SEA RANCHING OF *Holothuria atra*: STOCKING DENSITY AND TIME

RETNO HARTATI[1*], AMBARIYANTO[1], MUHAMMAD ZAINURI[2], AND WIDIANINGSIH[1]

[1]*Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Kampus Tembalang, Semarang 50275, Indonesia*

[2]*Department of Oceanography, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Kampus Tembalang, Semarang 50275, Indonesia*

Received 28 December 2018/Accepted 28 January 2020

ABSTRACT

Strong market demand, uncontrolled exploitation and/or the inadequate fisheries resource management have caused the overexploitation of sea cucumbers. Hence, sea ranching is suggested as an intervention to overcome, if not minimize, this problem. Since stocking density is the most important consideration in sea cucumber rearing, this study is aimed at discovering the best stocking density for the ranching of *Holothuria atra*. *H. atra* individuals were taken from the Panjang Island, Jepara waters and reared in cages at the bottom of Teluk Awur waters, Jepara with a density of 30, 20, or 10 individuals per cage measuring 2 × 2 × 1.8 m (with bottom area of 4 m^2). The stocking of *H. atra* was carried out three times, starting from the time of cage installation, then at the second, and finally at the third months after installation. Characteristics of the bottom sediment (i.e., chlorophyll a, b, phaeophytin, and total carotene) of the sea cucumber habitat and water quality in the cages were also measured monthly. This study showed that the growth of *H. atra* fluctuated. The low stocking density yielded a higher weight gain per individual than the high stocking density. The highest weight gain was observed at the stocking density of 10 individuals/cage in the second stocking month. The highest survival rate was recorded at stocking density of 30 individuals/cage (93%) in the third stocking month, considering that these sea cucumbers were only reared for three months. The highest mortality occurred at stocking density of 20 individuals/cage. Low survival rate of 45% occurred at the first stocking time or after the fifth month of rearing. Fission was observed among *H. atra* reared in the cages, resulting in smaller organisms. Among the water quality parameters, the concentration of chlorophyll a, b, phaeophytin, and carotene in the sediment fluctuated with the rearing duration due to the feeding and bioturbation of sea cucumbers. The results of this study suggested that low stocking density of *H. atra* during the second stocking month yielded a higher growth rate.

Keywords: fission, growth, sea cucumber, sediment, survival

INTRODUCTION

Utilization of invertebrate fisheries resources have recently expanded in catch and value, worldwide (Anderson *et al.* 2011). One increasingly harvested marine invertebrate species is the sea cucumber, locally known in Indonesia as "teripang", "trepang", "timun laut", or "gamat" (Hartati *et al.* 2015). Strong market demand resulting in uncontrolled exploitation, and/or inadequate fisheries management have led to the overfishing of many marine species, including the sea cucumber (Ambariyanto 2017). Hence, to overcome this problem, sea ranching was suggested.

Sea ranching is essentially an effort to release/grow cultured or wild juveniles into the natural habitat and harvest them when they reach optimum commercial size (Purcell *et al.* 2012a, b). Some advantages of sea ranching include nominally lower inputs, as the processes between release and harvest are largely left to food availability in the habitat, and the minimum level of care in sea cucumber rearing. These practices were able to yield a marketable size of the sea cucumber. However, published studies

Corresponding author, email: retnohartati.undip@yahoo.com

on sea ranching of sea cucumber in Indonesia is very limited. Attempts at culturing sea cucumber is mainly grow-out practices, especially for *H. scabra* (Pangkey *et al.* 2012). Similar studies on the ranching of sea cucumber had been done in several countries mainly for *H. scabra*, such as; in Philippines (Juinio-Meñez *et al.* 2012), Australia (Bowman 2012), and Papua New Guinea (Hair *et al.* 2016). Studies for other species were also done in Ecuador and Mexico for *Isostichopus fuscus* (Mercier *et al.* 2012) and Portugal for *H. arguinensis* (Domínguez-Godino *et al.* 2015). Consequently, to avoid the overexploitation of the natural populations of *H. atra*, further research on its sea ranching prospect is necessary. Such study would also provide knowledge to ensure a better understanding and application of programs in marine conservation, population genetics, and connectivity patterns.

A new trend emerging among Indonesian fisherfolks is the cultivation of sea cucumbers in sea pens. Sea ranching practices provide a way to restore damaged fisheries without having to legalize no-take zones or establish fishing rights for sea cucumbers. The sea ranching of sand fish *H. scabra* sea cucumber has given the fisherfolks additional activity i.e., rearing small sea cucumbers harvested from the wild in sea pens until they reach a marketable size (Juinio-Meñez *et al.* 2012; Hair *et al.* 2016). Among the recent published and unpublished data on sea cucumber from mariculture programs in the Indo-Pacific providing hatchery production, the use of juveniles (for experimental sea or pond farming, sea ranching, and/or stock enhancement reasons), and proponents information, very little came from Indonesia (Purcell *et al.* 2012a,b). This might be due to a lack of international publications on sea cucumber research in Indonesia. Moreover, for conservation purposes, no published work is available on the sea ranching of sea cucumbers in Indonesia. With the declining natural stock of sea cucumbers (Hartati *et al.* 2015), sea ranching technology has become urgent for the continuity of its production and conservation Considering that *H. atra*, locally known as "teripang hitam"/loly fish, provides a source of protein for human consumption as well as bioactive molecules for marine pharmaceuticals. The species is also ecologically important for their sediment bioturbation and remineralization, and also demonstrate a specific reproduction scheme (i.e., asexual reproduction through natural fission). Since stocking density plays a very critical role in aquaculture growth, the objectives of this study, therefore, was to determine the best stocking density and stocking time on the rearing of sea cucumber *H. atra*, and their specific effects on the species performance and characteristics of the bottom sediment in the cages used.

MATERIALS AND METHODS

Some 180 individuals of *H. atra*, weighing 100 - 150 g, were taken from Panjang Island, Jepara Waters. Bottom cages measuring 2 × 2 × 1.8 m were installed in Teluk Awur Waters, Jepara with a geographic position of 06°37'43.8" S and 110°38'31.7" E. The habitat has a muddy sand substrate with sea grasses and seaweeds and is known to be a suitable location for the rearing of *H. atra* (Hartati *et al.* 2017a).

The stocking densities applied were 30, 20, and 10 individuals/cage (equivalent to 7.5, 5, and 2.5 individuals/m^2), with three different stocking times i.e., when the cages were first installed, then at the second and the third months after installation. The stocking density applied in the study is based on the works of Lavitra *et al.* (2010a) on *H. scabra* and Xing *et al.* (2012) on *Apostichopus japonicas*. Stocking density is affected by behavioral performance due to competition for area and food. The sea cucumber weights were measured monthly to determine their growth performance and their numbers were counted at a determined time interval to calculate the survival rate.

Samples of bottom sediment were taken monthly to measure the biomass of the micro phytobenthic organisms through their photosynthesis pigment (chlorophyll a, b, phaeophytin; and carotene). Biomass of microphytobenthos were measured as concentration of chlorophyll a, b based on Jonge *et al.* (2012) and Kuczynska *et al.* (2015), phaeophytin (method of Montani *et al.* 2012) and carotene (method of Androuin *et al.* 2018). Water quality parameters (e.g., temperature, salinity, pH, and dissolved oxygen) were also measured using a water quality checker *in situ*.

The growth rate (weight gain) was calculated following Xing *et al.* (2012):

$$\text{Weight gain} = W_2 - W_1 \quad (1)$$

W_1 = weight of *H. atra* at T_{n-1} (grams)
W_2 = weight of *H. atra* at T_n (grams)

The survival rate was calculated as follows:

$$SR\ (\%) = (N_t/N_0) \times 100\% \quad (2)$$

N_t = Number of *H. atra* alive at the end of the experiment
N_0 = Number of *H. atra* stocked at the beginning of the experiment

RESULTS AND DISCUSSION

The Growth and Survival Rates of *H. atra*

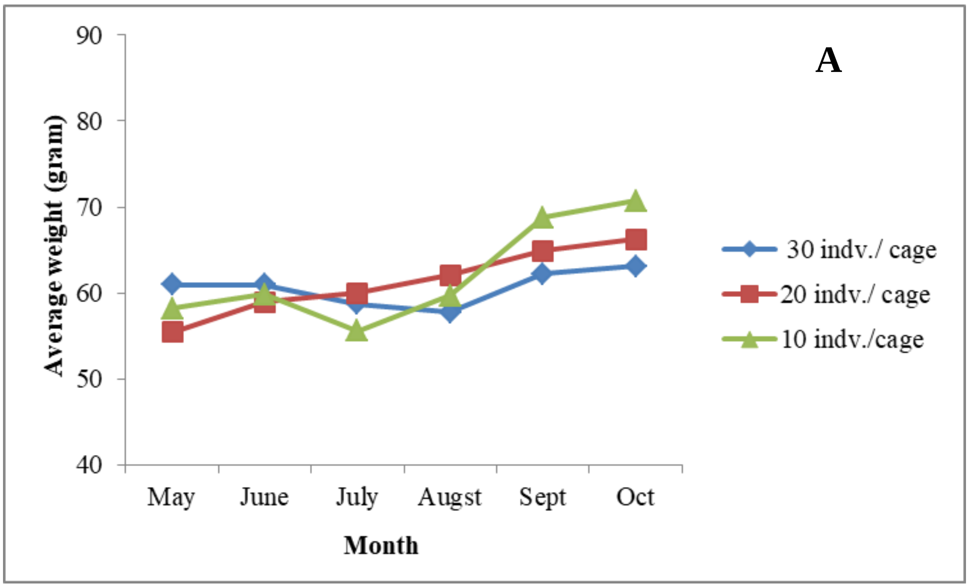
Weights of *H. atra* were measured at the beginning of the study and every month thereafter, until the study was completed. Weight gains were influenced by both the stocking density and time. Notably, high stocking density affected the weight of the reared sea cucumber. Likewise, stocking on the second month after installation of the bottom cage also resulted in higher weight gains than at other time of stocking (Fig. 1).

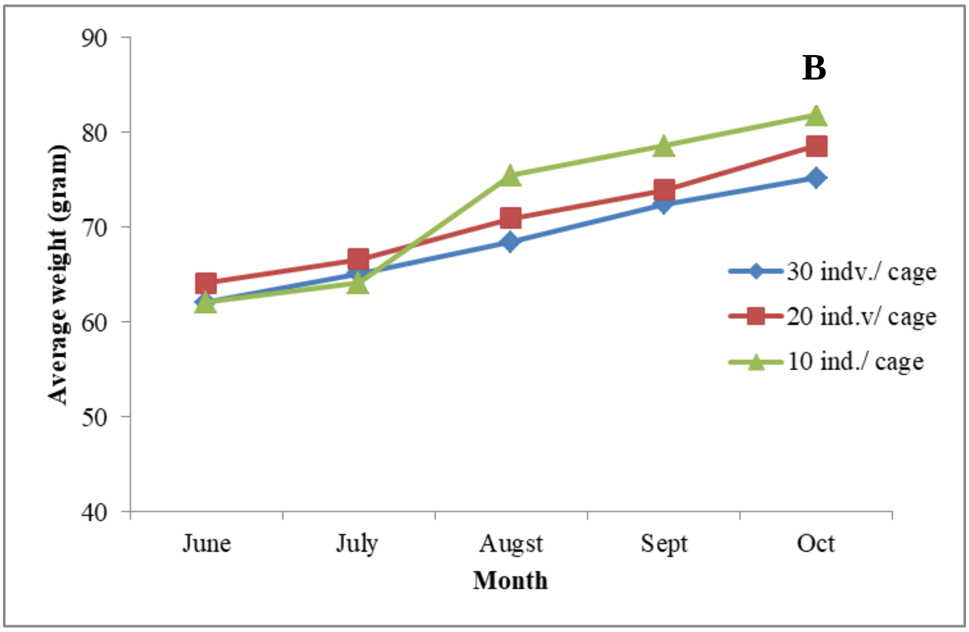
The growth of *H. atra* was calculated based on weight gain at the end of the study. The low stocking density yielded a greater weight gain than did the higher stocking densities. The highest increase was observed at the density of 10 individuals/cage at the second stocking. The lowest weight gain was recorded at the stocking of 30 individuals/cage at the first stocking (Fig. 2).

Stocking density remarkably affected the aquaculture growth. At all times of stocking, the lower stocking density (i.e., 10 and 20 individuals/cage) produced higher weight gains than the higher stocking density (e.g., 30 individuals/cage). The highest increase in weight (19.69 g) occurred at the stocking of 10 individuals/cage and the lowest (2.1 g) occurred at the stocking of 30 individuals/cage. Stress due to high stocking density greatly affected the growth performance of the sea cucumbers *H. arguinensis* and *H. mammata* (Domínguez-Godino & González-Wangüemert 2018). Stocking density had also adversely affected the growth of sea cucumber *Apostichopus japonicas*, not by decreasing the water quality or by food competition, but rather through the role of density as an environmental stress factor (Pei *et al.* 2012). This impact of high density led to greater variations in body weights, which also occurred in this study.

Stress caused by crowding or overcrowding can stimulate the endocrine system of small-sized individuals, increasing cortisol levels in coelomic fluid and, in turn, accelerating energy consumption, modifying the energy budget model, and ultimately playing a negative role in the growth and composition biochemistry of these individuals (Pei *et al.* 2012). In this study, the growth was also lowered by the effects of fission, which made the body size smaller. At high enough densities, sea cucumbers stop growing. The density value at which sea cucumbers can no longer grow is called the critical biomass value (CBV) (Lavitra *et al.* 2010a). The CBV for *H. scabra* was 650 g/m^2, whereas that for juvenile *H. atra* is 250 g/m^2 to 350 g/m^2. CBV is strongly influenced by the availability of food. *H. atra* is a deposit feeder (Asha *et al.* 2015; Hartati *et al.* 2019b), so these organisms digest organic materials largely from sediments, which continue to decrease during maintenance. Sea cucumbers mainly eat microphytobenthos (algal epiphyte) (Paga´n-Jime´nez *et al.* 2019) and bacteria (Lavitra 2010b) and the presence of sunlight reaching the bottom of the waters will support rapid growth of microphytobenthos which in turn will support sea cucumber growth (MacTavish et *al.* 2012). In this study, overcrowding caused fission which in turn slowed the growth. Hence, this study suggests the use of optimum stocking density to prevent problems of overcrowding, which will result in low growth and/or variations in body size, deformation of body shape, and the phenomenon called "rotten stomach" (thickened stomach wall) (Seeruttun *et al.* 2008).

The stocking time generally influenced the growth of *H. atra* which for the first, second and third stocking periods, were reared for five, four, and three months, respectively. Longer rearing of five months led to less weight gain of 2.1 - 12.52 g versus the second stocking time of four months which recorded 13.1 - 19.69 g weight gain. The second stocking provided an opportunity for the cage sediments to be overgrown with the microphytobenthos thereby preparing the sediments for sea cucumber habitation. At the third stocking, the maintenance duration of three months presented an average weight increase ranging from 5.43 g to 8.86 g.





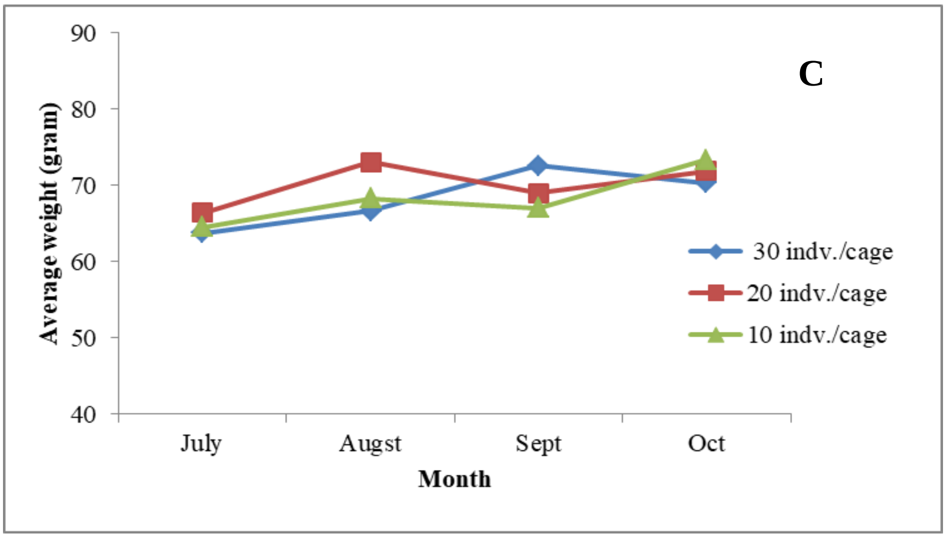


Figure 1 Average weight of *H. atra* reared at different stocking times and densities
Notes: A = stocking at Month 0 from the start of installation of the bottom cages; B = Month 1 after cages were installed; and C = Month 2 after cages were installed.

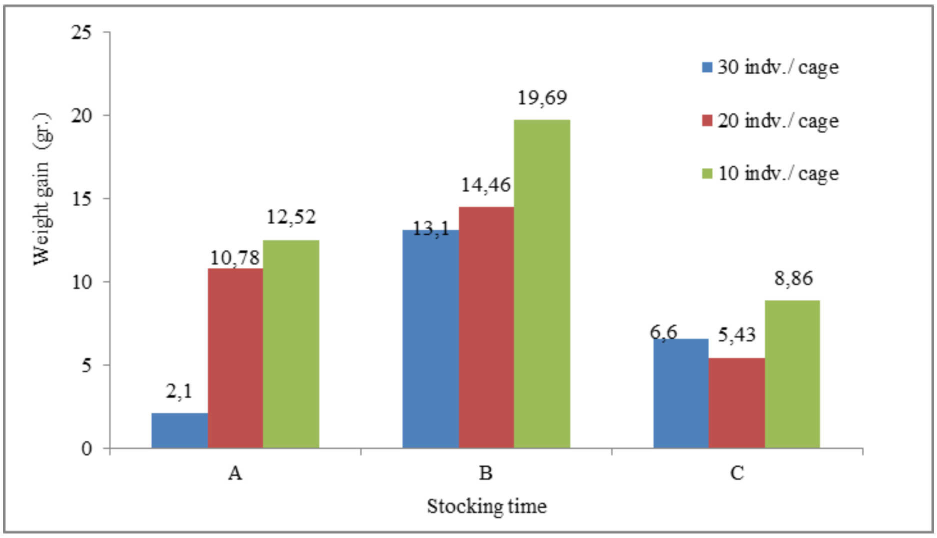


Figure 2 Weight growth of *H. atra* reared at different stocking times and densities
Notes: A = stocking at Month 0 from the start of installation of the bottom cages; B = Month 1 after cages were installed; and C = Month 2 after cages were installed.

The number of live *H. atra* differed according to stocking density. Remarkable fluctuations occurred in the number of sea cucumbers calculated as survival rates that registered 100% at the 10 individuals/cage stocking density at the third stocking (Fig. 3). This likely occurred because of the fission phenomenon (asexual reproduction) among the sea cucumbers reared in cages.

The highest survival rate of *H. atra* occurred in the 30 individuals/cage stocking density, including 93% in the third stocking, which meant that sea cucumbers can be maintained for only three months (Fig. 4). The lowest survival rate of 45% occurred at the density of 20 individuals/cage in the first stocking or in the fifth rearing month.

During their rearing period in cages, the survival rates of sea cucumbers fluctuated. Survival rate at the 30 individuals/cage stocking density was higher than that at the other stocking densities. The third stocking time also provided a high survival rate because rearing took place for only three months. The high survival rate might be caused by the existence of a fission process that increased the number of living individuals (Fig. 3), but resulted in smaller physical sizes (Fig. 2).

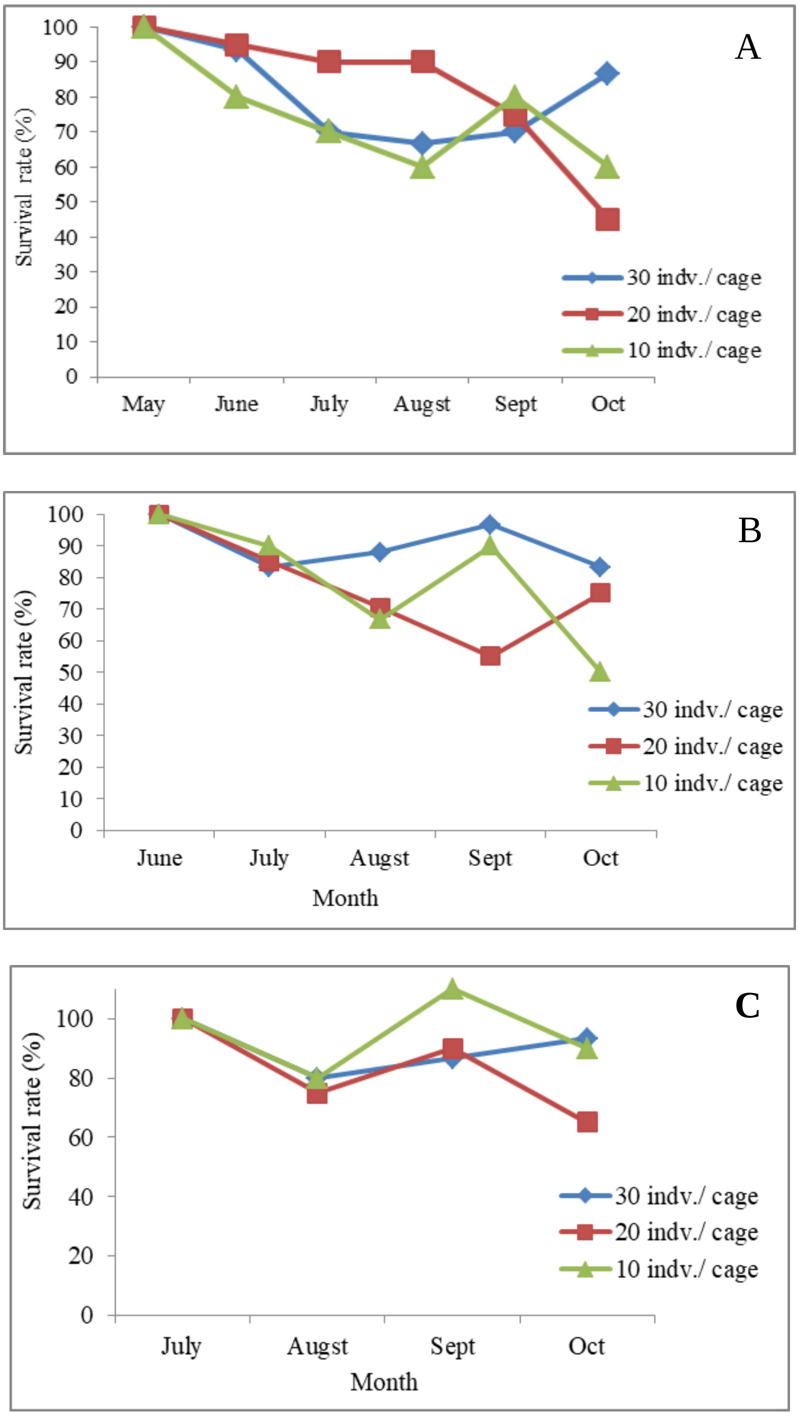


Figure 3 Survival rates of *H. atra* reared at different stocking times and densities
Notes: A = stocking at Month 0 from the start of installation of the bottom cages; B = Month 1 after cages were installed; and C = Month 2 after cages were installed.

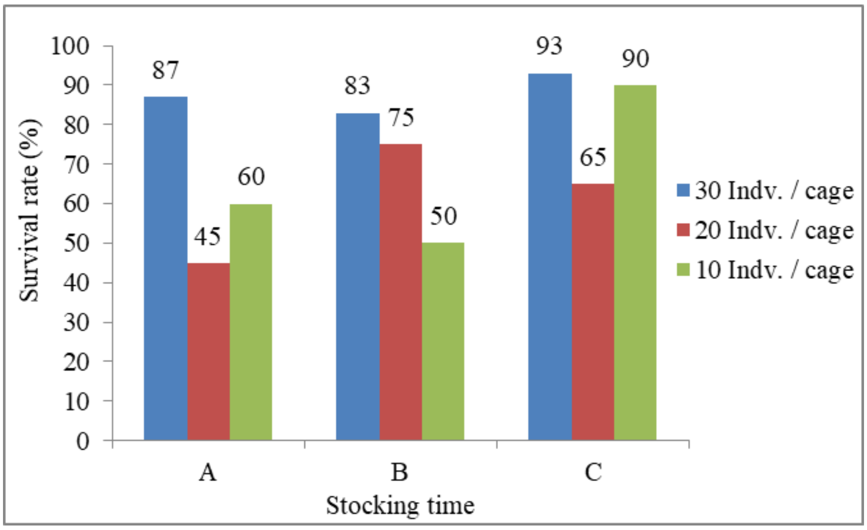


Figure 4 Survival rate of *H. atra* reared at different stocking times and densities
Notes: A = stocking at Month 0 from the start of installation of the bottom cages; B = Month 1 after cages were installed; and C = Month 2 after cages were installed.

Table 1 Number of *H. atra* fissions during cage rearing

Stoking time	Stocking density		
	30	20	10
Month 0	8	11	2
Month 1	8	9	8
Month 2	8	10	5
Total fissions	24	30	15

During its rearing period in cages with different stocking densities and stocking times, the sea cucumbers did participate in asexual reproduction or fission across all conditions (Table 1). Although most of these phenomena were not directly observed, the organisms that are results of fission can be distinguished from the original organisms (Fig. 5). Sea cucumbers naturally have a great potential to regenerate after the process of evisceration (expulsion of their internal organs). Evisceration occurs due to physiological changes or in response to various external factors. During evisceration, sea cucumbers secrete a large portion of their internal organs and, within a certain period of time, such will grow back and continue to live normally (Hartati *et al.* 2016). Regeneration also occurs after the process of natural asexual reproduction by fission as well as during fission stimulation (Hartati *et al.* 2016). This process produces new individuals or regeneration in the posterior or anterior part.

Fission is a naturally occuring process (Fig. 5); first is the narrowing of the part that will divide into two portions (Fig. 5A). After splitting (body fission), the new individual closes its wound in the division section (fission plane) (Fig. 5B) and the initial regeneration appears as a bulge in the fission plane. Then, regeneration of the body part happens (Fig. 5C). Ultimately, this fission process contributed to high survival rates but produced smaller individuals of sea cucumber.

Generally, in this study, the more densely populated cages experienced more fission than did the non-dense ones, but stocking time did not significantly affect the number of fission organisms (Table 1). Fission is a phenomenon that is highly dependent on population density (Asha & Diwakar 2015; Asha *et al.* 2015; Hartati *et al.* 2019a). In nature, fission is vital in maintaining populations of some types of holothurians to compensate for their mortality and migration (Dolmatov 2014).

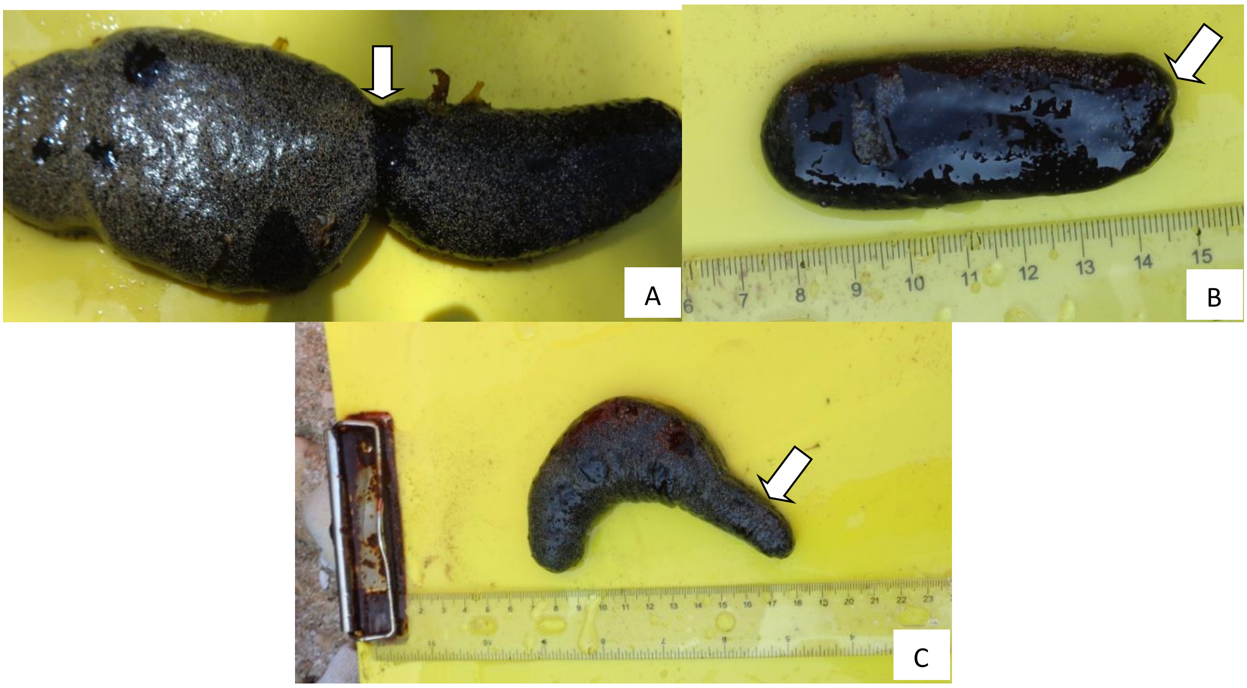


Figure 5 Sea cucumbers that are undergoing fission (A); those that have just undergone fission, where the cleavage wound has been closed (B); and the regenerated missing part of the body (C)
Note: → arrow mark k is the position of fission/fission plane and regeneration

The biomass of the microphytobentic food sources of *H. atra* were measured as concentration of chlorophyll a, b, phaeophytin, and total carotene levels in the sediment (Figs 6 & 7).

The concentration trends of chlorophyll a, b, phaeophytin, and total carotene were almost the same in all cages; high in the first month of rearing, decreasing in the second and third months of rearing, increasing in the fourth month, and then decreasing again in the last or fifth month (Figs 6 & 7).

Microphytobenthic or benthic microalgae are groups of photoautotrophic microorganisms that inhabit the surface layers of shallow aquatic ecosystems sediments such as diatoms, cyanobacteria, and other chlorophytes (Paga´n-Jime´nez *et al.* 2019). In shallow coastal waters, the microphytobenthos significantly contribute to the metabolic systems of primary producers (Hardiso *et al.* 2013). These microphytobenthos include important food sources for meiofauna such as sea cucumbers (Mfilinge & Tsuchiya 2016; Hartati *et al.* 2017a,b; Paga´n-Jime´nez *et al.* 2019). Holothurians can assimilate low content of organic matter as live diatoms, bacteria and detritus by passing sediment through their gut system (Tolon *et al.* 2015), thereby causing fluctuations in the concentrations of microphytobenthos (Hartati *et al.* 2019b). The microphytobenthos biomass is measured as chlorophyll content in the sediments (Du *et al.* 2017) and the ratio of total chlorophyll c and b to chlorophyll a can be a good indicator of the amount of diatomic and green algal biomass (Cartaxana *et al.* 2016).

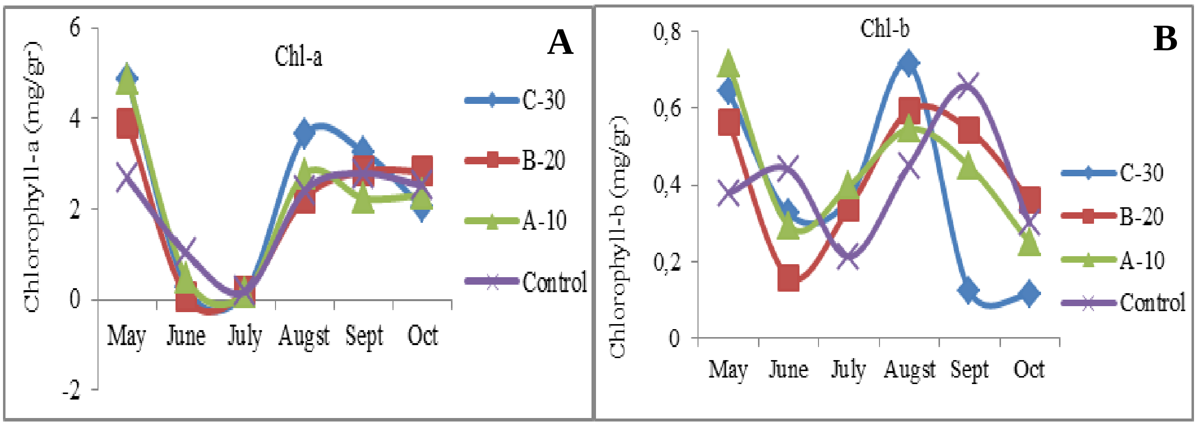


Figure 6 Concentration of chlorophyll-a (A) and chlorophyll-b (B), in the cage sediments of *H. atra* reared at different densities

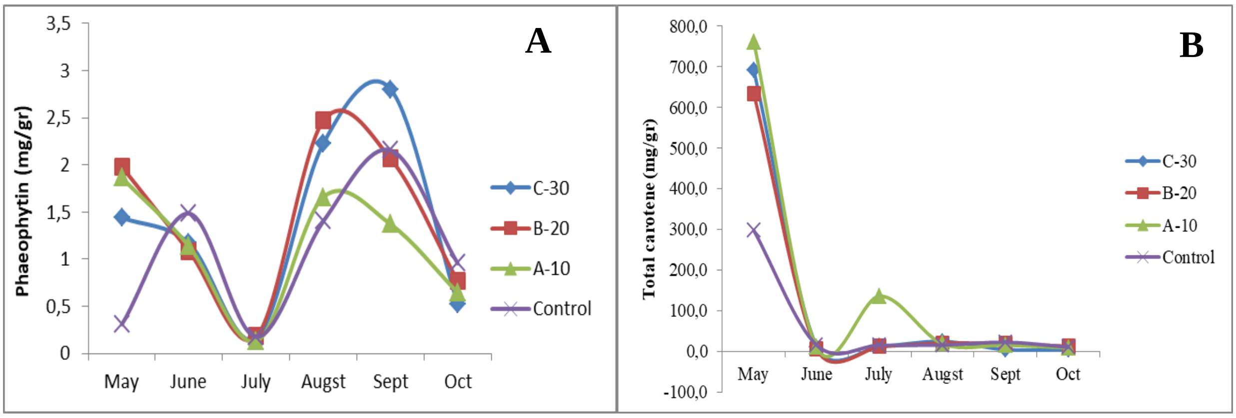


Figure 7 Concentration of phaeophytin (A) and total carotene (B) in the cage sediments of sea cucumber *H. atra* reared at different densities

Bottom sediment is one of the basic components influencing sea cucumber habitat preferences. Hence, studies of its characteristics are primarily important (Slater & Jeffs 2010). Rocky sediments should be avoided because sea cucumbers live in mud or sand and remain in the organic material for its lifetime. *H. atra* is a deposit feeder that swallows sediment along with organic matter (Robinson *et al.* 2013; Asha *et al.* 2015) and the microbial composition in the intestine of these adult holothurians constituted a greatly diverse microorganisms, such as bacteria, viruses, protozoa, and fungi colonizing their intestines (Paga´n-Jime´nez *et al.* 2019).

Studies have also shown that sea cucumbers swallow marine animal feces, including even their own feces (Barrio & Tuya 2013). In shallow waters (<10 m depth), *H. atra* prefers sea grass habitats with sediments of 15% to 25% gravel and coarse sand (0.7 - 1.2 mm), but not mud (Dissanayake & Stefansson 2012). *H. atra* was also found mainly in sand-dominated habitats (Hartati *et al.* 2017a). Its preference for specific habitat characteristics seems to be related to the feeding and security needs of *H. atra* (Hartati *et al.* 2017a,b).

Water quality parameters (e.g., temperature, salinity, pH, and dissolved oxygen) were also measured during the rearing of *H. atra* (Fig. 8). Water temperature is one most important parameter affecting growth and development of sea cucumbers as well as their distribution in the sea. Temperatures in seawaters tend to be stable at 28 - 30 °C throughout the study period (Fig. 8). The temperature between cages were not significantly different because the cages were close to one another. Since *H. atra* is a tropical species, they perform optimally in the higher temperature environments between 23 - 31 ºC (Buccheri *et al.* 2019). The aestivation process (adaptation to high temperatures) has not been widely studied in *H. atra*, but the species was observed to usually cover its body with sand during the day, which is thought to be a response to higher temperatures (Hartati *et al.* 2019a).

Sea cucumbers are stenohaline invertebrates that are unable to tolerate a wide range of salinity. Therefore, their settlement in river mouths, estuaries, and other bays where salinity falls below 10‰ during the monsoon season should be avoided. Although there is a small river that goes into the Teluk Awur Waters, it did not greatly affect the salinity around the cages which were located 500 m from the river mouth. Salinity levels in the Teluk Awur Waters ranging from 27.5‰ to 30.5‰ were still suitable for the sea cucumbers (Fig. 8).

Besides temperature and salinity, the water pH can also affect *H. atra*. Fluctuations in pH occur seasonally, in that the dry season is characterized by high water temperatures and low water movements/currents resulting in low pH values between 7.3 and 7.5, while pH increases to between 8.4 and 8.6 during the rainy season because of high water influx (Gullian & Preciat 2017). Generally, vertebrates have the ability to regulate osmotic pressure and create very good acid-base conditions and therefore, are able to cope with changes in pH better than invertebrates which have a lower regulatory capacity (Wittmann & Portner 2013). However, information about the effects of pH on sea cucumber is still limited.

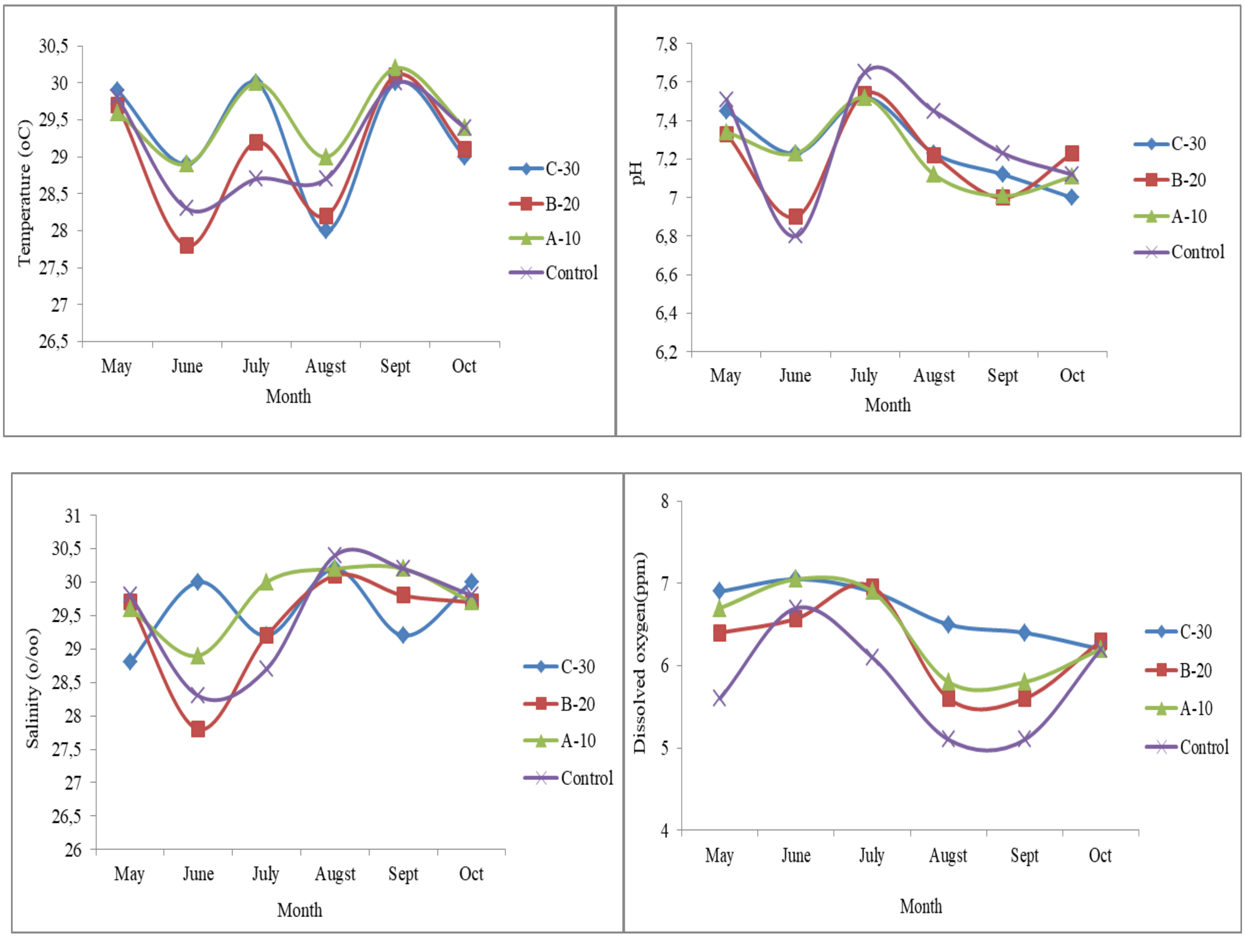


Figure 8 Average temperature, salinity, pH, and dissolved oxygen of the sea water surrounding the cages of *H. atra* reared with different stocking densities

Sea cucumbers respire with their tentacles, skin, and respiration trees by consuming a significant amount of water and absorbing the dissolved oxygen inside. The hypoxia (minimum amount of oxygen) limit of *H. leucospilota* was 3 mg O_2/L, wherein the sea cucumber released its cuverian tube to lower its energy needs in this aesthetic process (Zamani *et al.* 2018). Furthermore, the normal conditions of dissolved oxygen levels at >6 mg/L provide a

100% survival rate. For *A. japonicas*, dissolved oxygen was maintained at >5 mg/L (Xie *et al.* 2013). During the rearing period in this study, the dissolved oxygen content in the cages ranged from 6 mg/L to 7 mg/L (Fig. 8) and no signs of *H. atra* evisceration due to hypoxia was observed.

Sea cucumber rearing in bottom cages has been carried out previously for both *H. scabra* and *Stichopus hermannii* with no food addition (Purcell & Agudo 2013; Hartati *et al.* 2016). The maintenance of sea cucumbers with feeding greatly affected their growth performance (i.e., sea cucumbers grow faster when given additional feed) (Yokohama 2013) and Mahmoud *et al.* 2017). In this study, however, the *H. atra* were not given additional feed considering that the sediment/substrate would provide ample feed for the species. In sea ranching, the current sea cucumber management practices do not apply additional input so the existence of sea cucumbers depends largely on the availability of natural feed (Purcell *et al.* 2012). Therefore, choosing the right location for the rearing of *H. atra* is very crucial.

CONCLUSION

H. atra reared at low stocking densities of 10 individuals/cage at the bottom cages of the Teluk Awur Waters of Jepara, exhibited higher growth and survival rates. Stocking time in the second month after the cages were installed also resulted in higher growth, as the food organisms for the sea cucumber were already developed and thus, were made available. Moreover, the chlorophyll a, b, phaeophytin, and carotene levels fluctuated during the time of sea cucumber rearing due to their feeding and bioturbation activities.

ACKNOWLEDGMENTS

The authors would like to thank SEAMEO-BIOTROP for partly funding this present work through the DIPA BIOTROP 2018 of PhD grant and Universitas Diponegoro for also funding the chemical analyses through the International Publication Research (RPI) grant of Other Than APBN LPPM RKAT Universitas Diponegoro Year 2018-2019. Our gratitude also goes to Robertus TM, Elsa LA, Fabian PA and Mustagpirin for their help during the field work.

REFERENCES

Ambariyanto. 2017. Conserving endangered marine organisms: causes, trends and challenges. In IOP Conference Series: Earth and Environmental Science, 55(1): 012002. IOP Publishing. http://iopscience.iop.org/article/10.1088/1755-1315/55/1/012002

Anderson SC, Mills-Flemming J, Watson R, Lotze HK. 2011. Serial exploitation of global sea cucumber fisheries. Fish Fish 12:317-39.

Androuin T, Polerecky L, Decottignies P, Dubois SF, Dupuy C, Hubas C, Jesus B, Le Gall E, Marzloff MP, Carlier A. 2018. Subtidal Microphytobenthos: A Secret Garden Stimulated by the Engineer Species *Crepidula fornicata*. Front Mar Sci 5:475. doi: 10.3389/fmars.2018.

Asha PS, Diwakar K. 2015. Field observation of asexual reproduction by fission in sea cucumber *Holothuria atra*. Mar Fish Infor Serv, T & E Ser, 223&224:29-30.

Asha PS, Diwakar K, Santhanavalli G, Manissery MK. 2015 Comparative distribution and habitat preference of the sea cucumber *Holothuria atra* Jaeger at protected and unprotected sites in Thoothukudi region of Gulf of Mannar, south-east coast of India. Indian J Fish 62(1):52-7.

Dissanayake DCT, Stefansson G. 2012. Habitat preference of sea cucumbers: *Holothuriaatra* and *Holothuria edulis* in the coastal waters of Sri Lanka. J Mar Biol Ass of the UK 92(3):581-90.

Domínguez-Godino JA, Slater MJ, Hannon C, González-Wangüermert M. 2015. A new species for sea cucumber ranching and aquaculture: Breeding and rearing of *Holothuria arguinensis*. Aquaculture 438:122-8. http://dx.doi.org/10. 1016/j.aquaculture.2015.01.004

Domínguez-Godino JA, González-Wangüemert M. 2018. Does space matter? Optimizing stocking density of *Holothuria arguinensis* and *Holothuria mammata*. Aquacult. Res. 49(9): 3107-3115. doi: 10.1111/are.13773

Du G, Yan H, Dupuy C. 2017. Microphytobenthos and an indicator of environmental quality status in intertidal flats: Case study of coastal ecosystem in Pertuis Charentais, France. Estuar Coast Shelf Sci 196: 217-26. doi: 10.1016/ j.ecss.2017.06.031

Dulmatov IY. 2014. Asexual Reproduction in Holothurians. Hindawi Publishing Corporation The Scientific World Journal Vol. 2014, Article

ID 527234, 13 pages. http://dx.doi.org/10.1155/2014/527234

Gullian KM, Preciat TM. 2017. Effect of pH on temperature controlled degradation of reactive oxygen species, heat shock protein expression, and mucosal immunity in the sea cucumber *Isostichopus badionotus*. PLoS ONE 12(4):e0175812. https://doi.org/10. 1371/ journal. pone.0175812

Hair C, Mills DJ, McIntyre R, Southgate PC. 2016. Optimising methods for community-based sea cucumber ranching: Experimental releases of cultured juvenile *Holothuria scabra* into seagrass meadows in Papua New Guinea. Aquacult Rep 3:198-208. http://dx.doi.org/10.1016/ j.aqrep. 2016.03.004

Hardiso AK, Canuel EA, Anderso IC, Tobias CR, Veuger B, Waters MN. 2013. Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments. Biogeosci 10:5571-88. doi:10.5194/bg-10-5571-2013.

.Hartati R, Purwati P, Widianingsih. 2015. Field Guide. Timun Laut. Yogyakarta (ID): Pohon Cahaya.

Hartati R, Widianingsih, Endrawati H. 2016. The Growth of Sea cucumber *Stichopus herrmanni* after Transverse Induced Fission in Two and Three Fission Plane. Ilmu Kelautan IJMS Indonesian Journal of Marine Science 21(2):93-100.

Hartati R, Trianto A, Widianingsih. 2017a. Habitat characteristic of two selected locations for sea cucumber ranching purposes. 2017 IOP Conf Ser: Earth Environ Sci 55 012041. http://iopscience.iop.org/1755-1315/55/1/ 012041

Hartati R, Widianingsih, Trianto A, Zainuri M, Ambariyanto. 2017b. The abundance of prospective natural food for sea cucumber *Holothuria atra* at Karimunjawa Island Waters, Jepara, Indonesia. Biodiversitas 18(3):947-53.

Hartati R, Zainuri M, Ambariyanto A, Ayodya FP, Widianingsih W, Mustagfirin M, Soegianto A. 2019a. Initial assessment of *Holothuria atra* population in Panjang Islands**.** Eco Env Cons 25:S1-S6.

Hartati R, Ambariyanto A, Zainuri M, Widianingsih W, Supriyo E, Trianto A. 2019b. The concentration of chlorophyll-c in the bottom sediment of sea cucumber rearing cage. IOP Conference Series: Earth and Environmental Science 246 (2019) 012078. doi:10.1088/1755-1315/246/1/012078.

Jonge VN, de Boer WV, de Jong DJ, Brauer VS. 2012. Long-term mean annual microphytobenthos chlorophyll a variation correlates with air temperature. Mar Ecol Prog Ser 468:43-56. doi: 10.3354/meps09954

Kowalewska G, Witkowski A, Toma B. 1996. Chlorophylls c in bottom sediments as markers of diatom biomass in the southern Baltic Sea. Oceanologia 38(2):227-49.

Kuczynska P, Jemiola-Rzeminska M, Strzalka K. 2015. Photosynthetic Pigments in Diatoms. Mar Drugs 13:5847-81. doi:10.3390/md13095847

Lavitra T, Rasolofonirina R, Eeckhaut I. 2010a. The Effect of Sediment Quality and Stocking Density on Survival and Growth of the Sea Cucumber *Holothuria scabra* Reared in Nursery Ponds and Sea Pens. Western Indian Ocean J Mar Sci 9(2):153-64.

Lavitra T, Fohy N, Gestin P, Rasolofonirina R, Eeckhaut I. 2010b. Effect of water temperature on the survival and growth of endobenthic *Holothuria scabra* (Echinodermata: Holothuroidea) juveniles reared in outdoor ponds. SPC Bêche-de-mer Bulletin 30:25-8.

Mahmoud MAM, El-Saman MI, Babeker EA. 2017. Effects of Different Macroalgaediets on Growth Performance on Impatient Sea Cucumber *Holothuria impatiens* Forskaal 1775 (Echinodermata: Holothuroidea). J Fish Aqua Sci 12:177-83.

MacTavish T, Stenton-Dozey J, Vopel K, Savage C. 2012. Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically Enriched Coastal Sediments. PLoS ONE 7(11): e50031. doi:10.1371/journal. pone.0050031

Montani S, Tsutsumi H, Komorita T. 2012. Measurement of Benthic Microalgal Chlorophyll a and Pheo-pigments by Fluorometric Analysis. Workshop of The First Asian Marine Biology Symposium at Phuket, Thailand on 16-17 December 2012.

Pei S, Dong S, Wang F, Tian X, Gao Q. 2012. Effects of density on variation in individual growth and differentiation in endocrine response of Japanese sea cucumber (*Apostichopus japonicas* Selenka). Aquaculture 356-357;398-403. doi: 10.1016/ j.aquaculture. 2012.04.032

Purcell SW, Hair CA, Mills DJ. 2012a. Sea cucumber culture farming and sea ranching in the tropics: progress problems and opportunities. Aquaculture 368:68-81. http://dx.doi.org/ 10.1016/j.aquaculture.2012.08.053

Purcell SW, Mercier A, Conand C, Hamel JF, Toral-Granda MV, Lovatelli A, Uthicke S. 2012b. Sea cucumber fisheries: global analysis of stocks management measures and drivers of overfishing. Fish Fish 14:34-59. http://dx.doi.org/10.1111/j.1467-2979.2011. 00443.x.

Purcell SW, Agudo NS. 2013. Optimisation of Mesh Enclosures for Nursery Rearing of Juvenile Sea

Cucumbers. PLoS ONE 8(5): e64103. https://doi.org/10.1371/journal.pone.0064103

Slater MJ, Jeffs AG. 2010. Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*? J Sea Res 64:241-9.

Tolon T, Emiroglu D, Günay D, Saygi H. 2015. Effect of Sediment Grain Size on Growth Performance of Juvenile Sea Cucumber (*Holothuria tubulosa*). Turk J Fish Aquat Sci 15:561-5. doi: 10.4194/1303-2712-v15_2_43

Wittmann AC, Portner HO. 2013. Sensitivities of extant animal taxa to ocean acidification. Nat Clim Change 3:995-1001.

Xie X, Zhao W, Yang M. 2013. Combined influence of water temperature, salinity and body size on energy budget in the sea cucumber *Apostichopus japonicas* Selenka. Fish Sci 79:639-46.

Xing K, Liu S, Yang H, Zhang M, Zhou Y. 2012. Southward transplanted cage-culture of sea cucumbers *Apostichopus japonicus* in China's Shengsi Islands. SPC Beche-de-mer Information Bulletin #32:33-8.

Yokoyama H. 2013. Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages: Potential for integrated multi-trophic aquaculture. Aquaculture 372–375:28-38.

Zamani NP, Assidqi K, Madduppa H. 2018. Study of the Tolerance of Black Sea Cucumber *Holothuria leucospilota* to Hypoxia Stress. Pertanika J Trop Agric Sc 41(3):1511-21.