

令和 7 年 4 月 17 日現在

機関番号：17102

研究種目：若手研究

研究期間：2023～2024

課題番号：23K17048

研究課題名（和文）Assessing the rainfall and tide effect on the presence and exposure of microplastics to zooplankton in subtropical estuaries intensely polluted by plastics

研究課題名（英文）Assessing the rainfall and tide effect on the presence and exposure of microplastics to zooplankton in subtropical estuaries intensely polluted by plastics

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交付決定額（研究期間全体）：（直接経費） 5,000,000円

研究成果の概要（和文）：2年間の調査により、タイ湾北部の7地点から採取された動物プランクトンがマイクロプラスチックを摂取していることが明らかになりました。分析手法の標準化を目指す国際的な取り組みと連携し、異なる3種のカイアシ類（コペポダ）計486個体について、マイクロプラスチックの摂取状況を分析しました。平均体長は1372;1613マイクロメートルの範囲でした。すべてのサンプルからマイクロプラスチック粒子が検出されており、これらのプラスチック粒子が海洋食物網における栄養移行の可能性を示しています。主に検出されたポリマーはポリプロピレンとポリエチレンテレフタレートでした。粒子の平均サイズは約50ミクロンでした。

研究成果の学術的意義や社会的意義

My research enhances global understanding of microplastic pollution by developing standardized methods and generating comparable data. It supports policy, raises awareness, and empowers communities and researchers; especially in South East Asia; through collaboration, training, and data sharing.

研究成果の概要（英文）：A two-year study revealed that zooplankton from the 7 points across the upper Gulf of Thailand ingest microplastics. Working with international efforts to standardize analytical methods, a total of 486 individuals of 3 different species of copepods were analyzed for ingestion of microplastics. Average body size ranged between 1372 and 1613 micrometers. Microplastic particles were found in all samples, making these plastic particles available for trophic transfer in the marine food web. Polypropylene and polyethylene terephthalate were the predominant polymers. The average size of particles was around 50 microns.

研究分野：microplastics

キーワード：microplastics zooplankton marine copepods

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1. 研究開始当初の背景

Nowadays, plastics are considered one of the most relevant pollutants worldwide, as since 1950, the large scale of plastic production has dramatically increased, reaching 367 million tons in 2020 [1]. When plastic waste is wrongly discarded in the environment will be weathered (heat, UV rays, winds, waves) and fragmented forming microplastics (MPs), defined as plastic particles less than 5 mm in diameter [2]. Nowadays, MPs are ubiquitous in the environment. It was estimated that between 4 and 12 million tons of plastic waste gets into the ocean from coastal regions [3] and that 24.4 trillion pieces of MPs float in the world's upper oceans [4]. Due to the small size, MPs are bioavailable to many species, including zooplankton, and could be ingested involving bioaccumulation and biomagnification mechanisms across food webs. Laboratory studies have shown that zooplankton species can ingest MPs, causing adverse effects such as reduced feeding behavior, growth, and fecundity [5,6].

Nowadays, the information about MPs abundance (particles m⁻³) is still limited, particularly in Southeast Asia countries [4], where large amounts of mismanaged plastic waste are discharged through rivers [7]. Furthermore, to assess the impact on zooplankton, it is necessary to determine ecotoxicological experiments based on accurate information about the actual concentrations of MPs in the environment and determine the ingestion rate by the organisms. Particularly, highly polluted rivers as the Chao Phraya River, and its corresponding estuary in Thailand, presented a good case of study to understand the effect of plastic pollution on these organisms. Previous studies find significant MPs concentrations in water samples [8] therefore a high interaction between MPs and zooplankton is hypothetically expected. Furthermore, the effect of short-term environmental variables as rainfall and fortnightly tidal currents on MPs presence was documented [8; 9]. Nevertheless, the combined effects of these parameters on MPs ingestion were not studied to the date in Thailand or internationally.

This study aimed to understand how rainfall influenced MPs abundance (0.3-5 mm; particles/m³) and characteristics and determine to which extent mesozooplankton organisms (0.2-20 mm) are exposed in nature to MPs ingestion (particles/ individual).

This will allow us to improve laboratory experiments and determine the exposition risk of their predators. Furthermore, to identify which environmental MPs are ingested in the field and whether this is representative of their composition in seawater, proposing new ways of addressing global plastic pollution on marine systems using zooplankton ingestion as an indicator tool.

2. 研究の目的

One of the major threats of marine plastic pollution is that MPs could be ingested by many organisms, including zooplankton, involving bioaccumulation and biomagnification mechanisms across marine food webs. Previous studies investigated MPs ingestion by zooplankton [10,11,12,13,14,15]. But when it comes to understand the overall effect of rainfall and currents on MPs bioavailability to marine organisms, there is still a lack of information. This information will be crucial for developing accurate policies, using zooplankton ingestion as indicators for policymakers; particularly considering the new global agreement to abate plastic pollution addressed at the last UNEP Assembly (Nairobi, Kenya, 2022).

The primary objectives of this study are to:

1. Quantify MPs Abundance and Characteristics in the upper Gulf of Thailand (GoT): This includes determining the size, shape, and polymer types of MPs present in seawater.
2. Assess Zooplankton Exposure to MPs: Investigate the extent to which zooplankton organisms are exposed to MPs in their natural environment. This will improve the accuracy of laboratory experiments and help determine the exposure risk to their predators.
3. Identify Ingested Environmental MPs: Determine the types of MPs ingested by zooplankton in the field and assess whether these are representative of the MPs composition in seawater.

Analyzing MPs in water samples and those ingested by zooplankton will elucidate the natural exposure of these organisms, thereby refining laboratory experiments and assessing the exposure risk to higher trophic levels. Understanding the mechanisms behind MPs ingestion involves characterizing the environmental MPs consumed by zooplankton and comparing this to the MPs found in seawater. This comprehensive analysis will contribute significantly to our knowledge of MPs pollution in the GoT and support the development of targeted mitigation strategies.

3. 研究の方法

Sample Collection (FY2023-2024)

Sampling locations in the Gulf of Thailand (GoT) were selected covering the upper Gulf of Thailand. Zooplankton samples were collected using a Bongo plankton net equipped with a flowmeter (330 μm mesh size). The net was towed at a speed of 2 knots for approximately 40 minutes. The collected zooplankton was transferred into clean glass flasks. Water samples were collected using a Neuston net with a flowmeter (330 μm mesh size), and the net content was released into clean stainless-steel flasks.

Sample Processing (FY2023)

Zooplankton species identification was conducted using established taxonomic literature. Zooplankton abundance was determined by counting individuals in three 5 mL subsamples using a stereomicroscope and a Bogorov chamber. For microplastic examination, zooplankton individuals from the replicate samples were picked out with forceps and rinsed with Milli-Q water to remove any attached plastic particles. Based on zooplankton abundance, individuals of the principal taxa were processed for organic matter digestion following our own protocol to avoid MP sample damage [23]. This two-step digestion process includes an alkaline digestion (10% KOH at 40°C for 72 hours) followed by oxidative digestion (30% H₂O₂ Fe (II) solution, applied three times every 20 minutes at 60°C).

Water samples were processed by oxidative digestion, in the same way as zooplankton samples. The resulting MPs were filtered using an 8- μm pore size PTFE filter. Visually larger particles were picked up with forceps and stored in clean glass Petri dishes for further analysis.

Quantification and Characterization

Filters were analyzed using a Micro-FTIR Nicolet iN10 infrared microscope (Thermo Fisher Scientific) to quantify MPs and identify plastic polymers. Each particle was photographed and classified by shape, maximum length (mm), and color, following established guidelines [23]. To ensure quality assurance and quality control (QA/QC), blank samples were analyzed alongside zooplankton and water samples to monitor contamination. Sample preparation and analysis were conducted under a clean bench using cotton lab coats and gloves to avoid contamination. All surfaces were cleaned with 70% ethanol before use, glass and metal equipment was used where possible, and reagents were filtered beforehand. All equipment was cleaned with Milli-Q water before use, and samples were kept covered when not in use.

Data Analysis and Publication

Statistical analyses are being conducted to determine intra- and inter-sample differences. Data analysis, publication, and dissemination of results will be carried out during 2025.

4. 研究成果

Despite increasing global information about microplastic ingestion by zooplankton, data remains limited for particle sizes under 300 μm (SMPs) and regions such as Southeast Asia, a known hotspot for plastic debris. Over two years, this research investigated the presence and ingestion of microplastics (MPs) by zooplankton in the upper Gulf of Thailand. Two sampling campaigns were conducted across 12 points during the wet and dry seasons. The study aimed to standardize microplastic analysis methodologies in collaboration with international initiatives.

A total of 486 individuals of 3 different species of copepods were analyzed, revealing plastic particles in all samples. All particles were fragments, predominantly under 50 μm in size, with

polymer types primarily being polypropylene (PP) and polyethylene terephthalate (PET) suggesting that single use plastic products are the main pollution sources in the GoT. The final values for the number of particles ingested per individual and number of particles/m³ per site and seasons will be informed by end of FY 2025.

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5. 主な発表論文等

〔雑誌論文〕 計1件（うち査読付論文 1件/うち国際共著 1件/うちオープンアクセス 1件）

1. 著者名 Alfonso, M. B., Lindsay, D. J., Arias, A. H., Nakano, H., Jandang, S., & Isobe, A	4. 巻 905
2. 論文標題 Zooplankton as a suitable tool for microplastic research	5. 発行年 2023年
3. 雑誌名 Science of the Total Environment	6. 最初と最後の頁 167329
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.scitotenv.2023.167329	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕 計1件（うち招待講演 1件/うち国際学会 0件）

1. 発表者名 Maria Belen ALFONSO
2. 発表標題 Zooplankton as bioindicators of microplastic pollution
3. 学会等名 Thematic Workshop on Microplastic Impacts on Marine Ecosystems (JICA- DMCR) (招待講演) (招待講演)
4. 発表年 2025年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

6. 研究組織

氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考
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7. 科研費を使用して開催した国際研究集会

〔国際研究集会〕 計0件

8. 本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関
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