Characterization of the sand particle size from natural habitat and the digestive tract, as a basis in determining the grain size of sand suitable for a substrate of sea cucumber (*Holothuria scabra*) culture semiclosed system

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Abstract. The decline in production has prompted researchers in various countries to continue to study factors supporting the success of *Holothuria scabra* culture. This research aims to evaluate the characteristic of nutrients, size of a sand fraction of the sediment from natural habitats, and the digestive tract, as a basis to determine the grain size of sand suitable for substrates of sea cucumbers culture semi-closed system. The result showed that the highest fraction in the digestive tract of sea cucumber with a wet weight of 5-20 g was clay, 50-70 g of sea cucumbers was fine sand, size of 100-120 g as medium and fine sand, size of 200-220 g was the clay fraction, and in natural habitat was fine sand and clay. Medium sand to clay as a dominant fraction to be found was 86.83±1.22-96.74±0.60%. Smaller sea cucumbers tended to absorb a high amount of coarse sand, while larger sea cucumbers tended to absorb a higher amount of fine sand fraction. Meanwhile, protein, fat, and crude fibre content in the natural habitat sediment was low, so that nutrient enrichment and/or artificial feeding on the substrate is important, to provide for the growth of sea cucumbers if its culture efforts use a semiclosed system.

1. Introduction

The sea cucumber *Holothuria scabra*s one of the highest commercial values in Asia. In general, sea cucumbers are traded as food. Data on BPS-Statistics Indonesia shows that the export volume of Indonesian sea cucumber products continues to decline drastically. It was recorded that the export volume in 2016 was 2,003.78 t, while the export volume in 2012 was 411.878 t [1]. The stock of wild sea cucumber has been declining [2]. The increasing demand and overfishing have encouraged researchers in various countries to continue to study on biological information and other production factors that can support the success of farming activities so that the production of sand sea cucumbers can be supported by cultured activities [3]. Sea cucumber *H. scabra* is considered to be the most suitable species for aquaculture production because the seeds can be produced at the hatchery.

Sea cucumbers are found in greater abundance In the coastal waters with muddy sand substrate, especially which have seagrass. Conversely, in waters with rocky substrates, sea cucumber abundance will be very less, when the days are bright, usually found on the sidelines of the rocks. Its
daily cycle in a sandy substrate, sea cucumbers will be buried in the substrate to escape predators, and when it is dark they will appear on the surface of the substrate to obtain food [4].

Sediment preference is an important factor for the success of sea cucumber culture. Sand is one of the components of the substrate that must be present in the sea cucumber habitat. Sand will affect the level of friability of the substrate, the abundance of bacteria and other microphytobenthos, and act as a buffer to maintain the water quality of the culture environment. Sea cucumbers will dig the surface of the substrate using the mouth by coordinating the work of muscles, chalk rings, and tentacles. They have buccal tentacles that can directly swallow the surrounding mud and sand [5]. This epifauna is very selective in choosing the size of the sand particle fraction to get the nutrients it needs into its digestive tract. Sand muddy is an ideal substrate for sea cucumbers it provides high material organically, easy to consume, and for burying [4, 6]. However, other studies suggested that sea cucumbers prefer coarser sand particles than fine particle size [7, 8].

Characterization of the sand particle size contained in the digestive tract of various wet body weights of seacucumbers and natural habitats needs to be known, as a basic illustration for determining the appropriate substrate preference for sea cucumbers culture in ponds. Engineering of size sand fraction making up the substrates is required to streamline the absorption of nutrients and microorganisms associated with sediments, as well as the media in which cucumbers can burrow in it.

The new contribution of the study was to evaluate the character of nutrients, size sand fraction of the sediment from natural habitats, and the digestive tract, as a basis in determining the grain size of sand suitable for a substrate of sea cucumbers *Holothuria scabra* culture semiclosed system.

2. Material and methods

2.1 Research location

The research was conducted in the waters of Lalowaru Village, Southeast Sulawesi Province, from March to July 2018. This water has a high abundance of sea cucumbers, and as a place for fishermen to collect sea cucumber throughout the year, at Southeast Sulawesi. Therefore, the water quality characteristics of these waters are considered appropriate as a reference for assessing the suitability of water quality parameters for sea cucumber culture.

2.2 Sample collection

Sediment samples for the natural habitat of sand sea cucumbers were taken using a pipe that was stuck vertically on the surface of the substrates. Three replications of natural habitat sediment samples were taken from selected location points which were considered to be representative of the natural habitat of sea cucumbers. The sediment samples in the digestive tract were obtained from four size classes based on the wet weight of sea cucumbers, ie: 5-20 g, 50-70 g, 100-120 g, and 200-220 g. Each class of sea cucumber size was taken 3 replicate samples.

2.3 Texture analysis of sediment fraction

The texture of sediment fraction analysis used the sieve analysis method, at the laboratory of productivity and aquatic environment of the Department of Aquatic Resources Management, FPIK IPB. The samples were dried at 105 °C using an oven for 3 hours, then cooled in an exicator for 45 minutes. Subsequently, 3% hydrogen peroxide solution was added to 50 g samples, then crushed until slightly smooth. Furthermore, the samples were sieved using a stratified sieve, sizes 2 mm, 1 mm, 0.25 mm, 0.5 mm, and 0.125 mm. The samples retained on each sieve level were collected using aluminium foil that had been labelled for use oven-dried. The weight of the sample was weighed as the weight of fractions. The escaped sediment fraction was placed in a 1000 mL cylindrical tube for analysis of the sludge fraction using the pipette method. The samples were homogenized with 2 mL of 3% hydrogen peroxide for 24 hours. After 24 hours, the sediment was stirred again until it was homogeneously suspended again. Water sampling was done first using a 20 ml pipette volumetric at a depth of 10 cm. Water samples included in the aluminium plate which has been known weight for dried in an oven for 3 hours at a temperature of 105 °C. After cooling for 45 minutes using an exicator, the sample weight was weighed as a fraction of <50 μm. Furthermore, the water sample in
the cylinder was shaken again until it was homogeneous, after being allowed to stand for 3 hours 27 minutes at a temperature of 28°C, a second collection was made. The water sample dried in an oven for 3 hours at a temperature of 105°C. After cooling for 45 minutes using an exicator, the sample weight was weighed as a fraction of < 2 μm.

2.4 Nutrient sediment
Analysis of nutrient of sediment was carried out at the Fish Nutrition Laboratory of Department of Aquaculture, Faculty of Fisheries and Marine Science-IPB. Measurement of water content using the oven method, the ash content using the dry ashing method, protein using Kjeldahl method, fat content using methods of Soxhlet, and crude fibre content was calculated from the difference of the analysis of the other components, using the formula:

\[
\text{Crude fiber (\%) = 100\% - (moisture content + protein content + ash content + fat content)}\%
\] [9].

2.5 Macromineral sediment
The measured macromineral parameters of the sediment were nitrogen (TN), phosphorus (TP), potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg). The measurement of nitrogen and phosphorus parameters used the spectrophotometric method, while the K, Ca, Na, and Mg parameters used the extraction method using the AAS (atomic absorption spectroscopy) [10].

2.6 Statistical Analysis
The mean difference composition sediment fraction was evaluated using one way ANOVA and Duncan’s test, the level of significance was p<0.05. A comparison of nutrient and macromineral composition of sediment on coarse and fine substrates was evaluated descriptively.

3. Results and discussion
3.1 Result
The grain size profile of the sediment in the digestive tract of sea cucumbers is shown as a composition of five fractions, then for flexibility in its application to engineering on sea cucumbers culture media, it is reduced to a composition of three fractions. The distribution of five-grain size fractions of sediment in the digestive tract of sea cucumbers consists of coarse sand (1-2 mm), medium sand (0.25-1 mm), fine sand (0.05-0.25 mm), silt (0.002-0.0 5 mm), and clay (<0.002) [11]. In this classification, there are three dominant grain sizes in all size classes of sea cucumbers, namely: clay, fine sand, and medium sand. Clay as the fraction that had the highest percentage was found in the digestive tract of sea cucumbers with wet weight sizes of 5-20 g and 200-220 g, namely: 29.98±4.61%, and 35.60±4.99%.

![Figure 1](image_url)

**Figure 1.** The proportion of five-grain size fractions in the digestive tract of various size classes and natural habitats (Lalowaru Waters) of sea cucumbers. Different letters above the colour of the data blocks show a significant difference (Duncan’s test, p<0.05).
Furthermore, fine sand is the sediment fraction that has the highest proportion of cucumbers sizes of 50-70 g, 100-120 g, and sediment in its natural habitat, i.e.: 35.81±2.41%, 33.93±1.54%, and 34.43±4.13%, respectively. The proportion of clay was not significantly different compared to the medium sand in the sediment of the digestive tract of 50-70 g sea cucumber and fine sand fraction in the sediment of natural habitats (p>0.05). Likewise, the proportion of fine sand was not significantly different than medium sand in the digestive tract of 5-20g and 100-120 g of sea cucumbers (p>0.05) (Figure. 1).

Based on the classification of three fractions, the distribution of coarse, medium, and fine sand of sediment fractions in the digestive tract and natural habitat of sea cucumbers shows the same pattern. The proportion of fine sand sediment fraction in the digestive tract of sea cucumbers was significantly higher than the other two fractions (Fig. 2). The 5-20 g of sea cucumber absorbs a significantly higher coarse sand fraction than the others size classes, the 100-120 g of sea cucumber absorbs a significantly higher amount of medium sand fraction, and the 200-220 g of sea cucumber absorbs a significant higher of fine sand than the sea cucumber another size classes.

![Figure 2. Distribution of coarse, medium, and fine sand of sediment fractions in the digestive tract of various wet weight sizes of sea cucumbers and natural habitat. Different letters above the colour of the data blocks show a significant difference (Duncan's test, p <0.05).](image)

Table 1. Nutrient composition and gross energy of sea cucumber natural habitat

| Nutritional Components | Proximate Composition (%) | (g / Kg) |
|------------------------|----------------------------|---------|
| Water content          | 26.89 ± 0.22               | 268.90 ± 2.16 |
| Ash content            | 69.63 ± 0.41               | 696.33 ± 4.13 |
| Protein                | 0.21 ± 0.01                | 2.07 ± 0.06 |
| Fat                    | 0.53 ± 0.06                | 5.30 ± 0.56 |
| Crude fibre            | 2.74 ± 0.28                | 27.40 ± 2.78 |
| GE (kcal/ 100 g)       | 17.41 ± 0.59               | 174.10 ± 5.90 |

Information: GE (Gross energy) 1 g protein = 5.6 kcal GE, 1 g fat = 9.4 kcal GE, 1 g crude fibre = 4.1 kcal GE (Watanabe, 1988).
The proportion of coarse sand fraction was found to be higher in the digestive tract of sea cucumbers, which always live in groups, can lengthen and shorten their bodies, causing them to suddenly disappear from the natural habitat of sea cucumbers. In this study, the proportion of sediment grain size predominately found in the gastrointestinal tract and the natural habitat of sea cucumbers can be used as a basis in determining the size of grains of sand that is suitable for sea cucumbers substrate. The use of sand as a substrate has a positive effect on the growth of sea cucumber [13]. Furthermore, Watanabe et al. [14], suggested that the feed for consumption of sea cucumbers be mixed into the sand. Meanwhile, Altamirano et al.[8], mortality occurred in sea cucumber juveniles after 2 weeks maintained in ponds with muddy substrate characteristics, compared to sea cucumbers on muddy sand and coarse sand that had a high survival rate. This shows the positive effect of the sand fraction on increasing the survival of sea cucumbers in natural habitats. Sand incorporated in formulated diets for H. scabra did not promote growth in the same way, so that the optimal grain sizes of the sand provided as a substrate need to be identified to maintain favourable substrate conditions for intensive sea cucumber culture [13]. The behaviour of sea cucumbers, which always live in groups, can lengthen and shorten their bodies, causing them to suddenly disappear from the culture vessel, out into public waters through torn nets. Incidents like this often occur when sea cucumbers are cultivated using pen culture method in open water, while in public waters, these animals are most hunted. Enlargement of the penculture method in open water which experiences crop failure has often occurred. 70% of juvenile sea cucumbers have died due to predation by Thalamita crenata crabs that enter culture by tearing nets [8]. The limited growth of natural food in the sediment is exacerbated by the difficulty of artificial feeding by the tidal currents of seawater so that in this condition it is difficult for farmers to maintain the culture of sandfish.

The results of this study indicated that the sediment in the digestive tract in four size classes of wild sea cucumbers (wet body weight: 5-20 g, 50-70 g, 100-120 g, and 200-220 g), found sand fractions with different proportions (Figures 1 and 2). Based on an analysis of five sediment fractions, the highest fraction in each size class of sea cucumbers were: 5-20 g namely clay fraction (29.98±4.61%), 50-70 g size: fine sand fraction (35.81±2.41%), the size of 100-120 g found two the highest fraction of medium and fine sand (33.93±1.54% and 32.78±0.99%), and the size of 200-220 g is the clay fraction (35, 60 ± 4.99%). This showed that there were three sizes of sediment grains, namely: clay (diameter: <0.002 mm), fine sand (diameter: 0.05-0.25 mm), and medium sand (0.25-1 mm diameter) as the dominant fraction in the gastrointestinal tract of sandfish. Although the proportions of the three fractions were found to be significant differences in several size classes of sea cucumbers, however, the difference in proportions was not always high (Figure 1). The silty fraction (diameter: 0.002-0.05 mm) was identified as the second-lowest proportion of the five group size fraction, where except for sea cucumber size class 5-20 g and 100-120 g, the value was significantly higher than the proportion of grain size of a coarse sand fraction (lowest proportion). The loss of sediment fraction due to the assimilation activity of organic matter by the body of sea cucumbers was not observed in this study, where the sediment was taken from all parts of the sea cucumber digestive tract.

The proportion of coarse sand fraction was found to be higher in the digestive tract of sea cucumbers compared to sediments in natural habitats, and smaller sea cucumbers absorb the grain size of coarse sand sediment with a higher amount than sea cucumbers with larger sizes. This is thought to
be related to the characteristics of the sediments in which it lives. When nutrients are available in limited quantities, sea cucumbers will filter the sediment that can be swallowed to get the nutrients they need. Statistically, the amount of coarse sand fraction found in the digestive tract of sea cucumbers of 5-20 g was significantly higher than the size classes others (p <0.05). According to Robinson et al. [13] stated that sand in feed does not function as a digestive aid. It still needs to be further investigated whether this was related to the level of organic matter assimilation by sea cucumber so that it accumulated the coarse fraction with a higher amount.

Large sea cucumbers seem to like the substrate layer which contains a lot of clay particle fractions. In natural habitats, juvenile sea cucumbers are generally found in areas that tend to be coarser, while consumption-sized sea cucumbers are generally found in locations where the substrate texture tends to be smoother and plenty of seagrasses. H. scabra mostly absorbs muddy particles with a particle size of 125-250 µm, this indicates that H. scabra prefers a muddy soil layer to a sandy bottom [15]. Furthermore, the composition of the sediment particle size in the intestines of sea cucumbers, namely: 0.09 mm of diameter fraction size: 9.07%, 0.125 mm fraction: 15.5%, 0.25 mm fraction: 42.1%, fraction of 0.5 mm: 8.66%, 0.7 mm fraction: 14.15%, 1 mm fraction: 6.16% and 1.68 mm diameter fraction size: 4.2%. The phenomenon of larger sea cucumbers which are mostly found on sandy mud or fine substrates and a significantly higher fraction of fine and medium sand was found in the digestive tract, presumably related to the higher availability of natural food on fine substrates. The number of bacteria in the finer sediment is higher than the sediment with a coarser texture [16, 17]. The results of this study also found that the substrate where larger sea cucumbers (>200 g) were dominant found, namely fine texture, had higher nitrogen and magnesium content compared to a coarse texture where smaller sea cucumber (<100 g) were dominant found (Tables 2).

Based on the three fraction classification, in general, that the medium sand fraction (diameter: 0.25-1 mm) and a fine sand fraction (diameter: <0.25 mm) were the dominant fractions found in the digestive tract of sea cucumbers, the same pattern was found in the composition of the sediment fraction of the natural habitat of sea cucumbers, which the proportion of medium sand was 17.34±2.05%, the proportion of fine sand was 79.40±2.58%, while the proportion of coarse sand was 3.26±0.59%. The results of this study were different from the identification of sediment fractions by Plotieau et al.[2], where the grain size fraction <0.5 mm is the dominant fraction in the substrate of sea cucumber culture. We found that the size fraction <1 mm was the dominant fraction in the digestive tract of sea cucumbers. The results of Baskar's research [15], although, he did not mention the size of sea cucumbers, however, the results of this study were more or less the same as what he reported, namely the proportion of the size fraction <1 mm was 95.84%. This study recommends the use of the dominant sand grain size according to sea cucumber size class as the main component in the preparation of artificial substrates and/or mixtures when giving artificial feed formulations to sea cucumbers. Through this utilization, the provision of additional nutrient sources and/or artificial feed in the substrate for sea cucumber cultivation is expected to be optimally absorbed by sea cucumbers. Also, it is hoped that this utilization can maintain the natural function of sand to maintain water quality and as a medium for growing natural food for sea cucumbers, in a semi-closed system.

4. Conclusion

Medium sand-clay as a dominant fraction found in the sediment of tract digestive and the natural habitat of sea cucumber ranges 86.83±1.22-96.74±0.60%. The highest fraction of sediment in the digestive tract of sea cucumbers H. scabra with 5-20 g of wet body weight is the clay fraction (29.98±4.61%), 50-70 g: fine sand (35.81±2.41%),100-120 g: medium and fine sand (33.93±1.54% and 32.78±0.99%), 200-220 g: the clay fraction (35.60±4.99%), and at the sediment of the natural habitat, fine sand and clay found as the two highest fractions, amounting to 34.43±4.13 and 32.90±4.29%. Meanwhile, protein, fat, and crude fibre content in the natural habitat sediment was low, so that nutrient enrichment and/or artificial feeding on the substrate is important, to provide for the growth of sea cucumbers if its culture efforts use a semiclosed system.
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