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Mineral content in local feed ingredients used by fish farmers in four different regions of Tanzania

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Abstract

This study investigated the content of selected minerals (P, Ca, K, Na, Mg, Fe and I) in local feed ingredients used by tilapia fish farmers in Tanzania. Analyses were performed on 26 local feed ingredients collected at four different geographical locations in Tanzania (Dar es Salaam, Morogoro, Mbeya and Mwanza). The samples were taken randomly from fish farmers, fish feed producers, fingerling producers and animal feed shops or centers near fish farms in each region. The results showed a wide range of mineral concentrations. The highest levels of P was found in fish skeletons (17.8 g kg^{-1}), of Ca in limestone (107.3 g kg^{-1}), of K in gallant soldier (51.0 g kg^{-1}), of Na in marine shrimp (*Exhippolysmata oplophoroides)* (11.7 g kg^{-1}), of Mg in prawn head waste (4.2 g kg^{-1}), of Fe in azolla (2355 mg kg^{-1}) and of I in full fat soybean (447 mg kg^{-1}). The data on mineral content in feed ingredients can be used as a platform for better-targeted feed formulation for tilapia farming systems. In conclusion, the data suggest that if more than two ingredients are used in the diet, this may be sufficient to meet the mineral requirements of all cultured tilapia species and their hybrids, without inclusion of any mineral premix.

Keywords: mineral requirements; feedstuffs; fish pond; aquaculture; tilapia

Introduction

In Tanzania, fish farmers raise tilapia (*Oreochromis niloticus*) in extensive systems and/or semi-intensively through fertilisation of the fish culture system (ponds and tanks) and provision of supplementary feeds (Chenyambuga *et al.,* 2014). To date, the majority of tilapia fish farmers in Tanzania feed their fish with locally available feedstuffs of both plant and animal origin. However, in order to formulate nutritionally appropriate supplementary feeds for fish, accurate data on the nutrient content of different dietary components are required. There are some limited data available (based on proximate analysis) on the major nutrient composition (*i.e.* crude protein, crude fat, crude fibre and ash) of commonly used feed ingredients in Tanzania (FAO, 1987). However, information on the mineral content of locally available feed ingredients in Tanzania is lacking.

Minerals play an important role in the maintenance of normal metabolic and physiological functions in fish and other animals, which include muscle contraction, blood clotting, enzyme activity, transmission of nerve impulses, metabolism and electrolyte balance (Tacon, 1987; Dato-Cajegas and Yakupitiyage, 1996; Assey *et al.*, 2009). Moreover, lack of essential minerals in the fish diet may lead to mineral deficiency, resulting in conditions such as anaemia, osteoporosis, stunted growth and genetic disorders (Tacon, 1987; Dato-Cajegas and Yakupitiyage, 1996; Fumio *et al.,* 2012; Bhandari and Banjara, 2015).

Iron (Fe) and iodine (I) are limited in large areas of Tanzania and deficiency of these elements causes anaemia and goitre in humans. Therefore, Fe and I were analysed in addition to the macro-minerals calcium

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(Ca), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). In fish, Fe is involved in regulatory mechanisms controlling blood formation and respiration, hormone synthesis, fatty acid mechanism (Brody, 1994) and maintaining the integrity of the epithelium (Naser, 2000). Fish can absorb some trace minerals such as Fe not only from the diet offered, but also from their external aquatic environment through the gills (Watanabe *et al.,* 1997). The effects of limited access to I in fish are not well described, but the effects of I deficiency in fish are suggested to be similar to those in humans (NRC, 2011).

The content of Fe and I, as well as other minerals may vary in the fish diet and in the aquatic environment, therefore the aim of the present study was to investigate the mineral content of local feed ingredients used by tilapia fish farmers in Tanzania and to compare the levels measured against suggested requirements for tilapia.

Material and Methods

General

Mineral analysis was performed, according to methods described by AOAC (1990), at the food and soil science laboratories of Sokoine University of Agriculture, Tanzania. The following AOAC (1990) methods were used: Ca (968.08); P (965.17), Mg (968.08); K (956.01); Na (956.01); I (935.14); and Fe (968.08).

Samples and sample preparation

A total of 26 local feed ingredients (Table 1), collected during a field survey at four different geographical locations in Tanzania (Dar-es-Salaam, Morogoro, Mbeya and Mwanza region) (Fig. 1), were analysed for their mineral content. The samples were obtained from fish farmers, local fish feed producers, fingerling producers and animal feed centres located near fish farms in each region. In brief, 5 different local feedstuff samples (each weighing 200g) were obtained from 5 out of 20 randomly selected aqua-farmers or animal feed centres in three districts per region, depending on geographical zone, availability, specificity and climate conditions. Therefore, a total of 60 samples (15 per region) of the 26 different local feedstuffs were collected for the present study.

The collected samples of each feedstuff were pooled and sub-sampled. Briefly, the pooled sample of each feedstuff was spread out on a clean plain surface marked into quarters, and two opposite quarters were taken and mixed. This process was repeated until the two quarters selected comprised the desired amount of 100-200 g. The sub-samples were then sun-dried for 48 h, packaged and transported to the laboratory for mineral analysis. Prior to mineral analysis, samples were milled in a blender (JYL-D020 Powerful Multifunctional Blender Food Processor, Joyoung, China) and sieved by hand to pass through a sieve with 1.0mm circular openings.

For analysis of Ca, P, Mg, K, Na and Fe, 1.0 g of milled, homogenised sample was placed into a weighed porcelain crucible, which was placed in an incinerator and ignited at 450 °C until white or grey ash was obtained (Jorhem, 2000). The ash was dissolved in 10 ml of 10 % hydrochloric acid and the suspension was then filtered (No. 1 Whatman ashless filter paper, GE's Whatman Grade 40; 1440-090) prior to analysis.

For analysis of I, 2.0 g of milled sample was placed in a 25 ml Erlenmeyer flask and 10ml of deionised water was added. The mixture was shaken for 10 minutes using an orbital shaker (Baird & Tatlock, Multishaker, UK), diluted to 25 ml with deionised water and filtered (No. 1 Whatman filter paper, GE's Whatman Grade 40; 1440-090) prior to analysis.

Mineral analysis

The filtrate from both types of analysis described above was subjected to atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, UNICAM 199 AA Spectrometer, Cambridge CBI 2PX, England) for determination of Ca, Mg and Fe content, with absorbance reading at 422.7 nm for Ca, 285.2 nm for Mg, and 248.3 nm for Fe, according to the manufacturer's instructions. Standard solutions for atomic absorption spectrophotometry of each mineral were prepared by serial dilution of an appropriate stock solution. The standard solutions for Ca ($CaCl_2$) contained 0, 5.0, 10.0, 15.0 and 20.0 mg l^{-1} Ca; the standard solutions for Mg ($MgCl_2.6H_2O$) contained 0, 0.5, 1.0, 1.5 and 2.0 mg l^{-1} Mg; and the standard solutions for Fe ($FeCl_3.6H_2O$) contained 0.0, 5.0, 10, 20, and 40 mg l^{-1} Fe.

The content of K and Na in the filtrate was determined using a digital flame analyser (2655 Digital flame analyser, Chicago, USA) according to the manufacturer's instructions. Standard solutions for K and Na were prepared by serial dilution of an appropriate stock solution. The standard solution for K (KCl) contained 0.5, 2.5, 5.0 and 10.0 mg l^{-1} K and the standard solution for Na (NaCl) contained 0.5, 2.5, 5.0 and 10.0 mg l^{-1} Na. Phosphorus and I content in the filtrate was determined using an UV spectrophotometer (BIOMETE 6, WI53711, USA) with absorbance reading at 884.0 nm for P (AOAC, 1990), and at 665.6nm for I according to Narayana *et al.* (2006). The standard solutions for P (KH_2PO_4) contained 0, 0.1, 0.2, 0.4 and 0.8 ppm P and the standard solutions for I (KIO_3) contained 0, 5.0, 10.0, 15.0, 25.0 and 30.0 mg I/l.

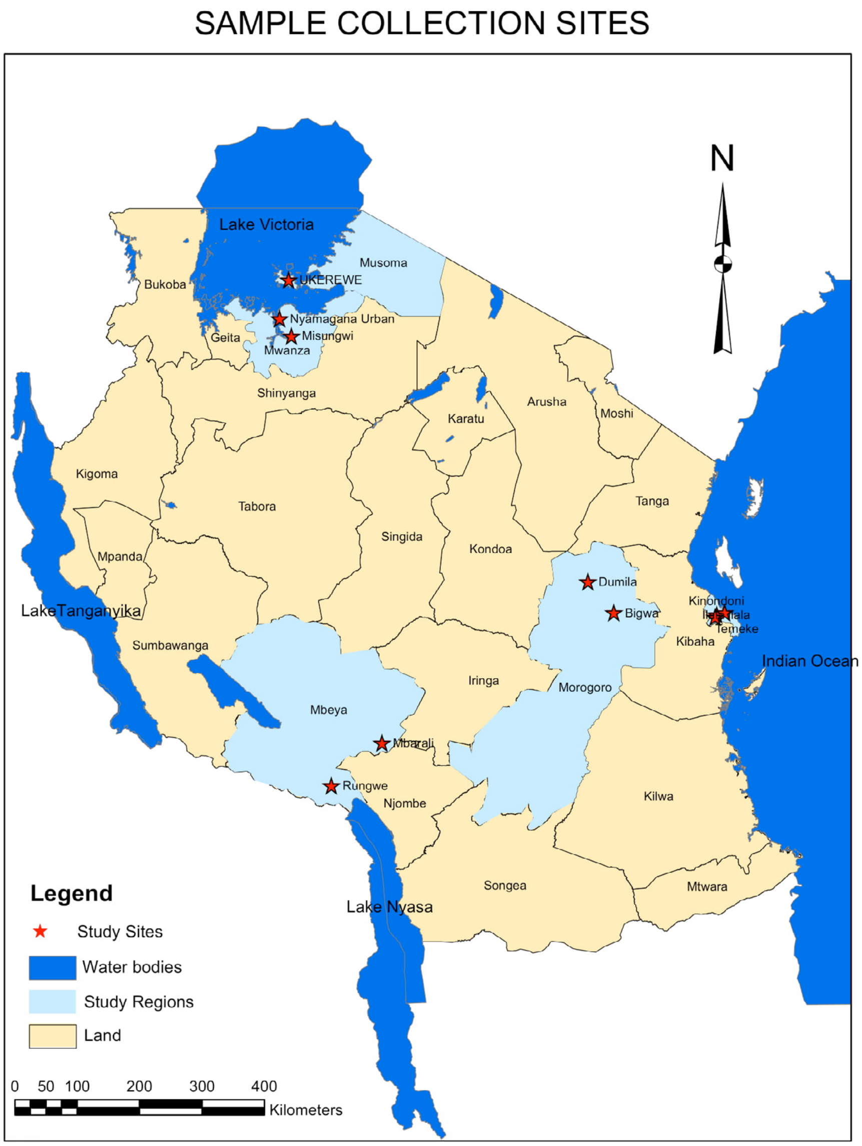

Figure 1. Map of Tanzania showing the location of the sampling sites in the four study regions: Dar es Salaam, Morogoro, Mbeya and Mwanza.

Results

The 26 feedstuff samples analysed were classified into the following five categories: Animal by-products; Agricultural by-products; Plant leaves and weeds; Aquatic plants; and Others (Table 1). The mineral content in each sample and in the different categories are presented in Table 1 and 2, respectively.

Animal by-products, except for cattle blood, had a high content of P, Ca, K, Na, Mg, Fe and I (Table 1). The mineral content in the ingredients of animal origin ranged between <0.1 and 26.6 g kg^{-1} for Ca; between 0.9 and 17.8 g kg^{-1} for P; between 2.4 and 11.7 g kg^{-1} for Na; between 94 and 370 g kg^{-1} for Fe; and between 3 and 294 mg kg^{-1} for I (Table 2).

Agricultural by-products were high in P, K, Fe and I, but low in Ca, Na and Mg (Table 1). However, one exception

Table 1. Concentration (per kg dry matter) of different minerals in local feed ingredients used by small-scale tilapia fish farmers in Tanzania.

Sample	P (g)	Ca (g)	K (g)	Na (g)	Mg (g)	Fe (mg)	I (mg)
Animal by-products							
Cattle blood	0.9	<0.1	3.2	8.5	<0.1	202	3
Fly maggot	6.6	1.4	11.1	3.6	2.2	370	167
Sardines	10.9	7.6	10.8	2.4	1.3	142	118
Marine shrimp	8.3	5.4	12.4	11.7	3.2	97	58
Freshwater shrimp	9.9	16.3	11.3	4.7	1.4	328	294
Prawn head waste	12.3	26.6	5.9	5.5	4.2	223	63
Fish frames	17.8	18.5	3.9	4.7	1.6	94	14
Agricultural by-products							
Full fat soybean	4.5	0.6	16.0	<0.1	<0.1	401	447
Soy bean	5.6	0.4	12.8	0.6	1.9	61	93
Sunflower seed cake	5.1	1.4	10.5	0.2	<0.1	146	66
Cotton seed cake	5.7	0.5	15.1	<0.1	<0.1	65	4
Maize bran	7.8	0.06	9.3	<0.1	<0.1	87	60
Rice polish	11.3	1.5	10.6	<0.1	<0.1	160	32
Wheat pollard	14.2	40.9	9.9	6.0	<0.1	78	41
Plant leaves and weeds							
Moringa leaf	3.2	8.4	14.0	0.8	3.6	95	84
Chinese vegetable	3.4	10.6	51.5	6.9	3.8	838	137
Cassava leaf	4.7	1.6	51.1	<0.1	<0.1	245	165
Taro leaf	3.2	3.2	46.5	0.1	1.6	143	89
Gallant soldier	5.6	4.9	51.0	0.2	<0.1	217	13
Sweet potatoes	0.8	0.1	7.7	<0.1	<0.1	66	15
Aquatic plants							
Azolla	5.8	1.5	31.5	5.6	1.9	2355	179
Water lettuce	4.8	13.6	33.2	4.3	0.9	229	77
Duckweed	14.3	44.8	9.3	6.7	<0.1	2265	60
Others							
Spent brewer's yeast	3.3	1.2	3.7	0.2	0.3	52	157
Seashells	0.5	93.8	0.4	3.1	2.7	320	6
Limestone	0.2	107.3	0.5	0.5	1.1	316	8

Table 2. Range of concentrations (per kg dry matter) of different minerals within the five categories of fish feedstuffs investigated.

Mineral	Animal by-products	Agricultural by-products	Plant products	Aquatic plants	Others
P (g)	0.9 - 17.8	4.5 - 14.2	0.8 - 4.7	4.8 - 14.3	0.2 - 3.3
Ca (g)	<0.1 - 26.6	0.06 - 40.9	0.1 - 10.6	1.5 - 44.8	1.2 - 107.3
K (g)	3.2 - 12.4	9.3 - 16.0	7.7 - 51.5	9.3 - 33.2	0.4 - 3.7
Na (g)	2.4 - 11.7	<0.1 - 6.0	<0.1 - 6.9	4.3 - 6.7	0.2 - 3.1
Mg (g)	<0.1 - 4.2	<0.1 - 1.9	<0.1 - 3.8	<0.1 - 1.9	0.3 - 2.7
Fe (mg)	94 - 370	61 - 401	66 - 838	229 - 2355	52 - 320
I (mg)	3 - 294	4 - 447	13 - 165	60 - 179	8 - 157

was wheat pollard, which was high in Ca and Na. The mineral content in the agricultural by-product ingredients ranged between 4.5 and 14.2 g kg^{-1} for P; between 0.06 and 40.9 g kg^{-1} for Ca; between 9.3 and 16.0 g kg^{-1} for K; between <0.1 and 6.0 g kg^{-1} for Na; between <0.1 and 1.9 g kg^{-1} for Mg; between 61 and 401 mg kg^{-1} for Fe; and between 4 and 447 mg kg^{-1} for I (Table 2).

Plant leaves and weeds showed a similar pattern as the agricultural by-products, with high values for P, K, Fe and I, and low values for Na and Mg, while the Ca content was high in wheat pollard and low in sweet potatoes (Table 1). However, moringa leaves and Chinese vegetable showed high values for Mg and Ca.

Aquatic plants were high in all minerals, except for Ca in azolla and Mg in duckweed (Table 1). In general, the Ca content was higher in aquatic plants than in agricultural by-products.

In the group 'Others', seashells and limestone showed a high content of Ca and Fe, while spent brewer's yeast showed a high I content (Table 1).

Discussion

The availability of good-quality fish feed for tilapia fish farmers is a major concern in the development of the aquaculture industry, not only in Tanzania but also across the entire East Africa region. Ideally, in addition to providing protein and fat, the feed should also supply the essential minerals needed for high performance and health. The results from the present study revealed a wide range of mineral concentrations in feed ingredients used by tilapia fish farmers in Tanzania.

The high content of P, Ca, K, Na, Mg, Fe and I in most animal by-products was in agreement with the findings of previous studies (Balogun and Akegbejo-Samsons, 1992; NRC, 1998; Chiba, 2009; Herdt *et al.,* 2000; Khan *et al.,* 2015). However, deviating results have also been reported (NRC, 2011; Odesanya *et al.,* 2011; Carter *et al.,* 2015). It should be noted that cattle blood was low in most minerals analysed, except for Na and Fe.

The mineral content in agricultural by-products (such as soybean, sunflower seed cake and maize bran) was comparable to that reported for other samples of the same feed ingredients collected in a study in Tanzania (Mutayoba *et al.,* 2011) and reviewed in a study by Chiba (2009). In general, the agricultural by-products analysed in the present study were high in K, while they had a low content of both Na and Mg. A low content of Na in local feedstuffs collected in Western Kenya has been reported previously by Onyango *et al.* (2018). Amongst the agricultural by-products analysed, wheat pollard had a high content for most minerals analysed, but in particular Ca and P.

The mineral content in plant leaves and weeds was comparable to that found in agricultural by-products in the present study. This is in agreement with previous findings (Bhanderi *et al.,* 2016; Onyango *et al.,* 2018). However, varying data have also been reported for commonly used plant leaves in fish farming and in the human diet (Dada and Owonu, 2010; Caunii *et al.,* 2010; Mutayoba *et al.,* 2011; Awol, 2014; Sun *et al.,* 2014; Temesgen *et al.,* 2016).

A very high content of Fe (>2200 mg kg^{-1} dry matter (DM)) was found in the aquatic plants azolla and duckweed. Moreover, water lettuce and duckweed showed a high content of Ca, in accordance with previous findings (Anand and Pereira 2006; Heaton, 2015).

However, varying results have been reported (Rodriquez *et al.,* 2000; Tripathi *et al.,* 2010; Wasangu *et al.,* 2013; Iram *et al.,* 2015; Adelakun *et al.,* 2016). Different growing conditions, genetic factors, geographical zone, efficiency of mineral uptake and stage of maturity can explain differences between studies (Mayer and Gotham, 1951; Bhowmik *et al.,* 2012; Izzati, 2017; Onyango *et al.,* 2018).

In general, the mineral content in spent brewery yeast, sea shells and limestone was in agreement with other studies (Chiba, 2009; NRC, 2011; Alibegović-Zečić *et al.,* 2011; Sacakli *et al.,* 2013; Amorim *et al.,* 2016; Heuzé *et al.,* 2017). Variation in mineral content between studies on seashells can be due to contamination, type and origin of shells, and for limestone on the type and form of limestone used.

Globally, variation in mineral content from one geographical area to another can be due to several factors, such as the variety of plants, stage of plant maturity, soil fertility or culture environment, soil type, mineral concentration of the soil/water, and climate conditions. In addition, processing, storage and possible contamination of samples prior to analysis may have an impact (Berger, 1996; Jumba *et al.,* 1996; Wobeto *et al.,* 2006; Steenkamp and McCrindle, 2014; Abdulkarim *et al.,* 2016; Onyango *et al.,* 2018). It is well documented that soil mineral content varies widely between different geographical zones in Tanzania, due to the presence of volcanic mountains, the Great Rift Valley and several plains and mountains with differences in elevation (Funakawa *et al.,* 2012).

In fish, some minerals can be absorbed from the surrounding water through the gills (Watanabe *et al.,* 1997). This has an impact on the mineral supply and the need for dietary minerals to meet physiological requirements (Robinson *et al.,* 1987). However, the mineral content in water can vary depending on the source (freshwater or seawater). For example, the Fe content in fresh water has been found to range between 0.06 and 44 µg l^{-1}, while that in seawater between 10 and 1400 µg l^{-1} (Terech-Majewska *et al.,* 2016).

Iodine can be absorbed by fish from the surrounding water through the gills, but freshwater fish depend more on a dietary source of I than seawater fish (Watanabe *et al.,* 1997). However, overall absorption of I in fish is determined by stress exposure, age, physiological condition and dietary supply (Terech-Majewska *et al.,* 2016). Moreover, Ca, Mg, K, Na and Fe are readily absorbed through the oral epithelia, gastro-intestinal tract, skin, fins and gills of fish (Tacon, 1987; Cooper and Burry, 2007; Kopp *et al.,* 2013; Terech-Majewska *et al.,* 2016). Absorption of minerals in fish is also dependent on the form in which these minerals are present (organic or inorganic), if they are free or bound (i.e. phytic acid, other minerals), sources (dietary or water) and route of entry (Tacon, 1987). Absorption of Ca is facilitated by dietary lactose and high gastric acidity, absorption of P in plant material is facilitated by the enzyme phytase, which hydrolyses inositol-phosphate to inositol and phosphoric acid, and absorption of inorganic P salts is facilitated by high gastric acidity (Tacon, 1987). However, absorption of Fe is depressed by high dietary intake of phosphate, calcium, phytate, copper and zinc. Moreover, absorption of Fe (non-haem iron) is enhanced by reducing substances such as vitamin C (Tacon, 1987; Terech-Majewska *et al.,* 2016).

The content of P in all feed ingredients analysed, except for limestone, met or was above the requirements of blue tilapia (Robinson *et al.,* 1987). However, for Nile tilapia the level of P supplied by sweet potato would be too low (Furuya *et al.,* 2008). Moreover, only marine shrimp, freshwater shrimp, prawn head waste, fish skeletons, maize bran, rice polish, wheat pollard and duckweed would cover the P requirements of

Table 3. Mineral requirements (per kg feed dry matter) of different tilapia species (NRC, 2011).

Species	P (g)	Ca (g)	K (g)	Na (g)	Mg (g)	Fe (mg)
Blue tilapia	0.3-0.5[1]	1.7-10[1]			0.2-0.7[6]	
Nile tilapia	2.1-7.1[2]				0.07-3.2[7]	24.7-200[4]
Red tilapia	7.6-7.9[3]					23.6-209[4]
Hybrid tilapia		0.6-10.7[4]	0.5-9.7[4]	1.5-1.6[5]	0.03-0.57[6]	

[1]Robinson *et al.,* 1987; [2]Furuya *et al.,* 2008; [3]Phromkunthong and Udom, 2008; [4]Shiau and Hsieh, 2001; [5]Shiau and Lu, 2004; [6]Lin *et al.,* 2013; [7]Dabrowska *et al.,* 1989.

red tilapia (Phromkunthong and Udom, 2008). For Ca, cattle blood, fly maggots, all agricultural products except wheat pollard, cassava leaf, sweet potato, azolla and spent brewer's yeast were all too low in Ca to meet the requirements of blue tilapia (Robinson *et al.,* 1987). All feed ingredients analysed, except seashells, would meet the K requirements of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Shiau and Hsieh, 2001). The Na content in all analysed animal by-products and aquatic plants in the present study met, or was above the requirements for juveniles hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) (Shiau and Lu, 2004). Moreover, not only would animal by-products and aquatic plants meet the Na requirements for hybrid tilapia fish, but also chinese vegetables (plant origin) and sea shells (others). The analysed Mg content in all animal by-products (except cattle blood meal), as well as soy bean, moringa leaves, Chinese vegetables, taro leaves, azolla, water lettuce, spent brewer's yeast, seashells and limestone was sufficient to cover the Mg requirements of blue tilapia (Furuya *et al.,* 2008), Nile tilapia (Dabrowska *et al.,* 1989) and hybrid tilapia (Lin *et al.,* 2013). All feed ingredients would cover the minimum Fe requirements of Nile tilapia and red tilapia (Shiau and Hsieh, 2001). There are no established values for the I requirements in tilapia (NRC, 2011). However, the I content in all feed ingredients analysed would cover the minimum dietary level of 2.8 mg I kg^{-1} recommended for fish in general (Watanabe *et al.,* 1997). Therefore, if more than two local feed ingredients are used in the diet, this may prove sufficient to meet the mineral requirements of all cultured tilapia species and their hybrids (Table 3), without the use of any mineral premix. According to the findings from the present study, apart from animal by-products, the incorporation of wheat pollard with other ingredients such as maize bran, rice polish or other agricultural by-products to supplement the food of farmed tilapia species has proven to meet their mineral requirements for growth and health. The majority of fish farmers in Tanzania usually use maize bran and rice polish as a basic feed component in diets to tilapia. It would therefore be sufficient to add either wheat pollard, Moringa leaf or Chinese vegetables to the feed components above to ensure that the feed contains enough Mg and other minerals to meet the mineral requirement for tilapia.

Conclusions

The analyses performed in this study revealed a wide range of mineral concentrations in feed ingredients used by tilapia fish farmers in Tanzania. These novel data can be used as a platform for better-targeted feed formulation for tilapia farming systems. The data suggest that using more than two ingredients in the diet may prove sufficient to meet the mineral requirements of all cultured tilapia species and their hybrids, without the need for any mineral premix.

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Small-scale milkfish (*Chanos chanos*) farming in Kenya: An overview of the trends and dynamics of production

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Abstract

A number of donor-funded projects by NGOs, government departments and faith-based organizations have piloted milkfish farming along the coast of Kenya at different scales with the aim of addressing poverty and food security at the community level. This paper provides an overview of the history of milkfish farming, organisation of operations, funding, farmers trends, and production dynamics, using both secondary and primary data. Primary data were obtained from quantitative and qualitative assessments covering 26 community milkfish farming groups, while secondary data were obtained from the grey literature and donor-funded project reports. Additional primary data were obtained through 9 key informant interviews covering three coastal Counties, and focus group discussions (8-10 members) at all the community milkfish farms. The study established that milkfish farming could be traced back to the early 1980's as a by-product of the prawn farming intervention by FAO, and later in other private farms that practised prawn farming. Community/village based small-scale milkfish farming was initiated about two decades ago using organised community groups (OCGs) as the entry point. The number of OCGs undertaking milkfish farming fluctuated with availability of donor funds and increased significantly between 2007 and 2015 with an increase in earthen pond farming area from 0.9ha to 6.8ha over this period. However, the scale of production remained low. Annual milkfish production increased from less than 50kg in 2005 to a maximum of 3.2 tons in 2015. Milkfish farming has not attained production levels that can address poverty and food security issues; a fact that was associated with the lack of reliable milkfish seed for stocking culture facilities. Harvests from existing farms are sold at the farm gate and in nearby market centres with a few communities venturing into opening fish shop outlets to sell their produce.

Keywords: Milkfish; scale; farming; dynamics; production

Introduction

The contribution of aquaculture to total fish production has steadily increased over the years due to the recruitment of additional species of aquatic animals and plants for culture, leading to this food production sector becoming the fastest growing in the world (FAO, 2016). Globally, aquaculture accounted for 44.1% of total production from capture fisheries and aquaculture in 2014, increasing from 31.1% in 2004 and 42.1% in 2012 (FAO, 2016; FAO, 2012). Tilapia, milkfish, catfish, carps and marine molluscs have been observed to contribute significantly to global aquaculture output from fresh and marine waters, (FAO, 2017; Naylor *et al*., 2000). Aquaculture employs a large number of people, especially in developing countries (FAO, 2003; Rana *et al*., 2009; FAO, 2012).

Whereas aquaculture production is increasing globally, there are large regional differences. In 2010 significant food fish aquaculture production took place in Asia (53.3 million tons, representing 89 % of world production) and Europe (2.5 million tons, 4.2 %) while Sub-Saharan Africa contributed only 0.6 % of the global output (FAO, 2012). Unlike many Asian countries, Sub-Saharan Africa has a limited history in aquaculture, which still remains largely under developed (Brummett and Williams, 2000; FAO, 2012).

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As mariculture development in the Western Indian Ocean countries remains slow (Troell *et al.*, 2011), there is a need for investments in research and extension to ensure development of the sector in order to improve income and food availability for poor coastal communities (Mirera, 2011a). However, at the same time, the integrity of the environment must be maintained (Ronnback *et al.*, 2002; Mirera, 2009; Mirera and Ngugi, 2009; Mirera, 2016). In this context there is a conflict between the mariculture methods commonly employed in Sub-Saharan Africa and traditional capture fisheries, as mariculture is based to a large extent on collecting seed stock (Milkfish, mud crabs, prawns) and feed from the wild (de Boer and Longamane, 1996; Mgaya *et al.*, 1999; Carle'n and Olafsson, 2002; Rice, 2003; Mirera, 2011a).

To counter these negative impacts, it is preferable to cultivate low-trophic-level marine species like milkfish, even though they fetch a low price and are not suitable for export. Globally, milkfish production is ranked 9th in quantity produced and contributes 3.63% of world aquaculture production, excluding China (FAO, 2017).

Milkfish culture has attracted considerable attention for marine finfish farming in East Africa because it tolerates wide environmental conditions, and seed is available from the wild (Mirera, 2011a). Some progress has been made in milkfish research in the western Indian Ocean (WIO) by establishing seasonal growth rates in earthen ponds, feed formulation and composition, response to feeds in laboratory conditions, fingerling occurrence, and tolerance to varied water quality in intertidal ponds (Mirera, 2016, 2011a; Mirera and Ngugi, 2009; Mirera, 2007; Mwaluma, 2003; Mwangamilo and Jiddawi, 2003; Mmochi *et al.*, 2002).

This study provides information on the history and organisation of milkfish farming in Kenya, the scale and dynamics of production, farming systems, factors influencing the industry, production trends and marketing systems.

Materials and methods

Kenyan milkfish farming is diverse and is influenced by the history of milkfish farming, farming systems used, inputs and scale, approaches to milkfish farming, gender, literacy, funding aspects, trends in production, culture area and marketing of the harvest. A combination of methods was employed to obtain primary and secondary data that was analysed to provide results discussed in this study.

Secondary data

Data from several project reports, farm records and the grey literature was collected in an attempt to better understand production per unit area, changes in farming area and groups, annual production statistics and farming systems. The reports were sourced from different organisations and projects that have undertaken milkfish farming along the coast of Kenya. Data and reports were obtained from the Kenya Marine and Fisheries Research Institute (KMFRI), Kwetu Training Centre, Coastal Oceans Research and Development in the Indian Ocean, East Africa (CORDIO-EA), State Department of Fisheries, Aquaculture and Blue Economy, OCGs where farming has taken place (farm records), and donors that have directly implemented projects at a local level. Data from these sources were combined with that from published literature to calculate production per unit area, trends in different areas, and annual production and donor support over time.

Primary data

Semi structured interviews were administered during focus group discussions with farmers and key informants to collect quantitative and qualitative data. Focus group discussion were conducted in 26 OCGs (OCGs have 20-70 members) undertaking milkfish farming in the three counties of Kilifi, Mombasa and Kwale. This tool provided descriptive statistics on the farmers, gender distribution among the OCGs, literacy levels, age, farming systems, types and sources of inputs like feed and seed, stocking cycles, scale of production, annual production trends and marketing aspects of the harvested milkfish.

The focus group discussions enabled a deeper understanding of small-scale milkfish farming in Kenya since it was designed to verify estimated productivity data in different project reports and the grey literature.

Key informant interviews engaged major players in the small-scale milkfish farming industry from each of the farming counties. The key informant interviews were used to capture how an individual relates different variables to each other within a system as a way of exploring individual beliefs (Axelrod, 1976). The tool aimed to establish the drivers behind milkfish farming in Kenya, lessons learnt, and community perceptions on the scale of milkfish farming in relation to the eradication of poverty and ensuring food security. The key informants were drawn from the communities and NGOs working in the respective counties. Semi-structured interviews using closed and open-ended questionnaires were used to collect data from the key informants. The questions focused on the scale of production of milkfish, role of funding, lessons learnt, main drivers informing participation in milkfish farming, challenges, management of milkfish enterprises, and market systems.

Results

History of milkfish farming in Kenya

The history of milkfish farming on the coast of Kenya is three pronged: (a) Private/ none governmental organizations (NGO); (b) government departments; and (c) community interventions. Private farmers, NGOs and government departments became aware of the potential for milkfish farming through the FAO-funded prawn farm project at Ngomeni, Kenya in the early 1980s. Milkfish production was an un-intended by product from the initial prawn farms. It was considered a low value fish that was given to farm workers and the local community for free or at minimal prices. Almost two decades after the collapse of the FAO prawn farm, Mwaluma (2003) working at the Kwetu Training Centre on mud crab experiments, observed milkfish fingerlings in an intertidal mud crab pen that had limited water exchange at neap tides, and recommended investigation of the species for aquaculture.

The study led to the development of small-scale community milkfish farming that followed a different approach to that of the FAO and private prawn farming enterprises. In agreement with Mwaluma (2003), communities exploiting intertidal mangrove resources observed fish in the intertidal mangrove pools at neap tides where there was minimal tidal exchange. During such periods children fished in the pools to obtain fish for domestic consumption. Some of these areas were deepened in Makongeni village, Gazi Bay with support from local conservation and development NGOs, leading to diverse fish communities in the artificially created mangrove pools (Table 1). Thus, small-scale fish farming developed in intertidal earthen ponds of around 120m^2 in size. The ponds were fertilised with organic manure (Table 2). The initial farming process established that 33.3% of the species stocked did not survive to harvest, and only 20% recorded significant growth, including milkfish (Table 3).

Farming systems and management

Milkfish is farmed in earthen ponds that are constructed in intertidal mangrove flats free of mangrove trees. The ponds are fed naturally by the rising tides, and drained completely at harvest during low tides using standpipes fixed at the bottom of the ponds. Water height in the ponds is modulated using overflow pipes that are fitted with screen nets to control predators and fish escapes from the ponds at high spring tides. More than 80% of the farmers stock milkfish extensively and fertilize ponds using organic manure. However, all the farmers feed their fish with formulated feeds and fertilize ponds using organic manure if donor funding is available.

Farmers use seine nets in the natural mangrove channels/pools to obtain fingerlings for stocking ponds. Previously, farmers had challenges in identifying the culture species at stocking, but constant training has

Table 1. Fish species found at Makongeni, Gazi Bay Intertidal pools during a random survey using screen nets.

Local name (Swahili)	Common name	Scientific name/family
Mkizi	Mullets	*Crenimugil crenilabils*
Kole kole	Trevally	*Carangoides orthogranus*
Kidara	Jack/Trevally	*Carangoides dinema*
Chaa	Silver belly	*Gerres sp.*
Ngagu	Grunt	*Terapon Jarbua*
Bunju	Puffer fish	*Arothon spp.*
Kiunga	Snapper	*Lutjanus argentimaculatus*
Kamba	Prawns	*Peneaus Monodon*

Table 2. Species of fish stocked in Makongeni intertidal earthen ponds to test survival and growth.

Local Name	Common Name	Scientific/family name
Mwatiko	Milkfish	*Chanos chanos*
Mkizi	Mullets	*Crenimugil crenilabis*
Chaa	Silver belly	*Gerres spp.*
Bunju	Puffer	*Arothron spp.*
Nyembe nyembe	Sweeper	*Pempheris oualensis*
Ngagu	Grunt	*Terapon Jarbua*
Kole kole	Trevally	*Carangoides orthogramus*
Chuchungi	Halfbeak	*Hermiramphus spp.*
Kamba	Prawns	*Peneaus monodon*
Kisumba	Barracuda	*Sphyraenidae*
Kiunga	Red snapper	*Lutjanus argentimaculatus*
Tuguu	Surgeon	*Acanthuridae*
Pamamba	Grunters	*Haemulidae*
Dizi	Scavangers	*Lethrinidae*
Kidara	Jack/Trevally	*Carangoides dinema*

enabled farmers to perfect their identification skills and they are now able to stock only milkfish as the preferred species. Due to seasonality and the inability to obtain enough fingerlings, 70% of the existing fish ponds in each group have one crop in a year, and in most cases the stocking densities are low (1.5-2 fish/m^2). Farmers are only able to produce two crops per year and at a stocking density of 3 fish/m^2 when there is donor funding to mobilise resources for seining of fingerlings at the required time. Information from focus group discussions indicated that farmers in OCGs are able to attain 100% attendance in weekly farmers meetings if donor funding is available, while only 40-50% attendance is registered in the absence of donor support.

More than 70% mortality was experienced in the farms at inception of community milkfish farming due to poor location of fishponds and predation from carnivorous fish and birds. Currently the farmers lose 30-40% of the stocked fish due to bird predation and poor water quality, especially in the earlier stages of production, and water management to control extreme water quality parameters at neap tides during the dry season (January–March). Key informant interviews indicated that losses of fish through bird predation and poor water management could be reduced to below 15% if donor funding is available to employ guards.

Farming and production dynamics

Small scale milkfish farming is undertaken in the three coastal Counties of Kwale, Mombasa and Kilifi. The number of OCGs participating in milkfish farming has increased over time from 9 in 2007 to 26 in 2015 (289%). Consequently, the area covered by milkfish ponds increased from 0.9ha in 2007 to 6.8ha in 2015 (Figure 1).

Despite the expansion in the number of communities involved, ponds and culture area, production per square meter has remained significantly low with slight increments over the years, while the quantity produced is inconsistent and related to the availability of donor funds (Figure 2, 3; Table 5). Production per unit area has varied between 0.06-0.2kg fish/m^2 in 2007 and 0.08-0.45kg fish/m^2 in 2015, with increasing annual production. Consequently, total milkfish production has increased from less than 0.05 tons annually in 2005 to more than 3.2 tons in 2015, generating direct income of USD7,600 per year. In comparison with other mariculture species on the coast of Kenya, milkfish production accounts for about 13.2% of the total production from mariculture (Figure 4).

Table 3. Summary of fish stocked, mortalities observed and average size (g) harvested after six months of culture in earthen ponds at Makongeni, Baraka Conservation group mariculture site.

Local name	Common name	Stocking size (g)	Mortality noted	Average size at harvest (g)
Mwatiko	Milkfish	8	No	350
Mkizi	Mullet	5	No	28
Chaa	Silver belly	20	Yes	10.5
Bunju	Puffer	22	Yes	-
Nyembe Nyembe	Sweeper	10	Yes	-
Ngagu	Surgeon	8.5	Yes	21.3
Kole kole	Trevally	11.5	Yes	-
Chuchungi	Halfbeak	4	No	15
Kamba	Prawns	3	No	40
Kidara	Jack/Trevally	2.5	Yes	-
Kiunga	Red snapper	5	Yes	96
Pamamba	Grunters	6	Yes	80
Dizi	Scavengers	3	No	15
Tuguu	Surgeon	2.5	Yes	28.1
Kisumba	Barracuda	5	Yes	-

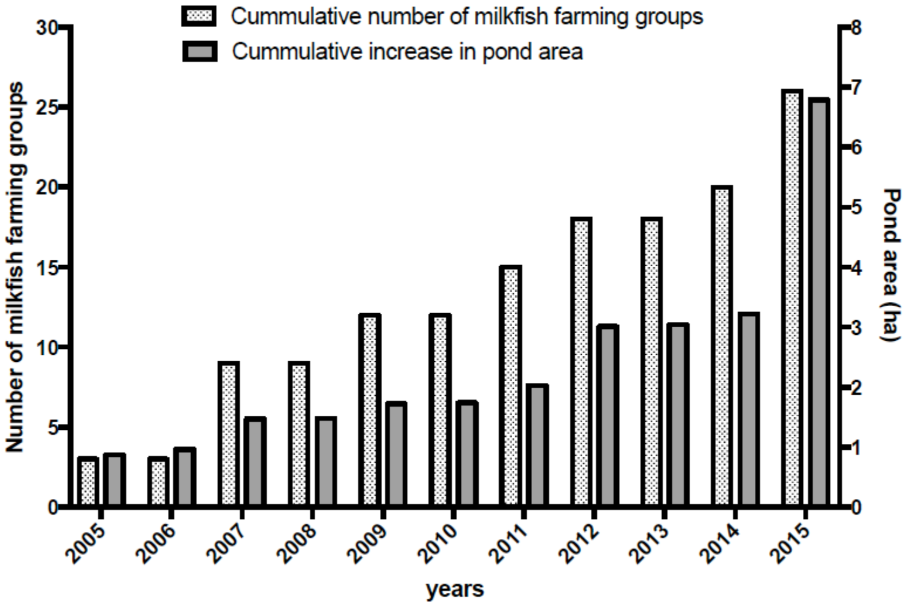

Figure 1. Cumulative increase in (a) number of communities undertaking milkfish farming, and (b) pond area used along the Kenyan coast. (Source: Mirera, 2007; Mirera and Ngugi, 2009; Grey literature from Kwetu training centre mariculture reports, community production records, KCDP baseline assessment statistics, State of mariculture WIOMSA report)

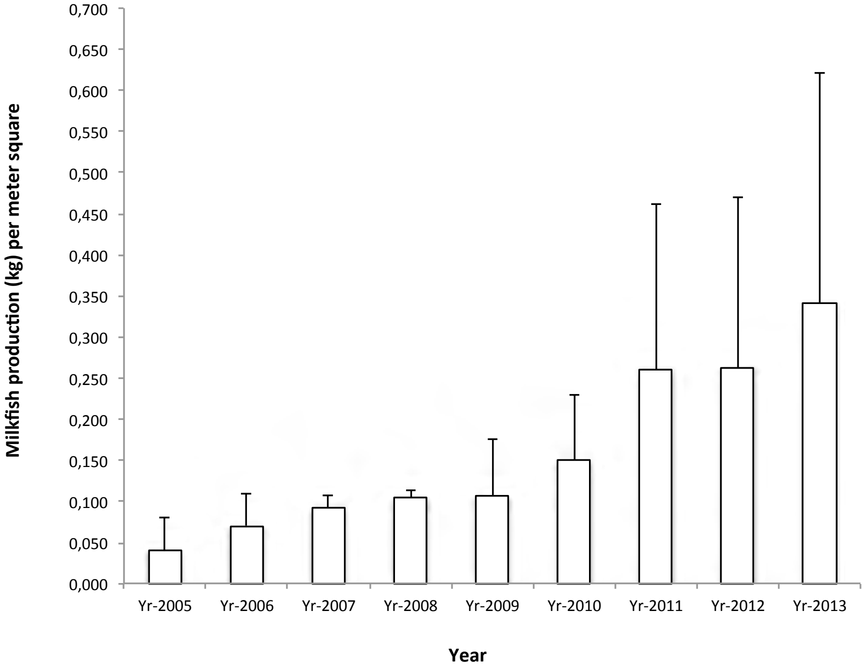

Figure 2. Change in small-scale community-based milkfish (*Chanos chanos*) production (kg/m^2) along the coast of Kenya between 2005 and 2013. (Source: Mirera, 2007; Mirera and Ngugi, 2009; Grey literature from Kwetu training centre mariculture reports, community production records, KCDP baseline assessment statistics, State of mariculture WIOMSA report)

An increment in the number of entrants into milkfish farming and changes in farming area between 2005 and 2013 was observed to be directly proportional to the number of new donors supporting milkfish farming (Table 4). Several donors supported development of milkfish farming in 2007 leading to a higher number of entrants and a large percentage of the area under milkfish farming (33.3%). Also, the amount of funds available for milkfish farming research had a direct impact on the increase in production (kg/m^2), but did not significantly impact on the number of communities participating in farming, or an increase in culture area. Donor or research support increased the spread of milkfish farming technology to other

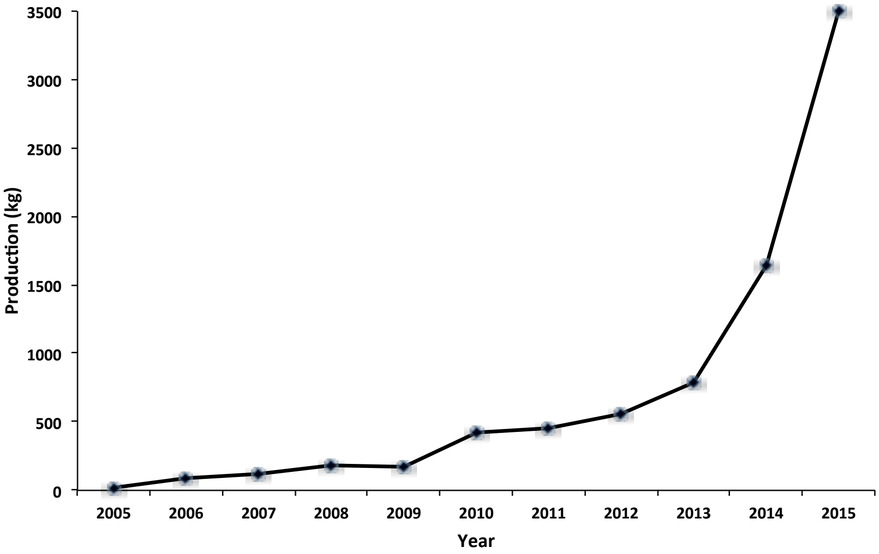

Figure 3. Trend in milkfish production along the coast of Kenya between 2005 and 2015. The figures are based on the total harvests by the different villages along the coast of Kenya. (Source: Mirera, 2007; Mirera and Ngugi, 2009; Grey literature from Kwetu training centre mariculture reports, community production records, KCDP baseline assessment statistics, State of mariculture WIOMSA report)

Table 4. Details of new community entrants into milkfish (*Chanos chanos*) farming in relation to source of funds for development and research between 2005 and 2013. (Source: project related reports and KCDP baseline survey report)

Year	Percentage of new entry into milkfish farming	New development funds available to support milkfish farming	New research funds available for milkfish farming
Base year 2005	11.5	Sida Sarec through CORDIO EA,	None
2006	0	None	None
2007	23.1	Toyota conservation, Diakonie – Tsunami, FAO, Rufford grant, WWFEFN	WIOMSA, CHE
2008	0	None	None
2009	11.5	ReCoMaP, UNDP	None
2010	0	None	None
2011	11.5	CAST Italy	NCST
2012	11.5	CDTF/KCDP	KCDP
2013-2015	30.8	KCDP/FAO	KCDP

* FAO – Food and Agricultural Organisation of the United Nations, UNDP – United Nations Development Program, KCDP – Kenya Coastal Development Program, CDTF – Community Development Trust Fund, ReCoMaP- Regional Coasts Management Programme, NCST – National Council for Science and Technology, CHE – Commission for Higher Education, WIOMSA – Western Indian Ocean Marine Science Association, CORDIO EA – Coastal Oceans Research and Development Indian Ocean, East Africa.

areas. For example, milkfish farming at Kibokoni was initiated through a National Council for Science and Technology (NCST) funded research project in 2013, while CAST introduced milkfish farming at Ihaleni.

Socio-economic aspects of milkfish farming

Milkfish farming is the most common type of mariculture activity practised along the coast of Kenya, compared to seaweeds, artemia, mud crab or prawn farming. Milkfish farming is practised in the intertidal mangrove flats which are designated government lands in all coastal counties except Lamu and Tana River. Through the Kenya Forest Service (KFS), the goverment has introduced user rights in intertidal mangrove flats for aquaculture development, as a strategy to improve mangrove management while developing the aquaculture sector in the country. In all farming areas milkfish culture is extensive and farmers operate on a small-scale level using conventional fish farming methods in ponds ranging between 120m^2 and 1,200m^2.

OCGs have a mixed composition of men and women. Percentage of women representation in milkfish farming ranged between 40 – 80 % depending on counties; the highest being in Kwale, Kilifi and Mombasa respectively. The mean age of farmers in OCGs was 40 years with the eldest being 79 and the youngest 18. Most farmers were middle aged (36-65), while youths (18-35) comprised 37%, and the elderly (above 66) formed 3 %. About 63.7% of the farmers had attained a primary level of education and 2.8% had no education at all. 28.2 % had obtained secondary education, 2.5 % madrassa (Islamic education), and 2.8% tertiary education.

Milkfish farming supports more than 1 400 people directly, and about 3000 indirectly, through small-scale businesses, seed collection, and Motocycle “poda poda” transport. It was observed that milkfish farming had led to the development of infrastructure such as roads, which were non-existent in some communities before the milkfish farming project (e.g. Kibokoni). The percentage of children going to school also improved over the years in some villages where milkfish farming was introduced.

Small-scale milkfish farming initiatives along the coast of Kenya have been facilitated by government and NGOs which have directly funded the projects

Table 5. Aquaculture production and employment in selected villages along the coast of Kenya between January 2005 and December 2013.

Village	**Aquaculture production (kg)**									**Employment (on farm-jobs) 2013 x (a, b)**
	2005	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	
Makongeni baraka shelf help group	4.6	15.7	45.8	56.9	33	256	150	350	480	20 (4, 16)
Kwetu Training Centre (NGO)	12.2	41.46	-	18	70	100	160	80	60	8 (6, 2)
Majaoni youth development group	-	30	76	91.65	48	30	95	32	40	13 (10, 3)
Abent youth group	-	-	-	10	22	31	45	48	60	17 (13, 4)
Kibokoni, Umoja self-help group	-	-	-	-	-	-	-	38	116	70 (15, 55)
Ihaleni Conservation group	-	-	-	-	-	-	-	8	25	20 (13, 7)
Total	16.8	87.2	121.8	176.6	173	417	450	556	781	148 (61, 87)

Source: Mirera, 2007; Mirera and Ngugi, 2009; Grey literature from Kwetu training centre mariculture reports, community production records, KCDP baseline assessment statistics.
Note: x (a, b) = Total (Men, Women).

or offered guidance while doing research and training/extension. About 81% of the milkfish farmers had received support for their interventions, while 19% had not received any support (Table 4).

A market for milkfish is locally available even though prices are low (2.4 – 3.5 USD/kg). Most of the harvested milkfish are sold at the farm gate, and in rare cases in local markets and in fish shops owned by the farmers themselves that have been developed through support from the Kenya Coastal Development Project (KCDP). The local farm gate and fish shop market includes consumers from within the villages, dealers, hotels and fish mongers.

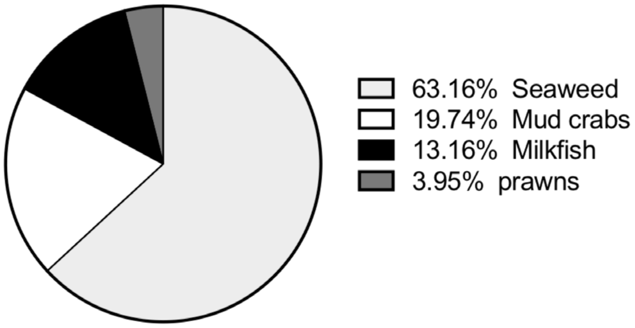

Figure 4. Current annual mariculture production in Kenya showing percentage contribution by species. (Source: State of mariculture WIOMSA report)

Discussion

History of milkfish farming in Kenya

Coastal communities have fished in intertidal mangrove pools for fish and shellfish for centuries to meet their subsistence needs. Over the years, communities have developed significant traditional knowledge concerning their environment. However, with increasing population and the increasing number of widows and orphans, intertidal resource harvesting in Kenya has in recent years experienced entry of children and women to meet the emerging and expanding family needs (Mirera 2011b; Mirera *et al.*, 2013). This increase in pressure on resources is likely to lead to serious problems of over-exploitation in the future. Even though most fishers are aware of the consequences of juvenile fish and shellfish collection for food in the mangrove pools, few are ready to take action – an example of the tragedy of the commons (Hardin, 1968).

Although marine fish farming is generally a small-scale activity in Kenya, it has the potential of becoming one of the highest producing sectors if the initial FAO commercial prawn farming interventions could be effectively developed (Mirera, 2011b). Fish farming in intertidal earthen ponds has rapidly developed over the last one and half decades as a small-scale intervention (Mirera, 2011b). However, milkfish was initially a by-product from private prawn farm activities and no studies were done to understand growth and survival of the fish. In this study together with previous studies, several species were observed to use mangroves as nursery grounds (Mirera *et al.*, 2010; Huxham *et al.*, 2004). Of the 15 species stocked in this study, milkfish, grunters and mangrove red snapper recorded good growth in intertidal earthen ponds. Milkfish had the highest growth of the three and has been found in large numbers within the mangrove creek channels in previous research studies in Kenya (Mirera, 2011a).

Initial attempts of small-scale milkfish farming involved the use of small earthen ponds (120m^2) constructed in open intertidal mangrove flats (Mwaluma, 2002; Mirera and Ngugi, 2009; Mirera, 2011a). The sizes of ponds remained constant from inception until 2015 when commercial milkfish ponds of 1,200m^2 were constructed with support from the KCDP. Thus, the scale of milkfish production has largely remained at the pilot stage and only minimal production was attained from the many development interventions dedicated towards milkfish farming in Kenya. According to Woltering *et al.* (2019), a major problem that undermines efforts to achieve scale in development projects is the fact that pilot projects are set up and managed in very controlled environments that do not reflect the reality at scale. Research and development organizations working on poverty alleviation and food security face growing scrutiny to demonstrate a return on investment from their work, especially in developing countries (Hurst *et al.*, 2017; Moyo, 2010)

Farming systems and management

For the last four decades milkfish has mainly been farmed in intertidal earthen ponds. During spring high tides when intertidal mangrove flats are flooded with ocean water, milkfish ponds are allowed to fill using overflow pipes and standpipes. A number of fish species enter the ponds with this sea water, and initially farmers stocked both herbivorous and carnivorous fish together in the same ponds. In the absence of milkfish hatcheries that are able to supply quality seed, all the farmers stocked mixed fish species in ponds, and had limited ability to identify milkfish and separate them from other species. Predation between species was therefore high leading to high mortalities in culture ponds. Also, the more sensitive species died due to fluctuations in water parameters like salinity and temperature which were not effectively monitored at the initial stages of small-scale milkfish farming.

Management of milkfish earthen ponds has previously been minimal and limited to preparation of ponds for stocking using lime, fertilisation with organic manure, stocking, sampling and harvesting of fish. This led to low production of fish per unit area. However, with improved management that included water exchange at spring high tides, artificial fertilizers, feeding, proper fish identification at stocking, and predation management, production per unit area and production capacity has been enhanced. Feeding of milkfish has been progressively improved from the use of non- formulated feeds such as wheat bran to the use of formulated feeds, although the quality of these formulated feeds still needs to be improved (Mirera, 2008). Improved management has resulted in higher production and the consequent increase in fish sales at the farm gate or in the OCGs shop outlets.

Even though management of earthen ponds has improved and some positive progress observed in milkfish production, significant effort is required to attain quantities that can adequately contribute to food security and poverty alleviation in Kenya. Woltering *et al.* (2019) underscored the fact that agricultural innovations, such as improved seed, better management systems and practices, and collaborations need to be tested at pilot scale, and once successful, they must be scaled-up to address the envisioned size of the problem. However, in the case of milkfish farming in Kenya, most projects never scale-up to achieve the expected impact, since support ends after the demonstration phase (Cooley and Howard, 2019; Spicer *et al.*, 2018). This could be associated with the fact that the number of donors and projects has doubled, while the project terms and budgets have significantly reduced in the last decades (Cooley, 2018).

In order to allow milkfish farming to contribute to food security, growth and development in the long term, it should be sustainable - not only technically feasible and economically viable but also environment-friendly, socially equitable and introduced at the required scale. Challenges that need to be addressed to allow sustainability include ensuring constant seed supply, quality feed, increasing production per unit area, and increasing the scale of production (Woltering *et al.*, 2019; Platon *et al*, 2006).

Production dynamics

Small-scale aquaculture has been seen as a way of improving food security and other welfare aspects of poor coastal communities (Ahmed *et al.*, 1999; Ahmed and Lorica, 2002). Being a capital-intensive venture, aquaculture development needs financial support to be effective in poor households in coastal East Africa. In the current study, the increment in number of aquaculture farmers (groups) and farming area was found to be closely related to the increase in donor funds supporting milkfish farming, especially through livelihood and mangrove conservation projects. This suggests that milkfish farming could contribute meaningfully to the food and nutritional status of people through income, employment and consumption linkages (Ndanga *et al.*, 2013; Holdren, 2011; Ahmed and Lorica, 2002).

In Africa, government and donor-driven aquaculture projects have most often targeted small-scale farmers in an attempt to ensure food security at the household level (Jamu *et al.*, 2012; Brummett *et al.*, 2008; Hishamunda, 2007; Moehl *et al.*, 2005). The same has happened in Kenya, where despite the increase of culture area under milkfish farming over time, production/m^2 and the scale of production has remained low with small and inconsistent increments over the years dependent on the availability of donor funds. It should also be noted that the presence of research funds significantly increased milkfish production/m^2, but did not increase the scale of production. Observing these trends, Beveridge *et al.* (2013), Brummett *et al.* (2008), and Hecht (2006) argued that the extensive nature of aquaculture systems in Africa has limited investments in the sector and thus impacted supply of quality inputs and the scaling-up of enterprises.

The current study observed that there were annual increments in milkfish production over the years until 2013, but still less than 1 ton of fish was produced, a fact that was associated with the small-size of ponds used in the farming, an inability to stock fish to the required densities, and poor feeding strategies. This indicates that most interventions in Kenya have either remained at pilot or research scale, and have not been able to move to the next level of meeting the food security objective. Despite the importance of scaling research or pilot projects to meet development objectives, successful examples are scarce; a factor discouraging further donor funding into research and development. Scaling is assumed to occur spontaneously or organically when pilot scale projects are successful (Wigboldus and Brouwers, 2016; Chandy *et al.*, 2013). According to Buntaine *et al.* (2013), this is a result of donor impatience to see on-the-ground results that directly link adoption to impact, thus encouraging research and development projects related to agriculture to focus on simple and visible inputs and outputs, rather than on form and function (Maru *et al.*, 2018; Spicer *et al.*, 2014).

Rey-Valette *et al.* (2008) argue that aquaculture production and sustainability is a continuous process; a 'journey' rather than a destination in terms of a sustainable, final and ideal aquaculture product. Compared to the Philippines, for example, milkfish culture is in its infancy in Kenya (Mirera, 2009; Bombeo-Tuburan and Gerochi, 1988). Milkfish farming can be traced back to the 1940's in south-east Asia when production was relatively low at around 0.007kg/m^2, but this has increased progressively over time to 0.06kg/m^2 and the industry is considered sustainable (Bombeo-Tuburan and Gerochi, 1988).

Socio-economic aspects of milkfish farming

Since its initiation, community milkfish farming has been embraced as a major livelihood activity and a motivator for the conservation of mangrove forests along the coast of Kenya. Indeed, the co-management approach has been adopted in community milkfish farming; as advocated for mangrove systems in the Forest Act of 2007 and 2016, respectively. According to Slater *et al.* (2013), the social and economic drivers that determine if farmers choose aquaculture as a livelihood option include gender establishments, social network strength, material style of life and the time available for a supplementary livelihood. Another key driver of milkfish farming along the coast of Kenya is population growth. The coastal region of Kenya has a population of 3.3 million people, growing at the rate of 2.9% per annum according to the 2009 census (Republic of Kenya, 2010). In addition to this natural population growth, the coastal areas also experience significant immigration due to the diverse livelihood opportunities like fishing, tourism, business and hospitality. This large population depends heavily on the catch of the small scale and subsistence fishers, whose catch has declined over time (Malleret-King *et al.*, 2003; Mangi and Roberts, 2006). To meet the fish deficits and be able to feed families milkfish farming has been embraced mainly by the youth and women. This finding is similar to that observed elsewhere where the growing strength of domestic markets due to a rising demand for fish by middle class populations is cited as one of the factors enabling aquaculture development in Africa (FAO, 2016; Tschirley *et al.*, 2015; Hecht, 2006).

Significant progress has been made in developing technologies for improved milkfish farming, but there are still limitations hindering development of the industry. This study found that most milkfish farming is carried out in the intertidal areas that are 95% government-owned, with minimal private land ownership. Even though the communities have traditionally used these areas, they lack tenure rights to encourage them to invest or significantly modify these areas for aquaculture development. Further, most areas lack legal access routes due to blockage by private investors who own the sea front. The situation is further confused by conflicting mandates and legislation concerning control of areas where milkfish farming takes place. For example, intertidal areas are the responsibility of the Kenya Forest Service if they are bordering or contain mangrove trees, while the National Environment Management Authority, Ministry of Lands and Planning, and the Department of Fisheries Development, Aquaculture and Blue Economy currently also claim authority over the same areas. The new Constitution of Kenya (2010) which created a system of National and County government provides another dimension of uncertainty with regards to sea-based aquaculture areas. Indeed, FAO (2016) underscores the significance of land tenure in development of agriculture, fisheries and forestry. The uncertainty with regard to land tenure in coastal Kenya is considered a major hinderance to the scaling-up of donor-dependent interventions, ensuring that they remain temporary and struggle to reach economic sustainability, as alluded to by Woltering *et al.* (2019).

Milkfish market and trade

Marine fish forms a popular delicacy in the traditional diet of the coastal people in Kenya. According to Karuga and Abila (2007), the main market segments for capture marine fish in Kenya include the domestic/institutional fish market, the domestic processed fish market, the export processed market, and the fishmeal market, with household fresh fish market and hotel/restaurant fish markets being important outlets. However, no market value chain assessment has been done for farmed fish (freshwater and marine) at the coast. This study established that farmed milkfish market outlets were mainly from the farm gate, targeting household members and the surrounding villages, and fish are sold fresh. Recently, with increased production, some farmers are diversifying markets to local shop outlets to reach other clientele, often in the frozen form. Farmers have opened shop outlets and store milkfish in cooler boxes, selling to community members, local hotels or institutions like schools, colleges or companies. These market outlets offer the highest potential for growth, and therefore income generation to the farmers, and thus may need to be nurtured in the future.

The household fresh fish market segment tends to be most useful to the villagers in need of daily food, as it operates throughout the year, irrespective of season. The prices for farmed fish vary according to season with higher prices (2.5-3.0 USD/kg) realized during the season when capture fisheries are less active, compared to the seasons when capture fisheries are more active (1.8 – 2.5 USD/kg). To capitalize on this, farmers could schedule their harvest and sell produce when prices are better. The market for milkfish, mainly relying on demand from local communities, appears to be more resilient, as it is not affected by the market dynamics associated with tourist hotel industry or export market, for example. This suggests that farmed marine fish could have enormous market potential if the current domestic segment can be fully exploited, in addition to opening other market segments available for marine fish along the coast and inland.

Conclusion

The findings of this study show that milkfish farming is undertaken through OCGs. Milkfish production has progressively increased in terms of quantity produced and area farmed, while production per unit area is still low. Farming is practiced at subsistence level, and extensively, contributing more to the food security of the communities, rather than to economic gains. Milkfish farming needs to be scaled-up for economic benefits. Entry into milkfish farming is mainly driven by existence of donor funds rather than benefits gained from successful interventions; a fact that has led to stagnation of production, despite significant efforts from development/ conservation organizations and government. Current production is sold either at the farm gate or in local outlet shops, initiated by farming groups.

Milkfish farming is faced with challenges such as relying on wild caught seed, leading to the inability to stock ponds at appropriate stocking densities. There are also challenges related to fish feeds with most being produced locally and containing inadequate nutrients. There is a need to assess the existing extension frameworks and provide appropriate options that can address the existing challenges of low production and dependency on donor funds if the milkfish farming sector is to grow. Further, a more thorough analysis of the economics of rural, small-scale milkfish farming is required to understand the current status and trajectory. With the enhanced production and availability of input supplies (mainly seed and feed), it is suggested that the milkfish industry can provide sufficient food and income requirements to local fish farmers in Kenya.

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Natural recovery of mangroves in abandoned rice farming areas of the Rufiji Delta, Tanzania

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Abstract

Natural recovery of mangroves in abandoned rice farming areas is important for reviving its multiple ecosystem services including climate change mitigation. This study aims at understanding the natural recovery rate and pattern of mangroves in the abandoned rice farming areas of the Rufiji Delta. Mangrove areas were stratified into early and intermediate succession as well as old growth forest. Forty-five nested plots, fifteen at each site, were randomly selected, where plant and soil data were collected. Based on the Importance Value Index, the most abundant tree species in the early succession was *Barringtonia racemosa*, a mangrove associated tree species having a value of 67.9. The mangrove *Avicennia marina* was the most abundant in both intermediate succession and old growth forest having a value of 170.7 and 163.1, respectively. Pairwise comparison of means indicated a significant change ($p < 0.05$) of structural parameters with fallow age. No significant change ($p > 0.05$) was detected in the measured soil properties among the three succession categories. The findings demonstrate that even a period of up to 15 years would not allow full recovery of structural attributes for a mangrove forest converted to agricultural land, with grass cover being among factors limiting the recovery due to obstruction of propagule dispersal.

Keywords: Succession; structural attributes; soil properties; fallow; abundance

Introduction

The mangrove ecosystem in the Rufiji Delta, Tanzania, has over the years been severely affected by a complex set of factors, including climate change and anthropogenic pressures, triggering their loss (Wagner and Sallema-Mtui, 2016). The anthropogenic threats include over harvesting of mangrove trees (for fuel wood, building materials), conversion of mangrove areas for other uses, such as salt works, human settlements and agriculture, and in particular rice farming (Semesi, 1992; Mangora *et al.,* 2016). Rice farming is reported to be a major cause of mangrove cover loss in the delta. Nindi *et al.* (2014) estimated that 4,140 ha of mangroves was cleared for rice farming between 1989 and 2010. Most recently (Monga *et al.*, 2018) reported the loss of mangroves due to rice farming at about 7,298 ha between 1991 and 2015, implying an annual loss of about 304 ha per year.

Similar trends in mangrove losses due to conversion into other uses are reported from other places. In particular, South-East Asia has been in the spot light where conversion for aquaculture has led to unprecedented losses of mangroves, with about 22,000 ha lost between 2000 and 2012 (Richards and Friessa, 2016). Likewise, in Guinea (West Africa) about 64,000 ha has been converted to rice farming (Adesina and Baidu-Forson, 1995). Mangrove deforestation also has implications for the provision of ecosystem services (Richards and Friessa, 2016), as it results in a loss of carbon (Lagomasino, 2019) and other biodiversity supported by the mangroves.

Despite these reported losses, gains from natural recruitments have also been reported to compensate the losses, reducing the net loss. (Lagomasino *et al.*, 2019; Monga *et al.*, 2018). Monga *et al.* (2018) reported an average mangrove cover gain of 109 ha

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from 1991 to 2015 in the Rufiji delta. Mangrove forest gain occurs through natural regeneration (Lagomasino, 2019) and planting (Monga *et al.*, 2018).

Rice farming has been practiced in the Rufiji Delta in areas close to fresh water sources by converting the mangrove forest to farmland (Lagomasino, 2019). Due to poor agronomic knowledge, the farming approach in the delta is of a shifting nature where farms are cultivated in a rotation of 3-5 years before farmers move on to open new farm fields by clearing mangrove forests (Monga *et al.*, 2018), and leaving the rice farm fallow. A field is referred to as fallow when it is left uncultivated (Wezel and Haigis, 2002). Natural recovery in the fallow fields has been observed taking place along different trajectories. While in some areas natural regeneration may take place immediately after abandonment (1 year), others remain infested with weeds.

This study aimed to assess the pattern of natural recovery on the abandoned rice farms and how successive transitions back to mangrove stands takes place through analysis of the sediment characteristics, forest species composition and structural attributes. Since succession recovery implies a return to the original type of mangrove forest (Lugo, 1980), understanding the pattern of natural recovery in abandoned agricultural areas is important to inform management decision and plans, particularly with regards to restoration initiatives. The vegetation components in the abandoned rice fields vary on spatial and temporal scales depending on the number of years the land has been left without agricultural activities (Iwara *et al.*, 2014). Moreover, species composition of secondary forest often fails to converge with that of nearby mature forest (Letcher and Chazdon, 2009). Undertaking this kind of a study is important as it will assist in making decisions on whether to attempt restoration or not, as restoration is recommended when an ecosystem can no longer self-correct, or self-renew (Lewis III, 2005).

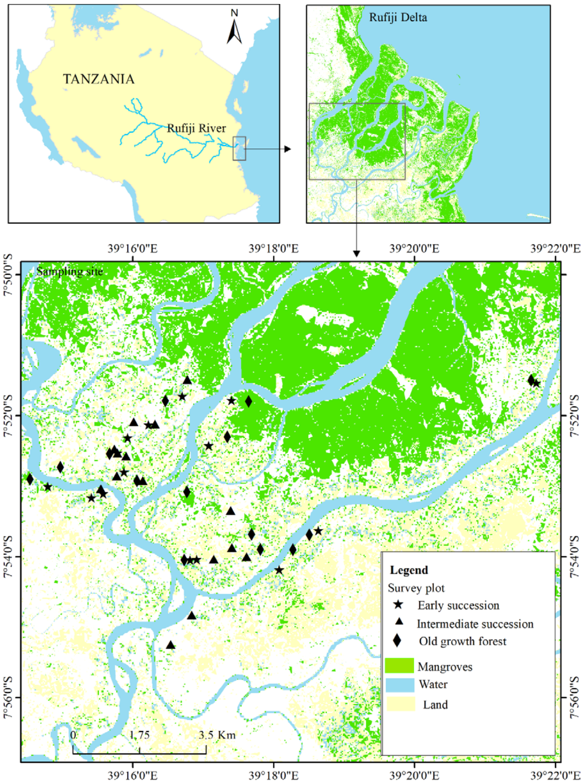

Figure 1. Map showing location and survey plot layout in the study area of the Northern Rufiji Delta.

Materials and methods

Study area

The Rufiji Delta is located between latitudes 7°50’ and 8°03’ S and longitudes 39°15’ and 32°17’E7.47° E (Fig. 1). The delta is the culmination of the Rufiji River basin that drains about 20% of Tanzania, extending over an area of approximately 177,000 km^2 (Mwalyosi, 2018). This critical habitat supports diverse estuarine, coastal and marine ecosystems that are ecologically interconnected. One of the key components of the Delta is the largest mangrove area in Tanzania covering about

45,519 ha (Monga *et al*, 2018). Geologically, the Delta is underlain by Cretaceous and Jurassic limestones and shales, which are overlain by alluvial sand, silt, and clay deposited from the Tertiary period to the present (Mwansasu, 2016). Based on data from the nearby Kilindoni station (Mafia Island District), the Rufiji Delta is characterised by two rainy seasons with rainfall ranging from 750 mm to 1250 mm per annum. The short rains usually start in October and end in December, and the long rains are from February to May. Average temperature in the Delta ranges from 13 °C to 41°C.

There are eight mangrove tree species occurring in the Delta, namely *Avicennia marina*, *Bruguiera gymnorhiza, Ceriops tagal, Heritiera littoralis, Lumnitzera racemosa, Rhizophora mucronata, Sonneratia alba* and *Xylocarpus granatum* (Lupembe, 2014; Wagner and Sallema-Mtui, 2016). The current population data from the ward offices of Sarale and Mwambao, shows that the population in the villages adjacent to the rice farming areas of the Delta is around 25,788. The distribution of people in the villages is as follows: Mtunda A (4900), Kikale (1822), Ruaruke B (4109), Mchungu (1396), Mdunda B (2581), Mfisini (2281), Nyamisati (2601), Kiomboni (3472) and Nchinga (2625). The major livelihood activities of these communities are rice farming (mainly farming in the Rufiji Delta), fishing and mangrove cutting. Other crops grown include cassava, pulses and legumes, coconuts, cashew nuts and mangoes, which are used for home consumption and as a source of income. Rice farming in the Rufiji Delta started in the 1970s with the preferred cultivation areas being along the main rivers and fringes of mangrove areas, where seasonal floods bring fresh water to the fields (Mwansasu, 2016).

Study design

A chronosequence method was used to study plant succession as described by Lagerström (2009). Accordingly, the targeted study area was categorized into three age classes: (i) Early succession, where rice farming activities have recently ceased (for less than five years) and natural plant recovery has started; (ii) Intermediate succession, areas abandoned for about 10 - 15 years; and (iii) Old growth forest, areas with mangrove trees of over 20 years and that have not been cleared for rice farming. The later was considered as reference forest as described by Lewis III, (2005). This succession age categorization was facilitated by local knowledge. A total of 45 nested plots (11.6, 2 and 0.5 m radii) were randomly established, with 15 plots for each succession category.

Field data collection

Vegetative measurements and data processing

In the main plots (radius of 11.6 m), all trees with ≥ 5 cm stem diameter were measured for diameter at breast height (DBH) (DBH, defined as 1.3 m above the ground) and height using a diameter tape and a Suunto hypsometer respectively. For *R. mucronata* trees, stem diameter measurements were taken at 30 cm above the highest prop root. Subplots of 2 m radius were established at the centre of the main plot, and within the subplot, DBH and heights of small trees (0.5 - 4.9 cm stem diameter) were measured. Only saplings/juvenile (trees less than 0.5 cm stem diameter) were counted. Non-tree species such as grasses and climbers were counted within a plot of 0.5 m. Species richness was determined as the total number of species represented in the plot. Importance Value Index (IVI) was used as a measure of the abundance and ecological success of the plant species. In a given plant community, the higher the IVI, the more abundant and successful, and importance of the role of a vegetation species (Shrestha *et al.,* 2000; Reddy *et al.,* 2008; Winata *et al.,* 2017). The IVI for each individual plant species was determined using the folowing formula (Mueller - Dombois and Ellenberg, 1974; Munishi *et al.,* 2007; Nzunda, 2008):

$$IVI = RD + RF + RDo \quad \text{...} (1)$$

Where:
RD = 100 (Number of individuals of the species/Number of individuals of all species)
RDo = 100 x Total basal area of the species/Total basal area of all species
RF = 100 x Number of occurrences of the species/ Number of occurrences of all species

Vegetation density (woody and non-tree species)

Number of plants per hectare for trees and non-tree species were computed as:

$$N = \frac{ni}{a} \quad \text{...} (2)$$

Where: N = Number of plants per hectare; n_i = number of plants counted; and a = plot area in ha

Basal area

Basal area (m²/ha) was calculated from measured DBH for all woody stems in each plot expressed as:

$$gi = \frac{\Pi dbh^2}{4} \quad \text{...} (3)$$

$$G = \frac{\sum gi}{A} \text{..} (4)$$

Where: G = Basal area per plot (m^2ha^{-1}); DBH = Diameter at Breast Height (m);
Π = Pi; A = Plot area (ha); gi = Basal area of a tree/shrub (m^2)

Soil sampling and laboratory treatments

Soil samples were collected at the centre of the plot by retrieving a soil core to a depth of 100 cm using a stainless steel gouge auger (AMS Inc., American Falls, Idaho, USA). These soil samples were collected at different depth ranges (0 - 30, 30 - 60 - 100 cm), and three soil samples of 5 cm length were extracted at each depth range of 15 - 20, 40 - 45 and 70 - 75 cm, hereafter referred to as L_1, L_2, and L_3 respectively. Each sample was divided into two sub-samples, with some oven dried at different temperatures to determine sediment characteristics. Organic matter content was determined by the loss ignition method, with samples placed in a muffle furnace (AAF 11/7, Wolf Laboratories Limited, UK) at 540°C for 5 hours.

The soil sub-samples for the determination of nitrogen, phosphorus and potassium were oven dried at 105 ± 0.5°C until constant weight was attained (typically 72 hours) using a Universal Oven (UN30 Memmert GmbH + Co. KG, Germany). The dry samples were sieved through a 2 mm sieve to obtain a fine earth fraction. Total Nitrogen (TN) was determined using the Kjeldahl method. The sample was first subjected to a sulfuric acid digestion to convert ON compounds to NH_4+. Secondly, the converted NH_4+, along with any NH_4+ that was originally present, was further converted to NH_3 using an alkali distillation process. The NH_3 liberated was quantified to determine the TN in the original digest. A separately determined value for NH^3 and NH^{4}+ was then subtracted from the value obtained by the Kjeldahl method, and the difference was considered to be the mineralizable, or potentially plant-available nutrients (Klute, 1986).

Extractable phosphorus was determined by the Bray-1 method by shaking 1g of dried soil in 10 ml of 0.025 M HCl and 0.03 M NH_4F for 5 minutes. Phosphorus was determined on the filtrate by the molybdate-blue method using ascorbic acid as a reductant. Colour development was measured using a UV VIS

Table 1. Plant species richness and dominance in the studied sites in the Rufiji Delta.

Plant type	Names Family	Species	Succession stage		Old growth Forest IVI
			Early IVI	Intermediate IVI	
Trees	Acanthaceae	*Avicennia marina*	58.9	170.7	163.1
	Rauraceae	*Heritiera littoralis*	25.5	14.2	131.8
	Rhizophoraceae	*Brugueira gymnorrhiza*	14.7	19.3	53.4
	Rhizophoraceae	*Ceriops tagal,*	-	13.0	21.6
	Rhizophoraceae	*Rhizophora mucronata*	-	-	5.7
	Lecythidaceae	*Barringtonia racemosa*	67.9	83.7	17.7
Non-tree plants	Palmae	*Phoenix reclinata*	12.5	37.7	28.1
	Poaceae	*Pothriochlo aglabra*	91.2	64.7	0
	Apocynaceae	*Derris trifoliata*	51.5	63.9	34.8
	Pteridaceae	*Acrostichum areum*	12.9	32.6	112.0
	Cyperaceae	*Fuirena zambesica*	78.4	87.8	0
	Poaceae	*Penisetum spp*	189.4	124.1	0
	Fabaceae	*Sesbaniam acrantha*	12.8	13.1	0
	Cyperaceae	*Cyerus latifolius*	26.4	65.3	0

spectrophotometer (Bray and Kurtz, 1945). The concentration of potassium was analyzed using an AAS flame spectrophotometer (Van Reeuwijk, 2002). Soil samples were extracted with a 1 M NH4OAc solution at pH7.00. The soil solution slurry was shaken for 2 h, and the solution was separated from the solid by centrifugation.

Data analysis

Statistical analysis of the data was carried out by using IBM SPSS Statistics 20. One - way analysis of variance (ANOVA) was used to test the variation of means of structural parameters and soil characteristic values from the early and intermediate succession stages as well as the old growth forest sites. Pair wise tests of the mean values of data sets collected from the three different sites were carried out using Least Significant Differences ($P < 0.05$).

Results

Floristic composition

A total of six tree species of three different families were recorded (Table 1). The highest plant species richness was found in the old growth forest, followed by the intermediate succession and then the early succession sites. Among the tree species recorded, one was a non – mangrove tree species (*Barringtonia racemose*). The mangrove species, *Rhizophora mucronata*, was only found in the old growth forest, and *Ceriops tagal* in the intermediate succession. *Barringtonia*

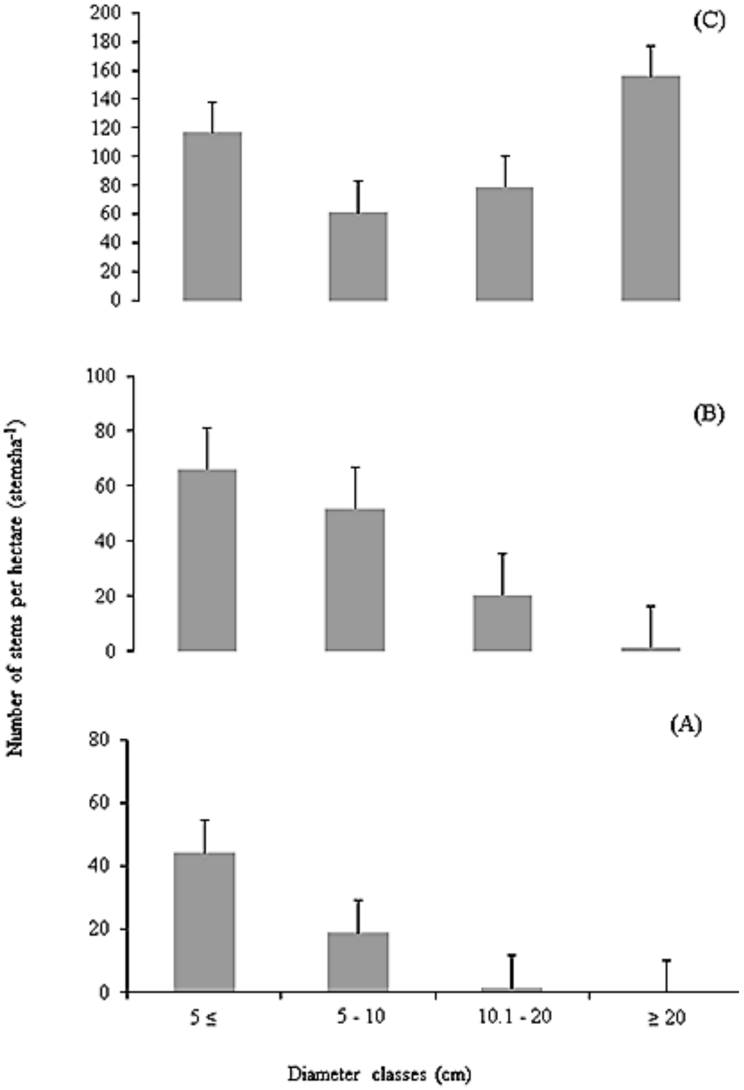

Figure 2. Number of stems per hectare across diameter classes of the different succession categories studied in the Rufiji Delta. (A) = Early succession; (B) = Intermediate succession; and (C) = old growth forest.

racemosa, Avicennia marina, Heritiera littoralis and *Bruguieragy mnorrhiza* were found in the early, intermediate and old growth forest. *Avicennia marina* was the most abundant mangrove tree species in the intermediate and old growth forest. However, in the early succession, *B. racemosa* was the most abundant (Table 1).

For non - tree plant species, the intermediate succession had the highest species richness, followed by the early succession, while the old growth forest had the least. *Phoenix reclinata, Derris trifoliata* and *Acrostichum areum* were the most common species found in both early and intermediate succession sites (Table 1). Among the non - tree plants, *Penisetum spp* was the most abundant species in the early and intermediate succession while *Acrostichum areum* was the most abundant in the old growth forest.

Diameter size class distribution, stem density and basal area of trees

In general, as the mangrove aged, tree sizes increased, while stem density per diameter size class decreased successively, depicting a reverse 'J' shape (Fig. 2). A larger diameter size of ≥ 20 cm was mostly recorded in the old growth forest, with a decrease in the intermediate succession, and was completely absent in the early succession site (Table 2). However, tree height increased significantly (p = 0.001) with mangrove age. The trend was similar to that of stocking parameters (stem density and basal area) which also increased with mangrove age.

Soil characteristics

The highest values of Soil Organic Carbon (SOC) in the old forest and intermediate succession sites were recorded in the topmost soils (0 - 30 cm depth),

Table 2. Structural attributes of trees and soil nutrients in the in the studied sites in Rufiji Delta; values are means and standard deviation for three sites: old growth; intermediate; and early succession. Means with different letters are significantly different at $p < 0.05$.

Variables	Unit	Old growth Forest (mean ± SD)	Sites		Test of variation	
			Intermediate (mean ± SD)	Early (mean ± SD)	F	P
Stem density	(stems ha^{-1})	417 ± 162^{a}	334 ± 147^{b}	98 ± 97^{c}	18.50	P = 0.001
Basal area	(m^{2} ha^{-1})	17.67 ± 6.11^{a}	0.98 ± 1.43^{b}	0.12 ± 0.24^{c}	115.20	P = 0.001
Height	(m)	16.41 ± 5.00^{a}	4.78 ± 2.47^{b}	2.74 ± 1.44^{c}	120.13	P = 0.001
Overall TN	(%)	0.15 ± 0.06	0.15 ± 0.06	0.13 ± 0.06	1.99	P = 0.597
Total NitrogenL$_1$	(%)	0.17 ± 0.07	0.19 ± 0.06	0.15 ± 0.07	1.949	P = 0.155
Total Nitrogen L$_2$	(%)	0.14 ± 0.07	0.14 ± 0.05	0.12 ± 0.05	0.296	P = 0.745
Total Nitrogen L$_3$	(%)	0.13 ± 0.05	0.13 ± 0.05	0.13 ± 0.05	0.114	P = 0.892
Overall P	(mgKg^{-1})	10.07 ± 6.92	9.04 ± 6.53	10.04 ± 10.40	0.79	P = 0.574
Phosphorous L$_1$	(mgKg^{-1})	10.62 ± 7.30	9.08 ± 6.10	10.00 ± 10.81	0.131	P = 0.877
Phosphorous L$_2$	(mgKg^{-1})	8.86 ± 6.33	7.74 ± 4.48	7.54 ± 4.48	0.283	P = 0.755
Phosphorous L$_3$	(mgKg^{-1})	10.74 ± 7.39	10.31 ± 8.55	11.81 ± 13.22	0.10	P = 0.905
Overall K	(%)	2.78 ± 1.15	2.06 ± 0.97	2.24 ± 0.93	5.902	P = 0.249
Potassium L$_1$	(%)	2.70 ± 1.18	1.78 ± 0.90	1.90 ± 0.71	4.23	P = 0.210
Potassium L$_2$	(%)	2.72 ± 1.09	2.10 ± 0.84	2.29 ± 0.84	1.794	P = 0.179
Potassium L$_3$	(%)	2.91 ± 1.25	2.32 ± 1.13	2.53 ± 1.07	1.002	P = 0.376
Overall SOC	(%)	5.97 ± 1.48	5.77 ± 1.25	5.70 ± 1.70	1.24	P = 0.480
Organic C L$_1$	(%)	6.06 ± 1.24	6.14 ± 1.24	5.51 ± 1.08	1.24	P = 0. 301
Organic C L$_2$	(%)	5.99 ± 1.45	5.62 ± 1.18	5.97 ± 2.23	2.12	P = 0.296
Organic C L$_3$	(%)	5.83 ± 1.76	5.53 ± 1.30	5.54 ± 1.68	0.170	P = 0.844

while at the early succession sites, the highest value was in the depth range of 30 to 60 cm. This was the same Total Nitrogen (TN) (Table 2). However, values for Phosphorus and Potassium were highest at deeper soil 60 to 100 cm depths. There was no significant change ($p > 0.05$) in the values of the studied soil properties (Total Nitrogen, Phosphorus, Potassium) between old forest, intermediate and early succession sites.

Discussion

Vegetation recovery trajectory and structural development

The number of mangrove species recorded in the old growth forest site (Table 1) is less than that reported in earlier studies, which reported 8 true mangrove species in the Rufiji Delta (Semesi, 1992; Wagner and Sallema-Mtui, 2016; Mangora *et al.,* 2016). This implies that only six mangrove species grow well in the studied sites. The two missing species, *Sonneratia alba* and *Lumnitzera racemose*, are rare in the Rufiji Delta, probably due to their limited ecological range.

The general trend in the natural recovery of mangrove tree species shows that species richness increases with fallow age (Table 1). An increase of one species from early succession to intermediate succession was apparent; with the same observed between intermediated succession and old growth forest. This implies that regeneration in the fallow rice fields would result in the development of a forest similar to the old growth forest in terms of tree species composition. Likewise, Ball (1980) reported a successional trend that may lead to the development of a forest similar to the historical forest in Southern Florida, USA. In other forest types, Haripal and Sahoo (2011) reported an increase in species richness with age in the tropical dry deciduous forest in the Western part of Orissa, India. This indicates that different vegetation types may have a similar successional trajectory.

The findings of the present study on the natural recovery trajectory of tree species did not fully support the initial floristic composition succession model, which suggests that species present at the early succession stages will also occur in the subsequent stages (Almazan - Nunenz *et al.,* 2016). While species like *A. marina*, *H. littoralis* and *B. gymnorrhiza* were encountered in both the early and the intermediate succession stages, *R. mucronata* was encountered only in the old growth forest, implying failure of these mangrove tree species in contributing to natural recovery. This suggests that the initial floristic composition succession model might be applicable only if there are favourable conditions for natural recovery of the mangrove tree species. Such conditions include the presence of mother trees producing propagules, and the absence of hydrologic restrictions or blockages (i.e. dikes) which prevent natural waterborne transport of mangrove propagules to a restoration site (Lewis III, 2005). Ball (1980) reported that direction of winds and currents may prevent substantial transportation of propagules to a site where they can germinate in a mangrove forest of Southern Florida.

In terms of dominance, the results from the old growth forest (Table 1) are similar to those described for the entire coast of Tanzania (Njana *et al.,* 2017). However, other inventory studies in the Rufiji Delta indicate a shift of dominance from A. *marina* at early forest development stages (under story) to *C. tagal* at mature stages (over story) (Mangora *et al.*, 2016). In a study in an upland forest ecosystem, Haripal and Sahoo (2011) reported on the shift in dominance of species along the chronosequence of rice fallows in the Western part of Orissa, India. Similarly, in the present study there was a shift of dominance from *B. racemosa,* in the early succession to *A. marina* in the intermediate and the old growth forest. According to Guariguata and Ostertag, (2001), together with the presence of remnant trees that can strongly influence the rate of initial colonization through their effects on seed dispersal, sprouting ability determines the abundance of the species. Buoyancy of propagules and tidal regime in the Rufiji Delta may also influence the abundance of species. Two factors might have contributed to *B. racemosa* being the most abundant tree species in the early succession of the fallows. Firstly, the seed disposal strategy of the plant may play a role. The fruit of *B. racemosa* is coated with buoyant fibrous tissue which allows them to be carried great distances by water currents (Osman *et al,* 2015). When deposited in a favourable location, the seed germinates to form a plant. Secondly, *B. racemosa* has the ability to sprout from stumps. Guariguata and Ostertag, (2001) argued that sprouting is likely to be important in forest regeneration, as many species are capable of producing either sprouts originating from boles or branches (above ground sprouts) or subterranean tissues (root sprouts). In the present study, species like *B. racemosa* mainly regenerated through sprouting from stumps. According to Osman *et al.* (2015) *B. racemosa* is a mangrove-associated species which grows well in wet areas (fresh water swamps, riverbanks and lakes) and can tolerate slightly saline conditions. In the Rufiji Delta, the plant is found close to the fresh water inflow, where the main Rufiji river starts branching into distributaries, and the influence of saline water is reduced. Mangrove species such as *A. marina*, *H. littoralis, B. gymnorrhiza*, *C. tagal*, and *R. mucronata* are regenerated through buoyant seeds and their dispersal is facilitated by water movement, particularly during the highest tides. The influence of physical environmental factors on the relative competitive abilities of the species probably accounts for the distribution of mangroves in characteristic zonation patterns (Ball, 1980).

Non-tree species in the recovering and old growth forest, and their associates threats

In terms of species richness, while previous studies reported only one species (*Acrostichum areum*) growing in the reference old growth forest (Wagner and Sallema-Mtui, 2016), the present study found three species growing here (Table 1), among which *A. areum* and *D. trifoliata* were mangrove-associated species (Lobo and Krishnakumar, 2014; Raju and Kumar, 2016). Non - tree species do not fully correspond to the initial floristic composition succession model (Almazan- Nunenz, *et al*, 2016), as not all species present in the early succession stages persist to subsequent succession stages (Table 1). In terms of dominance, the trend showed that this changes with fallow age. For example, *Pennisetum spp.* (Poaceae family) was dominant in the early and intermediate succession while *A. areum* and *D. trifoliata* were dominant in the old growth forest. *Pennisetum spp* are usually perennial rhizomatous herbs and are often woody and tree like (Gibson, 2009). They are fast growing, form dense ground cover, have well-developed root systems and are able to grow at various contaminated sites, display moderate salinity tolerance, and have an extensive root system that can firmly hold the soil and withstand adverse environmental conditions (Radhakrishnan *et al*, 2006; Mane *et al*, 2011). They have an extremely effective seed dispersal mechanism, and low soil nutrient requirements which facilitates their colonization on fallow rice fields (Haripal and Sahoo, 2011). In the mangrove forests of Bangladesh, few additional invasive species that inhibit normal growth of mangroves were recorded (Biswas *et al.*, 2007). *A. areum* is the only fern in the mangrove ecosystems of the Indian coast, and the species is widely distributed throughout the mangrove forest irrespective of local ecological and environmental conditions (Lobo and Krishnakumar, 2014).

The presence of non - tree species has been noted to pose threats to the natural recovery of mangrove tree species. For example, in the Sundarbans mangrove forest of Bangladesh. The climber *D. trifoliata* has been reported to pose a threat to many regenerating seedlings owing to its aggressive twinning and strangulating habit. Dense populations of *D. trifoliata* form a cover over seedlings and saplings of *Heritera fomes, Excoecaris agallocha, Sonneratia apetala*, among others (Biswas *et al.,* 2007). Likewise, in the Rufiji Delta, this climber (Liana) suppresses the growth of regenerating tree species. It smothers mangrove trees and may eventually lead to their death. In areas with no trees, this species covers the ground (Plate 1). In addition to smothering, the climbers and grasses negatively affect transportation and germination of propagules by preventing access to the soil. This affects the natural regeneration of the deforested and degraded parts of the mangrove forest, as well as species richness and recovery rate in the rice fallows of the Rufiji Delta.

Structural development

The diameter size class distribution curves (reverse 'J' distribution curve) obtained in the present study (Fig. 2) corresponds to that earlier reported from the mangroves of Gazi Bay in Kenya (Githaiga, 2013). According to Phillip (1994), a reverse 'J' distribution curve is commonly associated with natural forests with active recruitment and which are recovering from anthropogenic disturbances. Such disturbances include selective removal of poles from the forests (Githaiga, 2013). The 'J' distribution curve from the present study indicates that there was active natural regeneration of trees in the early and intermediate sites, while in the old growth forest sites the mangrove forest is recovering from selective harvesting. The early successional recovery had a low DBH range compared to other successional stages (Fig. 2). The presence of a small number of mature trees in the intermediate succession site and their complete absence in the early succession site indicates that the tree sizes increased with fallow age. Likewise, Ball, (1980) reported that mature individuals of Avicennia germinans (DBH approximately 20 - 30 cm) were very rare in the in a Mangrove Forest of Southern Florida, USA. Likewise, in the Rufiji Delta, lager diameter size trees of ≥ 20 cm was mostly recorded in the old growth forest, decrease in the intermediate succession while completely absent in the early succession site.

The value of stem density found in the old growth forest (Table 2) was less than that earlier reported in previous studies (Lupembe. 2014; Mangora *et al.*, 2016). The low stem density in the present study is

Plate 1. Grasses and the climber *Derris trifoliata* covering mangrove trees and land surface of the fallow rice farming areas in the Rufiji Delta.

probably because of selective harvesting of mangrove for poles and timber. This area is surrounded by many people who depend on the mangrove forest products for domestic and commercial purpose. However, the value of basal area and height obtained from this study was higher than that reported earlier for al mangrove forests of Tanzania (Njana *et al.*, 2017). This implies that the old forest site studied was composed of larger tree sizes, as the larger the tree sizes, the larger the basal area. Similarly, Sukardjo and Yamada (1992) reported basal area and height at one site to be higher than four other sites in a *R. mucronata* plantation in Tritih, Central Java, Indonesia, as a result of variation in growth caused by differences in soil fertility.

The recovery trends in this study shows that structural attributes, stem density, basal area and height increased significantly with fallow age ($p = 0.001$), but they are much lower compared to the old growth forest (Table 2). This implies that for the mangrove forest of Rufiji Delta, a period of up to 15 years would insufficient to allow full recovery from losses from conversion to agriculture. However, Lugo (1980) reported that the rate of succession recovery after disturbances varies in the different types of mangroves and depends on he characteristics of nearby ecosystems and growth conditions at each site.

Soil properties

The TN, Available Phosphorus (AP) and SOC values obtained in the Rufiji Delta were less than that reported in the mangrove forest of the Apar Nature Reserve in Indonesia, where the TN was 0.34 ± 0.02 %, while SOC, and AP were 3.96 ± 0.18% and 19.87 ± 0.22 mg/kg, respectively, in an *Avicenia officinalis* pure stand (Sukardjo, 1994). TN was 0 0.97 ± 0.01 %, while SOC, and AP were 60. 27 ± 1.03% and 19.87 ± 0.22 ppm, respectively, in a *C. tagal* pure stand. Dasgupta *et al.* (2018) reported that SOC ranges between 0.9 to 1.4 % in mangrove soils of the Sundarbans, West Bengal, India. However, Hossain and Nuruddin (2016) reported TN and SOC to range between 0.09 - 0.97 % and 0.38 - 13.31 % respectively in different mangrove forests of the world. Therefore, the value of TN and SOC obtained from the present study in both intact and the regenerating forest were within the global estimates. The value of Potassium found in the Rufiji Delta was higher than that of 0.27± 0.02 found in *A. officinalis* and 0.17 ± 0.02 in *C. tagal* reported in the mangrove forest of the Apar Nature Reserve in Indonesia.

The studied soil properties from converted sites and intact forest does not follow the paradigm of significant declines following conversion (Allen,1985; Raich, 1983; Guariguata and Ostertag, 2001). Neither significant increase nor decline in soil nutrient properties of the fallows was found. This might be because there is a continuous inflow of soil sediments carried by the river from upstream. Alongi *et al.* (2005) found that sediment and associated elements, including nutrients within mangroves is a result of intense human activity along the south China coast. Walling (1999) showed that the change from natural vegetation to cultivation can increase soil erosion rates by an order of magnitude or more. Elevated sediment loading to estuarine and coastal environments can occur via a number of mechanisms, including urban development, and often results in increased turbidity and sedimentation rates in estuarine and coastal waters (Ellis *et al.,* 2004). However, high levels of radio nuclides suggest that these sediments originate from erosion of agricultural soils within the catchment (Alongi *et al.,* 2005). For instance, in Kenya it has been reported that the two main rivers (Athi- Galana/Sabaki and Tana) which drain into the Indian Ocean are reported to deliver several tonnes of sediments into the coastal areas (Okello, 2016). In the Rufiji Delta, sediments with nutrients from upstream have been carried by the Rufiji River and deposited in the delta. The Rufiji river has a basin which extends over an area of 177,000 km^2 with a mean annual discharge that ranges between 900 and 1,133 m^3/s (ASCLME, 2012; Mwalyosi, 2018). These sediments with nutrients might counterbalance the loss of nutrient in the study area.

Conclusions

The present study has demonstrated that mangroves in the abandoned rice farming areas of the Rufiji Delta are undergoing successional recovery. Favourable conditions for natural regeneration are the presence of mother trees as a source of propagules, absence of vegetation such as the climber *D. trifoliata* and grass cover that prevent natural waterborne transport of mangrove propagules to the restoration site and contact of the propagules with soils. Species dominance varied with the fallow age, with the non-mangrove species, *B. racemosa*, dominating in the early succession stage, but subsequently replaced by *A. marina* in the intermediate succession stage and the intact mangrove forest. The structural characteristics determined in this study demonstrate that a period of up to 15 years would not be sufficient to allow full natural recovery of the degraded areas as a result of clearance and conversion of mangrove areas for traditional rice farming. Anthropogenic disturbance during tree harvesting and rice farming in the study site had no effects on soil nutrients as well as SOC. Sediments with nutrients from upstream have been carried by the Rufiji River and deposited in the delta and have counterbalanced the loss of nutrients due to rice farming. Therefore, soil nutrients are not a factor that affects the natural recovery rate of the mangrove forest in the studied area. In the areas where natural recovery has not been successful, human intervention geared towards restoration is recommended. Such interventions would include removal of grass cover and or planting to facilitate the recovery of the tree species. Therefore, in the abandoned rice farming areas of the Rufiji Delta, both planting and natural regeneration restoration approaches are applicable, but assessment of potential sites are required to determine the best restoration approach. In addition, permanent sample plots should be established in these parts of the delta for long term monitoring of plant recovery rate.

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Assessing the effectiveness of LED lights for the reduction of sea turtle bycatch in an artisanal gillnet fishery – a case study from the north coast of Kenya

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Abstract

Artisanal gillnet fisheries exist throughout the world's oceans and have been responsible for high bycatch rates of sea turtles. Three sites on the north coast of Kenya, i.e. Watamu, Ngomeni, and Bwana Said, were studied with the overall objective of assessing the effectiveness of LED lights in the reduction of sea turtle bycatch in the bottom-set gillnet fishery. A total of 10 boats with pairs of control and illuminated nets were deployed during the study, with 56 turtles caught in control nets, while 30 were caught in illuminated nets. The mean catch per unit effort (CPUE) of target species was similar for both control and illuminated nets. In contrast, the mean CPUE of sea turtles was reduced by 64.3% in illuminated nets. This statistically significant decrease ($p < 0.04$) in sea turtle catch rate suggests that net illumination could be an effective conservation tool. Some useful data on fish catch rates with and without LED lights were also obtained, and interviews with fishermen suggested that they believe that the lights are effective at reducing marine turtle bycatch in their gill nets when set at night. The issues associated with implementing the use of LED lights included increased net handling times, equipment costs, and limited awareness among fishermen regarding the effectiveness of this technology. These challenges need the support of other stakeholders, especially national government, so as to implement this strategy of reducing turtle bycatch more widely.

Keywords: mortality; sea turtles; bycatch; small-scale fisheries; gillnet; LED lights

Introduction

Five species of sea turtle have been documented from Kenyan waters (Frazier, 1975). These are the green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), loggerhead turtle (*Caretta caretta*), olive ridley turtle (*Lepidochelys olivacea*) and leatherback turtle (*Dermochelys coriacea*). Some of these sea turtles are known to nest on sandy shores in Kenya, while most forage in a diverse range of marine habitats, which include coral reefs, seagrass meadows, and mangrove swamps off Kenya (Wamukoya *et al.*, 1997; Okemwa *et al.*, 2004).

As with global sea turtle populations, those in Kenya also face increasing anthropogenic threats that put the populations at risk (IUCN, 2007). Typical threats include entanglement in fishing gear, poaching and illegal trade of eggs, meat, and shells, coastal development which destroys nesting habitat and disorientates sea turtles, plastic pollution and other marine debris, and global warming. The primary risk to sea turtles is entanglement or capture in the gear used in commercial and artisanal fisheries (IUCN, 1995; Spotila *et al.*, 2000; Lewison *et al.*, 2004; Alfaro-Shigueto *et al.*, 2010). In Kenya a significant number of sea turtles, among them endangered green sea turtles, are killed each year from the purse seine and gillnet fisheries (Wamukota, 2009; Watamu Turtle Watch, 2014; Fisheries Department, 2014). Although it is illegal to capture sea turtles in Kenya, the incidental capture of these animals remains a major threat to turtle populations in the region (WWF, 2009; KMFRI, 2016).

Bycatch in fisheries has been recognised as a global threat to sea turtles (WWF, 2009). The interaction of

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small-scale coastal gillnet fisheries with sea turtles is documented to equal, or in some cases exceed, interactions with industrial pelagic fisheries (FAO, 2009). It has been estimated that over 70% of sea turtle interactions with fisheries worldwide end-up in capture (Molony, 2005; FAO, 2009; Alfaro-Shigueto *et al.,* 2011) and this poses a significant source of mortality for sea turtles (Hays-Brown, 2003; WWF, 2016). Watamu Turtle Watch in Kenya reported more than 273 turtle mortalities per annum related to interactions with fisheries within Watamu coastal fishing of Watamu (pers. comm, September 20, 2015). This rate of incidental capture and mortality poses a great threat to sea turtle management and population recovery and has thus been the focus of recent conservation work. Several studies have suggested that illuminating fishing nets with LED lights can reduce sea turtle capture by up to 40% without any significant impact on the catch of targeted fish (Wang *et al*., 2010, 2013). These studies used either light-emitting diode (LED) light sticks or chemical light sticks to illuminate portions of nets.

To help limit the negative impacts of fisheries, bycatch reduction technologies (BRTs) have been developed for a limited number of fisheries (Cox *et al*., 2007). Much effort has focused on the use of circle hooks in longline fisheries (Gilman *et al*., 2006; Serafy *et al*., 2012) and the use of Turtle Excluder Devices (TEDs) in shrimp trawl fisheries (Crowder *et al*., 1994, 1995; Watson *et al*., 2005; Lewison and Crowder, 2006; Read, 2007; Jenkins, 2011). However, the development of bycatch mitigation measures for gillnets, one of the most ubiquitous gear types, has been comparatively slow (Melvin *et al*., 1999; Gilman *et al*., 2006).

Kenya has an estimated 4,450 boats in its fisheries (KMFRI, 2016; Fisheries Department, 2016) that fish six days per week for target species such as the blue shark (*Prionace glauca)*, flounder species (*Paralichthys* spp.), guitarfish (*Rhinobatos planiceps*), kingfish (*Scomberomorus cavalla*), and shortfin mako shark (*Isurus oxyrinchus)*. Since five species of sea turtles make use of the same waters as the fishing vessels (Amiteye, 2002), it is critical and imperative to quantify sea turtle mortality and assess the potential of LED lights in reducing sea turtle captures as a bycatch reduction tool.

Clearly, protecting Kenya's sea turtles is extremely important for the global recovery of the species. This study sought to 1) quantify the present sea turtle capture rate in the gill net fisheries, 2) compare capture rates in gill net fisheries with unmodified and modified (gear illumination with LED lights) fishing gear, and 3) compare catch quantity and composition of target fish species in unmodified and modified fishing gear. It was hypothesised that LED lights would reduce sea turtle bycatch, and would not have any impact on the target species fished.

Methods

The Kenyan coast spans approximately 600 km in a north-north east to south-south west direction, between 1° and 4° S on the Indian Ocean (UNEP, 1998; Okwema, *et al*., 2004). The study was undertaken between December 2016 to December 2017 at Watamu, Ngomeni and Bwana Said landing sites, approximately 105 - 150 km north of Mombasa. The sites host a large gillnet fishery and an abundance of sea turtles.

Sensitization of fishermen about the project was undertaken at the start of the research period, and the Split-Block Sampling Design (Fisher, 1925; Box *et al*., 2005) used in data collection. Nets were operated as pairs so as to ensure the experimental and control nets were carried by the same boats to minimize the errors. The catch from the nets were categorized into three groups: target species (fish sold), bycatch (discarded fish), and other (catch kept by the fishermen for consumption or retained for bait in other unrelated fisheries). The turtle species, curved carapace length (CCL; notch to tip (cm)), the rate of turtle bycatch, and fate of by-catch was recorded. Live sea turtles were released in accordance with internationally recognized guidelines (Epperly *et al*., 2004). Turtle catch-per-unit-effort (CPUE) for each net was calculated as the number of turtles captured/([net length/100 m]) x ([net soak time/12 h]). Data were also collected on fishing method, gear type, design and operation, target fish catch composition, and weight. The total target species CPUE was calculated as the number of individuals of target species/([net length/400 m] x [net soak time/12 h]).

Out of 30 boat captains identified at the start of the study, 10 were randomly selected to participate in the net trials. Each of the boats was equipped with bottom-set gill nets. Six boats at Bwana Said, two boats at Watamu and two boats at Ngomeni Participated in the study, based on the relative importance of each landing site. After the project the boat captains were interviewed through a questionnaire, primarily to determine the effect of the LED lights.

The fishing boats were provided with paired sets of gillnets (1 and 2) at the start of the study. Group 1 nets (control) were to fish without any modification, and Group 2 nets were fitted with gear modification/illumination (Deep Drop LED Fishing light 2,100 ft Green) (Fig. 2A) placed every 15 meters along the gillnet float line. The gillnets were made of multifilament twine (Fig. 2B) and were composed of multiple net panels 50 m long by 3 m high. The number of gillnet panels set each evening varied, depending on the fishing crew, but averaged 5 panels per night. Nets were typically deployed in the late afternoon, soaked overnight, and retrieved the following morning. For each deployment, both the control and illuminated net were set.

Each boat captain then continued their fishing operations as per usual, fishing in the same areas for the same amount of time that they normally fished. Trained observers/fishermen recorded all catch and by-catch information. Plastic flipper tags were fixed to all sea turtles captured to determine recapture rates. Support items were provided for fishing boats used in the research and observers were given free LEDs as motivation to sustain interest in the research.

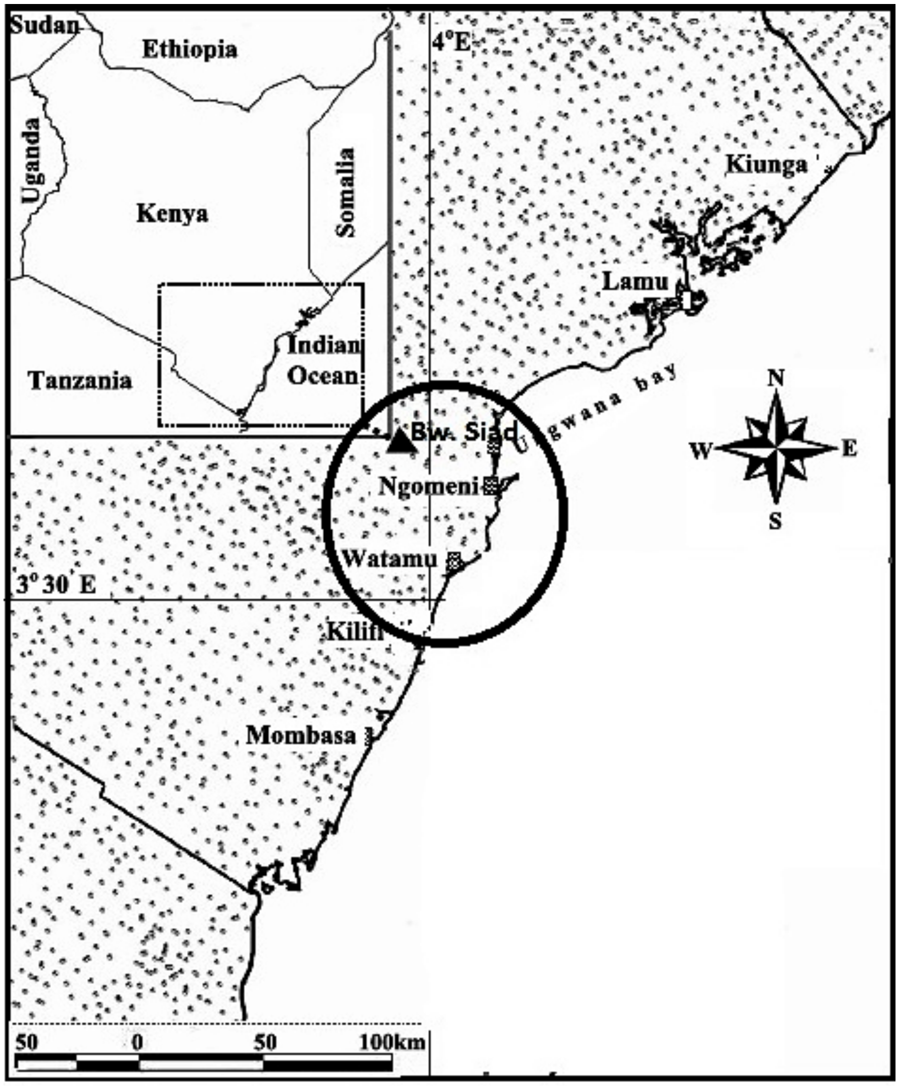

Figure 1. Map of the study area; Watamu, Ngomeni, and Bwana Said landing sites

In order to detect statistical differences between the catch rates for the control illuminated nets, the mean CPUE values for both were compared using a t-test. Additionally, 2-sample t-tests were used to analyse differences in body size for sea turtles and target fish between control and illuminated nets. The study used the randomization test to analyse the catch data and test the null hypothesis that there would be no difference in sea turtle catch rate, total target catch rate, and CPUE between experimental and control nets. Data

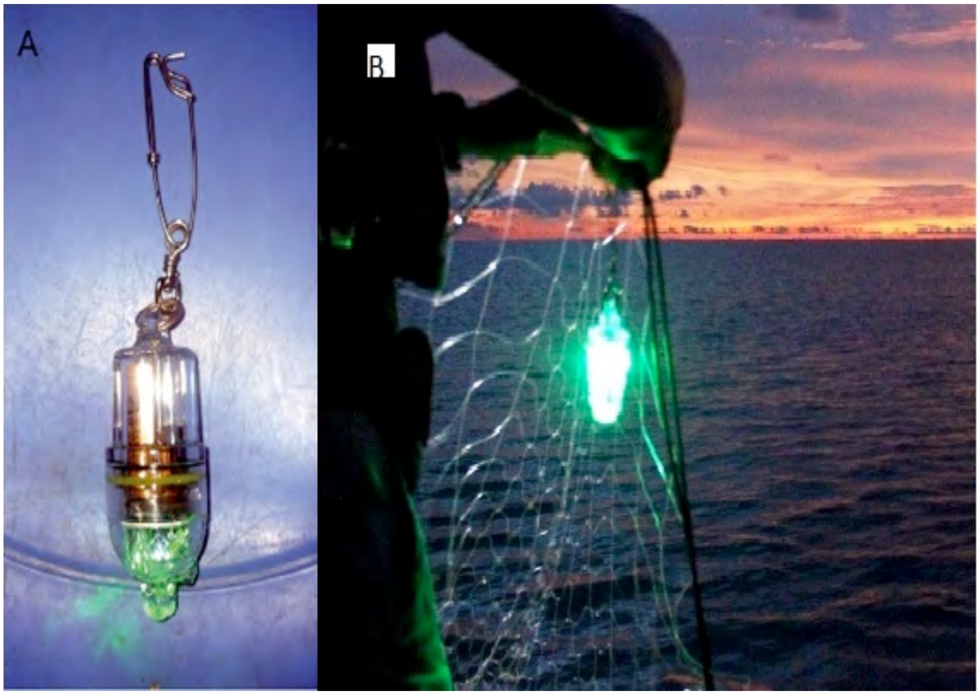

Figure 2. (A) Example of the LED light used during the study. (B) LED fitted on a bottom-set gillnet.

were re-sampled several times using the software RESAMPLING STATS for Excel (v. 4.0). This analysis measures the strength of evidence against a null hypothesis instead of estimating significance at a certain level.

Results

The study deployed 10 boats with paired control and experimental nets. The number of panels in each net varied slightly among boats and between trips as panels were sometimes added to increase target species catch, or were detached for repair. Therefore, net length varied, with control nets averaging 0.62 km ± 0.03 SE, while illuminated nets averaged 0.60 km ± 0.02 SE (Table 1). Soak time for control nets averaged 17.10 hrs ± 0.39 SE), while experimental nets averaged 17.40 hrs ± 0.39 SE (Table 1). Fishing effort was determined by combining net length and soak time (km x 24 hrs soak). The mean fishing effort averaged 0.41 ± 0.02 SE (km x 24 h) for control nets, while illuminated nets averaged 0.40 ± 0.01 SE (km x 24 h) (Table 1). A total of 86 sea turtles were caught during the study period. Of these, 56 were caught in the control nets constituting 41 green, 9 hawksbills, 5 loggerheads, and 1 olive ridley turtle. The illuminated nets caught 30 turtles of which 21 were green, 5 hawksbills and 4 loggerhead turtles.

Analysis with the two-sample t-test indicated that sea turtle CPUE was significantly higher in control nets (mean CPUE = 1.40 (± 0.16 SE) as compared with experimental nets (mean CPUE = 0.50 ± 0.06 SE, indicating a 64.3% reduction in mean catch rate (p = 0.04) (Table 3; Fig. 3). The paired nets were concurrently used to examine the effects of LED illumination on total target fish catch rates, composition and weight. A total of 12,987 individual target fish (46,581 kgs) were kept for market. Control nets caught 695 target fish (23,539 kgs) with a mean CPUE

Table 1. Summary of fishing effort by net type (control = without LED illumination, illuminated = with LED illumination) for paired gill net sets in the study area.

Net type	Sets	Set duration (h)		Net length (km)		Fishing effort (km x 24 h)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Control	80	17.10 ± 0.39	2.83-24.07	0.62 ± 0.03	0.32-1.28	0.41 ± 0.02	0.07-1.10
Illuminated	80	17.40 ± 0.39	3.75-24.33	0.60 ± 0.02	0.32-1.15	0.40 ± 0.01	0.09-0.75

Table 2. Summary of target species and sea turtles (number caught) by net type (control = without LED illumination, illuminated = with LED illumination).

Net type	Sets	Total effort (Km x 24 h)	Target species caught	Turtles caught
Control	80	48.96	695	56
Illuminated	80	47.71	603	30

Table 3. The outputs and mean catch per unit effort of target species and sea turtles (control = net without LED illumination, illuminated = net with LED illumination).

Response variable	Mean CPUE Control (mean ± SE)	Mean CPUE Illuminated (mean ± SE)	% diff.	p
Target Species	10.62 ± 0.71	10.35 ± 0.86	-2.5	0.78
Sea turtles	1.40 ± 0.16	0.50 ± 0.06	-64.28	0.04

of 10.62 ±0.71 SE, whereas experimental nets caught 603 target fish (23,042 kgs) with a mean CPUE of 10.35 ±0.86 SE (Table 3; Fig. 3), which was statistically similar (P = 0.78).

Fishermen interviewed after the completion of the project generally believed that the lights were effective at reducing marine turtle bycatch in their gillnets, although turtle bycatch was still present.

Discussion, conclusion and recommendations

Artisanal fishing in Kenya is a major source of income for more than 300,000 people in coastal communities with few economic opportunities other than those related to fishing (Fisheries Department, 2014). These important fisheries also account for significant sea turtle mortality (WWF, 2009). The purpose of this study was to investigate bycatch measures to reduce turtle bycatch without compromising target

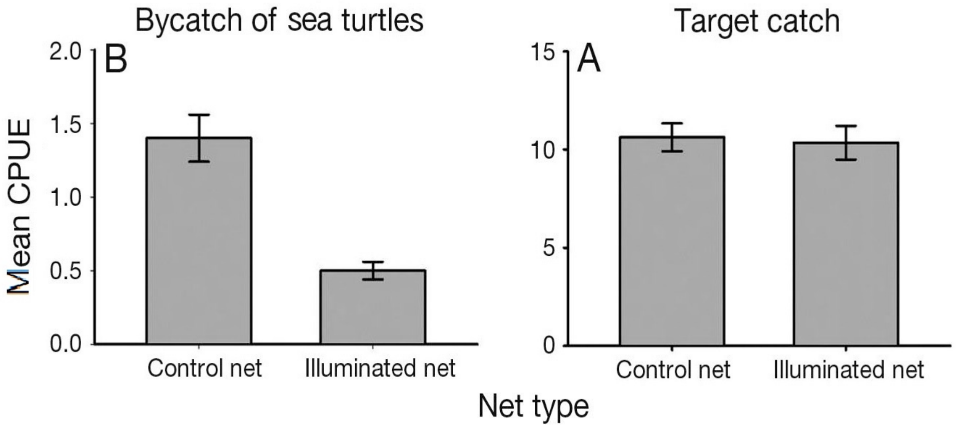

Figure 3. (A) Comparison of the mean CPUE of sea turtles between control and illuminated nets, and (B) comparison of the mean CPUE of target species between control (without LED illumination) and illuminated (with LED illumination) nets, showing no significant difference.

catches. This study showed that green LEDs attached to bottom-set gillnets in northern Kenya considerably reduce sea turtle bycatch, without adversely affecting target species catch rates. This technique could potentially serve as an effective sea turtle bycatch reduction device (BRD) for this type of fishery. This study demonstrates that, if this intervention was well managed and widely implemented, it could potentially promote the long-term stability of both sea turtle populations and local fisheries.

Sea turtles interact with gillnets globally (Wallace *et al.*, 2010). It will therefore be important to replicate this study in multiple locations and fisheries to assess the effectiveness of net illumination with a variety of gears, environmental conditions, and catch compositions (Southwood *et al.*, 2008; Gilman *et al.*, 2010). In order to effectively implement this BRD or other mitigation methods, any future studies need to consider costs and implications for fishermen, impacts on the catch of their target species, and the effect on other bycatch species (Cox *et al.*, 2007). Trials of this BRD in small-scale fisheries could serve as an important step in the global conservation of sea turtles. The cost of LEDs spread across multiple years still represents an untenable amount for Kenya's artisanal fishers. This means that efforts are needed at national or international levels to leverage financial support if this BRD is to be broadly implemented. To encourage this support, it would be useful to calculate the approximate cost (LEDs, gear etc) of preventing a single sea turtle interaction. This could then also be used to compare the costs of alternative conservation measures such as fisheries closures, time-area based closures, and development of marine reserves (Balmford *et al.*, 2004; McClanahan *et al.*, 2006).

Notwithstanding the challenges of implementing net illumination in artisanal fisheries (e.g. cost, LED design, fisher awareness), the results from this study emphasize the effectiveness of controlled fisheries experiments for the testing of bycatch reduction measures in artisanal gillnet fisheries. Future studies on net lighting should examine possible effectiveness as a multi-taxa technological tool for seabirds and marine mammals as these animals also rely on visual cues to a large extent (Jordan *et al.,* 2013; Martin and Crawford 2015). In addition, continued development of LED lights and their power sources could improve their efficiency and ensure optimal performance. Solar powered LEDs could also be developed in order to reduce the cost and waste associated with batteries. Fishermen involved in the trials were mainly positive and provided essential feedback, which included the suggestion that LED light sticks should be designed specifically for net fisheries. Such continuous associations with fishermen and their communities will be critically important for the continued development and testing of net illumination as well as other bycatch reduction strategies for artisanal fisheries.

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Biophysical factors influencing the choice of nesting sites by the green turtle (*Chelonia mydas*) on the Kenyan coast

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Abstract

The green turtle (*Chelonia mydas)* is the most common sea turtle nesting along Kenya's coastline. Varying biophysical factors influence choice of nesting sites where eggs are laid. This study was conducted between February and November 2016 and was designed to establish the relationship between numbers of *C. mydas* nests on the beach and biophysical variables such as vegetation cover, organic matter content (OMC), beach width, and beach slope. Multiple regression analysis was employed to assess the factors that contribute to the number of *C. mydas* nests on a beach. This analysis did not allow prediction of the number of nests in a statistically significant way ($F_{(4, 26)} = 0.094$, $p > 0.05$, $R = 0.120$). However, there was a decrease in the number of nests with increased vegetation cover (b = - 0.013), OMC (b = - 8.114), beach width (b = - 0.089) and slope of the beach (b = - 0.352). Approximately 70% of nests occurred on beaches with medium and fine sand. Beaches with intense human activities were associated with high OMC which significantly affected the number of nests on the beach. It is therefore recommended that existing laws should be enforced to control beach development and human activities along the Kenyan coast to protect nest sites. Additionally, long term monitoring should be put in place to evaluate the impact of human disturbance on the favourable biophysical factors influencing the number of nests on a beach, as a management tool in the conservation of *C. mydas*.

Keywords: Green turtle; *Chelonia mydas*; nest sites; nesting choice; Kenyan coast; conservation

Introduction

Green turtles (*C. mydas*) tend to prefer to nest in areas that possess specific beach qualities such as gentle slope, moderate vegetation cover, absence of barriers and pollution, medium sized sand particle, darkness and minimum human disturbance (Mortimer, 1990). However, Kenyan beaches have been adversely affected by anthropogenic activities that have the potential to impact negatively on this critical habitat in life cycle of *C. mydas*. Additionally, there is inadequate information on nest site ecology on the Kenyan coast, leaving institutions mandated to manage the species without a proper baseline for making decisions to conserve and protect them.

Data to determine sea turtle population nesting trends of is not sufficient in Kenya (National Marine Fisheries Service and US Fish and Wildlife Service, 2007). Turtle nesting, mortality, hatching and incidental catch is monitored at key sites along the Kenyan coast by government institutions, local Non-Governmental Organisations and Beach Management Units (BMUs - community groups registered by the Ministry of Fisheries). There is, however, inadequate information on *C. mydas* nesting on the coast of Kenya (Okemwa and Wamukota, 2006). The information on factors affecting turtle nesting is either inadequate or not harmonised, hampering conservation and management efforts (IUCN SSC, 1996). Demographic parameters are difficult to estimate owing to the long-term research required and the complexity of turtle life cycles. Animals that do not provide parental care have to ensure that a considerable number of their offspring survive to the next generation to keep the species viable, and natural selection favours individuals who make choices that enhance fitness of their offspring. Nest site selection can be

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defined as the placement of eggs by females at sites that differ from random sites within a delimited area (Wilson, 1998). The nest site that a female chooses will determine the level of success of hatchling emergence. Understanding habitat features which determine successful nest site selection are important in conservation and management of a particular species.

Reproductive biology has been identified as one of the key global research priorities for sea turtles. Important information required for each species includes determining the factors that underpin nest site selection, nesting behaviour, primary sex ratios produced (how they vary within or among populations), and the factors that are important for sustained hatchling production (Hamann *et al.,* 2010). An assessment of the distribution and status of critical nesting habitats and their protection both current and anticipated threats is crucial to the conservation of turtles. This is because management decisions must include precise assessment of population size, including determination of whether populations are stable, increasing, or declining (Eckert *et al.,* 1999). The Marine Turtle Conservation Strategy and Action Plan for the Western Indian Ocean (IUCN SSC, 1996), which includes the Sodwana Declaration, tasked Kenya to identify nesting and foraging habitats so as to assess, monitor, and regulate fisheries impacts on turtles, as this information was lacking for the country. Other information gaps identified included inadequate data and information on population status and structure, and underestimation of nest numbers due to incomplete coverage of nesting beaches (Okemwa *et al.,* 2004). Kenya's sea turtle strategy also identified factors that make turtles vulnerable such as slow growth rates, susceptibility to predation and low recruitment rates from juvenile to adult life stages (Kenya Wildlife Service, 2010). The objectives of this study were to identify and geo-reference nest sites, characterise biophysical factors influencing nest site choice, and to recommend management interventions to conserve and protect nest sites of *C. mydas* on the Kenyan coast.

Materials and methods

Sampling areas

The green turtle nesting site investigation was carried out along the Kenyan coast (Fig. 1) which is approximately 600 km long, stretching from 1^0 42' S to 4^0 40' S, bordering Somalia in the north and Tanzania in the south (UNEP, 1998). This study was conducted in the coastal counties of Kwale, Mombasa, Kilifi and Lamu from February to November 2016. Nesting data for the period from 2014 to 2016 was used to calculate the number of nests placed by *C. mydas* along the Kenyan coast. This data was obtained from various institutions and organisations engaged in turtle conservation such as Kenya Wildlife Service (KWS) for the Malindi and Watamu area, World Wide Fund for Nature (WWF) for the Lamu area, Baobab Trust (Kilifi and Mombasa areas), and BMUs (Kwale area). The data collected included number of nests, number of eggs laid, and number of hatchlings per nest site. The number of nests was obtained by recording the nests during regular patrols on the beach. The number of eggs laid was established by counting the number of eggs shells in nests after hatching. Biophysical factors investigated were vegetation cover, soil texture, OMC, width of the beach and slope of the beach. A total of 31 turtle nesting areas were investigated using a purposive sampling technique. The data was collected during low tide to allow for proper measurements of the beach variables at the nest sites. The beaches on which biophysical factors were investigated were classified as either occurring within or outside protected areas, vegetated or not, beaches with barriers to the sea, or open beaches, and those with open access or under cliffs.

Sampling Methods

Vegetation cover

Three quadrats measuring 1m by 1m were randomly established to determine the percentage of herbaceous foreshore vegetation cover at a nest site. The percentage vegetation cover was determined by a perpendicularly projected outline of the vegetation in the quadrat. For ease of estimation, the quadrat was divided into four equal quarters and the percentage vegetation cover was determined by addition of the estimated percentage vegetation cover in all the four quarters of the quadrat. Plants encountered in each quadrat were identified to species level (Abuodha *et al.,* 2003).

Organic matter content (OMC)

The Weight Loss on Ignition method (Agvise Laboratories, 2017) was used to determine the OMC of beach sand. Each of the soil samples was placed in the oven in aluminium foil to dry at 70°C for 72 hours. The sample was thereafter homogenised and three 5 g replicate sub-samples (Balance model Citizen, top loading analytical balance 0.1 mg) were put in a furnace of 450°C for 6 hours. The difference between the initial weight of the sample and final weight of the burnt sample represented the OMC. At 450°C the OMC was converted to carbon dioxide. The three replicates gave a mean for the OMC.

Soil texture

Three sets of soil samples were collected randomly from the nest site surface and at a depth of 50 cm. The three pairs of collected samples were sealed in a polythene bag and taken to the Kenya Marine and Fisheries Research Institute (KMFRI) laboratory. The sieve method was used to determine soil texture (Gee and Bauder, 1986). Each sample was dried at 70°C for 72 hours. A sample of 100 g was placed on a shaker (Eijkelkamp) for 10 minutes with sieves of 1.60 mm, 1.00 mm, 0.710 mm, 0.500 mm, 0.250 mm, 0.125 mm, 0.063 mm and 0.038 mm. The retained sample from each sieve was placed in a petri dish of known weight and weighed. The weight passing through each sieve was then converted into percentages of the total weight.

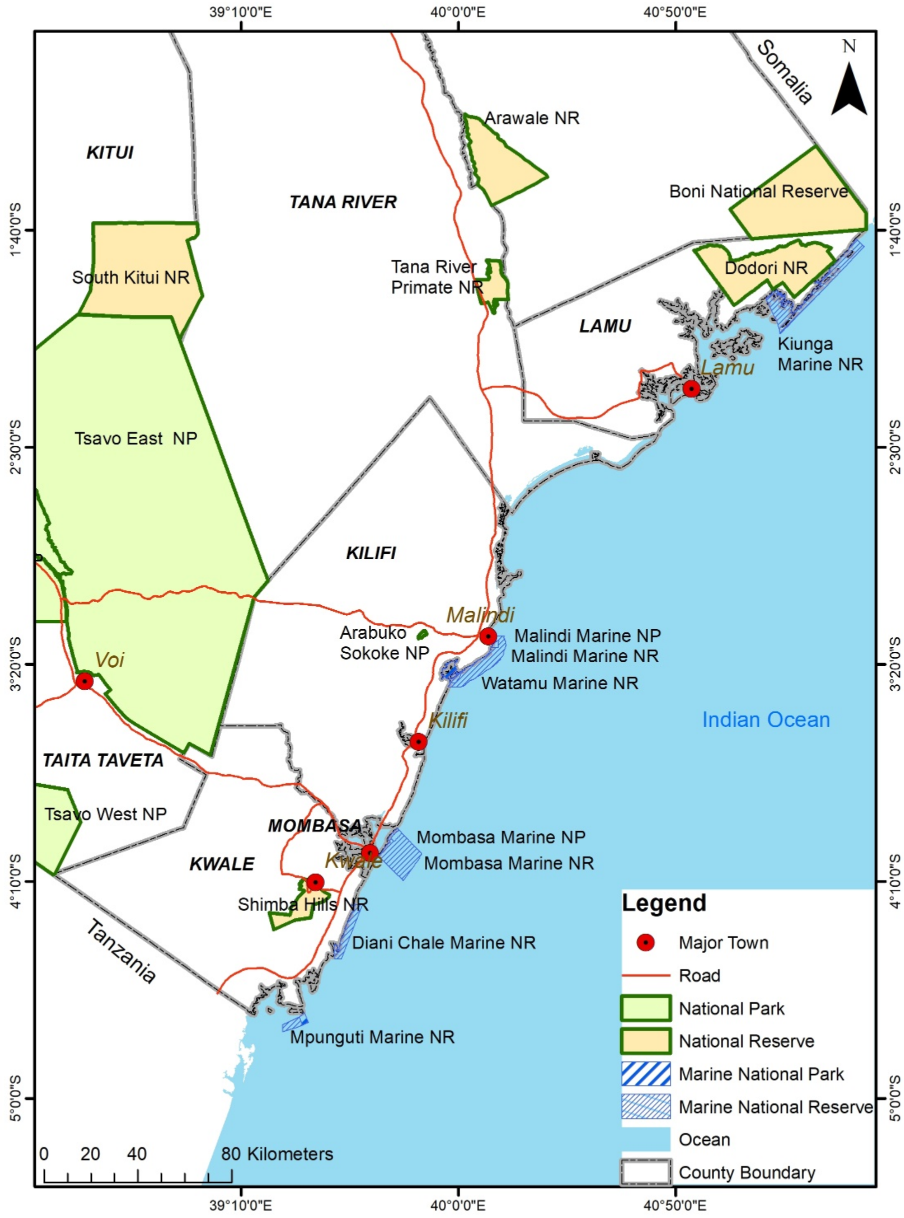

Figure 1. Kenyan coastline showing administrative boundaries and protected areas. (Source: KWS)

Width of the beach

The distance from the fringing vegetation on the beach to the low water mark was measured using a meter rule (Varela-Acevedo *et al.*, 2009) with the sampled nest site as a reference point. Two measurements of beach width were taken (in metres) to get an average estimate of the width.

Slope of the beach

The slope of the beach was measured from the sampled nesting site point to the low water mark. The Emery survey method (Emery, 1961) was used as shown in Fig. 2. This approach had the advantage of being inexpensive and the light equipment could easily be carried across various beaches. Using the data from the beach profiles recorded from the field, cumulative vertical elevation (y- axis) as a function of cumulative horizontal position (x-axis) was plotted revealing the actual beach profile. Slope was calculated by dividing the difference in elevation between any two adjacent points by the difference in horizontal distance between those two points. The elevation in degrees was calculated from cumulated distance against cumulated elevation using the formula below.

$$\tan \emptyset = \text{cumulated elevation}/\text{cumulated distance}$$

thus, $$\emptyset = \tan^{-1}(\text{cumulated elevation}/\text{cumulated distance})$$

Data analyses

Spatial data analyses were conducted using Arc-GIS 10 to map the distribution of *C. mydas* nest sites. A Garmin GPSMAP 64s was used to record the UTM coordinates of the nest sites during field surveys. The nest site coordinates were recorded on the entire coast from Funzi Island in the south (Kwale County) to Mwongo Sherriff in the north (Lamu County). Correlation was used to find the relationship of number of observed nests with biophysical factors. Pearson Chi-Square was performed to determine the association between conservation status of nesting areas and classification of nesting areas by beach type. Multiple Regression analysis was performed to identify the biophysical factors potentially influencing the number of nests recorded in 2016. The data were analysed at a confidence level of 95%, or $\alpha = 0.05$ using SPSS 23.0.

Results

Identification and geo-referencing of *C. mydas* nest sites

Coordinates collected by GPS showed the distribution of *C. mydas* nest sites along the Kenyan coast. Nesting sites in Lamu County had the highest number of nests per annum, while those in Mombasa had the lowest. Kilifi and Kwale had intermediate numbers of nests. In 2016 most turtle nesting occurred in areas which were not protected. Overall, areas within MPAs had fewer nests (42%) than those outside protected areas (58%) as shown by Fig. 3 and 4. All the nesting areas in Kwale County were outside protected areas because Kisite-Mpunguti MPA (Kwale County) has rocky beaches which were not suitable for *C. mydas* nesting. In Mombasa County, English Point and Nyali South were within the Mombasa MPA, and nesting was previously reported to occur at these locations but has decreased in the recent past. A large proportion of nest sites were outside MPAs in Kilifi County, and those within MPAs were found in tourist areas (Watamu Turtle Bay and Watamu Garoda Resort, within Watamu MPA). Malindi nest sites were all found in the Malindi MPA, and in Lamu County over 60% of nests were found within Kiunga Marine Reserve.

Types of beaches sampled

Nesting sites sampled were classified as those with barriers, open beaches, those under cliffs and those vegetated, as shown in Fig. 5. Nesting beaches with barriers constituted 12.9% (4 counts), open beaches 58.1% (18 counts), beaches under cliffs 3.2% (1 count) and those with vegetated beaches 25.8% (8 counts). In 2016 (Fig. 6)

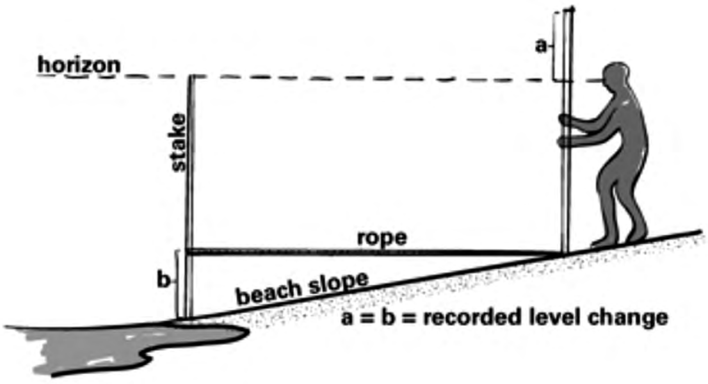

Figure 2. Emery Survey Method. (Source: Florida Centre for Instructional Technology, 2005)

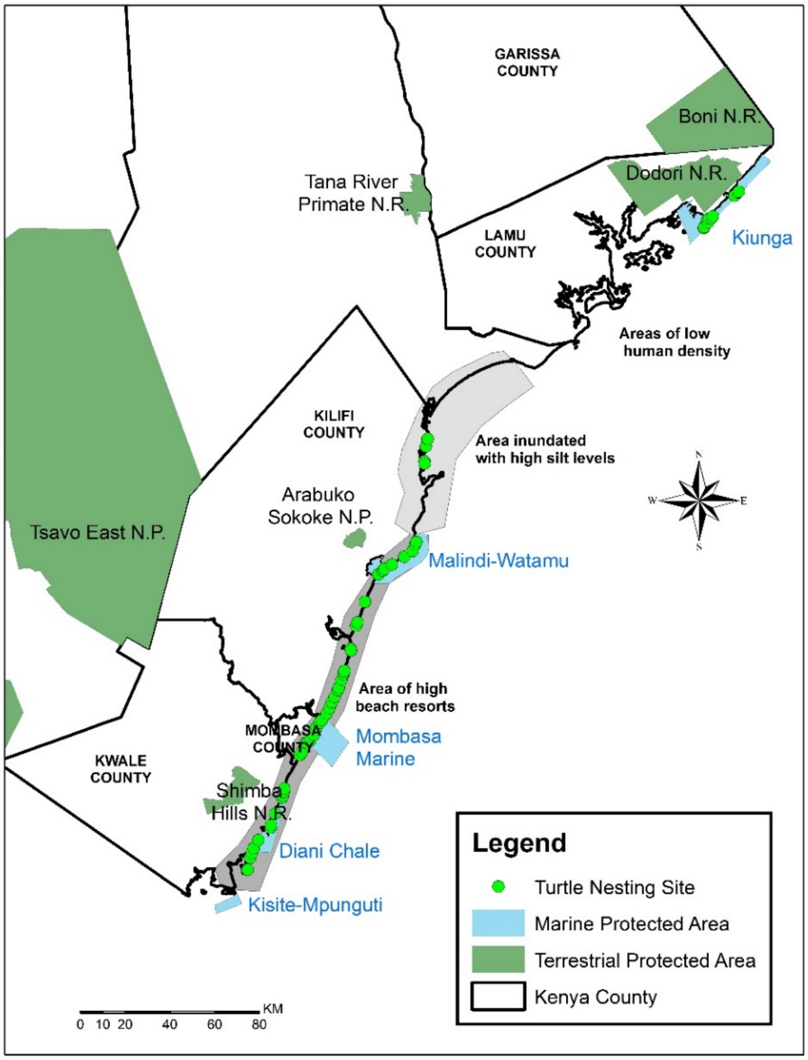

Figure 3. Nesting areas of C. mydas along the Kenyan coast.

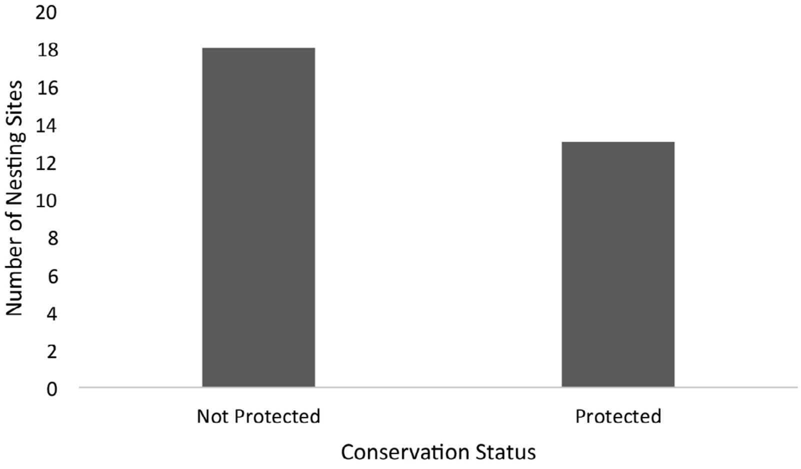

Figure 4. Conservation status of areas in which *C. mydas* nesting occurred in 2016.

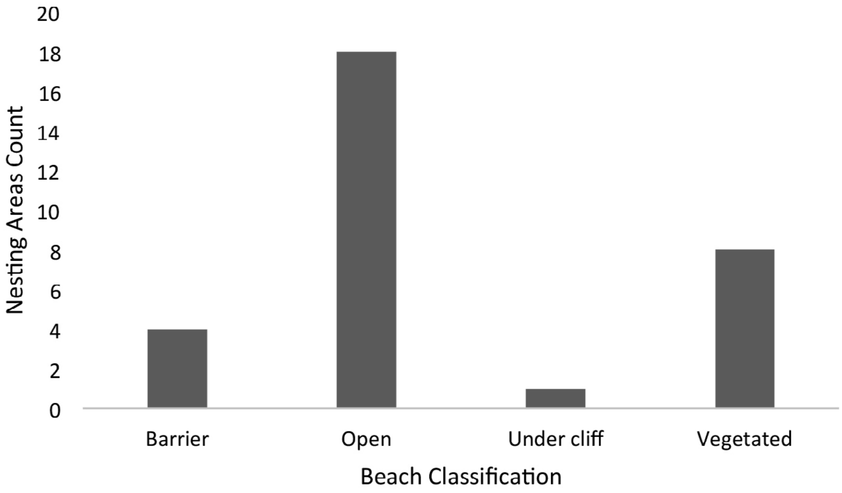

Figure 5. Classification of sampled nesting beaches in 2016 along Kenyan coast.

open beaches had most *C. mydas* nests at 85% (196 nests), followed by vegetated areas at 12% (28 nests), and those with barriers at 2% (4 nests). The beaches with the least nests were under cliff at 1% (2 nests). As shown in Fig. 7, most of the open and vegetated beaches occurred in unprotected areas. There were two nesting sites with barriers in areas which were protected, while unprotected areas had no beaches under cliffs. Results from the Pearson Chi-Square test performed to establish the association between conservation status of nesting areas and classification of the beaches, indicate that there was no statistically significant association between protected and unprotected areas, because they had similar beach types ($\chi2(3) = 1.625$, $p = 0.654$ ($p > 0.05$)). Cramer's V tests show that the association between the variables was acceptable at a value of 0.229.

Vegetation cover at the nesting sites

The important herbaceous vegetation observed on Kenyan beaches was dominated by *Halopyrum mucronatum, Ipomoea pes-caprae* and *Scaevola plumieri.* These herbaceous plants grow above the high water mark, however they are inundated with water during spring tides. The average vegetation cover was 47% in all the sampled nesting areas. The nest sites with vegetation cover of less than 11% included Malindi MPA in Kilifi County, Leopard Beach in Kwale County and Kongowale in Lamu County. The observed number of nests at these sites was 0, 5 and 1 respectively in 2016. The nest sites with high vegetation cover of over 85% were Msambweni House, Seascape (Kwale County) and Aga Khan English Point (Mombasa County). The observed numbers of nests were 3, 1 and 5, respectively. Table 1 gives the number of nests in 2016 at

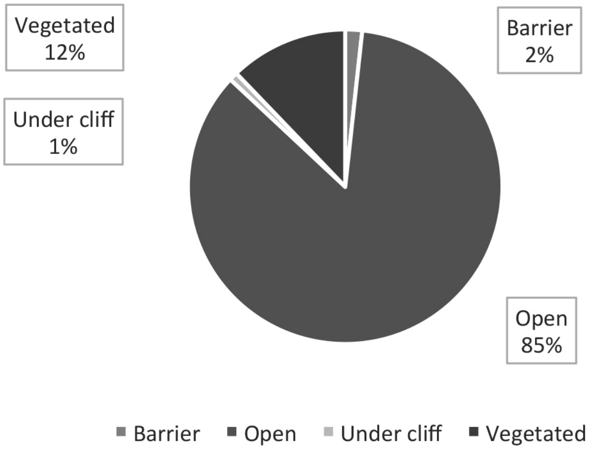

Figure 6. Percentage of number of nests found in 2016 per nest site classification.

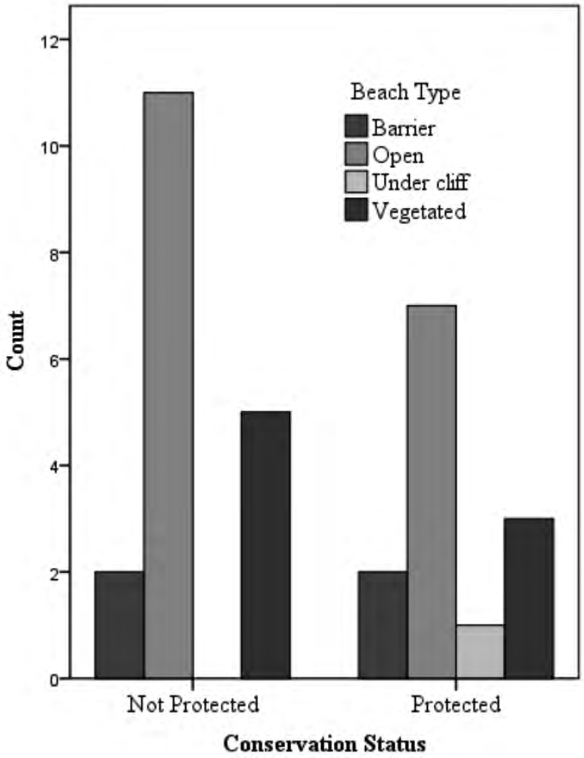

Figure 7. Comparison of conservation status and types of beaches in 2016.

various percentages of vegetation. Approximately 58% of nesting occurred within vegetation cover of 0-50%. Only 9% of nesting was observed to occur within vegetation cover of 76-100%. However, the Pearson product-moment correlation used to determine the relationship between vegetation cover and number of nests show that there was a weak, negative correlation between the two variables which was not statistically significant (r = -0.055, n = 31, p=0.767 ($p > 0.05$)).

Organic matter content of nest site soils

Samples collected showed that areas with high OMC included Watamu Garoda Resort (Kilifi County), Mwanabule (Lamu County), Msambweni House and Seascape (Kwale County) with over 0.2 g of OMC. Nest sites with less than 0.01g of OMC were Kongo Mosque (Kwale County), Nyali South (Mombasa County) and KSV (Lamu County). The average OMC in the sampled nesting areas was 0.11 g. Table 2 shows the amount of organic matter content in the soil at the nesting areas against number of nests observed along the Kenyan coast. 61.7% of nesting occurred in soils with 0.0500 - 0.0999 g of OMC. There were no observed nests in soils with 0.1500 - 0.1999 g OMC, while soils with 0.2000-0.2499 g of OMC had nesting of 7.4 %. The relationship between OMC in the soils and number of nests showed a weak, negative correlation between the variables which was not statistically significant (r = -0.109, n = 31, p = 0.561 ($p > 0.05$)).

Soil texture at nesting sites

All the soil samples were sandy without loam or clay content. Table 3 gives the average percentage of sand in the nesting areas. Most nests (69.81 %) occurred in

Table 1. Percentages of nests within vegetation cover along the beach.

Vegetation cover (%)	Number of observed nests	Percentage (%)
0 – 25	90	39
26 – 50	44	19
51 – 75	75	33
76 – 100	21	9

Table 2. Percentages of nests within ranges of soil organic matter content.

Organic Matter Content (g)	Number of observed nests	Percentage of nests (%)
0.0000 - 0.0499	57	24.78
0.0500 - 0.0999	142	61.73
0.1000 - 0.1499	14	6.09
0.1500 - 0.1999	0	0.00
0.2000 - 0.2499	17	7.39

Table 3. Percentages of soil texture classification at the nesting sites.

Very Course sand	Coarse Sand	Medium Sand	Fine Sand	Very Fine Sand	Silt
1.48 %	22 %	41.36 %	28.45 %	3.42 %	0.13 %

Table 4. Number of nests in relation to width of the beach.

Width of the Beach (m)	Number of observed nests	Percentage (%)
15 - 30	34	14.78
31 - 45	116	50.43
46 - 60	66	28.7
61 - 85	14	6.09

Table 5. Percentage of nests in relation to slope of the beach.

Slope of the Beach (º)	Number of observed nests	Percentage (%)
3.0-7.9	85	36.96
8.0-12.9	112	48.70
13.0-17.9	33	14.34

medium and fine sand, while very coarse sand had 1.48 %, coarse sand 22 %, very fine sand 3.42 %, and silt 0.13 % of observed nests. Kinondo nest site (Kwale County) had the highest amount of very coarse sand at 5.28 % at one observed nest, while the Bureni nest site (Kilifi County) had the highest amount of coarse sand at 75.63 % in 12 observed nests, KSV nest site (Lamu County) had 81.28 % of medium grained sand in 12 observed nests, and Nyali South nest site (Mombasa County) had the highest amount of fine sand at 85.96 with no observed nests in 2016, while Mwaepe (Kwale County) had the highest amount of very fine sand at 31.37 % in two nests in 2016.

Width of the beach at the nesting sites

The widest beaches were Kiwayuu and Chandani (Lamu County), Nyali South (Mombasa County) and Mwaepe (Kwale County) ranging between 61 and 85 m.

As shown in Table 4 these beaches had the least number of observed nests at 6.1 %. The narrowest beaches were Bureni in Kilifi County, and Massage Area and Kinondo in Kwale County. Highest nesting (79.1 %) was observed to occur within a beach width of 31 to 60 m. The average beach width was 44.4 m from the sampled nesting areas. There was a weak, negative Pearson's correlation which was not statistically significant ($r = -0.025$, $n = 31$, $p = 0.895$ ($p > 0.05$)), hence inconclusive evidence about the significance of the association between width of the beach and number of nests.

Slope of the beaches at the nesting sites

Most of the observed nests in 2016 had gentle slopes ranging between 8.0° - 12.9° (Table 5). These were Kitanga Kikuu, Mwanabule, Mongo Sherriff (Lamu County), and Mwanamia and Musumarini (Kilifi County). The average slope of nesting beaches was 9.44° with a higher number of nests (85.66 %) occurring on near flat to gentle slopes of 3.0 - 12.9°. Least nesting (14.34%) occurred on steep beaches of 13.0 - 17.9°. Pearson's correlation test established a weak, negative correlation between the variables which was not statistically significant ($r = -0.008$, $n = 31$, $p = 0.968$ ($p > 0.05$)).

Discussion

Identification and geo-referencing of *C. mydas* nest sites

Sea turtles have been known to nest along the Kenyan coast with over 90% consisting of *C. mydas* (Okemwa *et al.,* 2004). Green turtles have been documented to migrate over 2000 km between their feeding and nesting grounds (Read *et al.,* 2014). Lamu had a high numbers of nests in comparison with other parts of coastal Kenya. Long term studies from South Africa showed that MPAs are effective management tools in the conservation of loggerhead and leatherback sea turtles. The study however indicated that since future factors affecting nesting of sea turtles are stochastic, the presence of MPAs cannot effectively help to predict the status of future populations (Nel *et al.,* 2013). Sea turtle population have been observed to recover in protected areas globally, attributed to conservation efforts aimed at managing nesting areas and reduction of by-catch (Mazaris, *et al.* 2017). Tracking turtles with satellite tags has shown that *C. mydas* benefitted from protection offered by the Tortugas National Park, Florida, USA, with extended periods spent in the protected areas (Hart *et al.,* 2016). In Kenya, the MPAs are proportionally small in comparison to the whole length of the coast, perhaps explaining why most *C. mydas* (58.1%) nest in unprotected areas. However, a high number of nests were observed to occur in Lamu within Kiunga Marine Reserve. The legal notices that established MPAs in Kenya are not clear on the conservation status of beaches within MPAs, making it difficult to enforce laws to protect nest sites in the protected areas.

Vegetation cover at the nesting sites

Xavier *et al.* (2006) reported that *C. mydas* showed a preference of 76 % for nesting on sand dunes or vegetation zones. Nesting areas with very low vegetation cover (<10 %) causes nest cavities to collapse, while very high vegetation cover (>40 %) affected *C. mydas* digging success because of increased compactness of the surface layer associated with root systems. Moderate vegetation (10 - 30 %) assists in sand accumulation and nest site stabilisation (Chen *et al.,* 2007). Madden *et al.* (2008) reported that there was decreased egg mortality in the upper beach due to increased proximity to vegetation. Clutches laid under large bushes and trees had higher hatching and emergence success than those in other habitats (Zarate *et al.,* 2013). Beaches without vegetation have an effect on thermal regimes of nests that influences incubation and resulting sex ratios (National Marine Fisheries Service and US Fish and Wildlife Service, 2007). Most of the nesting sites observed on the Kenyan coast were on sandy beaches with vegetation cover. The mean percentage vegetation cover was 46.9 % ± 25.95 SD.

Organic matter content of nest site soils

Organic matter builds soil structure improving soil drainage and infiltration of water and air into the soil. It also increases the ion exchange capacity of soil and provides a buffering effect on soil pH (Gachene and Kimaru, 2003). OMC is an important factor in nest selection because soils with high OMC contain water-stable aggregates that bind soil particles together and are resistant to being broken down by the impact of water (Gachene and Kimaru, 2003). High OMC also supports vegetation cover that is sometimes crucial in nest placement. A high percentage of vegetation cover however makes it more difficult for the sea turtle to dig and lay eggs (Chen *et al.,* 2007). Stancyk and Ross (1978) reported that beaches with high OMC were often found near to human settlements and activities. High OMC was observed in Msambweni House and Seascape (Kwale County), Watamu Garoda Resort (Kilifi County) and Mwanabule (Lamu County) which all had intensive human activities. Weslawski *et al.* (2000) observed that one of the main threats to sandy beaches is eutrophication which leads to growth of invasive vegetation on the beach, affecting its ecology.

Soil texture

C. mydas dig in sandy beach soils to create cavities in which to lay eggs. Soil is made up of solid particles, water and air which are important in embryo development and determining the survival of the hatchlings. Soil texture determines soil workability, water-holding capacity, soil structure and nutrient retention (Gachene and Kimaru, 2003). *C. mydas* can nest in sites with a variety of soil particle sizes (Chen, *et al.*, 2007). Studies on Ascension Island show that the median number of trial nest holes dug per nesting emergence, and the mean particle diameter of the beach sands were positively correlated. Thus, turtles find it difficult to construct suitable nests in coarse and dry sand. The relationship between the average total survival of hatchlings and the sorting coefficients and mean particle diameters shows lowest survivorship in the most poorly sorted sand (Mortimer, 1990).

Width of the beach at the nesting sites

Narrow beaches are more vulnerable to the effects of climate change as sea level rise will wipe out possible nesting grounds of sea turtles (Fish *et al.,* 2005). Narrow beaches have high rates of erosion, especially if there are overhanging cliffs. Even on narrow beaches with minimal human activities, the number of nests will be low because of disturbance by wave action (Tsoukala *et al.,* 2015). The Magogo nest site in Lamu County had only two nests in 2016 despite being secluded with minimal human activities.

Slope of the beaches at the nesting sites

Studies in Mexico indicated that 65% of nesting occurred in two zones; one with a gradual slope and mean beach width of 38 m, and the other with a moderate beach slope with a beach width of 30 m (Zavaleta-Lizárraga and Morales-Mávil, 2013). In Florida, USA, Rizkalla and Savage (2011) showed that sea turtles prefer to nest in intermediate inclines. This was confirmed on the Kenyan coast where *C. mydas* showed a preference for gentle slopes with a mean of 9.44^0 ±3.24 SD, and a mean beach width of 44.40 m ±16.93 SD. Wood and Bjorndal (2000) reported that the mean slope of the beach for loggerhead turtles was 9^0. However, from the findings of this study, there was a weak negative correlation between slope and number of nests. The slope of the beaches in coastal Kenya are affected by winds associated with the south east monsoon (SEM) from December to March, and the north east monsoon (NEM) from April to September. Historical data shows that nesting of sea turtles in Kenya was high during the months of March to July.

Conclusions

The largest number of the green turtle nests was observed along the coastline of Lamu County. Nests were most common on the Island of Mwongo Sherriff within Kiunga MPA. A dwindling number of nests were observed in Mombasa and Kilifi Counties due to anthropogenic factors in the beach areas. Kwale County had intermediate observations with some areas having stable numbers of nests. Green turtles preferred to nest in areas with vegetation cover of less than 50 %, and 61.7% of nests occurred in areas with 0.0500-0.0999 g of OMC. The highest number of nests occurred on beaches with widths of 31- 60 m, while the least number of nests were on steep beaches with an inclination of 13.0 - 17.9°. Most nests occurred in medium and fine sand (69.81 %). Due to the removal of native beach vegetation to develop beaches, invasive plants were observed on some beaches, which act as barriers to nesting turtles. The invasive species need to be continually managed (removed) to allow other suitable beach vegetation to grow.

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Spirulina (*Arthrospira fusiformis*) as a potential protein source in practical diets for fry mariculture of Rufiji tilapia (*Oreochromis urolepis urolepis*)

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Abstract

The effects on growth performance, feed utilization, survival and whole fish body proximate composition of replacing fishmeal (*Rastrineobola argentea*) with a locally available spirulina species (*Arthrospira fusiformis*) as a protein ingredient in the feed of Rufiji tilapia (*Oreochromis urolepis urolepis*) fry was examined. Fishmeal replacement with *A. fusiformis* was carried out at 5%, 15%, 25%, 35% and 100% (S5, S15, S25, S35 and S100), and the effect of the replacement was compared with the control diet (S0, 0% spirulina). Fish fry were stocked at an average initial weight of 0.57 g at 10 fish per 0.1 m^3 and cultured for 60 days using full strength salt water (30-35 ppt). *A. fusiformis* was isolated from Momela Lake in Arusha National Park, Tanzania and propagated using selected culture media. It was observed that fish fry fed spirulina at a 5% fishmeal substitution level diet had better ($p<0.05$) final weight (8.48), average daily weight gain (0.132), specific growth rate (4.47), feed conversion rate (2.08) and protein efficiency ratio (1.37) compared to the control fish group. Growth performance in fish fed diet S15 was comparable with the control group ($p>0.05$). Spirulina supplementation had no effect on fish survival rates and meat quality. It appears that the Momella Lake spirulina may be an appropriate growth-stimulating plant protein when used as a feed additive in Rufiji tilapia mariculture.

Keywords: Rufiji tilapia; Spirulina; *Arthrospira fusiformis*; Growth performance; Meat quality; Mariculture

Introduction

Globally, aquaculture is currently the most rapidly growing food production sector, with an annual growth rate of more than 5.8% (FAO, 2018). In line with global aquaculture growth, there is an increased demand for nutritionally sufficient and economically affordable aqua feeds (Thilsted *et al.,* 2016). Protein remains the most expensive ingredient in these feeds and the most crucial factor affecting growth performance of fish (Jose *et al.,* 2007). Thus, one of the constraints to aquaculture sustainability and growth is the shortage of readily available cheap protein sources (Machena and Moehl, 2001). Fishmeal is the most preferred feed supplement in intensive and semi-intensive aquaculture systems (El-Saidy and Gaber, 2004). This is partly because it is considered most palatable and rich in essential amino acids, fatty acids, energy and minerals (Tacon, 1993; Hardy, 2010). However, because global fisheries have leveled off at a time when there is increasing demand to feed the growing human population and to supply a fast-expanding fish meal industry, the availability of fish meal is also limited (FAO, 2016). Moreover, from an environmental perspective, overfishing the wild fishery for fishmeal production is unjustifiable (Wijkström, 2009).

In the search for new, readily available protein sources, algae are attracting the attention of nutritionists as one possibility to supplement the world protein shortage, particularly in developing countries (Becker, 2007; Roy and Pal, 2015). Cultivated microalgae and cyanobacteria have long been integrated with hatchery production of many farmed finfish, shellfish and other commercially important aquaculture species (Muller-Feuge, 2010). Alongside these well-established microalgae uses in aquaculture hatcheries, there is currently a drive to substitute fishmeal with algae or cyanobacteria in formulated animal feeds, both for aquaculture and

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terrestrial animals (Nath *et al.,* 2012; Sheikhzaden *et al.,* 2012). One possibility is to utilize primary producers like spirulina (*Arthrospira spp.*), which supports huge populations of flamingos in Tanzania's soda lakes.

Spirulina are multicellular, filamentous, gram negative, non-toxic species of cyanobacteria belonging to two separate genera, namely *Spirulina* and *Arthrospira*, consisting of about 15 species (Habib *et al.,* 2008). Of these, *Arthrospira spp*. are the most common and widely available, possessing diverse biochemical compounds of biological and nutritional significance such as protein (55-70 %), minerals (calcium and iron) and vitamins (Habib *et al.,* 2008). It grows and thrives in warm alkaline lakes including the soda lakes of East Africa such as Elmenteita, Lishateni and Momela in Tanzania, and Nakuru in Kenya (Grant, 2006; Kaaya *et al.,* 2007). It can be harvested from such lakes and used directly to serve as a protein source in fish feeds, or to seed artificial mass production systems.

Several studies have evaluated *Arthrospira spp*. as a potential fish meal substitution in feeds for tilapia and other fish species (Lu *et al.,* 2002; Takeuchi *et al.,* 2002; Abdel-Tawwab and Ahmad, 2009; Dernekbasi *et al.*, 2010; Belal *et al.,* 2012; El- Sheikh *et al.,* 2014; Velasquez *et al.,*2016). The results show considerable variation in the degree of success for partial and complete replacement depending on the spirulina strains, farmed fish species as well as the growth stage of fish. In addition to their potential use in aquaculture as protein sources, *Arthrospira* species are reported to be a good immunnostilmulant in fish, poultry and rats (Abdelkhalek *et al.,* 2017; Abdel-Daim *et al.,* 2018; Kata *et al.,* 2018).

Tanzania is endowed with a long coastline, and farming the sea with high value marine species could be more profitable than land-based farms. According to the World Bank (2016), it is estimated that humans will soon run out of areas that will be able to produce enough food to provide for the entire population of earth, due to freshwater scarcity. The same report points out that continuous human population growth will lead to increasing competition for limited water resources, which are likely to become more constrained due to adverse climate change-associated effects such as drought and flooding. It would therefore make sense to explore new ways in which the ocean can be used to cultivate food in the future.

Rufiji tilapia (*Oreochromis urolepis urolepis*) occurs in the southeastern rivers, reservoirs and satellite lakes in Tanzania, where it is an economically important fish to the communities of these areas (Lamtane, 2008). This species has considerable potential for culture in saline waters in order to expand the farming area, and improve income and livelihoods of coastal communities.

Despite being found mainly in freshwater and brackish environments, *O. urolepis urolepis* can be cultured in salt water without adverse effects on growth and survival rates. For example, Nehemia *et al.* (2014a) reported 100 % survival and acceptable growth performance when *O. urolepis urolepis* was cultured using sea water at 35 ppt. Similar results were reported by Nehemia *et al.* (2014b) and John (2016). On the other hand, Ulotu *et al.* (2016) found best growth and survival rates at a salinity of 25 ppt when *O. urolepis urolepis* was cultured in water with salinity values of 5, 15, 25 and 35 ppt.

Rufiji tilapia, like other tilapiine species, is a typical omnivorous fish species. Therefore, it was hypothesized that spirulina may have potential as a protein source for *O. urolepis urolepis*. Spirulina (*A. fusiformis)* collected from Momela Lake was used in this study as a partial and complete fish meal substitute, and its effects on growth performance, survival rates and biochemical composition were tested on Rufiji tilapia fry cultured in full-strength salt water.

Material and methods

Collection and culture of spirulina

Water samples containing a mixture of microalgae was collected from the Big Momela Lake in Arusha National Park, Tanzania. After sampling, water samples were immediately transported in a cool box to the Department of Botany, University of Dar es Salaam for isolation. Isolation was carried out using serial dilution and streak plating microbiology techniques. Spirulina were identified according to Ciferri (1983) and Komarek and Lund (1990). Isolated spirulina was then cultured outdoors at the Institute of Marine Sciences in Zanzibar for three months.

Cultivation was carried out using ten 100 l half-metal drums lined with a plastic sheet to prevent rust (Fig. 1). Filtered tap water was left for 48 hours to allow for chlorine evaporation and used to make a culture medium composed of the following ingredients (Singh, 2006) per litre: sodium bicarbonate (8 g); potassium sulphate (0.5 g); sodium chloride (5g); magnesium sulphate (0.16 g); ferrous sulphate (0.05 g); Urea (0.2 g); and phosphoric acid (0.2 ml). Inoculation

Figure 1. *A. fusiformis* outdoor culture; (A) culture basin preparation; (B) culture unit.

started with 20 l culture medium inoculated with 1 l spirulina inoculum as a starter culture, followed by scaling-up to 90 l within 7 to 10 days (Son *et al.*, 2019). Daily measurements of various pond parameters such as temperature, light intensity and salinity were undertaken to monitor the wellbeing of the culture.

Microscopic examination was done daily to detect any abnormal morphological changes and the presence of contaminating organisms such as other algae and protozoa. Large contaminants like insects, plant leaves and birds were excluded by placing a wire mesh on top of the culture basins. Harvesting was carried out in the morning hours to ensure cool temperatures with enough sunshine to dry the product. After sun-drying, the spirulina was stored in cool, dry environment to preserve its quality.

Fish diets and experimental running

The proximate chemical composition of the feed ingredients used is presented in Table 1. Six isonitrogenous test diets (Table 2) were formulated to contain 35% crude protein. Fish meal and maize bran (obtained from the local market) were prepared with a cereal grinding machine, while spirulina was ground to powder form using a mortar and pestle. In the control set (S0), experimental fish were fed with a 100% fishmeal protein diet. In the remaining treatments, prior-cultured dried spirulina meal protein replaced 5% (S5), 15% (S15), 25% (S25), and 35% (S35) and 100% (S100) of fishmeal protein. The experimental culture system consisted of a series of eighteen 100 l plastic basins, divided into six triplicate treatments. The fish were cultured using seawater from the Pangani river estuary, pumped during the high tide to ensure high salinity of 33-35 ppt. Rufiji tilapia fry that had been raised in full strength sea water were obtained from the Institute of Marine Science Mariculture Centre (IMS-MC) hatchery at Pangani. Prior to the experiment, fish were acclimatized for two weeks while they were fed the control diet. Thereafter, the fish fry (0.57 ±0.01 g) were randomly distributed into six experimental groups in triplicate at a density rate of 10 fry per 0.1 m^3. Fish were fed at 5% of their body weight twice per day, at around 8:00 and 16:00. Complete water exchange was carried out every 10 days. Fish were group-weighed every 10 days and the amount of administered feed was adjusted accordingly. The experiment was conducted for 60 days.

Water quality monitoring

Physical parameters (temperature, pH, dissolved oxygen, and salinity) were checked daily using a hand-held thermo-pH meter (HANNA model no: HI 98128), oxygen meter (YIS Environmental model no: DO 200) and refractometer (EXTECH instruments model no: RF 20), respectively. Water samples for un-ionized ammonia analysis were collected twice a week in 500 ml plastic bottles and stored in a freezer at the IMS-MC before analysis in the laboratory. Concentration of ammonia in the water samples was determined according to the guidelines of UNESCO (1993).

Table 1. Chemical proximate composition (% Dry matter) of the ingredients used in experimental diets.

Ingredients	Dry matter	Crude protein	Crude lipid	Crude fiber	Ash
Fish meal	94.43	63.0	7.62	0	6.51
Spirulina meal	94.63	57.25	7.42	9.14	5.23
Maize bran	89.59	9.23	1.74	7.95	3.8

Table 2. Ingredient and proximate composition of the experimental diets.

	Diets					
Ingredients (g/100g)	**Control**	**S5**	**S15**	**S25**	**S35**	**S100**
Fish meal[a]	51.0	46.0	37.0	29.0	20.0	0.0
Spirulina meal	0.0	5.0	15.03	25.0	35.0	56.0
Maize bran	39.0	39.0	38.0	35.0	34.0	38.0
Sunflower oil	6.0	6.0	6.0	7.0	7.0	6.0
Binder (Cassava)	2.0	2.0	2.0	2.0	2.0	2.0
Premix[b]	2.0	2.0	2.0	2.0	2.0	2.0
Total	**100**	**100**	**100**	**100**	**100**	**100**
Proximate composition (% DM)						
Dry matter	92.22	92.23	91.19	91.87	92.1	92.45
Crude protein	35.19	35.29	35.0	35.19	35.39	35.2
Crude lipid	10.51	10.31	9.84	9.53	9.06	6.59
Crude fiber	3.18	3.14	3.14	3.06	2.98	2.7
Ash	4.78	4.74	4.67	4.6	4.53	4.09
NFE[c]	46.34	46.52	47.36	48.1	48.04	51.42
GE(kcal/kg)[d]	4937	4931.3	4906	4918.6	4884	4781.5

[a] Locally occurring Lake Victoria sardine (*Rastrineobola argentea*).

[b] Locally manufactured commercial premix (per kg mixture): vitamin: A, 500000 IU; D3, 1000000 IU; E,1500 IU; B1, 600 mg; B2, 2500 mg; B6, 125 mg; B12, 7.5 mg; K,1250 mg; C, 200 mg. Minerals: 1.5 mg, $CuSO_4$; 90 mg, $MnSO_4$; 300 mg, MnI_2; 70 mg, ZnO; 5500 mg, $C_6H_5NO_3$; 5000 mg, $C_{18}H_{32}CaN_2O_{10}$.

[c] NFE (Nitrogen free extract) = 100 - (crude protein + crude lipid + crude fiber + ash).

[d] GE (gross energy): calculated using conversion factors 5.65, 9.45 and 4.22 kcal/g for protein, lipids and carbohydrate respectively (NRC,1993).

Growth performance and feed utilization.

After the feeding trial, fish from each basin were collected, weighed, and counted. The parameters of fish growth and feed utilization were calculated according to the following equations:

Average daily weight gain (g/fish) (ADG)

$$\text{ADG} = (\text{W2} - \text{W1}) / \text{T} \quad \text{..........} 1$$

Where: W1 = Mean initial weight (g)
W2 = Mean final weight (g)
T= experimental period

Specific growth rate (SGR)

$$\text{SGR} = \left(\frac{\text{Ln W2} - \text{Ln W1}}{\text{T}}\right) \text{x} \, 100 \quad \text{..........} 2$$

Where: Ln = Natural Logarithm
W1 = Mean initial weight (g)
W2 = Mean final weight (g)

Feed conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Dry feed intake (g)}}{\text{Live weight gain (g)}} \quad \text{..........} 3$$

Where: Dry feed intake (FI) = Total feed consumed during the 60-day trial

Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Live weight gain (g)}}{\text{Protein intake (g)}} \quad \text{..........} 4$$

Where: Protein intake = % Crude protein x FI

Proximate analysis

Ingredients, practical diets and whole-body chemical composition were analysed according to AOAC (1984). Dry matter was calculated from weight loss after oven drying of the fresh samples at 105°C for 48 hrs continuously. Ash content was determined by incinerating

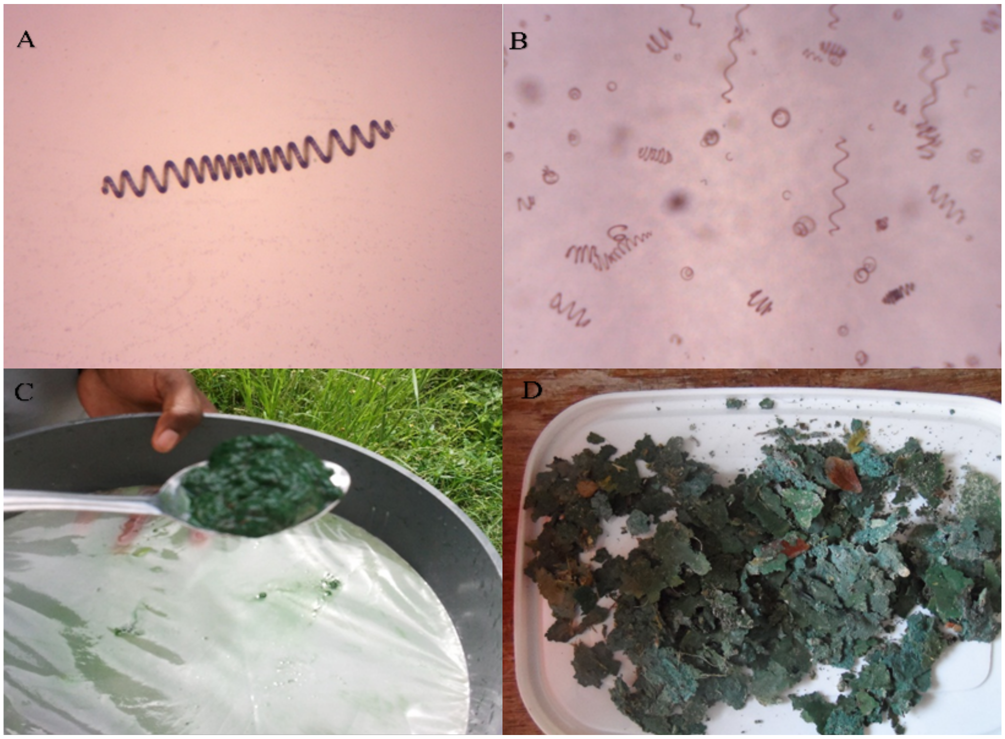

Figure 2. Morphology of spirulina, *A. fusiformis*. (A) x 10 magnification. (B) x 4 magnification. (C) fresh. (D) dried mass.

the fresh samples in a muffle furnace at 550 °C for 3 hrs. Crude protein (N X625) and crude fat was determined using micro kjeldahl and soxhlet extraction methods, respectively. Crude fiber was determined using an ANKOM fiber analyzer.

Data analysis

Results were recorded as means with standard error (± SE) calculated from the residual mean square. One-way analysis of variance (ANOVA) was used to determine differences between treatment means at $p < 0.05$. Post-hoc analysis was carried out where significant differences were detected between treatments by using Tukey's Honest Significant Difference (THSD) Test. Analysis was performed using Statistica software (Stat soft.) version 7 (Nunes *et al.*, 2015). Homogeneity of variance was checked using Levene's test.

Results

Spirulina identification

Cultured spirulina was identified based on microscopic characteristics and guidelines after Ciferri (1983), and Komarek and Lund (1990). The isolated spirulina were solitary with multicellular cylindrical trichomes. Most filaments were helical consisting of shorter than broader cells with clear and visible transverse cross walls (Fig. 2A and B).

General observations from the fish experiment

Fish fry in all treatments consumed their assigned experimental diets with full acceptance. Cultured spirulina had a proximate composition of 57.25% CP, 94.63% DM, 7.42% EE, 9.14% fiber and 5.35 % ash content (Table 1). Ingredients and proximate composition of the experimental diets are shown in Table 2. Water

Table 3. Physical-chemical parameters of water during experimental period.

Parameter	Minimum	Maximum	Mean ± SE
Temperature (°C)	24.5	29.3	27.1 ± 0.13
pH	7.5	8.2	7.9 ± 0.02
Unionized ammonia (mg/l)	0.007	0.13	0.02 ± 0.01
Dissolved oxygen (mg/l)	5.61	8.71	6.79 ± 0.21
Salinity (ppt)	33	35	33.4 ± 0.1

Table 4. Growth performance, feed utilization and survival rate of Rufiji Tilapia, *O. urolepis urolepis* fry, fed on experimental diets (mean ± SE, n=3).

	Experimental diets					
Parameter	**S0**	**S5**	**S15**	**S25**	**S35**	**S100**
I W (g)	0.57 ± 0.0^{a}	0.58±0.01^{a}	0.58 ±0.02^{a}	0.58±0.01^{a}	0.56±0.01^{a}	0.57±0.01^{a}
FW (g)	7.25±0.01^{a}	8.48±0.01^{b}	6.65 ±0.0^{ac}	6.31±0.01^{c}	5.98±0.02^{c}	3.78±0.02^{d}
WG (g)	6.68±0.01^{a}	7.9±0.01^{b}	6.07±0.02^{ac}	5.73±0.09^{c}	5.42±0.02^{c}	3.203±0.02^{d}
ADG (g fish^{-1}day^{-1})	0.111±0.0^{a}	0.132±0.01^{b}	0.101 ±0.02^{ac}	0.1±0.02^{c}	0.09±0.03^{c}	0.053±0.02^{d}
SGR (%g day^{-1})	4.23±0.01^{a}	4.47±0.03^{b}	4.07 ±0.02^{ac}	3.99±0.02^{c}	3.94±0.03^{c}	3.14±0.03^{d}
FI (g feed fish^{-1})	14.95±0.01^{a}	16.47±0.02^{b}	14.04±0.02^{ac}	13.51±0.01^{cd}	12.84±0.01^{d}	9.1±0.003^{e}
FCR	2.24± 0.0^{a}	2.08±0.0^{b}	2.31±0.0^{ac}	2.35± 0.0^{c}	2.39±0.01^{c}	2.58±0.01^{d}
PER	1.28 ± 0.0^{a}	1.37 ± 0.0^{b}	1.24 ± 0.0^{ac}	1.21 ± 0.0^{c}	1.19±0.03^{c}	1.0 ± 0.02^{d}
Survival rate (%)	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100 ±0.0^{a}	100 ±0.00^{a}

Values in the same row having different superscripts are significantly different ($p < 0.05$). Where: IW stands for initial weight, FW for final weight, ADG for average daily gain, SGR for specific growth rate, FCR for feed conversion ratio and PER for protein efficiency ratio.

quality parameters ranged from 24.5-29.3 °C, 7.5 - 8.2, 0.007-0.013 mg/l, 5.61-8.71 mg/l and 33-35 ppt for temperature, pH, unionized ammonia, dissolved oxygen (DO) and salinity, respectively (Table 3).

Growth performance

Growth performance of Rufiji tilapia fed with different experimental diets are presented in Table 4 and Fig. 3. Replacement of fishmeal by spirulina at different levels had a significant effect (p < 0.05) on the fish growth (FW, AWG, ADG and SGR). Fish fed with the S5 diet showed significantly (p<0.05) higher growth than those fed the control diet, S0. The lowest fish growth was observed in fish fed with the S100 diet. There was an increasing trend in FW, AWG, ADG and SGR in the S5, S0, S15, S25, S35 and S100 treatments respectively. Fish fed with the S15 diet had comparable (p>0.05) growth to those fed with the control diet, S0. Similarly, there was no statistical difference (p>0.05) in growth performance between fish fed with S15, S25 and S35 diets, the three diets however performed significantly higher (p<0.05) compared to S100. No fish mortality was observed during the whole experimental period.

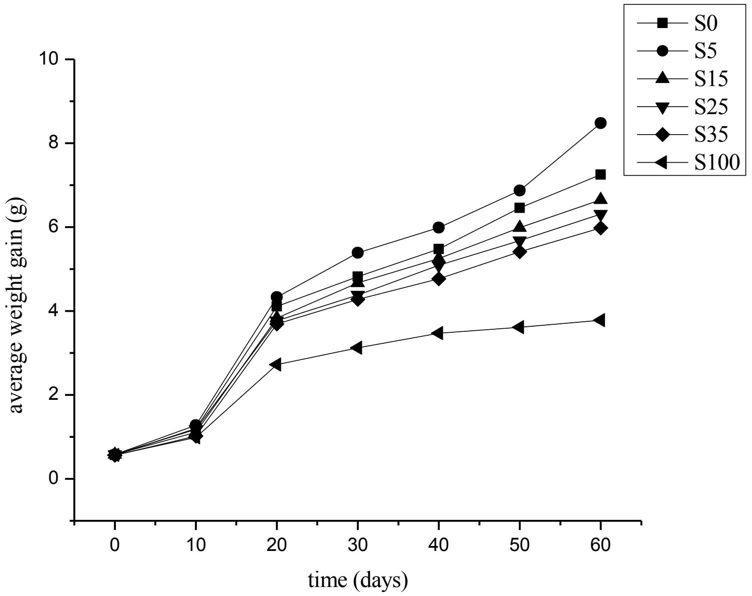

Figure 3. Changes in weight gain (g) of Rufiji tilapia, *O. urolepis urolepis* fry fed different levels of spirulina for 60 days.

Table 5. Chemical proximate composition (% on DM basis) of whole body of Rufiji tilapia, *O. urolepis urolepis*, before and after the experiment (mean ± SE, n=2).

Treatment	Dry matter	Crude protein	Crude lipid	Ash
Initial	92.64± 0.33^{a}	54.84± 0.22^{a}	15.61± 0.11^{a}	38.55± 0.24^{a}
S0	92.72± 0.16^{a}	50.71± 0.44^{a}	14.65± 0.18^{a}	20.96± 0.62^{b}
S5	93.05± 0.33^{a}	52.56±1.44^{a}	15.07± 0.41^{a}	20.17± 0.15^{b}
S15	95.18± 0.43^{a}	51.34± 1.25^{a}	14.72± 0.00^{a}	21.14± 1.38^{b}
S25	96.73± 0.21^{a}	53.48± 0.03^{a}	14.57± 0.51^{a}	20.29± 0.73^{b}
S35	95.70± 0.3^{a}	45.50± 4.4^{a}	12.8± 1.23^{b}	19.7± 0.290^{b}
S100	94.85± 0.26^{a}	51.18± 0.01^{a}	14.70± 0.00^{a}	21.35± 0.00^{b}

Values in the same column having different superscripts are significantly different ($p < 0.05$)

Feed utilization

A similar trend to growth performance was observed in the feed utilization parameters FCR and PER (Table 5). The lowest and highest FCR was exhibited in fish fed on S5 and S100 diets respectively, with the opposite trend observed in terms of PER. Both FCR and PER differed significantly ($p<0.05$) among experimental diets. There was no significant difference ($p>0.05$) in feed utilization between fish fed on S0 and S15, and also between S15, S25, S35 treatments. The lowest ($p<0.05$) feed utilization values were observed in fish fed on the FM free diet (S100).

Fish biochemical composition

Results of carcass composition (DM, CP, CL and ash) of the whole-fish body at the start and the end of the experiment on fish fed fishmeal-spirulina substituted diet at different levels are presented in Table 5. There were no significant differences ($p> 0.05$) in terms of CP, DM and total ash content among experimental diets, except for CL which was significantly higher in the carcass of fish fed with the S35 diet.

Discussion

The potential of using single cell protein ingredients such as spirulina in fish feeds can be assessed on the basis of its protein content. Results from studies examining the nutritive value of spirulina vary greatly (Shah *et al.,* 2017). Madkour *et al.* (2012) reported protein content values (dry mater basis) of 37.79 to 47.1 %, and 52% when spirulina was cultured in reduced cost and synthetic media, respectively. Evaluating biochemical composition of spirulina using different culture media, Marrez *et al.* (2014) reported protein content ranging from 49.5 to 59 % dry matter. A 57% crude protein content of the spirulina used in this study was within the ranges reported by Alvarenga (2011) and Belal *et al.* (2012), but differed from Yilmaz (2012), who reported crude protein ranges of up to 65%. The reasons for this variation could be attributed to the fact that spirulina in the present study was cultured under ambient conditions compared to the more controlled laboratory condition in the other studies.

Growth performance of Rufiji tilapia fed with spirulina supplementation at 5% inclusion were significantly higher than those fed with the control diet. Moreover, fish fed with 15% spirulina inclusion diet had comparable growth to those fed with the control diet. This indicates that spirulina inclusion of up to 15 % improved digestibility and feed intake, considering that spirulina is a single cell protein with no cell wall, and also contains high content of various nutrients such as vitamins and minerals (Habib *et al.,* 2008; Abdel-Tawwab and Ahmad, 2009). Similar findings were reported by Lu *et al.* (2002) when feeding raw spirulina to *Oreochromis niloticus* larvae at the onset of exogenous feeding. Additionally, *O. niloticus* was reported to display better weight gain and specific growth rates when fed with a 5 g spirulina kg^{-1} diet (Abdel-Tawwab and Ahmad, 2009).

Apart from tilapia, spirulina has also been reported to improve growth in other fish species. Dernekbasi *et al.* (2010) for example, reported superior growth when spirulina was supplemented at 40% in practical diets for guppy, *Poecilia reticulata*. Rainbow trout (*Oncorhynchus mykiss*) fed with 7.5% spirulina in formulated feed showed significantly higher weight gain than with the fishmeal control diet (Teimouri *et al.,* 2013). Similarly, 5% fishmeal replacement with spirulina in diets of Parrotfish (*Oplegnathus fasciatus*) resulted in the highest weight gain compared to other treatments (Kim *et al.*, 2014). On other hand, Ungsethaphand *et al.* (2010) did not find any significant differences in the growth performance of fish fed diets supplemented with spirulina at different levels to those fed the fishmeal control diet.

Improved fish growth has also been reported from studies that used other algal species to substitute fishmeal protein (Sarker, 2016). Tartiel *et al.* (2008), replacing fish meal with a combination of *Chlorella spp.* and *Scenedesmus spp.* in diets for *O. niloticus* at 10, 25, 50 and 70 % replacement levels, found that growth performance, FCR and protein productive were significantly higher in fish feed diets containing 50% algae. Similarly, Walker and Berlinsky (2011) reported improved feed utilization and growth in juvenile Atlantic cod (*Gadus morhua*) fed with 15% fishmeal substitution with a combination of dried *Nannochloropsis sp. and Isochrysis sp.*. Vizcaíno et al. (2016) also concluded that *Tetraselmis suecia* and *Tisochrysis lutea* could replace up to 15% fishmeal in diets of gilthead sea bream fry.

In the present study, both FCR and PER increased with spirulina supplementation up to 15% inclusion. This is in agreement with Watanabe *et al.* (1990) and Takeuchi *et al.* (2002) who also found that feed supplemented with spirulina powder improved the feed conversion ratio in striped jack, *Pseudocaranx dentex*. Also, Belal *et al.* (2002) reported better FCR and PER when a 5 g spirulina kg^{-1} diet was fed to *O. niloticus.* However, higher spirulina inclusion may result in poor feed utilization. This was found to be the case in the present study where both FCR and PER were negatively affected with higher and complete fishmeal replacement with spirulina. Similar results were reported by El-Sayed (1994) and Sharma and Panta (2012) who found that substitution with spirulina beyond 30% negatively affected fish growth. Additionally, Takeuchi *et al.* (2002) found that juvenile tilapia fed solely on the alga showed lower PER than commercial diets. On the other hand, Ungsethaphand *et al.* (2010) noted that feed utilization of hybrid red tilapia was not affected by spirulina supplementation. These variations might be attributed to differences in the spirulina concentration, the form of spirulina (raw or dried), fish species and size, as well as rearing condition.

Proximate biochemical composition of any edible organism forms an important aspect in food nutrition. The nutritive composition of fish can be greatly influenced by the type of feed they consume (Edea *et al.*, 2018). In the present study, there were no significant differences in body crude protein and dry matter between fish groups fed spirulina and control diets. This indicates that spirulina supplementation did not compromise protein synthesis in fish. Similarly, Olvera *et al.* (1998) found that crude body protein and dry matter in *O. mosambicus* were not clearly affected by spirulina inclusion. Also, red tilapia fed with spirulina diets at 0, 5, and 10% levels did not show significant differences in carcass proximate composition compared to those fed on the control diet (Ungsethaphand *et al.*, 2010). On the other hand, lower lipid content in fish body fed 35% spirulina in the present study could be linked to a decrease in fish appetite, resulting in lower feed intake and nutrient utilization, which could lead to decreased lipid synthesis and deposition.

Tilapia is able to exist in a wide range of water temperature (from 12 to 42 °C), but the temperature to which they will adapt on sudden transfer depends on the temperature to which they were acclimated (Avella *et al.*,1992). According to Popma and Lovshin (1995), the lethal lower temperature for most tilapia species is 10 - 11°C, while stress and diseases outbreaks occur at 37 - 38°C. In the present study, water temperature, dissolved oxygen and ammonia levels were within acceptable ranges reported by Makori *et al.* (2017), while pH (7.5 - 8.2) was within ranges reported by Ross (2000) who found pH tolerance in tilapia to be as low as 3 and as high as 11, and with the optimum range being from 7 - 9. On culturing Rufiji tilapia in salt water, Nehemia *et al.* (2014a) concluded that up to 35 ppt salinity was acceptable, which is within the range (33 – 35 ppt) in which fish were cultured in the present study.

Conclusions

The present study evaluated the potential use of a locally sourced spirulina, *A. fusiformis*, as a protein source in practical diets for Rufiji tilapia mariculture. The choice of this microalga was based on its relatively high protein content, local availability in soda lakes, and favorable climatic condition for its culture in Tanzania. The conclusion drawn from the present study is that *A. fusiformis* from Momela Lake can replace up to 15 % of the fishmeal protein in feeds for *O. urolepis urolepis* without adverse effects, and the best fish growth was observed at the 5% level.

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Stock status and some biological aspects of *Lethrinus lentjan* (Lacapede, 1802) from the south coast of Kenya

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Abstract

The Pinkear Emperor (*Lethrinus lentjan,* Laćepède, 1802) is among the three demersal species dominating fish catches in the artisanal fishery on the Kenyan coast. Available data indicate that total landings of *L. lentjan* have declined over the past decade due to possible over-exploitation. This paper provides a brief account of the stock status of this species and biological aspects including length-weight relationship, body condition, and reproduction from a total of 575 fish specimens sampled. Sampling was done at Msambweni, Shimoni, Majoreni and Vanga fish landing sites on the south coast of Kenya from September 2016 to February 2017. Individual total length (TL, cm) and body weight (BW, g) was measured on site while some specimens were dissected for sex and gonad maturity analysis. Mortality, exploitation rate, length-weight relationship, condition factor, fecundity, and size at maturity (L_{50}) were determined. Length frequency analysis indicated that *L. lentjan* was exploited above the optimum level, at $E = 0.55$ (exploitation rate). Growth was allometric with the length exponent ($b = 2.95$) being significantly <3. Mean fecundity was 89,573 ± 9,841 eggs/female/year. Gonadosomatic index was highest in January (2.08 ± 0.20) suggesting a possible peak spawning period at this time. The study provides a brief scientific overview of *L. lentjan* as a baseline for future in-depth biological studies of this species along the Kenyan coast.

Keywords: Stock status; length-weight relationship; condition; reproduction; allometric growth; south coast of Kenya

Introduction

Lethrinids are among the most commercially important group of fishes on many tropical coasts (El Gammal, 1988) and are considered a good source of animal protein for the communities of the western Pacific and the Indian Ocean (Carpenter and Allen, 1989). Hicks and McClanahan (2012) reported that *L. lentjan* accounted for 22% of the total catch on the south coast of Kenya and forms one of the top 3 species landed along with *Siganus sutor* (Valenciennes, 1835) and *Leptoscarus viagiensis* (Quoy and Gaimard, 1824). However, as reported for other species, there is a concern that landings of this species along the Kenyan coast have declined over the years (Kaunda-Arara *et al.*, 2003). Moreover, *L. lentjan* is exploited at levels that have led to both recruitment and growth overfishing (Hicks and McClanahan, 2012). These findings are consistent with those of a more recent study that examined decadal trends in Kenyan artisanal fisheries revealing that the decline of catches in the coral reef fisheries have quadrupled since the mid-1980s (Samoilys *et al.*, 2017). Few studies on stock status and biological aspects have been conducted on fish species in Kenya (Nzioka, 1979; Ntiba and Jaccarini, 1990; Kaunda-Arara and Ntiba, 1997; Kulmiye *et al.*,

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2002; Kaunda-Arara *et al.*, 2003) Generally, no comprehensive stock assessment and biological studies of *L. lentjan* have been conducted on the Kenyan coast. This presents a challenge to management efforts, as adequate data and information is not available (Fondo and Sigurðsson, 2004). This study therefore provides a brief overview of biological aspects of *L. lentjan* on the south coast of Kenya as a baseline for in-depth future studies on this important commercial species.

Figure 1. A map showing the location of the landing sites of Mkunguni, Shimoni, Majoreni and Vanga on the south coast of Kenya.

Materials and Methods

Data Collection

This study was conducted at Vanga, Mkunguni, Shimoni and Majoreni fish landing sites on the south coast of Kenya (Fig. 1). Fish samples were collected in the months of September through to February 2017. Individual fish TL in cm and BW in g were measured and LWR and condition factor determined. Some fish specimens were dissected to determine sex and gonad maturity using macroscopic observation. Determination of gonad maturity followed the guidelines from Ntiba and Jaccarini (1990) and those modified by Kulmiye *et al.* (2002). The whole ovary of each fish specimen was excised, weighed and preserved in Bouins solution in labelled containers for 48 hours. The gravimetric method of Holden and Raitt (1974) was then used to estimate the total fecundity of each fish specimen.

Data Analyses

The LWR by sex was expressed as a logarithmic transformation of the equation:

$W = aL^b$ (Pauly, 1983)

where W is the body weight, L is the total length, 'a' is the intercept, and 'b' is the slope of the regression line. Condition factor was calculated using the formula for relative condition factor:

$(Kn)\text{:}Kn = W/\hat{W}$

where W is the weight of an individual fish and $\hat{W} = aL^n$ is the computed length-specific mean weight from the LWR as described in Le Cren, (1951).

Fishing mortality (F), Exploitation Rate (E) and growth parameters K and Asymptotic Length L_∞ were estimated using Length-Frequency Analysis (LFA) in the

FAO ICLARM Stock Assessment Tool (FiSAT) II package (Gayanilo *et al.*, 2005). ELEFAN 1, a sub-package in FiSat II was used to estimate the von Bertanlanffy growth parameters K and L_∞ as applied by Kaunda-Arara *et al.* (2003). Gonadosomatic Index (GSI) was calculated using the formula described in Wooton (1990):

GSI = Gonad Weight/(Body Weight x 100).

The fish specimens were sorted according to size classes of 4 cm interval and the number of individuals of each maturity stage recorded according to size class. Gonad maturity stage III and above were considered sexually mature for both males and females. The percentage of mature individuals in each length class was then calculated and cumulated. Delta Graph Win (Ver. 5.6.2) software was then used to determine the length at massive maturity L_{50} by curve fitting the data to the following logistic equation as applied by Obota *et al.* (2016):

L_{50}: $M\ (TL) = 100/\ (1+\exp\ (-a*(x-b)))$

where '*a*' is a constant and '*b*' is the L_{50}.

Statistical Analyses

The differences in mean *Kn* and GSI between sexes and between months sampled were tested using the non-parametric Kruskal-Wallis test. Post hoc pair-wise comparison was used to confirm the actual differences between variables. These tests were conducted using the statistical software STATISTICA version 7. The relationship between fecundity and TL of females was analyzed using regression, and the same analysis was conducted for the relationship between BW and fecundity. The Analysis of Co-Variance (ANCOVA) test was used to test whether there was any significant difference between the slopes of the regression of the LWR for the males and the females. Data were subsequently pooled to give one regression equation for both male and female specimens. The slopes were then tested for significant difference from 3.0 using a one sample t-test as described in Townend (2013). Chi-square test of goodness was used to test whether the sex ratio of the population conformed to the ideal sex ratio of 1:1. These tests were conducted using MINITAB statistical software version 17. Significance level for all tests was assigned at $p < 0.05$.

Results and Discussion

A total of 575 fish specimens were sampled from September 2016 to February 2017. Results of some population parameters of this species derived from the length frequency are presented in Table 1. The growth coefficient K of 0.25/yr obtained in this study suggests that the growth of *L. lentjan* in Kenyan waters is much slower than that recorded in the waters of Tanzania (1.00/yr) (Benno, 1992) and Yemen (0.48/yr) (Aldonov and Druzhinin, 1979). Enberg *et al.* (2008) attributed differences in the rate of growth to differences in environmental parameters such as temperature, seasonality and ecological parameters, particularly population density and anti-predatory behavior. As these parameters were not investigated in the present study, it is possible that any or a combination of these could explain the observed differences. According to Gulland (1971), the exploitation rate (E) indicates the level of exploitation of a stock; lightly exploited ($E < 0.5$) and strongly exploited ($E > 0.5$), based on the assumption that fish are optimally exploited when $F = M$ or $E = 0.5$. Therefore, the E value of 0.55 determined in this study indicates that the exploitation rate for the *L. lentjan* stock on the south coast of Kenya is slightly higher than this. However, it is noted that Hicks and McClanahan (2012) reported much higher exploitation values of $F = 4.29$ and $E = 0.82$ for *L. lentjan* in Kenyan waters, which may be related to the fact that samples in their study were largely collected within the lagoon and inshore fishing grounds.

Table 1. Estimated growth, mortality and exploitation parameters for *Lethrinus lentjan* on the south coast of Kenya.

Parameter	Value
Asymptotic length ($L\infty$)	55.00
Curvature of growth (K)	0.25
Total mortality (Z)	1.32
Natural mortality (M)	0.60
Fishing mortality (F)	0.72
Exploitation rate (E)	0.55

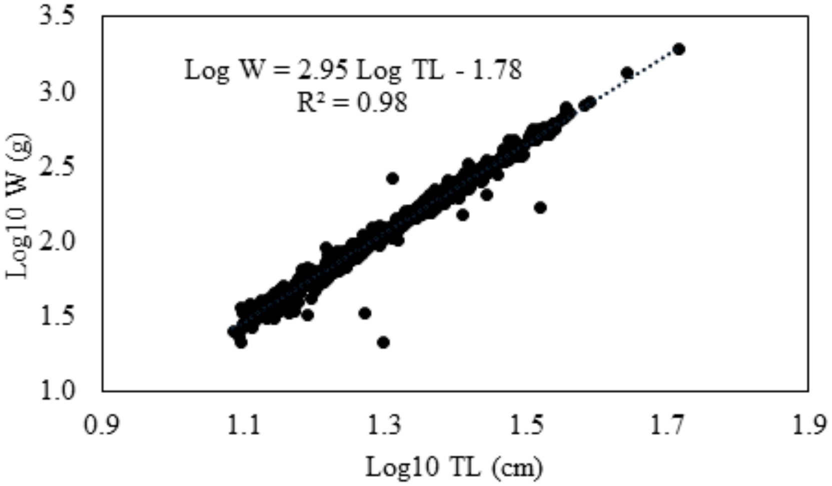

Figure 2. Length-weight relationship based on pooled data for *Lethrinus lentjan* sampled on the south coast of Kenya during the study period.

Mixed sex data was used to determine the LWR (Fig. 2) described by the equation Log W = 2.95 Log TL - 1.78 (n = 413, r2 = 0.98, $p < 0.05$). The *b* value for mixed sex in this study was 2.95 while that for males (n = 154) and females (n = 259) was 2.92 and 2.97, respectively. These *b* values were significantly less than 3 (t = -50.81, $p < 0.05$) indicating negative allometric growth; that is, the fish became slimmer as length increased (Riedel *et al.*, 2007). Letourneur *et al.* (1998) also reported *b* values of less than 3 for this species in the coral reefs and lagoons of New Caledonia. However, Mbaru *et al.* (2010), reported a *b* value of 3.183 for *L. lentjan* on the Kenyan coast for samples collected throughout the year. Samples in the present study were only collected during the North East Monsoon (NEM) season. According to Biswas (1993) and Armin *et al.* (2005) *b* values for the same species may vary due to geographical differences, seasonal variations in environmental parameters such as temperature, salinity, nutrition, gonadal development and physiological state at the time of sampling. These factors are likely to explain the observed differences.

The *Kn* for females ranged from 0.62 - 1.40 with a mean of 1.00 ± 0.01, while that of males ranged between 0.34 and 2.16 with a mean of 1.03 ± 0.01. Results of the Kruskal-Wallis test indicated significant differences in mean *Kn* between months for males (df = 5, H = 15.91, p

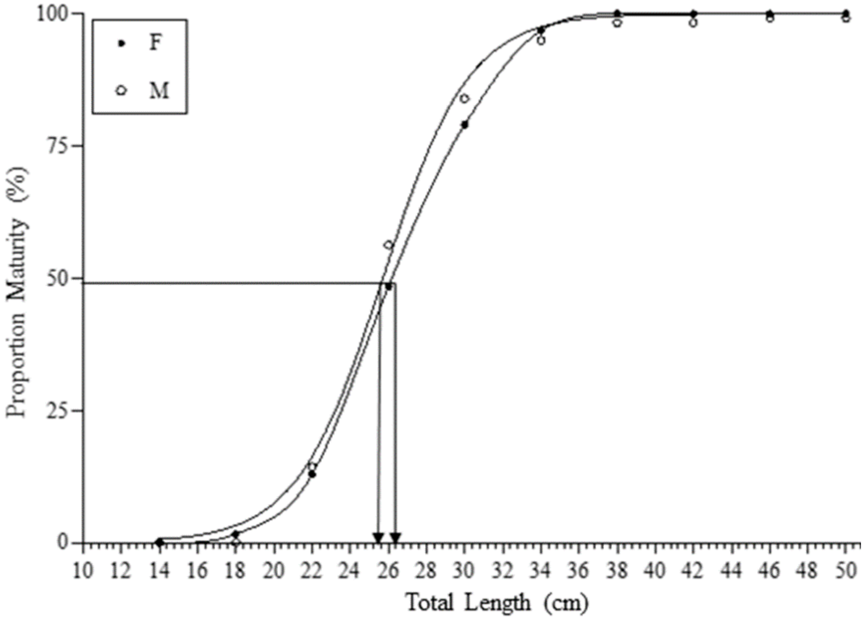

Figure 3. Percentage proportion of mature *Lethrinus lentjan* in relation to their Total Length sampled on the south coast of Kenya over the study period. Arrows indicate value of $L_{50.}$

< 0.05) and females (df = 5, H = 14.84, $p < 0.05$). This is indicative that both males and females were in a good physiological state. The variation in *Kn* for both males and females during the study may be due to several factors such as differences in maturity stages, type of food consumed, the extent of muscular development and amount of fat reserve (Barnham and Baxter, 2003).

The mean TL of *L. lentjan* was 21.54 ± 0.26 cm and the average weight was 180.74 ± 7.26 g. The smallest specimen measured 12.20 cm and weighed 25.30 g, whereas the largest individual measured 52.0 cm and weighed 1,913.0 g. Length at 50% maturity (L_{50}) for male and female *L. lentjan* was 25.8 cm. and 26.2 cm, respectively (Fig. 3). A total of 54 males out of 154 (35%) and 85 females out of 259 (32.82%) had attained this length. This was different from other studies on *L. lentjan* where, for example, Toor (1964) reported an L_{50} of 30 cm and 28.7 cm standard length (SL) for males and females, respectively in Indian Ocean waters, while Grandcourt *et al.* (2011) reported an L_{50} of 24.6 cm and 27.7 cm fork length (FL) for males and females, respectively in the southern Arabian Gulf. These differences could be related to fishing pressure on this species in various areas as suggested by Lappalainen *et al.* (2016) who noted that L_{50} is a potential indicator of fishing pressure in fish stocks. However, environmental factors such as food availability may also have an effect (Reznick, 1993). The smallest female with ovaries in a mature condition (Stage III and IV) measured 21.6 cm and weighed 160.4 g, while the smallest male in a mature condition (Stage IV) measured 18.7 cm and weighed 93.5 g.

Only 35% of the male and 32% of the female specimens in this sample had attained the L_{50} (25.80 cm and 26.2 cm, respectively) indicating that the majority of the fish were caught before attaining maturity. This suggests growth overfishing characterized by small immature fish in the catch (Amponsah *et al.*, 2016). This is probably due to the use of beach seines within lagoons in some fishing grounds at Majoreni, one of the sampling sites in this study. The beach seines used by fishers at Majoreni are less than 2-inch mesh size, with a 'kakaban' code end which limits the escape of juveniles. Fishing in Majoreni and Vanga mostly occurs in mangrove swamps and shallow inshore fishing grounds which are critical habitats for juveniles (Carpenter and Allen, 1989; Kimirei *et al.*, 2011). On the other hand, fishers in Shimoni and Mkunguni usually exploited deeper waters outside the reef, and therefore landed bigger sized fish compared to Majoreni and Vanga.

The mean GSI for females was higher (0.97 ± 0.08) than for males (0.21 ± 0.22). Differences were highly

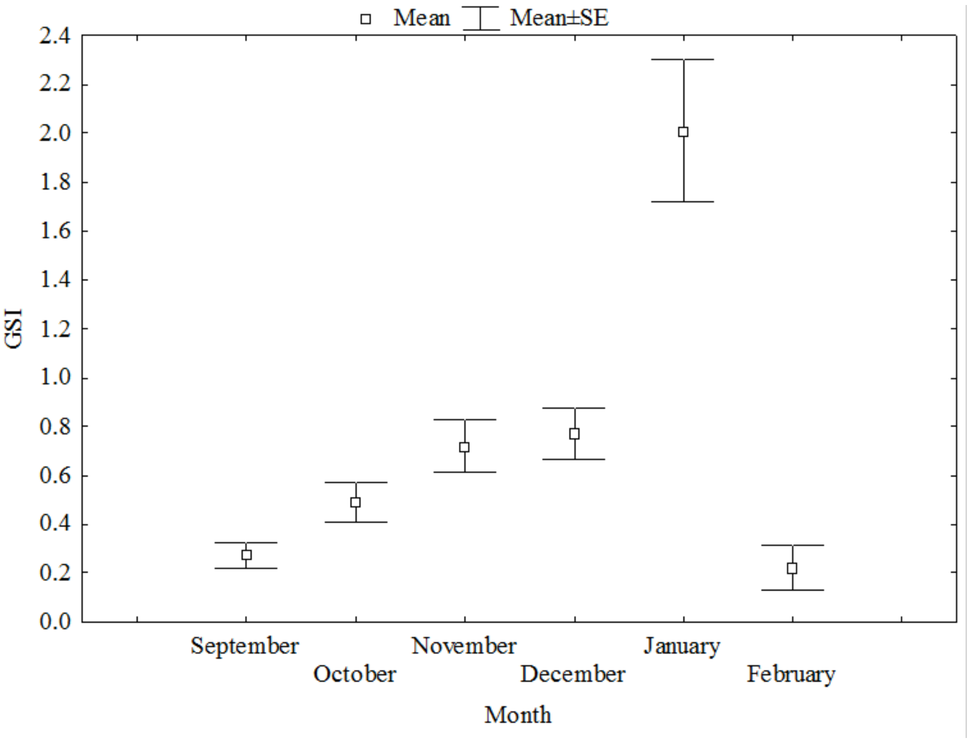

Figure 4. Mean ± SE gonadosomatic index of *Lethrinus lentjan* on the south coast of Kenya over the study period.

significant (H = 37.91, df = 1, $p < 0.05$). Differences also existed in the mean monthly GSI for combined sexes (H = 70.12 df = 5 $p < 0.05$). An increasing trend was observed from September peaking in January, and a decline in February. January had the highest GSI, with a mean of 2.08 ± 0.20) (Fig. 4), and February had the lowest mean GSI of 0.22 ± 0.08. Monthly variations in GSI provide a reasonable indicator of reproductive seasonality of fish with spawning time often identified from changes in the GSI (Arruda *et al.*, 1993). This is based on the assumption that gonad weight increases during times of spawning due to the swelling and ripening of oocytes (Taylor and McIlwan, 2012). This explains the steady increase in GSI for the species in this study from September peaking in January, and a sudden decline in February. Females sampled in January had the highest GSI suggesting that they had ripe oocytes and were ready to spawn. This compares well with the January-February peak spawning reported for the species in east African reefs by Nzioka (1979) and Currey *et al.* (2009) in the Australian Great Barrier Reef. In other studies, *L. lentjan* has been reported to spawn almost throughout the year with two main peaks (Mobiha, 1991). Since sampling did not cover the entire year in the present study, this could not be confirmed from the data collected. However, the occurrence of individuals in different gonadal maturity stages in all monthly samples over the study period suggests that *L. lentjan* is a multiple spawner. Similar results were reported by Currey *et al.* (2009) for this species.

Fecundity ranged between 90 to 380,364 eggs with a mean of 89,573 ± 9,841 eggs/female/year. The females ranged from a TL of 20.6 to 52.0 cm and 124.4 g to 1,913.0 g in individual BW. This differs from the findings of Toor (1964) who reported that the fecundity for *L. lentjan* in Indian Ocean waters ranged between 12,146 – 77,922 eggs. This variation could be due to several environmental factors such as temperature, sunlight, and weather (Jonsson and Jonsson, 1999). Simple linear regression of fecundity and TL, and fecundity and BW showed a weak significant positive relationship, even after log transformation (i.e. $\text{Log } F = 6.24 \text{ Log } TL - 4.43$ ($n = 94$, $r^2 = 32.50$, $p < 0.05$) and $\text{Log } F = 2.00 \text{ Log } BW - 0.378$ ($n = 94$, $r^2 = 32.70$, $p < 0.05$)). A total of 413 individuals were sexed; 154 males (37.29%) and 259 females (62.71 %). There were more females than males (Table 2), and females also dominated throughout the length classes with a female:-male sex ratio of 1: 0.59, which was significantly different from the expected 1:1 (Chi square test, $\chi 2 = 26.70$, df = 1, $p < 0.05$). The frequency of occurrence of males and females in the various size classes all showed no significant difference ($p < 0.05$ in all cases) in sex ratio, except in size classes 20.00 - 23.99 and 24.00 - 27.99 cm. Results indicate that males attained smaller sizes than females, with the largest male being in the 36.00 - 39.9 cm size class while the largest female was in the 48.00 - 52.99 size class.

This study established that *L. lentjan* in the waters of the south Kenyan coast had a relatively slow growth

Table 2. Sex ratio of various size classes of *Lethrinus lentjan* sampled on the south coast of Kenya over the study period.

Size class	No. of males	No. of females	Sex ratio	Chi-square value	P-value
12	15	23	1: 0.65	1.68	0.19
16	46	67	1:0.69	3.90	0.05
20	24	48	1: 0.5	8.00	0.01
24	30	67	1: 0.45	14.11	0.00
28	24	35	1: 0.66	2.05	0.15
32	14	13	1: 1.08	0.04	0.85
36	1	4	1: 0.25	1.80	0.18
40	0	0	-	-	-
44	0	1	-	-	-
48	0	1	-	-	-
Total	154	259	0.59:1	29.70	0.00

rate compared to other regions. The stock was also being exploited above the optimal rate, and this, coupled with the fact that the species is a slow grower, can easily lead to overexploitation. Growth overfishing was also apparent catches made up of over 60% immature individuals. Higher GSI values in January suggest peak spawning at this time, however, the occurrence of individuals in various gonadal maturity stages in monthly samples is indicative of multiple spawning. There is a need to enforce fishing regulations such as restriction on mesh size and destructive fishing gears such as beach seines commonly used in some parts of the Kenyan coast. This will prevent the capture of juvenile fishes and instead allow them to recruit into the fishery. Mesh size and gear type restrictions will also avoid damage to critical habitats such as sea grasses and corals that are important for fish survival. In addition, there is a need for research on gear selectivity to enable recommendation of suitable mesh sizes. There is also need for longer term studies on reproduction of this species covering the whole year and to cover other parts of the Kenyan coast to establish whether the species is a multiple spawner.

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The biology of Goldsilk Sea Bream (family: Sparidae) from the inshore waters of north coast Kenya

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Abstract

The biology of the sparid, *Acanthopagrus berda* (goldsilk sea bream) was investigated using catch samples obtained from artisanal fishers at selected fish landing sites in the Marereni and Ngomeni fishing areas on the north coast of Kenya. This species has not previously been studied in Kenya although it forms significant proportions of artisanal fish landings and is also a promising aquaculture species. In order to contribute to knowledge of this species, the length-weight relationship, condition factor and feeding preference for prey items were studied in a total of 751 specimens sampled from July 2013 to July 2014. Length-weight relationships for male and female individuals from both fishing areas were strongly correlated (Males: Marereni - $R^2 = 0.920$, Ngomeni - $R^2 = 0.983$; Females: Marereni - $R^2 = 0.966$, Ngomeni - $R^2 = 0.941$). The condition factor of mixed sexes was not significantly different between the two fishing areas (Marereni 2.15 ± 0.08; Ngomeni 2.05 ± 0.02) at $p > 0.05$. Gut content analysis recorded a total of 5 taxa in the diet (gastropods, molluscs, detritus, crustaceans and fish). The overall sample was dominated by female individuals with an overall sex ratio of females (405 individuals) to males (338 individuals) of 1:0.8 being significantly different at $p < 0.05$. The species exhibited isometric and positive allometric growth patterns, indicative of the physiological well-being of this species on the north coast of Kenya. Gonadal maturation occurred throughout the year with peaks in July, August and September. However, more work is needed on the biology, distribution, spawning grounds, behavior, and migration patterns along the Kenyan coast.

Keywords: goldsilk sea bream, biology, north coast of Kenya

Introduction

The family Sparidae, commonly known as sea breams or porgies, inhabit both tropical and temperate coastal waters worldwide (Randall *et al.*, 1997) where the genus *Acanthopagrus* prefers shallow waters, specifically estuarine environments. In southern Africa this fish family has been reported to comprise a total of 41 species, of which 25 are endemic (Smith and Heemstra, 1986). Many sparids have been shown to be hermaphroditic, with both male and female gonads developing simultaneously (Smale, 1988; Jakobsen, 2009). Some individuals change sex from male to female (protandrous) or from female to male (protogynous) (Randall, 1995). They typically consume a wide variety of benthic prey with substantial amounts of plant material (Sarre *et al.*, 2000; Mariani *et al.*, 2002; Tancioni *et al.*, 2003).

The Sparidae include many species of commercial, recreational and aquaculture importance (Mongile *et al.*, 2014; Rahim *et al.*, 2017). The goldsilk sea bream (*Acanthopagrus berda* Forsskål, 1775) is widely distributed in the tropical Indo-Pacific region; from South Africa to India, northern Australia and Japan. As with many species of seabreams, *Acanthopagrus* sp. is considered a commercially important food fish, with good potential for aquaculture and recreational fishing throughout several regions of the world, including China, southeast Asia, Africa, the United Kingdom and the United

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States of America (Rahim *et al*., 2017). *A. berda* has the potential to change sex and is one of several protandrous sparids (Garratt, 1993). This species prefers habitats with a bottom characterized by gravel or rubble and water depths of between 20 and 500 m. Abundance of the species varies with water depth with juveniles being gregarious and adults solitary (FAO, 1981).

A. berda is an important component of the artisanal fishery throughout the Western Indian Ocean region, and especially in Kenya, where its stock status and biology is poorly understood. This species comprises a significant proportion of demersal fish landings on the north coast of Kenya, while a few specimens have occasionally been reported on the south coast. Several species belonging to the genus *Acanthopagrus* are harvested throughout the Indo-Pacific region. In Kuwait, *Acanthopagrus* sp. are landed in large numbers by commercial fishers using stake nets, fish traps, fish pots and trawl nets. They are also targeted in the recreational fishery which is becoming increasingly common (Nicola, 2001). In South African estuaries, *A. berda* is targeted by a variety of fishing gears including hook and line, gill nets and traditional fish traps. Specifically, in Kwa Zulu Natal, *A. berda* is caught in both recreational and subsistence fisheries on large estuaries, comprising one of the five most abundant species caught (James *et al*., 2001). In Kenya, *A. berda*

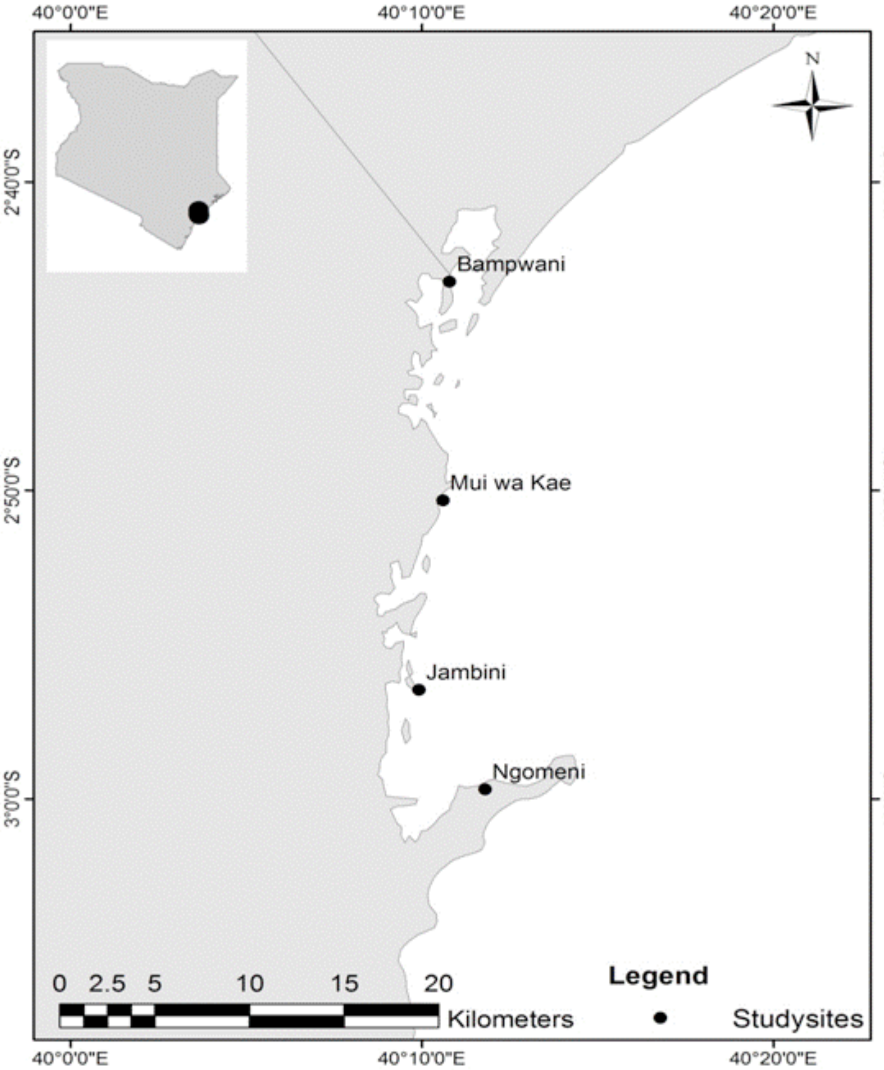

Figure 1. Map of part of the north coast of Kenya showing the sampling stations of Ngomeni and Jambini fish landing sites in the Ngomeni fishing area, and Mui-wa-kai and Bampwni fish landing sites in the Marereni fishing area.

is also associated with estuaries, both in the juvenile and adult phases of its life cycle. Adult individuals of this species have been rarely observed in the oceanic environment. Several studies on this species have been carried out in South Africa where reproductive and feeding biology has been well documented (Wallace, 1975; Wallace and van der Elst, 1975; Garratt, 1993; Eschmeyer et al., 2016). Similar studies are needed in Kenya, where this species is growing in commercial importance in the artisanal fishery, and is a promising candidate for aquaculture production.

Critical physiological functions of an organism such as growth and reproduction are dependent on energy derived from food. Therefore, food is the basic determinant of fish species distribution and abundance (Wootton, 1992; Anderson and Neumann, 1996). Research on food, feeding and ecology are fundamental in understanding the physiological functions of fish in relation to ecosystems (Hajisamaea *et al.*, 2003). This helps in understanding competition and predation effects on fish community structure (Krebs, 1999). Food and feeding habit studies help in determining the nutritional requirements of a fish species, its interaction with other organisms, and its aquaculture potential (Santos and Borges, 2001). Further, food and feeding habits are a vital part of biological and taxonomic studies in fish. The present study therefore examined the length-weight relationship, condition factor, and feeding preference for prey items of *A. berda*, both to fill scientific knowledge gaps, and to lay a foundation for formulating species management recommendations in Kenya and the Western Indian Ocean region as a whole.

Materials and Methods

The Study Area

This study was conducted on the north coast Kenya in the fishing areas of Ngomeni (comprising the Ngomeni village and Jambini fish landing sites), and Marereni (comprising Mui-wa-kae and Bampwani fish landing sites), within Malindi-Ungwana Bay, the largest bay in Kenya (Fig. 1). The study area extends from 0.6462° S, 38.4061° E to 2° 58' 32" S, 40° 1' 29" E. Two large rivers, Sabaki River to the south and Tana River to the north, drain into the bay providing nutrient enrichment to the bay ecosystem. The common artisanal fishing gears in the study area are seine nets, gill nets, intertidal weirs, hand lines and spear guns, targeting both pelagic and demersal fisheries resources. Artisanal fishing activities are regulated by both river discharge and ocean tides, as well as the north east monsoon (NEM) and south east monsoon (SEM) seasons. Artisanal catches are generally observed to be higher during the dry NEM season as compared to the wet SEM season. Higher catches of *A. berda* are however, associated with the wet season.

Fish Sampling and Data Analysis

Fish specimens were sampled from artisanal fishers on a monthly basis for a period of 1 year from July 2013 to July 2014, covering both the NEM and SEM seasons. For each specimen, the total length (TL, cm) was measured to the nearest 0.1 cm using a fish measuring board. Measurements were taken from the snout tip (mouth closed) to the tip of the longest caudal fin (Anam and Mostarda, 2012). Body weight (BW, g) was measured to the nearest 0.1 g using a top loading balance (Ashton Meyers, model 7765). Sex of each specimen was determined by macroscopic examination of the gonads in the laboratory. Maturity stages of males and females were assigned macroscopically according to the descriptions of Owiti and Dadzie (1989) (Table 1), while spawning period was established by analyzing the composition and proportion of gonad maturity stages. The proportion of male and female individuals was used to calculate the sex ratio. Differences in sex ratio was analyzed using a non-parametric Chi-square (χ^2) test.

The length-weight relationship (LWR) was determined using the power curve: $W = aL^b$ (Le Cren, 1951) where: W = fish weight in grams; L = fish total length in cm; and a and b are regression constants. A straight line relationship was provided by the formula:

$$\text{Log } W = \log a + b\log L$$

Coefficient parameter of determination (R^2), a and b were calculated by least-squares regression.

The condition factor (K) was calculated according to Fulton (1904) and Wootton (1990) from the relationship:

$$K = \frac{100W}{L^3}$$

Where: K = condition factor; W = total body weight (g); L = total length (cm); 100 and 3 = constants. The difference in mean K between the years was analyzed using a non-parametric Kruskal Wallis test.

Finally, food items in the guts were identified to taxon level following Richmond (2011). The percentage composition of each (taxon) food item by season

Table 1. Identification of gonad maturity stages by description of macroscopic appearance of gonads in both male and female *Acanthopagrus berda* sampled on the north coast of Kenya.

Maturity Stage	Male	Female
I. Immature	Never reproduced before, gonads small and close to vertebral column, undiscernible to naked eye, testes threadlike, transparent and colourless or grey, occupy about 1/3 of body cavity.	Never reproduced before, gonads are small and undiscernible to naked eye, ovary contains no developed oocytes, transparent, colourless or grey, occupy about 1/3 of body cavity.
II. Maturing	Testis ribbon-like structures, slightly bigger than Stage I, greyish-white in colour, occupy about 1/2 of ventral cavity.	Firm and ribbon like with slight increase in size, pink in colour, Oocytes not discernible to naked eye, occupy about 1/2 of body cavity.
II b. Regenerating/Resting	After each spawning cycle, macroscopic aspects of testes in regenerating phase are very similar to those in immature phase, but transverse section tends to be larger and the gonad wall thicker, they tend to be more opaque than immature gonads, occupy about 1/2 of ventral cavity.	After each spawning circle, macroscopic aspects of regenerating ovaries are very similar to those in immature phase, but transverse section tends to be larger, gonad wall thicker, they tend to be more opaque than immature gonads, reddish with blood capillaries, occupy about 1/2 of ventral cavity.
III. Maturing	Broad and thick, dark white in colour, blood vessels visible externally to naked eye, milt oozes out from cut surfaces and occupying 3/4 of abdominal cavity.	Broad and thick, red or reddish brown, blood vessels visible externally, Oocytes visible through ovary wall, occupying 3/4 of abdominal cavity.
IV. Mature	Testes white, occupying almost 9/10 of abdominal cavity, sperm flows following application of slight pressure.	Ovary very large, occupying almost 9/10 of abdominal cavity, very thin ovarian membrane. Oocytes easily visible and expelled on application of slightest pressure.
V. Ripe/Running	Fully distended occupying almost entire abdominal cavity, exudes milt on slight pressure.	Fully distended with granular surface occupying almost entire abdominal cavity.
VI. Spent	Testes shrunken and flaccid, occupy about 1/2 of ventral cavity, walls are harder and wrinkled, no milt oozes out on pressure and blood vessels still visible externally.	Ovary not fully empty, occupy about 1/2 of ventral cavity, residual oocytes present. Flaccid and red in colour, ovary wall thick.

and fish length size class were calculated based on the number of occurences in all sampled fish guts.

Results

A total of 751 specimens were sampled with the highest number from Ngomeni (201), followed by Jambani (195), Mui wa kae (180) and Bampwani (175). More specimens were sampled in the NEM season (464) than the SEM season (287). More females (454) were sampled compared to male individuals (297). The majority of males were within the size range of between 14 and 22 cm, while the majority of female individuals ranged from 15 and 29 cm (Fig. 2). Results of 2-way ANOVA, however, indicated no significant difference in sizes between sexes (df = 1; f = 0.400; p = 0.527).

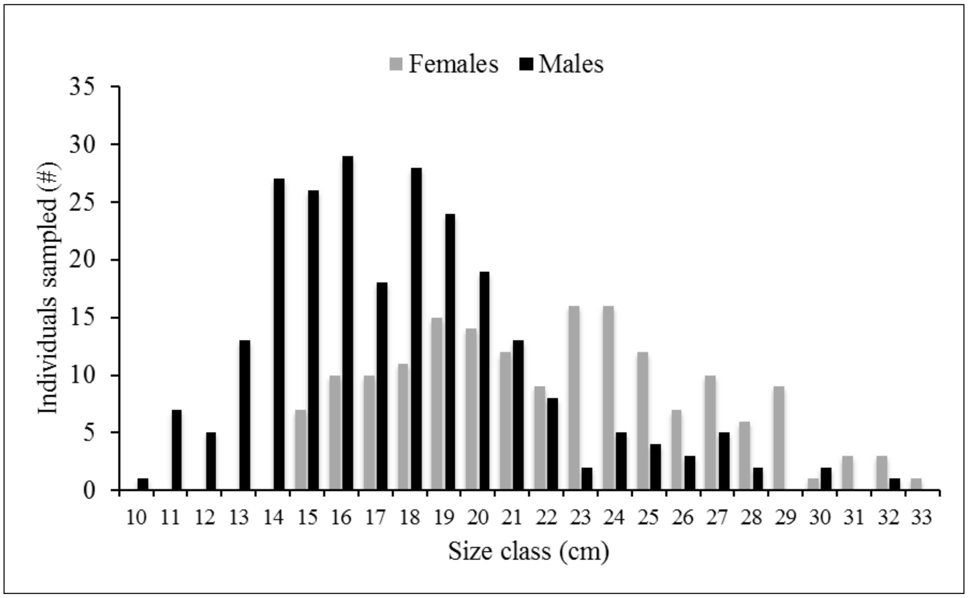

Figure 2. Length frequencies of *Acanthopagrus berda* individuals by sex, sampled on the north coast of Kenya during the study period.

The length-weight (LWR) parameters computed by sex resulted in *b* values of 2.9673 for male individuals, and 2.9941 for female individuals, with an overall *b* value of 2.988 for combined sexes. These *b* values are indicative of a positive allometric growth pattern. The LWR of both male and female individuals (Fig. 3) fitted the expected exponential growth curves (R^2 = 0.9571 for males; 0.9654 for females; and 0.9676 for combined sexes).

The condition factor (*K*) of both male and female individuals of *A. berda* was highest in the months of July and October 2013 (2.15 ± 0.08 each), compared to (2.05 ± 0.02) observed in the month of March 2014 (Fig. 4). However, there was no significant annual difference in *K* (Kruskal Wallis test: p = 0.052).

Both mature and immature gonads were observed throughout the year in different numbers (Table 2).

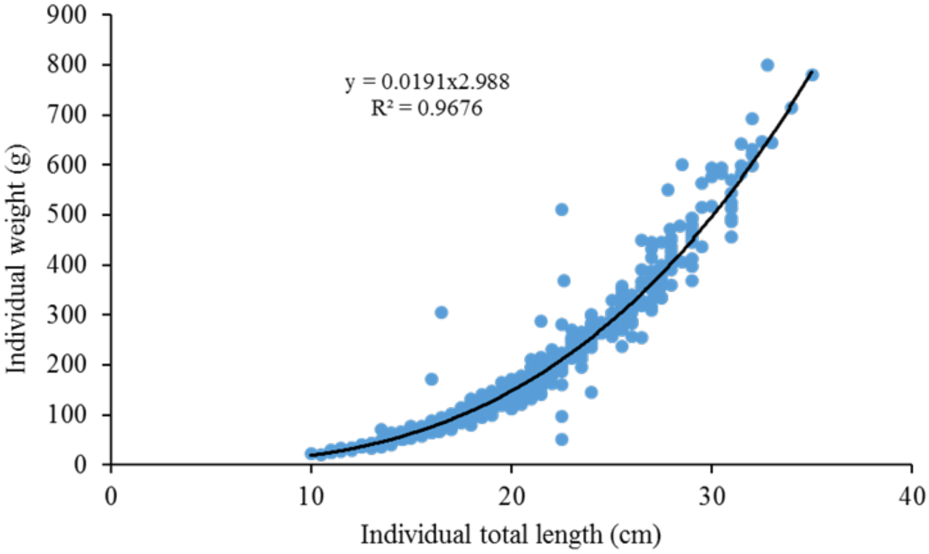

Figure 3. Length-weight relationship of combined sexes of *Acanthopagrus berda* individuals sampled on the north coast of Kenya during the study period.

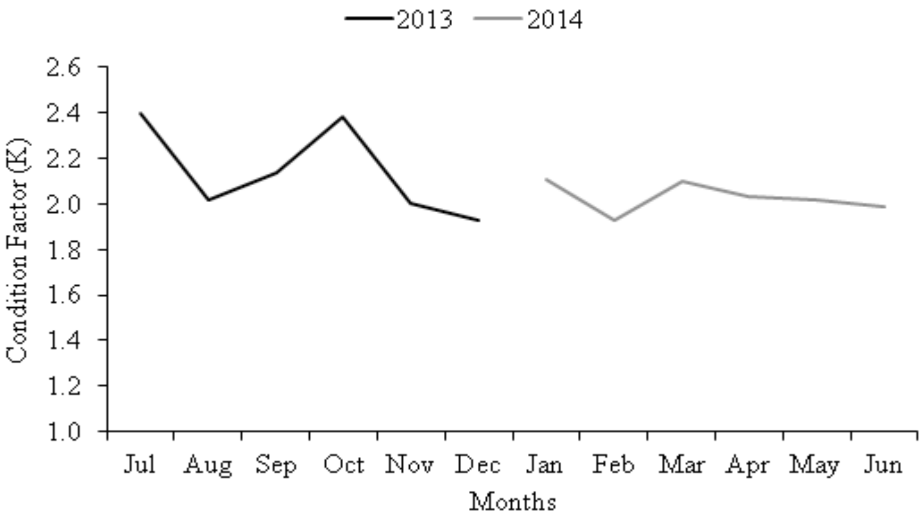

Figure 4. Condition factor (K) of male and female *Acanthopagrus berda* individuals observed during the study period on the north coast of Kenya.

Three peaks of mature gonads occurred in the months of July, August and September (Fig. 5). The majority of immature gonads were observed from October, November, December and January, with the month of December being the peak for immature gonads. The least mature and immature gonads were observed between February and June.

Male to female sex ratio was 1:1.1 for 2013 (n = 201 and 228, respectively). This was not significantly different from the expected 1:1 (χ^2 = 1.699; p = 0.192). The male to female sex ratio in 2014 was 1: 0.5 (n = 204 and 110, respectively). This differed significantly from the expected 1:1 (χ^2 = 28.140; p = 0.000). The overall male to female sex ratio of 0.8:1.0 (338 males and 405 females) was also significantly different (χ^2 = 6.042; p = 0.014).

A total of six food items (taxa) were identified, with a number of empty guts observed (Fig. 6). Seasonal differences in gut content was also observed, with more guts containing food items during the dry NEM season compared to the wet SEM season. The preference food (PF) index was highest for gastropods (25.2%),

Table 2. Monthly distribution of gonad maturity stages for both sexes of *Acanthopagrus berda* sampled on the north coast of Kenya over the study period.

Months	Maturity Stages					
	I	II	II b	III	IV	V	VI
January	28	25	1	21	4	1	2
February	4	10	2	13	2	2	1
March	6	6	0	21	2	1	1
April	4	11	2	15	2	1	0
May	2	8	3	11	2	8	3
June	5	10	1	1	1	12	9
July	4	5	1	22	7	16	23
August	1	1	0	12	7	12	8
September	19	37	0	22	5	19	15
October	20	14	0	8	0	0	13
November	22	34	0	0	0	0	27
December	48	32	25	0	0	0	3

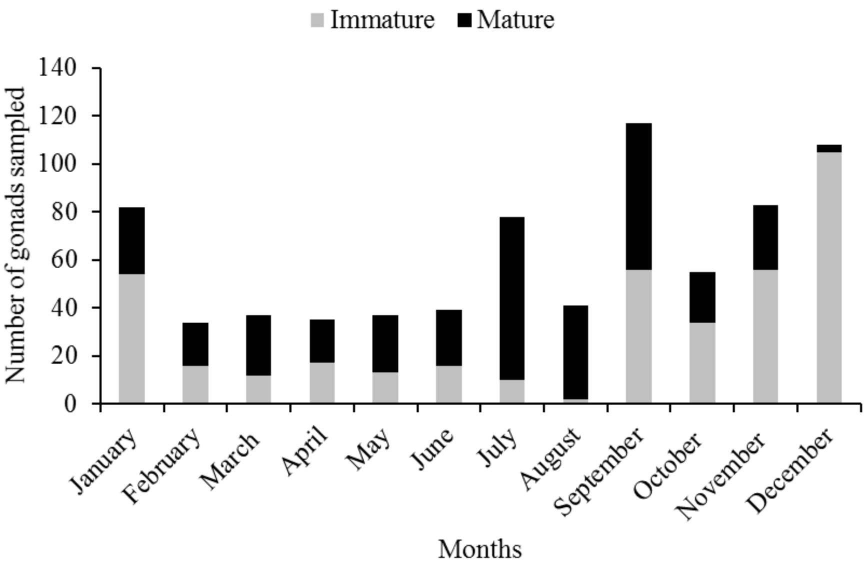

Figure 5. Monthly distribution of gonad maturity (immature and mature stages) of both sexes of *Acanthopagrus berda* sampled on the north coast of Kenya.

followed by crustaceans and fish at 22.8% and 16.2%, respectively. Relatively low values of FP index were recorded for detritus (13.5%), mollusks (13.1%), and mixed mollusk and gastropod prey (9.2%). Analysis of food items taken by different size classes of fish showed that the majority of the guts containing food items came from fish ranging between 16 to 21cm and between 22 to 27 cm. Most guts contained gastropods, mollusks and detritus (Fig. 5). Fewer guts contained food items such as crustaceans, fish and mixed mollusk-gastropods. Empty guts were recorded in some specimens from size classes that ranged between 10 and 15 cm, 16 and 21 cm, and 22 and 27 cm.

Discussion

The present study established key population parameters and biological characteristics of the sparid, *Acanthopagrus berda*, on the north coast of Kenya. It was found that the *b* exponents of the length-weight relationships (individual sexes and combined sexes) were all close to 3, indicative of an isometric growth pattern. Similar values of *b* were reported for other sparids in

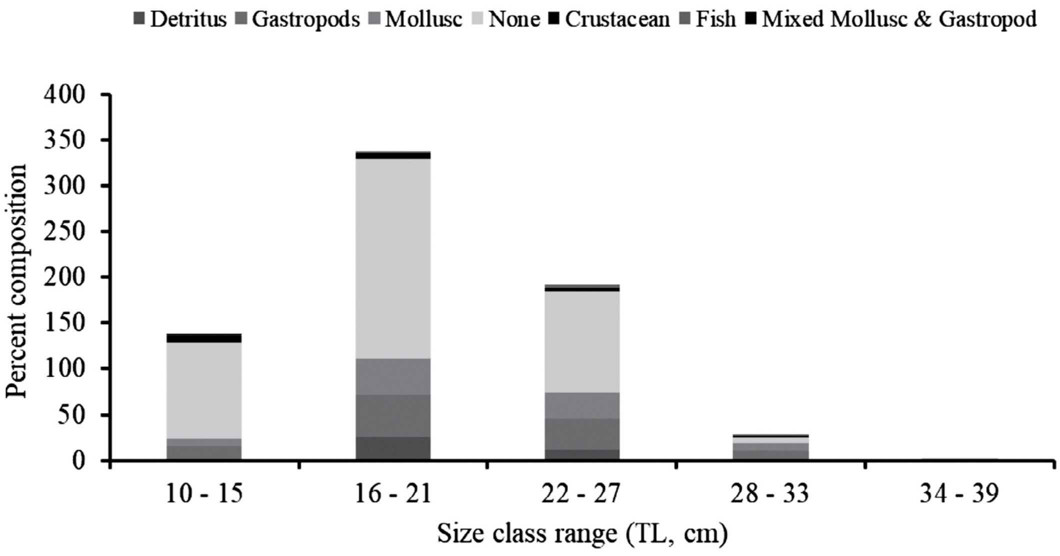

Figure 6. Composition of food items (taxa) in stomachs of *Acanthopagrus berda* sampled from the north coast of Kenya over the study period.

Kenya by Aura *et al.* (2013), and in Nigeria by Omogoriola *et al.* (2011), while b varied slightly from the same sparid species studied in Kuwait (Vahabnezhad *et al.*, 2017). Biological parameters in fishes, including length-weight relationships, are affected by factors such as prevailing environmental conditions, health condition, season, and population differences (Morey et al., 2003; Faroese, 2006). The condition factor (*K*) in *A. berda* indicated good physiological condition of the individuals sampled during the study period. Observed annual differences in *K* for this species may be attributed to variations in physico-chemical conditions.

The monthly occurrence of mature gonad stages (III and above) indicates that *A. berda* spawns throughout the year (Fig. 4). Previous studies by Wallace (1975), and Kyle (1986), documented that sparids mature between May and August in South Africa, with a peak spawning period observed in May and June. This compares favourably with the work of Garratt (1993) who reported differential maturity levels of ripe and running gonads between sexes of 8.8:1 (males to females) in Kosi Estuary, South Africa.

The presence of more mature males at the time when most females were immature may suggest that the males were caught before the end of the sex change process. Differences in sexual maturity between males and females indicates that females mature at stage III, at a time when males are in ripe and running condition. Several fish species have been reported to exhibit prolonged spawning periods lasting for between 7-9 months in the Western Indian Ocean region (Qasim, 1973). This scenario has also been confirmed to occur in other sparid species such as *Acanthopagrus latus* in the Persian Gulf, where spawning took place from February to June (Vahabnezhad *et al.*, 2017).

Sex composition of the sampled fish was characterized by more females than males (405 females against 338 males). This may be attributed to behavioral differences during sex change, or to one sex being more vulnerable to certain fishing gears. Hirpo, (2013) attributes the prevalence of female tilapia in catches to sexual segregation during spawning, activity differences, gear type and fishing sites.

This study established that gastropods, detritus, mollusks, crustaceans and fish displayed the highest FP index in the *A. berda* specimens sampled. This is in agreement with previous studies conducted along the west African coast that classified sparids as carnivorous feeding chiefly on crustaceans, fish and mollusks (FAO, 1981). Based on the wide range of food items observed, including detritus, *A. berda* may be referred to as "opportunistic feeder" (organisms that eat any available food item) in the absence of preferred food items. A seasonal difference in diet was observed with a greater variety of food items in the guts during the NEM compared to the SEM season. This may be attributed to higher water transparency in the NEM season that may improve visual location of prey items (Nyunja *et al.*, 2002).

Conclusion

The study established that *A. berda* occurring along the Kenyan coast exhibited positive allometric and isometric growth patterns, and were in good physiological condition. The species was found to spawn throughout the year with July, August and September being the peak spawning months. Males dominated the sample at immature stages, but overall females were more dominant. *A. berda* is a carnivorous bottom feeder that feeds mainly on gastropods and mollusks, but may also take detritus, crustaceans and fish in the absence of preferred food. It is recommended that longer term investigations of the life cycle and ecology of *A. berda* are carried out to confirm the spawning patterns found in this study. More work needs to be conducted on the biology, distribution, spawning grounds, behavior, and migration patterns of the species along the entire Kenyan coast. There is also a need for further research on the taxonomy of *A. berda*, including analysis of molecular genetic variation, to confirm relationships both within Kenya and in the western Indian Ocean region as a whole.

Acknowledgements

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Sometimes hard to swallow: Attempted feeding on a porcupinefish results in death of both predator and prey

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Abstract

Predator-prey relationships are critical components of population dynamics across all ecosystems. Interactions between predators and dangerous prey are especially likely to result in a co-evolutionary arms race. To avoid predation, porcupinefishes (Diodontidae) present a suite of physical and chemical defences, including spines, inflation, and the potent neurotoxin, tetrodotoxin, which is concentrated in the internal organs. A failed predation attempt is described here on a longspined porcupinefish, *Diodon holocanthus*, by a benthopelagic predator, *Carangoides fulvoguttatus*, resulting in the death of both the predator and the prey.

Keywords: asymmetrical selection; co-evolution; coral reef; ecology; fishes; Indian Ocean; predation; Zanzibar

Predator-prey relationships are critical components of population dynamics and ubiquitous across all ecosystems (Gokhale and Wignall, 2019). They are often presented as an evolutionary arms race; selection pressures within these relationships are bi-directional and can be asymmetrical (Brodie and Brodie, 1999). For example, the *life-dinner principle* characterizes a race between predators and prey: if predators lose, they may be able to hunt again, but if the prey is caught and eaten, this removes it from the system and eliminates future reproductive potential. Therefore, the consequences are much more severe for the prey than for the predator (Dawkins and Krebs, 1979). Predator-prey relationships are also shaped by interactions between intra and inter-specific behavioural types (e.g. aggressive vs. docile; Chang *et al.*, 2017). The type of predator-prey interaction that is most likely to result in a co-evolutionary arms race is in systems involving dangerous prey (Brodie and Brodie, 1999).

Porcupinefishes (Diodontidae) are circumtropical and inhabit a large variety of shallow water ecosystems (Nelson *et al.*, 2016). They are slow swimmers that rely upon a suite of anti-predator adaptations: spines, pronounced inflation, and the potent neurotoxin tetrodotoxin, which is concentrated in the internal organs (Brainerd, 1994; Wainwright *et al.*, 1995; Bane *et al.*, 2014). This assemblage of defense mechanisms should effectively limit predation, however, a case is reported here where a benthopelagic fish attempted to prey upon an adult longspined porcupinefish (*Diodon holocanthus*), and died in the process. A yellow-spotted trevally, *Carangoides fulvoguttatus* (Carangidae, approximately 1 m TL) was retrieved, dead and floating at the surface off the east coast of Zanzibar, on 11 December 2018 (Fig. 1a). Upon close examination, a large longspined porcupinefish (approximately 30 cm TL) was found in the buccal cavity (Fig. 1b). The porcupinefish was barely alive, semi-inflated, with its spines lodged between the trevally's gill arches, and was thus unable to extricate itself from the mouth of its predator (Fig. 1c).

Considering the effectiveness of the suite of defensive strategies employed by porcupinefish, was this a naive predation attempt or a failed case of intentional consumption by an overly aggressive trevally? The literature reports a relatively high diversity of predators

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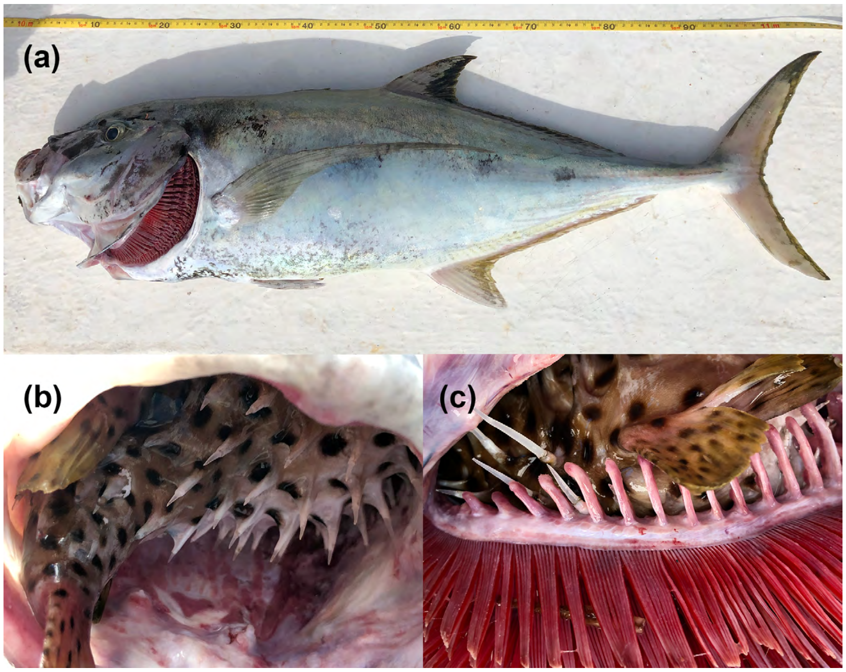

Figure 1. (a) Adult yellowspotted trevally, *Carangoides fulvoguttatus* (approx. 1 m TL), found dead, floating at the ocean surface off the east coast of Zanzibar. (b) *Diodon holocanthus* (approx. 30 cm TL) trapped in the buccal cavity, as viewed through the mouth of the trevally. (c) Detail of *Diodon holocanthus*, as viewed from beneath the operculum.

that feed upon juvenile porcupinefishes, including the pelagic and reef fishes *Coryphaena hippurus*, *Epinephelus itajara, Galeocerdo cuvier, Haemulon plumieri, Lutjanus analis, L. cyanopterus, L. griseus, L. synagris, Peprilus paru, Sphyraena barracuda* and *Tylosurus crocodilus* (Randall, 1967; Oxenford and Hunte, 1999). In May 2017, photographs circulated on the internet of a lemon shark, *Negaprion brevirostris*, dead on a beach in the Maldives, with a porcupinefish stuck in its mouth (Weisberger, 2017). Other marine animals also interact with porcupinefishes; there is a documented case of a bottlenose dolphin found dead with a slender-spined porcupinefish (*Diodon nichthemerus*) lodged in the posterior pharynx and upper esophagus (Byard *et al.*, 2010), as well as reports of them being preyed upon by killer whales, *Orcinus orca*.

Porcupinefishes are slow swimmers that are regularly observed out in the open, including up in the water column, and are thus easy for predators to target. They depend upon physical (spines, inflation) and chemical (tetrodotoxin poison) defences to avoid this predation. Although selection pressure is usually stronger on the prey, predators that capture dangerous prey will experience a severe consequence with a high predictability, and therefore also face strong selection pressure (Brodie and Brodie, 1999). Predators will respond evolutionarily, and in the arms race model, can, in time, evolve mechanisms to overcome the physical defences of porcupinefishes and variable resistance to their internal toxins, as in other taxa (Geffeney *et al.*, 2002). Therefore, this predation attempt could have been intentional, reinforced by the trevally's (hypothetical) prior experiences of successfully feeding on smaller porcupinefishes. The documentation of mortalities in individuals from other species that have attempted to prey upon porcupinefishes supports the assertion that this is not an uncommon event.

The longspined porcupinefish's spines, one of its primary defense mechanisms, locked the fish inside the predator and prevented it from escaping. Is this a case where an anti-predator adaptation has gone too far? Spines evolved as a defence mechanism (Shono *et al.*, 2018), ostensibly to prevent the fish from being taken into the mouth of a predator. However, spines are not necessarily a guarantee of protection (Willman, 2007). In the case of the porcupinefishes, if the fish is swallowed head first, it will easily slide into the predator's mouth, due to the orientation of the spines and their biomechanical erection mechanism (Brainerd, 1994). Nevertheless, selection pressure might be stronger for the prey here as well. Following Brodie and Brodie (1999), the two main factors that shape the asymmetrical selection between prey and predators are the consequence of individual interactions and predictability of the consequence. Porcupinefishes are very characteristic in their shape and behavior, so the predictability of the consequence to the predator is therefore high. Differences at the individual level, including predator aggressiveness, and individual variability in prey behaviour, interact to impact predator foraging performance (Chang *et al.*, 2017). More aggressive predators perform better when facing unpredictable prey behaviours, while more docile predators perform better with more predictable prey (Chang *et al.*, 2017). Previous successful consumption, or an overly aggressive behaviour might have encouraged the trevally's attempt, resulting in a serious and unpredicted consequence. In this case, despite an asymmetrical selection pressure, the result was the same for both predator and prey - neither survived the interaction.

Acknowledgements

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Broadcast spawning in *Porites lutea* at Reunion Island (Western Indian Ocean)

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Keywords: coral; spawning; Porites; Reunion Island

The scleractinian *Porites lutea*, commonly found on back reefs, lagoon and fringing reefs, is an important reef-building coral in the western Indian Ocean (WIO). Despite its widespread distribution, little is known of its reproductive biology. Here, *in situ* observations of broadcast spawning of *P. lutea* on a shallow reef flat of Reunion Island are reported on.

Spawning in *P. lutea* was observed in January 29th 2016 on the reef flat of Etang Salé (~1m deep, Reunion Island, 21°16'°00"S, 55°19'55"E, Figs.1a, b), five days after the full moon, at 19h30. At least six large colonies (>50cm in diameter) of *P. lutea* were observed simultaneously releasing either sperm or oocytes (Figs. 1a, b). During spawning, eggs were released in clumps (Figs. 1c, d). No other coral was observed spawning on this night. An additional night observation was conducted the following day, but no spawning was observed.

Gonochorism and a similar timing of reproduction with respect to the full moon were reported previously in *P. lutea* on the reef flat of la Saline (Planch'Alizé, Réunion Island), where spawning occurred on December 29th 2007, 5 days after the full moon (Denis *et al.*, 2011). This mode and timing of reproduction (December to early February, 2-5 days after the full moon), has also been observed in colonies of *P. lutea* and *P. Lobata* at similar latitudes (20-23°S) in eastern and western Australia (Kojis and Quinn, 1982; Baird *et al.*, 2011; Stoddart *et al.*, 2012). This suggests a consistent pattern of reproduction in *P. lutea* in environments exposed to a similar temperature regime. Further study is required to understand spawning cues in *P. lutea* and document its reproduction in Reunion Island.

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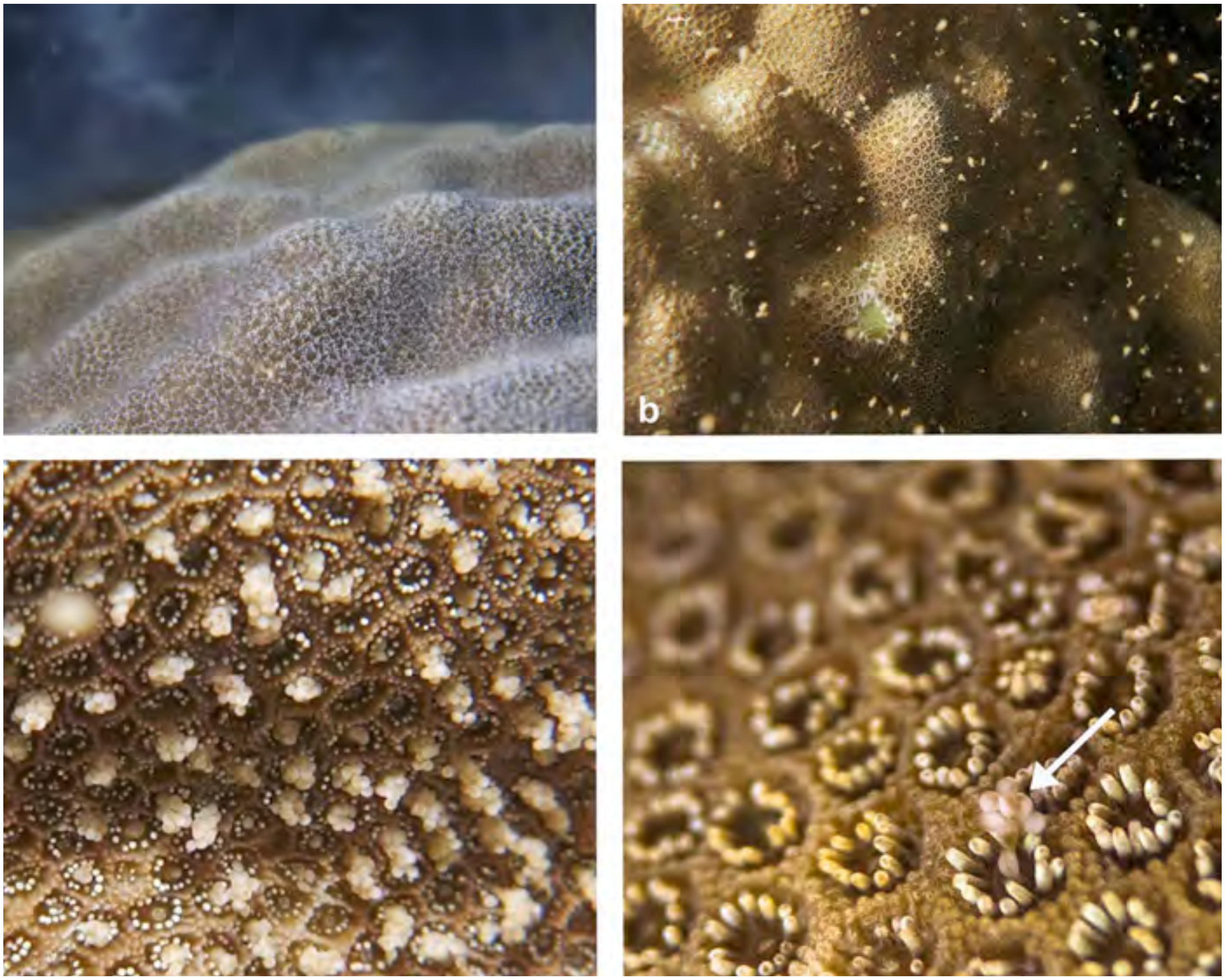

Figure 1. Male (a) and female (b, c, d) colonies of *Porites lutea* during spawning on the reef flat of Reunion Island in January 2016. In female colonies, oocytes are expelled in clumps (c), showing a dark green pigmentation (d), probably due to the presence of zooxanthellae.

Re-description and new records of a poorly known leucosiid crab, *Merocryptus boletisculpta* Zarenkov, 1994 (Crustacea, Decapoda, Brachyura)

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Abstract

Sixty-nine specimens of the rare leucosiid crab *Merocryptus boletisculpta* are reported from Walters Shoal, an isolated seamount in the southwestern Indian Ocean. The species has previously only been known from the type series collected during the R/V Vityaz 1988-1989 expedition. The species is re-described, illustrated, and colour photographs are provided.

Keywords: Leucosiidae; *Merocryptus*; Walters Shoal; southwestern Indian Ocean; rare species; seamoun

Introduction

Walters Shoal is an isolated seamount in the southwestern Indian Ocean (about 400 and 600 nautical miles distant from Madagascar and South Africa, respectively) and reaching to within 18 m of the surface. Exploratory fishing on the seamounts of the southwestern Indian Ocean, including Walters Shoal, began in the 1970s, followed by frenetic commercial exploitation (Collette and Parin, 1991). Few decapod crustaceans were collected by previous expeditions to Walters Shoal. Five species, including an endemic snapping shrimp, *Alpheus waltervadi* Kensley, 1969, were described from the haul of a rock dredge taken at a single site (Stn 381, 33°13'S, 43°51'E, 38–46 m, 30.08.1964) by R/V Anton Bruun during the International Indian Ocean Expedition (Kensley, 1969, 1981). A leucosiid crab, *Merocryptus boletisculpta* Zarenkov, 1994, was described from material collected off Mozambique (Stn 2626, 24°39'S, 35°31'E, 22.11.1988) and on Walters Shoal (Stn 2753, 33°13'S, 43°53'E, 22.12.1988) by the R/V Vityaz 1988-1989 expedition to the western Indian Ocean, as well as a few other decapod species identified in stomach contents of fish collected during that expedition (Collette and Parin, 1991; Zarenkov, 1994). A serendipitous haul of 19 frozen spiny lobsters obtained from a Spanish trawler fishing on Walters Shoal resulted in the description of *Palinurus barbarae* Groeneveld, Griffiths and Van Dalsen, 2006 (Groeneveld *et al.*, 2006).

In 2017, the Muséum national d'Histoire naturelle, Paris (MNHN) and the Institut de Recherche pour le Développement (IRD) collaborated in the 'Walters Shoal' Expedition on board the R/V Marion Dufresne. This oceanographic expedition was a part of the project "Conservation and Sustainable Use of Underwater Mountain Ecosystems and Hydrothermal Sources in the South West Indian Ocean, beyond Areas of National Juridiction", led by the Global Program for the Marine and Polar Environment of the IUCN (International Union for the Conservation of Nature) and supported by the French Fund for the Global Environment (FFEM).

During this cruise, 69 specimens of a single species, *M. boletisculpta*, were collected in 15 hauls between 217 m and 668 m (37 were taken at one site alone). The species has not been recorded since Zarenkov's original description but proved to be ubiquitous on Walters Shoal. It is re-described and illustrated here. The specimens are deposited in the Musèum national d'Histoire naturelle, Paris (MNHN).

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Material and methods

The specimens reported on here were collected by dredging on the summit and slopes of the seamount during the MD208-Walters Shoal expedition, on board R/V *Marion Dufresne* from 24 April to 18 May 2017 (see https://expeditions.mnhn.fr/campaign/waltersshoal). After sorting of the sampled material on board, some specimens were photographed to record their colour patterns, and the entire haul was preserved in 80% ethanol.

Measurements provided (in mm) are of the carapace length measured along the mid-line from the frontal margin to the posterior margin. The following abbreviations are used: G1 = gonopod 1; G2 = gonopod 2; DW = Warén dredge; Ph = photographed; and Stn = station.

Systematics

Family **LEUCOSIIDAE** Samouelle, 1819
Subfamily **EBALIINAE** Stimpson, 1871
Genus ***MEROCRYPTUS*** A. Milne-Edwards, 1873
Merocryptus boletisculpta Zarenkov, 1994

Ebalia tuberosa (Pennant)? var.; Stebbing, 1910: 337.
Ebalia tuberculata; Barnard, 1950: 367, fig. 70a-e. (Not *Ebalia tuberculata* Miers, 1881).
Merocryptus boletisculpta Zarenkov, 1994: 108-111, figs 8e, 9.

Material examined

Walters shoal, Indian Ocean. N.O. Marion-Dufresne Cruise MD208, Stn DW4877, 33°10'S, 43°49'E, 217–256 m, 01.05.2017, 1 male 5.0 mm (MNHN-IU-2016-9500); Stn DW4877, 33°10'S, 43°49'E, 217–256 m, 01.05.2017, 1 male 6.5 mm (MNHN-IU-2017-3471); Stn DW4877, 33°10'S, 43°49'E, 217–256 m, 01.05.2017, 1 female 5.6 mm, Ph (MNHN-IU-2016-9499); Stn DW4878, 33°09'S, 43°50'E, 221–256 m, 01.05.2017, 1 male 4.8 mm (MNHN-IU-2017-11340); Stn DW4880, 33°17'S, 43°51'E, 275–318 m, 01.05.2017, 1 male 5.0 mm (MNHN-IU-2017-3014); Stn DW4881, 33°16'S, 43°50'E, 377–382 m, 02.05.2017, 1 male 7.0 mm, Ph (MNHN-IU-2016-9496); Stn DW4881, 33°16'S, 43°50'E, 377–382 m, 02.05.2017, 1 male 6.3 mm, Ph (MNHN-IU-2016-9497); Stn DW4885, 33°17'S, 43°55'E, 272–380 m, 03.05.2017, 1 female 6.9 mm, Ph (MNHN-IU-2016-9490); Stn DW4885, 33°17'S, 43°55'E, 272–380 m, 03.05.2017, 1 female 6.6 mm (MNHN-IU-2017-3085); Stn DW4886, 33°17'S, 43°56'E, 573–582 m, 03.05.2017, 2 females 5.4, 7.7 mm (MNHN-IU-2017-3616); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 1 male 4.7 mm, Ph (MNHN-IU-2016-9500); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 1 male 8.1 mm, Ph (MNHN-IU-2016-9410); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 1 female 8.1 mm, Ph (MNHN-IU-2016-9409); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 2 females 7.2, 8.6 mm (MNHN-IU-2017-3325); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 1 female 3.8 mm (MNHN-IU-2017-11841); Stn DW4887, 33°17'S, 43°57'E, 599–640 m, 03.05.2017, 16 males 5.2-8.7 mm, 16 females 4.0-7.8 mm (MNHN-IU-2017-3084); Stn DW4888, 33°10'S, 43°57'E, 299–311 m, 03.05.2017, 1 female 7.8 mm (MNHN-IU-2017-11338); Stn DW4889, 33°09'S, 43°58'E, 353–465m, 03.05.2017, 1 male 6.1 mm (MNHN-IU-2017-3315); Stn DW4890, 33°09'S, 43°59'E, 492–588 m, 04.05.2017, 1 male 7.9 mm, Ph (MNHN-IU-2016-9421); Stn DW4890, 33°09'S, 43°59'E, 492–588 m, 04.05.2017, 4 males 5.1–8.4 mm, 1 female 7.9 mm (MNHN-IU-2017-3322); Stn DW4891, 33°12'S, 44°01'E, 650–653 m, 04.05.2017, 3 males 8.0–9.0 mm, 1 female 4.6 mm (MNHN-IU-2017-3532); Stn DW4892, 33°12'S, 44°01'E, 624–646 m, 04.05.2017, 1 female 8.2 mm, Ph (MNHN-IU-2016-3721); Stn DW4894 33°09'S, 43°50'E, 199–261 m, 05.05.2017, 3 males 5.1–6.8 mm, 2 females 5.6, 6.1 mm (MNHN-IU-2017-3312); Stn DW4895, 33°09'S, 43°49'E, 238–283 m, 05.05.2017, 1 male 4.6 mm (MNHN-IU-2017-3080); Stn DW4898, 33°09'S, 44°01'E, 652–668 m, 06.05.2017, 1 ovigerous female 11.4 mm, Ph (MNHN-IU-2016-9465); Stn DW4898, 33°09'S, 44°01'E, 652–668 m, 06.05.2017, 1 female 5.5 mm, Ph (MNHN-IU-2016-9498).

Description

Carapace (Fig. 1, 2) subhexagonal in outline, slightly longer than wide in male, slightly wider than long in female; upper surface closely paved with flattened, round granules of various sizes; prominent, oblique epibranchial ridges intersecting at median ridge. Front produced, slightly concave medially, bilobed. Postfrontal section of median ridge concave; gastro-cardiac region raised, with 2 anteriorly bent conic tubercles on gastric region, 1 tubercle on cardiac region. Intestinal region markedly raised, well demarcated, rounded (Fig. 1G). Hepatic region markedly convex. Pterygostomian margin convex, with triangular median tooth, visible in dorsal view. Lateral margin projecting, bidentate bearing mushroom-like granules, more distinct in females. Epibranchial ridge conspicuous, running obliquely from gastric tubercle to posterolateral angle of epibranchial region, proximally lined with low granules, distally with mushroom-like granules. Metabranchial region concave, paved with mushroom-like granules; margin concave. Posterior margin distinctly bilobed in males, lobes rounded, closely paved with flattened granules; only slightly

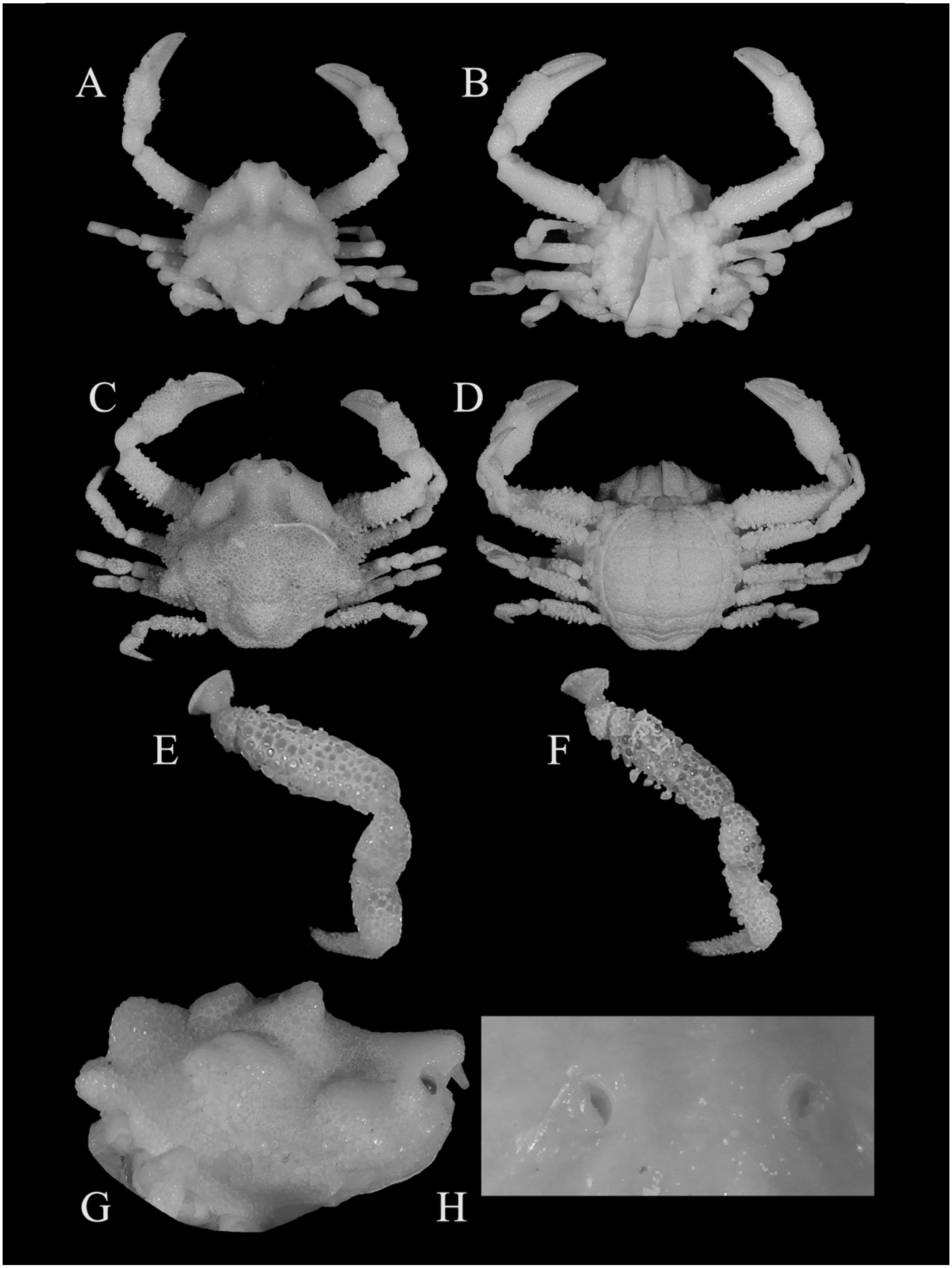

Figure 1. *Merocryptus boletisculpta* Zarenkov, 1994. A, B, E, G, male (4.0 mm) (9.0 mm), (MNHN-IU-2017-3532); C, D, F, H, female (9.0 mm), (MNHN-IU-2017-3532).
A, C, overall habitus; B, D, ventral view, E, F, last ambulatory leg; G, lateral view of carapace; H, female sternopleonal cavity and vulvae.

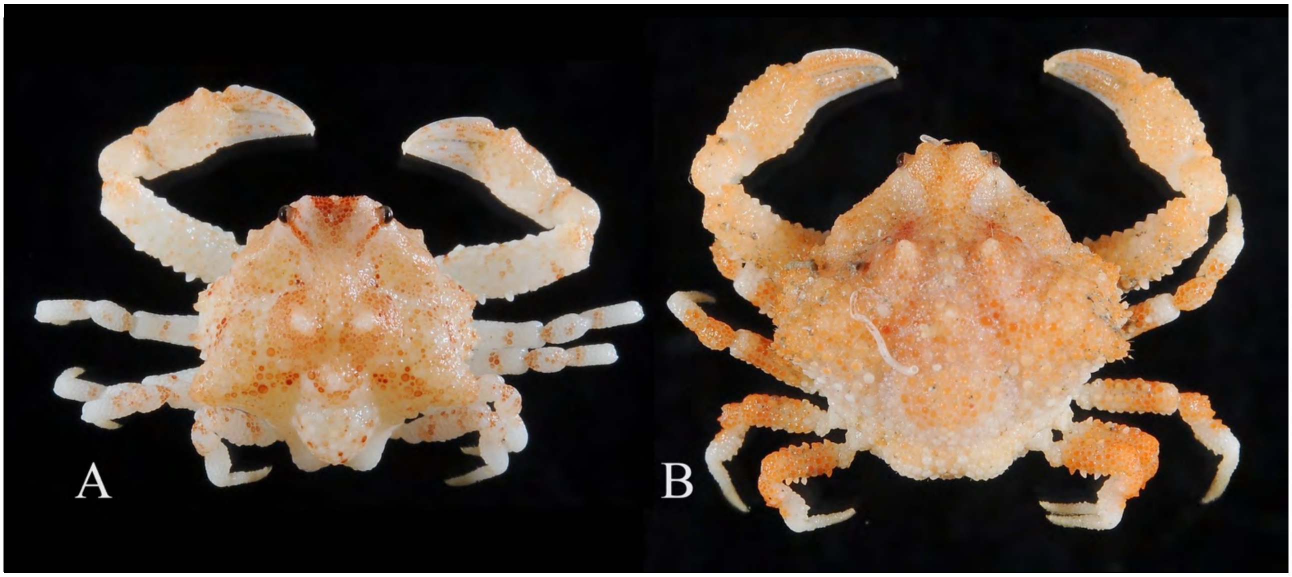

Figure 2. Colour in life. *Merocryptus boletisculpta* Zarenkov, 1994. A, male (6.3 mm) (MNHN-IU-2016-9497); B, ovigerous female (11.4 mm) (MNHN-IU-2016-9465).

concave in females (Fig. 1A, C). Ocular peduncle very short. Orbital margin trifissured. Antennule folded into oblique fossa; basal segment occupying lower part of fossa. Antennae small, slender, basal antennal article inserted in orbital hiatus. Anterior margin of efferent branchial channel produced, convex, laterally notched, separated by narrow groove from lower orbital margin. Third maxilliped (Fig. 1B, D) closely paved with flat-top mushroom-like granules of various sizes; exopod with narrow longitudinal groove.

Cheliped (Fig. 1A, C) entirely covered with granules of various sizes and shapes. Merus subcylindrical, elongate, with prominent, conic, mushroom-shaped granules on inner, outer margins, more numerous in females, scattered among flattened, rounded granules. Carpus rounded, paved with flattened granules. Palm rounded, slightly shorter than fingers, with conic granules on upper, lower margins; fingers with minute granules arranged in longitudinal rows, outer margins prominently cristate, cutting edges unevenly denticulate. Ambulatory legs slender; decreasing in size posteriorly; merus with prominent conic mushroom-shape granules along upper, lower margins, more numerous in females; propodus with conic granules on margins; dactylus setose, covered with conic granules, tip corneous (Fig. 1E, F).

Male thoracic sternites covered with flattened granules of various sizes (Fig. 1B). Abdominal cavity not reaching to buccal cavern; sutures interrupted medially. Male pleon (Fig. 1B) paved with flattened granules; fused somites, composed of third to sixth somites, elongate, distally convergent; telson elongate, triangular, proximally with triangular denticle. Female abdominal cavity reaching to buccal cavern; abdomen (Fig. 1D) entirely covered with closely set granules of various sizes; somites fused, rounded, convex, divided by pair of shallow longitudinal grooves; telson tongue-shaped. G1 slender, slightly sinuous, with short setae on distal half of both margins; tip acuminate, with short stiff setae on mesial margin. G2 short, convex, distally lanceolate, tip acute. Vulvae relatively large, obliquely placed next to mesial end of suture between sternites 5/6, almond-shaped, (Fig. 1H).

Colour

Carapace, chelipeds pale orange variously mottled with white, speckled with darker orange granules; ambulatory propodi, dactyls white (Fig. 2).

Remarks

Barnard (1950: 368) considered the specimen collected off 'Cape Natal' and listed by Stebbing (1910: 337) as *Ebalia tuberosa* (Pennant)? var., as the eastern Atlantic *Ebalia tuberculata* Miers, 1881 "Pending direct comparison of actual specimens". However, his description and illustrations conform to *Merocryptus boletisculpta* Zarenkov, 1994, described from several specimens collected on Walters Shoal and off Mozambique. The present material differs from the specimen depicted (Zarenkov, pl. 9, fig. 1) in having male lateral margin bidentate, and posterior margin distinctly bilobed but less produced in specimens of comparable size. The specimens agree with the type description and illustrations in all other characters.

Geographical distribution

Known from the type series locations: Stns 2626 and 2753 (Mozambique and Walters Shoal, respectively), but presented as "Madagascar underwater ridge, depth 320-480 m" (Zarenkov, 1994: 111), and from Durban, South Africa, depth 86-155 m. Our material extends the depth range to 668 m.

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