BIODIVERSITAS
Volume 25, Number 12, December 2024
Pages: 4664-4676

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251203

DNA barcoding of intertidal barnacles as potential bioindicators of microplastic pollution in Seribu Islands and Jakarta Bay, Indonesia

MIFTAKHUL SEFTI RAUFANDA, ROMANUS EDY PRABOWO♥, AGUS NURYANTO
Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno 63, North Purwokerto, Banyumas 53122, Central Java, Indonesia.
Tel.: +62-281-638794, ♥email: romanus@unsoed.ac.id

Manuscript received: 16 May 2024. Revision accepted: 2 December 2024.

Abstract. *Raufanda MS, Prabowo RE, Nuryanto A. 2024. DNA barcoding of intertidal barnacles as potential bioindicators of microplastic pollution in Seribu Islands and Jakarta Bay, Indonesia. Biodiversitas 25: 4664-4676.* Barnacles, with their sessile nature and filter-feeding behavior, hold significant potential as bioindicators of microplastic pollution. Due to the diverse morphotypes across barnacle species, DNA barcoding is a reliable technique for accurate taxonomic identification. This research aimed to determine intertidal barnacle species and identify potential bioindicators of microplastic pollution in Seribu Islands and Jakarta Bay, Indonesia. Barnacle samples were collected from seven locations using purposive sampling. Microplastic characteristics were analyzed visually and polymer-type testing was performed using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). Data was analyzed using Pearson correlation and bioconcentration factors to determine potential microplastic bioindicator species based on three criteria. The significance level was set at $p<0.05$ and all statistical analyses were performed using SPSS. Four species of intertidal barnacles were identified in Seribu Islands and Jakarta Bay, namely *Amphibalanus amphitrite*, *Striatobalanus amaryllis*, *Amphibalanus zhujiangensis*, and *Newmanella radiata*. DNA barcoding was used to determine the first three species, while morphological analysis identified the fourth species. The microplastic particle count varied among the species, with *A. amphitrite* showing the highest concentration at 42-53 particles/g. Due to its clear taxonomy, ease of surveying, and wide distribution, *A. amphitrite* has strong potential as a bioindicator of microplastic pollution, as it can accumulate more than other species.

Keywords: Barcoding, barnacles, bioindicators, microplastics, Seribu Islands

INTRODUCTION

Barnacles, a group of marine crustaceans with over 1,000 species distributed globally (Xu et al. 2020), occupy a wide range of substrates, including limestone, mollusk shells, coral, mangrove roots, turtle shells, and whale skin along intertidal zones of temperate and tropical coastlines (Pochai et al. 2017). These crustaceans possess several attributes that make them excellent bioindicators of microplastics, such as they are sessile, filter-feeding, cosmopolitan, easy to collect, have a sessile adult life, exhibit relative tolerance to contaminants, and can reflect changes in contaminant levels in the aquatic environment (Vaezzadeh et al. 2021). Microplastics (MPs) are plastic particles less than 5 mm that contaminate water columns, sediment, and biota of marine and freshwater environments (Coyle et al. 2020). Their small size makes them easily ingestible by various marine organisms. Over 300 marine species, ranging from large mammals to small crustaceans have been reported to consume microplastics (Yu et al. 2021), such as brown shrimp (*Crangon crangon* Linnaeus 1758) (Devriese et al. 2015), mussels (*Mytilus edulis* Linnaeus 1758), and oysters (*Saccostrea cucullata* Born 1778) (Li et al. 2018).

Bioconcentration is a concept used in ecological risk assessment to determine the level of pollutant transport within the food web. Bioconcentration factors can predict the level of contamination, such as microplastics, based on the concentration of pollutants in the surrounding environment (Miller et al. 2023). A study by Xu et al. (2020) identified four barnacle species in Hong Kong waters, namely *Amphibalanus amphitrite* Darwin 1854, *Fistulobalanus albicostatus* Pilsbry 1916, *Tetraclita japonica* Pilsbry 1916, and *Capitulum mitella* Linnaeus 1758. Microplastic accumulation in these barnacles ranged from 0 to 8.63 particles per g of wet weight, with fibers being the most common type. The research also indicated a positive correlation between sediment microplastics and *A. amphitrite*, suggesting its potential as a bioindicator for microplastics.

Bioindicator species must meet four main criteria, i.e. (i) tractable taxonomy; (ii) ease of detection and observation, (iii) broad distribution but specialized species; and (iv) diversity patterns that reflect other groups (Syaripuddin et al. 2015). The taxonomic identity of barnacles can be determined through morphological identification using shell compartments and soft body parts. However, this method is challenging due to the diverse morphotypes within most barnacle genera. Molecular identification using DNA barcoding is necessary to ascertain taxonomic identity with certainty. This technique validates cryptic species, which may exhibit similar morphology but have genetic differences. DNA barcoding employs standard markers for animal characterization, specifically cytochrome c oxidase subunit 1 (COI), as COI has highly variable fragments that can differentiate species

with identical morphology, such as members of *Balanus amphitrite* Darwin 1854 group (Chen et al. 2014; Riani et al. 2021).

The geographical proximity of Jakarta Bay and the waters of the Seribu Islands to the National Capital, Jakarta, means various anthropogenic activities significantly influence these waters. According to Cordova and Nurhati (2019), approximately 23±7.1 tons/day of waste entered Jakarta Bay with 37% being plastic waste (8.32±2.44 tons). Indonesia produces around 200,000 tons of plastic waste annually, with about 36% ending up in the sea (Cordova and Nurhati 2019; Wiadnyana et al. 2021). This region is a confluence point for household waste carried by rivers. The fisheries sector is notably impacted by marine plastic pollution, which can affect the productivity, survival, profitability, and safety of fisheries activities (Sianggaputra et al. 2022). This study aimed to determine the taxonomic identity of barnacles found in Seribu Islands and Jakarta Bay using the DNA barcoding method and to identify potential intertidal barnacle species suitable as bioindicators of microplastic pollution in Seribu Islands and Jakarta Bay, Indonesia.

MATERIALS AND METHODS

Sampling sites and laboratory examination

Sampling was carried out in September 2023 at seven sampling points which were selected at the Seribu Islands and Jakarta Bay, Indonesia (Table 1; Figure 1). Physical characteristics of microplastics, such as size, shape, and color, in barnacle, water, and sediment samples were analyzed at the International Tropical Marine and Earth Sciences Laboratory (ITMEL), Universitas Jenderal Soedirman, Purwokerto, Indonesia. Chemical characteristics of microplastics in barnacle, water, and sediment samples were analyzed at the Faculty of Mathematics and Natural Sciences Integrated Laboratory, Universitas Negeri Yogyakarta, Indonesia.

Table 1. Sampling site notation and name

Notation	Location	Coordinates (Latitude: S; Longitude: E)
1	Harapan Island floating restaurant	5° 38’919’’ 106° 34’736’’
2	Panggang Island floating net cage	5° 44’678’’ 106° 36’078’’
3	Pramuka Island pier	5° 44’554’’ 106° 36’818’’
4	Untung Jawa Island pier	5° 58’716’’ 106° 42’290’’
5	Muara Angke Estuary pier	6° 06’270’’ 106° 46’330’’
6	Ancol Beach	6° 07’160’’ 106° 50’956’’
7	Marunda Beach	6° 05’543’’ 106° 57’804’’

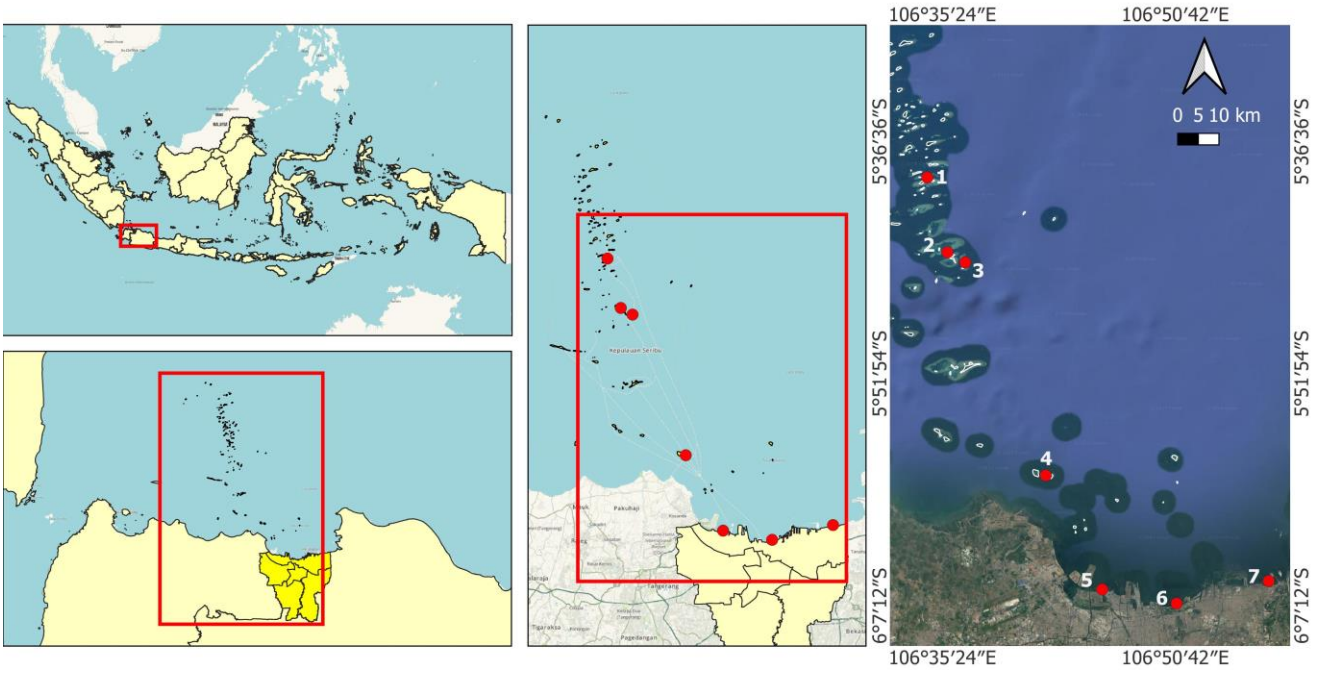

Figure 1. Sampling locations in the Seribu Islands and Jakarta Bay, Indonesia. 1. Harapan Island floating restaurant; 2. Panggang Island floating net cage; 3. Pramuka Island pier; 4. Untung Jawa Island pier; 5. Muara Angke Estuary pier; 6. Ancol Beach; 7. Marunda Beach

Procedures

Morphological identification

Fifty individual barnacle samples of each species were collected from the research locations. The barnacles were detached from the substrate using a scraper, placed in sample bottles, and treated with 96% alcohol to minimize contamination during transportation and storage. To ensure the integrity of ingested microplastics, the sample bottles containing barnacles were refrigerated, transported back to the laboratory, and then frozen at -20°C prior to further identification (Xu et al. 2020).

Morphological identification of the barnacles was conducted by observing shell characteristics. The process began by separating the hard and soft parts of the barnacles, followed by observations using a stereo microscope and photographing the observations. Barnacle species were identified based on shell morphology (Tsang et al. 2015). Subsequently, the barnacle specimens were preserved in 96% absolute ethanol. This step aimed to group identical samples into morphospecies, which required further validation using molecular characteristics (Riani et al. 2021).

Genetic identification

Genomic DNA was extracted using the gSYNTM DNA Extraction Kit (Geneaid, GS300) according to the manufacturer's protocol. Polymerase Chain Reaction (PCR) amplification of the COI gene was performed using MyTaq HS Red Mix (Bioline, BIO-25048) and the universal primer pair LCO1490: 5'-GGT CAA CAA ATC ATA AA G ATA ATT GG-3' and HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Chen et al. 2014). The final 25 µL PCR mixture comprised 12.5 µL of 2x MyTaq Red Mix, 0.4 µM of each primer, template DNA (10-5 ng), and ddH_2O up to 25 µL. The amplification protocol started with an initial denaturation at 95°C for 1 minute, followed by 35 cycles of denaturation at 95°C for 10 seconds, annealing at 52°C for 15 seconds, and extension at 72°C for 15 seconds.

One specimen from each morphotype was sent to a sequencing service company for DNA barcoding. Genomic DNA isolation and COI marker amplification were performed at PT. Genetika Science Indonesia, Jakarta. Bi-directional sequencing of the COI gene was conducted at 1st BASE Asia in Kuala Lumpur, Malaysia.

Measurement of microplastics in barnacles

The length and width of the barnacle shells were measured using calipers. The soft tissue of the barnacles was separated from the shells using forceps, and the wet weight of the tissue was recorded. Groups of five barnacles were collected and placed in a 100 mL beaker as one replicate. A 10% potassium hydroxide (KOH) solution was added to each beaker, totaling 60 mL per sample (Rochman et al. 2015). The solution was then incubated at 40°C for 48 hours and subsequently filtered through Whatman paper No. 42. The filter paper was placed in a clean petri dish for further analysis (Xu et al. 2020).

Water and sediment sample collection

Water sample collection was conducted during low tide as the waters receded towards high tide. The water sampling method followed the procedure used by Md Amin et al. (2020). Water samples were collected using the pour method with a bucket, taking 100 liters of water that passed through a plankton net. GPS was used to record the coordinates of the initial and final sampling positions (Viršek et al. 2016). Water sampling was performed horizontally at the surface at each station along the transect (Zhang et al. 2017). The plankton net was rinsed with water from the mouth towards the cod end to filter any microplastic particles that adhered to the net. All water samples were transferred into sample bottles, then tightly sealed and stored in a cool box. Care was taken to minimize microplastic contamination from other sources during storage (Viršek et al. 2016; Hiwari et al. 2019).

Sediment samples were collected using a shovel. Approximately 400 g of sediment was collected at each research location and the coordinates of each location were recorded using GPS. Any organic material collected with the sediment was discarded. The samples were placed in pre-labeled plastic bags and stored in a container box for further analysis in the laboratory (Mauludy et al. 2019).

Extraction of microplastics in water and sediment

Microplastic content in water samples was analyzed according to Masura et al. (2015). A 14 mL water sample was transferred into a test tube and dried in an oven at 80°C for 24 hours. Subsequently, organic material was digested using 30% H_2O_2 with a 1:1 volume ratio to oxidize the biological material, followed by incubation in an oven at 80°C for 24 hours. The resulting clear solution was then dried on Whatman filter paper no. 42 using a vacuum pump (Gewert et al. 2017).

Sediment samples were dried in an oven at 74°C for 24 hours. A 200 g sediment sample was transferred into a glass beaker and mixed with 600 mL of concentrated NaCl. The mixture was stirred with a glass stirrer for 2 minutes and left undisturbed for 1 hour to allow the sediment to settle. This process was repeated twice to separate microplastics from the sediment, resulting in a natant and supernatant. The supernatant containing microplastic particles was carefully transferred into another beaker. The sample was then filtered using a vacuum pump and Whatman filter paper no. 42, and the filter paper was stored in a petri dish (Claessens et al. 2011; Cordova et al. 2018; Mu et al. 2019).

Observation of visual characteristics of microplastics in barnacles, water, and sediment

The visual properties of microplastics were examined based on their morphological characteristics, such as shape, size, and color. Microplastics collected on filter paper were observed using a stereo microscope. Subsequently, measurements of the microplastic samples were taken using the Optilab Advance 2.2 tool and Optilab Viewer software, followed by further analysis using Image Raster software (Anu et al. 2017). Measurements were based on the longest side of each microplastic particle. Microplastic

particles were categorized into size ranges: <100 μm, 100-500 μm, 500-1,000 μm, 1,000-1,500 μm, 1,500-2,000 μm, 2,000-2,500 μm, 2,500-3,000 μm, 3,000-3,500 μm, 3,500-4,000 μm, 4,000-4,500 μm, and 4,500-5,000 μm (Ding et al. 2018). Visual recognition employs a standard size and color sorting system (SCS System), effectively categorizing plastic pieces based on size and shape (Crawford and Quinn 2017).

Observation of chemical characteristics of microplastics in barnacles, water, and sediment

Microplastics from barnacle soft tissue samples that had been visually observed were further analyzed for chemical or polymer composition using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) with a Shimadzu IRSpirit-T model equipped with QATR. FTIR produces a spectrum for each analyzed particle, which is then compared with reference spectra recorded in the instrument's library to identify the type of polymer (Cutroneo et al. 2020). The microplastic samples were placed in the sample holder before analysis (Viršek et al. 2016). Validation of ATR-FTIR was performed by identifying polymers based on the presence of specific absorption bands (Jung et al. 2018).

Data analysis

All species were determined based on external shell morphology, including the pattern of the parietes and opercular plates, as described by Chan and Prabowo (2009). Each generated chromatogram was checked and assembled using CodonCode Aligner v. 10.0.1. A similarity search of the sequences was conducted with the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/). For phylogenetic analyses, additional sequences of related species within the group were retrieved from GenBank, along with *Ibla cumingi* Darwin 1851 as an outgroup. All sequences were aligned in MEGA XI using ClustalW and checked for deletions, insertions, and stop codons (Tamura et al. 2021). Phylogenetic trees were reconstructed using three methods: Neighbor-Joining (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP). The NJ tree was generated using the Kimura 2-parameter model, while the ML tree used the optimal nucleotide substitution model based on the lowest Bayesian Information Criterion (BIC) score. The MP tree was constructed using the Tree-Bisection-Regrafting (TBR) algorithm with search level 1, starting from initial trees generated by random sequence addition (10 replicates). Branch lengths for the MP tree were calculated with the average pathway method. For each tree reconstruction method, bootstrap support was calculated with 1000 replicates to evaluate clustering robustness, with all reconstructions performed in MEGA XI.

The abundance of microplastic particles in barnacles, water, and sediment was calculated and characterized based on shape, size, and color. The data were analyzed to determine significant differences between microplastics in barnacles, water, and sediment. The normality of the data was assessed using the Shapiro-Wilk test. Pearson Correlation was used to analyze the correlation between the amount of microplastics in the environment (water and sediment) and the bodies of barnacles. Additionally, the correlation between the number and size of ingested microplastics and the body length of barnacles was tested using Pearson Correlation. The level of significance was set at $p<0.05$. All statistical analyses were performed using SPSS.

The bioconcentration factor (BCF) was calculated to indicate the ability of barnacles to absorb and accumulate microplastics from the environment. BCF was determined using the formula (Hu et al. 2019): $BCF = C_{biota}/C_{environment}$ where C_{biota} represents the concentration of microplastics in the body tissue of barnacles and $C_{environment}$ represents the concentration of microplastics in the aquatic environment (water and sediment).

RESULTS AND DISCUSSION

Species identification based on morphological characters

Shell morphology is crucial for barnacle identification and serves as a foundation for taxonomic and phylogenetic studies (Tsang et al. 2015). Morphological observations revealed four distinct morphotypes. Species 01 was found at four locations, i.e. Muara Angke Estuary Pier, Ancol Beach, Marunda Beach, and Untung Jawa Island pier. Morphological observations showed that the shell is smooth, conical, white with vertical purple striations, and lacks horizontal striations (Figure 2.A). These characteristics match *A. amphitrite*, as described by Chan and Prabowo (2009), which features a six-piece shell, conical or rounded, smooth and white with vertical purple markings and no horizontal striations.

Species 02 was found at Panggang Island floating net cage and Harapan Island floating restaurant. Morphological observations indicated that SP2 has cone-shaped parietes, white with a pink radiating pattern (Figure 2.B). These characteristics correspond to *Striatobalanus amaryllis* Darwin 1854, as described by Chan and Prabowo (2009), which has a flat shell base and parietes with red-purple longitudinal lines.

Species 03 was found at two locations: Panggang Island floating net cage and Harapan Island floating restaurant. Morphological observations showed that SP3 has purplish parietes with a smooth surface and dark purple lines (Figure 2.C). These features align with *Amphibalanus zhujiangensis* Ren 1989, as identified by Chan and Prabowo (2009), characterized by purplish parietes with smooth surfaces, dark purple lines, and narrow, long spurs.

Species 04 was found at three locations: Pramuka Island pier, Panggang Island floating net cage, and Harapan Island floating restaurant. Morphological observations revealed that SP4 has a rough outer shell, low conical shape, and white parietes (Figure 2.D). These traits match *Newmanella radiata* Bruguière 1789, as described by Chan and Prabowo (2009), characterized by green parietes with four blue striped plates, separate parietes, a calcareous base, a low conical to cyclindroconic shape, and 1 to 3 rows of tubes on the wall.

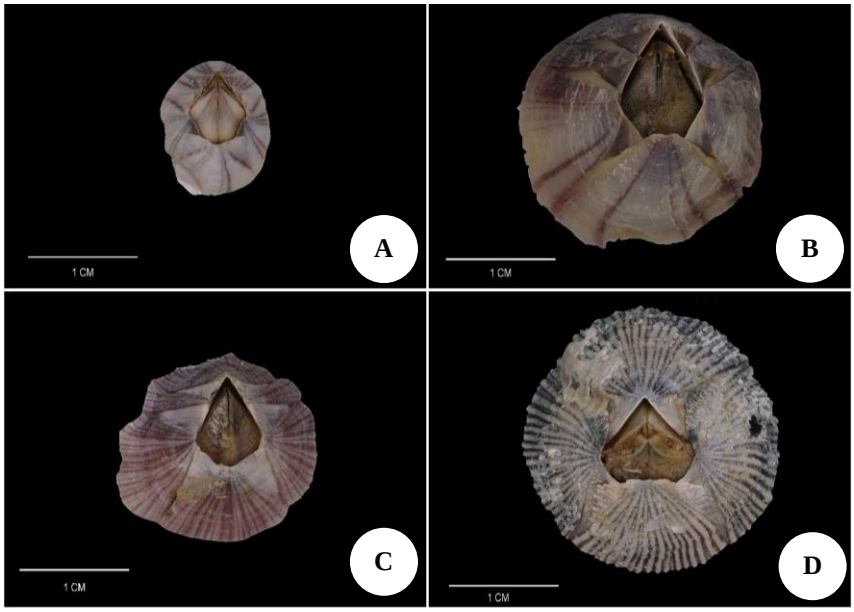

Figure 2. Morphological characteristics of barnacle found at the research location A. *Amphibalanus amphitrite*; B. *Striatobalanus amaryllis*; C. *Amphibalanus zhujiangensis*; D. *Newmanella radiata*

Table 2. Sequence identity of closest relatives of the four isolated barnacle species found in GenBank Using BLAST

Species	Query cover (%)	E-value	Identity (%)	Reference species (Accession number)	Accession
Amphibalanus amphitrite Darwin 1854	97	0.0	99.39	*Amphibalanus amphitrite* (OM943430.1)	PQ498734
Striatobalanus amaryllis Darwin 1854	96	0.0	99.55	*Striatobalanus amaryllis* (MN690055.1)	PQ498735
Amphibalanus zhujiangensis Ren 1989	97	0.0	99.85	*Megabalanus tintinnabulum* (KU204355.1)	PQ498736
Newmanella radiata Bruguière 1789	98	0.0	99.11	*Liriomyza trifolii* (EU219614.1)	PQ498737

The four species of barnacles obtained from the seven research locations were morphologically identified as follows: *A. amphitrite* (Muara Angke Estuary pier, Ancol Beach, Marunda Beach, and Untung Jawa Island pier), *S. amaryllis* (Panggang Island pier and Harapan Island floating restaurant), *A. zhujiangensis* (Panggang Island floating net cage and Harapan Island floating restaurant), and *N. radiata* (Pramuka Island pier, Panggang Island floating net cage, and Harapan Island floating restaurant).

DNA barcodes

The initial examination of the genetic similarity of the samples to the data in GenBank is as follows: species 01 has a genetic similarity of 99.39% to *A. amphitrite*, species 02 has a genetic similarity of 99.55% to *S. amaryllis*, and species 03 has a genetic similarity of 99.85% to *Megabalanus tintinnabulum* Linnaeus 1758 (Table 2). The standard percentage value of species similarity in the BOLD system is typically between 96-100% (Bhattacharjee et al. 2012; Abedin et al. 2021). This range indicates a high level of sequence similarity at the species level, allowing confident species identification. Researchers often use a similarity cutoff of ≥97% for species identification in databases like GenBank and BOLD (Nuryanto et al. 2018; Djoemharshjah 2023). The BOLD system, along with the BIN system, groups similar sequences into clusters representing potential species. Several previous investigations related to DNA barcoding of barnacle species have reported the use of threshold values as species limits, including *Balanus balanus* Linnaeus 1758 and *Semibalanus balanoides* Linnaeus 1767 (99%) (Walczyńska et al. 2019), *A. zhujiangensis* (99%) (Jaberimanesh et al. 2019), and *Amphibalanus reticulatus* Utinomi 1967 and *Amphibalanus variegatus* Darwin 1854 (99%) (Riani et al. 2021). Therefore, an identity similarity of 99% in this study is considered the most reliable threshold value for DNA barcoding of barnacle species.

Species 04 exhibited a high genetic similarity to *Liriomyza trifolii* Burgess 1880. Consequently, the COI gene cannot be used to validate Species 04 as *N. radiata*, given its 99.11% similarity to the species *L. trifolii* (leafminer fly). This barcoding sample is likely a contaminant due to its similarity to a non-target species. This statement is supported by Shokralla et al. (2014), who noted that the presence of DNA sequence signals from competing sources at low concentrations can often cause Sanger-based sequencing of organisms to fail to produce accurate DNA barcodes. Various standard protocols are implemented to reduce or eliminate the risk of cross-contamination during DNA extraction, PCR amplification, and sequencing (Boessenkool et al. 2012).

Nevertheless, some sources of non-target contamination cannot be entirely avoided. Organisms captured in large quantities and stored in a medium, such as ethanol will experience DNA dispersion throughout the preservation liquid (Hajibabaei et al. 2012). Insect hairs and scales can also be transferred unnoticed from one specimen to another prior to tissue subsampling and individual DNA barcoding. If the entire specimen is sampled, it may also contain larvae and eggs of parasitoid insects (e.g., wasps and flies), which can introduce non-target DNA sequence data (Shokralla et al. 2014).

A COI gene phylogram was created with sequences of closely related COI genes from each species obtained from GenBank. The phylogenetic trees constructed using the Neighbor-Joining (Figure 3.A), Maximum Likelihood (Figure 3.B), and Maximum Parsimony (Figure 3.C) methods included 44 nucleotide sequences encompassing all codon positions (1st, 2nd, 3rd) as well as noncoding regions. Data points with gaps and missing information were excluded, resulting in a final dataset of 639 positions. Across all three analyses, the results consistently demonstrated that Species 01, morphologically identified as *A. amphitrite*, clusters with reference sequences from GenBank, confirming congruence between morphological and genetic data. Similarly, Morphospecies 02, identified as *S. amaryllis*, exhibits consistent clustering with GenBank reference sequences, corroborating its morphological classification. In contrast, Species 03, initially classified as *A. zhujiangensis* based on morphology, clusters with reference sequences for *M. tintinnabulum*, indicating a genetic relationship that deviates from its morphological identification. This discrepancy underscores the importance of integrating both morphological and molecular approaches for accurate species identification.

One of the criteria used to assess the potential of a bioindicator is tractable taxonomy, which refers to the ease of species identification by non-experts. These criteria are evaluated through the success of DNA barcoding (Syaripuddin et al. 2015). This research obtained four barnacle species, which were morphologically identified as *A. amphitrite*, *S. amaryllis*, *A. zhujiangensis*, and *N. radiata*. The number and quality of available reference sequences significantly impact the speed and accuracy of DNA barcoding processes. Reference sequences are crucial for matching and identifying unknown sequences to known species. The DNA barcode reference library for *A. amphitrite* is expanding the fastest, with more than 70,000 DNA barcodes facilitating faster sequence matching. This is followed by *S. amaryllis* (26 DNA barcodes), *M. tintinnabulum* (23 DNA barcodes), and *N. radiata* (9 DNA barcodes) (NCBI 2024). Based on these results, it can be suggested that *A. amphitrite* is a strong candidate for becoming a bioindicator species, because it meets the tractable taxonomy criteria.

Accumulation of microplastic in barnacle

Microplastics were detected in 84% (279 out of 331) of the barnacle individuals studied. The average amount of microplastics in four species of barnacles from various regions ranged between 1 and 53 particles per g (wet weight) and 0.33 to 2.19 particles per individual. Among the four species, *Amphibalanus amphitrite* had the highest amount of microplastics, with 42 to 53 particles per g and 0.42 to 1.48 particles per individual, followed by *A. zhujiangensis* (6 to 13 particles per g, 1.03 to 1.72 particles per individual), *Newmanella radiata* (1 to 9 particles per g, 0.33 to 2.19 particles per individual), and *S. amaryllis* (1 to 3 particles per g, 1 to 1.14 particles per individual) (Table 3).

These results align with the findings of Xu et al. (2020), which reported that among four species found in Hong Kong, *A. amphitrite*, *F. albicostatus*, *T. japonica*, and *C. mitella*. *A. amphitrite* had the highest abundance of microplastics, ranging from 0.3 to 10.3 particles per g. Another study by Thushari et al. (2017) reported 0.23 to 0.43 microplastic particles per g in *A. amphitrite* from three locations in Thailand. According to Xu et al. (2020), differences in microplastic abundance between species may result from variations in their ability to ingest or excrete microplastics. Pasternak and Achituv (2007) explained that *A. amphitrite*, at normal feeding speeds, increases water flow through the mantle cavity for better filtration of microscopic food. At higher speeds, there is a greater opportunity to capture particulate food with large cirri, which can be rotated and extended to enhance particle capture efficiency.

Table 3. Morphometry and abundance of microplastics in barnacles

Species	Location	N	Average length of barnacles (cm)	Average wet weight of barnacle (g/ind.)	Abundance (particles/g)	Abundance (particles/ind.)
A. amphitrite	Muara Angke Estuary pier	50	0.7±0.13	0.14±0.07	53	1.48
	Ancol Beach	50	0.6±0.1	0.07±0.03	40	0.56
	Marunda Beach	50	0.6±0.12	0.06 ±s 0.04	50	0.66
	Untung Jawa Island pier	50	0.7±0.13	0.05±0.03	42	0.42
N. radiata	Pramuka Island pier	21	1±0.22	0.24±0.12	9	2.19
	Panggang Island floating net cage	6	0.7±0.21	0.20±0.15	2	0.33
	Harapan Island floating restaurant	7	0.9±0.1	0.31±0.11	1	0.43
S. amaryllis	Panggang Island floating net cage	7	1±0.3	0.40±0.46	3	1.14
	Harapan Island floating restaurant	18	1.2±0.2	0.93±0.62	1	1.00
A. zhujiangensis	Panggang Island floating net cage	43	0.7±0.12	0.13±0.06	13	1.72
	Harapan Island floating restaurant	29	0.8±0.1	0.16±0.10	6	1.03

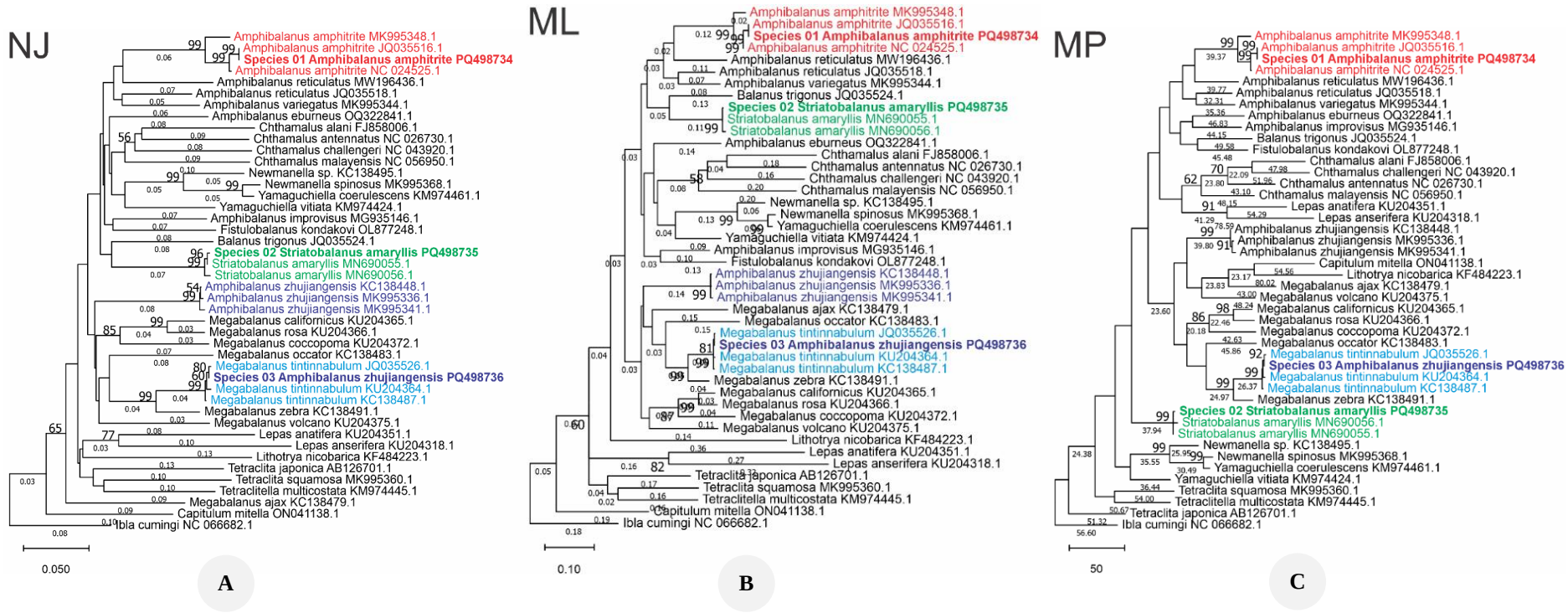

Figure 3. A. The phylogenetic tree is depicted using the Neighbor-Joining method, presenting the optimal tree. Bootstrap values from 1000 replicates indicate the robustness of the clustering, shown next to the branches. Branch lengths represent evolutionary distances calculated using the Kimura 2-parameter model, expressed as the number of base substitutions per site; B. The phylogenetic tree is depicted using the Maximum Likelihood method with the General Time Reversible model, showcasing the tree with the highest log likelihood (-7898.43). Bootstrap percentages indicate the robustness of the clustering, displayed next to the branches. Initial trees for the heuristic search were generated using the Neighbor-Joining method based on pairwise distances estimated via the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was applied to model evolutionary rate differences among sites, with five categories (+G, parameter = 2.0934), allowing for some sites to be evolutionarily invariable ([+I], affecting 59.78% of sites). The tree is drawn to scale, with branch lengths representing substitutions per site; C. The phylogenetic tree was reconstructed using the Maximum Parsimony method, displaying the most parsimonious tree with a length of 2053. This tree's metrics include a consistency index of 0.226011 (0.224121), a retention index of 0.491031 (0.491031), and a composite index of 0.110978 (0.110050) for all sites and parsimony-informative sites (values in parentheses). Bootstrap percentages, shown adjacent to the branches, reflect the clustering support from 1000 replicates. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences. Branch lengths were calculated using the average pathway method and are in the units of the number of changes over the whole sequence. They are shown below the branches

The visual characteristics of microplastics in barnacles were observed in various shapes, sizes, and colors. The results of these visual observations revealed three forms of microplastics, namely fibers, fragments, and films (Figure 4). Among the four barnacle species studied, fibers dominated the microplastics (83.68%), followed by fragments (16.02%) and films (0.30%). These findings are consistent with Xu et al. (2020), which showed that fibers were the most dominant form of microplastic of 95.7%, with the remainder being fragments of 3.4%, and pellets of 0.8%. Thushari et al. (2017) also demonstrated that microplastic particles accumulated in samples of *B. amphitrite*, *Saccostrea forskahlii* Gmelin 1791, and *Littoraria* sp. were in the form of fragments and synthetic fibers.

Fibers primarily originate from clothing and textiles and are the most commonly found microplastics in marine environments and biota (Lu et al. 2024). Synthetic fibers reportedly account for nearly 90% of the microplastics found in global coastal ecosystems (Surana et al. 2024). The widespread use of synthetic clothing in metropolitan populations and the fast fashion industry contributes to the increased presence of synthetic fibers in the environment. Domestic washing of synthetic textiles contributes up to 35% of the total microplastic fibers released into the environment (Mishra et al. 2020). Fiber and fragment-type microplastics are formed due to the fragmentation of larger plastic waste in the ocean, driven by factors such as photo-oxidation, surface waves, and water current turbulence (Kavya et al. 2020). On the other hand, film-type microplastics are sourced from single-use plastic bags, which have a low resistance to degradation when exposed to seawater and sunlight (Coyle et al. 2020).

This result is reinforced by Ardiansyah et al. (2022), who emphasized that the semi-enclosed waters with various activities in Jakarta Bay make it vulnerable to receiving plastic waste from multiple sources, such as river estuaries, households, offices, industries, fisheries, ports, and transportation. Jakarta Bay is highly susceptible to serving as a repository for waste originating from both land and sea, transported by tidal currents into bays, estuaries, and the Seribu Islands. Primary sources of marine waste include aquaculture and capture fisheries (buoys and nets), shipping (plastic pellets), transportation (tires), cosmetics (microbeads), and retail (plastic bags, bottles, packaging) (Cordova and Nurhati 2019).

The sizes of microplastics in the four barnacle species ranged from 60 to 4,700 µm, with an average size of 1156 µm (Figure 5). Overall, the amount of microplastics decreased with increasing size, with the most commonly found size category being 100-500 µm. According to Xu et al. (2020), the average fiber length found in barnacles is 953.7 µm, while the diameter of fragments is 1743.7 µm. Goldstein and Goodwin (2013) identified the smallest microplastic size limit in barnacles at 300 µm, achieved through dissection and microscopic examination of barnacle intestines. Generally, the microplastic size limit varies depending on the methodology used by different research teams (Li et al. 2019).

In this study, barnacle tissue was extracted using 10% KOH, and microplastics were filtered through Whatman no. 42 filters with a pore size of 2.5 µm, allowing detection of microplastics as small as 2.5 µm. The sizes of microplastics varied as follows: 60.84-4739.06 µm in *A. amphitrite*, 182.33-4,407.90 µm in *N. radiata*, 103.83-2877.01 µm in *S. amaryllis*, and 64.46-4,028.94 µm in *A. zhujiangensis* (Figure 5).

The Pearson correlation test between barnacle body length, microplastic size, and total microplastics in each species is shown in Table 4. A significant correlation ($p<0.05$) was found for *N. radiata* between barnacle body length and microplastic size, as well as between barnacle body length and total microplastics. These findings contrast with Xu et al. (2020), who explained that smaller barnacle species might accumulate more microplastics due to reduced efficiency in removing them from the digestive tract. Similarly, Yu et al. (2021) suggested that smaller microplastics remain longer in the digestive tract of barnacle larvae.

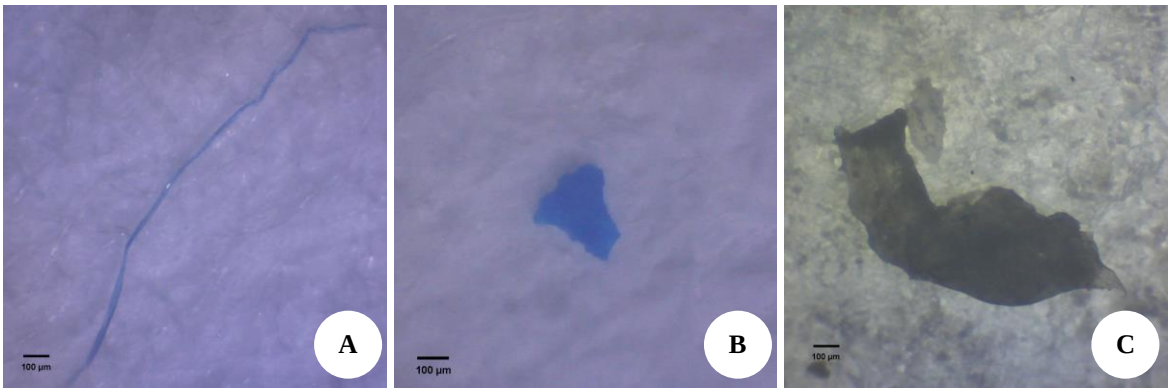

Figure 4. Various forms of microplastics are found in barnacles. A. Fiber; B. Fragments; C. Film

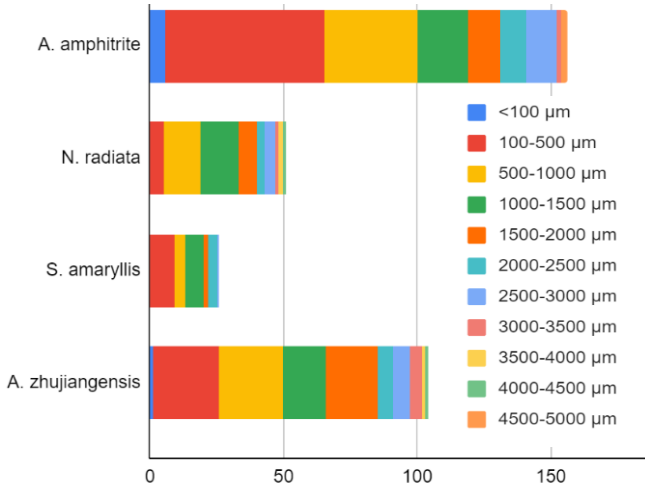

Figure 5. Size distribution of microplastics found in each barnacle species

Additionally, barnacles on muddy beaches were found to ingest microplastics more efficiently than those on rocky and coral reefs. There is limited experimental evidence supporting the correlation between barnacle body size and microplastic consumption (both microplastic size and total microplastics). This discrepancy suggests the need for further research, particularly bioaccumulation experiments comparing microplastic consumption and elimination across different barnacle species, to clarify the causes of interspecific differences in microplastic retention.

Microplastics found in the four species of barnacles were predominantly black (46.59%), followed by blue (39.76%), red (8.90%), and other colors (4.75%) (Figure 6). The variety of colors in microplastics is primarily due to prolonged exposure to sunlight, which causes oxidation and subsequent color changes (Azizah et al. 2020). Over time, this exposure can fade the colors of microplastics, eventually turning them light or even white. This color change can also result from chromophore products produced during the oxidation of microplastics (Li et al. 2023). The black microplastics are likely derived from polystyrene (PS) or polypropylene (PP). In contrast, blue and red microplastics, particularly fibers, mostly originate from domestic wastewater, such as laundry water from residential areas and effluents from wastewater treatment plants (Wen et al. 2018; Azizah et al. 2020).

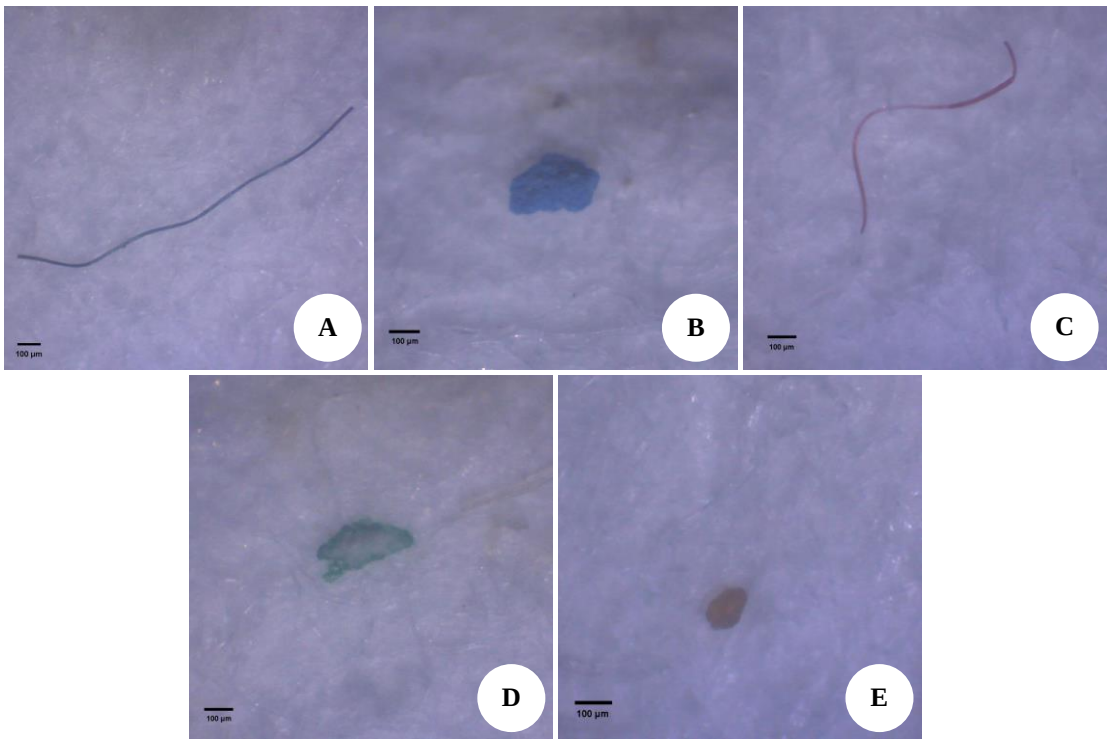

Figure 6. Color of microplastics found in barnacles. A. Black; B. Blue; C. Red; D. Green; E. Orange. Scale bars represent at 100 µm

Table 4. Pearson correlation test results between microplastic size and barnacle length and total microplastics in barnacles

Species	Pearson correlation of barnacle body length-MPs size	Pearson correlation of barnacle body length-total MPs
A. amphitrite	r = - 0.218; p>0.05 (p = 0.176)	r = 0.214; p>0.05 (p = 0.185)
N. radiata	r = 0.391; p<0.05 (p = 0.022)	r = 0.541; p<0.05 (p = 0.001)
S. amaryllis	r = - 0.150; p>0.05 (p = 0.475)	r = 0.030; p>0.05 (p = 0.887)
A. zhujiangensis	r = 0.061; p>0.05 (p = 0.610)	r =- 0.003; p>0.05 (p = 0.978)

A total of 64 microplastic particles were visually identified and subsequently analyzed using FTIR-ATR. The FTIR-ATR test results identified eight microplastic polymers, i.e. Polyurethane (PU) (65.38%), Polypropylene (PP) (7.69%), Polyvinyl chloride (PVC) (7.69%), Polyethylene (PE) (3.85%), Polyethylene terephthalate (PET) (3.85%), Polyamide (PA) (3.85%), Polyethylene vinyl acetate (PEVA) (3.85%), and Nylon (3.85%) (Figure 7). PU (Polyurethane) is currently the sixth most widely used polymer worldwide, with an annual production of nearly 18 million tonnes. Its excellent thermal insulation performance and mechanical properties make rigid PU foam a common thermal insulation material (Ma et al. 2023).

Petroleum-based microplastics include polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene terephthalate (PET). PP has emerged as a prominent microplastic due to its extensive production, widespread utilization, and inadequate disposal practices, especially during the global COVID-19 outbreak (Mishra et al. 2020). PP plastic is widely used in producing single-use items such as packaging, ropes, bottles, caps, fishing tools, carpets, strapping, drinking straws, and personal care products, including masks, gloves, and hair nets, particularly during the pandemic (Jeyavani et al. 2024).

Polyamide (Nylon) is derived from household waste, clothing, fishing, and industrial goods. Nylon is stiff but less flexible, making it resistant to damage and resulting in smaller microplastics. Polyamide is often used in anchor fibers, which is why this type of microplastic is prevalent in marine ecosystems (Choi et al. 2022). PET is generally used as a primary material for making plastic bottles, beverage and food packaging, and other packaging products. PET is favored for its lightweight, durability, and resistance to heat and water (Martín et al. 2017). Polyvinyl Chloride (PVC) is used for pipes, medical packaging, raincoats, children's toys, plastic wraps, detergent bottles, blood bags, and medical equipment (Turner and Filella 2021).

Relationship between microplastics in barnacles, water, and sediment

Bioconcentration Factor (BCF) is a metric used to assess the accumulation of pollutants from the environment into organisms (Zhu et al. 2020). In this study, BCF values were calculated to determine the extent of microplastic accumulation in barnacles from different environmental factors and locations. The highest level of microplastic accumulation in barnacles from sediment was observed in *A. amphitrite* from Marunda Beach. In contrast, the highest level of microplastic accumulation in barnacles from the water was found in *A. amphitrite* from Muara Angke Estuary pier (Table 4). When microplastics enter the aquatic environment, they can either remain suspended in the water column or sink into the sediment, depending on their density, composition, and shape (Tien et al. 2020). The number of microplastics at the bottom of the sediment can be influenced by gravitational forces, currents, wave movements, and density (Laksono et al. 2021). The density of microplastics increases due to the attachment of clay minerals and biota to their surfaces, causing them to sink (Anderson et al. 2016). Consequently, microplastics can be detected in surface water and sediments in rivers, lakes, and oceans (Rodrigues et al. 2018).

Two main factors may explain the highest accumulation of microplastic pollutants in barnacles from Marunda sediments and Angke waters. The first factor is the primary source of microplastics. Marunda and Angke are part of Jakarta Bay, a semi-enclosed water body with high levels of anthropogenic activity, including dense residential areas, shops, and fishing activities, which contribute to the release of waste and wastewater. Microplastics, as part of pollutants flowing from rivers, can be pushed into the open sea when they reach river mouths (Priscilla and Patria 2020). The second factor is sediment density. According to Pratama et al. (2023), Marunda sediments are dominated by sand. This argument is supported by van Cauwenberghe et al. (2015), who stated that there is a strong relationship between the abundance of microplastics and the fine fraction in sediment, suggesting that microplastics tend to accumulate in depositional areas (Table 5).

The Pearson correlation test for the abundance of microplastics in the four species of barnacles with the abundance of microplastics in water and sediment is shown in Table 6. A significance value ($p<0.05$), a strong positive correlation ($r = 1$) was found between the abundance of microplastics in the species *S. amaryllis* and *A. zhujiangensis* and the abundance of microplastics in the water. This correlation suggests that microplastics accumulating in the water column are more likely to affect these barnacle species. This finding is supported by Thushari et al. (2017), that barnacles use a filter feeder mechanism by extending their cirri to create a water flow. During feeding, water containing suspended particles, food materials, and plastic waste enters the body cavity without selection and accumulates in the body. Furthermore, Xu et al. (2020) noted that barnacles exhibit less food selectivity than bivalves, leading to greater microplastic accumulation in barnacles.

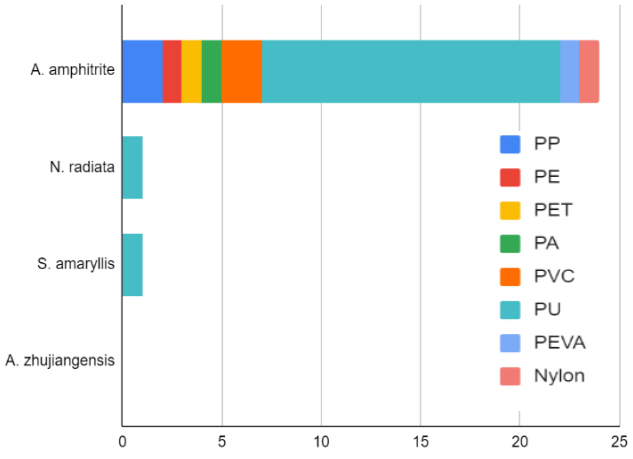

Figure 7. Microplastic polymers found in barnacles

Table 5. Abundance of microplastics in barnacles, water, sediment, and calculation results of bioconcentration factors

Species	Location	Abundance (particles/g)	Sediment abundance (particles/g)	Water abundance (particles/g)	Sediment BCF (particles/g)	Water BCF (particles/g)
A. amphitrite	Muara Angke Estuary pier	53	0.18	0.143	293.651	369.630
	Ancol Beach	40	0.117	0.381	341.880	104.987
	Marunda Beach	50	0.033	0.286	1515.152	174.825
	Untung Jawa Island pier	42	0.09	0.262	466.667	160.305
N. radiata	Pramuka Island pier	9	0.047	0.143	195.035	64.103
	Panggang Island floating net cage	2	0.06	0.238	25.000	6.303
	Harapan Island floating restaurant	1	0.073	0.071	17.676	18.174
S. amaryllis	Panggang Island floating net cage	3			45.833	11.555
	Harapan Island floating restaurant	1			14.730	15.145
A. zhujiangensis	Panggang Island floating net cage	13			217.949	54.945
	Harapan Island floating restaurant	6			85.616	88.028

Table 6. Pearson correlation test between microplastic size and barnacle length and total microplastics in barnacles

Species	Pearson correlation of barnacle MPs abundance-water MPs abundance	Pearson correlation of barnacle MPs abundance-sediment MPs abundance
A. amphitrite	r = - 0.795 ; p>0.05 (p = 0.205)	r = 0.197 ; p>0.05 (p = 0.803)
N. radiata	r = 0.036 ; p>0.05 (p = 0.977)	r = -0.918 ; p> 0.05 (p = 0.260)
S. amaryllis	r = 1 ; p<0.05 (p = 0)	r = -1 ; p<0.05 (p = 0)
A. zhujiangensis	r = 1 ; p<0.05 (p = 0)	r = -1 ; p<0.05 (p = 0)

Potential of intertidal barnacle species as bioindicators of microplastic pollution

The most common species found across all locations were *A. amphitrite* (4 locations are Muara Angke Estuary pier, Ancol Beach, Marunda Beach, Untung Jawa Island pier), *N. radiata* (3 locations are Pramuka Island pier, Panggang Island floating net cage, Harapan Island floating restaurant), *A. zhujiangensis* (2 locations are Panggang Island floating net cage, Harapan Island floating restaurant), and *S. amaryllis* (2 locations are Panggang Island floating net cage, Harapan floating restaurant). Among these, *A. amphitrite* was most commonly found in two habitats: the pier (Angke, Untung Jawa) and rocky beaches (Ancol, Marunda). This finding is supported by Xu et al. (2020), who explained that habitat versatility is one reason *A. amphitrite* may serve as a better potential bioindicator for microplastics than other barnacle species. *A. amphitrite* occurs in various habitats, including mudflats, docks, and embankments, providing greater flexibility in monitoring microplastic pollution across different aquatic habitats. In this study, *A. amphitrite* was found in intertidal locations, while the other three species were found in subtidal locations. Additionally, only *A. amphitrite* was found in locations with the highest abundance of microplastics. The findings are corroborated by laboratory exposure experiments conducted by Xu et al. (2023) on the resistance of A. *amphitrite* to microplastics. The study demonstrated that exposure to environmentally relevant concentrations of microplastics did not result in a significant lethal or sub-lethal response (Yu and Chan 2020). The tolerance of *A. amphitrite* to microplastics allows this species to become a bioindicator of microplastic pollution.

In conclusion, the intertidal barnacle species identified in the Seribu Islands and Jakarta Bay, Indonesia using DNA barcoding are *A. amphitrite*, *S. amaryllis*, and *M. tintinnabulum*. Meanwhile, another species, *N. radiata*, was only identified morphologically. The accumulation of microplastics in the bodies of each intertidal barnacle species found in Jakarta Bay and the Seribu Islands varies. *A. amphitrite* had the highest abundance of microplastics, with 42-53 particles/g. Among the four species found in the Seribu Islands and Jakarta Bay, *A. amphitrite* shows the strongest potential as a bioindicator of microplastic pollution. This is due to its clear taxonomy, high tolerance to microplastic pollution, and its ability to accumulate more microplastics compared to other species.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Finance through the Indonesia Endowment Fund for Education (LPDP) for the Master's Program. We extend our gratitude to the lecturers from the Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia for their valuable insights and knowledge shared during this research. Additionally, we would like to thank the Seribu Islands National Park Center, under the auspices of the Ministry of Environment and Forestry Republic of Indonesia, for granting the permit to collect barnacle, water, and sediment samples in the Seribu Islands.

REFERENCES

Abedin M, Eshik M, Punom N, Begum M, Rahman M. 2021. Genetic characterization of swamp eel of Bangladesh through DNA barcoding and RAPD techniques. J Asiat Soc Bangladesh Sci 46 (2): 117-131. DOI: 10.3329/jasbs.v46i2.54408.

Anderson JC, Park BJ, Palace VP. 2016. Microplastics in aquatic environments: Implications for Canadian ecosystems. Environ Pollut 218: 269-280. DOI: 10.1016/j.envpol.2016.06.074.

Anu O, Rampe HL, Pelealu JJ. 2017. Struktur sel epidermis dan stomata daun beberapa tumbuhan suku euphorbiaceae. Jurnal MIPA 6 (1): 69-73. DOI: 10.35799/jm.6.1.2017.16160. [Indonesian]

Ardiansyah, Damar A, Hariyadi S, Machfud. 2022. Current source and distribution pattern of plastic waste leakage in the estuary of Jakarta Bay. IOP Conf Ser 950: 012057. DOI: 10.1088/1755-1315/950/1/012057.

Azizah P, Ridlo A, Suryono CA. 2020. Microplastics in sediment on Kartini Beach, Jepara Regency, Central Java. J Mar Res 9 (3): 326-332. DOI: 10.14710/jmr.v9i3.28197. [Indonesian]

Bhattacharjee MJ, Laskar BA, Dhar B, Ghosh SK. 2012. Identification and re-evaluation of freshwater catfishes through DNA barcoding. PLoS ONE 7 (11): e49950. DOI: 10.1371/journal.pone.0049950.

Boessenkool S, Epp LS, Haile J, Bellemain E, Edwards M, Coissac E, Willerslev E, Brochmann C. 2012. Blocking human contaminant DNA during PCR allows amplification of rare mammal species from sedimentary ancient DNA. Mol Ecol 21 (8): 1806-1815. DOI: 10.1111/j.1365-294X.2011.05306.x.

Chan BKK, Prabowo RE. 2009. Crustacean fauna of Taiwan: Barnacles, Volume 1-Cirripedia: Thoracica excluding the Pyrgomatidae and Acastinae. National Taiwan Ocean University, Keelung City. DOI: 10.13140/2.1.1043.7921.

Chen HN, Tsang LM, Chong VC, Chan BK. 2014. Worldwide genetic differentiation in the common fouling barnacle, *Amphibalanus amphitrite*. Biofouling 30 (9): 1067-1078. DOI: 10.1080/08927014.2014.967232.

Choi S, Kim J, Kwon M. 2022. The effect of the physical and chemical properties of synthetic fabrics on the release of microplastics during washing and drying. Polymers 14 (16): 3384. DOI: 10.3390/polym14163384.

Claessens M, de Meester S, van Landuyt L, de Clerck K, Janssen CR. 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar Pollut Bull 62 (10): 2199-2204. DOI: 10.1016/j.marpolbul.2011.06.030.

Cordova MR, Hadi TA, Prayudha B. 2018. Occurrence and abundance of microplastics in coral reef sediment: A case study in Sekotong, Lombok-Indonesia. AES Bioflux 10 (1): 23-29. DOI: 10.5281/zenodo.1297719.

Cordova MR, Nurhati IS. 2019. Major sources and monthly variations in the release of land-derived marine debris from the Greater Jakarta area, Indonesia. Sci Rep 9: 18730. DOI: 10.1038/s41598-019-55065-2

Coyle R, Hardiman G, O'Driscoll K. 2020. Microplastics in the marine environment: A review of their sources, distribution processes, uptake and exchange in ecosystems. Case Stud Chem Environ Eng 2: 100010. DOI: 10.1016/j.cscee.2020.100010.

Crawford CB, Quinn B. 2017. Microplastic collection techniques. In: Crawford CB, Quinn B (eds). Microplastic Pollutants. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-809406-8.00008-6.

Cutroneo L, Reboa A, Besio G, Borgogno F, Canesi L, Canuto S, Dara M, Enrile F, Forioso I, Greco G, Lenoble V, Malatesta A, Mounier S, Petrillo M, Rovetta R, Stocchino A, Tesan J, Vagge G, Capello M. 2020. Correction to: Microplastics in seawater: Sampling strategies, laboratory methodologies, and identification techniques applied to port environment. Environ Sci Pollut Res 27: 20571. DOI: 10.1007/s11356-020-08704-5.

Devriese LI, van der Meulen MD, Maes T, Bekaert K, Paul-Pont I, Frère L, Robbens J, Vethaak AD. 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar Pollut Bull 8 (1-2): 179-187. DOI: 10.1016/j.marpolbul.2015.06.051.

Ding J-F, Li J-X, Sun C-J, He C-F, Jiang F-H, Gao F-L, Zheng L. 2018. Separation and identification of microplastics in digestive system of bivalves. Chin J Anal Chem 46: 690-697. DOI: 10.1016/s1872-2040(18)61086-2.

Djoemharshjah I. 2023. DNA barcoding *Clithon* sp. (Gastropoda: Neritidae) from Badur beach, Madura, Indonesia, based on COI gene molecular marker. Biodiversitas 24 (5): 2779-2787. DOI: 10.13057/biodiv/d240531.

Gewert B, Ogonowski M, Barth A, MacLeod M. 2017. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. Mar Pollut Bull 120 (1-2): 292-302. DOI: 10.1016/j.marpolbul.2017.04.062.

Goldstein MC, Goodwin DS. 2013. Gooseneck barnacles (*Lepas* spp.) ingest microplastic debris in the North Pacific Subtropical Gyre. PeerJ 1: e184. DOI: 10.7717/peerj.184.

Hajibabaei M, Spall JL, Shokralla S, van Konynenburg S. 2012. Assessing biodiversity of a freshwater benthic macroinvertebrate community through non-destructive environmental barcoding of DNA from preservative ethanol. BMC Ecol 12: 28. DOI: 10.1186/1472-6785-12-28.

Hiwari H, Purba NP, Ihsan YN, Yuliadi LP, Mulyani PG. 2019. Condition of microplastic garbage in sea surface water at around Kupang and Rote, East Nusa Tenggara Province. Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia 5 (2): 165-171. DOI: 10.13057/psnmbi/m050204. [Indonesian]

Hu C, Yang X, Gao L, Zhang P, Li W, Dong J, Li C, Zhang X. 2019. Comparative analysis of heavy metal accumulation and bioindication in three seagrasses: Which species is more suitable as a bioindicator?. Sci Tot Environ 669: 41-48. DOI: 10.1016/j.scitotenv.2019.02.425.

Jaberimanesh Z, Oladi M, Nasrolahi A, Ahmadzadeh F. 2019. Presence of *Amphibalanus eburneus* (Crustacea, Cirripedia) in Gomishan Wetland: Molecular and morphological evidence of a new introduction to the southern Caspian Sea. Reg Stud Mar Sci 25: 100469. DOI: 10.1016/j.rsma.2018.100469.

Jeyavani J, Al-Ghanim KA, Govindarajan M, Nicoletti M, Guilherme Malafaia and Baskaralingam Vaseeharan. 2024. Bacterial screening in Indian coastal regions for efficient polypropylene microplastic biodegradation. Sci Tot Environ 918: 170499. DOI: 10.1016/j.scitotenv.2024.170499.

Jung MR, Horgen FD, Orski SV, Rodriguez CV, Beers KL, Balazs GH, Jones TT, Work TM, Brignac KC, Royer S-J, Hyrenbach KD, Jensen BA, Lynch JM. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Mar Poll Bull 127: 704-716 DOI: 10.1016/j.marpolbul.2017.12.061.

Kavya ANVL, Sundarrajan S, Ramakrishna S. 2020. Identification and characterization of micro-plastics in the marine environment: A mini review. Mar Pollut Bull 160: 111704. DOI: 10.1016/j.marpolbul.2020.111704.

Laksono OB, Suprijanto J, Ridlo A. 2021. Microplastic content in sediments in Bandengan waters, Kendal Regency. J Mar Res 10: 158-164. DOI: 10.14710/jmr.v10i2.29032. [Indonesian]

Li HX, Ma LS, Lin L, Ni ZX, Xu XR, Shi HH, Yan Y, Zheng GM, Rittschof D. 2018. Microplastics in oysters *Saccostrea cucullata* along the Pearl River estuary, China. Environ Pollut 236: 619-625. DOI: 10.1016/j.envpol.2018.01.083.

Li J, Lusher AL, Rotchell JM, Deudero S, Turra A, Bråte ILN, Sun C, Shahadat Hossain M, Li Q, Kolandhasamy P, Shi H. 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ Pollut 244: 522-533. DOI: 10.1016/j.envpol.2018.10.032.

Li R, Wang J, Deng J, Peng G, Wang Y, Li T, Liu B, Zhang Y. 2023. Selective enrichments for color microplastics loading of marine lipophilic phycotoxins. J Hazard Mater 459: 132137-132137. DOI: 10.1016/j.jhazmat.2023.132137.

Lu H-C, Smith JL, Ziajahromi S, Leusch FDL. 2024. Microplastics and other anthropogenic fibres in large apex shark species: Abundance, characteristics, and recommendations for future research. Chemosphere 349: 140957. DOI: 10.1016/j.chemosphere.2023.140957.

Ma Q, Fu K, Zhang J, Li M, Han X, Chen Z, Ma L, Chang C. 2023. New bio-based polyurethane (PU) foams synthesized using crude glycerol-based biopolyol and humin-based byproducts from biomass hydrolysis. Indus Crops Prod 205: 117548. DOI: 10.1016/j.indcrop.2023.117548.

Martín DM, Magdi Ahmed M, Rodríguez M, García M, Faccini M. 2017. Aminated polyethylene terephthalate (PET) nanofibers for the selective removal of Pb(II) from polluted water. Materials 10 (12): 1352. DOI: 10.3390/ma10121352.

Masura J, Baker J, Foster G, Arthur C. 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Waters and Sediments. NOAA Marine Debris Division, Silver Spring, Maryland. DOI: 10.25607/OBP-604.

Mauludy MS, Yunanto A, Yona D. 2019. Microplastic abundances in the sediment of coastal beaches in Badung, Bali. Jurnal Perikanan Universitas Gadjah Mada 21 (2): 73-78. DOI: 10.22146/jfs.45871.

Md Amin R, Sohaimi ES, Anuar ST, Bachok Z. 2020. Microplastic ingestion by zooplankton in Terengganu coastal waters, Southern South China Sea. Mar Pollut Bull 150: 110616. DOI: 10.1016/j.marpolbul.2019.110616.

Miller ME, Motti CA, Hamann M, Kroon FJ. 2023. Assessment of microplastic bioconcentration, bioaccumulation and biomagnification in a simple coral reef food web. Sci Tot Environ 858: 159615. DOI: 10.1016/j.scitotenv.2022.159615.

Mishra S, Singh RP, Rath CC, Das AP. 2020. Synthetic microfibers: Source, transport and their remediation. J Water Process Eng 38: 101612. DOI: 10.1016/j.jwpe.2020.101612.

Mu J, Qu L, Jin F, Zhang S, Fang C, Ma X, Zhang W, Huo C, Cong Y, Wang J. 2019. Abundance and distribution of microplastics in the surface sediments from the northern Bering and Chukchi Seas. Environ Pollut 245: 122-130. DOI: 10.1016/j.envpol.2018.10.097.

NCBI [National Center for Biotechnology Information]. 2024. GenBank Overview. National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/genbank/

Nuryanto A, Pramono H, Kusbiyanto, Ghifari MI, Andareswari N. 2018. Barcoding of fin clip samples revealed high exploitation of *Plectropomus leopardus* in the Spermonde Archipelago. Biosaintifika 10 (3): 629-635. DOI: 10.15294/biosaintifika.v10i3.16142.

Pasternak Z, Achituv Y. 2007. Feeding behavior of shallow-water barnacles from the Mediterranean and Red Seas. J Crustac Biol 27: 543-547 DOI: 10.1651/s-2785.1.

Pochai A, Kingtong S, Sukparangsi W, Khachonpisitsak S. 2017. The diversity of acorn barnacles (Cirripedia, Balanomorpha) across Thailand's coasts: The Andaman Sea and the Gulf of Thailand. Zoosyst Evol 93 (1): 13-34. DOI: 10.3897/zse.93.10769.

Pratama DP, Atmodjo W, Wirasatriya A. 2023. The influence of longshore currents on the process of shoreline change at Marunda Beach, North Jakarta. Indonesian J Oceanogr 5 (2): 108-117. DOI: 10.14710/ijoce.v5i2.16198. [Indonesian]

Priscilla V, Patria MP. 2020. Comparison of microplastic abundance in aquaculture ponds of milkfish *Chanos chanos* (Forsskål, 1775) at Muara Kamal and Marunda, Jakarta Bay. IOP Conf Ser Earth Environ Sci 404 (1): 012027. DOI: 10.1088/1755-1315/404/1/012027.

Riani S, Prabowo RE, Nuryanto A. 2021. Molecular characteristics and taxonomic status of morphologically similar barnacles (*Amphibalanus*) assessed using the cytochrome c oxidase 1 gene. Biodiversitas 22 (3): 1456-1466. DOI: 10.13057/biodiv/d220349.

Rochman CM, Tahir A, Williams SL, Baxa DV, Lam R, Miller JT, Teh FC, Werorilangi S, Teh SJ. 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci Rep 5 (1): 14340. DOI: 10.1038/srep14340.

Rodrigues MO, Abrantes N, Gonçalves FJM, Nogueira H, Marques JC, Gonçalves AMM. 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). Sci Tot Environ 633: 1549-1559. DOI: 10.1016/j.scitotenv.2018.03.233.

Shokralla S, Gibson JF, Nikbakht H, Janzen DH, Hallwachs W, Hajibabaei M. 2014. Next-generation DNA barcoding: Using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. Mol Ecol Resour 14 (5): 892-901. DOI: 10.1111/1755-0998.12236.

Sianggaputra MD, Sagita A, Pratama CD. 2022. Model aktor dalam pengelolaan sampah plastik laut di Kawasan Pesisir Jakarta. Jurnal Kebijakan Perikanan Indonesia 14 (2): 87-98. DOI: 10.15578/jkpi.14.2.2022.%25p. [Indonesian]

Surana D, Vinay, Patel P, Ghosh P, Sharma S, Kumar V, Kumar S. 2024. Microplastic fibers in different environmental matrices from synthetic textiles: Ecotoxicological risk, mitigation strategies, and policy perspective. J Environ Chem Eng 12 (2): 112333. DOI: 10.1016/j.jece.2024.112333.

Syaripuddin K, Sing KW, Wilson JJ. 2015. Comparison of butterflies, bats and beetles as bioindicators based on four key criteria and DNA barcodes. Trop Conserv Sci 8 (1): 138-149. DOI: 10.1177/194008291500800112.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Thushari GGN, Senevirathna JDM, Yakupitiyage A, Chavanich S. 2017. Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. Mar Pollut Bull 124 (1): 349-355. DOI: 10.1016/j.marpolbul.2017.06.010.

Tien C-J, Wang Z-X, Chen CS. 2020. Microplastics in water, sediment and fish from the Fengshan River system: Relationship to aquatic factors and accumulation of polycyclic aromatic hydrocarbons by fish. Environ Pollut 265: 114962. DOI: 10.1016/j.envpol.2020.114962.

Tsang LM, Chu KH, Achituv Y, Chan BK. 2015. Molecular phylogeny of the acorn barnacle family Tetraclitidae (Cirripedia: Balanomorpha: Tetraclitoidea): Validity of shell morphology and arthropodal characteristics in the systematics of Tetraclitid barnacles. Mol Phylogenet Evol 82: 324-329. DOI: 10.1016/j.ympev.2014.09.015.

Turner A, Filella M. 2021. Polyvinyl chloride in consumer and environmental plastics, with a particular focus on metal-based additives. Environ Sci 23 (9): 1376-1384. DOI: 10.1039/d1em00213a.

Vaezzadeh V, Thomes MW, Kunisue T, Tue NM, Zhang G., Zakaria MP, Affendi YA, Yap FC, Chew LL, Teoh HW, Lee CW. 2021. Examination of barnacles' potential to be used as bioindicators of persistent organic pollutants in coastal ecosystem: A Malaysia case study. Chemosphere 263: 128272. DOI: 10.1016/j.chemosphere.2020.128272.

van Cauwenberghe L, Devriese L, Galgani F, Robbens J, Janssen CR. 2015. Microplastics in sediments: A review of techniques, occurrence and effects. Mar Environ Res 111: 5-17. DOI: 10.1016/j.marenvres.2015.06.007.

Viršek MK, Palatinus A, Koren Š, Peterlin M, Horvat P, Kržan A. 2016. Protocol for microplastics sampling on the sea surface and sample analysis. J Vis Exp 118: 55161. DOI: 10.3791/55161.

Walczyńska KS, Søreide JE, Weydmann-Zwolicka A, Ronowicz M, Gabrielsen TM. 2019. DNA barcoding of *Cirripedia* larvae reveals new knowledge on their biology in Arctic coastal ecosystems. Hydrobiologia 837: 149-159. DOI: 10.1007/s10750-019-3967-y.

Wen X, Du C, Xu P, Zeng G, Huang D, Yin L, Yin Q, Hu L, Wan J, Zhang J, Tan S, Deng R. 2018. Microplastic pollution in surface sediments of urban water areas in Changsha, China: Abundance, composition, surface textures. Mar Pollut Bull 136: 414-423. DOI: 10.1016/j.marpolbul.2018.09.043.

Wiadnyana NN, Krismono, Widodo. 2021. Towards Sustainable Fisheries Management in Jakarta Bay and Seribu Islands. AMAFRAD Press, Jakarta. [Indonesian]

Xu X, Fang JKH, Wong CY, Cheung SG. 2023. Experimental accumulation of microplastics in acorn barnacle *Amphibalanus amphitrite* and its use in estimating microplastic concentration in coastal waters. Front Mar Sci 9: 1081329. DOI: 10.3389/fmars.2022.1081329.

Xu XY, Wong CY, Tam NFY, Liu HM, Cheung SG. 2020. Barnacles as potential bioindicator of microplastic pollution in Hong Kong. Mar Pollut Bull 154: 111081. DOI: 10.1016/j.marpolbul.2020.111081.

Yu SP, Chan BKK. 2020. Effects of polystyrene microplastics on larval development, settlement, and metamorphosis of the intertidal barnacle *Amphibalanus amphitrite*. Ecotoxicol Environ Saf 19: 110362. DOI: 10.1016/j.ecoenv.2020.110362.

Yu SP, Nakaoka M, Chan BK. 2021. The gut retention time of microplastics in barnacle naupliar larvae from different climatic zones and marine habitats. Environ Pollut 268: 115865. DOI: 10.1016/j.envpol.2020.115865.

Zhang W, Zhang S, Wang J, Wang Y, Mu J, Wang P, Lin X, Ma D. 2017. Microplastic pollution in the surface waters of the Bohai Sea, China. Environ Pollut 231: 541-548. DOI: 10.1016/j.envpol.2017.08.058.

Zhu X, Qiang L, Shi H, Cheng J. 2020. Bioaccumulation of microplastics and its in vivo interactions with trace metals in edible oysters. Mar Pollut Bull 154: 111079. DOI: 10.1016/j.marpolbul.2020.111079.