Cuttlefish (*Sepia pharaonis* Ehrenberg, 1831) as a bioindicator of microplastic pollution

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Abstract. Microplastics (MPs) have recently become a significant issue in ocean pollution, threatening food safety and human health. *Sepia pharaonis* or cuttlefish is one of the marine commodities consumed by humans requiring further analysis to determine whether or not they are contaminated by microplastics to evaluate their potential use as bioindicators. The abundance and characteristics (shape, size, and colour) of MPs were observed in the outer body, gills, intestines, and the correlations between MPs and total body length, mantle length, age, and sex were analysed. All samples were collected from the fish market surrounding the Fish Auction Market at Muara Angke Port, North Jakarta, Indonesia. MPs particles were found in the outer body and gills (226/mL) and the intestines (208/mL), with fibre, fragment, and film shapes, a size range of < 0.25 to 2.0 mm, and colours including black, transparent, blue, green, red, and yellow. The total MPs did not correlate (t_{count}1.401 < t_{table} 2.145) with the total body length, and there was also no difference in MPs between mantle length/age classes ($p > 0.05$). Female cuttlefish had higher levels of MPs than males. All cuttlefish samples were contaminated with MPs. However, there is no evidence yet as to whether the MPs will cause the cuttlefish to die or become harmful to human health, and further studies are needed on these potential impacts.
Key Words: Sepiidae, bioindicators, MP categories, mantle size, sexual disparity.

Introduction. Cuttlefish (*Sepia* sp.) has been a leading consumer product and fishery commodity in Indonesia, with a recorded production for 2012-2017 of 120 thousand tons (KKP 2018) and increasing trends to 2018 (FAO 2020). The species *Sepia pharaonis* Ehrenberg, 1831 is the main catch of fishermen in Muncar (Banyuwangi) and is also found in the Arafura Sea and the waters around Bali, Java, Kalimantan, Maluku, Papua, Sulawesi, and Sumatra (Ghofar 1999; Barrat & Allcock 2012; Setyohadi et al 2016; Tirtadanu & Suprapto 2016). Cuttlefish are a target or saleable by-catch in fisheries using trawls, special tools, or light-assisted gears including traps (Roper et al 1984). The cuttlefish caught in Javanese waters are then traded, often through the Muara Angke fish market in Jakarta.

The seas around Java Island have been contaminated by microplastics (MPs), which have been found in both the water and in sediments (Manalu et al 2017; Dwiyitno et al 2018; Lie et al 2018; Septian et al 2018; Widianarko & Hantoro 2018; Asadi et al 2019; Cordova et al 2019; Handyman et al 2019; Rahmad et al 2019; Yona et al 2019; Falahudin et al 2020; Priscilla & Patria 2020). MP particles have a size smaller than 5 mm and come from cosmetic products (e.g. facial scrubs), the plastics industry, pre-production resin pellets, microbeads in cosmetics, toothpaste, micro-sized powders for textile coatings, drug delivery media, macro plastic degradation, and the washing of synthetic textiles (Cole et al 2011; GESAMP 2015; He et al 2018; De Falco et al 2019; Dowarah & Devipriya 2019). Plastic waste tends to end up in the oceans and cause problems in the marine environment (Eriksen et al 2014; He et al 2018). Small and persistent MPs in the environment can contaminate various marine organisms (Bessa et al 2018); they can thus affect the safety of seafood products and the health of humans who consume them (Van Cauwenberghe & Janssen 2014; Barboza et al 2018).

Both invertebrates and vertebrates have been reported as contaminated by MPs (Desforges et al 2015; Davidson & Dudas 2016; Nelms et al 2019). Previous studies have shown that molluscs, cnidarians, annelids (Sfriso et al 2020), crustaceans, and small fish (Barboza et al 2018) have been contaminated with MPs in the form of fragments, fibers, films, and pellets (Naji et al 2018). Furthermore, in Indonesia MP contamination has been reported in bivalves (Fachruddin et al 2020), echinoderms (Lolodo & Nugraha 2019), molluscs (Angganararas 2019) and crustaceans (Restiani 2017). Reported impacts of MP consumption on marine organisms includes physical damage to the digestive organs, interference with breathing in the gills, and even death (Cole et al 2013; Su et al 2019), as well as reduced reproduction and disorders of the energy metabolism (Lei et al 2018).

Microplastic contamination can occur in the digestive organs of molluscs, including cuttlefish (cephalopods of the genus *Sepia*). Research on cultivated and wild caught *Sepia officinalis* found MP contamination in the stomach, digestive tract, and digestive glands (Oliveira et al 2020). Contamination in molluscs (in specific organs, individual sizes and sex), and the characteristics of MPs (type, shape, and size) can be used to provide information on environmental waste management, as pollution bio-indicators, and for food safety risk evaluation related to Indonesia's marine resources. However, there were no reports on MPs in cuttlefish from Indonesia. Therefore this research was conducted to evaluate the abundance and characteristics (shape, size, and colour) of MPs in cuttlefish caught in Indonesian waters around Java, as well as the correlation between mantle length, age, and sex with MPs found in the body surface, gills, and intestines, in order to determine whether cuttlefish can be used as a bioindicator and to evaluate the implications for food safety management.

Material and Method

Sample collection and preparation. A sample of 16 *S. pharaonis* specimens was obtained from the Muara Angke fish market (North Jakarta, Indonesia) from March to April 2020. The *S. pharaonis* sold in Muara Angke fish market are caught from the sea areas of Pemalang, Batang (Central Java Province), and Lambuan Panimbang (Banten Province). The sample criteria included no signs of decay, mantle colour and pattern still clearly visible, external and internal organs complete (Reid et al 2005). The samples were then frozen to -20^{o}C (Oliveira et al 2020) before analysis of morphological characters and MPs. Morphological characters were examined to determine sex, total length, mantle length, and age. Sex is determined by the size of the cuttlebone which is wider in females than males, as one function of the cuttlebone is as a place for eggs, as well as based on the reproductive system, with females having ovaries and oviduct glands while in males there are testes present (Huang et al 2018; Gestal et al 2019; Guerra-Marrerro et al 2019). The approximate age of *S. pharaonis* can be determined based on the length of the mantle, which increases with age (Nabhitabhata & Nilaphat 1999; Neethiselvan 2001). The *S. pharaonis* specimens were divided into 3 age/size classes: 5.6, 6, and 6.3 months.

Laboratory analysis. Analysis of MPs in cuttlefish comprised the following stages: (i) isolation of microplastics from outside of cuttlefish body; (ii) dissection; and (iii) lysis of digestive tissue with chemicals (Lusher et al 2017). (i) MPs were isolated from the outside of the body by flushing the whole body with 20 mL of saturated saline solution. (ii) The body was dissected to remove the gills and intestines/digestive organs which were then transferred to a Petri dish to prevent contamination from the air. (iii) MPs were isolated from organs using HNO_3 biogenic compounds as oxidizing agents (Claessens et al 2013). Gills and intestines were immersed for 2 days at room temperature in HNO_3 (70%) with a ratio of 1:5, enabling the separation of less dense particles such as MPs in the intestines and gills. The HNO_3 suspension was diluted using NaCl in a ratio of 1:4. The liquid in each organ was removed using a 1 mL dropper and the process was repeated 3 times.

Microplastic quantification and identification. Identification of MPs was based on shape, size, and colour of the particles, each having different characteristics and levels of toxicity, while the colour can resemble natural prey. MP shapes were divided into fibers, fragments, and films (Jiang et al 2018). MPs from each organ were identified and isolated from the liquid collected using light microscopy (400 x magnifications) and measured using an Olympus BX 50 (10x magnification). The MPs were divided into 5 size classes adapted from Abbasi et al (2018): < 0.25, 0.25-0.50, 0.51-2, 2-5, and > 5 mm. MP colour was divided into transparent, black, blue, red, green, and yellow (Abidli et al 2019). The number, shape, size, and colour of the MPs found were recorded and documented.

Data analysis. The composition of MPs found on the outside of the body, in the gills and the intestines was analysed descriptively based on shape, size, and colour. MP abundance was expressed as the number of MP particles per mL calculated by the equation:

$$\text{MP abundance} = \frac{\text{Number of MPs in organ}}{\text{Total MPs in all organs observed}} \times 100$$

The abundance of MPs by body and mantle length, age, and sex were analysed using one way Analysis of Variance (ANOVA) at the 95% confidence level ($p < 0.05$). Correlation of cuttlefish total body length with total MPs found was analysed using linear regression. Data analysis used IBM SPSS software v. 20.0 and Microsoft Excel.

Results and Discussion

MP abundance. The relative abundance of MP particles differed between the body surface, gills, and intestines of *S. pharaonis*. The outside of the body and gills had a higher relative abundance (34%) with a total of 226 particles mL^{-1} each compared to the digestive tract/intestines (32%) with a total of 208 particles mL^{-1} (Figure 1). These results indicate that MPs have polluted the environment or habitat of the cuttlefish, so that they could be used as a bioindicator.

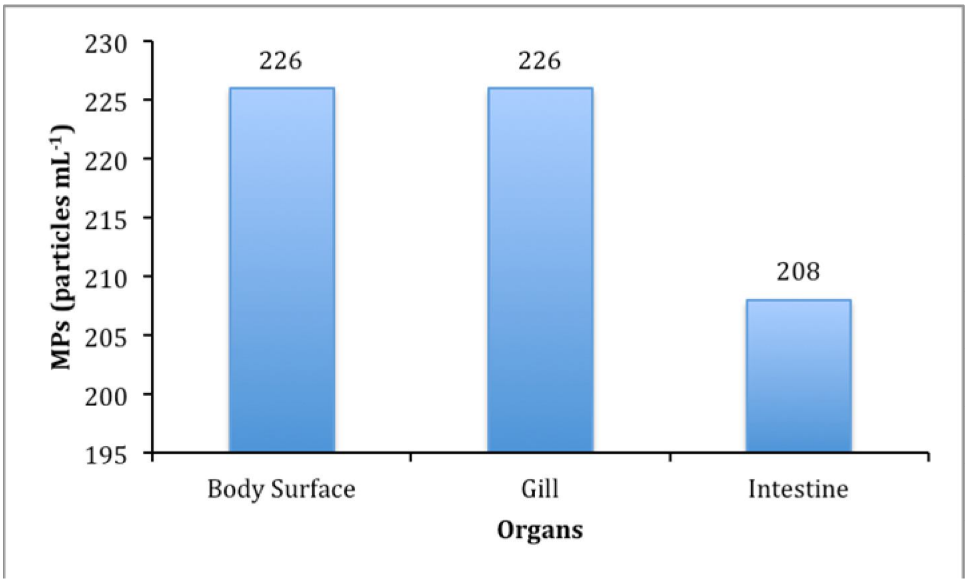

Figure 1. Distribution of MPs in *S. pharaonis* organs.

Marine organisms consumed by humans such as squid, cuttlefish, and octopus (Cephalopods) have been found to be contaminated with MPs in the internal and external organs. MPs have been reported in squid stomachs, with at least one particle in 12% of the 160 squid (*Dosidicus gigas*) examined by Rosas-Luis (2016); in the shells and body cavities (gills, ovaries, intestines and oviducts) of *Argonauta nouryi* with a total of 914 particles in one individual (Alejo-Plata et al 2019); and in the digestive tract of *Sepia officinalis* with 208 particles in six individuals (Oliveira et al 2020). Furthermore, MP particles were found in body tissue of 9 types of shellfish obtained from a fish market in

China with a density of 2.1-10.5 MPs g^{-1} and 4.3-57.2 MPs $individual^{-1}$ (Li et al 2015) as well as in the gills and digestive tract of *Carcinus maenas* due to consuming contaminants (Watts et al 2014). Seafood sold at the Paotere fish market, South Sulawesi, was found to be contaminated with MPS in the intestines of 28% of marine organisms tested (Rochman et al 2015), and demersal seafood and pelagic fish in Persia had 828 MP particles attached to the skin, muscles, gills, intestines, and liver (Abbasi et al 2018).

Contamination by MPs in *S. pharaonis* is caused by a combination of cuttlefish behaviour, contaminated habitat, movement of particles in the sea, and handling after capture before being traded. Contamination on surface parts of the body is thought to occur through MPs becoming attached due to mucus production by mucosal skin cells. The mucus layer around a cephalopod's body has a glue-like texture and in cuttlefish it is present on the surface of the mantle, suckers, and other parts of the body (Accogli et al 2017). Furthermore, unhygienic handling can lead to contamination of the materials used for preserving the catch; MP contamination on the outside of animals can also come from secondary contamination related to storage, sale, or transportation of fisheries catch sold on the fish market (Chan et al 2019).

Contamination occurs in the gills during respiration when seawater contaminated by MPs enters the gill lamellae during the absorption of O_2. This process has a high potential to enter the blood circulation system because the two hearts are connected to the gills so that MPs could enter the mantle cavity, which would pollute the outer and inner organs of *S. pharaonis*. MPs entering the gills through the ventilation process can then become attached to the gills (Watts et al 2014). Contamination found in the intestines is thought to have entered through respiration, a contaminated environment, consuming MPs resembling natural prey and prey that has been contaminated by MPs. The volume of food and liquid that enter the digestive tract will increase the amount of MP particles that enter the body accidentally and stick to the digestive tract of marine life (Priscilla et al 2019). Furthermore, MP consumption in marine invertebrates is related to the way they eat and MP particles concentrations in the area where they live (EFSA 2016).

Sepia pharaonis lives in the demersal zone and is an active predator (Sasikumar 2013) with a wide depth distribution including the deep sea, migrating to shallower waters at night and during the breeding season (Tehranifard & Dastan 2011). The behaviour of *S. pharaonis* can lead to MP contamination. Consumption of MPs is influenced by the environment and is more prevalent in bottom-dwelling organisms compared to pelagic organisms (Koongolla et al 2020) such as *Platycephalus indicus* and *Saurida tumbil,* accumulating on the skin, in muscles, gills, and digestive organs (Abbasi et al 2018). MP particles are more abundant in the deep sea compared to surface waters in the Atlantic Ocean, Mediterranean Sea, and Indian Ocean (Woodall et al 2014); this is caused by the degradation and fragmentation of plastics from various sources that become MPs and are carried to the open ocean (GESAMP 2015) then deposited on the seabed.

MPs on the body surface and in the internal organs of *S. pharaonis* indicate that their habitat has been contaminated. Previous studies have indicated that waste found in several areas around Java is dominated by plastics, including MPs (Maharani et al 2018; Rahmad et al 2019). Java is the most populous island in Indonesia, and this human population is a potential source of waste carried to the oceans. Furthermore, the waters around Java have some of the highest potential fisheries resources in Indonesia, with *S. pharaonis* being included in the 3 top export commodities from Indonesia (KKP 2018). Marine debris is thought to have an impact on the ecosystem, potentially affecting the abundance of *S. pharaonis* as well as toxic effects on these cuttlefish and the health of humans who consume them.

Characteristics of MPs. Three types of MP shape (fibre, fragment, and film) were found. Fibre was the predominant shape on the body surface, in the gills and in the intestines of the cuttlefish specimens examined, with 410 particles followed by fragments (211 particles), and film (38 particles). Characteristics of fibres are thin and string-like, elongated and cylindrical; fragments are hard, jagged, and irregular, while films are soft, and thin, often transparent. MP particles colours found were blue (9%), yellow (3%), red (6%), green (9%), black (44%), and transparent (29%), with black and transparent predominating. MPs found were mostly in sizes of < 0.25, 0.25-0.50 and 0.51-2 mm, with very few particles > 5 mm. The largest MPs found measured 0.438, 0.430 and 0.586 mm while the smallest measured 0.024, 0.026, and 0.022 mm (Figure 2).

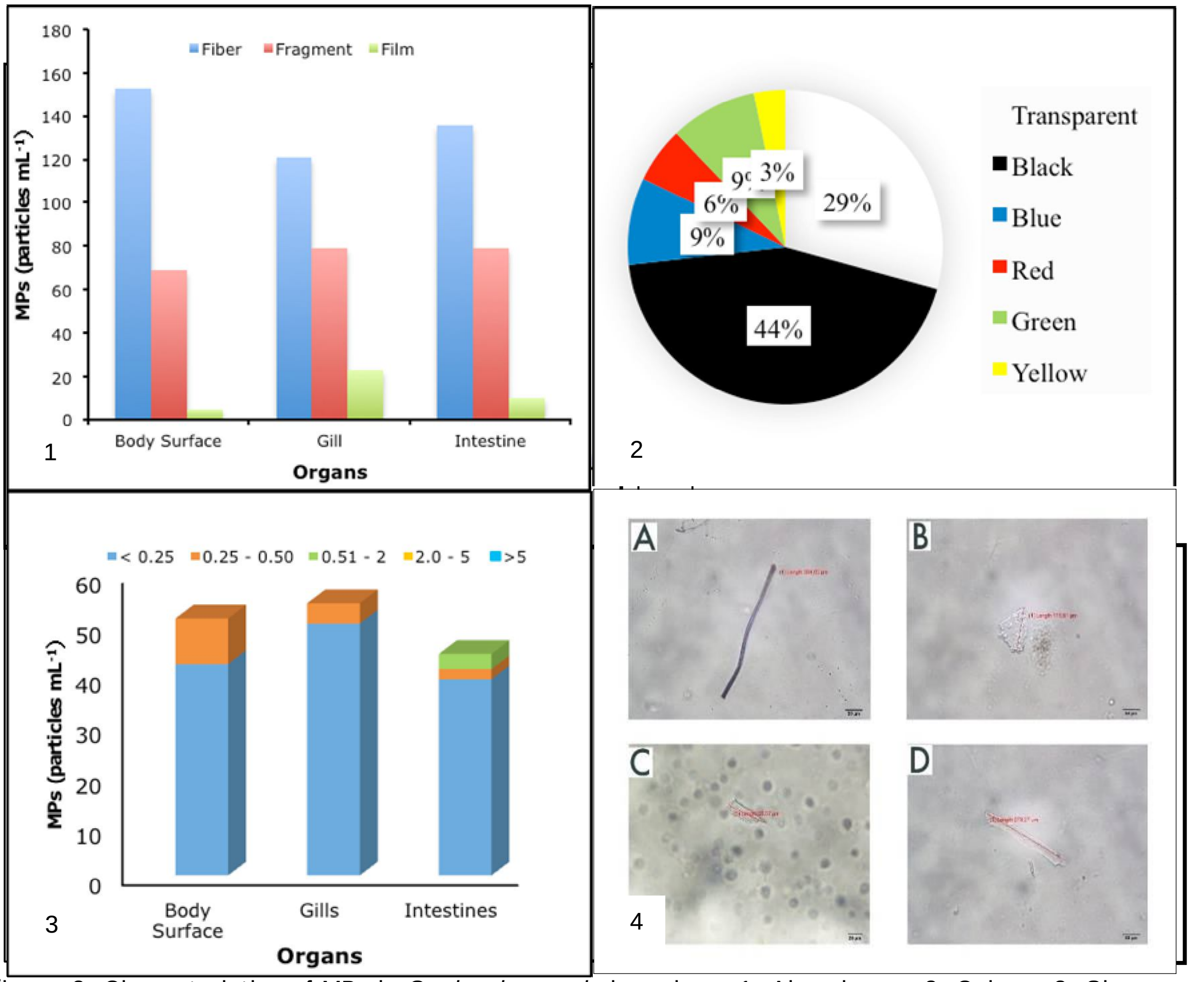

Figure 2. Characteristics of MPs in *Sepia pharaonis* based on: 1. Abundance; 2. Colour; 3. Size; and 4. Shape [scale µm: A - fibre, B & C - fragments; D - film].

MPs in fibre form are reported to be mostly found in sediments (Sathish et al 2019), water (Priscilla et al 2019), and marine organisms (James et al 2020), one of which is *S. pharaonis*. Shapes of MPs in cuttlefish were also found to be dominated by fibers, fragments, and microfilms by Oliveira et al (2020). The primary sources of fibre are materials from the textile industry and fishing equipment made from polypropylene (PP) and polyethylene (PE), all of which contribute to producing MPs in marine waters (Kor et al 2020). Meanwhile, fragments and films found were a mixture of polypropylene (PP) and polyethylene (PE) polymers derived from larger plastics which have been broken into debris over a long time. Fragments and films have been found from types of plastic waste such as damaged plastic bags, film (agricultural plastic waste), and floating plates (Zhou et al 2018). Plastics come in various shapes and types which cause the MPs found to have different sizes. Factors affecting MP size include the many physical and chemical characteristics that can affect the degradation of MPs (Septian et al 2018). Furthermore, the smaller the MP particles size, the greater the chance of being accidentally ingested by marine organisms, as seen by the way in which small particles can enter several internal organs of *S. pharaonis*.

The colour characteristics of MPs are similar to some foods or natural prey, so they can attract predators to eat. Colour of ingested MPs is associated with prey colour similarity where blue MPs can be mistakenly ingested by some copepod species, while white or transparent MPs are more often ingested by oysters and shellfish and black, white, light, and blue colours can be mistakenly ingested by marine fish (Tata et al 2020). Therefore, identification of MP colours can determine the potential for MP contamination based on a given organisms preferred behaviour (Wright et al 2013). Colours found in fish are dominated by black, green, and blue MPs in the Persian Gulf (Abbasi et al 2018) and blue, transparent and black MPs in the Mondego estuary, Portugal (Bessa et al 2018). MP colours are very diverse, whether they are found in sediments, water, or the organs of various organisms. Colour variability in MP particles can be attributed not only to the various origins of plastic waste but also to changes due to weathering (Sathish et al 2019).

Contamination by MP particles of various shapes can occur in fisheries, tourism, and human populations. Lack of awareness among the public about the dangers of MPs and the improper management of plastic waste more generally is a driver for particles pollution that will end up in the ocean. Human activities, tourism, and fishing are related to the distribution of macroplastics and MPs in the waters (Maharani et al 2018; Dowarah & Devipriya 2019). The distribution of plastic waste around Java Island varies in intensity from 30 to 70% with MPs of various shapes found in sediments, water, fish, and shellfish and dominated by fragments (Dwiyitno et al 2018). MPs in the shape of fibres, fragments, and films originating from household waste and anthropogenic activities were also found to be distributed in the seas around Tunda, Pangandaran, and Banyuurip Islands (Maharani et al 2018; Septian et al 2018; Ayuningtyas et al 2019).

Plastic equipment used in modern fishing is a contributor to MP pollution. Equipment used includes ropes, buoys, nets, fish boxes, gloves, straps, and others (Dowarah & Devipriya 2019). Other MP sources include inappropriate waste processing in the textile industry or household waste. Studies by Napper & Thompson (2016) show that 700,000 fibers will be released from washing 6 kg of cloth and enter the aquatic realm, including marine ecosystems. Textile industry spreads plastic fibre particles containing chemicals hazardous to the environment which can be toxic in water (Koongolla et al 2020) with the shapes and sizes of the particles affecting their toxicity to various organisms (Naji et al 2018). One potentially fatal impact on marine organisms is intestinal obstruction which occurs because MPs cannot be degraded (Kor et al 2020).

The size of different MPs affects the site of contamination in marine organisms. MPs found in gills and digestive tracts are often larger than those found in other organs, where the abundance and variation of MPs in the digestive organs can indicate variations in the amount and type of food consumed (Abbasi et al 2018). MPs particles in sizes of 50 and 100 µm were found in filter feeding invertebrates (Sfriso et al 2020), > 0.01-1 mm in bivalves, gastropods, and cephalopods (Abidli et al 2019), 0.5-1 mm in the digestive tract of the fish *Mugil cephalus* (Avio et al 2015) and 4-10 cm plastics were found in the stomachs of the squid *Dosidicus gigas* (Rosas-Luis 2016). The size of organisms affects the size of MPs consumed. Small organisms, e.g. anchovies and spiny oysters, were found to have MPs < 200 µm in their digestive tracts (Kazour et al 2019). Other results indicate that particles size does not always have a clear correlation with fish size because particles size differs in almost every specimen observed (Foekema et al 2013). Furthermore, small organisms such as *Danio rerio* have been found with MPs of various sizes and *Caenorhabditis elegans* were found with particles of 1 µm size (Lei et al 2018).

Due to their small size, MPs have a high capacity for movement so that they have great potential to enter the ecosystem and food chain. Size can affect bioavailability in the aquatic environment because MP surfaces can absorb pollutants that enter the water (Kor et al 2020) and attract and accumulate toxic metals (Sathish et al 2019). The hydrophobic properties of MPs can make it easier for organic contaminants to become attached to the particles and increase the MPs' surface area (Aliabad et al 2019).

Biological parameters and MPs contamination. The *S. pharaonis* sample obtained consisted of 7 males (43.75%) and 9 females (56.25%) with a total body length ranging from 31 to 58.5 cm and a mantle length of 8.5 to 14.5 cm. MP abundance was not significantly different between mantle length and age classes (ANOVA: $p > 0.05$), and the regression of total MPs against total body length showed no significant correlation (Figure 3.1; t_{count} 1.401 < t_{table} 2.145). However females were significantly more contaminated than males (Figure 3.2; ANOVA: $p < 0.05$). A previous study (Sbrana et al 2020) showed that MP contamination was more common in males than females of the fish *Boops boops*. Furthermore, they found that the males had more MP particles compared to females with 0.9 and 0.8 fibres g^{-1}, respectively and posited that differing levels of MP contamination by sex could be caused by differential habitat use, different behaviour between males and females, and anthropogenic stress.

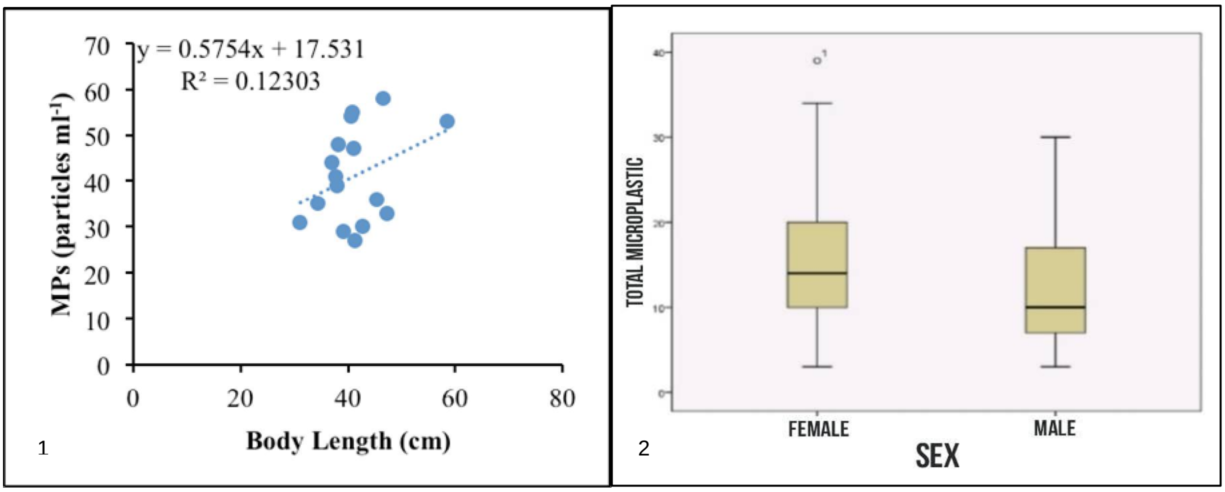

Figure 3. Correlation of body length with total MP abundance (1) and boxplots of total MP contamination in males and females (2).

Food safety. The use of plastics and other materials containing plastics has triggered various environmental polemics, many stemming from the accumulation of garbage and waste management systems that have not been well designed. The plastic pollution that is occurring in Indonesian waters is threatening various aspects of life. Plastics that have been degraded into MPs in the environment can be very dangerous, threatening the safety of seafood in Indonesia. As the plastic degradation process reaches new peaks, the abundance of MPs is set to become much greater than that of plankton in the ocean (Jovanović 2017). Small MPs have the potential to enter the bodies of marine organisms and enter the food chain. The lowest levels of trophic chains such as filter feeders and herbivores show the highest levels of MP contamination (Sfriso et al 2020).

Early prevention is needed, including through further studies on MP pollutants that enter the organisms that are consumed by humans. Humans have been found to have consumed up to 11,000 particles per year from molluscs contaminated with MP pollutants in European countries with high levels of shellfish consumption (Van Cauwenberghe & Janssen 2014). Consumption of shrimp (*Crangon crangon*) is estimated to have contaminated humans at levels from 15 to 175 MP particles per person per year (Devriese et al 2015). Several institutions have carried out MP research in seawater, sediment, and marine organisms used for human consumption. Research around Java Island (Indonesia) found MPs with various shapes, sizes, colours, raw plastics, and addictive substances contained therein (Hastuti et al 2014; Deng et al 2017; Widianarko & Hantoro 2018; Rahmadhani 2019; Azizah et al 2020; Sarasita et al 2020). Complex compound content in MPs will be hazardous for the marine environment and marine products obtained from our seas.

The MP particles found in *S. pharaonis* indicate that several areas in the Java Sea have experienced MP pollution. Indonesian marine products are very abundant but have been contaminated with MPs which have a high potential to damage natural ecosystems (KKP 2018; Widianarko & Hantoro 2018; Sarasita et al 2020). The decline in marine organism populations, health, and changes in behaviour coupled with bioaccumulation of pollution at higher trophic levels pose risks to humans who consume marine organisms (Barboza et al 2018; Carbery et al 2018). In particular, MPs could accumulate in the human body and cause health problems. Studies using mice (*Mus musculus*) exposed to MPs show that MPs can build up in the liver, kidneys, and intestines, causing several adverse effects including liver, energy, and lipid metabolism disorders, oxidative stress, and neurotoxic responses (Deng et al 2017). These cases raise concerns about the cellular toxicity of ingested MPs to humans, especially with respect to liver cells.

Content of active substances, accumulated toxins, and pathogenic microorganisms in MPs can be complex substances that will be very dangerous in the environment. The accumulation of toxic substances on MP particles can cause decreased appetite, decreased bodyweight, stunted growth, disorders of the reproductive system, reduced mobility, and even death of organisms consuming these MPs (Wang et al 2020). Furthermore, biomagnification and bioaccumulation of MP particles can occur in the human body, potentially disrupting human health, with effects including skin irritation, respiratory problems, diseases of the blood, disorders of the nervous system and consciousness, problems with digestion, and disorders of the reproductive system (Carbery et al 2018).

MP pollution should be a concern and efforts should be made to reduce the use of plastic in all aspects of human life. It is estimated that the amount of plastic production will increase 100-fold by 2050 if current trends continue (Rochman et al 2015). The Indonesian Ministry of Health, BPOM, and FAO-WHO have not regulated safe limits for MPs on food (Prasetyowati 2019 Litbangkes 2019). Prevention of MP contamination in seafood that is consumed requires a reference or stipulation by the government regarding the action level (AL) value (Widianarko & Hantoro 2018). This value is used to determine the standard limit or maximum threshold. If the MP concentration exceeds the threshold, actions can be taken to withdraw the product from the market or destroy the contaminated material. Monitoring should also be carried out in coastal and aquaculture areas. If the AL value exceeds the threshold, then a temporary closure should be implemented until there is a decrease in the AL value in the cultivation or fishing area.

Another solution is to start with public awareness in reducing plastic use and disposing of it appropriately. Furthermore, the government provides regulations regarding plastic use including bans on certain types and/or uses and supports managing plastic waste and hazardous materials. These efforts must be supported and implemented correctly by various parties to keep the natural environment intact. Food safety could then be maintained with good quality and quantity of marine products, creating a healthy life for sea life and humans.

Conclusions. MP contamination in *S. pharaonis* was found on the outside of the body, gills, and intestines. Characteristic shapes of MPs found were fibres, fragments, and films; size classes were dominated by the smallest (< 0.25 mm) and largest (> 0.586 mm) classes; and the predominant MP colours were black and transparent. There were no differences in MP contamination between mantle length, age, and total body length classes. Furthermore, no correlation was found between total body length and MP contamination. MP contamination was higher in females than in males. MP contamination in *S. pharaonis* indicates a threat to seafood safety, and further studies are needed on MPs in marine organisms, seawater, and sediment to determine the safe threshold for marine organisms. Thus, cuttlefish could be used as a bioindicator for MP contamination.

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References

Abbasi S., Soltani N., Keshavarzi B., Moore F., Turner A., Hassanaghaei M., 2018 Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. Chemosphere 205:80-87.

Abidli S., Lahbib Y., Trigui El Menif N., 2019 Microplastics in commercial molluscs from the lagoon of Bizerte (Northern Tunisia). Marine Pollution Bulletin 142:243-252.

Accogli G., Scillitani G., Mentino D., Desantis S., 2017 Characterization of the skin mucus in the common octopus *Octopus vulgaris* (Cuvier) reared paralarvae. European Journal of Histochemistry 61(3):204-214.

Alejo-Plata D. C. M., Herrera-Galindo E., Cruz-González D. G., 2019 Description of buoyant fibers adhering to *Argonauta nouryi* (Cephalopoda: Argonautidae) collected from the stomach contents of three top predators in the Mexican South Pacific. Marine Pollution Bulletin 142:504-509.

Aliabad K. M., Nassiri M., Kor K., 2019 Microplastics in the surface seawaters of Chabahar Bay, Gulf of Oman (Makran Coasts). Marine Pollution Bulletin 143:125-133.

Angganararas M., 2019 [Microplastic identification in squid from traditional markets in Semarang]. BSC thesis, Soegijapranata Catholic University, Semarang, 67 pp. [in Indonesian]

Asadi M. A., Ritonga Y. A. P., Yona D., Hertika A. M. S., 2019 Vertical distribution of microplastics in coastal sediments of Bama Resort, Baluran National Park, Indonesia. Nature Environment and Pollution Technology 18(4):1169-1176.

Avio C. G., Gorbi S., Regoli F., 2015 Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Marine Environmental Research 111:18-26.

Ayuningtyas W. C., Yona D., Julinda S. H., Irawati F., 2019 [Abundance of microplastic in the waters Banyuurip, Gresik, Jawa Timur]. Journal of Fisheries and Marine Research 3(1):41-45. [in Indonesian]

Azizah P., Ridlo A., Suryono C. A., 2020 [Microplastic in sediment of Kartini beach Jepara Regency, Central Java]. Journal of Marine Research 9(3):326-332. [in Indonesian]

Barboza L. G. A., Vethaak D. A, Lavorante B. R. B. O., Lundebye A. K., Guilhermino L., 2018 Marine microplastic debris: an emerging issue for food security, food safety and human health. Marine Pollution Bulletin 133:336-348.

Barratt I., Allcock L., 2012 *Sepia pharaonis*. The IUCN Red List of Threatened Species 2012: e.T162504A904257.

Bessa F., Barría P., Neto J. M., Frias J. P. G. L., Otero V., Sobral P., Marques J. C., 2018 Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine Pollution Bulletin 128:575-584.

Carbery M., O'Connor W., Palanisami T., 2018 Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environment International 115:400-409.

Chan H. S. H., Dingle C., Not C., 2019 Evidence for non-selective ingestion of microplastic in demersal fish. Marine Pollution Bulletin 149:110523.

Claessens M., Van Cauwenberghe L., Vandegehuchte M. B., Janssen C. R., 2013 New techniques for the detection of microplastics in sediments and field collected organisms. Marine Pollution Bulletin 70(1-2):227-233.

Cole M., Lindeque P., Halsband C., Galloway T. S., 2011 Microplastics as contaminants in the marine environment: a review. Marine Pollution Bulletin 62(12):2588-2597.

Cole M., Lindeque P., Fileman E., Halsband C., Goodhead R., Moger J., Galloway T. S., 2013 Microplastic ingestion by zooplankton. Enviromental Science and Technology 47(12):6646-6655.

Cordova M. R., Purwiyanto A. I. S., Suteja Y., 2019 Abundance and characteristics of microplastics in the northern coastal waters of Surabaya, Indonesia. Marine Pollution Bulletin 142:183-188.

Davidson K., Dudas S. E., 2016 Microplastic ingestion by wild and cultured Manila clams *Venerupis philippinarum* from Baynes Sound, British Columbia. Archives of Environmental Contamination and Toxicology 71:147-156.

De Falco F., Di Pace E., Cocca M., Avella M., 2019 The contribution of washing processes of synthetic clothes to microplastic pollution. Scientific Reports 9:6633.
Deng Y., Zhang Y., Lemos B., Ren H., 2017 Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Scientific Reports 7:46687.
Desforges J. P., Galbraith M., Ross P. S., 2015 Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Archives of Environmental Contamination and Toxicology 69:320-330.
Devriese L. I., van der Meulen M. D., Maes T., Bekaert K., Paul-Pont I., Frère L., Robbens J., Vethaak A. D., 2015 Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Marine Pollution Bulletin 98(1-2):179-187.
Dowarah K., Devipriya S. P., 2019 Microplastic prevalence in the beaches of Puducherry, India and its correlation with fishing and tourism/recreational activities. Marine Pollution Bulletin 148:123-133.
Dwiyitno, Wibowo S., Januar H. I., Andayani F., Yusuf G., Barokah G. R., Putri A. K., 2018 [Threats of marine debris and microplastic contamination in aquatic environment and fishery products]. Research and Product Processing Center Marine and Fisheries Biotechnology, No. PB04-4-2018. Jakarta. [in Indonesian]
EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2016 Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 14(6):e04501.
Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Ryan P. G., 2014 Plastic pollution in the World' s Oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLOS ONE 9(12):e111913.
Fachruddin L., Yaqin K., Iin R., 2020 [Comparison of two methods of analyzing microplastic concentrations of green mussels, *Perna viridis*, and their application in ecotoxicological studies]. Jurnal Pengelolaan Perairan 3(1):1-13. [in Indonesian]
Falahudin D., Cordova M. R., Sun X., Yogaswara D., Wulandari I., Hindarti D., Arifin Z., 2020 The first occurrence, spatial distribution and characteristics of microplastic particles in sediments from Banten Bay, Indonesia. Science of the Total Environment 705:135304.
FAO, 2020 Fishery statistical collections: fishery commodities and trade. Available at: http://www.fao.org/fishery/statistics/global-commodities-production/en. Accessed October, 2020.
Foekema E. M., De Gruijter C., Mergia M. T., van Franeker J. A., Murk A. J., Koelmans A. A., 2013 Plastic in North Sea fish. Environmenrtal Science and Technology 47(15):8818-8824.
GESAMP, 2015 Sources, fate and effects of MP in the marine environment: a global assessment. IMO, FAO, UNESCO-IOC, UNIDO, WMO, IAEA, UN, UNEP, UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Reports and Studies GESAMP No. 90:96 pp.
Gestal C., Pascual S., Guerra A., Fiorito G., Vieites J. M., 2019 Handbook of pathogens and diseases in cephalopods. Springer International, 230 pp.
Ghofar A., 1999 Indonesian seas cephalopods: a collection of fisheries laboratories Diponegoro University. Journal of Coastal Development 2(2):347-354.
Guerra-Marrero A., Jimenez-Alvarado D., Hernandez-García V., Curbelo-Muñoz L., Castro-Hernandez J. J., 2019 Cuttlebone morphometrics and sex identification of *Sepia bertheloti* (d'Orbigny, 1835) from the Central-East Atlantic. Helgoland Marine Research 73:10.
Handyman D. I. W., Purba N. P., Pranowo W. S., Harahap S. A., Dante I. F., Yuliadi L. P. S., 2019 Microplastics patch based on hydrodynamic modeling in the North Indramayu, Java Sea. Polish Journal of Environmental Studies 28(1):135-142.
Hastuti A. R., Yulianda F., Wardianto Y., 2014 [Spatial distribution of marine debris in mangrove ecosystem of Pantai Indah Kapuk, Jakarta]. Bonorowo Wetlands 4(2):94-107. [in Indonesian]

He D., Luo Y., Lu S., Liu M., Song Y., Lei L., 2018 Microplastics in soils: analytical methods, pollution characteristics and ecological risks. Trends in Analytical Chemistry 109:163-172.

Huang J. D., Lee S. Y., Chiang T. Y., Lu C. C., Lee M. F., 2018 Morphology of reproductive accessory glands in female *Sepia pharaonis* (Cephalopoda: Sepiidae) sheds light on egg encapsulation. Journal of Morphology 279(8):1120-1131.

James K., Vasant K., Padua S., Gopinath V., Abilash K. S., Jeyabaskaran R., Babu A., John S., 2020 An assessment of microplastics in the ecosystem and selected commercially important fishes off Kochi, south eastern Arabian Sea, India. Marine Pollution Bulletin 154:111027.

Jiang C., Yin L., Wen X., Du C., Wu L., Long Y., Liu Y., Ma Y., Yin Q., Zhou Z., Pan H., 2018 Microplastics in sediment and surface water of West Dongting Lake and South Dongting Lake: abundance, source and composition. International Journal of Environmental Research and Public Health 15(10):2164.

Jovanović B., 2017 Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environmental Assessment and Management 13(3):510-515.

Kazour M., Jemaa S., Issa C., Khalaf G., Amara R., 2019 Microplastics pollution along the Lebanese coast (Eastern Mediterranean Basin): occurrence in surface water, sediments and biota samples. Science of the Total Environment 696:133933.

KKP, 2018 [Indonesian fishery productivity evaluation of the 2017 budget implementation]. Directorate General of Strengthening the Competitiveness of Marine and Fishery Products, Jakarta, 49 pp. [in Indonesian]

Koongolla J. B., Lin L., Pan Y. F., Yang C. P., Sun D. R., Liu S., Xu K. R., Maharana D., Huang J. S., Li H. X., 2020 Occurrence of microplastics in gastrointestinal tracts and gills of fish from Beibu Gulf, South China Sea. Environmental Pollution 258:113734.

Kor K., Ghazilou A., Ershadifar H., 2020 Microplastic pollution in the littoral sediments of the northern part of the Oman Sea. Marine Pollution Bulletin 155:111166.

Lei L., Wu S., Lu S., Liu M., Song Y., Fu Z., Shi H., Raley-Susman K. M., He D., 2018 Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans.* Science of the Total Environment 619-620:1-8.

Li J., Yang D., Li L., Jabeen K., Shi H., 2015 Microplastics in commercial bivalves from China. Environmental Pollution 207:190-195.

Lie S., Suyoko A., Effendi A. R., Ahmada B., Aditya H. W., Sallima I. R., Arisudewi N. P. A. N., Hadid N. I., Rahmasari N., Reza A., 2018 Measurement of microplastic density in the Karimunjawa National Park, Central Java, Indonesia. Ocean Life 2(2):54-58.

Lolodo D., Nugraha W. A., 2019 [Microplastics on sea urchine from Sumenep Labak Gili Islands]. Indonesian Journal of Marine Science and Technology 12(2):112-122. [in Indonesian]

Lusher A. L., Welden N. A., Sobral P., Cole M., 2017 Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods 9:1346-1360.

Maharani A., Purba N. P., Faizal I., 2018 Occurrence of beach debris in Tunda Island, Banten, Indonesia. E3S Web of Conferences 47:04006.

Manalu A. A., Hariyadi S., Wardiatno Y., 2017 Microplastics abundance in coastal sediments of Jakarta Bay, Indonesia. AACL Bioflux 10(5):1164-1173.

Nabhitabhata J., Nilaphat P., 1999 Life cycle of cultured pharaoh cuttlefish, *Sepia pharaonis* Ehrenberg, 1831. Phuket Marine Biological Center Special Publication 19(1):25-40.

Naji A., Nuri M., Vethaak A. D., 2018 Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environmental Pollution 235:113-120.

Napper I. E., Thompson R. C., 2016 Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Marine Pollution Bulletin 112(1-2):39-45.

Neethiselvan N., 2001 A new species of cuttlefish *Sepia ramani* sp. nov. (Class: Cephalopoda) from Tuticorin Bay, southeast coast of India. Indian Journal of Marine Sciences 30:81-86.
Nelms S. E., Barnett J., Brownlow A., Davison N. J., Deaville R., Galloway T. S., Lindeque P. K., Santillo D., Godley B. J., 2019 Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Scientific Reports 9(1):1075.
Oliveira A. R., Sardinha-Silva A., Andrews P. L. R., Green D., Cooke G. M., Hall S., Blackburn K., Sykes A. V., 2020 Microplastics presence in cultured and wild-caught cuttlefish, *Sepia officinalis.* Marine Pollution Bulletin 160:111553.
Prasetyowati H., 2019 [Facts about microplastics]. InsIDE Media Inspirasi dan Ide Litbangkes 27:6-9. [in Indonesian]
Priscilla V., Patria M. P., 2020 Comparison of microplastic abundance in aquaculture ponds of milkfish *Chanos chanos* (Forsskål, 1775) at Muara Kamal and Marunda, Jakarta Bay. IOP Conference Series: Earth and Environmental Science 404:012027.
Priscilla V., Sedayu A., Patria M. P., 2019 Microplastic abundance in the water, seagrass, and sea hare *Dolabella auricularia* in Pramuka Island, Seribu Islands, Jakarta Bay, Indonesia. Journal of Physics: Conference Series 1402(3):033073.
Rahmad S., Purba N., Agung M., Yuliadi L., 2019 [Characteristic of microplastic debris at estuary of DKI Jakarta]. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan 8(1):9-17. [in Indonesian]
Rahmadhani F., 2019 [Identification and analysis of microplastic content in pelagic and demersal fish as well as sediment and sea water in the waters of Mandangin Island, Sampang Regency]. Undergraduate Thesis, Sunan Ampel State Islamic University, Surabaya, 69 pp. [in Indonesian]
Reid A., Jereb P., Roper C. F. E., 2005 Cuttlefishes. In: Cephalopods of the world: an annotates and illustrated catalogue of cephalopod species known to date. Jereb P., Roper C. F. E. (eds). FAO, Rome, Species Catalogue for Fishery Purposes No. 4, Vol. 1, pp. 56-152.
Restiani B. R., 2017 [Initial study of microplastic in sediment, water, and white shrimp from pond in Tapak Semarang]. Undergraduate Thesis of Food Technology. Soegijapranata Catholic University, Semarang, 57 pp. [in Indonesian]
Rochman C. M., Tahir A., Williams S. L., Baxa D. V., Lam R., Miller J. T., Teh F. C., Werorilangi S., Teh S. J., 2015 Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Scientific Reports 5:14340.
Roper C. F. E., Sweeney M. J., Nauen C. E., 1984 Cephalopods of the world: an annotated and illustrated catalogue of species of interest to fisheries. FAO, Rome, Fisheries Synopsis No. 125, vol. 3, 277 pp.
Rosas-Luis R., 2016 Description of plastic remains found in the stomach contents of the jumbo squid *Dosidicus gigas* landed in Ecuador during 2014. Marine Pollution Bulletin 113(1-2):302-305.
Sarasita D., Yunanto A., Yona D., 2020 [Microplastic abundance in four different species of commercial fishes in Bali Strait]. Jurnal Iktiologi Indonesia 20(1):1-12. [in Indonesian]
Sasikumar G., 2013 Fishery and biology of pharaoh cuttlefish *Sepia pharaonis*. Central Marine Fisheries Research Institute, Mangalore, pp. 7-10.
Sathish N., Jeyasanta K. I., Patterson J., 2019 Abundance, characteristics and surface degradation features of microplastics in beach sediments of five coastal areas in Tamil Nadu, India. Marine Pollution Bulletin 142:112-118.
Sbrana A., Valente T., Scacco U., Bianchi J., Silvestri C., Palazzo L., de Lucia G. A., Valerani C., Ardizzone G., Matiddi M., 2020 Spatial variability and influence of biological parameters on microplastic ingestion by *Boops boops* (L.) along the Italian coasts (Western Mediterranean Sea). Environmental Pollution 263:114429.
Septian F. M., Purba N. P., Agung M. U. K., Yuliadi L. P. S., Akuan L. F., Mulyani P. G., 2018 [Microplastic spatial distribution in sediment at Pangandaran Beach, West Java]. Jurnal Geomaritim Indonesia 1(1):1-8. [in Indonesian]

Setyohadi D., Sunardi, Mukhlis N., Cahya N. C., 2016 Cuttlefish (*Sepia* spp.) identification and biological analysis of a dominant cuttlefish species landed in Muncar, Banyuwangi Regency, East Java. Research Journal of Life Science 3(2): 109-118.

Sfriso A. A., Tomio Y., Rosso B., Gambaro A., Sfriso A., Corami F., Rastelli E., Corinaldesi C., Mistri M., Munari C., 2020 Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). Environment International 137:105587.

Su L., Deng H., Li B., Chen Q., Pettigrove V., Wu C., Shi H., 2019 The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of East China. Journal of Hazardous Materials 365:716-724.

Tata T., Belabed B. E., Bououdina M., Bellucci S., 2020 Occurrence and characterization of surface sediment microplastics and litter from North African coasts of Mediterranean Sea: preliminary research and first evidence. Science of the Total Environment 713:136664.

Tehranifard A., Dastan K., 2011 General morphological characteristics of the *Sepia pharaonis* (Cephalopoda) from Persian gulf, Bushehr region. International Conference on Biomedical Engineering and Technology 11:120-126.

Tirtadanu, Suprapto, 2016 [Distribution of squid (Loliginidae) and cuttlefish (Sepiidae) caught trawling in the Arafura Sea]. Pelagic Fisheries Management National Seminar. Marine Resources Exploration and Management, Fakultas Perikanan dan Ilmu Kelautan, Universitas Brawijaya, pp. 77-81. [in Indonesian]

Van Cauwenberghe L., Janssen C. R., 2014 Microplastics in bivalves cultured for human consumption. Environmental Pollution 193:65-70.

Wang W., Ge J., Yu X., 2020 Bioavailability and toxicity of microplastics to fish species: a review. Ecotoxicology and Environmental Safety 189:109913.

Watts A. J. R., Lewis C., Goodhead R. M., Beckett S. J., Moger J., Tyler C. R., Galloway T. S., 2014 Uptake and retention of microplastics by the shore crab *Carcinus maenas.* Environmental Science and Technology 48(15):8823-8830.

Widianarko B., Hantoro I., 2018 [Microplastic in seafood from the north coast of Java]. Soegijapranata Catholic University Press, Semarang, 88 pp. [in Indonesian]

Woodall L. C., Sanchez-Vidal A., Canals M., Paterson G. L. J., Coppock R., Sleight V., Calafat A., Rogers A. D., Narayanaswamy B. E., Thompson R. C., 2014 The deep sea is a major sink for microplastic debris. Royal Society Open Science 1:140317.

Wright S. L., Thompson R. C., Galloway T. S., 2013 The physical impacts of microplastics on marine organisms: a review. Environmental Pollution 178:483-492.

Yona D., Sari S. H. J., Iranawati F., Bachri S., Ayuningtyas W. C., 2019 Microplastics in the surface sediments from the eastern waters of Java Sea, Indonesia. F1000Research 8:98.

Zhou Q., Zhang H., Fu C., Zhou Y., Dai Z., Li Y., Tu C., Lou Y., 2018 The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. Geoderma 322:201-208.

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