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研究課題名（英文）Microplastic pollution impact on the Gulf of Thailand: insights from water samples assessment and ingestion by zooplankton as complementary tools

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研究成果の概要（和文）：2年間の研究で、タイ湾のシー・チャン島の動物プランクトンがマイクロプラスチック（MP）を摂取することを明らかにしました。国際的な取り組みと協力して分析手法を標準化し、カラノイド類のC. furcatusが平均0.41個の粒子を摂取しました。摂取されたマイクロプラスチックの主なポリマータイプはポリプロピレン（PP）、エチレン・プロピレン・ジエン・モノマー（EPDM）、ポリエチレン（PE）でした。海洋食物網での栄養移動に利用可能である470.2個の粒子が推定されました。降水量が摂取に与える影響は不明瞭で、沿岸域での摂取量が高い傾向が示されました。

研究成果の学術的意義や社会的意義
The research results deepen our understanding of microplastic pollution's impact on marine ecosystems. By examining microplastic ingestion by zooplankton and standardizing analysis methods internationally, the study provides valuable insights for assessing risks to marine life

研究成果の概要（英文）：Over a span of two years, this research delved into the presence and ingestion of microplastics (MPs) by zooplankton in the Gulf of Thailand, with a specific focus on Si Chang Island. Through six sampling campaigns conducted between August 2022 and April 2023, encompassing various seasons and tidal intervals, the study aimed to standardize microplastic analysis methodologies in collaboration with international initiatives. Analysis of samples in the second year unveiled that the calanoid Centropages furcatus ingested an average of 0.41 ± 0.13 particles per individual, predominantly polypropylene (PP), Ethylene-Propylene-Diene-Monomer (EPDM), and Polyethylene (PE) fragments, with an retention rate of 470.2 particles per cubic meter and available for trophic transfer in marine food webs. Findings suggested inconclusive effects of rainfall on microplastic ingestion, with higher values near coastal areas.

研究分野：microplastics

キーワード：microplastics　plastic pollution　marine　zooplankton

１．研究開始当初の背景

Plastics are now recognized as one of the most significant pollutants globally. Since the 1950s, the large-scale production of plastics has surged dramatically, reaching 390 million tons in 2021 [1]. Improperly discarded plastic waste persists in the environment due to its low degradation rate. Over time, plastics undergo weathering from heat, UV rays, wind, and waves, breaking down into microplastics (MPs), which are plastic particles less than 5 mm in diameter [2]. MPs have become ubiquitous in the environment. It is estimated that between 4 and 12 million tons of plastic waste enter the ocean from coastal regions annually [3], with approximately 24.4 trillion pieces of MPs floating in the upper layers of the world's oceans [4].

The small size of MPs makes them bioavailable to a wide range of species, including zooplankton, leading to ingestion and subsequent bioaccumulation and biomagnification through food webs. Laboratory studies have demonstrated that zooplankton can ingest MPs, resulting in adverse effects such as reduced feeding behavior, stunted growth, and decreased fecundity [5,6].

Despite the increasing awareness, information about the abundance of MPs is still limited, particularly in Southeast Asia [4]. Field documentation of MP ingestion by zooplankton has primarily been confined to regions like China and the Arctic [12-17]. Moreover, only a few studies have explored the relationship between the MPs ingested by zooplankton communities and those present in seawater [18,16]. This geographical limitation and methodological differences make comparisons challenging. In Thailand, only one study [19] has recorded zooplankton ingestion of MPs, with additional studies focusing on fish [20] and shellfish [21,22].

This project aimed to bridge the knowledge gap regarding MPs' presence in seawater and their ingestion by zooplankton. Understanding this relationship is crucial for assessing the current pollution state of marine systems, both in the Gulf of Thailand (GoT) and globally. This is particularly important given the significant amounts of mismanaged plastic waste discharged into the area via rivers [7].

To evaluate the impact of MPs on marine organisms, it is essential to conduct ecotoxicological experiments based on accurate data about the actual concentrations of MPs in the environment and the ingestion rates by organisms. Such information will provide insights into whether the MPs ingested by marine life are representative of those present in their environment.

２．研究の目的 purpose

Reducing plastic pollution requires identifying primary hotspots and mitigating the main sources of pollution. Tourism and inadequate waste management have been identified as major contributors to plastic pollution [8]. In Thailand, where tourism is a significant economic driver, studies have documented the presence of microplastics (MPs) on beaches and in the Chao Praya estuary [9,10]. However, data on MPs in the marine environment, particularly in the ocean, is limited [11]. Therefore, this project aims to address this gap by enhancing our understanding of MPs' presence and impact on the marine systems of Thailand. This information is vital for developing effective policies, especially in light of the new global agreement to combat plastic pollution established at the UNEP Assembly in Nairobi, Kenya, in 2022.

The primary objectives of this study are to:

1. Quantify MPs Abundance and Characteristics in the 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 (FY2022-2023)

Sampling locations in the Gulf of Thailand (GoT) were selected, with sample collection carried out between December 2022 and July 2023. Zooplankton samples were collected using a 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 (N=26) 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 followed the same digestion protocol 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 dished 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 [24,25]. 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 were conducted to determine intra- and inter-sample differences. Data analysis, publication, and dissemination of results was carried out in the project's second year (FY2023).

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. This study aimed to characterize the size, shape, and polymer types of SMPs ingested by Centropages furcatus in Si Chang Island, a coral reef conservation area (upper Gulf of Thailand), along with those MPs present in water samples. Over two years, this research investigated the presence and ingestion of microplastics (MPs) by zooplankton in the Gulf of

Thailand, specifically focusing on Si Chang Island. Six sampling campaigns were conducted between August 2022 and April 2023, covering various seasons and tidal intervals. The study aimed to standardize microplastic analysis methodologies in collaboration with international initiatives.

A total of 750 individuals of Centropages furcatus were analyzed, revealing 309 plastic particles with an average ingestion value of 0.41 ± 0.13 particles per individual, one of the highest recorded values. All particles were fragments, predominantly under 50 μm in size, with polymer types primarily being PP (71%), followed by EPDM (16%) and PE (7%). Up to 470.2 particles per cubic meter were estimated to be retained by this calanoid species, potentially available for trophic transfer. The effect of rainfall on SMPs ingestion was inconclusive, with a non-significant trend toward higher ingestion values near coastal areas compared to offshore areas, suggesting a decrease in particle exposure due to runoff effects. In conclusion, our study reveals significant ingestion of SMPs by copepod C. furcatus in the Gulf of Thailand, with ingestion rates notably higher than those reported in previous studies. Utilizing an improved analysis technique, we observed a considerable abundance of SMPs in zooplankton individuals, particularly during the wet season and near coastal areas, but in average higher during the dry season in the coastal area, suggesting a runoff contribution of plastic particles and a dilution effect. Polypropylene (PP) emerged as the most prevalent plastic polymer type ingested by C. furcatus, with variations observed in polymer composition and diversity between survey points and seasons, likely influenced by rainfall and runoff dynamics.

Regarding floating MPs obtained by neuston net surveys, the analysis revealed MP abundance ranging from 0.02 to 42.46 particles per cubic meter, also registered polymer types such as PP, PE, PE/PP, and PS, with a lack of PVC, the same as those ingested by copepods. Precipitation, wind, and current direction induced by monsoons significantly influenced MP abundance and distribution, presenting notable seasonality. Data analysis of MP colors and polymer types suggested diverse sources of plastic particles.

Based on the results, a proposal for the generation, sources, and pathways for MPs in the Gulf of Thailand was presented: (1) plastic wastes exposed to strong UV light during the dry season fragment around rivers, and (2) heavy rains wash away these particles during the wet season. This proposal is applicable to tropical regions, including the Gulf of Thailand. The study concluded that ocean currents induced by monsoons and unique climatic conditions contribute to the generation and distribution of MPs in the ocean surrounding Southeast Asian countries. Coral reef ecosystems are particularly threatened by MPs in these areas, highlighting the need for increased MP monitoring in coral ecosystems in Thailand and worldwide. For more details about the results of this projects, please refer to the following publications:

- **Alfonso et al**., 2023 Zooplankton as a suitable tool for microplastic research. Science of the Total Environment 905 167329
- Nakano et al., 2024 Influence of monsoon seasonality and tidal cycle on microplastics presence and distribution in the Upper Gulf of Thailand. Science of the Total Environment 920: 170787
- **Alfonso et al**., 2024 Small microplastic ingestion by the calanoid Centropages furcatus in the Gulf of Thailand. Science of the Total Environment 930: 172837

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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　2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

１．著者名 Nakano, H., Alfonso, M. B., Jandang, S., Phinchan, N., Chavanich, S., Viyakarn, V., & Isobe, A.	４．巻 920
２．論文標題 Influence of monsoon seasonality and tidal cycle on microplastics presence and distribution in the Upper Gulf of Thailand	５．発行年 2024年
３．雑誌名 Science of the Total Environment	６．最初と最後の頁 170787
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.scitotenv.2024.170787	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5件（うち招待講演　5件／うち国際学会　3件）

１．発表者名 Maria Belen Alfonso, Haruka Nakano
２．発表標題 Practical overview of microplastics sampling and analytical methods
３．学会等名 Onsite Regional Training Course on Marine Debris and Microplastics Sampling Collection and Analysis（招待講演）（国際学会）
４．発表年 2022年

１．発表者名 Maria Belen Alfonso
２．発表標題 Ocean plastic pollution: What's next?
３．学会等名 Young researchers presentations in the meeting with the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.（招待講演）
４．発表年 2022年

１．発表者名
Maria Belen Alfonso

２．発表標題
Efforts against Marine Plastics Pollution including Harmonization of Monitoring Methods (MOEJ)

３．学会等名
EuroQCharm meeting, Amsterdam（招待講演）（国際学会）

４．発表年
2022年

１．発表者名
Maria Belen Alfonso, Haruka Nakano, and Suppakarn Jandang

２．発表標題
Highlights of Key Activities in the SATREPS Project during FY2023

３．学会等名
meeting with JICA representatives at Thailand（招待講演）

４．発表年
2023年

１．発表者名
Haruka Nakano, Maria Bleen Alfonso and Suppakarn Jandang

２．発表標題
Addressing plastic pollution: COPS’ international research activities

３．学会等名
KYUDAI NOW Thailand（招待講演）（国際学会）

４．発表年
2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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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

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			
タイ	Chulalongkorn University			
アルゼンチン	Instituto Argentino de Oceanografia			