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Ingestion of Microplastics in the Planktonic Copepod from the Indonesian Throughflow Pathways

(Pengambilan Mikroplastik dalam Kopepod Planktonik dari Laluan Arus Lintas Indonesia)

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ABSTRACT

Zooplankton are vulnerable to microplastics in the waters due to their indiscriminate feeding habits. Zooplankton consumption of microplastics affects microplastic accumulation and transmission in the marine ecosystem. Therefore, it is essential to know the intake and transmission by different group sizes of zooplankton in natural seawater. This study documented for the first time the levels of microplastics found in three sizes of copepods along the Indonesian Throughflow (ITF) pathways. The ingestion rates were 0.028, 0.023 and 0.016 n/ind for group sizes copepod 1000-2000 µm, 500-1000 µm and 200-500 µm, respectively. There was no significant distinction in the microplastics concentrations of the three groups of copepod classes along the ITF pathway ($p>0.005$). Fiber microplastics were the most dominant in the body of copepods, constituting 87.22% of ingested microplastics. In terms of the chemical composition of the microplastic, a total of 7 polymers were detected in copepods in the ITF pathway. The three predominant polymer types identified were polyvinyl butyral (PVB), polyvinyl ether maleic anhydride (PVEMA) and polyester (PES) (27%, 27% and 20%, respectively). This study provides the critical parameters of the microplastic in copepods in the ITF pathway and is an essential basis for further ecological risk assessments of microplastics in biota species.

Keywords: Copepod; Indonesian throughflow; microplastic; zooplankton

ABSTRACT

Zooplankton terdedah kepada mikroplastik di perairan kerana tabiat pemakanan mereka yang tidak memilih. Pengambilan mikroplastik oleh zooplankton menjejaskan pengumpulan dan pemindahan mikroplastik dalam ekosistem marin. Oleh itu, adalah penting untuk mengetahui pengambilan dan pemindahan mengikut saiz kumpulan zooplankton yang berbeza di dalam air laut. Kajian ini pertama kalinya mendokumenkan tahap mikroplastik yang ditemui dalam tiga saiz kopepod di sepanjang laluan Arus Lintas Indonesia (ITF). Kadar pengingesan adalah 0.028, 0.023 dan 0.016 n/ind untuk saiz kumpulan kopepod masing-masing adalah 1000-2000 µm, 500-1000 µm dan 200-500 µm. Tiada perbezaan ketara dalam kepekatan mikroplastik tiga kumpulan kelas kopepod di sepanjang laluan ITF ($p>0.005$). Mikroplastik gentian adalah yang paling dominan dalam badan kopepod, membentuk 87.22% daripada mikroplastik yang tertelan. Dari segi komposisi kimia mikroplastik, sejumlah 7 polimer telah dikesan dalam kopepod dalam Laluan ITF. Tiga jenis polimer utama yang dikenal pasti ialah polivinil butiral (PVB), polivinil eter malik anhidrida (PVEMA) dan poliester (PES) (masing-masing 27%, 27% dan 20%). Kajian ini menyediakan parameter kritikal mikroplastik dalam kopepod dalam laluan ITF dan merupakan asas penting untuk penilaian risiko ekologi lanjutan mikroplastik dalam spesies biota.

Kata kunci: Arus Lintas Indonesia (ITF); kopepod; mikroplastik; zooplankton

INTRODUCTION

The accumulation of plastic waste in marine environments has raised concerns about its potential impact on marine species. Microplastics (<5 mm) have the most significant influence on marine organisms (Ugwu, Herrera & Gómez 2021). Several modelling studies have generated estimations indicating the number of microplastics existing on the surface of the ocean may potentially range from 1.7 to 4.85 trillion pieces (Cózar et al. 2014; Eriksen et al. 2014; Lebreton et al. 2018; Van Sebille et al. 2015). Marine organisms may mistakenly perceive tiny microplastics as food substances. Numerous studies have demonstrated that the ingestion of microplastics has adverse effects on reproductive processes, growth patterns, health, and behavior of marine biota (Bucci, Tulio & Rochman 2020; Rochman et al. 2013). In marine ecosystems, microplastics have been ingested by a variety of organisms, including fish (Davison & Asch 2011), seabirds (Van Franeker 2011), sea turtles (Duncan et al. 2019), marine mammals (Ortega-Borchardt et al. 2023), and zooplankton (Botterell et al. 2019).

Zooplankton are crucial for distributing and transferring energy in ecosystems but are highly susceptible to microplastic contamination (Botterell et al. 2019). They are crucial food chain components, serving as primary consumers for many aquatic organisms and as a nutrition source for larger predators. Furthermore, zooplankton, which refers to the diverse organisms that float or drift in aquatic ecosystems, has been widely recognized as a valuable bioindicator for assessing the ecological status of these environments. Due to their sensitivity to environmental changes and crucial role in the food web, planktonic communities can provide valuable information about the health and functioning of aquatic ecosystems. Zooplankton are at risk of consuming microplastics due to their filter-feeding behavior and inability to distinguish between plastic particles and natural food sources (Boerger et al. 2010). This vulnerability is due to their lack of selectivity in food intake, as they cannot differentiate between plastic particles and natural food sources (Moore 2008). As a result, many studies have utilized plankton to monitor water quality, identify pollution sources, and assess the impact of human activities on marine environments (Araujo, Dias & Bonecker 2017; Hemraj et al. 2017). The laboratory experiments showed that zooplankton could consume specific quantities of microplastics found within the water column, as described by Cole et al. (2019, 2015, 2013) and Setälä, Fleming-Lehtinen and Lehtiniemi (2014). Ingestion of microplastics by zooplankton has also been proven in several natural waters (Amin et al. 2020; Cedervall et al. 2012; Desforges, Galbraith & Ross 2015; Fibbe et al. 2023; Lima et al. 2023; Sun et al. 2017; Zavala-Alarcón et al. 2023; Zheng et al. 2021).

Copepods are the most prevalent type of zooplankton, and they can be discovered in saltwater and freshwater environments. Copepods are crucial in connecting primary producers with higher trophic levels across marine ecosystems (Vroom et al. 2017). The empirical investigations have demonstrated that when copepods are exposed to elevated levels of microplastics, various alterations occur in their feeding behaviour and prey selection (Cole et al. 2019, 2015, 2013). Nevertheless, studies regarding copepods ingesting microplastics in natural environments still need to be explored.

The Indonesian Throughflow (ITF), also known as Arus Lintas Indonesia (ARLINDO), facilitates the transport of low-latitude Pacific water into the Indian Ocean. The main entry point for Pacific waters into the Indonesian Seas is through the Makassar Strait. A smaller amount also enters through the Lifamatola Passage, the South China Sea, and the Karimata Strait (Susanto et al. 2013; 2010). The Makassar Strait is the pathway for approximately 80% of the total ITF (Gordon et al. 2010; Li et al. 2018).

The microplastics can be transported via ocean currents because they are light (Kane et al. 2020) and it is believed that the ocean water masses travelling through the ITF route area may contain microplastic pollutants. This suspicion is strengthened by the most recent study report concerning the prevalence of microplastics in the open water along the main gate of the ITF route (Manullang et al. 2024; Yuan et al. 2023). Furthermore, the studies conducted along the coast of ITF pathways have reported the presence of microplastics in various habitats within the Makassar Strait, including seagrass habitats (Tahir et al. 2020), salt ponds (Tahir et al. 2019), estuary areas (Wicaksono, Tahir & Werorilangi 2020), and coastal areas (Kama, Rahim & Yaqin 2021; Sawalman et al. 2021). The presence of microplastic had also documented in the marine organism such as anchovies (Ningrum & Patria 2022); milkfish (Amelinda et al. 2021); fish (Rochman et al. 2015; Tahir et al. 2020); benthic (Tahir et al. 2020, 2019). The presence of microplastics in coastal areas of the Bali Strait region was reported by nd Germanov et al. (2019). In this study, we investigated the presence of microplastic pollutants in the three different sizes of planktonic copepods from the primary pathway of the ITF.

MATERIALS AND METHODS

SAMPLE COLLECTION

The collection of zooplankton samples was conducted within the framework of the TRIUMPH (Throughflow Indonesian Seas, Upwelling and Mixing Physics) oceanographic expedition. The TRIUMPH project is joint research encompassing multiple disciplines. It involves scientists from the National Agency for Research and Innovation (BRIN) in Indonesia, the First Institute of Oceanography (FIO) in China, and the University of

Maryland in the United States of America. The research expedition was conducted on the R/V Baruna Jaya VIII, operated by BRIN, from January 5 to April 1, 2021. The sampling covered a total of 10 stations, which are strategically distributed across four distinct oceanographic regions: (a) the ITF threshold in the north Makassar Strait (consisting of three stations), (b) the Makassar Strait (comprising three stations), (c) the Alas Strait (1 station) and (d) Lombok Strait (comprising three stations) (Figure 1). The zooplankton samples were vertically collected by a NORPAC plankton net (mesh size: 200 µm) from a depth of 300 meters to the surface. The NORPAC net was deployed once at each station. After collection, the zooplankton samples were transferred into a sealed glass container. Samples were transferred from the sampling net to the sample container through a repetitive washing mechanism using distilled water. Samples have been preserved with a 90% ethanol solution for long-term storage.

LABORATORY ANALYTICAL PROCEDURE

The collected zooplankton samples are separated using a stainless-steel tweezers under an Olympus SZX7 stereo microscope to isolate copepods in the laboratory. The copepods were classified into three distinct size categories: 200-500 µm, 500-1000 µm, and 1000-2000 µm, throughout the visual sorting process. Each class consists of 200 individuals in each size category from each station. This measurement quantity qualifies the minimum requirement specified by the International Council for the Exploration of the Sea (ICES 2015) for assessing microplastics in organisms. The specimens selected underwent a rinsing process using filtered water, followed by placement in 20 mL glass vials. Through a double rinsing process, the chosen individuals were meticulously observed using a microscope to verify the absence of any microplastic particles attaching to their bodies. This meticulous examination ensures that only the microplastics ingested by the biota are included in the final count (Amin et al. 2020; Karami et al. 2017). The sample sorting process with

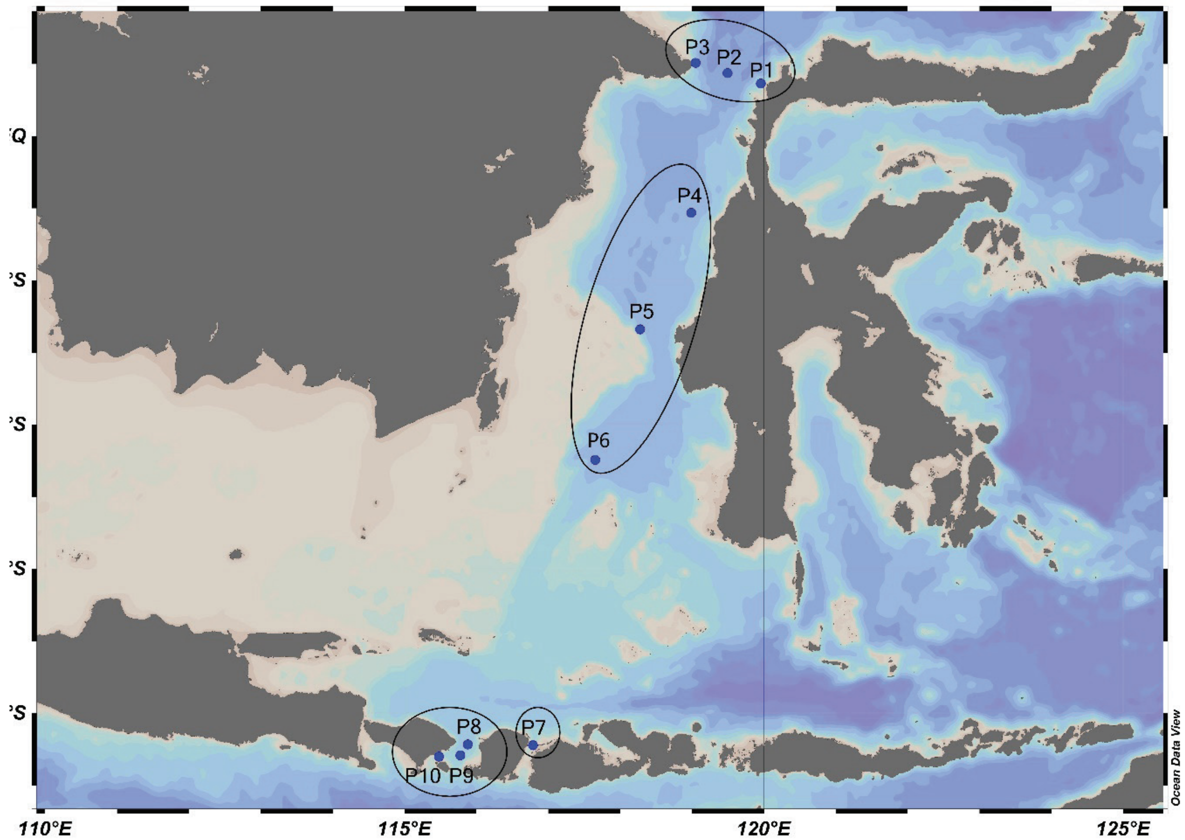

FIGURE 1. Zooplankton was collected from 10 stations spread over four oceanic areas along the ITF pathways. These are (a) the ITF Threshold (input) in the North Makassar Strait (P1, P2, P3); (b) Makassar Strait (P4, P5, P6); (c) Alas Strait (P7) and (d) Lombok Strait (P8, P9, P10)

a microscope was equipped with a glass box to cover the microscope lens to minimize air contamination. Each sample was digested with 3-5 mL of a 30% H_2O_2 solution and then heated in an oven at 40 °C for 24 h until all organic tissue was wholly destroyed. Previous research has extensively reported that 30% H_2O_2 can degrade organic materials (Cordova, Riani & Shiomoto 2020; Desforges, Galbraith & Ross 2015). Subsequently, the gathered substance was subjected to vacuum filtration using GF/C filter paper (Whatman, diameter: 47 mm, pore size: 0.47 µm). Then, the filter paper was carefully transferred into a sterile Petri dish and securely sealed with aluminum foil until the particles were examined microscopically. The samples were characterized using an Olympus SZX7 stereo microscope fitted with a camera capable of magnifying the pieces within the range of 40-50X.

The microplastic particles were classified into different types (i.e., fragment, fiber, film, pellet) and colors. Subsequently, the dimensions of the microplastics were measured and classified into several size categories as follows: (i) 50–100 µm, (ii) 100–200 µm, (iii) 200–500 µm, (iv) 500–1000 µm., and (v) 1000–2000 µm. Determining microplastic abundance for each group at each station involved the calculation of the ratio between the number of microplastics discovered in copepods and the number of copepods that consumed them (microplastics/zooplankton).

A polymer test was conducted on a representative sample using a Horiba LabRAM HR Evolution Raman Spectrometer. We randomly selected 20% of samples number from each size group of copepods and analyzed a minimum of 20% of the particles present in the subsample using Raman spectroscopy. It was decided that the 20% proportion is equal to the total number of samples, given that the experiment procedure included a blank method and then the degradation of organic compounds. The previous study also set aside about 15% to 20% of the sample to identify polymers (Cordova, Riani & Shiomoto 2021; Falahudin et al. 2020).

Particles were examined directly on the filter surface using an optical microscope. The Raman microscope has three objective lenses, allowing for various magnification levels. Particles were first observed using a 10X objective lens before being examined at two additional magnifications (50X, 100X). Afterward, the laser beam was directed toward the surface of the chosen particle. The samples underwent analysis with a 785 µm diode laser, utilizing energy levels ranging from 10% to 50%. Recorded spectra spanned a spectral wavenumber range from 200 to 3200 cm^{-1}. The spectral characteristics obtained from each sample were then compared with standard polymers using a reference Micro-Raman Spectrometer. Polymer specimens for comparison were chosen based on the highest percentage values in the Horiba micro-Raman spectrometer library. The match value on average is over 60%.

STATISTICAL ANALYSIS

This study reported microplastic concentrations in units of particles or items per individual zooplankton (n/ind). Descriptive statistics were applied to comprehensively depict microplastic quantities categorized by type, colour, and size. Furthermore, the mean proportion of each microplastic type was determined based on the total amount of plastic material collected. The Kolmogorov-Smirnov test was applied to evaluate the assumption of normality. One-way ANOVA was performed to compare the average microplastic concentrations in four oceanographic locations for each station. A nonparametric analysis of variance, the Kruskal-Wallis test was applied to determine the microplastic concentrations in the three copepod size groups. MINITAB 21 was used for each data analysis. Ocean Data View 4 (http://odv.awi.de) was used to prepare maps and contour plots.

AIRBONE AND CONTAMINATION CONTROL

All of the research equipment was given a thorough cleaning with distilled water and three times rinses. The bottle samples, petri dishes, steel filters, sample vials and beaker glasses were heated to 200 °C for 24 h to remove any traces of possible plastic to prevent contamination during the sampling process. During the sample transfer from plankton net to bottle sample, nitrile gloves were used, the empty bottle sample was placed and the bottle was left open as a control sample. Several procedures were implemented during the laboratory analysis methods to avoid contamination. The samples were processed in a distinct laboratory where other operations were carried out to avoid air contamination. The air vents in the analysis room were blocked off so that the chamber would take in less air from the outside. The digestion process was carried out inside a fume hood to keep the air as clean as possible (Van Cauwenberghe et al. 2013). In addition, samples were kept covered to minimize the airborne deposition, while during the analysis, a sterile filter paper was placed where the work was being done as a control sample (Cordova, Riani & Shiomoto 2021).

RESULT

CHARACTERISTICS OF MICROPLASTICS IN COPEPOD SAMPLES ALONG THE ITF PATHWAY

The present study provided evidence that copepods, which play a crucial role in the ITF food web, have been found to ingest microplastics. A total of 6000 individual copepods were isolated along the ITF pathways, which included the north Makassar Strait, Makassar Strait, Alas Strait and Lombok Strait (Figure 1). A total of 133 particles were found from these specimens. The ingestion of microplastics by copepods on the ITF pathway is illustrated in Figure 2. The predominant particles seen in the study were classified as fibers (87.22%) (Figure 3). The observed particles exhibited the following color distribution: transparent (32.33%), blue (27.82%), red (13.53%), blue (9.77%), green (6.77%), yellow (6.02%), and purple (3.76%) (Figure 3). The findings indicated that the predominant fraction of particles, precisely 29.32%, consists of microplastics within the 200-500 µm size range. This range size is followed by particles within the 1000-2000 µm size range, accounting for 25.56% of the total. Additionally, particles within the size range of 100-200 µm constitute 24.06% of the sample. The 50-100 µm size comprises 11.28% of the particles, while the 100-200 µm size accounts for 9.77% (Figure 3).

Twenty percent of the separated particles from 20% of the total samples were examined using a micro-RAMAN spectrometer. The analysis of Raman spectra indicated that the isolated particles exhibited a close correspondence with the reference spectra. The reference spectra encompassed a comprehensive set of seven polymer types, namely polyvinyl butyral (PVB), polymethyl vinyl ether-co-maleic acid (PVEMA), polyisobutylene (PIB), polyurethane foam (PUR), polyester (PES), polyether sulfone (PESU) and polyacrylic acid/carboxy vinyl polymer (PAA) (Figure 3). The three predominant polymer types identified in this study were PVB (27%); PVEMA (27%) and PES (20%). The absence of particles on the blank sample suggests no airborne contamination or laboratory-related issues.

THE MICROPLASTIC CONCENTRATION IN COPEPOD SAMPLES ALONG THE ITF PATHWAY

Microplastic particles were detected at all stations where copepods were collected (Figure 4). The mean number of microplastic particles consumed per copepod samples was

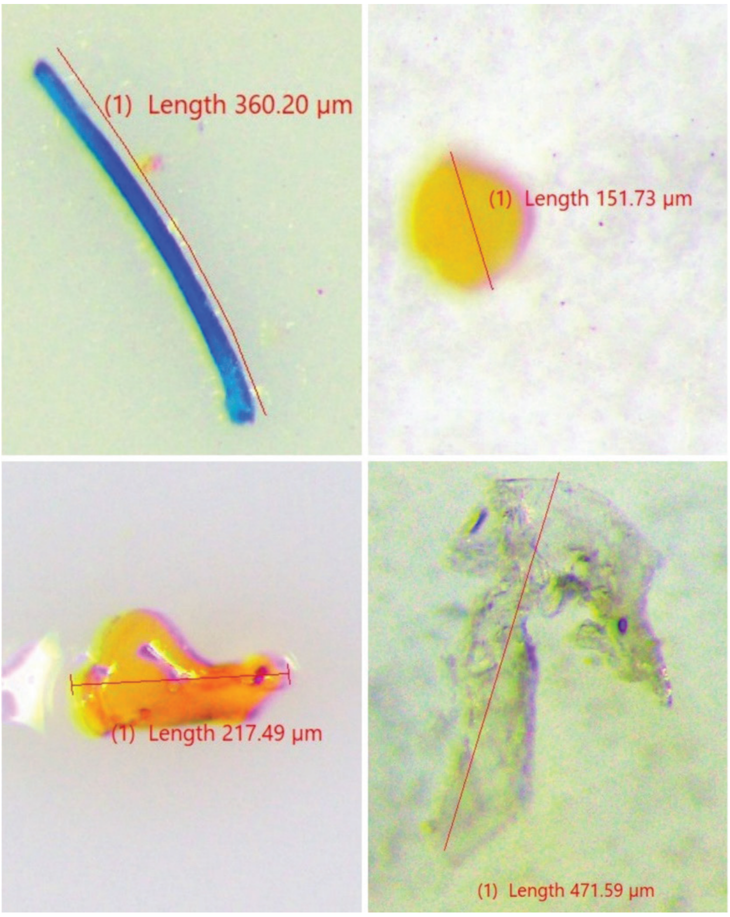

FIGURE 2. Microplastic particles were found in copepod samples collected along the ITF pathway. (a) fiber, (b) pellet, (c) fragment, and (d) film

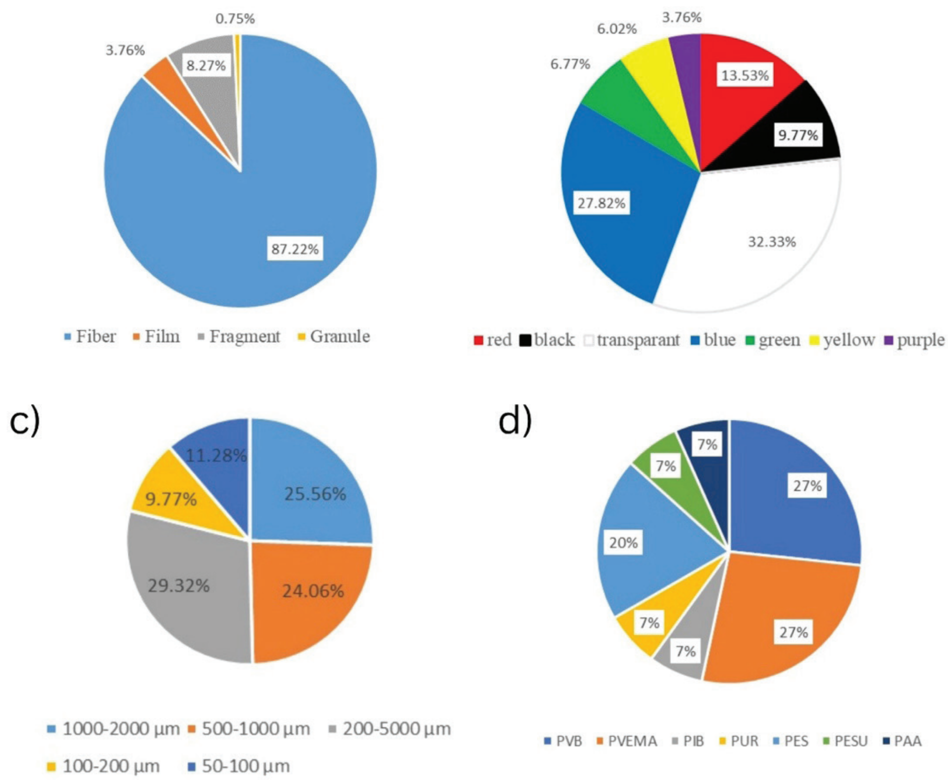

FIGURE 3. Characteristics of microplastics found in copepods from the ITF pathway. (a) The majority of microplastics found in this study were fibers (87.22%), (b) Color composition of microplastics, (c) Percentage of the size of microplastics, and (d) Types of polymers found in copepods

0.022 (SE±0.009) n/ind, comparable to an average of 1 plastic particle for every 45 copepods. The ITF threshold area, specifically the north Makassar Strait, had the most significant accumulation of microplastics, as evidenced by a concentration of 0.028 n/ind (equal to one plastic particle per 36 copepods). In addition, the average microplastic concentration in the oceanographic region of the Makassar Strait (P4, P5, P6) was 0.018 n/ind (1 plastic particle for 55 copepods). The average microplastic concentration in the oceanographic regions of the Alas Strait was 0.015 n/ind (equivalent to 1 plastic particle per 65 copepods). The average microplastic concentration in the oceanographic regions of the Lombok Strait (P8, P9, and P10) was 0.022 n/ind (equivalent to 1 plastic particle per 45 copepods) (Figure 5). An analysis of variance (ANOVA) was conducted to assess the variations in microplastic concentrations among four distinct oceanographic locations: the northern ITF entrance of the Makassar Strait (P1, P2, P3), Makassar Strait (P4, P5, P6), Alas Strait (P7) and Lombok Strait (P8, P9, P10). The ANOVA analysis showed no statistically significant difference in the mean microplastic concentration among the four oceanographic locations ($p=0.553$). Nevertheless, it is essential to acknowledge that the levels of microplastic concentrations are notably elevated at the ITF threshold, located in the north Makassar Strait.

THE LEVELS OF MICROPLASTICS IN THREE DISTINCT SIZE CATEGORIES ALONG THE ITF PATHWAY

The mean microplastic concentration was 0.016 (SE±0.008) n/ind, equivalent to one particle per 64 individual copepods in the 200-500 μm copepod group. The mean microplastic concentration in the 500-1000 μm size copepod group was 0.023 (SE±0.015) n/ind, corresponding to one particle per 44 individual copepods. Similarly, the mean microplastic concentration was 0.028 (SE±0.015) n/ind, indicating the presence of approximately one plastic particle per 36 copepods in the 1000-2000 μm copepod group (Figure 6). The concentration of microplastics seems related to the

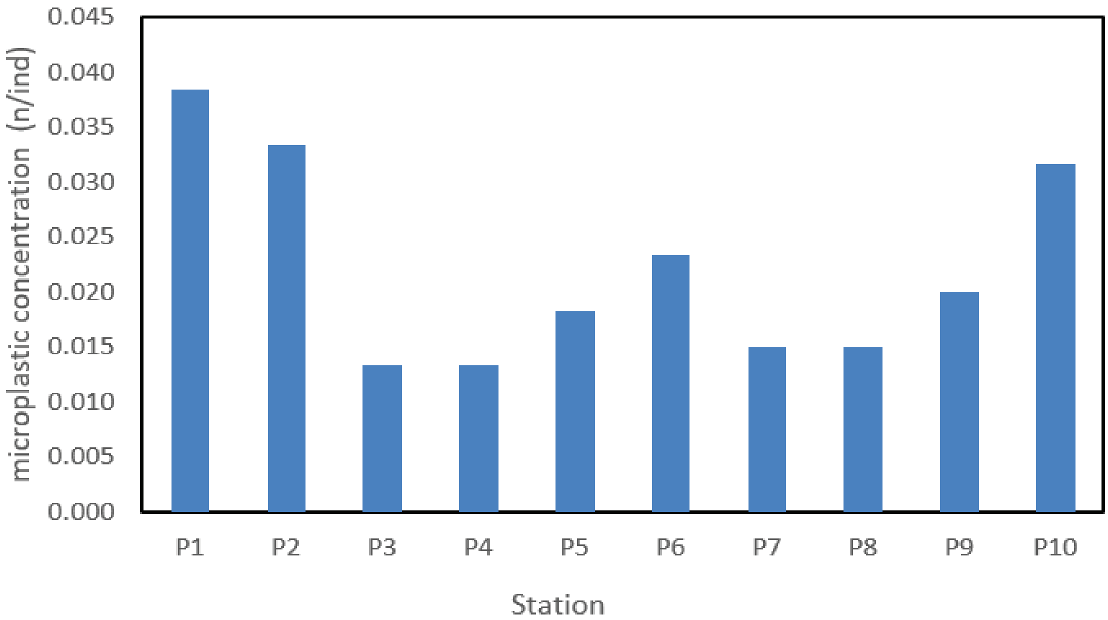

FIGURE 4. Concentration of microplastics in copepods along the ITF/ARLINDO pathway at 10 stations

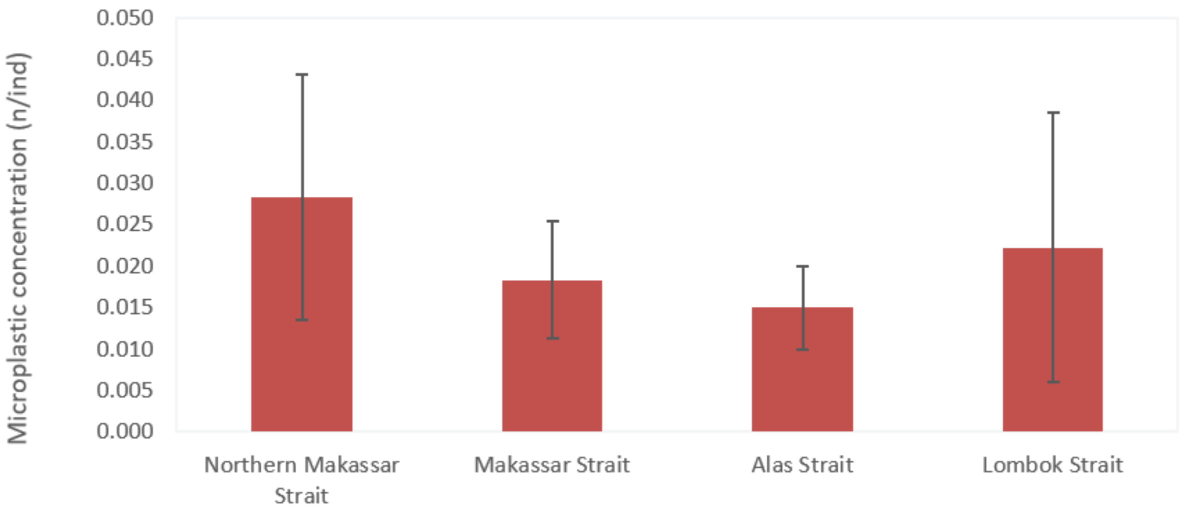

FIGURE 5. The average concentration value of microplastics ingested by copepods differs between the four oceanographic regions of the ITF pathway. There is no statistically significant difference between the microplastic concentration values, but the north Makassar Strait region has higher microplastic concentration values

amount of microplastics that collect up in their body. Microplastic accumulation increases by the size of the copepod. The Kruskal-Wallis test was used to ascertain if there were significant differences in the microplastic concentrations among the three groups of copepods. The Kruskal-Wallis test is a nonparametric alternative to the analysis of variance, commonly employed to examine the differences among three or more distinct populations. An analysis of variance was conducted to assess the statistical outcomes of the Kruskal-Wallis test (H). The obtained results indicate that the significant value (H) was 3.51, with 2 degrees of freedom (DF) and a probability (P) of 0.173. Based on the obtained probability value of 0.173, which is lower than the predetermined significance threshold of 0.05, it may be inferred that, at a 5% significance level, there is no statistically significant distinction in the concentrations of the three groups of copepod classes along the ITF pathway ($p>0.005$).

The concentration values of microplastics for each size class of copepods in different oceanographic regions are presented in Figure 7. The concentration values of microplastics exhibited variability. An analysis of variance was conducted to assess variations in microplastic concentrations among four oceanographic locations: the northern ITF entrance of the Makassar Strait (P1, P2, P3), Makassar Strait (P4, P5, P6), Alas Strait (P7) and Lombok Strait (P8, P9, P10). The objective was to compare these locations to the average microplastic concentration across different size classes. The ANOVA analysis results indicate no statistically significant difference in the mean microplastic concentration among the four oceanographic zones.

DISCUSSION

The present study provided evidence that copepods, which play a crucial role in the ITF food web, have been found to ingest microplastics. Our findings indicate that fiber is the most microplastic type that copepods ingest (87%) with small contribution of fragment (8%). Zooplankton in aquatic environments cannot differentiate between organic food and plastic materials (Boerger et al. 2010). Microplastics shaped like fibers have a similar structure to filamentous algae commonly seen in aquatic habitats (Cole et al. 2013). As a result, copepods may mistakenly perceive higher levels of microplastics in the water column as edible substances. Fibers are widely recognized as the predominant type of microplastic prevalent in the open ocean environment (Choy et al. 2019; Kanhai et al. 2017; Lusher et al. 2015; Obbard et al. 2014; Peng et al. 2018). The predominant fiber type ingested by copepod were reported in the Southwest Coast of India (Rashid et al. 2022); Tampa Bay (Fibbe et al. 2023); Northeast Pacific Ocean (Desforges, Galbraith & Ross 2015); Yellow Sea (Zheng et al. 2021), Northeast Brazil (Lima et al. 2023) and northern South China Sea (Sun et al. 2017). In contrast, other studies reported that fragments type of microplastic in greater numbers were ingested by copepods in the Black

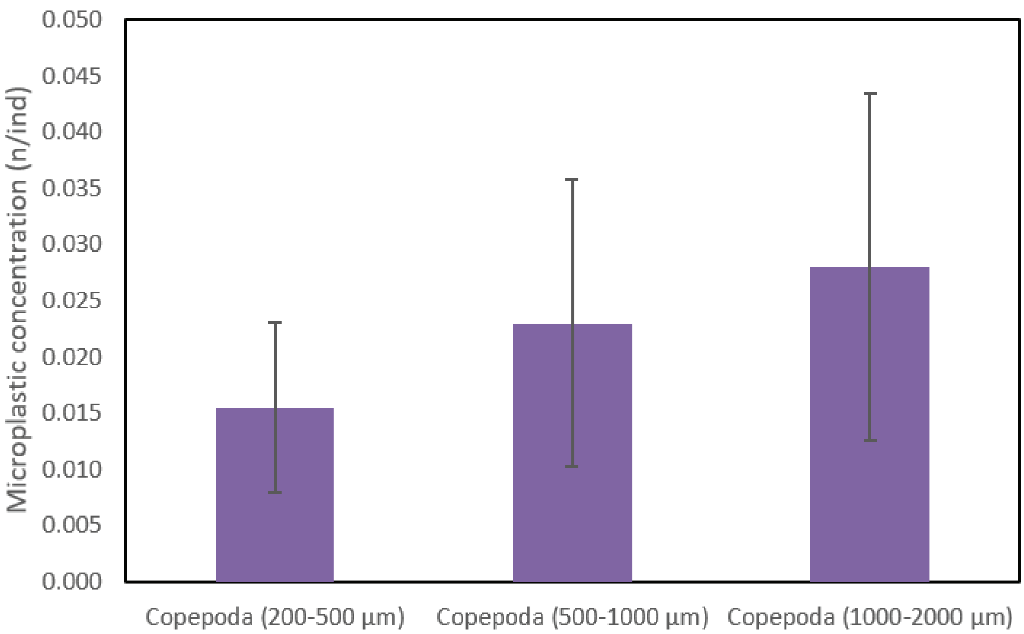

FIGURE 6. The mean concentrations of microplastics ingested by the three sizes of copepods studied. Microplastic value was observed to increase with copepod size

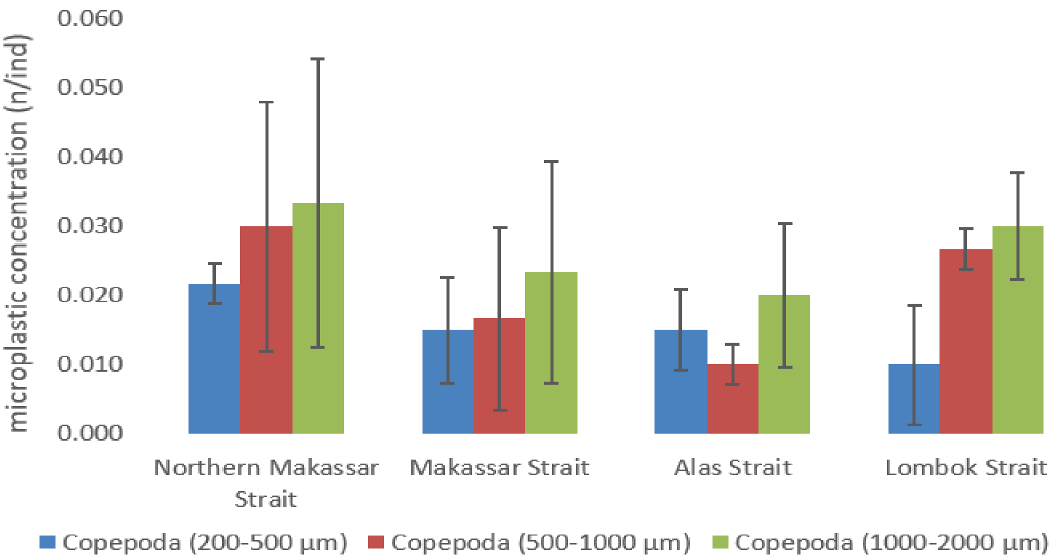

FIGURE 7. The ingestion of microplastics by copepods exhibits variation throughout the four oceanographic zones along the ITF pathway

Sea (Aytan, Esensoy & Senturk 2022); Central Mexican Pacific (Zavala-Alarcon et al. 2023); Fram Strait, Arctic Ocean (Botterell et al. 2022). This different pattern may happen because copepods can ingest various microplastics in the marine ecosystem. However, their consumption of these microplastics depends on the type and prevalence of them available in their environment. The previous investigation of microplastic concentration in the water column in the main pathway of the ITF showed a similar composition of microplastics in the water column to those found in the copepods in this study (Manullang et al. 2024). The expected laundry wastewater may be a significant source of microplastic fibers in marine environments. As per the research carried out by Napper and Thompson in (2016), it was determined that more than 700,000 fibers are released during a single wash when washing acrylic fabrics. These fibers are microscopic and invisible to the naked eye but can accumulate and cause long-term environmental harm. Additionally, Vassilenko et al. (2019) found that the number of microfibers lost during the washing of textiles can vary greatly, with anywhere between 9,777 and 4,315,371 microfibers being lost in every 1 kg of textile washed.

This study identified PVEMA, PVB, and PES as the most common polymers. PVEMA polymer is commonly utilized as a detergent builder, a stabilizer in medical applications, and for food packaging purposes (Demir et al. 2017; Shahbazi et al. 2014). PVB is a material that finds widespread use in the automotive and architectural industries (McKeen 2016). On the other hand, the PES polymer is widely employed in various industries, including construction, textile, automotive, medical, and electrical industries. Furthermore, polyester is extensively used to produce packaging materials like bottles and other containers (Camlibel 2018; Zughaibi & Steiner 2021).

According to the findings of this study, copepods found along the ITF pathway had consumed microplastics. This study found that the mean concentration of microplastics in copepods was 0.022 n/ind. This concentration is 48 times lower than the concentration of microplastics found in the water column in the ITF (Manullang et al. 2024). The number of microplastics per individual in this study is relatively similar to other studies in the Central Mexican Pacific (0.02) (Zavala-Alarcon et al. 2023); Northeast Pacific Ocean (0.026) (Desforges, Galbraith & Ross 2015); Black Sea (0.008-0.024) (Aytan, Esensoy & Senturk 2022); Tampa Bay (0.015) (Fibbe et al. 2023) and Eastern Arabian Sea (0.03) (Rashid et al. 2021). However, the ingestion rate of copepods in the ITF was lower than the ingestion of copepods in the Fram Strait, Arctic Ocean (0.01 to 0.21) (Botterell et al. 2022), Southern South China Sea (0.13) (Amin et al. 2020) and the Yellow Sea (0.21) (Zheng et al. 2021) (Table 1).

Copepods are the most prevalent zooplankton found in the ITF pathway. They play a critical role in the food chain. These tiny creatures serve as a major food source

TABLE 1. Copepods ingestion rate of microplastic reported across the world

No	Location	Microplastic concentration (n/ind)	Shape dominant	References
1	ITF Pathway	0.022±0.009	Fiber	This study
2	The Yellow Sea	0.21 ± 0.10	Fiber	Zheng et al. (2021)
3	The central Mexican Pacific	0.02	Fragment	Zavala-Alarcon et al. (2023)
4	Pacific Ocean	0.026	Fiber	Desforges, Galbraith & Ross (2015)
5	Black Sea	0.008-0.024	Fragment	Aytan, Esensoy & Senturk (2022)
6	Arctic Ocean	0.01 to 0.21	Fragment	Botterell et al. (2022)
7	Tampa Bay	0.015	Fiber	Fibbe et al. (2023)
8	The southwest coast of India	0.01 - 0.11	Fiber	Rashid et al. (2022)
9	Arabian Sea	0.03 ± 0.01	Pellet	Rashid et al. (2021)
10	South China Sea	0.13	Fragment	Amin et al. (2020)

for marine organisms, including fish and whales. The presence of microplastics in the ITF pathway is cause for concern, as it could have a ripple effect throughout the food chain. If the copepod population declines, it could have a devastating impact on the marine ecosystem. Although the research has not established the exact effects of microplastics on the zooplankton or the larger species that consume them, studies conducted in the laboratory have shown several sublethal effects resulting from the ingestion of microplastics in copepods. Cole et al. (2013) provided empirical evidence that ingesting microplastics, particularly those in *Centropages typicus* copepods, reduced appetite. This study highlights the impact of microplastics on the feeding behavior of marine organisms. Furthermore, studies on the impact of microplastics on the copepod *Tigriopus japonicus* have demonstrated that microplastics significantly influence the copepod's reproductive rate and survival. This finding emphasizes the potential long-term effects of microplastics on the copepod population, which could have far-reaching consequences for the marine ecosystem (Besseling et al. 2014; Lee et al. 2013; Yu et al. 2020).

It was observed that copepods with a size range of 200-500 µm had the lowest ingestion rate, followed by copepods with a size range of 500-1000 µm and copepods with a size range of 1000-2000 µm. The studies suggest that this trend can be attributed to biological dilution effects, which occur when larger copepods compete with smaller ones for microplastic particles (Desforges, Galbraith & Ross 2015). Consequently, the availability of microplastics decreases for the smaller copepods. Additionally, feeding behavior also has a role in influencing the rate of ingestion (Rashid et al. 2021). Larger copepods, in particular, may require more prey to meet their nutritional needs, resulting in a higher risk of ingesting microplastics. They can ingest plastic when they mistaken the plastic particles for food or consume prey that has already ingested microplastics. However, did the higher levels of microplastics in the 1000-2000 µm size copepods come from the contaminated consumption of smaller copepods? This assumption still needs further laboratory research. The potential transfer of microplastics into the food web, especially in the zooplanktonic group, remains largely unanswered. However, laboratory studies had shown the transfer of microplastic pollutants from fish to lobsters (Murray & Cowie 2011); blue mussel shellfish to lobster crabs (Farrell & Nellson 2013); copepods to jellyfish (Costa et al. 2020); brine shrimp to small fish yellow croaker (Kim et al. 2022), and copepods to seahorses (Domínguez-López et al. 2022). Setälä, Fleming-Lehtinen and Lehtiniemi (2014) reported plastic transfer in zooplanktonic food webs. *Mysis* spp. shrimp were reported to contain microplastics after being fed copepods and worms contaminated with plastic. However, the results of this research are a serious concern that the size of the biota affects the amount of microplastics in the concentration of microplastics in the body. The higher probability of plastic can be influenced by a greater level of consumption and the need for food by biota, so there is a greater possibility of ingesting microplastics and the possibility that the food consumed has been contaminated by plastic. The transfer of microplastics in natural waters from plastic-contaminated zooplankton to high trophic biota was reported by Desforges, Galbraith and Ross (2015). Plastic-contaminated zooplankton is thought to have migrated to salmon off the coast of North America.

The copepod samples in this study were collected from four distinct oceanographic zones along the primary pathway of the ITF. These regions include the north Makassar Strait, which serves as the entrance point of the ITF, as well as the Makassar Strait, Alas Strait, and Lombok Strait. There is no statistically significant variation in the levels of microplastic uptake by copepods across the four examined locations. Similar to this study, there was no spatial pattern to ingestion rates in Tampa Bay (Fibbe et al. 2023), Fram Strait, Artic (Botterell et al. 2022), Black Sea (Aytan, Esensoy & Senturk 2022), Southern South China Sea (Amin et al. 2020), South China Sea (Sun et al. 2017), and northeast Pacific Ocean (Desforges, Galbraith & Ross 2015). However, the north Makassar Strait oceanographic region has a larger value of microplastics, as depicted in Figure 7. It is hypothesized that the water mass derived from the Pacific Ocean contains a substantial quantity of microplastics. The ingestion of microplastics by copepods in the ITF pathway is believed to result from water mass movements from the Pacific Ocean and anthropogenic activities around the ITF areas. Due to their minuscule mass, microplastics are highly susceptible to being carried by ocean currents. The ITF traverses the Makassar Strait, primarily influenced by enormous volumes of water from the Pacific Ocean. Prior research in the Pacific Ocean region documented elevated microplastic concentrations, which peaked at 12.83 n/L in the water column (Peng et al. 2018). It is also hypothesized that human activities on land significantly contribute to microplastics along the ITF pathway, which copepods in these waters mistaken for food. Microplastics in the marine environment primarily originate from human activities conducted on land (Browne et al. 2011).

CONCLUSION

The present study confirms that copepods in the ITF pathway are mistaking microplastics for food and ingesting them. The microplastic ingestion rate by copepods along the ITF pathways is relatively similar to the Pacific Ocean and other studies for copepods in the natural waters worldwide (except in the Yellow Sea and South China Sea where their concentrations are higher). Copepods ingested significantly more fibers than fragments. The ingestion rate of copepods is directly proportional to their body size. This study was the initial investigation of the ingestion of microplastic in the zooplankton across the Indonesian seas. This comprehensive study represents a significant milestone in our understanding of microplastic ingestion by copepods in the waters of Indonesia. The findings provide crucial baseline data for future research in this vital area. Future studies should further investigate the potential for and impacts of trophic transfer of microplastics across Indonesian seas and multiple trophic levels from the ITF pathways.

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