Microplastic ingestion in the black sea cucumber *Holothuria leucospilota* (Brandt, 1835) collected from Rambut Island, Seribu Islands, Jakarta, Indonesia

K B Wicaksono¹, M P Patria¹* and A Suryanda²

¹ Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia, Kampus FMIPA-UI, Depok 16424, Indonesia
² Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Rawamangun, Jakarta Timur, 13220, Indonesia

*mpatria@sci.ui.ac.id

Abstract. The research on microplastic ingestion in the black sea cucumber *Holothuria leucospilota* (Brandt, 1835) collected from Rambut Island, Kepulauan Seribu, DKI Jakarta aims to determine the amount and types of microplastic in sea cucumbers, acknowledge the organ or component of the sea cucumber with the highest amount of microplastic particles, and know the correlation between the amount of microplastics found in the sediment ingested and intestines of the sea cucumber. Samples of sea cucumbers were collected from 3 different sites, which were the west, east, and south region of Rambut Island. The sampling technique used in this study was the purposive random sampling method. The analysis of microplastic amount and types was done by isolating microplastics in each sample. The sea cucumber’s respiratory tree and intestine was dissolved in 65% HNO₃ solution, whereas separation by size and density by immersion in saturated NaCl solution was performed on the consumed sediment samples. The respiratory tree contained the most amount of film, i.e. 4.7 particles/g. Fibre were dominant in the intestine and the consumed sediment, i.e. 2.34 and 1.4 particles/g respectively. There was a positive correlation between the amount of microplastic in the sediment ingested and intestines of the sea cucumber.

1. Introduction

Marine plastic pollution is an impact from human activities which are also threatening marine life [1]. The estimated amount of plastic waste in the sea reaches 4.8—12.7 million tonnes each year, and Indonesia is the second largest country with the most amount of marine plastic waste after China [2]. Plastic waste could be degraded or fragmented into smaller particles called microplastics, after going through physical, chemical, or biology processes [3]. Microplastics could be defined as plastic particles less than 5 mm in size [4]. They could come from break downs of larger plastic particles, or plastic manufactured in small sizes [5]. Their small sizes, mostly similar to planktons and other low trophic organisms, makes them possible to disrupt the food chain [6]. Microplastics are found ingested by organisms like pelagic and demersal fishes [7], loggerhead sea turtle *Caretta caretta* [8], Norwegian lobster *Nephrops norvegicus* [9], and even the true's beak whale *Mesoplodon mirus* [10].

Microplastics suspended in sediments are easily consumed by deposit feeders, like sea cucumbers [11]. *Holothuria leucospilota* are one of the sea cucumber species easily found in Seribu Islands [12].
They consume organic particles suspended in sediments, such as planktons, meiofauna, and sea phanerogams [13]. Holothurians susceptibility to ingest microplastics made them proposed as an indicator species for sediment microplastic pollution [14]. This study was conducted to quantify microplastic ingestion, and the amount of microplastic particles in the respiratory tree and intestine of the sea cucumber located in Seribu Islands, specifically at Rambut Island.

2. Materials and methods

2.1. Study area
Samples used in this study was collected in an area located at the west, south, and east side of Rambut Island, in the intertidal zone. The water has an average depth of 3.3 m. Sediments consists of sand and reef flats. Sampling site coordinates were 106°41’20.47”E and 5°58’33.28”S, 106°41’31.72”E and 5°58’40.63”S, 106°41’43.80”E and 5°58’30.98”S. Environment parameters (Table 1) showed that it supported the growth of Holothurians.

| Site | Depth (m) | Temperature (°C) | pH | Salinity (ppt) | Transparency (m) |
|------|-----------|------------------|----|----------------|------------------|
| 1    | 3         | 28               | 7  | 37             | 3                |
| 2    | 4         | 29               | 7  | 37             | 3.6              |
| 3    | 3         | 28               | 7  | 37             | 3                |

2.2. Sample collection
Samples of *H. leucospilota* was collected with the purposive random sampling method. Fifteen individuals were collected, which were five individuals with at each location. Individuals were put in glass jars containing 70% alcohol for preservation. The wet weight of each *H. leucospilota* was measured in the laboratory. Wet weight of the sediment ingested, intestine, and respiratory tree was also measured (Table 2).

2.3. Microplastic analysis
Sediment ingested by the sea cucumber were dried in the oven (60°C) for 24 hours and sieved through a 5 mm pore diameter mesh to separate big particles [12]. Sediment was mixed with saturated NaCl solution. This mixing aims to float microplastic particles suspended in the sediment by density separation. Microplastic density separation works on low density plastics like polystyrene, polyethylene, and polypropylene. The mixture was homogenized for 2 minutes and left for 1 hour. Supernatant produced was put through a 38µm mesh [15]. Microplastic particles were observed optically using a microscope.

The intestine and respiratory tree were isolated using a knife, and put into Beaker glasses. They were soaked in a 65% HNO₃ solution for 24 hours to dissolve any organic materials. The solution was diluted 10 times from its original volume with aquadest. Microplastic particles were piped into a Sedgewick Raftet counting chamber and observed with a microscope.
Table 2. Wet weight of sea cucumber individuals, respiratory tree, intestine, and sediment ingested.

| Study sites | Sea cucumber (g) | Respiratory tree (g) | Intestine (g) | Sediment ingested (g) |
|-------------|------------------|----------------------|---------------|-----------------------|
| 1           | 92               | 2                    | 4             | 15                    |
|             | 80               | 1                    | 2             | 6                     |
|             | 80               | 2                    | 2             | 11                    |
|             | 91               | 2                    | 5             | 18                    |
|             | 77               | 4                    | 3             | 13                    |
| 2           | 127              | 5                    | 7             | 25                    |
|             | 95               | 4                    | 4             | 14                    |
|             | 68               | 1                    | 2             | 9                     |
|             | 103              | 3                    | 3             | 22                    |
|             | 94               | 4                    | 4             | 10                    |
| 3           | 78               | 1                    | 1             | 3                     |
|             | 70               | 2                    | 10            | 12                    |
|             | 160              | 8                    | 18            | 34                    |
|             | 65               | 4                    | 11            | 10                    |
|             | 34               | 1                    | 6             | 6                     |

3. Results and discussion

The amount of microplastic found in each organ and the ingested sediment differs (Table 3). Out of the 15 sea cucumbers analysed, microplastic particles has the highest amount in the respiratory tree, followed by the intestine, and sediment ingested by the sea cucumber. The respiratory tree dominantly contained plastic films with an average amount of 4.7 particles/g organ. Plastic fragments and pellets were more abundant in the intestine and sediment ingested by the sea cucumber. However, plastic films in the respiratory tree were found 8.5 and 14.7 times the amount of plastic films in both the intestine and sediment ingested. Plastic fibres and films are low density microplastics that could be found floating in the water [12]. Those particles could enter the sea cucumber’s respiratory tree during their respiration process because they extract oxygen by absorbing water through their anus [16]. That causes microplastic particles entanglement to the sea cucumber’s respiratory tree. Fragments and plastic pellets tend to sink and settles in the sediment, so both of those types of plastic are not commonly found in respiratory organs. Similar results were shown in the green clam *Perna viridis*, where fragments were found in the lowest amount whereas pellets were not found at all [17]. The highest amount of microplastic found in the crab *Carcinus maenas* was also in the respiratory organ, two hours after exposure to microplastic particles [18].

Tabel 3. Microplastic amount and types in organ of sea cucumber.

|                     | Microplastics amount (particles/g) |
|---------------------|------------------------------------|
|                     | Fiber    | Film    | Pellet   | Fragment | Foam    |
| Respiratory Tree    | 1.95     | 4.7     | 0.04     | 0.13     | 0       |
| Intestine           | 2.34     | 0.55    | 1.46     | 0.62     | 0       |
| Sediment Ingested   | 1.40     | 0.32    | 0.63     | 0.87     | 0.1     |

The correlation between microplastic amount in the intestine and sediment ingested was tested with the Spearman rank correlation test. It showed positive results (P=0.445), indicating that the accumulation of microplastics in tissues could happen. Microplastic particles contain some compounds which binds to fatty tissues of marine biotas [16]. Plastic films were not abundant in the intestine and sediment ingested, but fibre and pellets are found in high amounts. This suggest that plastic could be degraded to smaller sizes, so it increases the chance of plastic to stick or tangled to organs [18]. Plastic films found in both intestines and sediment consumed could come from floating films that went through biofouling.
processes. Biofouling process could happen for a couple of days until it finally sinks and settle at the sediment [19]. This long of a process could be the cause of the low amount of plastic films consumed by the sea cucumbers. Foam was only found in the sediment ingested. Foam and fragments maybe has low sticking forces to the organs. Each plastic polymer has different sticking forces or different surface adhesion. In addition, temperature gives impact to sticking force [20]. Film, pellets, and fibre possibly has a lower melting point than foam and fragments, so the three kinds of microplastic has a tendency to stick more to organs because they melt and get sticky.

Microplastic allegedly causes digestive blockage and damages tissues of the biotas accidentally consuming them [19]. Other than that, the feeding of 20,000 plastic particles/kg dry sediment to sea cucumber *Holothuria sanctori*, causes the decrease of eating frequency of the sea cucumber [21]. The feces production of sea cucumbers with purposely given mixture of sediment and microplastics are significantly lower than sea cucumbers given normal sediment [12]. The toxic effect of microplastics could be caused by the stress of plastic consuming itself, additive leakage from microplastics, and the pollutant on them, such as POPs (persistent organic pollutants) and heavy metal [4].

4. Conclusion
Microplastic types found in the sea cucumber consists of fibres, films, pellets, fragments, and foams. This study found that the respiratory tree contained the highest amount of microplastic particles, and the sediment consumed contained the lowest. The positive correlation between the microplastic amount in the intestines and sediment consumed shows that there is a possible accumulation of plastic particles from the environment. Microplastics and the compounds inside it could impact marine biota health, so it must be further studied.

Acknowledgment
This research was funded by the Grant of Indexed International Publications for Universitas Indonesia Students Final Projects (PITTA) 2018, and supported by the Coastal and Marine Resources: Environment and Management Research Group, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia.

References
[1] Derraik J G B 2002 *Mar. Pollut. Bull.* **44** 9
[2] Jambeck J R, Geyer C, Wilcox T R, Siegler M, Perryman A, Andrady R, Narayan and Law K L 2015 *Science* **347** 6223
[3] Browne M A, Galloway T and Thompson R 2007 *Integr. Environ. Assess. Manag.* **3** 559-61
[4] Andrady A L 2011 *Mar. Pollut. Bull.* **62** 1596-605
[5] Andersson E 2014 *Microplastics in the oceans and their effect on the marine fauna* (Swedia: SLU Departement of Biomedical Sciences and Veterinary Public Health) p 14
[6] Browne M A, Dissanayake A, Galloway T S, Lowe D M and Thompson R C 2008 *Environ Sci Technol* **42** 5026-31
[7] Lusher A L, McHugh M and Thompson R C 2013 *Mar. Pollut. Bull.* **67** 94-9
[8] Pham C K, Rodriguez Y, Dauphin A, Carriço R, Frias J P G L, Vandeperre F, Otero V, Santos M R, Martins H R, Bolten A B and Bjorndal K A 2017 *Mar. Pollut. Bull.* **121** 222-9
[9] Murray F and Cowie P R 2011 *Mar. Pollut. Bull.* **62** 1207—1217
[10] Lusher A L, Hernandez-Milian G, O’Brien J, Berrow S, O’Connor I and Officer R 2015 *Environ. Pollut.* **199** 185-91
[11] Graham E R and Thompson J T 2009 *J. Exp. Mar. Biol. Ecol.* **368** 22-9
[12] Assidqi K 2015 *The physiological impact of microplastics on HolothuriaLeucospilota* (Bogor: Institut Pertanian Bogor) p 38
[13] Wulandari N, Krisanti M and Elfidasari D 2012 *Unnes J. Life Sci.* **1** 134-9
[14] Miliou A, Höfer S, Maridakis C, Almeida M and Cox R2016 *Rapp.Comm. Int. Mer. Médit.* **41** 232
[15] Claessens M, De Meester S, Van Landuyt L, De Clerck K and Janseen C R 2011 Mar. Pollut. Bull. 62 2199-204
[16] Marine Education Society of Australasia 2015 Sea Cucumbers [online] retrieved from http://www.mesa.edu.au/echinoderms/echino05.asp
[17] Fathoniah I 2017 Kelimpahan mikroplastik pada kerang hijau Perna viridis, air, dan sedimen di Kamal Muara, Jakarta Utara (Depok: Universitas Indonesia) p 30
[18] Farrell P and Nelson K 2013 Environ. Pollut. 177 1-3
[19] Wright S L, Thompson R C and Galloway T S 2013 Environ. Pollut. 178 483-92
[20] Abbot S 2018 Particle adhesion [online] retrieved from https://www.stevenabbott.co.uk/practicaladhesion/particles.php
[21] Grossman J L 2014 Evaluating the potential of microplastics ingestion to harm the sea cucumber Holothuria sanctori (Hamburg: University of Hamburg) p 100