *G. paucidentatus*, and *A. maximus*, are not cavernicolous but occasionally visit the caves.

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