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ORIGINAL RESEARCH ARTICLE

Microplastic contamination and growth pattern of oyster; Crassostrea gigas in a coastline

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ABSTRACT

BACKGROUND AND OBJECTIVES: Oysters (Crassostrea gigas) are one food source commonly consumed by the community and an important commodity with high economic value. Environmental issues, such as microplastics, have become a worldwide concern for its implications for aquatic organisms, especially oysters. This study aims to identify the microplastics and growth patterns of oysters in Aceh Province, Indonesia's north and east coasts. This study aims to determine which oysters are suitable for consumption and food health based on research locations along the east-north coast of Aceh Province.

METHODS: The locations in this study include nine regencies/cities, which are directly facing the Malacca Straits. Microplastic isolation from oysters using 10 percent potassium hydroxide and incubation process were done to dissolve the organic materials. The growth pattern was analyzed to determine the growth rate of oysters at each study location.

FINDINGS: The results of this study showed that oysters in all sampling locations were contaminated with microplastics, with a high prevalence of 48 percent found in Langsa, followed by Aceh Timur and Pidie each (40 percent), Banda Aceh (38 percent), Aceh Utara (32 percent), Aceh Besar and Bireun (30 percent), Lhokseumawe (12 percent), and Aceh Tamiang (8 percent). The analysis of the growth patterns revealed that the growth of oysters at each location was not optimal (b <3 or negative allometric).

CONCLUSION: In 500 oyster samples collected, 139 were contaminated with microplastics. The most dominant type of microplastic contaminating oysters is fiber up to 170 particles, followed by films 28 particles, and fragments 19 particles. Negative allometric growth pattern might correlate with microplastics that contaminate the waters and enter the oyster's digestive organs. The results of this study reveal that oysters consumed by people have been contaminated with microplastics, so stakeholders must carry out socialization for early prevention to be realized.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
52	4	5

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INTRODUCTION

Plastic overuse in daily activities has become a serious environmental problem worldwide (Singh and Sharma, 2016; Nielsen *et al.*, 2020). It is estimated that every year, around 155–266 million tons of plastic are produced, with 19–23 million tons ending up in the sea (Onoja *et al.*, 2022), leading the plastic waste the most dominant type of waste, which is around 80 percent (%) of all waste in the sea (Alabi *et al.*, 2019). Plastic waste accumulating in the sea threatens the survival of marine biota, as it can be swallowed or entangled around the biota (Kurtela and Antolovic, 2019). Marine biota that ingests plastic waste will disrupt their digestive system and cause death (Cole *et al.*, 2011). The plastic waste entangled in the marine biota will interfere with the growth and development process, movement, and reproductive and eating activities (Hammer *et al.*, 2012; Vermeiren *et al.*, 2016). Fragmented plastic waste will form microplastics with a size <5 millimeter (mm) (Patti *et al.*, 2020). Microplastics can be divided into two groups: the primary microplastics originating from polyethylene fibers found in beauty products, cleansers, toothpaste, etc., and secondary microplastic originating from decomposing larger plastics which then accumulate in the sea (Moore, 2008). Microplastics can enter the digestive system of a marine organism due to an error in the food identification process or the existence of a food chain associated with microplastics (Mercogliano *et al.*, 2020). Microplastics can also enter the gill organs, causing blockages in the biota's blood flow, disrupting the oxygen distribution, antioxidant capacity, oxidation, neurotoxicity, and immune response (Kim *et al.*, 2021). Microplastics in aquatic organisms can also affect the immune system, causing tissue damage, nervous system disorders, and a negative impact on reproduction (Wang *et al.*, 2021). Generally, high concentrations of microplastics in fish, mollusca, or marine biota can also impact humans through the food chain, causing health problems in the community (Browne *et al.*, 2013). Danopoulos *et al.* (2020) showed that the highest microplastic content found in shellfish has an average amount of 0–10.5 microplastics per gram (MPs/g), followed by 0.1–8.6 MPs/g in crustaceans and 0–2.9 MPs/g in fish. This content value influences the rate of microplastics spread to humans because shellfish are generally consumed whole, unlike fish (except for small ones), which are first separated from their stomachs and intestines, where microplastics are stored in the fish's body. *Crassostrea gigas* is one kind of shellfish susceptible to microplastic contamination. Oysters have a way of eating as a filter feeder (Kasmini *et al.*, 2019a). Adult oysters can filter up to 50 gallons of seawater daily (Koenig, 2018). This fact showed that oysters are more susceptible to microplastic contamination than other marine biotas, especially in waters that microplastics have polluted, including Indonesia. With the status of the second largest producer of plastic waste in the world (Eko and Dwiyitno, 2020), Indonesia's sea waters have been recorded as contaminated with microplastics. Microplastic contamination in Indonesia's seawater was shown in the research of Meirenno *et al.* (2022) in Tambak Lorok, Semarang City, which found that microplastics contaminated oysters. Research on microplastic contamination in oysters is still relatively rare in Indonesia, especially in Aceh waters. This research is crucial considering that Aceh produces and consumes a high amount of oysters and can potentially become an export commodity from Aceh (Razali, 2019). Monitoring this issue to maintain the quality of oysters is crucial. Aceh waters have been recorded as contaminated with microplastics. The research results of the Nusantara River Expedition Team, who conducted a water quality and microplastic contamination test on the Krueng Aceh River from upstream (Aceh Besar) to downstream (Banda Aceh), concluded that the river had been contaminated with microplastics, with the concentrations getting high as the water flows to the downstream (Habibi, 2022). The same conclusion was also obtained based on research at Krueng, Langsa, wherein the river had been contaminated with microplastics and was considered unfit for consumption (Waspada, 2022). Microplastics in the river will flow into the sea, contaminating Aceh's sea waters. Hence, it has the potential to contaminate oysters, especially in the north-east coast of Aceh. This study aims to identify microplastic content in oysters in the north-east coast of Aceh. The current study aims to identify microplastics and growth patterns of *Crassostrea gigas*. This study was carried out in Aceh Province, Indonesia's north and east coasts in 2022.

MATERIALS AND METHODS

Time and location

This study was conducted along the north and east coasts of Aceh Province, which led to the water of the Malacca Strait (Fig. 1). Sample collection was done from June to August 2022. The microplastic identification process was conducted at the Marine Biology Laboratory, Faculty of Marine and Fisheries, Universitas Syiah, Banda Aceh, Indonesia. The description of the numbers in the figure can be seen in Table 1.

Sample collections

Purposive sampling determined the sampling locations in each district along the north and east of Aceh Province. Fifty oyster samples were collected in each visited district. Sampling was carried out using shovels, machetes, and knives to separate oysters from its attachment to hard media such as stone, wood, and concrete. The collected samples were then placed into plastic samples and coded according to the sampling location. The packaged samples were stored in a styrofoam box and then transported to the Laboratory for further analysis.

Microplastic extractions

Before the extraction process, the total length of the sample was measured using a digital caliper (Error = 0.01 mm), and the weight was measured using a digital scale (Error = 0.01 g). Accordingly, the oysters were dissected to separate the shell and flesh, soaked in a 10% potassium hydroxide (KOH) solution (1:10 ratio), and then incubated for 12 hours at 60 degrees Celsius (°C) (Karami *et al*., 2017). The meat that decomposes into liquid is filtered using Whatman filter paper (No. 540) assisted by a vacuum

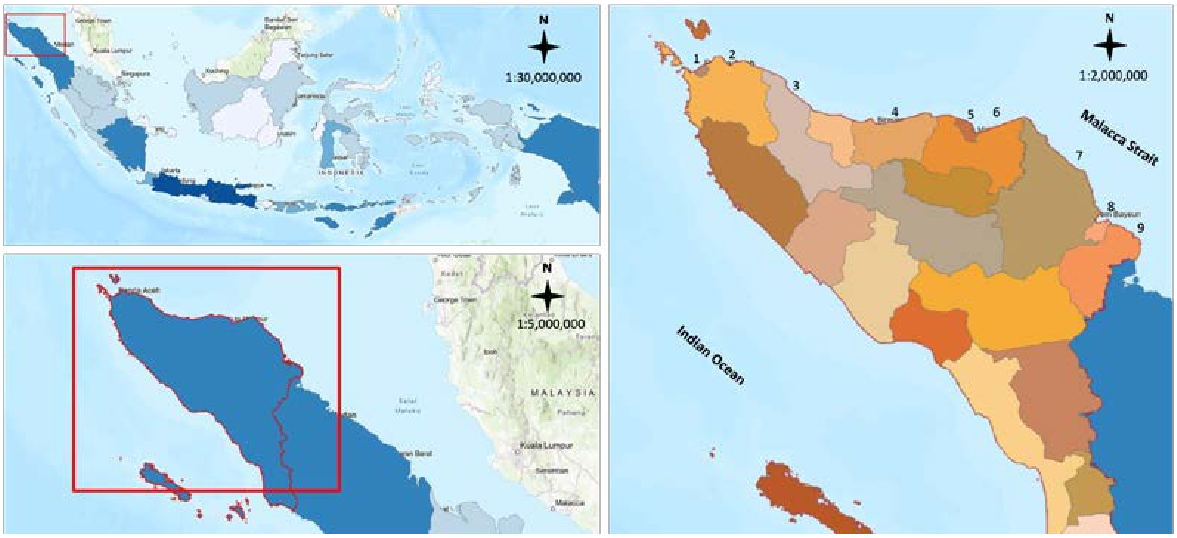

Fig. 1: Geographic location of the study area in the north and east coasts of Aceh Province in Indonesia.

Table 1: Location and coordinates of the study.

No.	Location	Coordinate
1.	Banda Aceh	5^{0}33’1.782”N 95^{0}17’12.128”E
2.	Aceh Besar	5^{0}37’18.258”N 95^{0}23’53.407”E
3.	Pidie	5^{0}24’18.663”N 95^{0}56’6. 853”E
4.	Bireuen	5^{0}14’1.493”N 96^{0}41’42. 54”E
5.	Lhoksemawe	5^{0}9’54.772”N 97^{0}8’32.899”E
6.	Aceh Utara	5^{0}11’46.199”N 97^{0}22’5.571 “E
7.	Aceh Timur	4^{0}40’32.543”N 97^{0}55’0.465”E
8.	Langsa	4^{0}51’89.082”N 98^{0}01’55.416”E
9.	Aceh Tamiang	4^{0}45’52.175”N 98^{0}13’89.004”E

machine. The compound results from that filter were then incubated at 50^{0}C for 5 hours to evaporate the liquid on the filter paper. The dried filter paper was stored in a petri dish for microplastic identification through a microscope examination.

Observational parameters

a. *Intensity and prevalence of microplastic*

Microplastic particle identification and measurement were carried out using binocular (Zeiss Primo Star) and stereo (Meiji Techno EM-32) microscopes. Calculation of microplastic particles was carried out based on the type (fragments, fibers, and films), according to Song *et al*. (2015). The prevalence and intensity were analyzed using the formula Karami *et al*. (2018) and Roch *et al*. (2019), where Prevalence = Total sample contaminated by microplastic/Total sample x 100; Intensity = Total amount of microplastic/Total sample contaminated by microplastic.

b. *Length-weight relationship (LWRs)*

Length-weight relationship analysis was done using Linear Allometric Model (LAM) using Eq. 1 (Muchlisin *et al*., 2010).

$$W = a.L^b \quad (1)$$

Where,

W = oyster weight (g), L = oyster length (mm), a and b = constant.

RESULTS AND DISCUSSION

500 oyster samples (50 samples from each location), with a total length between 14–98 mm and a range of weight between 6.3–95.42 g, were collected during the study at 9 research locations. Based on the size of the oysters collected during the study, it was estimated that they were 1–2 years old, according to the previous study of Lili *et al.* (2019b). The results from the analysis showed that oysters collected in Langsa accumulated microplastics reaching 48%, followed by Aceh Timur and Pidie each 40%, Banda Aceh 38%, Aceh Utara 32%, Aceh Besar and Bireun 30% each, Lhokseumawe 12%, and Aceh Tamiang 8% (Table 2).

The 139 oysters contaminated with microplastics yielded a total of 28 films, 170 fibers, and 19 fragments, where detailed data on microplastic types by location, as Table 3 shows. The dominance of fiber-type microplastics is possibly due to the high concentration of these contaminants in the waters at the research location, originating from laundry house waste. Previous research revealed that fiber-type microplastics generally come from polyester textiles for people's clothing during washing (S Cesa *et al*., 2017; Stanton *et al*., 2019; Chai *et al*., 2020). Film-type microplastics originate from agricultural

Table 2: Prevalence of microplastic contamination in oysters based on sampling location.

Location	Sample (n)	Total Length (mm)	Weight (g)	Accumulation (individu)	Prevalence (%)
Banda Aceh	50	31–81 64.82 ± 0.83	10.41–46.84 21.73 ± 7.11	19	38
Aceh Besar	50	35–61 46.44 ± 0.67	6.62–55.5 15.17 ± 8.44	15	30
Pidie	50	34–98 54.08 ± 1.39	6.3–68.77 22.84 ± 14.77	20	40
Bireun	50	33–62 47.08 ± 0.74	9.34–51.85 23.82 ± 8.92	15	30
Lhokseumawe	50	26–59 40.38 ± 0.74	8.55–37.81 20.92 ± 7.39	6	12
Aceh Utara	50	34–72 51.78 ± 1.04	10.19–62.24 27.49 ± 13.35	16	32
Aceh Timur	50	34–93 66.8 ± 1.43	8.99–95.42 43.31 ± 20.51	20	40
Langsa	50	14–79 50.1 ± 1.07	9.4–63.52 21.04 ± 9.52	24	48
Aceh Tamiang	50	43–82 61.68 ± 0.96	15.03–57.35 33.39 ± 10.03	4	8

Table 3: The amount of microplastic type that accumulated in oysters.

Location	Microplastic (particles/location)		
	Film	Fiber	Fragment
Banda Aceh	16	30	1
Aceh Besar	1	16	1
Pidie	0	38	0
Bireun	0	14	1
Lhokseumawe	0	10	0
Aceh Utara	0	20	0
Aceh Timur	2	24	1
Langsa	9	14	15
Aceh Tamiang	0	4	0
Total	28	170	19

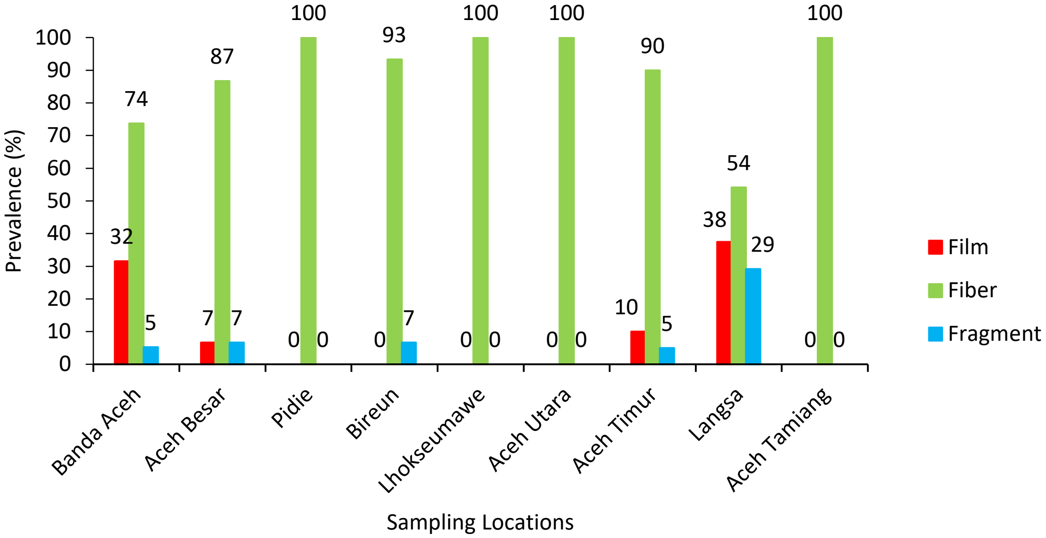

Fig. 2: The prevalence value of microplastic in oysters based on the type of microplastic.

activities (agricultural mulch film) and are released into the waters during rainfall (Qi *et al.*, 2020; Ren *et al.*, 2021). Fragment-type microplastics are derived from mechanical properties, crystallinity, and crack propagation from various activities on roads, buildings, and waters (Julienne *et al.*, 2019; Gaylarde *et al.*, 2021). Based on the research location, the intensity of microplastics in oysters showed that the highest film type was found in Banda Aceh (0.84 microplastics/oyster), the highest fiber type was found in Pidie (1.90 microplastics/oyster), and the highest type of fragment was found in Langsa (0.63 microplastics/oyster) (Table 4). Data showed that 139 of 500 total oyster samples collected had been contaminated by microplastic. From those 139 oysters, fiber-type microplastics dominated oysters with prevalence values between 54%–100%, followed by film-type between 0%–38% and fragments between 0%–29% (Fig. 2).

These results also indicate that fiber-type microplastics have contaminated oysters in all study locations. The results of the LWRs analysis showed that the oyster growth pattern was negative allometric (b < 3) (Table 5 and Fig. 3). It can be concluded that the growth of oysters is not optimal in all research locations. The highest b-value was shown in Pidie (b = 2.1) and the lowest was obtained in Lhokseumawe (b = 0.1). Previous research reported that microplastics could impair and inhibit the growth of *C. gigas* oysters, depending on the size of the microplastic particles (Bringer *et al.*, 2020a). Other studies also reported the significant effect of microplastic contamination

Table 4: Microplastic intensity in oysters based on the type of microplastic and sampling location.

Location	Sample (n)	Accumulation (individu)	∑ Microplastic			Intensity (Microplastic/oyster)		
			Film	Fiber	Fragment	Film	Fiber	Fragment
Banda Aceh	50	19	16	30	1	0.84	1.58	0.05
Aceh Besar	50	15	1	16	1	0.07	1.07	0.07
Pidie	50	20	0	38	0	0	1.90	0
Bireun	50	15	0	14	1	0	0.93	0.07
Lhokseumawe	50	6	0	10	0	0	1.67	0
Aceh Utara	50	16	0	20	0	0	1.25	0
Aceh Timur	50	20	2	24	1	0.10	1.20	0.05
Langsa	50	24	9	14	15	0.38	0.58	0.63
Aceh Tamiang	50	4	0	4	0	0	1.00	0

Table 5: Analysis of LWRs, including minimum value, maximum value, mean, and standard deviation.

Location	Sample (individual)	TL (cm)	W (g)	b	R^2
Banda Aceh	50	5.1–8.1 (6.5 ± 0.7)	10.4–46.8 (21.7 ± 7.1)	0.8	0.1
Aceh Besar	50	3.5–6.1 (4.7 ± 0.7)	6.6–33.5 (14.4 ± 6.1)	1.6	0.3
Pidie	50	3.4–9.8 (5.4 ± 1.4)	6.3–68.8 (22.8 ± 14.8)	2.1	0.8
Bireun	50	3.3–6.2 (4.7 ± 0.7)	9.3–51.8 (23.8 ± 8.9)	1.5	0.5
Lhokseumawe	50	2.6–5.9 (4.0 ± 0.7)	10.8–37.8 (21.0 ± 7.3)	0.1	0
Aceh Utara	50	3.4–7.2 (5.2 ± 1.0)	10.2–62.2 (27.5 ± 13.3)	1.4	0.4
Aceh Timur	50	3.4–9.3 (6.7 ± 1.4)	8.9–95.4 (43.3 ± 20.5)	1.9	0.6
Langsa	50	3.2–7.9 (5.1 ± 0.9)	9.4–63.5 (21.0 ± 9.5)	1.4	0.4
Aceh Tamiang	50	4.3–8.2 (6.2 ± 09)	15.0–57.3 (33.4 ± 10.0)	1.1	0.3

TL = total length, W = body weight, b = coefficient of growth pattern, R = coefficient of determination

on valve activity and daily growth of *C. gigas* (Bringer *et al.*, 2021).

Fig. 4 reveals that the observed growth of oysters was similar to the predicted results. Microplastic pollution in Aceh waters is apprehensive and requires more attention, as most of the oysters had an accumulation of microplastics internally. Oyster meat separated from the shell is generally directly consumed by humans. All parts of the oyster meat are not discarded, allowing the microplastics to be transferred from the food route. According to Van-Cauwenberghe and Janssen (2014), it is estimated that Europeans can be contaminated with 11,000 microplastics within a year due to consuming contaminated oysters. Microplastics that enter humans through food, in the long term, have the potential to pose health risks, especially cancer (Sabilillah *et al.*, 2023). According to Teng *et al.* (2019), the main factor for microplastic accumulation in oysters is the oyster's natural way of feeding, namely the filter feeder. The analysis showed that microplastics had contaminated 139 of 500 oyster samples collected. Another report by Maulana *et al.* (2023) revealed that fish distributed in Aceh (the current study location) were also contaminated with microplastics in the digestive tract of fish. The prevalence value based on location shows that the highest value was found in Langsa (48%), and the lowest was found in Aceh Tamiang (8%). The phenomenon of microplastic accumulation in oysters also occurs in other regions of the world,

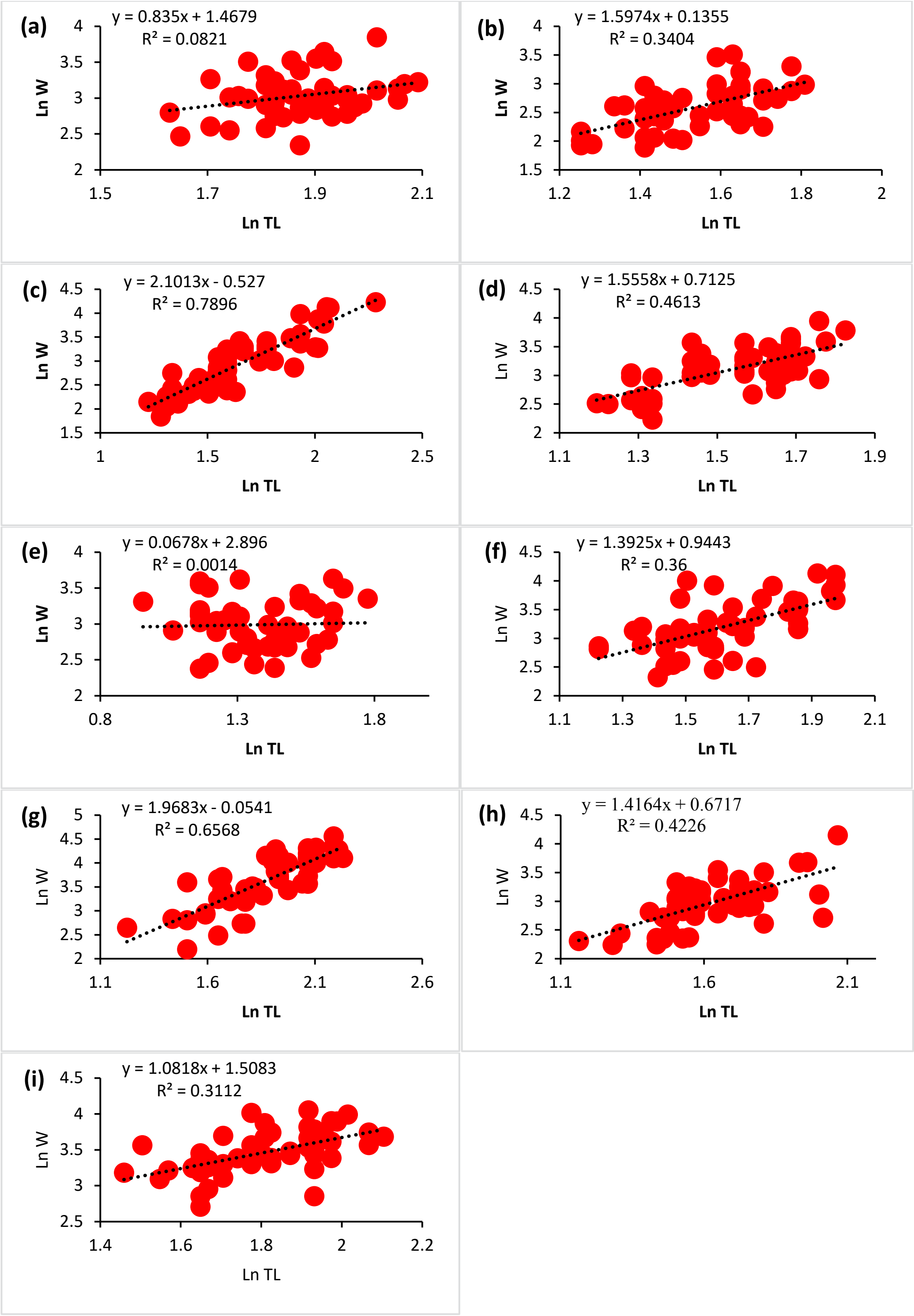

Fig. 3: LWRs of oyster based on sampling location, (a) Banda Aceh, (b) Aceh Besar, (c) Pidie, (d) Bireuen, (e) Lhokseumawe, (f) Aceh Utara (g) Aceh Timur, (h) Langsa, and (i) Aceh Tamiang

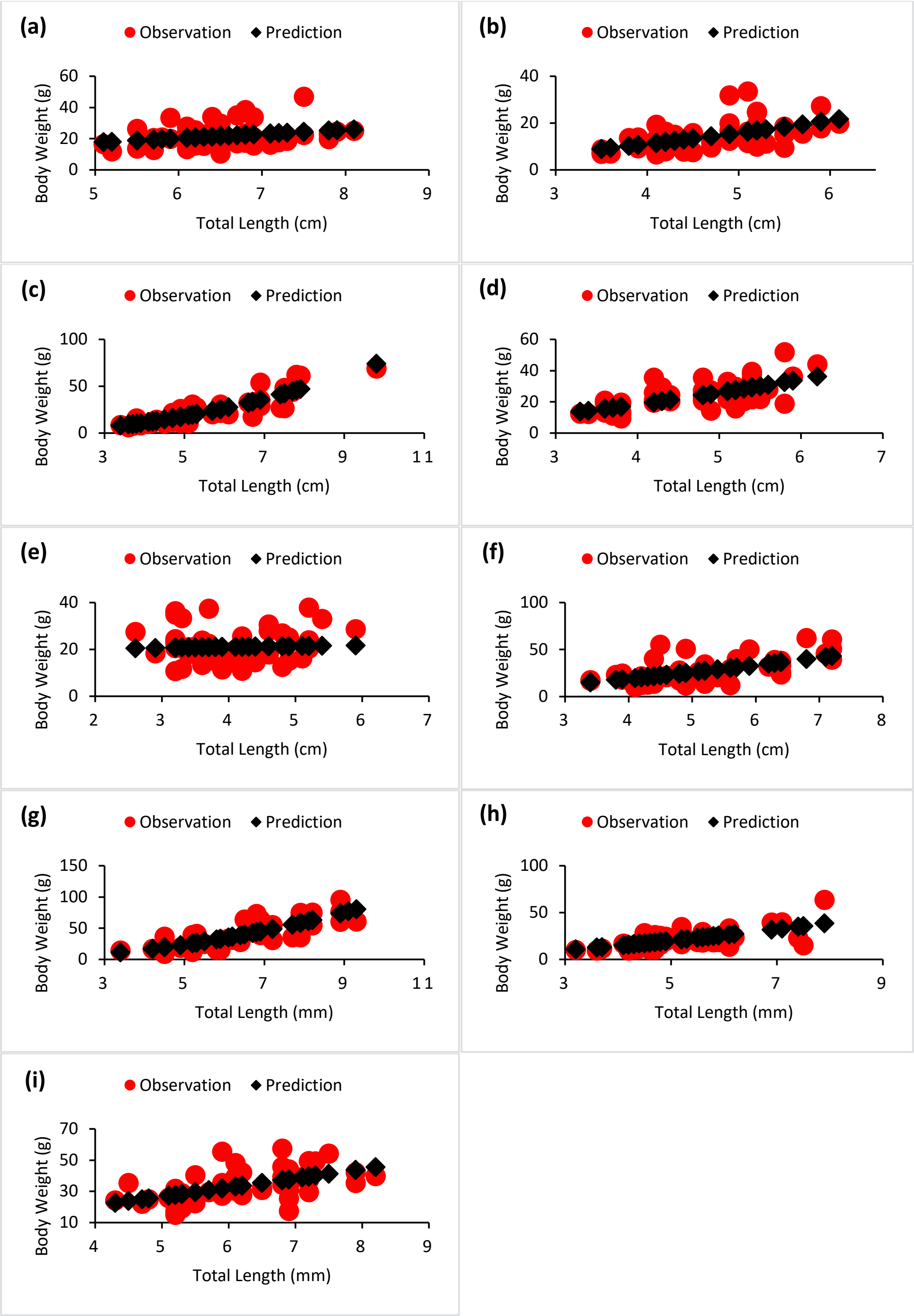

Fig. 4: Growth pattern based on sampling location, (a) Banda Aceh, (b) Aceh Besar, (c) Pidie, (d) Bireuen, (e) Lhokseumawe, (f) Aceh Utara (g) Aceh Timur, (h) Langsa, and (i) Aceh Tamiang

such as China (Li *et al*., 2018; Teng *et al*., 2019; Wang *et al*., 2021b), Australia (Jahan *et al*., 2019), and Taiwan (Liao *et al*., 2021). The microplastic polymers that contaminated the oysters in China are cellophane (CP), polyethylene (PE), and polyethylene terephthalate (PET). According to Wootton *et al*. (2022), oysters in Australia were exposed to polyethylene microplastic from aquaculture activity nets. Liao *et al*. (2021) revealed PET accumulation in Taiwan's oysters. Other studies revealed the prevalence of microplastics found in *Crassostrea gigas* reaches up to 63% in the Salish Sea, USA (Martinelli *et al*., 2020), 91% in Bahía Blanca Southwestern Atlantic (Severini *et al*., 2019), and 100% in southern Brazil (Saldaña-Serrano *et al*., 2022). Corami *et al*. (2020) also conducted a study in Venice Lagoon, Italy, which aimed to identify microplastics (<100µm in size) in the gills and hepatopancreas of *Crassostrea gigas*. This study's results showed that contamination prevalence values reached 41% and 12% for each organ. The highest intensity values for film, fiber, and fragment microplastics were obtained as 0.84, 1.90, and 0.63 microplastics/oyster, respectively. Saldaña-Serrano *et al*. (2022) revealed 0.33–0.75 microplastic/oyster in the form of fiber type were found in *Crassostrea gigas* from the six cultivation sites in southern Brazil, which was lower than the findings of this study. Dang *et al*. (2022) revealed that microplastics had contaminated *Crassostrea gigas* with an intensity value of 18.54 microplastics/oyster in Danang Bay, Vietnam. Oyster growth patterns in all study sites showed negative allometric values ($b < 3$). This fact represents that oysters got insufficient food, as they required. The microplastics ingested by the oysters may cause disturbances in their digestive process because microplastics are non-nutritional materials. These results are confirmed by previous studies, which reported the negative impact of microplastics, including hepatic stress, intestinal alteration, and digestive organ damage in Bivalvia and fish (Hoyo-Alvarez *et al*., 2022; Hollerova *et al*., 2023). Oyster larvae contaminated with microplastics can suffer from growth disorders such as malformations and abnormalities (Bringer *et al*., 2020b). Microplastic contamination also causes increased mortality in adult oysters, besides causing decreased locomotor activity and developmental abnormalities in oyster larvae (Bringer *et al*., 2022).

CONCLUSIONS

The study results revealed that microplastics had contaminated oysters throughout the north-eastern region of Aceh Province, with the highest prevalence reaching 48% found in Langsa and the lowest found 8% in Aceh Tamiang. Based on the intensity value, fiber-type microplastics dominate with a value of 1.33 particles/oyster. Fiber-type microplastics that contaminate oysters were found in all research locations, where the highest was found in Pidie with 38 particles and the lowest in Aceh Tamiang with 4 particles. Fragment-type microplastics contaminate oysters only in five research locations (Aceh Besar, Aceh Timur, Banda Aceh, Bireun, and Langsa), where the highest was found in Langsa with 15 particles and nil in 4 other research locations (Aceh Tamiang, Aceh Utara, Lhokseumawe, and Pidie). Film-type microplastics contaminated oysters only in four research locations (Aceh Besar, Aceh Timur, Banda Aceh, and Langsa), where the highest was found in Banda Aceh with 16 particles and nil in 5 other research locations (Aceh Tamiang Aceh Utara, Bireun, Lhokseumawe, and Pidie). The presence of microplastics in oysters can be potentially transferred to humans via food since humans eat oysters as a whole except for the shell, which is separated from the oyster's body. Conversely to fish, organs that are generally contaminated with microplastics, such as the digestive tract, gills, and several other organs, are removed when the fish is cleaned to minimize the transfer of microplastics to humans through the mouth. Thus, the transfer of microplastics to the human body is greater if people eat oysters than fish. Although the direct impact of microplastics on the contaminated human body is inconclusive, several studies have revealed that microplastics that accumulate in the human body have a negative impact in the long term. In the growth patterns analysis, the development of oysters at each study location was not optimal ($b < 3$ or negative allometric). This phenomenon might correlate with microplastics contaminating the waters and entering the oyster's digestive organs. Based on the results of the current study, oyster farming technology in the pond area must be developed, where the water used in the ponds is filtered to minimize microplastic contamination.

AUTHORS CONTRIBUTIONS

L. Kasmini developed the study concepts, survey, data collection and analysis, and approved the manuscript's final draft. A.S. Batubara performed survey, data collection and processing, and prepared the manuscript's first draft.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and, or falsification, double publication and, or submission, and redundancy have been completely witnessed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS

µm	Micrometer
%	Percent
°C	Degrees Celsius
a	Constanta
b	Constanta
CP	Cellophane
g	Gram
KOH	Potassium hydroxide
L	Length
LAM	Linear allometric model
LWRs	Length-weight relationship
mm	Milimeter
MPs	Microplastics per gram
No.	Nomor
PE	Polyethylene
PET	Polyethylene terephthalate
W	Weight

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