**ACCEPTED MANUSCRIPT**

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

Hartati R, Ambariyanto, Zainuri M, Widianingsih

DOI: 10.11598/btb.……..

To appear in : BIOTROPIA Issue

Received date : 27 December 2018
Accepted date : 28 January 2020

**This manuscript has been accepted for publication in BIOTROPIA journal. It is unedited, thus, it will undergo the final copyediting and proofreading process before being published in its final form.**

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

**Retno Hartati[1,2*], Ambariyanto[1], Muhammad Zainuri[1] and Widianingsih[1]**

[1]Departement of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro, Semarang 50275, Indonesia

[2]Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP), Bogor 16134, Indonesia

[*]Corresponding author, e-mail: retnohartati.undip@yahoo.com

## ABSTRACT

Strong market demand and uncontrolled exploitation and/or the inadequate management of fisheries have caused many stocks of sea cucumbers to be overexploited. One suggested effort to overcome this problem is sea ranching. Stocking density is the most important consideration in sea cucumber rearing; therefore, this present work is aimed at elucidating the best stocking density for sea ranching of *Holothuria atra*. *H. atra* was taken from the Panjang Island, Jepara waters and reared in bottom cages in Teluk Awur waters, Jepara with a density of 30, 20, or 10 individuals per cage measuring 2 m × 2 m × 1.8 m (with bottom area of 4 $m^2$). Stocking times of *H. atra* were at the initial time of cage installation, the second and the third months after installation. Bottom sediment characteristics (i.e., chlorophyll a, b, phaeophytin, and total carotene) of the sea cucumber habitat and water quality in the cages were measured monthly during the study. The results showed that growth of *H. atra* fluctuated; low stocking density yielded a higher weight gain than high stocking density did. The highest weight gain was present in the density of 10 individuals/cage in the second stocking month. The highest survival rate of *H. atra* was seen in the condition of 30 individuals/cage (93%) at the third stocking month, which means that these sea cucumbers were only reared for three months. The highest mortality occurred at a density of 20 individuals/cage with the survival rate being low (45%) at the first stocking time or in the fifth month of rearing. There was fission evidence 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 according to the time of sea cucumber rearing caused by their feeding and bioturbation. The study results suggested to stock *H. atra* at low density during the second stocking month to get higher growth.

**Keywords**: chlorophyll, fission, growth, sediment, survival

## INTRODUCTION

In recent decades, invertebrate fisheries have expanded in catch and value worldwide (Anderson *et al.*, 2011). One increasingly harvested marine invertebrate group is the sea cucumber. In Indonesia, sea cucumber is called “teripang”, “trepang", “timun laut”, or “gamat” (Hartati *et al.*, 2015). Strong market demand and uncontrolled exploitation and/or inadequate fisheries management have led to many marine species, such as sea cucumber stocks becoming heavily overfished (Ambariyanto, 2017). One option suggested to overcome this problem is sea ranching.

Sea ranching is essentially an effort to release/grow cultured or wild juveniles into natural habitat and harvested when they reach commercially optimal size (Purcell *et al.*, 2012). There are some advantages of sea ranching, for example, inputs are nominally lower, as the processes between release and harvest are largely left to availability of food in the habitat, and the level of care to sea cucumber reared is minimum. But this practice is still able to yield a marketable sea cucumber size. However, to our knowledge, published studies on sea ranching of sea cucumber in Indonesia is very limited Attempt to culture sea cucumber in Indonesia is mainly grow out practices, especially for *H. scabra* (Pangkey *et al.*, 2012). Concerning with sea ranching of sea cucumber, there are similar studies had been done in several countries mainly for *H. scabra* suh as in Philippines (Juinio-Meñez *et al*., 2012), Australia (Bowman, 2012), and Papua New Guinea (Hair *et al*., 2016). While studies for other species were done in Ecuador and Mexico for *Isostichopus fuscus* (Mercier *et al*., 2012) and Portugal for *H. arguinensis* (Domínguez-Godino *et al.*, 2015), Therefore, to avoid the overexploitation of natural populations, further research on the sea ranching of sea cucumbers is necessary. Such study would also be able to provide knowledge to ensure a better understanding and to apply in marine conservation, population genetics, and connectivity patterns.

There is a new trend among fishermen in Indonesia that is cultivating sea cucumbers in sea pens. Sea ranching practices provide a way to restore damaged fisheries without having to formalize no-take zones or establish fishing rights for sea cucumbers. In the case of sand fish or *H. scabra* (Juinio-Meñez *et al*., 2012, Hair *et al*., 2016) said that sea ranching of sea cucumber give additional activity for fishers, i.e. rearing small sea cucumbers harvested from the wild in sea pens until they reach a marketable size. As has been gathered by Purcell *et al*. (2012a,b), regarding both the recent published data and unpublished data from mariculture programs on sea cucumber in the Indo-Pacific that provide 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. This might be due to a lack of international publications on sea cucumber research in Indonesia. Moreover, in the case of conservation, 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), it is urgent to conduct works on sea cucumber sea ranching for the continuation of production and conservation. Here, we propose sea ranching for *H. atra* (local name: "teripang hitam"/loly fish) because they represent a source of protein for human consumption as well bioactive molecules for marine pharmaceuticals, are ecologically important for their sediment bioturbation and remineralization, and also demonstrate a specific reproduction scheme (i.e., asexual reproduction through natural fission). Stocking density is a very important factor that affects growth in aquaculture. Therefore, the objectives of this study were to determine the best stocking density and stocking time for sea cucumber *H. atra* ranching. Specifically, this paper will discuss the effects of stocking density and stocking time on the performance of *H. atra* and the characteristics of the bottom sediment in the cages used.

## MATERIALS AND METHODS

*H. atra* (180 individuals) were taken from Panjang Island, Jepara waters with sizes of 100 g to 150 g. Bottom cages measuring 2 m × 2 m × 1.8 m were installed in Teluk Awur waters, Jepara with a geographic position of 06$^o$37'43.8"S and 110$^o$38'31.7"E. A previous study (Hartati *et al*, 2017a) determined that this area is a suitable location for cages of *H. atra*, as the habitat has a muddy sand substrate with sea grasses and seaweed.

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 time, i.e., when the cages were first installed, the second and the third months after the cages were installed. The stocking density is based on Lavitra *et al*. (2010a) for *H. scabra* and Xing et al. (2012) for *Apostichopus japonicas*, and assumed to be affected by behavioral performance due to competition for area and food. Sea cucumber weights were measured monthly to determine their performance and the number of sea cucumbers alive at each time point was counted to determine their 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 measure as concentration of chlorophyll-a, -b according method of 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 as follows (Xing *et al*., 2012)::

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

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

$W_2$ = weight of *H. atra* at $T_n$ (grams)

Survival rate was calculated as follows:

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

$N_t$= Number of *H. atra* alive in the end of the experiment

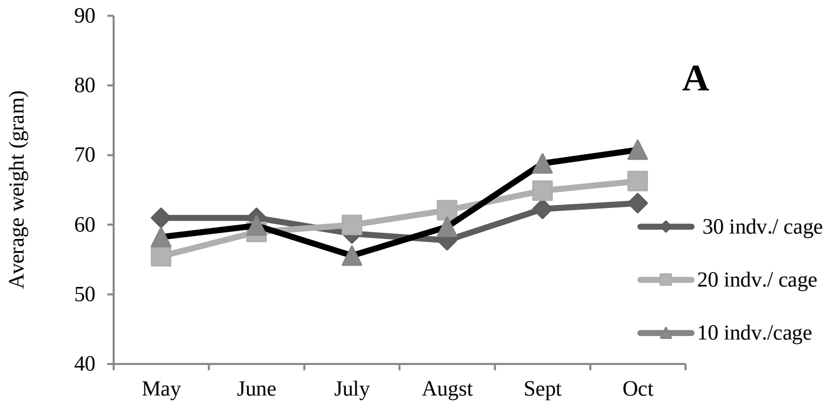
$N_0$ = Number of *H. atra* stocked at the beginning of the experiment

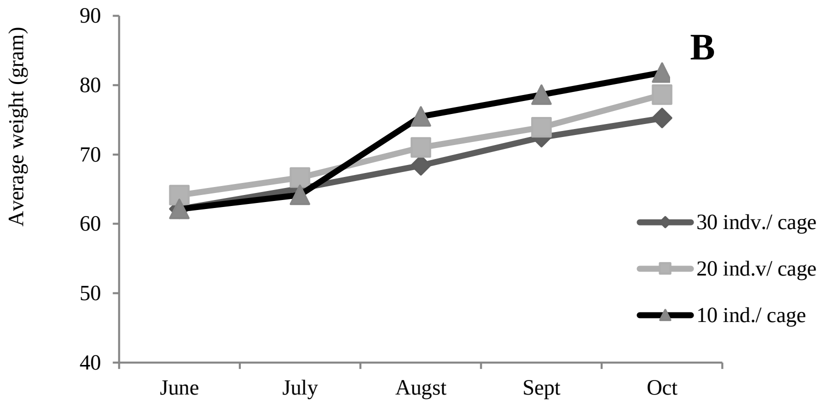
## RESULTS AND DISCUSSION

### The Growth and Survival Rates of *H. atra*

During the rearing period, the weights of *H. atra* were measured at the beginning of the study and every month thereafter until the study was completed. Increases in the weights of sea cucumbers were shown to be influenced by both the stocking density and time of stocking. Notably, a high stocking density affected the weight of the reared sea cucumber. Likewise, stocking on the second month after the bottom cage was installed was found to be higher weight gain than any of other time of stocking (Figure 1).

Sea cucumber growth was calculated based on the weight gain of *H. atra* at the end of the study and tended to fluctuate, in that low stocking density yielded a greater weight gain than did the higher stocking densities. The highest increase was obtained in the density of 10 individuals/cage at the second stocking. The lowest weight gain was seen with the stocking of 30 individuals/cage at the first stocking (Figure 2).



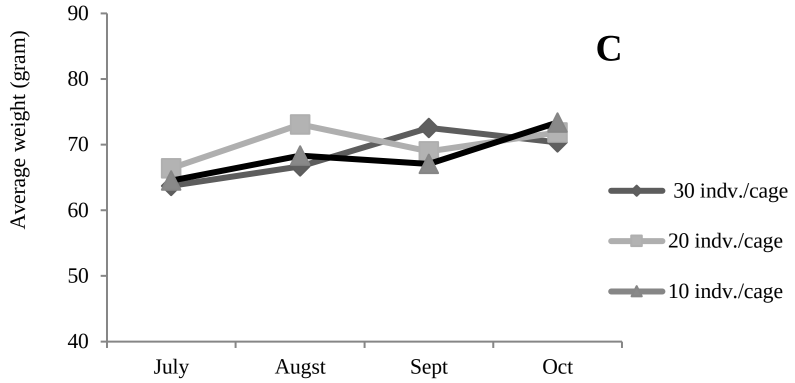



Figure 1. Average weight of *H. atra* reared at different stocking times and densities (A = stocking at Month 0 from the start of installation of the bottom cages, B = Month 1 after cages were installed, and C = Month 2 after cages were installed).

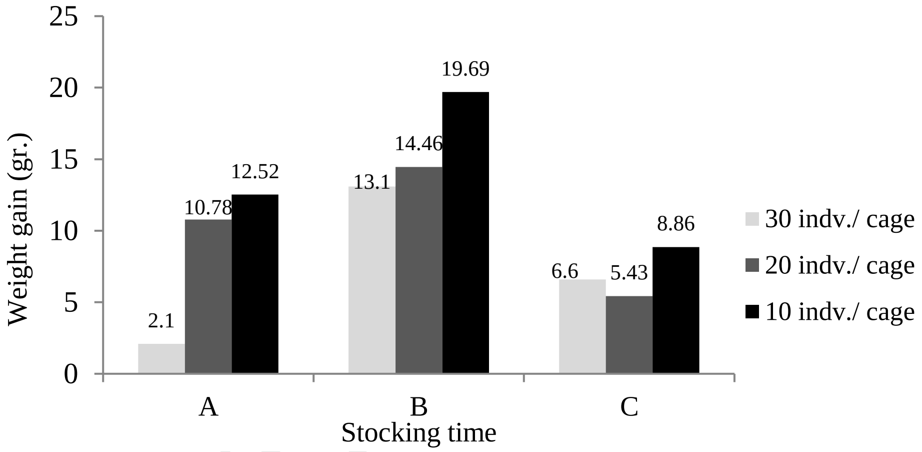



Figure 2. Growth of weight of *H. atra* reared at different stocking times and densities (A = stocking at Month 0 from the start of installation of the bottom cages, B = Month 1 after cages were installed, and C = Month 2 after cages were installed).

Stocking density is a very important factor that affects growth in aquaculture. At all times of stocking, lower stocking density (i.e., 10 and 20 individuals/cage) culminated in a higher weight than did higher stocking densities (e.g., 30 individuals/cage). The highest increase in weight (19.69 g) occurred with the stocking of 10 individuals/cage and the lowest (2.1 g) occurred with the stocking of 30 individuals/cage. As noted in the study of Domínguez-Godino and González-Wangüemert (2018), stress due to high stocking density is thought to be a major factor affecting the growth and performance of the sea cucumbers *H. arguinensis* and *H. mammata*. Separately, according to the results of research by Pei *et al*. (2012) on *Apostichopus japonicas,* stocking density has an adverse effect on sea cucumber growth, not through decreasing water quality or food competition but rather through the role of density as an environmental stress factor. The impact of high density also leads to a greater variation in body weight, which is the same thing that happened in this study.

The 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). It seems that, 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* is 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 growth of sea cucumbers (MacTavish et *al.*, 2012). The results of this study suggest to use the optimum stocking density to prevent problems such as overcrowding, which will cause low growth and/or variations in body size, deformation of the body shape, and the phenomenon "rotten stomach" (thickened stomach wall) (Seeruttun *et al*., 2008). In this study, overcrowding caused fission, which in turn slowed the growth.

Stocking time in general also influenced the growth of *H. atra*: for first, second and third stocking periods, sea cucumbers were reared for five, four, and three months, respectively. Longer rearing (five months) led to less weight gain (2.1–12.52 g) versus the second stocking time (four months; 13.1–19.69 g). The second stocking provides an opportunity for sediments in the cages to be overgrown with members of the microphytobenthos such that the sediments are often more readily prepared for sea cucumber habitation as compared with at first stocking. At the third stocking, the maintenance duration of three months presented an average weight increase ranging from 5.43 g to 8.86 g.

The numbers of *H. atra* alive at the end of the study were different according to stocking density. Fluctuations in the number of sea cucumbers occurred, which, when calculated based on the survival rate, were remarkable in some cases due to exceeding 100% (occurring in the 10 individuals/cage stocking density at the third stocking). This likely occurred because of the phenomenon of fission (asexual reproduction) in the sea cucumbers reared in cages. The survival trends of sea cucumbers stocked in dense stocking conditions and at different stocking times during maintenance are presented in Figure 3.

The highest survival rate of *H. atra* at the end of the study occurred in the 30 individuals/cage stocking density, including 93% in the third stocking, which meant that sea cucumbers were only maintained for three months. The highest mortality occurred at the density of 20 individuals/cage with the survival rate being 45% in the first stocking or in the fifth month of rearing. Detailed survival rate results are presented in Figure 4.

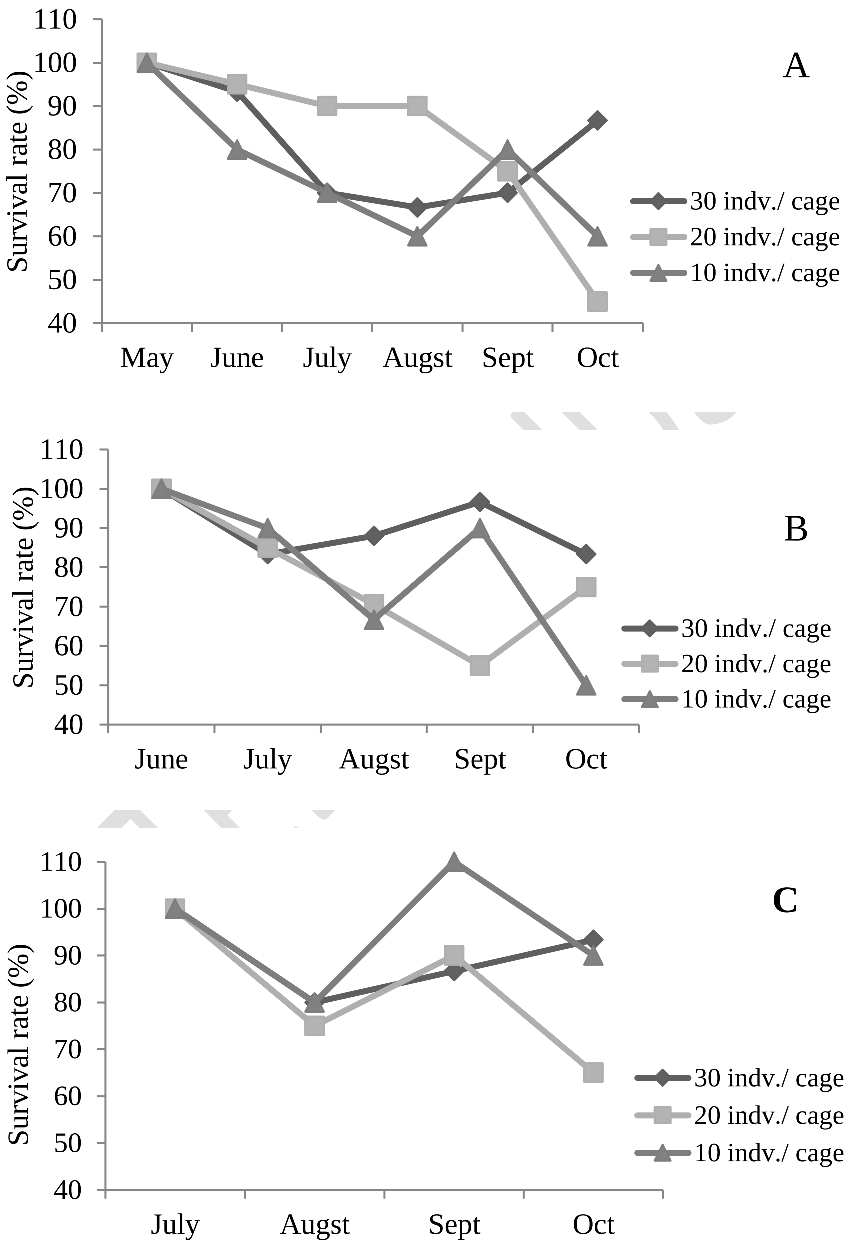



Figure 3. Survival rate of *H. atra* reared at different stocking times and densities (A = stocking at Month 0 from the start of installation of the bottom cages, B = Month 1 after cages were installed, and C = Month 2 after cages were installed).

The survival rates of sea cucumbers during rearing in cages fluctuated. Based on stocking density and stocking time, the survival rate at the 30 individuals/cage stocking density was higher than other stocking densities. Based on stocking time, the third stocking still provided a high survival rate because rearing took place for only three months. The high survival rate is thought to be due to the existence of a fission process that increased the number of organisms living (Figure 3) but resulted in smaller physical sizes (Figure 2).

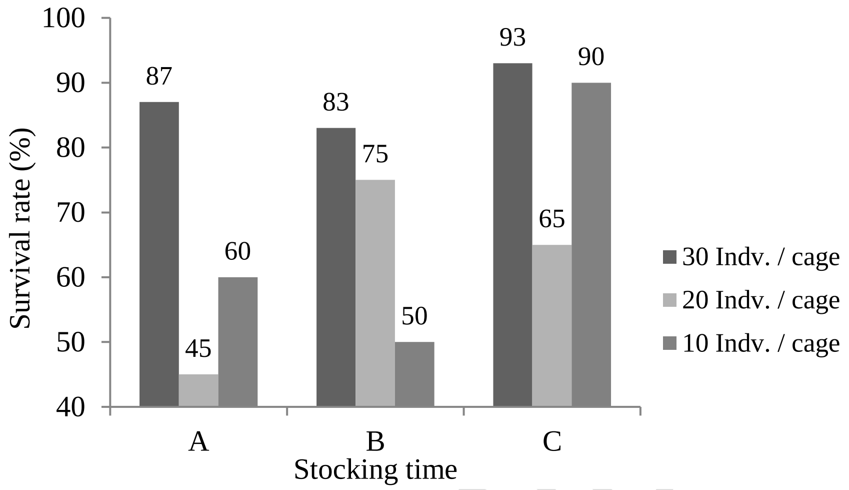



Figure 4. Survival rate of *H. atra* reared at different stocking times and densities (A = stocking at Month 0 from the start of installation of the bottom cages, B = Month 1 after cages were installed, and C = Month 2 after cages were installed).

During being reared in cages with different stocking densities and stocking times, 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 (Figures 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 they can 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 with regeneration in the posterior or anterior part.

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

| **Stoking time** | **Stocking density** | | |
|---|---|---|---|
| | **30** | **20** | **10** |
| 1 | 8 | 11 | 2 |
| 2 | 8 | 9 | 8 |
| 3 | 8 | 10 | 5 |

| | | | |
|---|---|---|---|
| Total fissions | 24 | 30 | 15 |

The process of fission naturally occurs as presented in Figure 5; first, a narrowing of the part of the body that will be divided into two portions is observed (Figure 5A). After splitting (fission), a new individual closes the wound in the division section (fission plane) (Figure 5B) and the initial regeneration appears as a bulge in the fission plane. Then, regeneration of the body part happens, as shown in Figure 5C. Ultimately, this fission process contributed to high survival rates but produced smaller individual sea cucumber organisms.

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

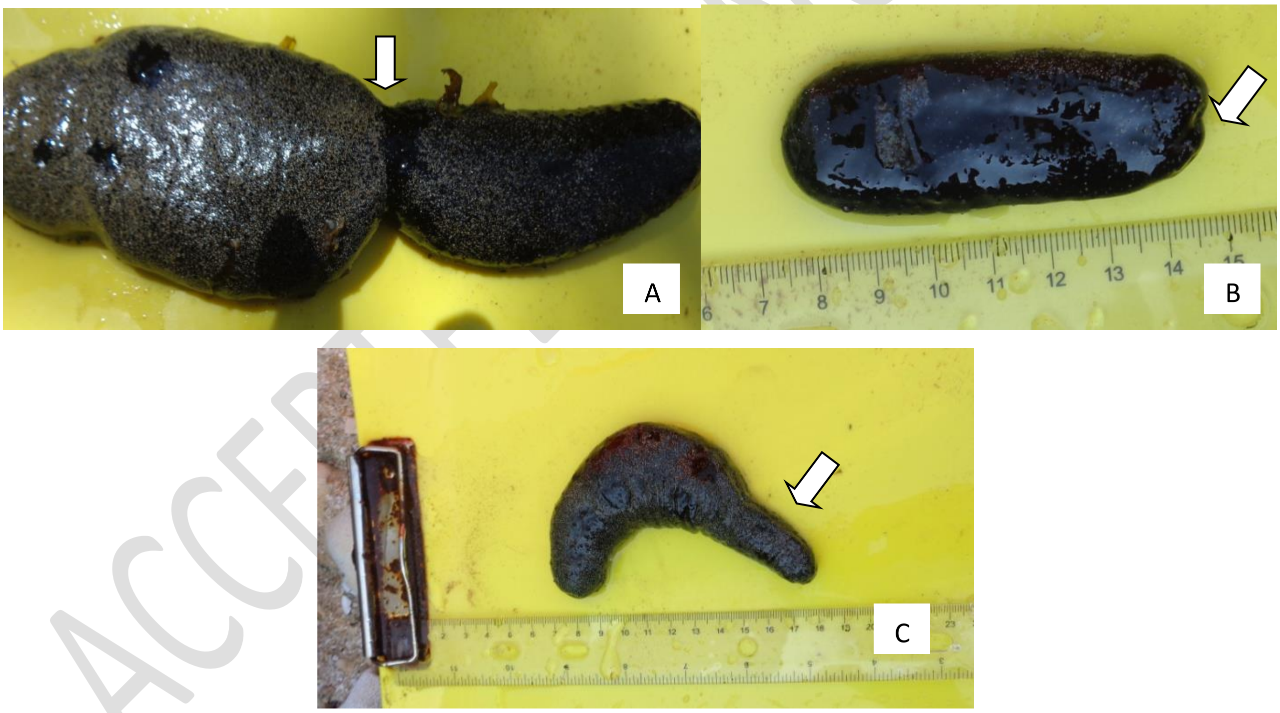



Figure 5. Sea cucumbers that are undergoing fission (A); those that have just undergone fission, where the cleavage wound has been closed (B); and the regenerated missing part of the body (C) (→ arrow mark k is the position of fission/fission plane and regeneration).

Microphytobentic biomasses as food sources of *H. atra* were measured as concentration of chlorophyll a, b, phaeophytin, and total carotene levels in the sediment. Results are shown in Figures 6 and 7.

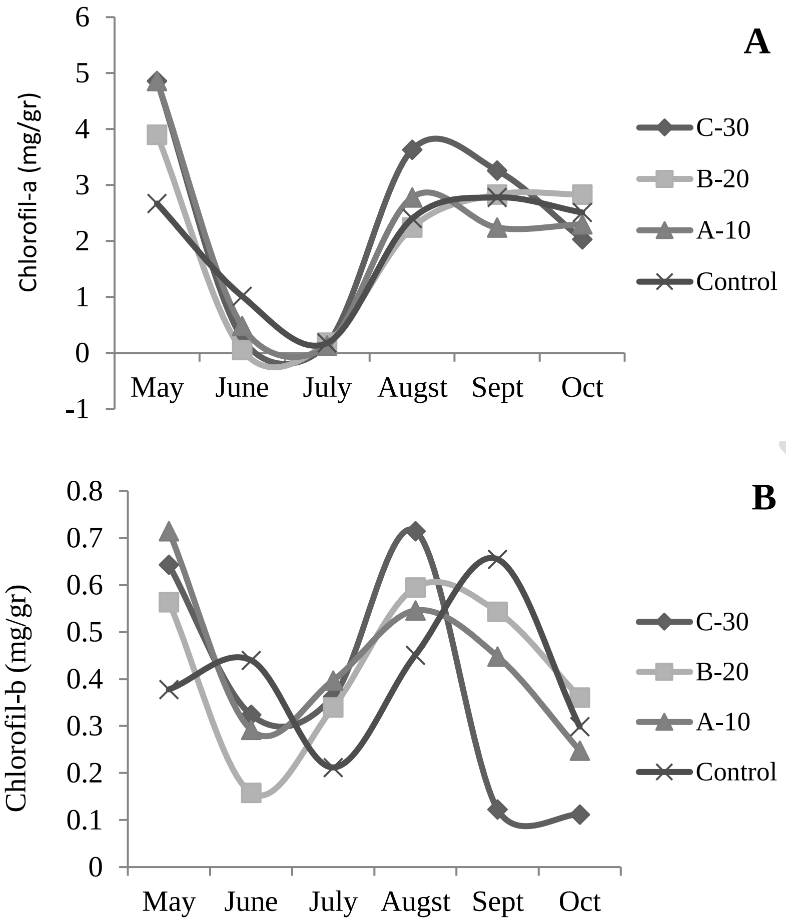



Figure 6. Concentration of chlorophyll-a (A) and chlorophyll-b (B), in the sediment of *H. atra* cages reared at different densities.

The trends in the concentrations of chlorophyll a, b, phaeophytin, and total carotene were almost the same in all cages—specifically, high in the first month of rearing, decreased in the second and third months of rearing, increased in the following (fourth) month, and then decreased again in the last (fifth) month of the study (Figures 6 and 7). Microphytobenthic or benthic microalgae are described as groups of photoautotrophic microorganisms that inhabit the surface layers of sediment of shallow aquatic ecosystems such as diatoms, cyanobacteria, and other chlorophytes (Paga´n-Jime´nez *et al*., 2019). In shallow coastal waters, the microphytobenthos plays an important role in the metabolic system by significantly contributing to primary producer (Hardiso*et al.* 2013). Furthermore, the microphytobenthos includes important food sources for meiofauna such as sea cucumbers (Mfilinge and Tsuchiya, 2016, Hartati *et al.*, 2017, 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). This consumption can cause fluctuations in concentrations of microphytobenthos, as stated by Hartati *et al.* (2019b). The biomass of microphytobenthos could be measured as chlorophyll content in the sediment (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)

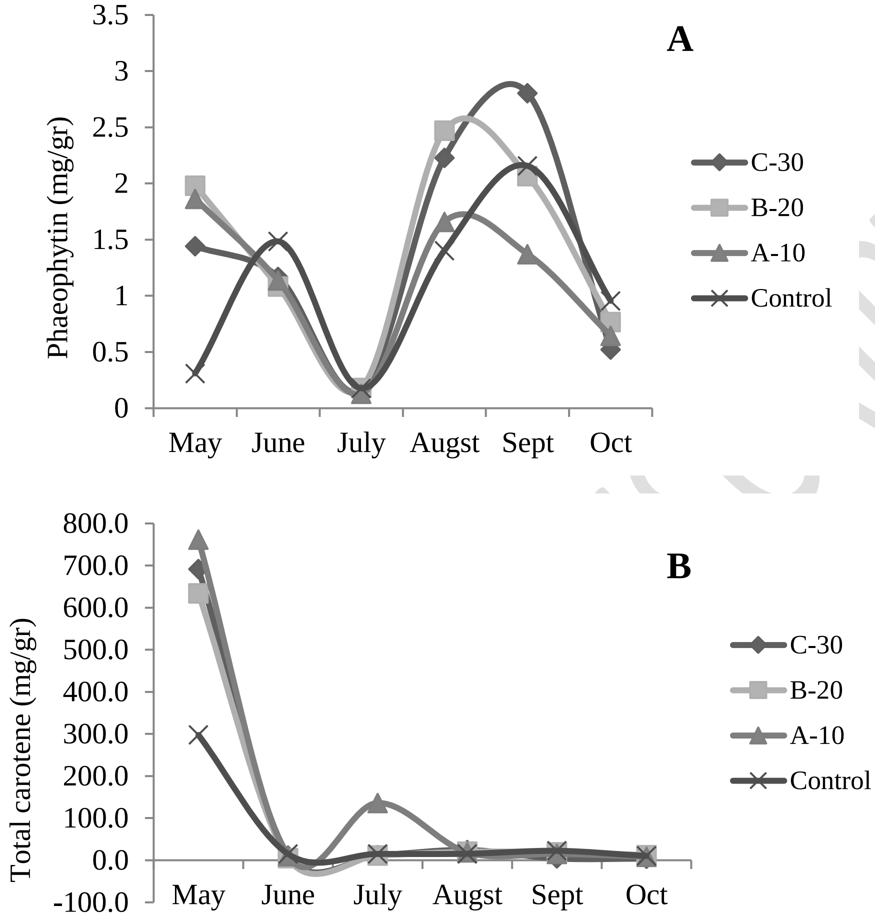



Figure 7. Concentration of phaeophytin (A) and total carotene (B) in the sediment of sea cucumber *H. atra* cages reared at different densities.

According to Slater and Jeffs (2010), bottom sediment characteristics constitute one of the important components that influence sea cucumber habitat preferences and, hence, studies of the characteristics of sea cucumber sediment are very important to complete. Rocky sediment should be avoided because sea cucumbers live in mud or sand and remain in the organic material in the same place. *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 adult holothurians have shown that they have a great diversity of microorganisms, such as bacteria, viruses, protozoa, and fungi that colonize their intestine (Paga´n-Jime´nez *et al*., 2019).

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

Water quality parameters (e.g., temperature, salinity, pH, and dissolved oxygen) during the rearing of *H. atra* were measured and are presented in Figure 8. Water temperature is considered to be one of the most important parameters affecting the level of growth and development of sea cucumbers as well as their distribution in the sea. Temperatures in seawaters tend to be stable throughout the study period at 28 to 30°C (Figure 8). The temperature between cages were not significantly different because the cages were close to one another. According to Buccheri *et al.* (2019) because *H. atra* is tropical species, they perform optimally in the higher temperature environments in between 23-31$^{o}$C. The process of aestivation (adaptation to high temperatures) has not been widely discussed in *H. atra*, but, usually during the day, *H. atra* will cover the body with sand, 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 placement in river mouths, estuaries, and other bays where salinity falls below 10 ppt during the monsoon season should be avoided. Although there is a small river that goes into the Teluk Awur waters, it did not affect the salinity around the cages, which were located 500 meters from river mouth. Salinity levels in the Teluk Awur waters are presented in Figure 8, which ranges from 27.5 to 30.5%, which is still suitable for the life of sea cucumbers.

In addition to temperature and salinity, water pH can affect the life of *H. atra*. pH fluctuations occur seasonally, in that the dry season is characterized by high water temperatures and low water movements/currents that produce 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 the entry of high water influx (Gullian and Preciat, 2017). Wittmann and Portner (2013) said that, in general, vertebrates have the ability to regulate osmotic pressure and very good acid–base conditions and therefore are better able to cope with changes in pH as compared with invertebrates, which have a lower regulatory capacity, but information about the effects of pH on sea cucumber is limited.

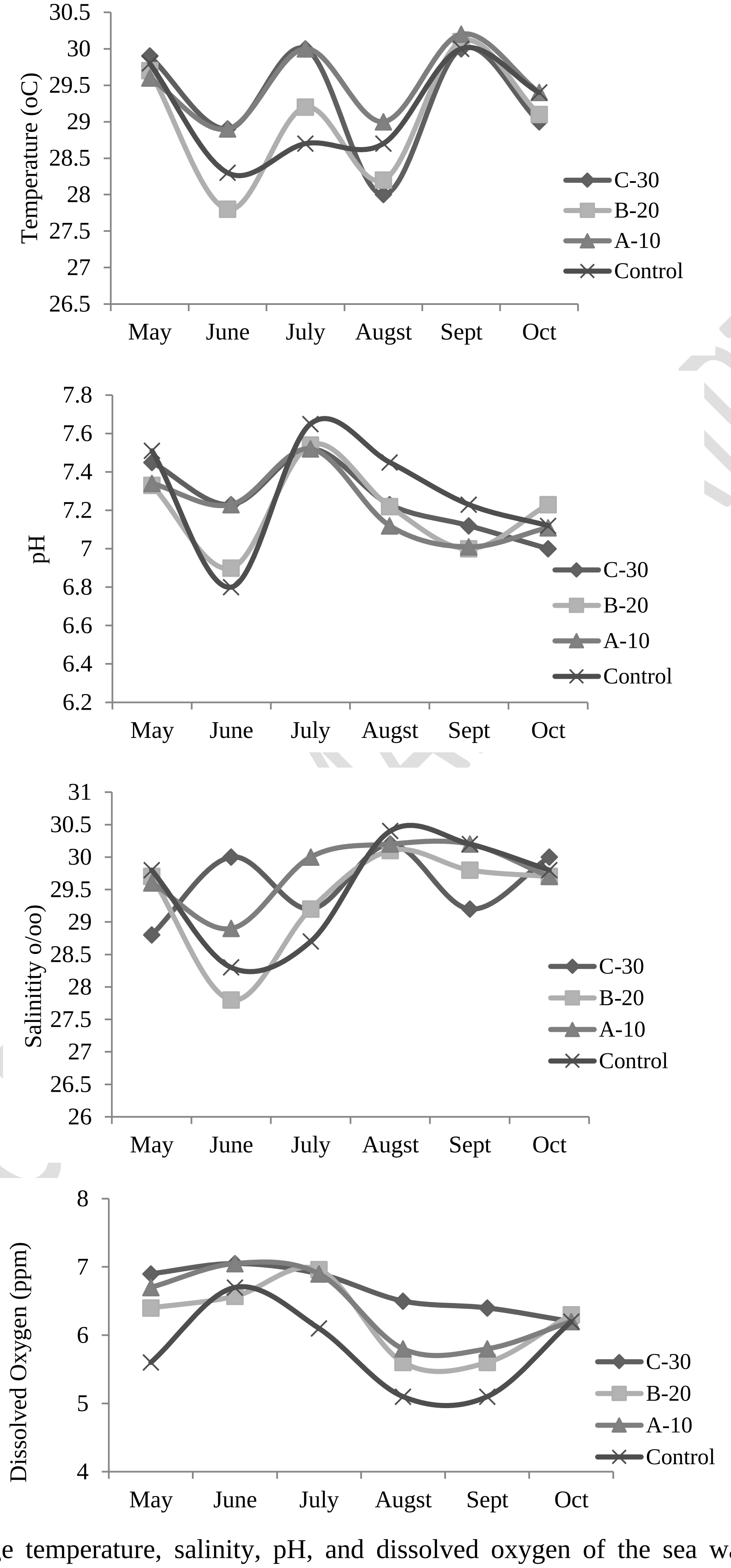



Figure 8. Average temperature, salinity, pH, and dissolved oxygen of the sea water in *H. atra* rearing cages with different stocking densities.

Sea cucumbers respire with their tentacles, skin, and respiration trees by consuming a significant amount of water and absorbing the dissolved oxygen inside. In their research, Zamani *et al*. (2018) found that the limit of hypoxia for *H. leucospilota* was 3 mg $O_2$/L, wherein the sea cucumber released its cuverian tube to lower its energy needs in its aesthetic process. Furthermore, it has also been said that the normal conditions of dissolved oxygen levels of> 6 mg/L provide a 100% survival rate. In the maintenance of *A. japonicas,* dissolved oxygen is maintained at > 5 mg/L (Xie *et al.* 2013). In this study, during the rearing period, the dissolved oxygen content in the cages ranged from 6 mg/L to 7 mg/L (Figure 8) and there were no signs of *H. atra* evisceration due to hypoxia.

The rearing of sea cucumbers in bottom cages has been carried out previously by Purcell and Agudo (2013), Hartati *et al*., (2016) for both *H. scabra* and *Stichopus hermannii* with no food addition. Although according to Yokohama (2013) and Mahmoud et al. (2017) the maintenance of sea cucumbers with feeding greatly affects growth performance (i.e., sea cucumbers grow faster when given additional feed), in the process of rearing sea cucumbers in this study, the *H. atra* were not given additional feed as it was thought that the sediment/substrate would provide ample feed for sea cucumbers. On the subject of sea cucumber maintenance with the aim of sea ranching, no input is given, so, in current practices, the life of sea cucumbers is left to/depends largely on the availability of natural feed (Purcell *et al*, 2012). Therefore, choosing the right location in this regard is very important to consider.

## CONCLUSION

*H. atra* reared in the bottom cages in the Teluk Awur waters of Jepara with low stocking densities (10 individuals/cage) presented higher growth and survival rates. Stocking time in the second month after the cages were installed also resulted in higher growth, as it allowed the food organisms available for the sea cucumber to be developed. Chlorophyll a, b, phaeophytin, and carotene levels fluctuated according to the time of sea cucumber rearing caused by their feeding and bioturbation activities.

## ACKNOWLEDGEMENTS

The authors wish to thank to BIOTROP for partly funding this present work through DIPA BIOTROP 2018 of Dissertation grant and Diponegoro University for also funding the chemical analyses through International Publication Research (RPI) grant of Other Than APBN LPPM

RKAT Universitas Diponegoro Year of 2018-2019. We also wish to thank to Robertus TM, Elsa LA, Fabian PA and Mustagpirin for their helps during 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 and Fisheries 12:317–339.

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, P. S. ., K. Diwakar, G. Santhanavalli, Manissery MK, 2015 Comparative distribution and habitat preference of the sea cucumber *Holothuria atra* Jaeger at protected and unprotected sites inThoothukudi region of Gulf of Mannar, south-east coast of India. Indian J. Fish., 62 (1): 52-57

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):581-590.

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: 122–128 . 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: 217-226. 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, D J Mills, R McIntyre, 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. Biogeosci10:5571–5588. doi:10.5194/bg-10-5571-2013

.Hartati R, Purwati P, Widianingsih. 2015. Field Guide.Timun Laut. Pohon Cahaya. Yogjakarta Indonesia.

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):947-953.

Hartati R, Zainuri M, Ambariyanto A, Ayodya FP, Widianingsih W, Mustagfirin, M., A. Soegianto. 2019a.. Initial assesment of *Holothuriaatra* 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 Conf. 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, 2012 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):227–249.

Kuczynska P, Jemiola-Rzeminska M, Strzalka K. 2015. Photosynthetic Pigments in Diatoms. Mar Drugs 13: 5847-5881; 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):153-164.

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 30:25-28

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. of Fish. and Aqua. Sci. 12:177-183.

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 Dec. 16-17 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). Aquaculture356-357:398-403. 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 64:241–249.

Tolon, T., D. Emiroglu, D. Günay, Saygi H. 2015. Effect of Sediment Grain Size on Growth Performance of Juvenile Sea Cucumber (*Holothuria tubulosa*). Turk J Fish Aquat Sci 15: 561-565. 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: 639-646.

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 #32:33-38.

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):1511-1521.