



Growth and survival rate of *Holothuria (Metriatyla) scabra* with feed from chicken waste in Menia Waters, Sabu Raijua District, East Nusa Tenggara, Indonesia

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Abstract. Fisheries resources are common property rights, so their management has open access, which means anyone can get involved. The fishery products obtained become uncertain and overfished, therefore to overcome the problem of overfishing the *Holothuria scabra* (sea cucumber) resources, this study experiments on pen culture techniques development. Cultivation of *H. scabra* in confined imprisonment, in addition to maintaining the sustainability of its resources, is also a new employment field for coastal communities that can provide added value in improving welfare. The objectives of the study were to determine: (a) the growth, (b) the survival rate, (c) the pattern of length versus weight increase, for the *H. scabra* found in the Menia waters. The method used is an experimental method of cultivation (pen culture). The results obtained from this study are: increase in the length with 1.5-6.35 cm and in the weight with 4-10.07 g weekly, for 60 days (8 weeks). The growth rate of *H. scabra* day⁻¹ for 30 days was between 0.15-0.93% and the average daily growth rate was 0.66±0.68%. Length relationship to weight (growth pattern) had an allometric coefficient $b > 3$. The body proximate analysis revealed contents of 68.59% protein, 4.09% fat and 4.85% carbohydrate. The survival rate of *H. scabra* was 100%. Average water quality parameters showed a temperature of 29-31°C, a salinity of 30-32 ppt, a pH of 7-8 and a DO of 4-5 mg L⁻¹, which are in the range of tolerance for *H. scabra* maintenance.

Key Words: allometric, protein content, fat, water content.

Introduction. Fisheries resources are common property rights, thus, the management is open access, which means anyone can manage it (Smith & Panayotou 1984). Therefore, there are often resource pressures that cause more capture (overfishing). The consequence is that the results obtained become uncertain (Elfidasari et al 2012). One solution to overcome this problem is the cultivation of species of sea cucumbers, such as sandfish (*Metriatyla scabra*) (Sukmiwati et al 2012). Their cultivation is accessible to the coastal communities, the technique being quite simple and the required inventory relatively small. In addition, market demand for these products is increasing from year to year, thus encouraging increased exploitation efforts (Darsono et al 1994). Conservation efforts and fulfillment of production needs must be carried out immediately through the cultivation of *Holothuria scabra*, so that the quality of *H. scabra* production needs are met and does not reduce the stock of *H. scabra* in nature (Hendri et al 2009).

The biological properties of typical *H. scabra* (a filter feeder) includes a living habitat with sand or mud with overgrown sea grass, relatively shallow waters and available food in the culture medium (Tuwo et al 2012). One of the important biological properties of *H. scabra* is that the body is elastic so that it is easy to straighten itself through very narrow gaps (Sugama et al 2019). In nature, *H. scabra* lives in sandy mud habitats located between seagrass and coral plants and abundant in organic material. Besides that, the existence of *H. scabra* is strongly influenced by available natural foods such as sea grass pieces, sand particles/coral fragments (Oedjoe & Eoh 2015). The growth and graduation of *H. scabra* life are very dependent on the feed is given. For this

reason, the present study was conducted to determine the growth and continuity of the life of *H. scabra* which were given artificial feed, in Menia waters, Sabu Raijua District, East Nusa Tenggara.

Material and Method

Research time and location. The present research has been carried out by the waters of Mania, Sabu Raijua District, in August-October 2015. The results of the study were analyzed in the laboratory of the Undana Faculty of Marine and Fisheries, and the Kupang State Polytechnic Biochemistry and Chemical Laboratory. The bottom of the water was sandy or muddy sand mixed with coral fragments and there were many aquatic plants such as seaweed or seagrass because many contain detritus as *H. scabra* food and are used as hiding places from predators.

Materials and tools. Based on the biological properties of *H. scabra*, suitable cultivation containers were installed (by fencing the cultivation area with 2 m brackets) around their native habitat. The following tools were used: confinement with a capacity of 50 m³ (5 x 5 x 2 m), bamboo, wood, net, nails, raffia rope or plastic rope, filter, digital oximeter, pH meter, hand refractometer, thermometer, digital scales, micrometers with an accuracy of 0.05, digital camera. The depth of each confinement was 0.5-1 m.

H. scabra specimens measuring 5-7 cm and weighing 40-60 g were stocked at a density of 6-8 individuals m⁻³ (300-400 specimens), while specimens heavier than 60 g (70-100 g) were stocked at a density of 4-6 tails m⁻³ (200-300 tails). The sample used came from the Menia waters, Sabu Raiju District. *H. scabra* was acclimatized for 1 day.

The type of feed given was chicken manure mixed with bran (1:1), it was given twice a day, namely at 6:00 a.m. and 5:00 p.m. The length and weight, the growth rate and the survival rate of *H. scabra* were measured on a weekly basis. To maintain the water quality of the research media, measurements of water quality parameters (temperature, DO, pH, salinity and brightness) were also carried out every week.

Research variable. The parameters observed in the study were the survival and growth (length and weight) rates and proximate analysis (protein, carbohydrate and fat contents) of *H. scabra* and the water quality parameters (DO, temperature, salinity and pH). The independent variables in this study are the *H. scabra* treatment parameters (the rectangular pen model, the food and the water quality), while the dependent variables are the survival rate, the growth rate and the length to weight allometric relationship of *H. scabra*.

Data analysis. *H. scabra* weight observations were used to calculate the growth rate by using the formula (Supriharyono 2000):

$$G = \left[\frac{W_n}{W_o} \right]^{1/n} - 1 \times 100\%$$

Where:

G - daily growth rate;

W_n - *H. scabra* weight on day n;

W_o - the initial weight of *H. scabra*;

n - age in days.

The survival rate (SR) was calculated by the formula (Supriharyono 2000):

$$SR = (N_t / N_o) \times 100$$

Where:

N_t - number of *H. scabra* still alive;

N_o - initial number of *H. scabra*.

The process of separating the *H. scabra* meat is shown in Figure 1.

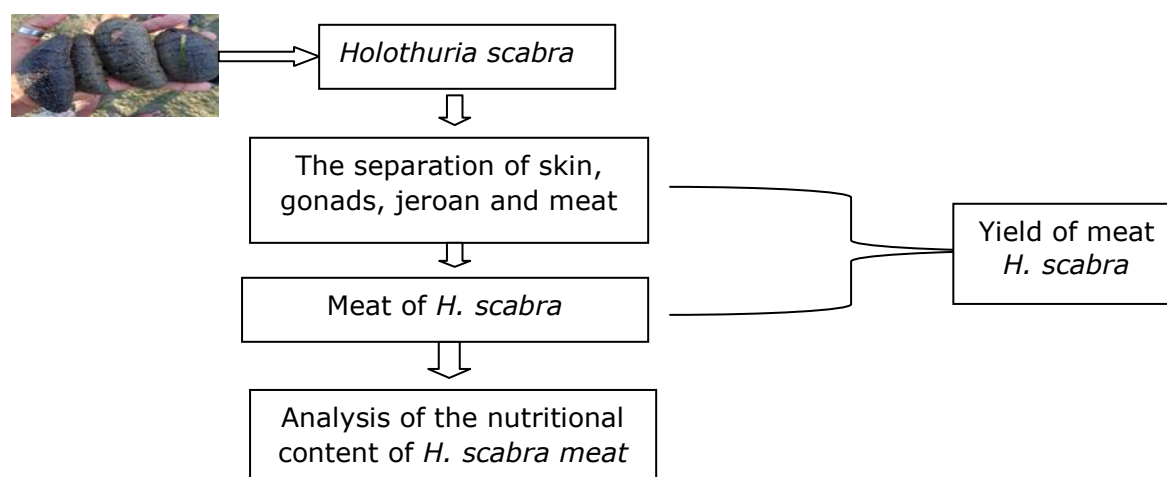


Figure 1. Flow diagram of *Holothuria scabra* meat yield.

Proximate analysis: fat, protein, water content of *H. scabra*. Protein concentrate was obtained by maceration, according to the Nurjanah's method (2008) with slight modifications. The maceration was performed by soaking *H. scabra* meat in a solvent for 24 hours, at refrigerator (temperature $\pm 4^{\circ}\text{C}$) in order to extract the protein concentrate. In the early stages, fresh *H. scabra* was cleaned and separated from unwanted parts, then cut and milled for size reduction. 100 g of *H. scabra* meat was placed in an Erlenmeyer flask, then soaked in acetone solvent at a ratio of 1:2 w/v and refrigerated for 24 hours. After the extraction completion, it was proceeded with the separation of the supernatant liquid phase from the precipitation residue using centrifugation (10,000 rpm for 15 minutes at 4°C). The precipitate obtained at this stage was then subjected to the freeze drying process. The observation of *H. scabra* protein concentrate produced (proximate analysis) included: total protein analysis, fat content, ash content, moisture content and carbohydrate. The concentrate that has been obtained was packed in plastic and aluminum foil and stored in a cool room at 4°C until further usage. The yield was calculated based on the percentage represented by the weight of *H. scabra* protein concentrate in the weight of the used fresh *H. scabra* meat, according to the formula (Karnila et al 2011):

$$\text{Yield} = \frac{\text{weight of } H. \text{ scabra protein concentrate produced}}{\text{weight of } H. \text{ scabra meat used}} \times 100\%$$

The measured chemical parameters (proximate analysis) included: total protein analysis using the Kjeldahl method, fat content with the Soxhlet method, ash and moisture contents (AOAC 2005), and carbohydrate (by difference).

Fat content was determined according to Ridhowati & Asnani (2015): *H. scabra* meat was chopped until a homogeneous state, than 100 g of sample placed in a blender were added with 200 mL of methanol and 100 mL of chloroform, and then mixed for 2 minutes. The protein content was calculated by the following equation (AOAC 2005):

$$\text{Protein content} = \frac{\text{mL NaOH blanko} - \text{mL NaOH sample}}{\text{sample weight (mg)}} \times \text{N NaOH} \times 14,008 \times 100\% \times \text{Fk}$$

Where:

Fk - conversion factor (100/16:6.25);

The conversion factor 6.25 was obtained from the average nitrogen in protein 16%.

Blanko - 50 mL distilled water and 40 mL 45% NaOH mix into the distillation flask;

N - normality NaOH.

Results. The three months observation results (August to October) showed a positive weekly evolution. The length increase every week for 60 days (8 weeks) was of 1.5 to 6.35 cm, starting from the initial average length of 6.50 cm, while the weight gain was of 4 to 10.07 g starting from the initial average weight of 70.56 g (Table 1).

Table 1 shows that the growth rate of *H. scabra* maintained in a rectangular confinement in Menia, Sabu Raijua waters was the highest at the fifth week ($2.73 \pm 0.1\%$), followed by the fourth week ($0.93 \pm 0.11\%$), sixth week (0.64%), third week ($0.43 \pm 0.01\%$), eighth week ($0.21 \pm 0.02\%$), second week ($0.18 \pm 0.02\%$) and first week ($0.15 \pm 0.01\%$). *H. scabra* specimens reached a weight of 200-250 g head⁻¹ at an average growth rate of $0.66 \pm 0.68\%$ day⁻¹. There was an increasing in the growth of *H. scabra* during the research period. This condition shows that the feed made from chicken waste is very suitable for the *H. scabra*. Since *H. scabra* consumes detritus which is rich in organic matter, chicken waste is good for them as it has detritus. As explained by Darman et al (2016), *H. scabra* are deposit feeders and suspension feeders. Meanwhile, Afrely et al (2015) stated that detritus is the main feed for this type of sea cucumber. Likewise, Komala (2015) the habitats of *H. scabra* are in areas that are rich in organic matter.

Table 1

Average weight and length of *Holothuria scabra* in Menia waters, Sabu Raijua District, for the 8 week experimental period

Week	Length (cm)	Weight (gr)	Growth rate (%)
0	6.50	70.56	-
1	7.01	75.19	0.15 ± 0.01
2	7.93	128.21	0.18 ± 0.02
3	10.01	141.13	0.43 ± 0.01
4	16.43	169.04	0.93 ± 0.11
5	20.67	240.17	2.73 ± 0.1
6	25.82	259.38	0.64 ± 0.1
7	31.54	262.09	0.09 ± 0.01
8	37.09	268.47	0.21 ± 0.02
Means	19.64	197.40	0.66 ± 0.68

Survival rate of *H. scabra*. *H. scabra* kept in a pen culture for 2 months (60 days) and fed with chicken manure and bran (1:1) has 100% survival rate, from the first week to the eighth week. This is due to the addition of chicken manure and bran as the main support for the successful cultivation of *H. scabra* (Table 1). Apart from that, it is supported by the clear, sloping and substrate conditions of Mania's waters with white sand and seagrass of the *Thalassia hemprichii* and *Enhalus acoroides* species, as described by Oedjoe & Eoh (2015). Thus, the natural and environmental conditions affect the development and survival rate of *H. scabra* (Padang 2015).

As stated by Padang et al (2017), the survival rate of *H. scabra* in rearing containers is influenced by the conditions of the aquatic environment such as sand, coral and seagrass substrates which usually have an abundance of detritus, diatoms. In addition, Aziz (1997) and Hendri et al (2009) explained that *H. scabra* is a sediment eater or suspense material eater, so its nutritional fulfillment is largely determined by the abundance of nutrients in the substrate.

The relationship between the length and the weight of the *H. scabra*. Length and weight of *H. scabra* in Mania waters were 13.07 cm and 126.84 g, respectively, with an allometric coefficient b of 3.01 and 3.14. The relationship of length and weight of *H. scabra* is shown in Table 2.

Table 2

The relationship between the lengths of the *Holothuria scabra*

<i>Parameter</i>	<i>Initial size</i>	<i>Final size</i>
Total length (cm)	6.5	19.64
Total weight (g)	70.56	197.40
Sx (long standard deviation)	0.072138	0.059667
Sy (severe standard deviation)	0.199158	0.15681
Correlation coefficient index (R^2)	0.938	0.934
Value b	b=3.14 b>3	b=3.01 b>3

Table 2 shows that the relationship between the length and the weight of *H. scabra* is in the positive allometric growth pattern ($b > 3$), which means that the length increase is not as fast as weight gain. The results are still in accordance with those reported by Mulfizar et al (2012), stating that animals which live in calm waters will produce high b values. This phenomenon may be caused by the slow-moving *H. scabra* behavior. Muchlisin et al (2010) stated that the size of b is also influenced by the behavior of aquatic animals, meaning that if aquatic animals are calm, the value of b is high when compared to aquatic animals that swim actively. This is related to the energy consumption for movement and growth (Bidawi et al 2012). While Manik (2009) and Mulfizar et al (2012) stated that in general the value of b depends on physiological and environmental conditions, such as temperature, pH, salinity, current velocity and sample measurement techniques. Ramadhani et al (2014) explained that the difference in b value not only occurs between different populations of the same species but also within the same population. Whereas De Robert & William (2008) stated that the value of b relates to the condition of aquatic animals and the condition of aquatic animals depends on food.

Yield of *H. scabra* meat. The yield is a parameter used to determine the economic value and effectiveness of a material. The *H. scabra* meat yield was calculated by comparing the percentage of *H. scabra* meat to the weight of whole *H. scabra*. It was taken at week 8 as many as 100 *H. scabra* with 5 times repetition (*H. scabra*/replication) (Table 3).

Table 3

Yield of *Holothuria scabra* meat

<i>Replication</i>	<i>Weight of H. scabra (g)</i>	<i>Weight of H. scabra meat (g)</i>	<i>Skin (%)</i>	<i>Innards (%)</i>	<i>Water and dirt (%)</i>	<i>Yield of H. scabra meat (g)</i>
1	11360	2680	14.36	4.07	57.84	23.73
2	11340	2670	14.48	4.09	57.88	23.55
3	11136	2568	14.36	4.07	58.51	23.06
4	11348	2674	14.53	4.09	57.82	23.56
5	11318	2659	14.33	4.15	58.03	23.49

Table 3 shows that the yield of *H. scabra* meat was 57.51%-58.51%. This yield is classified as low due to the high water content of sea cucumbers around 57.51%-58.51%. As stated by Fawzya et al (2016), the low yield of *H. scabra* meat is due to the high water content and meat that is left during the separation process. In addition, water and dirt reached 57.03-58.51% because *H. scabra* has the ability to eat by filtering water and eating sand or sediment particles and decaying food scraps as stated by Navaro et al (2013) and Oedjoe & Eoh (2015). Whereas, Karnila et al (2011) explained that *H. scabra*'s body consists of skin composed of chalk plates that are tightly joined to the body wall.

Nutrient content of *H. scabra*. Body analysis of *H. scabra* cultivated in the Mania waters, Sabu Raijua District revealed contents of: of 68.59% protein, 4.09% fat and 84.38% moisture (Table 4).

Table 4

Holothuria scabra nutrient content in the waters of Sabu Raijua

<i>Nutrient content</i>	<i>Percentage (%)</i>
Protein content	68.59
Fat content	4.09
Carbohydrate content	4.85
Water content (%)	84.38
Ash content (%)	12.25

Our findings concerning the *H. scabra* protein content of 68.59% are in accordance with Azam (2013), who reported a protein content in the range of 61-70%. Omran (2013) reported 43.43±0.2% protein for *H. scabra* from the Red Sea coast of Egypt, while Wen et al (2010) reported 39.8-60.2% (protein), 1.2-2.4% (fat), and 17.9-44.5% (ash). The protein content level of 68.59% in Mania Sabu Raijua waters is probably due to the clear and unpolluted waters. The values obtained are very close to the 76.94-90.81% reported by Chang-Lee et al (1989) and Chen (2003). Ridhowati et al (2015) also reported a *H. scabra* protein content of 82%. This is accordance with the conclusion of Nurjanah (2008), that fresh *H. scabra* contain high protein levels, especially in the meat. Furthermore, Karnila et al (2011) explained that proteins in the body are food reserves, stored in building and regulatory substances such as enzymes and antibodies (Bordbar et al 2011). Proteins in *H. scabra* have complete amino acids, both essential amino acids, and non-essential amino acids. Amino acids are very useful in protein synthesis process, in the muscle formation and in the formation of androgen hormones, namely testosterone, which plays a role in reproduction both to increase libido and spermatozoa formation (Karnila et al 2011). The composition of nutrients in *H. scabra* varies due to environmental factors, geographical location, season, place, area, type, weight, behavior and handling process (Salarzadeh et al 2012).

Discussion. Our findings revealed a fat content of 4.09% in fresh *H. scabra* meat, which is in accordance with Karnila et al (2011), who found 4.16% fat in fresh *H. scabra*, Azam et al (2013) found fat contents of 2-3%, while Haider et al (2015) stated that for some *H. scabra* fat levels was only 0.76-0.88%. This difference in fat content can be caused by many factors such as water conditions, climate, geographical location, behavior, handling procedures and food (Salarzadeh et al 2012). Nurjanah (2008) stated that *H. scabra* fresh meat contains considerable fat concentrations. The body of *H. scabra* consists of muscles and ossicles, which are places to store fat. Blood vessels are likely to spread fat throughout all parts of the body. The fat content of fresh *H. scabra* meat consists of saturated fatty acids and unsaturated fatty acids. Fredalina et al (1999) performed extraction of *H. scabra* fatty acids using PBS, which dominantly produced EPA (25.69%) and oleic acid (21.98%), while extraction using water resulted in DHA (57.88%) and linolenic acid (12.59%) content. According to Nurjanah (2008), *H. scabra* contain 0.119% linolenic acid and 0.128% arachidonic acid. The chemical content of *H. scabra* shows the superiority of its meat as a healthy food containing omega-3fatty acids (linolenic, EPA and DHA) and omega-6 fatty acids (linolenic and arachidonic).

The moisture content of *H. scabra* in the Mania waters, Sabu Raijua District is 84.38%, not much different from the research findings of Karnila et al (2011), namely 87.3%. This is presumably because the water habitat of *H. scabra*. According to Ayas et al (2011), the moisture content is an important component in food ingredients, because water can have an influence on appearance, text and taste.

The ash content level of meat *H. scabra* in Sabu Raijua waters was 12.62%. This result shows a higher value than the 11.53% obtained by Karnila et al (2011). The high level of ash is due to the microscopic chalk particles scattered in the epidermal layer of

the *H. scabra*, forming a cuticle which is a protective layer covered with chalk and thorns (Fechter 1969). Overall, from the results of the chemical (proximate) analysis of the *H. scabra* protein concentrate, it can be concluded that food based on *H. scabra* has a high nutritional content, which ultimately increases the added value of *H. scabra*.

Conclusions. The present research was successful in the development of pen culture experiments with *H. scabra* species. The results obtained from the 60 days (8 weeks) of observation are: a weekly length increase from 1.5 to 6.35 cm from the initial average length of 6.50 cm and a weight gain ranging from 4 to 10.07 g from of the average initial weight of 70.56 g. The daily growth rate of *H. scabra*, for 60 days, was 0.15-0.93% and the average daily growth rate was $0.66 \pm 0.68\%$. The length to weight relationship showed growth patterns of $b > 3$ (allometric coefficient). The proximate analysis revealed 68.59% protein, 4.09% fat and 4.85% carbohydrate contents. The survival rate of *H. scabra* was 100%. The average water quality parameters values were: a temperature of 29-31°C, a salinity of 30-32 ppt, a pH of 7-8 and a DO of 4-5 mgL⁻¹, which are in the range of tolerance for *H. scabra* maintenance.

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Conflict of interests. None reported.

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