

Macroalgae Diet Indicates Potential in Mariculture Production of Shoemaker Spinefoot Rabbitfish

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Abstract

Worldwide, fish feed is known to be costly in aquaculture production majorly contributed by the cost of fish meal which is widely used as a protein ingredient in fish diet causing a great problem for the development and growth of the aquaculture sector. Therefore, seeking other sources that will be satisfactory, such as alternative protein for fish feed and providing nutritional benefits at a lower price is very important. This study aimed to explore the nutritional profile of the most preferred naturally occurring marine macroalgae species commonly utilized as bait for the basket trap fishery targeting rabbitfish. Data was collected through random administration of questionnaires to 62 fishers from Mkunguni, Kibuyuni, and Kijiweni fish landing sites in South Coast Kenya. A total of 6 species of rabbitfish were identified as targets for basket trap fishers. These were: *Siganus stellatus*, *Siganus sutor*, *Siganus luridus*, *Siganus canaliculatus*, *Siganus argenteus* and *Siganus rivulatus*. Results showed that fishers mostly preferred three species of marine macroalgae as bait for Rabbitfish including *Chondrophyucus papillosus*, *Fischerella* sp, and *Chaetomorpha crassa*. The most preferred marine macroalgae were taken to the laboratory for proximate composition analysis. In terms of nutritional profile, all species were rich in carbohydrates $> 21.60 \pm 0.07$ (g/100g) and energy $> 245.65 \pm 0.58$ (g/100g) levels. Total fat content was the least 0.48 ± 0.01 (g/100g) whereas no significant difference > 0.05 was observed in moisture content. *Fischerella* sp accounted for the highest protein content levels 33.88 ± 0.02 (g/100g) compared to other species.

Introduction

According to Gong et al., (2019), aquaculture production on a global scale depends on more than 70% of formulated feed. Furthermore, Llagostera et al., (2019) argue that feeds account for 50-70% of total aquaculture production costs. Formulated feeds have various essential building blocks like proteins, amino acids, oils, vitamins, carbohydrates, and minerals which are sourced from varied substances (Ansari et al., 2021). Fish meal and soybean meal are the most fronted sources of protein whereas, wheat bran is widely used to provide carbohydrates to the cultured species. Ansari et al., (2021) assert that the proportion for inclusion of each ingredient in fish feed is largely determined by the type of species cultured and their respective growth stage. For instance, the juvenile stage demands a higher protein inclusion because of great metabolic growth as compared to other stages.

The high cost associated with fishmeal has prompted studies in recent years to develop alternative protein sources. Trials have been made to replace fishmeal with plant-based proteins and insect meal. Although Al-Thobaiti et al., (2017) claim that it is possible to substitute 20% of fishmeal with plant-based protein without compromising the growth performance, Hardy (2007) argues that plant-based protein is deficient in amino acid profile; hence it does not meet the fish requirements. Insect meal is quickly gaining traction as a promising fish feed (Shanthi et al., 2021). Thus, Tibbetts et al., (2017) argue that there is a need for the aquafeed industry to come up with sustainable feed sources that will supplement all nutritional requirements for cultured species

The increased demand for fish proteins over the recent years due to the surging global

population has propelled the expansion of the aquaculture and mariculture industry. As such, Hasan, (2009) reported that researchers from developing countries have continued to direct concerted efforts towards developing alternative aquafeed products from marine macroalgae to supplement the dietary requirements, especially the protein content of the cultured fish species. Globally, macroalgae are regarded to be one of the most widely distributed photosynthetic plants that can withstand varied weather conditions throughout the season (Ścieszka & Klewicka, 2019). Their ability to carry out photosynthesis enables them to be considered crucial primary producers that nourish the marine ecosystem and provide more than 40% of oxygen gas globally (Sun et al., 2017).

Descriptively, marine macroalgae are multicellular plants that inhabit intertidal/subtidal zones, especially along the rocky shores. Their structure is relatively complex and differs in terms of pattern and color from one species to another. According to Guo et al., (2022), some macroalgae species can grow up to a height of 60m and are usually the biggest in shape. From a botanical perspective, marine macroalgae can be broadly categorized into three groups depending on their structural color: red, brown, and green algae. The foliage color of these species is always derived from their constituent pigments like phycoerythrin in red algae, lithophane in brown algae, carotene, and lutein in green algae (Guo et al., 2022).

The distribution and location of marine macroalgae in the coastal regions differ between species due to diverging environmental factors. For instance, changes in seawater temperature are regarded to be a major factor influencing the distribution of marine macroalgae. Kumar (2020) asserts that springtime provides the most conducive water temperature for marine macroalgae to flourish and accumulate the highest nutrient proportion. Also, the varying environmental factors from one season to another, normally alter the sea surface and macroalgal habitat, prompting changes in the existing macroalgae community composition and structure (Jiang et al., 2019). Generally, the distribution of marine macroalgae in different localities offers unique environments that support essential sites for epiphytic and symbiotic relationships between

marine habitats and other marine organisms (Guo et al., 2022).

The high nutritional value associated with marine macroalgae has turned out to be a valuable asset in developing alternative animal and fish feeds as compared to conventional ones such as fishmeal. Worldwide, fish feed is known to be costly in aquaculture production majorly contributed by the cost of fish meal which is widely used as a protein ingredient in fish diet causing a great problem towards the development and growth of the aquaculture sector (FAO, 2020). Therefore, seeking other sources that will be satisfactory as alternative protein for fish feed and providing nutritional benefits at a lower price is very important (Arori et al., 2019). Throughout history, a wide variety of macroalgae species have been valued for their essential roles in culinary and medicinal applications. (Chandini *et al.*, 2008). According to Govardhan *et al.* (2023), macroalgae serve as a unique source of protein, although the protein content varies among different types. Proteins are crucial for a wide range of biological processes, serving as the foundation for enzymatic catalysis, transportation, storage, and mechanical support (Govardhan *et al.*, 2023). Lipids generally provide more energy than other biological compounds during the oxidation process as they serve to store living organisms (Govardhan *et al.*, 2023). Carbohydrates play a vital role in metabolism by providing energy for respiration and other essential processes (Gokulakrishnan *et al.*, 2015). Seaweeds contain the crucial minerals and trace elements vital for human nutrition, with macroalgal ash content typically registering high levels (Govardhan *et al.*, 2023). Macroalgae are enjoyed by up to 20% of the population in Asia, not only for their unique flavor but also for the numerous health benefits they offer.

Therefore, the use of macroalgae in fish feed has been recommended as different records have shown good responses towards growth, feed utilization, and higher survival rates (Hasan, 2009). The nutrient composition of several marine macroalgae along the Kenya coast has been reported. Most of them contained a significant amount of crude protein and crude lipids, among other essential nutrients for fish growth (Mwalugha et al., 2015). During fish feed formulation, proximate composition enables the balancing of various

nutritional components, especially crude protein (Arori et al., 2019). Further studies indicated that red seaweed (Rhodophyta) has higher nutritional value and higher protein content than brown and green seaweeds (Mohammadi., 2013; Mwalugha et al., 2015). Arori et al., (2019), conclude that a significant amount of crude protein is in seaweeds and thus recommended in fish feed formulation. Adequate macroalgae is required to feed individuals of species of the genus *Siganus* being herbivorous to maintain their biological activities (Abdel-Aziz & Ragab, 2017). However, no information is available on the most preferred macroalgal species by rabbitfishes along the Kenya coast thus requiring some further research work.

Materials and Methods

Study Area

Information on the marine macroalgae species that are naturally fed by rabbitfishes in the wild was collected from Kibuyuni, Msambweni, and Shimoni fishing areas of south coast Kenya situated at 4°38'23"S 39°20'21"E, 4°28'47"S 39°29'17"E and 4°38'52"S 39°22'57"E,

respectively (Figure 1). Shimoni is located 80 km south of Mombasa City. It is the main gateway to Wasini Island, seen from the Shimoni jetty and the Kisite Mpunguti National Marine Park and Reserve. Msambweni is a small fishing town in Kwale County of south-eastern Kenya situated 55.4 km south of Mombasa City and 46.5 km northeast of Lunga-Lunga on the Tanzanian border. Msambweni is characterized by sandy beaches, rocky outcrops, and low cliff tops and is relatively pristine. Kibuyuni is a village along the shores of the Indian Ocean that is popularly known for seaweed farming. According to Mirera et al., (2020), this area experiences tidal fluctuations throughout the day, and its sea surface is covered by various seagrass species. More than 70% of the total marine primary production is contributed by macroalgae where red algae *Eucheuma denticulatum*, *Kappaphycus alvarezii*, and *Kappaphycus striatum* are the most important species (Gustafsson & Sivard 2020). These algae prefer water areas that are not influenced by strong currents and winds particularly Shimoni, south coast of Kenya where the conditions are known to be favorable (Pereira & Yarish, 2008).

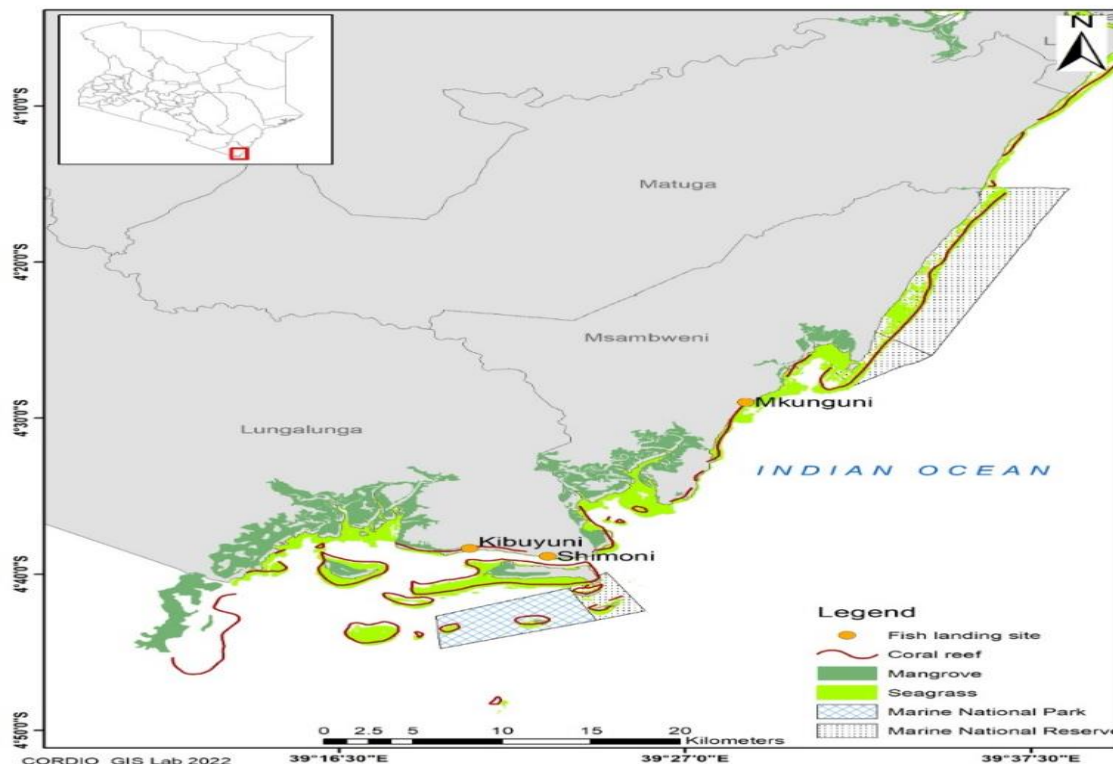


Figure 1. Map of the study area showing macroalgae sampling sites of Msambweni, Shimoni, and Kibuyuni in south coast Kenya

Data Collection

Survey on the Identification of Macroalgae for Feed Formulation

Marine macroalgae for feed formulation were identified following semi-structured interviews using questionnaires administered to fishers based on their fishing gear types to establish what macroalgal species were commonly utilized as bait for the basket trap fishery targeting rabbitfishes. Macroalgae species naturally fed by rabbitfishes were identified by the fishers using local names. Verification of the identified species was done by scientific names in the laboratory using an identification guide according to Al-Yamani et al., (2014). The most preferred macroalgae species as fish feed were subjected to proximate analysis in the laboratory. From the proximate analysis results, the best macroalgal species

were selected based on protein content as an alternative for commercial fish feeds.

Collection of the naturally occurring marine macroalgae species

The 3 most preferred algal species as bait for rabbitfish were then collected in large quantities from Mkunguni and Kibuyuni fishing areas, on the South Coast of Kenya (Plate 1). Harvested macroalgae underwent sorting, rinsing, and cleaning to remove any impurities. Samples of macroalgae were then packed and stored in freezers at a temperature of - 45°C in the laboratory(Plate 2) . After 12 hours, cleansing was done and later dried under indirect sunlight, ground, and finally dried in the oven. The most preferred macroalgae species as fish bait was dried under shade for about 4-5 days on dry racks to remove its mimosine content, which affects fish growth under experimental feeding.



Plate. 1 harvesting of naturally occurring marine macroalage species

Data and Statistical Analysis

Proximate Analysis of the Macroalgae Species

All descriptive statistics were performed using SPSS statistical software version 20 and Microsoft Excel. Nutrient composition of the fish diets was done following standard procedures in the laboratory. Feed formulation was computed using the Pearson Square Method. The most preferred macroalgae species were selected for proximate analysis in the laboratory to determine macromolecule

levels of protein, carbohydrates, fat content, crude fiber, energy, and moisture content that were used for fish feed formulation. Proximate analysis was done using a similar method according to Bhuiyan et al., (2016).

Determination of the moisture content

The percentage of moisture content in the sample was calculated according to AOAC (1975): where the sample was blended and a portion of the sample was weighed at 5g using an analytical balance. The oven was set at 105

degrees and the sample was dried for 4 hours. The sample was cooled in a desiccator. Finally,

the sample was weighed and percentage loss was calculated on drying.

$$\% \text{ moisture} = \{ \text{weight of original sample} - \text{weight of dried sample} \} / \text{Weight of original sample} \times 100 \dots\dots\dots (i)$$

Determination of total ash

A portion of the sample was weighed at 5g on preconditioned crucibles. The crucibles were then placed in a muffle furnace. The furnace was set at 550 degrees and ash for four hours. Crucibles were removed from the furnace

cooled in a desiccator and later weighed. The samples were then returned to the furnace for a further hour and weighed until the two successive weights showed negligible differences. Finally, the percentage ash content was calculated using the following formula according to Maynard (1970).

$$\% \text{ ash} = (\text{weight of ash} / \text{weight of sample}) \times 100 \dots\dots\dots (ii)$$

Determination of crude lipid

The percentage of crude lipid was calculated by the following formula according to Maynard (1970): where a portion of the sample was

weighed at 5 grams. A reflux fat determinator was then used to extract 50ml of petroleum ether. Evaporation of the solvent was followed. Oil content was later weighed and

$$\% \text{ crude fat} = (\text{corrected weight of fat} / \text{weight of sample}) \times 100 \% \dots\dots\dots (iii)$$

Determination of crude protein

The percentage of crude protein was calculated using the formula according to Bhuiyan *et al.*, (2016): where a portion of the sample was weighed at 5 grams. The sample was then placed in Kjeldahl tubes and added 7 grams of K₂SO₄ salt and 0.8 grams of anhydrous CuSO₄. Then 13 ml of conc H₂SO₄ was added

and digested for one hour at 420 degrees. Afterward, the sample was kept cooled to room temperature and later 20ml of water was added and distilled with 50ml of Boric acid solution at the evapodest receiving end. Finally titrated with 0.1N HCL to the pink endpoint. Titre value was recorded and protein content was calculated as follows

$$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times \text{Conversion factor} \dots\dots\dots (iv)$$

Where the Conversion factor for animals is 6.25 and that of plant origin is 5.90.

Levels of carbohydrates present in the sample were calculated following the formula according to AOAC (1975):

Determination of carbohydrates

$$\text{Carbohydrates} = 100 - \Sigma (\text{moisture, protein, fat, ash}) \dots\dots\dots (v)$$

Results

Determination of Naturally Occurring Marine Macroalgae Species fed by *S.sutor* in the Wild

Demographic characteristics of respondents

All respondents were artisanal fishers and the majority (30%) were within the range of 46-60 and 46-60 years old.

years with no formal education. Most respondents (17%) at the age of 36-45 years had attained a primary level of education, followed by those who were between 26 and 35 years old (Figure 2). The few respondents who attained a primary level of education were between 18 and 25 years old

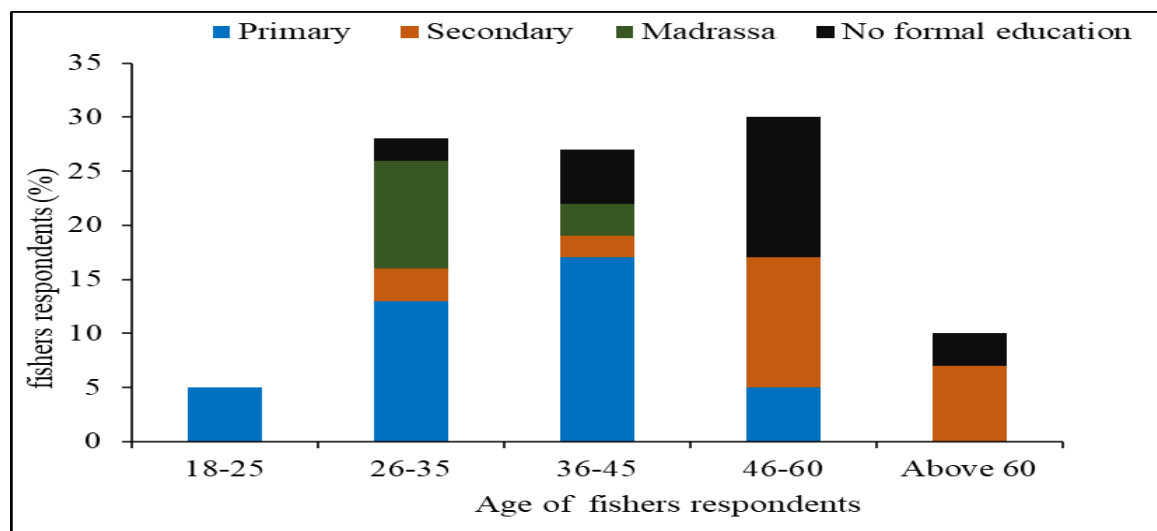


Figure 2. Age of respondents by education levels in south coast Kenya over the study period

Macroalgal Species Utilized as Bait by Basket trap Fishers

Different macroalgae species were utilized by basket trap fishers as bait for rabbitfish species as shown in Figure 3 and these include *Chondrophyucus papillosus* (red algae), *Fischerella*

sp., (blue-green algae) *Chaetomorpha crassa* (green algae), and other bait species that include seagrass and seaweeds. *Chondrophyucus papillosus* was the most utilized macroalgae species with the highest percentage of fisher’s response at 43%.

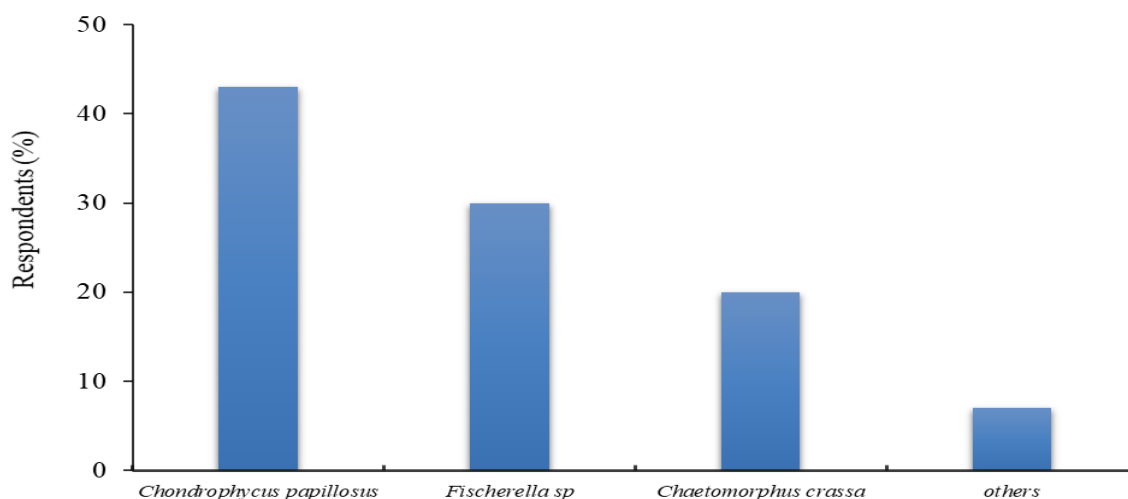


Figure 3. Macroalgae species utilized by basket trap fishers, south coast Kenya

Most Preferred Macroalgae Species by Rabbitfish Species

A total of 6 species of rabbitfish were identified as targets for basket trap fishers. These were: *Siganus stellatus* (brown-spotted spinefoot),

Siganus sutor (shoemaker spine foot), *Siganus luridus* (dusky spinefoot), *Siganus canaliculatus* (white-spotted spinefoot), *Siganus argenteus* (streamlined spinefoot) and *Siganus rivulatus* (marbled

spinefoot). These species of rabbitfish preferred different species of macroalgae species Figure (4). *Siganus sutor* and *Siganus stellatus* preferred *Fischerella sp* compared to *Chondrophyucus papillosus* and

Chaetomorpha crassa. Whereas *Chaetomorpha crassa*, green algae was widely preferred by all rabbitfishes. *S. stellatus* and *S. sutor* preferred all types of macroalgal species.

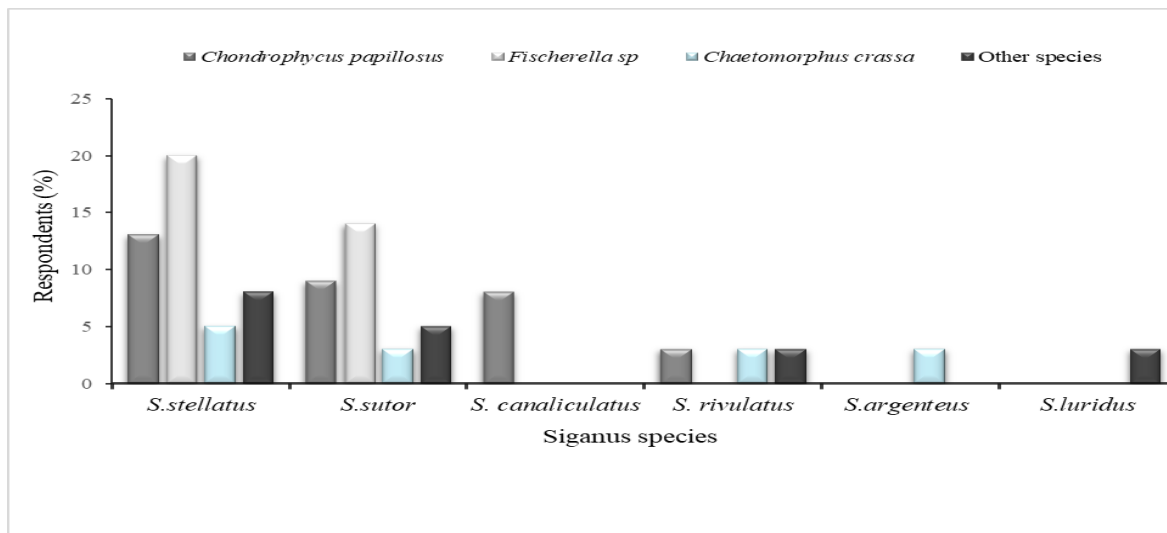


Figure 4. Most preferred macroalgae species by rabbitfish species

Determination of Proximate Composition of Macroalgae Species and Formulated Diets

Results of proximate analysis of selected macroalgae species

The results of the proximate analysis are presented in Table 1. Moisture content was highest for *Chondrophyucus papillosus* at 7.78 ± 0.02 followed by *Chaetomorpha crassa* and was lowest in *Fischerella sp*. The macroalgae species *Fischerella sp*. was the richest in total fat content at 7.34 ± 0.02 followed by *C. crassa* with the

lowest level recorded in *C. papillosus*. Protein levels were twice as high for *Fischerella sp*. at 33.88 ± 0.02 compared to those of *C. papillosus* and *C. crassa*. The crude fibre was more pronounced in *C. crassa* than in *C. papillosus* and *Fischerella sp*. Levels of carbohydrates were highest in *C. papillosus* compared to *C. crassa* and *Fischerella sp*. Energy levels for the three macroalgal species were similar at over 240 g/100g but were highest in *C. papillosus* followed by *Fischerella sp*.

Table 1. Results of mean \pm SE proximate composition of selected macroalgal species used as bait in basket traps

Test	<i>Chondrophyucus papillosus</i>	<i>Chaetomorpha crassa</i>	<i>Fischerella sp.</i>
Moisture Content (g/100g)	7.78 \pm 0.02	6.89 \pm 0.02	4.77 \pm 0.01
Total Fat Content (g/100g)	0.48 \pm 0.01	2.38 \pm 0.01	7.34 \pm 0.02
Protein Content (g/100g)	13.08 \pm 0.04	17.27 \pm 0.02	33.88 \pm 0.02
Crude Fibre (g/100g)	9.43 \pm 0.01	34.64 \pm 0.03	4.73 \pm 0.01
Carbohydrates (g/100g)	59.29 \pm 0.01	38.86 \pm 0.00	21.60 \pm 0.07
Energy (g/100g)	294.41 \pm 0.53	245.65 \pm 0.58	287.49 \pm 1.83

Discussion

Demographic characteristics of respondents

The majority of the respondents involved in macroalgae identification were within the range of 46-60 years old with no formal education. This implies that most of the respondents were adults with an average age of 50 years and therefore, knowledgeable on macroalgae species that have been used as bait in the basket trap fishery. The results of this study support findings by Mwakaribu et al., (2022) that basket trap fishery along the Kenya coast is associated with older fishers. This old age of fishers is characterized by no formal education and therefore, these fishers rely on traditional knowledge and use of traditional fishing gear such as the use of basket traps. Among this fisher category, small-scale fishing is considered to be the main source of income-generating activity for livelihood support. Most of them are married supporting relatively large families (Mwakaribu et al., 2022). These older fishers also have been observed to be more experienced with relatively many years of fishing experience (Mwakaribu et al., 2022). Few fishers who had attained primary level were between the ages of 18 and 25 years. This reflects that younger fishers did not prefer the use of basket traps as a result of the low experience associated with this age bracket for the basket trap fishery. Worldwide, knowledge of fish and fisheries gained at the local level may make a significant contribution to biological and ecological studies and is crucial

in the formulation of fisheries management measures.

Preferred macroalgae species as bait for rabbitfishes

The basket trap fishers used three types of macroalgae species as bait for rabbitfishes. The red algae (*Chondrophyucus papillosus*) was the most common and used by basket trap fishers as bait for rabbitfish due to its preference and availability. The macroalgae species *C. papillosus* was reported by the respondents to be the most preferred feed type by brown-spotted spinefoot (*Siganus stellatus*), whereas, the macroalgae species *Fischerella sp.* was most preferred by both *S. stellatus* and *S. sutor*. The green algae (*Chaetomorpha crassa*) was reported to be most preferred by all rabbitfish species. Latuconsina et al., (2023) reported that the white spotted spinefoot (*Siganus canaliculatus*) preferred macroalgae as feed with higher protein and soluble sugar content due to the species' elevated energy requirements. Furthermore, rabbitfishes prefer feeding on fibrous and flat macroalgae species than calcified ones indicating that the preference for a particular macroalgae feed by rabbitfishes is not only determined by nutritional content but also the morphological traits of the respective macroalgae species. Von Westernhagen (1974), revealed that the texture of the algal thallus is a key determinant factor in the choice of feed preference for rabbitfishes. Therefore, most siganids prefer crispy

macroalgae as feed and the ones with thin thallus which are easy to bite.

This study observed that the macroalgae species *Fischerella sp.* was collected from the protected shallow lagoons with other bait species such as squids, seagrass, and sea urchins by hand during low tide. This observation is supported by similar findings by Musembi et al., (2019) who found out that basket trap fishers collected different macroalgae species as bait from the shallow and sheltered lagoons. Latuconsina et al., (2023) reported that the white-spotted spinefoot (*Siganus canaliculatus*) preferred macroalgae as feed with higher protein and soluble sugar content due to the species' elevated energy requirements. Furthermore, rabbitfishes prefer feeding on fibrous and flat macroalgae species than calcified ones indicating that the preference for a particular macroalgae feed by rabbitfishes is not only determined by nutritional content but also the morphological traits of the respective macroalgae species. Westernhagen, (1974) revealed that the texture of the algal thallus is a key determinant factor in the choice of feed preference for rabbitfishes. Therefore, most siganids prefer crispy macroalgae as feed and the ones with thin thallus which are easy to bite.

Proximate analysis of selected macroalgae species

The level of protein was highest in *Fischerella sp.* at 33.88% surpassing the levels of *Chondrophyucus papillosus* (13.08%) and *Chaetomorpha crassa* (17.27%) by the double margin (Table 1) indicating that the protein content in these macroalgae species was within the recommended of 10 - 47% dry weight (Fleurence, 1999). The results also conquer with the findings of Tacon et al., (1989) who recommended dietary protein requirements for rabbitfishes especially *S. canaliculatus* to be 31% crude protein, 8% lipids, and 38% carbohydrates. The moisture content ranged between 4.77% and 7.78% for the studied macroalgae species. In extreme conditions, elevated moisture levels accelerate bacteria, molds, and yeast proliferation which in turn speeds up food spoilage. In terms of crude fibre, *Chaetomorpha crassa* in this present study

had the greatest content (34.64%) while *Chondrophyucus papillosus* and *Fischerella sp.* had the lowest contents of 9.43% and 4.73%, respectively. According to Siddique et al., (2013), the disparities in the composition of crude fibre among macroalgae can happen due to changes in photosynthetic activities and seasonal factors that affect both photosynthesis and nutrient uptake by macroalgae. Carbohydrates and energy were high in all the studied macroalgae species (Table 2) attributed to non-structural carbohydrates which act as energy storage units in macroalgae (Dunstan et al., 2002). Total fat was the lowest in chemical content recorded in all the studied macroalgae species. *Fischerella sp.* had the highest total fat content (7.34%) followed by *Chaetomorpha crassa* at 2.38% and *Chondrophyucus papillosus* with a lowest of 0.48%. All the results of proximate composition from this study except for *Fischerella sp.* concur with other works which reported that crude fat content in different investigated seaweeds was less than 5% (Mwalugha, et al., 2015) thus seaweeds are not regarded to be excellent sources of crude fats. Norziah and Ching, (2000); and Marinho-Soriano, et al., (2006) reported that different seaweed species have varying crude fat concentrations due to differences in growth phases, environmental factors, and geographic location.

Conclusion and Recommendations

The study explored the use of naturally occurring marine macroalgae as a potential source of alternative protein for fish feed in aquaculture production. In particular, the interest of this study was to find a possibility for developing Rabbitfish feed from the readily available macroalgae to substitute the commercial fishmeal whose dwindling supply and elevated prices make it unsustainable to most fish farmers. Naturally occurring marine macroalgae preferred as bait for Rabbitfish have been identified with the red algae (*Chondrophyucus papillosus*) dominating the bait type used in basket-trap fishery in Kenya. However, with each species of Rabbitfish having a preference for certain marine algal feeds, the brown-spotted spinefoot (*Siganus*

stellatus), was inclined towards feeding on *C. papillosus* whereas; *Siganus stellatus* and *Siganus sutor* preferred *Fischerella sp.*

Despite all species depicting recommended amounts of proteins required by *S. sutor* for optimal growth, *Fischerella sp* could be the best suited for preparing feeds for *S. sutor* culture due to its elevated protein content levels as compared to other macroalgae species. High carbohydrates and energy levels across the three macroalgae species indicated the importance of these feeds in supplementing *S. sutor* dietary requirements for biological and metabolic activities. Less significant variations in moisture content levels across the three macroalgae species equate to stable conditions existing in their natural habitats.

Proximate composition analyses of the selected naturally occurring marine macroalgae species presented essential data on their respective nutritional contents. Therefore, this study's findings map out a clear pathway for developing aqua feeds that are convergent with the nutritional profile of marine macroalgae species preferred by *S. sutor* in the wild. In doing so, fish farmers will be in a better position that optimize both the growth rate and health of the cultured *S. sutor*. Based on findings from the evaluation of the proximate composition of selected naturally occurring marine macroalgae, continuous evaluations, and assessments should be carried out to determine the right proportionate nutritional content required by *S. sutor* to optimize growth and enhance good health.

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