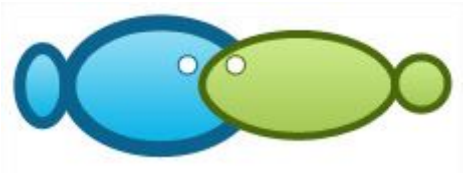

Morphometric and proximate analysis of mole crabs (*Hippa* genus) in Maluku Province, Indonesia

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Abstract. The use of mole crabs *Hippa* genus for food diversification and side income of coastal community in Indonesia is not yet a viable option, until recently. Therefore, more studies regarding the enormous potential of sand crabs *Hippa* genus are needed. This study aimed to thoroughly identify the morphologic aspect of three species of *Hippa* genus namely *Hippa marmorata*, *Hippa ovalis*, and *Hippa celaeno* and to conduct a nutrient analysis of those mole crabs species. The data collection was conducted in Silale Village, Ambon City in Maluku Province, Indonesia, in May 2019. For morphologic analysis, allometry analysis was used in this study. Whereas, for the proximate test, we used AOAC method to identify the composition of *Hippa* genus based on two treatment scenarios; the first one was a raw form (pre-processing), and the second one was fried mole crab (post-processing). The result of the study showed that all *Hippa* species have a linear relationship on growth based on morphometry analysis; while proximate analysis showed that mole crabs *Hippa* genus have rich amount of nutrition which makes mole crabs *Hippa* genus a potential alternative for a quality meal and economic sources. The implication of the study is to shed some light on true and great potential of *Hippa* genus to be used as diversification meal, an alternative source of nutrient, and revenue for the local coastal community especially in Maluku Province, as well as Indonesia in general.
Key Words: *Hippa*, food diversification, morphometric, proximate test.

Introduction. The abundance of marine biodiversity due to the strategic location of Maluku region – where it has been included on coral triangle – creates the availability and richness of marine species, especially fishes and other marine species. Maluku has 10.662,92 km shoreline (KKP 2013), where the habitat of various marine species and sand crabs are one among many. Mole crabs used to live on the sandy beach with a dynamic coastal area where sand, waves, and tides interact; thus, animals that inhabit the area have been adapted by being more mobile and be more opportunistic by feeding on other animals (Brown & McLachlan 2006). *Hippa* sp. namely *Hippa marmorata* (Hombron & Jacquinot, 1846) is living in tropical and subtropical sandy beaches in the Indo-Pacific region (Osawa et al 2010); the same home for other two *Hippa* species namely *Hippa ovalis* (A. Milne Edwars, 1862) (Bauchau et al 1987) and *Hippa celaeno* (De Man, 1896) (Haig et al 1986).

Mole crab – or "undur-undur" in local Indonesian name - has been utilized either for side dish and alternative sources of income. However, it is still very limited in a small coastal community in Java Island. There are not many studies available, in particular, to analyze the nutritional content of mole crabs in Indonesia. There are some studies regarding the omega-6 in mole crabs. Mursyidin et al (2002) investigated the omega-3 content in mole crabs. Astriana et al (2013) studied the use of mole crabs as food supplement for bird pets. In addition, Mashar & Wardiatno (2016) showed that mole crabs in Cilacap District have an economic value of IDR 64,500,000 per year on average. It is in line with the study by Mashar & Wardiatno (2013) stating that mole crab has a

tremendous potential for alternative source for economic income. In addition, mole crab (*Hippa* genus) also has high ecological value due to its role on food chain cycle which at a trophic level is the first consumer in the sand beach area.

The abundance of mole crab (*Hippa* genus) in Maluku Province is available in large number which can provide the vast opportunity for food diversification and can become another source for income to coastal community in the region. The food processing of mole crabs in Maluku is commonly done in basic ways such as boiling, steaming, baking, drying (Wenno 2013) and frying (Leimena 2015); although, these various types of food processing can affect the nutrient content of mole crabs. Hanifa (2014) stated that processing mole crabs by heating affects the nutritional value especially on protein, fat, carbohydrate, amino acids, fatty acids, and minerals. Furthermore, Hanifa (2014) and Leimena (2015) reported that the proximate levels were decreasing after boiling, steaming and roasting. The same results were shown by Wenno (2013), who reported proximate indicators value changed after boiling and drying. In addition, Hanifa (2014) also reported the same indication where levels of amino acids, fatty acids, minerals and cholesterol changed after boiling and steaming.

Wenner et al (1987) suggested that different types of the coastal area also could contribute to the different types and behavior of mole crabs from one coastal region to another. Thus, it is necessary to conduct a study on the *Hippa* genus because Mashar & Wardiatno (2013) mentioned the lack of research on the mole crabs in Indonesia. The limited research on mole crabs (*Hippa* genus) in particular for morphometrically aspects and the utilization of mole crabs especially in testing their nutrient contents both pre- and post-processing have triggered us to conduct this study. This study aimed to provide an analytical description of morphometric and proximate analysis of processing three *Hippa* genus namely *Hippa marmorata*, *Hippa ovalis*, and *Hippa celaeno*. This study was expected to provide needed information regarding *Hippa* genus and an overview of alternative marine resources in the form of mole crabs which have economic value and potential to serve as an alternative for food diversification, specifically for the coastal community in the Maluku region and all communities of coastal regions around the world.

Material and Method

Sample collection and study area. Data collection was conducted in Wayasel Beach, Silale Village, Nusaniwe Sub-district, Ambon City in Maluku Province, Indonesia, in May 2019 (Figure 1). Mole crabs are captured by villagers using foot (Figure 2) in the intertidal zone (highest tides and lowest tides) by putting their feet in the sand horizontally and pushing them into the sand with a slope of 10 degrees. They observe whether there is a movement in the sand indicating that mole crabs are available to be caught using bare hands. Furthermore, the mole crabs captured are put in a container filled with sand and seawater, before being washed and drained. A number of 86 crabs were taken for morphometric measurements (carapace length, width, body weight) and species identification. Whereas, 252 crabs (360 g) were taken for analysis where raw mole crabs (180 g) were for proximate analysis and the other half (180 g) were for proximate post-processing analysis (Figure 3).

Proximate analysis. Proximate chemical composition analysis was determined using standard procedures (AOAC 2005). Water and ash, fat, and protein content were analyzed using the oven method, the soxhlet method, and the Kjeldahl method, respectively. In addition, carbohydrate content was estimated by difference calculation, while mineral content was analyzed using the SSA method (AOAC 2005). Five (5) grams of mole crabs were put into the porcelain ash dish, which further gradually being placed in the furnace to a temperature of 600°C. The greying process was carried out until the ash was white. Afterwards, the porcelain ash dish was cooled for 30 minutes in a desiccator then weighed and counted. Ash content analysis was conducted at the Pattimura University Ambon Basic Chemistry Laboratory.

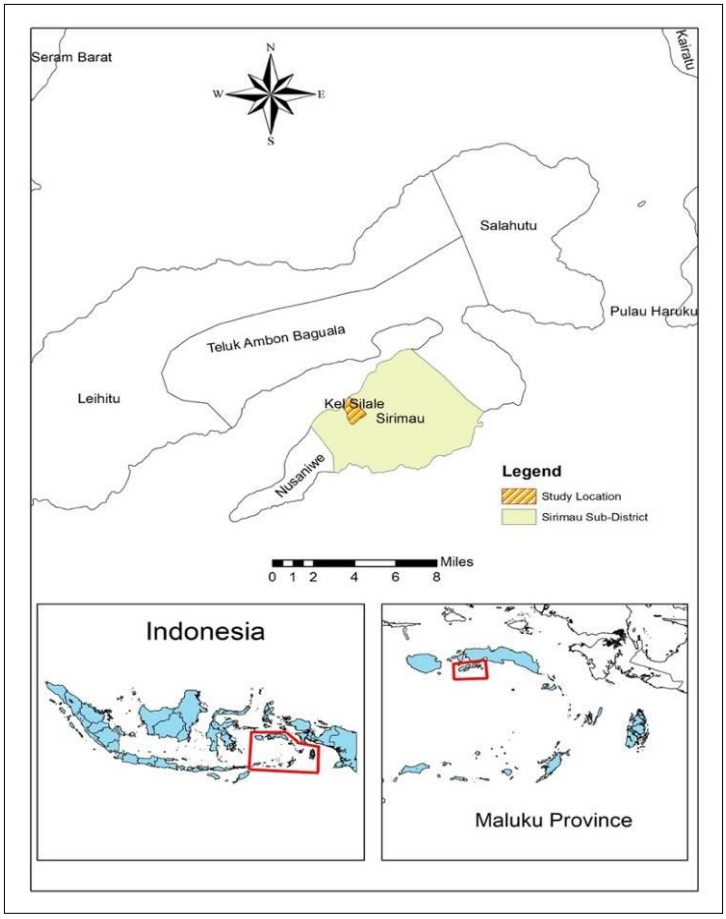

Figure 1. Study location.

Figure 2. Method of capturing mole crabs.

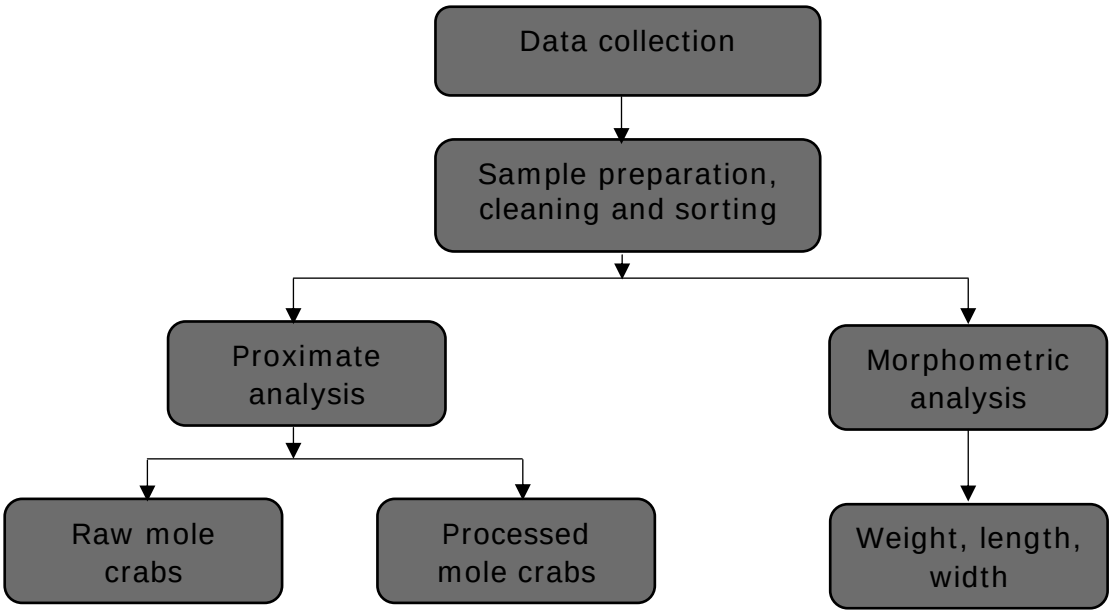

Figure 3. Research framework.

The mineral content of the mole crabs was placed into the Erlenmeyer added with 10 mL of concentrated nitric acid and 30 mL of concentrated hydrochloric acid before left it for a day. After that, the Erlenmeyer was heated at 200^{o}C approximately for 1 hour. Once it was done, 2 mL of concentrated nitric acid was added and the Erlenmeyer was then reheated until a clear solution was obtained. The solution was then transferred to 100 mL Erlenmeyer and distilled water and later being filtered. The destruction solution was then analyzed using the SSA (Atomic Absorption Spectrophotometer) method.

Analysis of fatty acids and cholesterol was determined using GC-MS, where 94.97 g of crushed mole crabs were powdered, and then wrapped in filter paper while the top was covered with cotton. The soxhlet extraction device was prepared and then wrapped and put into an extraction flask of 60% volume of petroleum ether. The sample was extracted until the liquid became colorless. The extract was obtained and then poured into Erlenmeyer and covered with filter paper. Afterwards, it was stored until the solvent has evaporated, cooled, weighed then the extract was put in a bottle for the fatty acid analysis.

Transesterification using an acid catalyst was carried out by inserting 10 mL of 15% boron trifluoride solution in methanol into a three-neck flask and adding 51.17 grams of sea retreat oil before refluxing it for 90 minutes above the water bath. The reflux results after being cooled were put into a separating funnel and then washed with 25 mL distilled water, before being extracted twice with 20 mL n-hexane after two layers were formed. The lower layer containing glycerol was separated while the mixture of methyl esters (top layer) was extracted with 10 mL n-hexane twice. The top layer was washed with distilled water until the pH was neutral. Then, ester layer was dried with anhydrous Na_2SO_4. The mixture was filtered, and the solvent evaporated at room temperature, the methyl ester mixture was analyzed.

Morphometric analysis. Hossain et al (2019) stated that morphometric analysis provides information regarding growth patterns, population dynamics, and well-being of individuals in particular environments. In this study, we used allometric to analyze the growth of mole crabs *Hippa* genus. The concept of allometry was initially developed in biology to see the changes in the relative dimensions of body parts and to see the correlation with changes in overall size (Gayon 2000). Figure 4 shows three species of *Hippa* genus used in the study which were obtained in Wayasel Beach, Silale village, which has the characteristic of sandy beach and fits the habitat of *Hippa* genus (Figure 5).

Figure 4. 1) *Hippa marmorata*; 2) *Hippa ovalis*; 3) *Hippa celaeno*.


Figure 5. The substrate of mole crab *Hippa* genus.

Results. The results showed that *H. marmorata* was heavier than the other two *Hippa* species namely *H. ovalis* and *H. celaeno.* In addition, the carapace length also showed that *H. marmorata* was lengthiest among three *Hippa* species. The same result was also shown in the width parameter where *H. marmorata* had wider body frame, followed by *H. ovalis* and *H. celaeno* (Table 1).

Table 1

Biometric data of *Hippa* genus (*Hippa marmorata*, *Hippa ovalis* and *Hippa celaeno*)

No	*Species*	*Min*	*Max*	*Average*	*Std Dev*
1	*Hippa marmorata*				
	Weight (g)	1	4.2	1.9	0.822
	Carapace length (cm)	1.2	1.9	1.8	0.307
	Width (cm)	0.8	1.6	1.1	0.238
2	*Hippa ovalis*				
	Weight (g)	0.9	2.8	1.5	0.472
	Carapace length	1.2	2.2	1.6	0.234
	Width (cm)	0.6	1.3	1.0	0.163
3	*Hippa celaeno*				
	Weight (g)	0.5	2	1.0	0.518
	Carapace length	1	2	1.3	0.318
	Width (cm)	0.5	1.2	0.7	0.224

As shown in Table 2, the results of the correlation between morphometry parameters showed that all parameters had positive correlation value. It indicates the linear relationship between all morphometry parameters, where the more significant size had direct and proportional relation to the length and weight of *Hippa* genus. Figure 6 showed that the growth analysis of the three *Hippa* species in this study had a linear relationship for carapace length and width, and between carapace length and weight.

Table 2

Pairwise Pearson correlation of morphometric parameters of *Hippa* genus

No	Species	Correlation		
		Weight and width	*Carapace length and weight*	*Carapace length and width*
1	*Hippa marmorata*	0.94	0.78	0.84
2	*Hippa ovalis*	0.81	0.90	0.85
3	*Hippa celaeno*	0.80	0.92	0.86

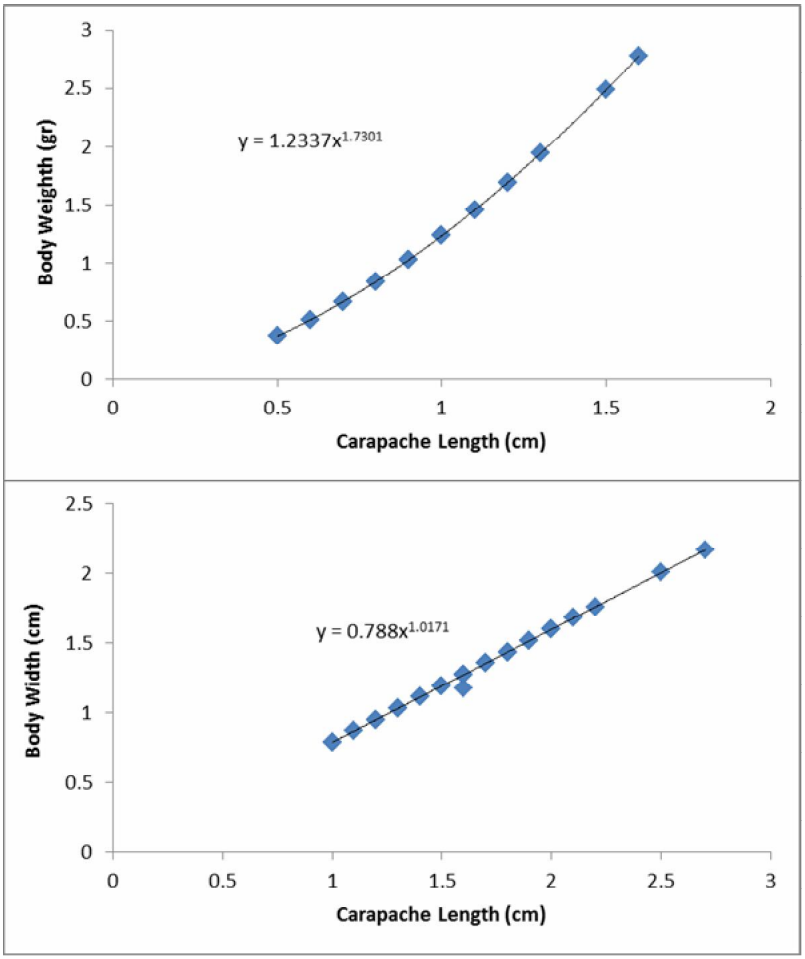

Figure 6. Allometry result.

Proximate analysis of mole crab (Hippa genus). Hanifa (2014) stated that the nutritional content of mole crabs had changed after undergoing food processing such as frying, steaming, and grilling. The results of the proximate analysis on the content of mole crab (*Hippa* genus) showed that there was a change in the protein, ash, and fat content after food processing, except in water and carbohydrate content (Table 3).

Atomic Absorption Spectrophotometer (AAS) analysis showed that Kalium content was higher than zinc. Kalium and zinc mineral contents in fresh mole crabs did not change significantly after being processed into crispy form (Table 4).

Table 3

The result of proximate analysis on mole crab (*Hippa* genus)

Composition (%)	*Mole crab (Hippa genus)*	
	Fresh (post processing)	*Crispy fried (post-processing)*
Water	72.22	21.17
Protein	17.21	20.24
Fat	1.33	20.03
Ash	2.63	35.31
Carbohydrate (by difference)	6.61	3.25

Table 4

Mineral content of mole crab (*Hippa* genus)

Mineral (mg L^{-1})	*Mole crab (Hippa genus)*	
	Fresh (post processing)	*Crispy fried (post-processing)*
Kalium (K)	33.57	33.18
Zinc (Zn)	0. 41	0.30

Table 3 shows the result of analysis using gas chromatography-mass spectrometry (GC-MS) indicating that the fatty acids in the mole crab after undergoing the heating process had a different value compared to fresh mole crabs (pre-processing). Fatty acids detected in fresh mole crab (*Hippa* genus) consist of 2 types of saturated fatty acids and four unsaturated fatty acids (Table 5).

Table 5

Fatty acid analysis of mole crab (*Hippa* genus)

Type of fatty acid	*Symbol*	*Sample (%)*	
		Fresh (post processing)	*Crispy fried (post-processing)*
Unsaturated			
Palmitoleic acid	C16:1	4.67	-
Linoleic acid (omega 6)	C18:2	3.48	8.63
Oleic acid (omega 9)	C18:1	10.67	25.94
Arachidonic acid (omega 6)	C20:4	3.42	-
Saturated			
Palmitic acid	C16:0	14.91	21.1
Stearic acid	C18:0	8.82	

Discussion. Water content has direct effect on food quality. The decrease of water content in crispy food can be influenced by oil used for frying and type of flour. Elinda (2007) explained that water on the surface turns to steam because of direct contact with cooking oil. This causes the concentration of water on the surface of the material (food) is always lower than the concentration of water inside. The mass of water is diffused to the surface as the final moisture content of the fried product. Thus, during frying process, the water content of the material evaporates. In addition, water and hot air come out of the product due to the heat from the oil. This process also increases fat content, which replaces the space due to water evaporation during frying. During the frying process, oil seeps into the surface and fills the space due to loss of water; thus, the volume of fat is balanced by the total volume of water that comes out from the fried material. Mallikarjunan et al (2017) stated that, during the frying process, there is a reduction in water content due to the evaporation of water.

Protein and ash contents in fresh mole crab increase after being processed into crispy caused by flour, eggs, seasonings and the processing. The higher the proportion of ingredient used, the higher the protein content of the product. The higher the ash

content, the higher the minerals contained in a product. Elinda (2007) stated that protein can be increased with the addition of other ingredients that contain protein, while the spread of insoluble minerals is associated with protein, especially in non-fat meats. Lean meat generally has a higher ash content. Analysis of carbohydrate content of fresh mole crabs using the by difference method shows carbohydrate levels decrease compared to the crisp. This change is influenced by other nutritional content such as water content, protein, fat, and ash when the nutrient content decreases, and carbohydrate levels increase. This result is supported by research from Siswanti et al (2017) stating that nutritional components influence carbohydrate levels calculated by difference. The lower content of the other nutritional components, the higher the carbohydrate content, and vice versa. Nutritional components that influence the amount of carbohydrate content include protein, fat, water, and ash.

Zinc is an essential component in the structure and function of cell membranes, serving as an antioxidant and protecting the body from the attack of lipid peroxidase. Furthermore, zinc plays a role in protein synthesis and transcription, i.e. in gene regulation, and in stabilizing the structure of proteins, such as insulin, alcoholic liver dehydrogenase, alkaline phosphate, and superoxide dismutase (Arifin 2008). Zinc deficiency can make the body stunted, undeveloped sex organs, swollen liver, and kidneys, and iron nutrient anemia. On the other hand, potassium plays a role in maintaining osmotic pressure and acid-base balance, maintaining osmotic pressure in intracellular fluid, helping activate enzyme reactions, such as pyruvate kinase, which can produce pyruvic acid in the process of carbohydrate metabolism.

Oleic acid (Omega 9) has an essential role in health, i.e. increasing HDL blood cholesterol or good cholesterol and reducing LDL cholesterol in the blood or lousy cholesterol. Oleic acid has higher protective power compared to omega-three and omega-six fatty acids. Oleic fatty acid also has the potential to block the production of eicosanoid compounds that are stimulants for tumor growth (Edison 2010). Arachidonic acid is the result of desaturation and elongation of linoleic acid, and Arachidonic acid is an essential fatty acid as a precursor for eicosanoids. Linoleic acid and α-linolenic acid are precursors in PUFA synthesis. Linoleic acid is used to maintain the structural parts of cell membranes and to make materials such as hormones called eicosanoids. Arachidonic acid is the result of desaturation and elongation of linoleic acid in animals. Desaturation is the process of adding double bonds to fatty acids with the help of enzymes, while elongation is an extension of two carbon chains. Arachidonic acid is an essential fatty acid as a precursor for eicosanoids (Aji 2011).

The results of fresh mole crab (pre-processing) and post-processed in the form of crisp were identified in which they contained the same amount of cholesterol. The cholesterol content of fresh mole crab was 7%, while crisp was 7.7%. Cholesterol is an essential molecule in animals that has the function of building and repairing cell membranes, precursors of steroid hormone biosynthesis, bile acids, and vitamin D. Federal dietary guidelines recommend limiting cholesterol intake to less than 300 mg day^{-1} (Samaha et al 2003). The threshold for healthy cholesterol levels of the human body is < 200 mg dL^{-1}. An average adult needs 1.1 g of cholesterol to maintain cell walls and other functions of organs in the body, which around 25-40% (200-300 mg). The source of cholesterol commonly comes from food, whereas the rest is endogenous (biosynthesis) mainly by the liver and small intestine (Resta 2016). Although, mole crabs are not recommended for those who have heart problem due to the cholesterol levels.

Conclusions. This study aimed to enrich the literature relating to the *Hippa* genus, especially *Hippa marmorata, Hippa ovalis* and *Hippa celaeno*. The results of this study are expected to be used as a reference in understanding and further study of the *Hippa* genus. The results of this study showed that *Hippa marmorata* has more significant physical characteristics than the other two species. Morphometry results and growth analysis with allometry also showed a linear relationship on the growth of each species. Relevant results from the study showed a significant nutrient content in mole crab *Hippa* genus. Mole crabs *Hippa* genus which has abundant unsaturated fatty acid makes mole crabs has become an alternative that has a potentially high economic value, an

alternative source of nutrition from fish, and an alternative source for income to the local community specifically in coastal sandy beach areas in general.

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