Rate and Efficiency of Organic Carbon Assimilation by Aquacultured Juvenile Sandfish *Holothuria scabra*

(Kadar dan Kecekapan Asimilasi Karbon Organik oleh Ikan Pasir Juvenil *Holothuria scabra* Akuakultur)

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ABSTRACT

Diet assimilation rate is crucial to the growth and survival of *Holothuria scabra* juveniles during culture. To understand the assimilation rate and efficiency we assess organic carbon assimilation, which is closely related to the growth and source of variations in diet. We conducted a two-factor experiment, i.e., juvenile origin (cultured and wild *H. scabra* juveniles), and diet treatment (one control with no additional diet, and three additional diets, i.e., rice bran, dried cow manure, and seagrass *Enhalus acoroides* extract). We monitored the amounts of each diet that the juveniles consumed and the fecal pellets they egested. The diet, sediment, body walls, and organic carbon content of the fecal pellets were measured using an elemental analyzer combined with an isotope ratio mass spectrometer. Exponential growth was seen in the juveniles fed with rice bran, which had a fecal pellet egestion of 0.12–0.21 gC/d. Stable isotope analysis showed that the contribution of the diet proportion to the growth of the sandfish did not exceed 30%. The range of the assimilation rate was 35.3–62.4 gC/d. The average assimilation efficiency of organic carbon was 43.6 ± 27.7% (max 57.9%). Considering the assimilation rate and efficiency, we suggest a feeding interval of once every two days or twice per week at a rate of 3–5% of the total *H. scabra* biomass for a juvenile culture system.

Keywords: Assimilation; growth rate; *Holothuria scabra*; organic carbon; rearing culture sandfish

ABSTRAK

Kadar asimilasi diet adalah penting untuk pertumbuhan dan kemandirian juvenil *Holothuria scabra* semasa pengkulturan. Untuk memahami kadar asimilasi dan kecekapan, kami menilai asimilasi karbon organik, yang berkait rapat dengan pertumbuhan dan punca variasi dalam diet. Kami menjalankan uji kaji dua faktor, iaitu, asal juvenil (juvenil *H. scabra* yang dikultur dan liar) dan rawatan diet (satu kawalan tanpa diet tambahan dan tiga diet tambahan; dedak padi, baja tahi lembu dan ekstrak rumput laut *Enhalus acoroides*). Kami memantau jumlah setiap diet yang diambil oleh juvenil dan pelet najis yang dikeluarkan. Diet, sedimen, dinding badan serta kandungan karbon organik pelet najis diukur menggunakan penganalisis unsur yang digabungkan dengan spektrometer jisim nisbah isotop. Pertumbuhan eksponen dilihat pada juvenil yang diberi makan dedak padi, yang mempunyai penghadaman pelet najis 0.12–0.21 gC/d. Analisis isotop stabil menunjukkan bahawa sumbangan perkadaran diet kepada pertumbuhan ikan pasir tidak melebihi 30%. Julat kadar asimilasi ialah 35.3–62.4 gC/d. Purata kecekapan asimilasi karbon organik ialah 43.6 ± 27.7% (maks 57.9%). Memandangkan kadar asimilasi dan kecekapan, kami mencadangkan sela pemakanan sekali setiap dua hari atau dua kali seminggu pada kadar 3-5% daripada jumlah biojisim *H. scabra* untuk sistem kultur juvenil.

Kata kunci: Asimilasi; *Holothuria scabra*; kadar pertumbuhan; karbon organik; ternakan ikan pasir

INTRODUCTION

Holothuria scabra, commonly known as the sandfish, is one of the most expensive species of tropical sea cucumber traded on global markets (Purcell, Williamson & Ngaluafe 2018). This biota has a high potential to be developed as a functional food with a high nutritional value (Bordbar, Anwar & Saari 2011; Mohammadizadeh et al. 2013; Sroyraya et al. 2017). *H. scabra* aquaculture has been developed at various scales in several countries,

including Australia, Philippines, Vietnam, Madagascar, Fiji, and Indonesia to meet growing demand and address the problem of overfishing (Bowman 2012; Duy 2012; Eeckhaut et al. 2008; Hair et al. 2011; Juinio-Meñez et al. 2017; Lavitra, Rasolofonirina & Eeckhaut 2010; Olavides, Rodriguez & Juinio-Me˜nez 2011).

However, the techniques used to cultivate sandfish face many challenges and obstacles that can reduce the growth rate and quality of the end product (Purcell, Hair & Mills 2012). Various critical issues occur during the development of juveniles, including eating behavior (Altamirano, Recente & Rodriguez 2017; Domínguez-Godino et al. 2015; Purcell, Hair & Mills 2012), early juvenile attachment (Battaglene, Seymour & Ramofafia 1999; Domínguez-Godino et al. 2015), juvenile release (Juinio-Meñez et al. 2017; Purcell 2004; Taylor et al. 2016), transportation (Robinson & Pascal 2012), predation (Hair et al. 2016; Robinson & Pascal 2012), stocking density (Domínguez-Godino et al. 2015; Purcell, Hair & Mills 2012), growing techniques (Domínguez-Godino et al. 2015), biophysical properties (Hair et al. 2016), and low survival rates (Purcell, Hair & Mills 2012).

Previous studies have focused on feeding behavior and the right source of food for sea cucumber species. They have investigated, for example, the food sources of juvenile *Aposticophus japonicus* (Shi et al. 2015) and sand as a substrate and food component for *H. scabra* (Robinson et al. 2013). Domínguez-Godino et al. (2015) examined appropriate food sources for *H. arguinensis*, while Chen et al. (2018) and Song et al. (2017) considered multiple food sources for *A. japonicus*. However, to date, knowledge related to *H. scabra* eating behavior (Sun et al. 2012) and appropriate food sources, especially in the juvenile phase (Purcell, Hair & Mills 2012), remains limited. Moreover, survival rates in *H. scabra* culture could be improved by increasing the assimilation rate of food components (Purcell, Hair & Mills 2012), knowing feed source preferences, and meeting nutritional requirements (Duy, Francis & Southgate 2017).

Organic carbon assimilation analysis provides one possible approach to determining the rate of feed assimilation. In this study, such analysis was carried out through stable isotope analysis of carbon and nitrogen (δ^{13}C and δ^{15}N). Stable isotope analysis has been widely used as a natural tracer to determine the rate of flux of organic matter in food web systems (Fry & Sherr 1989; Peterson, Howarth & Garritt 1985), the trophic level of organisms (Sun et al. 2012; Wahyudi et al. 2013), to identify the source and level of assimilation of nutrients

and organic matter (Piola, Moore & Suthers 2006), and the material origin of environmental parameters (Wahyudi & Afdal 2019; Wahyudi, Afdal & Meirinawati 2019; Wahyudi et al. 2019). Once identified, procedures and evidence relating to an increased assimilation rate can be incorporated into *H. scabra* cultivation standards to increase efficiency in cultivation and rearing, especially during the crucial growth phase (juveniles in the weight range of 10-20 grams).

Organic carbon assimilation analysis using stable isotopes has been applied, for example, to determine the primary food source of *A. japonicus* (Yokoyama 2013) and to calculate the absorption efficiency of food nutrients in *A. japonicus* fed with three different food composition (Gao et al. 2011). Xu et al. (2017) also studied the food network structure, food sources, and feed types of *A. japonicus*. Indriana, Wahyudi and Kunzmann (2018) looked specifically at *H. scabra* and found that it has insufficient potential for organic carbon assimilation. These findings need to be comprehensively and systematically tested to determine the value and efficiency of assimilation.

This research aimed to determine the assimilation rate of organic matter at the juvenile stage of the tropical sea cucumber species *H. scabra*. The assimilation of organic matter was investigated primarily from the perspective of different feed sources and possible juvenile growth rates. The results of the present research are expected to improve cultivation techniques for sea cucumbers (the juvenile enlargement process) to ensure more effective and efficient feeding during the crucial juvenile phase. Stable isotope analysis methods can also be applied to similar research to determine potential dietary sources for juveniles in the attachment phase, which is a critical stage in sea cucumber growth.

Materials and Methods

experimental setting

H. scabra juveniles were collected from three locations in Lombok, West Nusa Tenggara, Indonesia. Wild juveniles were collected from Kayangan Bay (Kayangan, East Lombok) and Batu Kijuk Beach (Sekotong, West Lombok). In addition, cultured juveniles were collected from the Labuhan Treng pond (Lembar, West Lombok) (Figure 1). The juveniles ranged from 10 to 15 grams in wet weight.

The experiment was performed using a two-factor design with the source of the juveniles and their diets as the variable factors. We used the codes S, K, and T for

the juveniles from Sekotong, Kayangan, and the Labuhan Treng pond, respectively. The diet sources comprised three main food ingredients used by farmers in the cultivation of sea cucumbers, namely rice bran, dry cow manure, and seagrass extract. The use of dry cow manure is considered safe; furthermore, it is an additional growth booster commonly used by local farmers (Indriana et al. 2017). A control treatment (without feeding) was included in the trial set, with the codes X, Y, Z, and C used for rice bran, dry cow manure, seagrass extract, and the control, respectively. Combinations of SKT juveniles and XYZC treatments were applied to 12 experimental sets, as follows: SX, SY, SZ, SC, KX, KY, KZ, KC, TX, TY, TZ, and TC. For example, code SX was an experimental tank containing *H. scabra* juveniles from location S (Sekotong, West Lombok) treated with a rice bran diet (X).

The growth trial was carried out over 10 weeks (65 days). During this period, the *H. scabra* juveniles were kept in 85-liter plastic tanks (bottom sand area of around 0.5 m^2). At the bottom of each tank, an even layer of sterile fine sand (particle size < 1 mm), 5 cm thick, was spread as a substrate. Each tank was stocked with 20 *H. scabra* juveniles. In nature, the biomass density (total of all individual weights) for *H. scabra* is around 200 and 250 g/m^2 (Battaglene, Seymour & Ramofafia 1999; Purcell & Simutoga 2008). However, some experimental studies have reported that *H. scabra* biomass may extend to 400-700 g/m^2 (Altamirano, Recente & Rodriguez 2017; Watanabe, Sumbing & Lebata-Ramos 2014) during culture. Our culture set indicates a density of 200-300 g/m^2, which is within the suggested range. This condition ensures that no density-dependent effects will influence the treatments, and that growth (or shrinkage) can only be attributed to the food items given to the *H. scabra*.

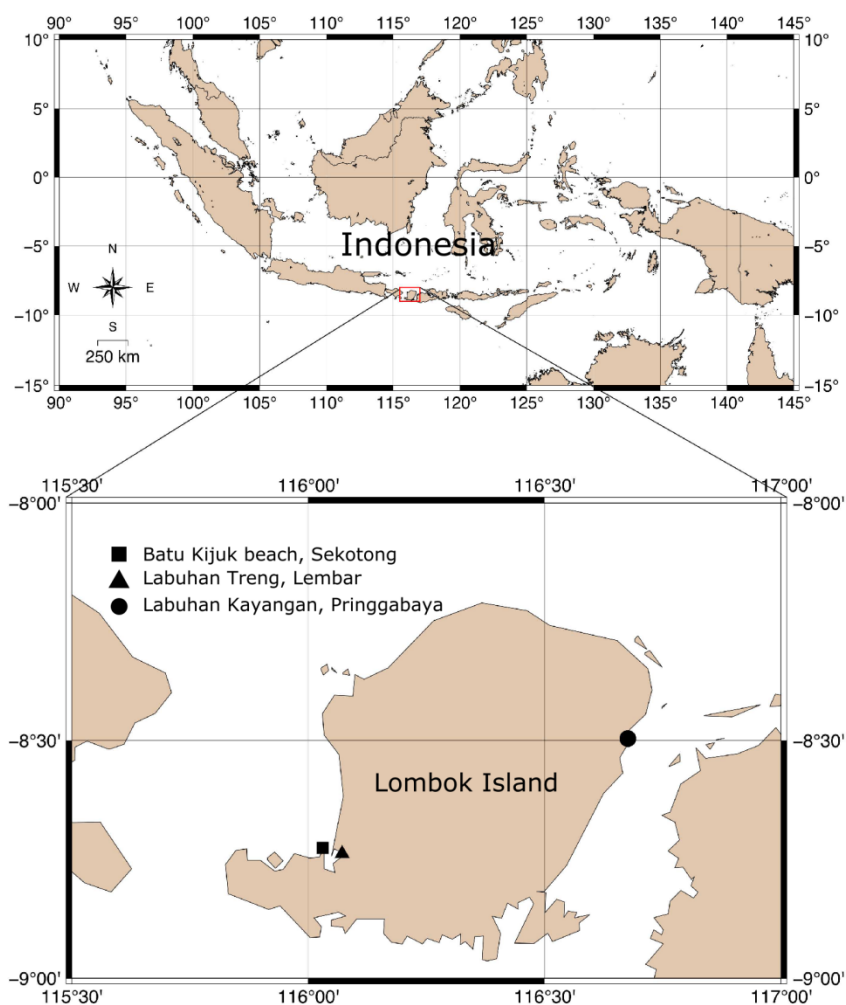Eight juveniles were chosen for weekly sub-

FIGURE 1. Juvenile collection sites on Lombok Island, i.e., Batu Kijuk beach, Sekotong (filled square), sandfish pond at Labuhan Treng, Lembar (filled triangle), and Labuhan Kayangan, Pringgabaya (filled circle)

sampling, and the remaining juveniles were reserved. The dietary source was given twice a week during the experimental period, at 10 grams (dry weight) per tank (equal to 20 grams wet weight or about 5% of the total biomass of the *H. scabra* juveniles in the tank). The total diet given was adjusted to the number of juveniles in each tank. Each day, 75% of the volume of the water in the tank was replaced with filtered seawater that had been sterilized with UV treatment. To meet the demand for dissolved oxygen, aeration was provided continuously with a blower backed up by a generator to prevent disruption to the air supply in the event of a power failure. The initial weight of each juvenile was measured before it was placed in the plastic tank.

SUB-SAMPLING AND MONITORING OF GROWTH

Concerning the sub-sampling for stable isotope and organic carbon analysis during the growth trial, *H. scabra* juveniles were sampled on days 0, 4, 11, 18, 25, 39, 53, and 65. The wet and dry weights of the *H. scabra* sub-sample were measured. Five replication samples (5n) were also taken for each type of diet treatment (rice bran, dry cow manure, and seagrass extract). A sample of fecal pellets was taken for stable isotope analysis and organic carbon analysis during the last week of each month during the growth trial. The pellets were sucked up through a hose connected to a bowl and then filtered and oven-dried for further analysis.

During the growth trial, the water quality was monitored by measuring the pH, salinity, temperature, nitrates, phosphates, and silicates. These measurements were taken twice a day, before and shortly after the water exchange. A portable water quality meter was used to measure the pH, temperature (Hanna instruments HI-9124), and salinity (Atago Master-S/Mill). Analysis of nitrate, phosphate, and silicate using a continuous flow analyzer (the San ++) was carried out as recommended in the device manual.

ORGANIC CARBON CONTENT AND STABLE ISOTOPE ANALYSIS

The oven-dried samples (i.e., diet sources, sea cucumber body walls, sediment, and fecal pellets) were packed into a tin capsule (Elemental Microanalysis D1104 10.5 × 9 mm) following the method used by Indriana, Wahyudi and Kunzmann (2018) and Wahyudi and Afdal (2019). The tissues sample were not delipidified following Indriana, Wahyudi and Kunzmann (2018) to ensure that the carbon assimilation will measure in total of the metabolic product. Further analysis was

conducted using an elemental analyzer/isotope ratio mass spectrometer (Thermo Delta plus XP coupled to a trace Gas Chromatography Ultra with a Combi PAL autosampler). The investigation was carried out at Iso-Trace Research, University of Otago, New Zealand. The diet sources and sea cucumber body walls were analyzed for both isotope ratio (δ^{13}C, δ^{15}N) and organic carbon content, while the sediment and fecal pellets were analyzed for organic carbon content and δ^{13}C only.

The stable isotope ratios of ^{13}C/^{12}C and ^{15}N/^{14}N are expressed as δ^{13}C and δ^{15}N. The isotopic ratios were normalized using the Vienna-Pee Dee Belemnite (VPDB) limestone standard and atmospheric nitrogen (N_2). The isotope ratio (R), given as the per-mill (‰) deviation from the standard value, was calculated using the following formula:

$$R = {}^{13}C/{}^{12}C \text{ or } R = {}^{15}N/{}^{14}N$$

$$\delta\ (‰) = ((R_{sample}/R_{standard}) - 1) \times 1000$$

The isotopic and elemental values were determined using laboratory reference materials (USGS-40 and USGS-41), and control materials (EDTA-OAS) were used to determine precision and accuracy. Analytical uncertainty for δ^{13}C and δ^{15}N is 0.16 and 0.06 ‰, respectively. Meanwhile, analytical uncertainty for % carbon and nitrogen are 0.45 and 0.03%, respectively.

We assumed the fractionation of the carbon isotope is 1 ‰ and the nitrogen isotope is 3.4 ‰ following Indriana, Wahyudi and Kunzmann (2018), Post (2002) and Wahyudi et al. (2013). We used a mixing model to determine the potential assimilation rate (see statistical analysis), with the following thresholds: 1) > 50% contribution of total diet sources to the body wall of *H. scabra* indicates a moderate to high assimilation rate; and 2) < 30% contribution of total diet sources to the fecal pellets of *H. scabra* indicates a moderate to high assimilation rate.

THE ASSUMPTION OF INTAKE-OUTPUT EQUALITY

Indriana, Wahyudi and Kunzmann (2018) found that *H. scabra* has a low potential assimilation rate and that diet sources should be homogenized and mixed with the sediment substrate. Similar research by Robinson et al. (2013) showed that sand substrate is part of the ingested diet of *H. scabra*. Evidence from stable isotope analysis (Indriana, Wahyudi & Kunzmann 2018) suggests that sea cucumbers ingest mixed substrate materials (i.e., sand) and diet sources and then absorb organic materials when the mixture reaches the intestine. As such, *H. scabra* has

a unique digestion mechanism: it ingests substances that contain organic materials from its diet sources, absorbs the organic materials, and then excretes the sediment as fecal pellets. During this process, all of the sediment ingested is returned to the environment/habitat. Given these mechanisms, we can assume that the dry weight of the ingested sediment is almost equal to the weight of the fecal pellets. Therefore, in this case, the intake ≈ the output. This assumption is used to calculate the assimilation rate and assimilation efficiency.

ASSIMILATION RATE AND EFFICIENCY

The assimilation rate is calculated using the following formula:

Assimilation rate (mg C/d) = [((s*a)+(o*a)-(f*a))]/t

where a is the dry weight of the ingested mixed sediment ≈ dry weight of excreted fecal pellets (in grams); o is the organic diet material (in mg C g^{-1}); s is the organic material from sediment substrate (in mg C g^{-1}); f is the organic material of fecal pellets (in mg C g^{-1}); and t is the time (in days).
Assimilation efficiency is calculated using the following formula:

Assimilation efficiency (in %) = [((s*a)+(o*a)-(f*a))/
((s*a)+(o*a))] * 100%

where a is the dry weight of ingested mixed sediment ≈ dry weight of excreted fecal pellets (in grams); o is the organic diet material consumed per day (in mg C $g^{-1}.d^{-1}$); s is the organic material from sediment substrate ingested per day (in mgC $g^{-1}.d^{-1}$); f is the organic material of fecal pellets excreted per day (in mg C $g^{-1}. d^{-1}$). This formula is derived from the conservative calculation method (Capone et al. 2008) where the assimilation efficiency (AE) = (Ingestion rate−Egestion rate)/Ingestion rate × 100%.

This calculation method differs from that used in previous studies, e.g., by Domínguez-Godino and González-Wangüemert (2019), and Nelson, MacDonald and Robinson (2012), which determined the absorption efficiency instead of the assimilation efficiency. Additionally, while the terms used are similar to Yu et al. (2014), the method used to calculate assimilation efficiency is different in this present study due to the different approaches to intake-output equality.

STATISTICAL ANALYSIS

We conducted Shaphiro-Wilk normality test for the data before conducting the parametric statistical analysis. The test shows p value>0.05; therefore, parametric tests can be conducted. We used a two-way ANOVA to determine whether the diet source or juvenile sample origin had any effect on the dependent variables (i.e., organic carbon assimilation rate and efficiency). Separately, we used a one-way ANOVA and Tukey contrasts to determine the differential effect of the juvenile origin or additional diet on the organic carbon assimilation rate and efficiency. The proportional contribution of the diet sources to the growth rate of *H. scabra* was determined using hierarchical Bayesian mixing model analysis with MixSIAR (Stock & Semmens 2016), following the method used by Wahyudi and Afdal (2019), but with a Markov chain Monte Carlo (MCMC) method using a 'very short' run to obtain Gelman diagnostics of < 1.05.

RESULTS

THE PROPORTIONAL CONTRIBUTION OF DIET SOURCES TO GROWTH RATE

During the rearing experiment, *H. scabra* growth showed an exponential trend (Figures 2 & 3). The increase in biomass indicates the use of the diet source for growth. While the rice bran treatment showed the highest growth rate compared to other diet treatments, generally, the provision of each additional dietary source led to an increase in growth (Figure 3). The *H. scabra* in the control treatment also showed growth but at a slower rate compared to the *H. scabra* with the diet treatments. We particularly noticed an average growth rate of 2.9 g WW/day (equal to 0.75 g DW/day) for the *H. scabra* juveniles with the rice bran diet. The exponential equation of the growth trend was used to estimate this value.

Moreover, the cumulative excretion rate of fecal pellets (Figure 4) illustrates the utilization of the diet source added to the rearing system (i.e., rice bran, cow manure, and seagrass detritus pulp). The excretion of fecal pellets thus indicates the feeding activities occurring within the rearing system. The *H. scabra* with the control treatment (i.e., with no diet added) showed the lowest fecal pellet egestion rate, i.e., a maximum of 2.6 g/day (equivalent to 0.07 g C/day). In contrast, an egestion rate of around 0.12-0.21 g C/d was seen in the tanks with an additional diet (rice bran, cow manure, and seagrass pulp). The *H. scabra* with the rice bran diet showed the highest fecal pellet egestion rate, followed by
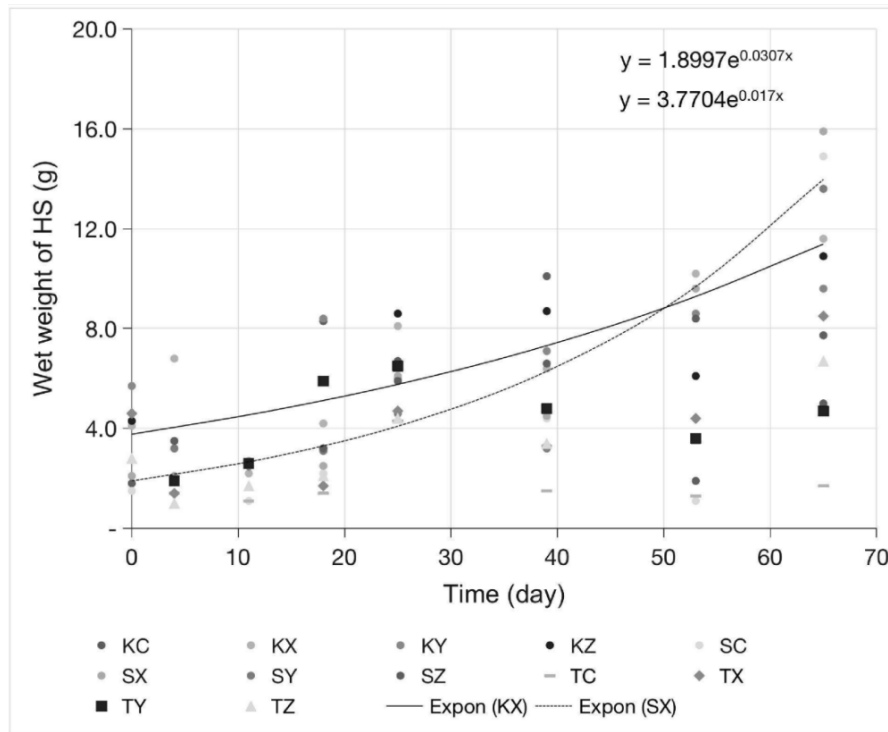
FIGURE 2. The growth rate of *H. scabra*, as shown by the wet weight of the juveniles. The codes S, K, and T are used for juveniles from Sekotong, Kayangan, and the Labuhan Treng pond, respectively. The codes X, Y, Z, and C are used for rice bran, cow manure, seagrass extract, and the control, respectively. We highlight the exponential growth rate of juveniles with the rice bran diet
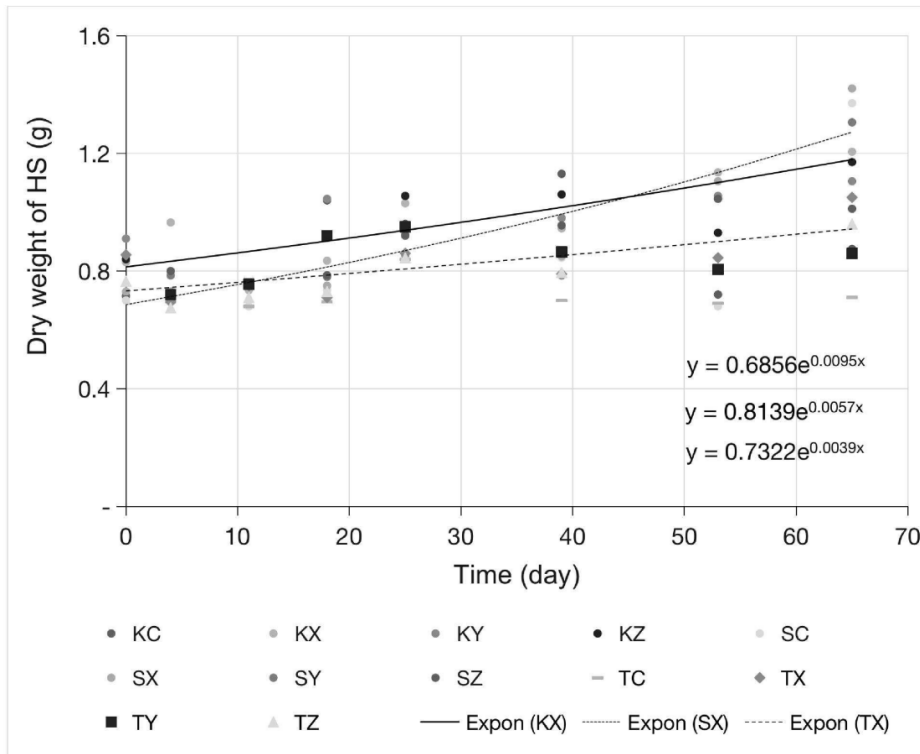

FIGURE 3. The growth rate of *H. scabra*, as shown by the dry weight of the juveniles. The conversion of wet weight to dry weight followed the process used by Skewes et al. (2004). The codes S, K, and T are used for juveniles from Sekotong, Kayangan, and Labuhan Treng pond, respectively. The codes X, Y, Z, and C are used for rice bran, dry cow manure, seagrass extract, and the control, respectively. We highlight the exponential growth rate of juveniles with the rice bran diet

the cow manure and seagrass pulp treatments. However, the origin of the juvenile samples seems likely to have had an insignificant impact on the treatments.

The bi-plot chart of stable isotopes indicates that the three diet sources, namely rice bran, seagrass pulp (*E. acoroides*), and dried cow manure, contributed to assimilation by *H. scabra* (Figure 5, left panel). We assumed the fractionation of the carbon isotope is 1 ‰ and the nitrogen isotope is 3.4 ‰. The chicken manure that was not used as a diet source does not show any

contribution, as indicated by its far higher position within the bi-plot than the *H. scabra* isotopic profile. According to the Bayesian mixing model analysis, the right panel of Figure 5 shows a double or triple peak of proportional contribution. Therefore, we calculated the mean values to obtain the proportional contribution of each diet to *H. scabra*. From this, average diet proportion contributions of 27, 28, and 30% were found for rice bran, seagrass, and cow manure, respectively (Figure 5).
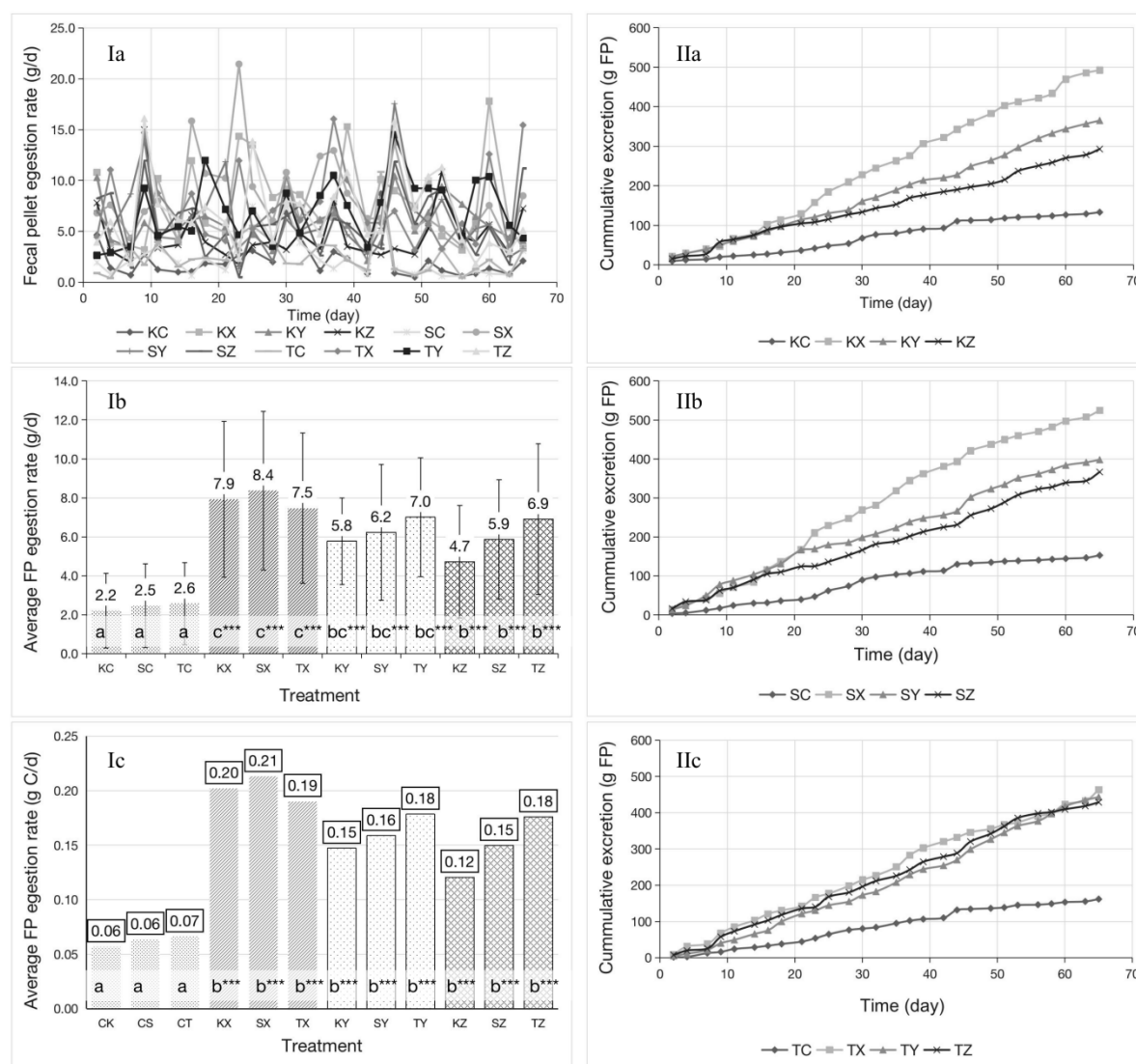
ORGANIC CARBON ASSIMILATION RATE AND

FIGURE 4. Fecal pellet (FP) egestion rate (column Ia) and the average FP egestion rate based on treatment in dry weight of fecal pellets (Column Ib) and organic carbon (Column Ic). The notations for the statistical analysis results are indicated at the bottom of bar charts Ib and Ic. Column II shows the cumulative egestion of fecal pellets for the three juvenile origins (Kayangan-IIa, Sekotong-IIb, and field pond-IIc, respectively). The codes S, K, and T are used for juveniles from Sekotong, Kayangan, and Labuhan Treng pond, respectively. The codes X, Y, Z, and C are used for rice bran, dry cow manure, seagrass extract, and the control, respectively
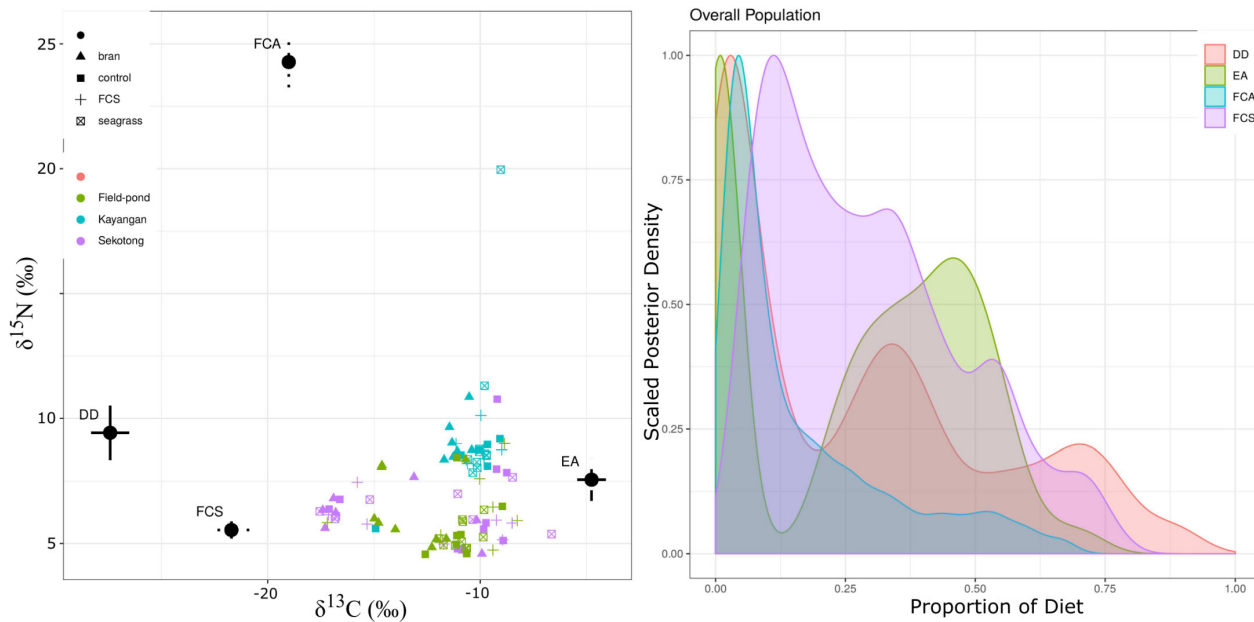
FIGURE 5. Stable isotope analysis: bi-plot of *H. scabra* isotopic value relative to the diet source (left panel); and proportion of diet fed to the *H. scabra* that shows potential organic matter assimilation (right panel). Codes: DD = rice bran; EA = seagrass *E. acoroides*; FCS = cow manure; and FCA = chicken manure (as additional data). $\delta^{13}C$ average value of juvenile from Sekotong < Kayangan = Field pond; $\delta^{15}N$ average value of juvenile from Field pond < Sekotong < Kayangan

EFFICIENCY

The organic carbon assimilation rates of the treatments are significantly different from the control. Furthermore, the juvenile origin or combination response of juvenile origin and diet treatment was found to have an insignificant effect on the organic carbon assimilation rate (two-way ANOVA; $p = 1.206E-12$; F value = 21.3515). The origin of the juveniles has no effect on the assimilation rate (one-way ANOVA; $p = 0.407$; F value = 0.902; Tukey Contrasts Cl95%). Meanwhile, the one-way ANOVA for treatment shows a significant difference among the variables ($p = 9.08E-13$; F value = 21.53; Tukey Contrasts Cl95%). The juveniles fed with rice bran (X) have a significantly higher assimilation rate compared to the juveniles fed with seagrass extract (Z), although both treatments are comparable to the juveniles fed with dry cow manure (Y); i.e., notated as X≥Y≥Z. The assimilation rates of the *H. scabra* with treatments are in the range of 35.31-62.40 gC/d, with an average of 49.6 gC/d (Figure 6).

Assimilation efficiency was found to respond significantly to the diet treatments compared to the control treatment, but not to juvenile origin (two-way ANOVA; $p = 2.03E-8$; F value = 13.9185). The *H. scabra* fed on an additional diet had a higher assimilation efficiency than the control without an additional diet. The juvenile origin was found to have no effect on the assimilation efficiency (one-way ANOVA; $p = 0.472$; F value = 0.754), while the diet treatment had a significant effect compared to the control (one-way ANOVA; p = 1.46E-8; F value = 14.15; Tukey contrast Cl95 %). The maximum assimilation efficiency of organic carbon by *H. scabra* juveniles was 57.9 %, with an average assimilation efficiency of 43.6 ± 27.7 % (Figure 7).

DISCUSSIONS
GROWTH RATE AND PROPORTIONAL CONTRIBUTION OF ADDITIONAL DIET

The organic matter and benthic microbiota available in the culture system are usually adequate to feed *H. scabra* (Ceccarelli, Logan & Purcell 2018); however, an additional diet is required in some cases (Robinson et al. 2013). There are two approaches to feeding *H. scabra*: the first is to provide an additional diet that can be directly consumed and digested (Ahmed et al. 2018); the second approach involves providing materials that can induce the growth of benthic microorganisms as natural feed sources (Sinsona & Juinio-Meñez 2018). Several materials are suitable as *H. scabra* feed, for example, shrimp feed (Mills et al. 2012), *Sargassum*
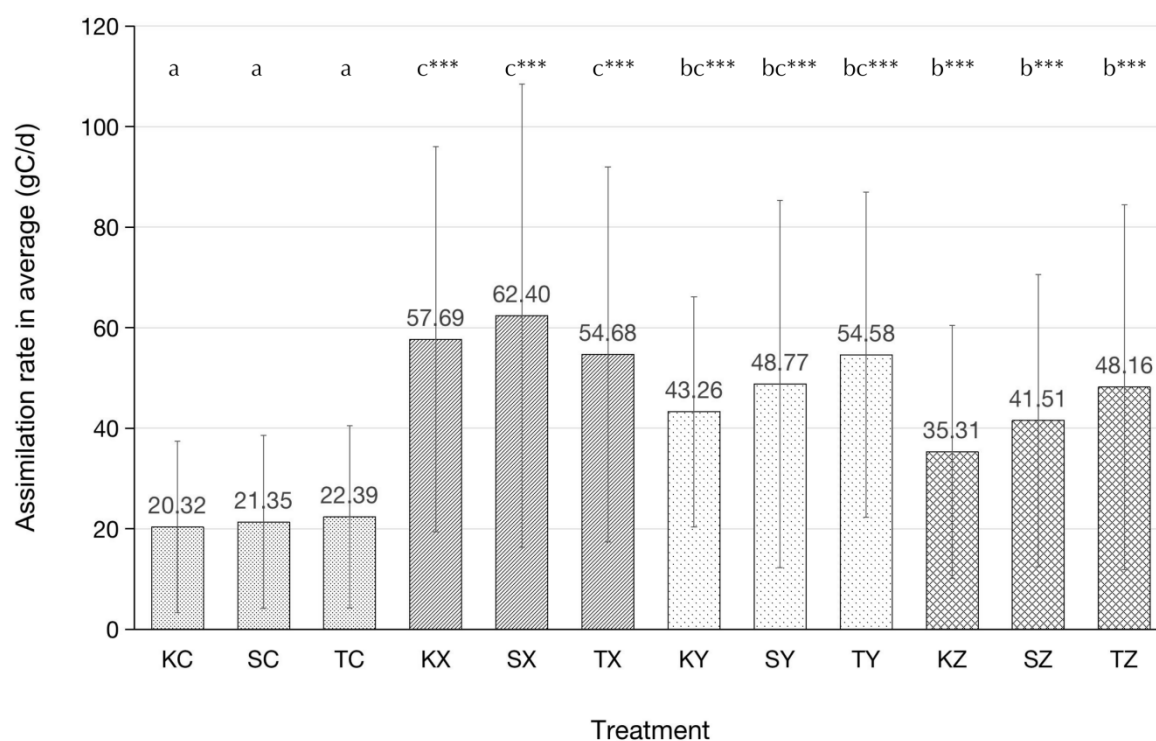
FIGURE 6. Organic carbon assimilation rate by *H. scabra* juveniles. The codes
S, K, and T are used for juveniles from Sekotong, Kayangan, and Labuhan
Treng pond, respectively. The codes X, Y, Z, and C are used for rice bran, dry
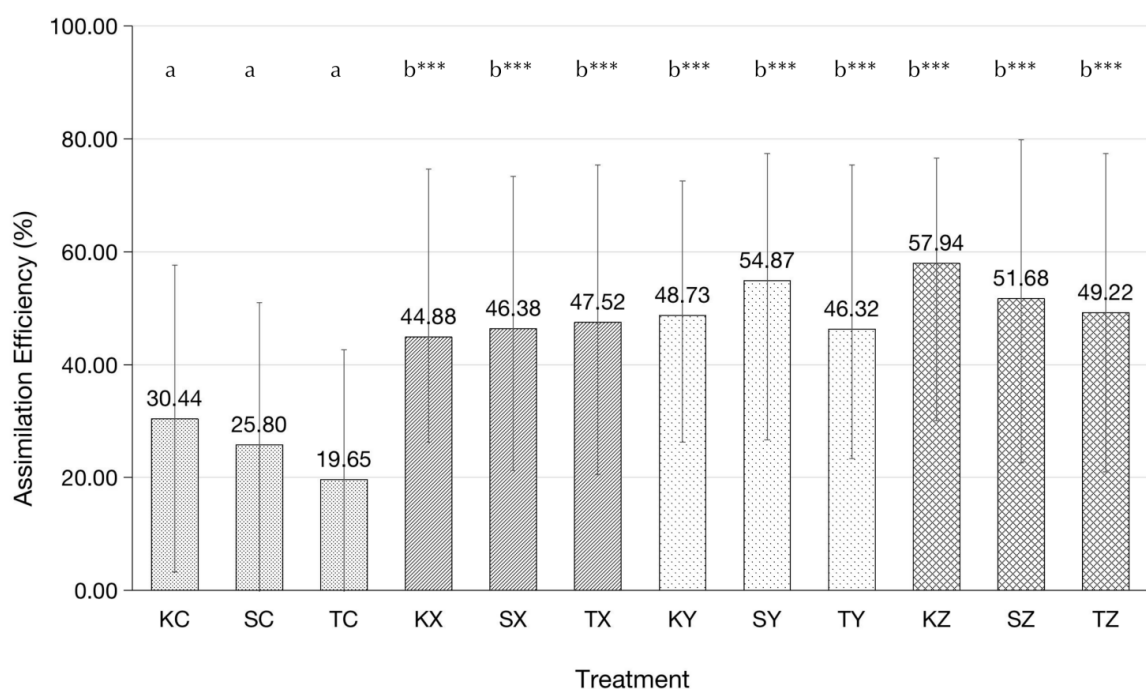cow manure, seagrass extract, and the control, respectively

FIGURE 7. Assimilation efficiency of organic carbon by *H. scabra* juveniles.
The codes S, K, and T are used for juveniles from Sekotong, Kayangan, and
Labuhan Treng pond, respectively. The codes X, Y, Z, and C are used for rice
bran, dry cow manure, seagrass extract, and the control, respectively. The
notations for the statistical analysis results are shown above the bar chart

(Liu et al. 2010; Sinsona and Juinio-Meñez 2018), benthos (Sembiring et al. 2017), aquacultural solid waste (Robinson et al. 2013), formulated feed (Ahmed et al. 2018), livestock manure, compost, tofu dregs, and rice bran. The amount of feed is usually around 3% of the total biomass of *H. scabra* in the tank or culture system, given every other day (Ahmed et al. 2018). Feeding can be undertaken by spreading the feedstuff directly on the substrate or mixing it evenly with the substrate (Indriana, Wahyudi & Kunzmann 2018; Sinsona & Juinio-Meñez 2018).

In the present experiment, the additional feed was given at 5% of the total biomass of *H. scabra* in the tank. While this was a greater amount than that suggested by Ahmed et al. (2018), we added it less frequently (twice per week). The diet amount and feeding strategy are likely to be essential in maintaining the water quality of the rearing system. Meirinawati et al. (2020) reported that temperature and salinity are the crucial physical parameters, while phosphate, nitrate, and ammonia are the essential chemical parameters. The addition of a diet will change the nutrient concentration (i.e., nitrate, silicate, and orthophosphate); however, physical factors such as temperature, salinity, and pH will continue to follow the environmental diurnal pattern (Meirinawati et al. 2020). It has been reported that the addition of cow manure will increase the silicate and orthophosphate concentration, though not significantly. The water quality dynamic is also likely to be affected by the fecal pellets excreted by the *H. scabra* population in the tank. Moreover, the organic carbon content of the egested fecal pellets will affect the C:N mol ratio of the rearing system (Panigrahi et al. 2019), although the C:N mol ratio was not measured in the present study.

By monitoring the increase in the wet weight of *H. scabra* and the cumulative egested fecal pellets, we can ensure that the additional diet increases the growth rate. Our results show the expected exponential growth rates for the three treatments. Indriana et al. (2017) reported that adding seagrass pulp or cow manure resulted in a growth rate of $0.15 \pm 0.03\%$ WW/day. It appears that the rice bran diet applied in our study led to a significant increase in growth rate (2.9 g WW/day). Considering that the juveniles had an initial average wet weight of 20 g, then a growth rate of up to 15% per day can be estimated. However, our estimation may be biased due to the dissimilarity of the juveniles' size and weight, along with the tank sub-sampling method used. It seems that the density of *H. scabra* also affects the growth rate. Namukose et al. (2016) showed that sea cucumbers cultured at low density had a mean growth rate of 0.80 g/day.

The stable isotope signature obtained shows the direct effect of the diets fed on *H. scabra* growth. The bi-plot results illustrate this pattern, even though the rice bran and seagrass pulp do not satisfy the criteria suggested by Post (2002) or Wahyudi et al. (2013) to be the carbon source of the *H. scabra*, i.e., 1.0 ‰ enrichment of carbon for the diet sources and 3.0 ‰ enrichment of nitrogen indicating the trophic level. The fact that these criteria of the stable isotope signatures are unmet demonstrates the low potential of organic carbon assimilation and also the low diet contribution. The bi-plot shows that the growth of *H. scabra* may not depend solely on the diet we added to the rearing system. Other organic matter sources may be used, obtained, for instance, from the benthic microorganisms present in the substrate.

The proportional contributions of the diets, as shown by the stable isotope signature (analyzed using the Bayesian mixing model), confirmed insignificant differences among the diet treatments of no more than 30%, thus indicating a low potential assimilation rate (contribution lower than the threshold of 50%). A similar result was reported by Indriana, Wahyudi and Kunzmann (2018) while Sinsona and Juinio-Meñez (2018) confirmed that as *H. scabra* digest a mixture of sediment substrate and added diet, the diet proportion will be lower than the total materials digested. This result indicates that 70% of the digested organic matter may be obtained from other sources within the rearing/culture system mixed with the substrate, e.g., decomposed organic materials, suspended particulate matter, and benthic microorganisms (Sinsona & Juinio-Meñez 2018). Mercier, Battaglene and Hamel (1999) reported that *H. scabra* could select sediment with high organic matter content, although they did not seem to have any specific diet preferences.

ASSIMILATION RATE AND EFFICIENCY FOR EVIDENCE-BASED FEEDING TREATMENTS IN REARING SYSTEMS

H. scabra can immerse themselves longer during the day when required to spend less time meeting nutritional needs. As such, *H. scabra* have better survival and growth prospects because they tend to bury themselves to avoid predators (Sinsona & Juinio-Meñez 2018). This behavior reflects the sufficient quality and availability of feed. However, additional feed must be incorporated carefully to prevent organic matter pollution. Overfeeding can raise the organic matter content above the tolerance limit and

may contribute to mortality (Mills et al. 2012) and the emergence of parasites (e.g., isopods and amphipods).

There are two possible routes by which dietary carbon content can enter the sea cucumber biomass. The first is via the diet or detritus ingested and digested by the sea cucumber. In this case, the digestibility of the diet is a determining factor in assessing the suitability of additional feed for sea cucumber aquaculture. Better digestibility will increase carbon assimilation because more carbon from the diet can be digested and deposited as sea cucumber biomass. Mathieu-Resuge et al. (2020) suggested that differential integration of carbon and nitrogen could result from the differential digestibility of carbon and nitrogen compounds in the dietary source. Adding rice bran, cow manure, or seagrass pulp to the diet can increase the assimilation rate and efficiency (Figures 6 & 7). Among these additional diets, no statistically significant difference was observed that affected the assimilation rate and efficiency. The significantly higher assimilation rate of the juveniles that consumed rice bran compared to those that consumed seagrass extract illustrates the dietary preferences of the *H. scabra* juveniles. This result was confirmed by the growth rate of the juveniles. However, cow manure and seagrass pulp seem to produce very similar assimilation efficiencies when fed to *H. scabra*. It is therefore likely that those diets have a comparable nutrient composition. Duarte (1990) reported that the median C:N:P ratio of seagrass leaves was 474:24:1, which represents a C:N ratio of almost 20. On the other hand, Wang et al. (2014) reported a C:N ratio of dairy manure of between 15 and 20.

Alternatively, carbon assimilation can take place via an indirect route. Carbon in the diet may also be assimilated by other organisms, such as benthic diatoms, bacteria, or benthic algae, which the sea cucumbers then consume. In this case, it should be ensured that the diet profile meets the requirement of those intermediary biotas. Sinsona and Juinio-Meñez (2018) demonstrated an example of this indirect diet utilization route by sea cucumbers. Their research showed that enriching sediment with *Sargassum* initiated the growth of microalgae in that sediment, which in turn increased the carbon sources and improved the growth and survival of juvenile *H. scabra*.

The low to medium assimilation rate of *H. scabra* will likely affect the whole culture system, i.e., through the enrichment of organic matter in the substrate. Namukose et al. (2016) demonstrated that a culture system with a density of *H. scabra* at 200 g/m^2 enhances seaweed growth (in integrated multi-trophic aquaculture) and can also reduce the organic matter content in the substrate. However, culture systems with lower or higher densities will lead to an increase in the organic matter content of the substrate. Our experiment used a density higher than 200 g/m^2, thereby creating the possibility that the substrate might contain increased organic matter. This condition is very likely to occur since our assessment showed a low assimilation rate.

The statistical analysis shows that juvenile origin does not have a significant effect on either the assimilation rate or efficiency. This result may have been affected by the similarity of the juvenile habitat origins on Lombok Island. One of the origins was a pond, which may also have a similar water and sediment profile. Another factor is that before conducting the experiment, the juveniles were acclimated to the conditions in the culture tank. As such, their feeding behavior will eventually change accordingly. While we may expect differences in the stable isotope profile of juveniles from different locations, this does not affect the assimilation rate and efficiency during the experiment periods.

Our results show an average assimilation efficiency for *H. scabra* of around 43.6 ± 27.7 %; thus, we may assume that only half of the additional diet will be used for their growth. This value is higher than the assimilation efficiency of *A. japonicus*, i.e., 13.78±5.83 - 15.13±7.75% (Yu et al. 2014). Comparing the absorption efficiency of *Cucumaria frondosa* (~70±3%; Nelson, MacDonald & Robinson 2012) and *Holothuria arguinensis* (~80%; Domínguez-Godino & González-Wangüemert 2019), it can be seen that the efficiency of *H. scabra* may be comparable, noting that assimilation efficiency is usually 3-6% lower than absorption efficiency (Drazen, Reisenbichler & Robison 2007).

Given that the total amount of added diet should equate to 3-5% of the total biomass of the *H. scabra* (Ahmed et al. 2018, and this study), and the average assimilation rate of 49.6 gC/d (assimilation efficiency <50%), we propose that *H. scabra* should not be fed too frequently. Meirinawati et al. (2020) highlighted that the addition of a diet booster will affect the water quality of the culture system in terms of the silicate, nitrate, and orthophosphate concentration; therefore, feeding too frequently will have a serious impact (e.g., nutrient over-enrichment). Our suggestion also confirms the findings of Ahmed et al. (2018) regarding feeding *H. scabra* every other day (i.e., an interval of once every two days). Alternatively, using a diet equal to 5% of the total *H. scabra* biomass, the feeding interval can be reduced to twice per week.

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