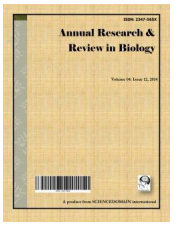

Annual Research & Review in Biology

28(2): 1-10, 2018; Article no.ARRB.42591
ISSN: 2347-565X, NLM ID: 101632869

Assimilation Dynamics of Different Diet Sources by the Sea Cucumber *Holothuria scabra*, with Evidence from Stable Isotope Signature

Lisa F. Indriana[1*], A'an J. Wahyudi[2] and Andreas Kunzmann[3]

[1]Research and Development Division for Marine Bio Industry, Indonesian Institute of Sciences LIPI, Lombok, Indonesia.
[2]Research Center for Oceanography, Indonesian Institute of Sciences LIPI, Jakarta, Indonesia.
[3]Leibniz Center for Tropical Marine Research (ZMT), Bremen, Germany.

Authors' contributions

This work was carried out in collaboration between all authors. Authors LFI and AK designed the study. Authors AJW and LFI performed the statistical analysis, wrote the methodology and wrote the first draft of the manuscript. Authors AJW and AK managed the analyses of the study. Authors LFI, AJW and AK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/42591
Editor(s):
(1) Dr. Paola Angelini, Department of Applied Biology, University of Perugia, Perugia, Italy.
(2) Dr. Eshrat Halim, University of Toronto, Canadian Diabetes Association, Canada and Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India.
(3) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
(4) Dr. Eugene A. Silow, Institute of Biology, Chair of invertebrate Zoology and Aquatic Ecology Sukhe-Baator str., 5, Irkutsk, Russia.
Reviewers:
(1) Mustafizur Rahman, Malaysia.
(2) Jiying Wang, Shandong Marine Resources and Environmental Institute, China.
(3) Jesiel Mamedes Silva, Federal University of Mato Grosso do Sul, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/25922

Short Research Article

Received 30th May 2018
Accepted 2nd August 2018
Published 17th August 2018

ABSTRACT

The sea cucumber *Holothuria scabra* has a high commercial value and a great potential to be cultivated. A thorough feeding strategy is needed to overcome juvenile rearing technique constraints. Stable isotope analysis can be used for determining diet sources of sea cucumbers that play a role as deposit feeders. This study aims to determine suitable diet sources and elucidate the potential of organic matter assimilation of *H. scabra* by combining three different mixed diets including 10% seagrass *Enhalus acoroides* bulk, 45% grass *Pennisetum purpureum* and 45% of cow feces (diet A); 20% seagrass *E. acoroides*, 40% grass *P. purpureum* and 40% of cow feces (diet B) and 33% seagrass *E. acoroides*, 33% grass *P. purpureum* and 33% cow feces (diet C) and

**Corresponding author: E-mail: lisaindriana23@gmail.com, lisa_indriana23@yahoo.com;*

identifying the fecal pellet isotopic properties and compare it to the diet sources and the surrounding sediment. Stable isotope signature of *H. scabra* and its prospected diet sources, altogether with sediments and fecal pellets were plotted in a conservative bi-plot $\delta^{13}C$ and $\delta^{15}N$. The isotopic value of fecal pellets and diet sources indicate a low assimilation rate, the organic matter contained in the sediment is similar to that of the diet sources. Sea cucumber feeds the bulk of the sediment and the detritus of the diet sources and then assimilates the organic matter as soon as the bulk enters the intestine. Due to the low assimilation rates, we suggest for the mariculture of *H. scabra* that the food should be homogenised and then mixed into the sediment, where the sea cucumber is cultured.

Keywords: Feed; seaweed; aquaculture; sediment; organic matter.

1. INTRODUCTION

Overfishing due to rising demand has led to the depletion of sea cucumber populations around the world [1,2,3,4]. Mariculture production of sea cucumber is one of the ways to solve the problem [1,4], moreover, it has the prospect to grow into an industry [5]. The sea cucumber *Holothuria scabra*, also called sandfish, has a high commercial value as preferred food in China, but also for medical and nutritive purposes and last not least as aphrodisiac [6,7,8] and consequently, lead to heavy exploitation [9]. Sandfish has a big potential to be cultivated in tropical regions [7,10], particularly in the Asia-Pacific area [1].

Hatchery techniques have various obstacles, which can decrease the benefit and reliability [5]. The issues which have constrained and hindered the development of culture methods in juvenile stadia were feeding behavior [5,11,12], settlement process of newly juveniles [11,13], release of juveniles [3,14], transportation [15], predation [15, 16], stocking density [5,11], rearing techniques [11], biophysical properties [16] and low survival rates [5].

Some studies have been conducted on the feeding strategy of sea cucumbers as part of overcoming the rearing technique constraints. Shi et al. [17] conducted a study about feed ingredients using mud, sand, diatoms and macroalgae powder for juvenile *Apostichopus japonicus*. The role of sand as a substrate and/or dietary component for *H. scabra* was investigated by [7]. Other studies were conducted especially for elucidating the diet requirements of *H. arguinensis* [11] and *A. japonicus* using macroalgae *Chaetomorpha linum*, seagrass *Zostera marina,* or biofloc supplement [18,19].

However, there is limited information about feeding behavior [20] and suitable diets, particularly in early juvenile stadia [5]. New studies are needed to increase growth and survival rates by improving the assimilation of feeding components [5], nutritional requirements and preferred diets [21].

Also for *H. scabra* juveniles feeding behaviour and diets ingredients are not well understood. Since it is difficult to conduct microscopic observations on the intestine content, a better approach is needed such as stable isotope analysis. This method can be used for determining diet sources of sea cucumbers that play a role as deposit feeders.

Stable isotope analysis is a useful method to trace the flow of organic matter in estuarine food webs and gives clues concerning its origins and transformations [22,23]. It is also a useful approach to determine the trophic relationships of aquatic ecosystems [20,24] and to track nutrient and organic matter source movement and assimilation [25]. Some studies using this kind of approach have been done with different aims such as the investigation the efficiency of co-culturing fish and *A. japonicus* with potential food sources [26], quantification of the absorption efficiency of *A. japonicus* with three different food ingredients [27] and also for the identification of the feeding type, food resources and food web structure of *A. japonicas cultured in* integrated multi trophic aquaculture (IMTA) systems [28].

This study aims to elucidate the potential of organic matter assimilation of *H. scabra, by* combining three different mixed diets (with focus on low cost ingredients) from terrestrial and marine resources. It is anticipated that we can conclude on effective diet ingredients by identifying the fecal pellet isotopic properties and compare it to the diet sources and the surrounding sediment.

2. MATERIALS AND METHODS

2.1 Sample Collection and Experimental Diets

Field samples of sea cucumber *Holothuria scabra* and corresponding natural sediments were collected from Sira, North Lombok, Indonesia. This location is a natural habitat of *H. scabra.* Body wall and intestine samples were taken after dissection then oven dried at 70°C. Dietary components such as seagrass *Enhalus acoroides,* napier *Pennisetum purpureum* and cow feces were collected from North Lombok, Indonesia, while *Laminaria digitata* was collected on Helgoland Island, Germany and dried in the laboratory of the Leibniz Center for Tropical Marine Research (ZMT) Bremen, Germany. Samples feces were then oven dried for 48 hours at 40°C.

Experimental samples were collected from a rearing system with 15 individuals of *H. scabra.* We used three so-called “happa” nets with 5 individuals each at the Klimahaus Bremerhaven, Germany for 5 days in a recirculation system. Three mixtures of diet formulas were used, namely 10% seagrass bulk, 45% grass and 45% of cow feces (diet A); 20% seagrass feed, 40% grass and 40% of cow feces (diet B) and 33% seagrass, 33% grass and 33% cow feces (diet C). Diets were added *ad libitum* every two days after a change of 50% sea water. Feces samples were collected through suction with a small hose every two days in each experiment.

2.2 Sample Preparation and Analysis

Feed, feces and sediment samples were fractionated and organic materials were homogenised at the chemical laboratory of ZMT. Sediment samples (fraction < 2mm) were homogenized by a planetary ball mill Retsch PM 100 for 50 minutes at 500 rpm. Organic material containing fibers was homogenized by using a Retsch ZM100 mill and centrifugation at 18,000 rpm with an 80 µm sieve. Carbon and nitrogen detection were conducted at the chemical laboratory ZMT using a Eurovector EA 3000 with a mass spectrometer.

The stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are expressed as $\delta^{13}C$ and $\delta^{15}N$, respectively. The isotopic ratios were normalised using Vienna-Pee Dee Belemnite (VPDB) limestone-standard and atmospheric nitrogen (N_2). Isotope ratio (R) calculation, given as per-million (‰) 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 (Low Organic Soil Standard OAS, Apfelblatt SRM 1515 and pepton). Stable isotopes were detected with a Delta plus coupled with Flash EA 1112 (Thermo Finnigan, USA), with a detection limit of 10 µgN, and a flow rate at EA of 100 ml/min.

2.3 Mixing Model Analysis

A hierarchal Bayesian mixing model analysis using MixSIAR [29] was used to determine the diet contribution to *H. scabra* and the fecal pellet. The analysis used 95% confidence intervals (95% CI), 'residual' error structure and 'uninformative/generalist' type. The mixing model was run using a 'long' run length of Markov chain Monte Carlo/MCMC [29]. In this study we used the seagrasses *Laminaria digitata*, *Thalassia hemprichii* and *Enhalus acoroides*, cow feces, a mixture of those ingredients (foodmix) and also the sediment as potential diet sources.

3. RESULTS AND DISCUSSION

Stable isotope signature of *Holothuria scabra* and its prospected diet sources, altogether with sediments and fecal pellets were plotted in a conservative bi-plot $\delta^{13}C$ and $\delta^{15}N$ (Fig. 1). Since there is no complete data set of the isotopic profile of an entire *H. scabra*, we used tissue samples from *H. scabra* intestine and body wall as proxy. In order to obtain a more comprehensive snapshot, also secondary data of stable isotope signature were included in the bi-plot. Stable isotope signature of *H. scabra* body wall and intestine (H.s.b.w and H.s.i.x) refer to [30]. Additional stable isotope signature of the prospected diets (*Enhalus acoroides*/ Ea2, *Thalassia hemprichi*/ Th and suspended particulate matter/ SPM) refer to [31].

Stable isotope signature of *H. scabra* body wall and intestine are considered to be low in this study compared to its diet sources, which does not meet with the conservative rule (common range) of the stable isotope analysis for food web and trophic level study. It is well known that enrichment of carbon and nitrogen stable

isotopes for consumers relative to their diet is 1 and 3.4‰ respectively [24,32,33]. Even the isotopic value of *H. scabra* obtained from secondary data [30] has lower $\delta^{15}N$ values compared to primary data of this study. Both [30] and this study use an acidification process for removing carbonate content from the samples that may affect the reduction of the nitrogen isotopic value [34]. Watanabe et al. [30] estimated that the mean $\delta^{15}N$ fractionation for the intestine (1.5‰) to be about half of the common range; meanwhile the mean $\delta^{13}C$ fractionation for the intestine (2.2‰) was about double the common range.

Although the isotopic value of the sea cucumber intestine is lower than expected, in fact lower than the offered diet sources and lower than the fecal pellets, we can still estimate the proportional contribution of the sources.

The stable isotope signatures of most sources (primary data, i.e. Laminaria, cow-feces, Ea1, food-mix) and the fecal pellets are very similar. This shows that sources such as *Laminaria*, cow-feces and foodmix are part of the fecal pellets and very likely have passed through the digestive tract, while sources such as *Enhalus acoroides, Thalassia hemprichii* and suspended particulate matter (SPM) are more distant. According to the bi-plot, we cannot discriminate the proportion of the incorporated sources. This is why we use the mixing model shown in the Figs 2-4.

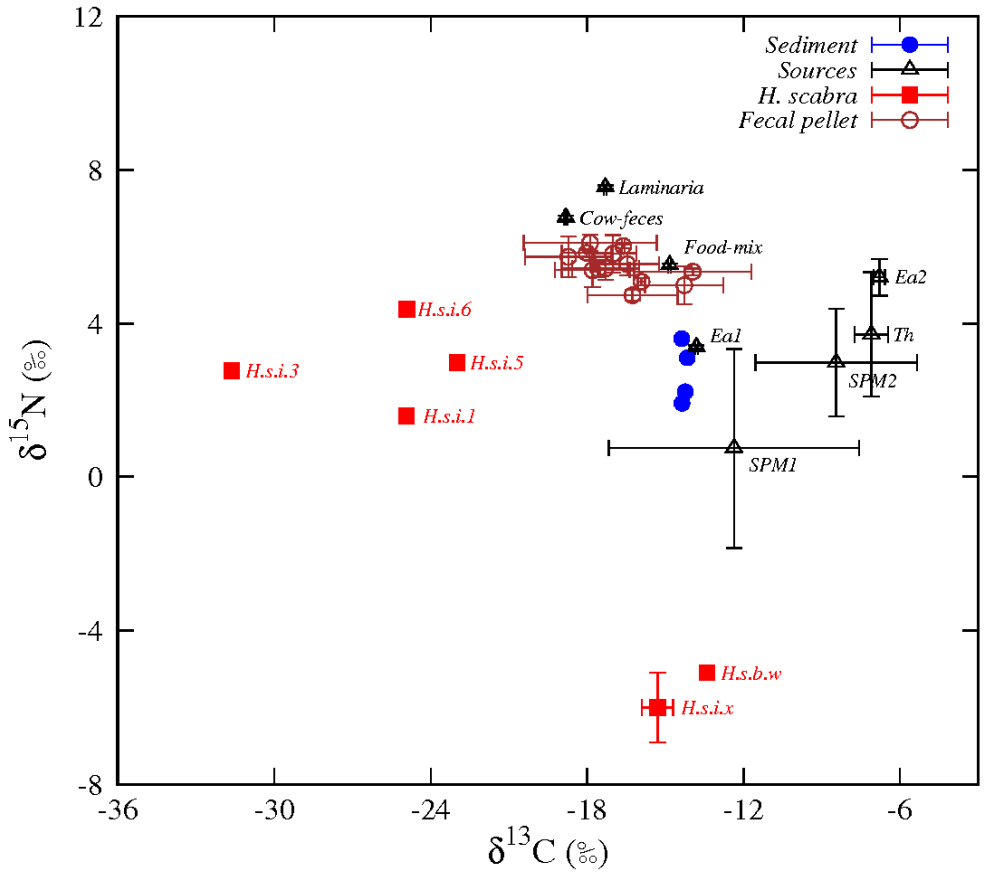

Fig. 1. Bi-plot of diet sources (*Enhalus acoroides*/ Ea1, cow feces, food-mix) relative to the *Holothuria scabra* (H.s.), sediment and fecal pellets. *Holothuria scabra* intestine (H.s.i.1, -3, -5, -6) are primary data (numbers 1, 3, 5 and 6 refer to different sampling locations). *Holothuria scabra* body wall (H.s.b.w), and H.s.i.x are secondary data following (30). While, *Enhalus acoroides* (Ea2), *Thalassia hemprichii* (Th) and suspended particulate matter (SPM) following [31]

The similar isotopic value of fecal pellets and diet sources also shows that no distinct fractionation occurs, indicating a low assimilation rate. Since also the sediment isotopic value is similar to that of the diet sources, it can be concluded that the organic matter contained in the sediment is similar to that of the diet sources. Therefore it is likely that detritus, as a result of processed diet sources, has a significant contribution to the sediment composition. These results confirm the previous study by [30] that indicates similar low assimilation patterns by comparing the isotopic value of *H. scabra*'s fecal pellet and its potential diet source (*Navicula ramossisima*).

Very similar diet proportions of 9-15% were identified, when fecal pellet and *H. scabra* intestine and body wall are considered together (Fig. 2). When only *H. scabra* samples are considered, the sediment alone (sediment I, III, V and VI, altogether) contribute up to 30-40% (Fig. 3), almost equal with other diet sources. When only fecal pellets are considered, the sediment has a higher proportional contribution than other sources (i.e. more than 60%) (Fig. 4). Looking only at the non-sediment sources, which are all very similar to each other, suggests that the sea cucumbers do not have a real preference of the diet. The preference of the diet source can be determined if the diet proportion reaches minimum 30% [24]. These criteria do not meet with the present study. Thus we expect that *H. scabra* may have different diet preference patterns.

Figs 3 and 4 show a shift in the proportional contribution of the sediment (i.e. a higher sediment contribution in the fecal pellets) as compared to other diet sources. This might indicate that in combination with a low assimilation rate, the sea cucumber consumes all of the diet sources altogether with the sediment as medium. However, only organic material is assimilated, while the sediment is defecated again. This is well in line with behavioral studies which classify *H. scabra* as a deposit feeder, burrowing in the sediment [35]. While it is known that *H. scabra* extracts the diet from the sediment [36], our study adds the fact that the sea cucumber feeds the bulk of sediment and the detritus of the diet sources, and then assimilates the organic matter as soon as the bulk enters the intestine. [36] reported that for farming sea cucumbers, sea mud should be added to the feeding formulation.

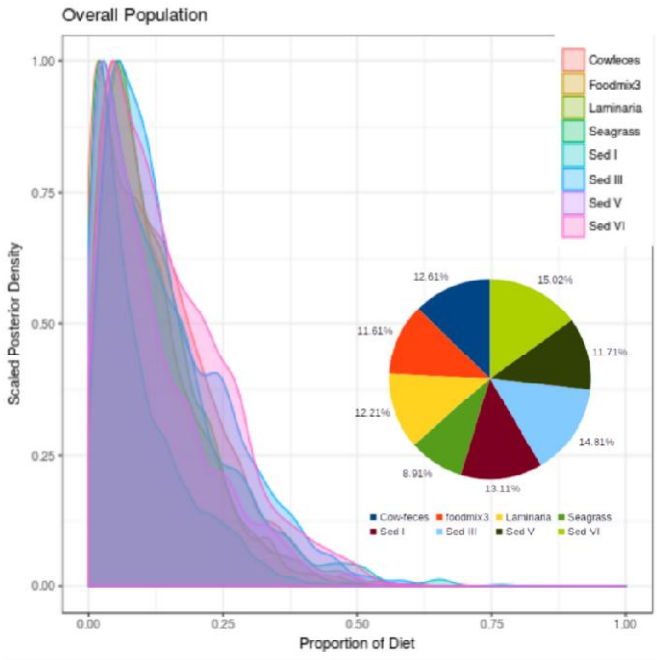

Fig. 2. Proportion of diet sources, when fecal pellets and *Holothuria scabra* intestine/body wall are considered altogether as dependent variable. Number I, III, V and VI of the sediment refer to different sampling sites similar to those where the sea cucumbers were obtained

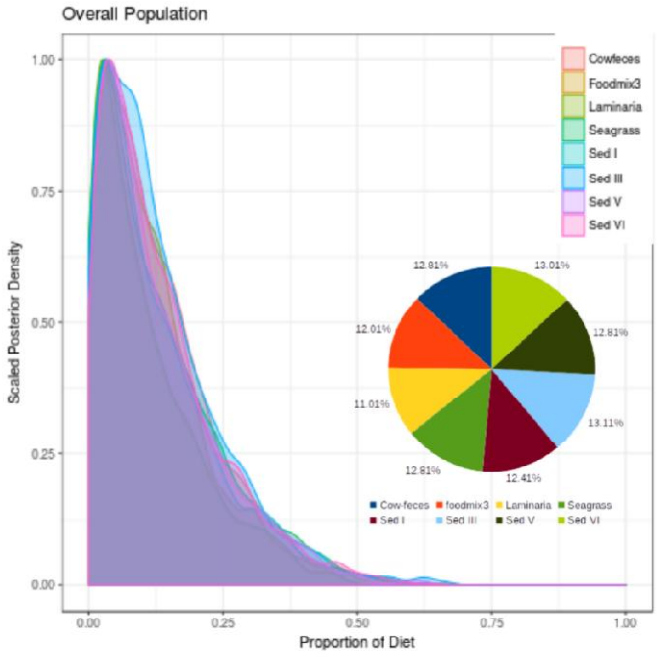

Fig. 3. Proportion of diet sources, when only *Holothuria scabra* intestine/body wall is considered

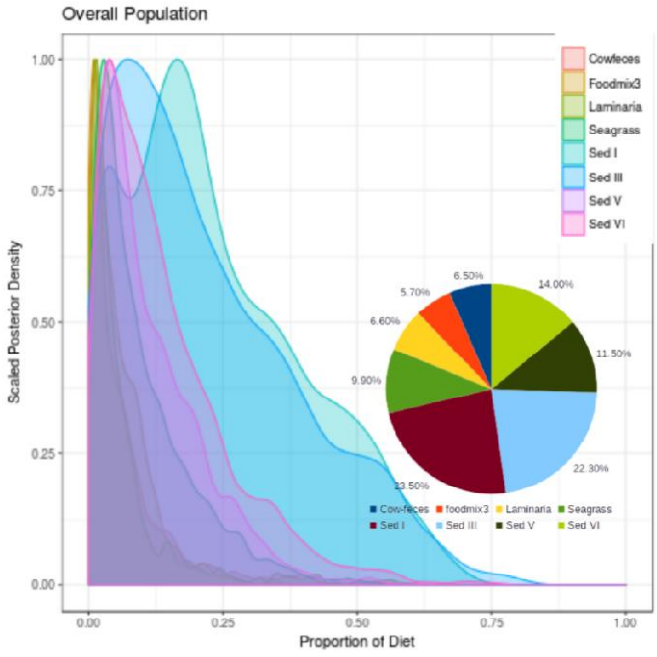

Fig. 4. Proportions of diet sources, when only fecal pellets are considered

Evidence of the low assimilation rate by *H. scabra* is also provided by analyzing the C/N ratio of the tissue compared to diet source, sediment and fecal pellet. The diet sources and fecal pellets have a similar distribution of C/N mol ratio (Fig 5), indicating that there are almost no chemical changes .during the digestion process. Some samples of the body wall of *H. scabra* and

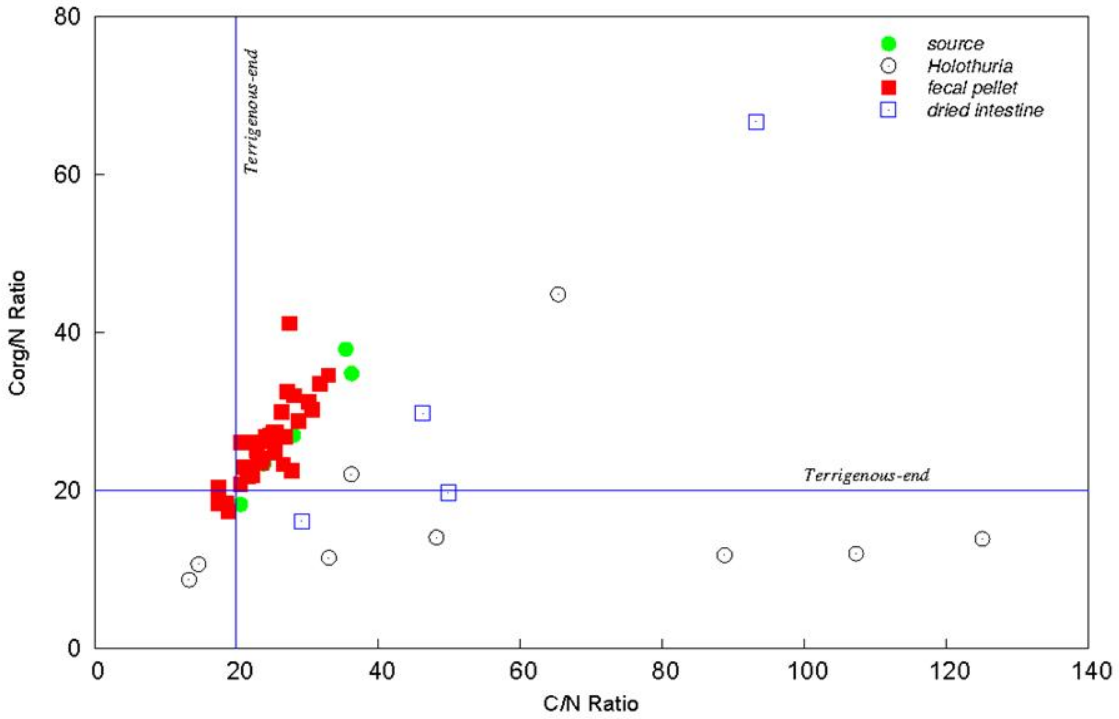

Fig. 5. Trend of C/N ratios of various diet sources, *Holothuria scabra* intestine and body *wall,* sediment and fecal pellets. A C/N ratio of more than 20 is considered to point at terrigenous-end members (terrestrial sources) according to [37]

the intestine show a C/N ratio value more than 80, indicating high concentrations of carbon. We suspect the carbonate (e.g. contributed by spicules may affect the carbon concentration, as also proposed by [30]. The C/N mol ratio of intestine and body wall of *H. scabra* tend to vary (Fig. 5), which confirms the wide range of the C/N ratio. Fig. 5 shows that C/N values of all diet sources, fecal pellets, sediments and *H. scabra* are more than 20, which indicates terrestrial sources/ terrigenous-end members [37]. This finding demonstrates a significant terrestrial material input to the *H. scabra* daily life.

Due to the low assimilation rates, we suggest for the mariculture of *H. scabra* that the food should be homogenized and then mixed into the sediment, where the sea cucumber is cultured. All of the low cost diet sources may be possible, since there are apparently no clear diet preferences. Most other sea cucumber species however, seem to be able to select diet with high nutrition from the environment [38].

4. CONCLUSIONS

The isotopic value of fecal pellets and diet sources indicates a low assimilation rate, the organic matter contained in the sediment is similar to that of the diet sources. Sea cucumbers ingest the bulk of the sediment and the detritus of the diet sources, and then assimilate the organic matter as soon as the bulk enters the intestine. Due to the low assimilation rates, we suggest for the mariculture of *H. scabra* that the food should be homogenized and then mixed into the sediment, where the sea cucumber is cultured. While we were not able to identify an optimum diet composition, due to a low assimilation rate, and due to analyzing only stable isotope signatures, we suggest additional experiments, also with other diet sources in order to optimize feed composition.

ETHICAL APPROVAL

Written ethical approval has been collected and preserved by the authors as the institutional standard.

ACKNOWLEDGEMENTS

This project was partly supported by a grant from the Centre for Science and Technology of the

Non-Aligned and Other Developing Countries (NAM S&T Centre) and the Leibniz Centre for Tropical Marine Research (ZMT), Bremen, Germany. We would like to thank technical staff at ZMT in Bremen and LIPI on Lombok for their support during experiments and Lutz Fischer at the Klimahaus Bremerhaven for the sea cucumber rearing facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Toral-Granda V, Lovatelli A, Vasconcellos M. editors. Sea cucumbers: A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper. No. 516. Rome, FAO. 2008;317.
2. Kinch J, Purcell S, Uthicke S, Friedman K. Population status, fisheries and trade of sea cucumbers in the Western Central Pacific. In Toral-Granda V, Lovatelli A, Vasconcellos M, editors. Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper. No. 516. Rome, FAO. 2008;7–55.
3. Juinio-Menez MA, Tech ED, Ticao IP, Gorospe JRC, Edullantes CMA, Rioja RAV. Adaptive and integrated culture production systems for the tropical sea cucumber *Holothuria scabra*. Fisheries Research. 2017;186:502–513.
4. Gonzalez-Wangüemert M, Dominguez-Godino JA, Canovas F. The fast development of sea cucumber fisheries in the Mediterranean and NE Atlantic waters: From a new marine resource to its over-exploitation. Ocean & Coastal Management. 2018;151:165-177. Available:https://doi.org/10.1016j.ocecoaman.2017.10.002
5. Purcell SW, Hair CA, Mills DJ. Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. Aquaculture. 2012;368–369:68–81.
6. Ochiewo J, Torre-Castro M, Muthama C, Munyi F, Nthuta JM. Socio-economic features of sea cucumber fisheries in southern coast of Kenya. Ocean & Coastal Management. 2010;53:192–202.
7. Robinson G, Slater MJ, Jones CLW, Stead SM. Role of sand as substrate and dietary component for juvenile sea cucumber *Holothuria scabra*. Aquaculture. 2013;392–395:23–25.
8. Ram R, Chand RV, Zeng C, Southgate PC. Recovery rates for eight commercial sea cucumber species from the Fiji Islands. Regional Studies in Marine Science. 2016;8:59–64.
9. Hamel JF, Conand C, Pawson DL, Mercier, A. The sea cucumber *Holothuria scabra* (*Holothuroidea*: *Echinodermata*): Its biology and exploitation as Beche-de-mer. Advances in Marine Biology. 2001;41:129-223.
10. Hair CA, Ram R, Southgate PC. Is there a difference between bêche-de- mer processed from ocean-cultured and wild-harvested sandfish (*Holothuria scabra*)? Aquaculture. 2018; 483:63–68.
11. Domínguez-Godino JA, Slater MJ, Hannon C, González-Wangüermert M. A new species for sea cucumber ranching and aquaculture: Breeding and rearing of *Holothuria arguinensis*. Aquaculture. 2015; 438:122–128.
12. Altamirano JP, Recente, CP, Rodriguez Jr JC. Substrate preference for burying and feeding of sandfish *Holothuria scabra* juveniles. Fisheries Research. 2017;186: 514–523.
13. Battaglene SC, Seymour JE, Ramofafia C. Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra*. Aquaculture. 1999;178:293–322.
14. Taylor AL, Nowland SJ, Hearnden MN, Hair CA, Fleming AE. Sea ranching release techniques for cultured sea cucumber *Holothuria scabra* (*Echinodermata*: *Holothuroidea*) juveniles within the high-energy marine environments of northern Australia. Aquaculture. 2016;465:109–116.
15. Robinson G, Pascal B. Sea cucumber farming experiences in south-west Madagascar. In: Hair CA, Pickering TD, Mills DJ, editors. Asia-Pacific Tropical Sea Cucumber Aquaculture, Australian Centre for International Agricultural Research. Canberra; 2012.
16. Hair C, Mills DJ, McIntyre R, Southgate PC. Optimising methods for community-based sea cucumber ranching: Experimental releases of cultured juvenile *Holothuria scabra* into seagrass meadows in Papua New Guinea. Aquaculture Reports. 2016;3:198–208.

17. Shi C, Dong S, Wang F, Gao Q, Tian, X. Effects of the sizes of mud or sand particles in feed on growth and energy budgets of young sea cucumber (*Apostichopus japonicus*). Aquaculture. 2015;440: 6–11.
18. Song X, Xu Q, Zhou Y, Lin C, Yang H. Growth, feed utilization and energy budgets of the sea cucumber *Apostichopus japonicus* with different diets containing the green tide macroalgae *Chaetomorpha linum* and the seagrass *Zostera marina*. Aquaculture. 2017;470: 157–163.
19. Chen J, Ren Y, Wang G, Xia B, Li Y. Dietary supplementation of biofloc influences growth performance, physiological stress, antioxidant status and immune response of juvenile sea cucumber *Apostichopus japonicus* (Selenka). Fish and Shellfish Immunology. 2018;72:143–152.
20. Sun ZL, Gao QF, Dong SL, Shin PKS, Wang F. Estimates of carbon turnover rates in the sea cucumber *Apostichopus japonicus* (Selenka) using stable isotope analysis: the role of metabolism and growth. Mar Ecol Prog Ser. 2012;457:101-112.
Available:https://doi.org/10.3354/meps09760
21. Duy NDQ, Francis DS, Southgate PC. The nutritional value of live and concentrated micro-algae for early juveniles of sandfish, *Holothuria scabra*. Aquaculture. 2017;473: 97–104.
22. Peterson BJ, Howarth RW, Garritt RH. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science New Series. 1985;227: 1361-1363.
23. Fry B, Sherr EB. $\delta^{13}C$ Measurements as indicators of carbon flow in marine and freshwater ecosystems. In: Rundel PW, Ehleringer JR, Nagy KA, editors. Stable Isotopes in Ecological Research. Ecological Studies (Analysis and Synthesis). New York: Springer; 1989.
24. Wahyudi AJ, Wada S, Aoki M, Hama T. Stable isotope signature and pigment biomarker evidence of the diet sources of *Gaetice depressus* (*Crustacea*: *Eubrachyura*: *Varunidae*) in a boulder shore ecosystem. Plankton and Benthos Research. 2013;8:55-67.
Available:http://doi.org/10.3800/pbr.8.55
25. Piola RF, Moore SK, Suthers, IM. Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary. Estuarine, Coastal and Shelf Science. 2006;66:255-266.
26. Yokoyama H. Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages - Potential for integrated multi-trophic aquaculture. Aquaculture. 2013;372–375:28–38.
27. Gao QF, Wang Y, Dong S, Sun Z, Wang F. Absorption of different food sources by sea cucumber *Apostichopus japonicus* (Selenka) (*Echinodermata*: *Holothuroidea*): Evidence from carbon stable isotope. Aquaculture. 2011;319:272-276.
28. Xu Q, Zhang L, Zhang T, Zhang X, Yang H. Functional groupings and food web of an artificial reef used for sea cucumber aquaculture in northern China. Journal of Sea Research. 2017;119:1–7.
29. Stock BC, Semmens BX. MixSIAR GUI User Manual. 2013;Version 3.1.
(Accessed January 2018)
Available:https://github.com/brianstock/MixSIAR/
Available:http://dx.doi.org/10.5281/zenodo.47719
30. Watanabe S, Kodama M, Sumbing JG, Lebata-Ramos MJH. Diet-tissue stable isotopic fractionation of tropical sea cucumber, *Holothuria scabra*. JARQ. 2013; 47(1):127–134.
31. Wahyudi AJ, Rahmawati S, Prayudha B, Iskandar MR, Arfianti T. Vertical carbon flux of marine snow in *Enhalus acoroides*-dominated seagrass meadows. Regional Studies in Marine Science. 2016;5:27-34.
Available:https://doi.org/10.1016/j.rsma.2016.01.003
32. DeNiro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta. 1978; 42:495–506.
33. DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta. 1981; 45:341–351.
34. Jacob U, Mintenbeck K, Brey T, Knust R, Beyer K. Stable isotope food web studies: a case for standardized sample treatment. Mar Ecol Prog Ser. 2005;287:251–253.
35. Mercier A, Battaglene SC, Hamel JF. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors.

Journal of Experimental Marine Biology and Ecology.1999;239:125-156. Available:https://doi.org/10.1016/S0022-0981(99)00034-9

36. Yu HB, Gao QF, Dong SL, Wen B, Hou YR, Ning LG. Utilization of corn meal and extruded soybean meal by sea cucumber *Apostichopus japonicus* (Selenka): Insights from carbon stable isotope analysis. Aquaculture. 2015;435:106–110.
37. Gilhooly WP, Macko SA, Flemings PB. Data report: Isotope compositions of sedimentary organic carbon and total nitrogen from Brazos-Trinity Basin IV (Sites U1319 and U1320) and Ursa Basin (Sites U1322 and U1324), deepwater Gulf of Mexico. Proceedings of the Integrated Ocean Drilling Program. 2008;308.
38. Yu Z, Zhou Y, Yang H, Ma Y, Hu C. Survival, growth, food availability and assimilation efficiency of the sea cucumber *Apostichopus japonicus* bottom-cultured under a fish farm in southern China. Aquaculture. 2014;426–427:238–248.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25922