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https://doi.org/10.5109/7388807

出版情報：九州大学大学院農学研究院紀要. 70 (2), pp.101-106, 2025. 九州大学大学院農学研究院
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **70** (2), 101–106 (2025)

An Exploratory Approach Using Wharf Roach Feces as a Non–Invasive Bioindicator: Microplastic Accumulation in Wharf Roaches in a Coastal Area of Fukuoka, Japan

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(*Received May 5, 2025 and accepted May 16, 2025*)

This study investigates the potential of wharf roach (*Ligia* spp.) feces as a bioindicator for non–invasive environmental monitoring to assess microplastic contamination in coastal ecosystems. From May to December 2024, monthly sampling was conducted at Nishinoura Fishing Port (Fukuoka, Japan), and microplastics were detected in both the digestive tracts and feces of wharf roaches. The concentrations of microplastics in both digestive tracts and feces exhibited similar seasonal trends, with a decrease during winter. Additionally, difference in the shape characteristics was observed between microplastics found in wharf roach samples and seawater. This study highlights the feasibility of using wharf roach feces as a non–invasive sample for monitoring microplastic accumulation in the coastal area, offering a potential method for assessing pollution levels in coastal environments.

Key words: *Ligia* spp, funamushi, feces, non–invasive bioindicator

INTRODUCTION

Environmental monitoring studies play a crucial role in environmental science, particularly in assessing pollution levels, understanding ecological dynamics of pollutants, and formulating conservation strategies. Among various monitoring approaches, measuring pollutant accumulation in living organisms (bioindicators) has been used as a vital technique for assessing pollutant dynamics and their impacts on the ecosystem (Burger, 2006). The use of bioindicators is particularly significant in coastal ecosystems, which serve as critical interfaces between terrestrial and marine environments.

Organisms inhabiting coastal areas are affected by pollutants directly discharged into coastal environments or transported via rivers and ocean currents. A wide range of chemical pollutants, including persistent organic pollutants (Honda *et al*., 2021a; Zhang *et al*., 2009), heavy metals (Li *et al*., 2022; Luo *et al*., 2022), and microplastics (Hantoro *et al*., 2019; Lee *et al*., 2025; Tanoiri *et al*., 2024), are introduced into coastal environments through anthropogenic activities and have been detected in organisms inhabiting these areas. To monitor coastal pollution effectively, various organisms have been suggested and used as bioindicators, such as mussels (Goldberg *et al*., 1978), squids (Ueno *et al*., 2003), and fish (Corsi *et al*., 2003; Hamada *et al*., 2024). Among them, wharf roaches (*Ligia* spp., commonly known as Funamushi in Japanese) have been recognized as potential bioindicators due to their ubiquitous presence in intertidal zones and their ability to bioaccumulate contaminants from their surroundings. Previous studies have demonstrated that wharf roaches accumulate organotins (Undap *et al*., 2013), polycyclic aromatic hydrocarbons (Honda *et al*., 2021a; Honda *et al*., 2018), heavy metals (Honda *et al*., 2021b), radionuclides (Qiu *et al*., 2017), and microplastics (Choi *et al*., 2023; Lee *et al*., 2025) in coastal environments.

Although biological sample–based measurements are essential for environmental assessments, they often require sacrificing organisms, raising ethical concerns. If the goal is ecosystem conservation, experimental methods should ideally be non–invasive for the organisms. In terrestrial environments, non–invasive monitoring methods using feces, saliva, or urine have been successfully implemented (Schilling *et al*., 2022), allowing researchers to collect valuable biological data without harming organisms. However, applying such non–invasive approaches in coastal ecosystems is challenging due to the proximity of water, where biological materials, such as feces and mucus, rapidly degrade or disperse.

To address this limitation, we explored the feasibility of using wharf roach feces as a non–invasive bioindicator in coastal ecosystems. Given the increasing concerns over microplastics contamination in coastal areas (Savoca *et al*., 2025), we selected microplastics as the target pollutant and assessed whether wharf roach feces could serve as an effective non–invasive bioindicator.

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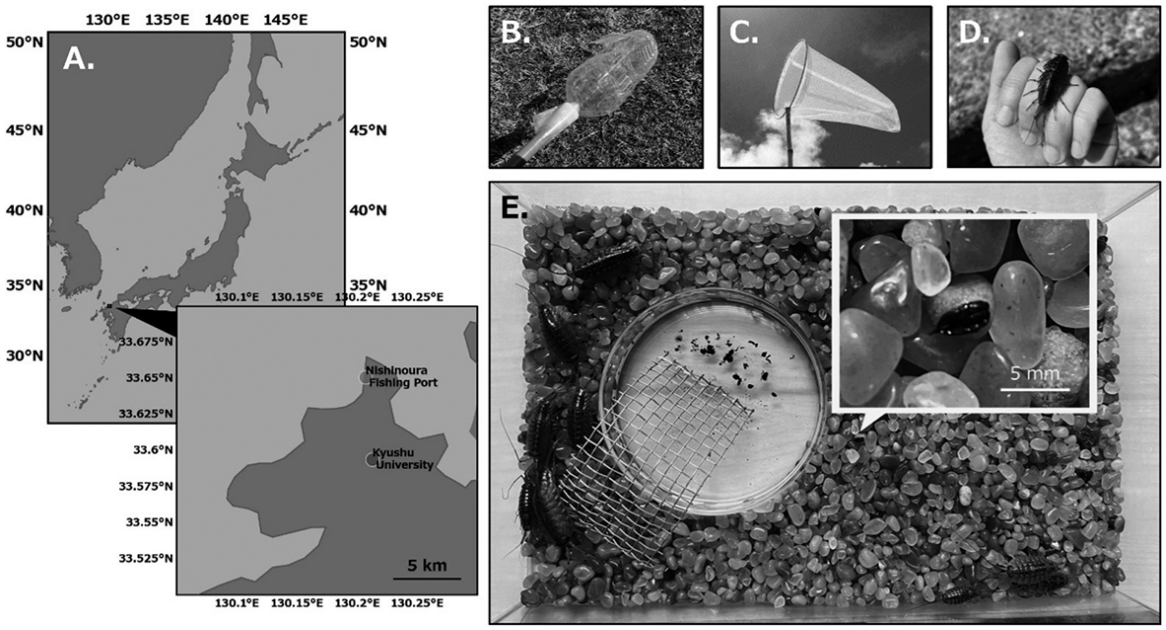

Fig. 1. Wharf roach capturing site and sample collection methods.
Nishinoura Fishing Port (Fukuoka, Japan) (A), the capturing methods examined in this study (B, a custom–made wharf roach capturing tool; C, an insect net; D, hand capturing), and the tank used for collecting wharf roach feces along with the collected feces (E).

MATERIALS AND METHODS

Chemical Reagents

Potassium hydroxide (KOH), hydrochloric acid (HCl), and sodium iodide (NaI) of guaranteed reagent grade were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Nile red (>98% purity) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Evaluation of Wharf Roach Capturing Methods

On May 7, 2024, a total of 179 wharf roaches (*Ligia* spp.) were captured at Nishinoura Fishing Port (Fukuoka, Japan) using three different methods: a custom–made wharf roach collection tool (a tool crafted from a plastic bottle) (64 individuals), an insect net (60 individuals), and hand capturing (55 individuals) (Fig. 1). The captured wharf roaches (length, 2.1–3.5 cm, width, 0.9–1.6 cm) were maintained in a glass tank (60 × 30 × 36 cm) for 24 hours. After their survival rates were recorded, they were returned to their original collection site.

Sample Collection at Nishinoura Fishing Port

From May to December 2024, monthly sample collection was conducted at Nishinoura Fishing Port (Fukuoka, Japan, Fig. 1A) during the low tide of the spring tide, with no sampling in June due to adverse weather conditions. Wharf roaches (*Ligia* spp.) inhabiting the port were collected for digestive tract sampling (4 individuals per month) and fecal sampling (30 individuals per month) (length, 3.0 ± 0.4 cm; width, 1.3 ± 0.2 cm; weight, 0.82 ± 0.26 g). Due to the absence of wharf roaches in the fishing port during winter, only 14 individuals (4 individuals for digestive tract sampling, 10 individuals for fecal sampling) were collected in November, and none were collected in December. Seawater samples were collected from the surface layer (10–30 cm depth) in the fishing port. A total of 10 L seawater was filtered through a stainless–steel sieve (mesh opening 53 μm, Nonakarikaki Co. Ltd., Tokyo, Japan), and the residues retained on the filter were collected in a glass bottle.

Of the collected wharf roaches, 4 individuals were humanely euthanized with CO_2 and dissected to isolate their digestive tracts (wet–weight, 118.3 ± 67.5 mg), which were then transferred to glass test tubes. For fecal sampling, 30 individuals were placed in glass tanks (30 × 20 × 14 cm) containing glass beads and artificial seawater (salinity 30) (10 individuals/tank) and maintained overnight at room temperature. The following morning, feces in the tank (Fig. 1E) were collected (total weight, 150.0 ± 50.1 mg/tank) in a glass test tube, and the wharf roaches were returned to their original collection site.

Microplastics Extraction and Detection

Wharf roach digestive tract, feces, and seawater residues were incubated in 50 mL of 10% KOH for 18–24 hours at 60°C (90 rpm, BC–730, Bio craft Co. Ltd., Tokyo, Japan). The treated solution was filtered through a hydrophilic PTFE membrane (5 μm, ϕ47 mm, Merck Millipore Corporation, MA, USA) and retained residues were incubated in 20 mL of 10% HCl for 1 hour at room temperature (90 rpm). The treated solution was then filtered through a stainless–steel sieve (mesh opening 53 μm), and the retained residues were subjected to density separation using saturated NaI solution (density, 1.6 mg/cm^3). The retained residues were incubated in

50 mL of saturated NaI solution for 18–24 hours, and precipitates were removed with a Pasteur pipette. The remaining solution was filtered through a hydrophilic PTFE membrane (5 μm, ϕ47 mm), and the retained residues were incubated in 20 mL of Nile red solution (10 μg/mL; the Nile red stock solution of 1 mg/mL in methanol was diluted with ultrapure water for use) for 1 hour at room temperature. The stained particles were captured on a glass fiber membrane (1.2 μm, ϕ47 mm, Whatman GF/C, Cytiva, MA, USA), and observed under a fluorescence microscope (BZ–X800, KEYENCE Corporation, Osaka, Japan) with a fluorescence filter (Ex, 545/25 nm; Em, 605/70 nm).

Microplastics Analysis

The images of Nile red–stained microplastics were analyzed using ImageJ (version 1.54 m) (Schneider *et al*., 2012) to obtain the minor and major Feret diameter for each particle. Following the method described by Ueda *et al*. (2024), which calculates the volume of microplastics assuming them to be ellipsoidal or fibrous, the weight of each plastic particle was estimated. Since the polymer type of the plastic particles could not be identified in this study, their density was assumed to be 1.00 g/cm^3 for the weight estimation. Particles with a maximum Feret diameter of less than 53 μm were excluded from the analysis.

Statistical Analysis

The statistical analysis in this study was conducted using R (ver. 4.4.3) (R Core Team, 2024). The packages used were multcomp (ver. 1.4–25) (Hothorn *et al*., 2008) and nparcomp (ver. 3.0) (Konietschke *et al*., 2015). For data assumed to follow a normal distribution (microplastic diameter), the Tukey–Kramer test was performed. For data not assumed to follow a normal distribution (microplastic concentration in wharf roach samples), the Steel–Dwass test or the Wilcoxon rank–sum test was conducted. A P–value of <0.05 was considered statistically significant. The graph visualization and microplastic weight estimation were performed using pandas (ver. 2.2.3) (McKinney, 2010; The pandas development team, 2024), numpy (ver. 1.26.4) (Harris *et al*., 2020), matplotlib (ver. 3.9.2) (Hunter, 2007), and seaborn (ver. 0.13.2) (Waskom, 2021) in Python (ver. 3.11.8) (Van Rossum and Drake, 2009).

RESULTS AND DISCUSSION

Wharf Roach Capturing Method

The custom–made capturing tool group had the highest capture efficiency (0.4 min/individual, 25 minutes for 64 individuals), followed by the insect net group (0.6 min/individual, 35 minutes for 60 individuals), and the hand capturing group (1.4 min/individual, 75 minutes for 55 individuals). On the other hand, after 24 hours of wharf roach collection, the highest survival rate was observed in the hand–collected group (94.5%, 52/55), followed by the insect net group (88.3%, 53/60), and the custom–made capturing tool group (79.7%, 51/64).

The custom–made capturing tool is designed to scrape wharf roaches off the quay wall, which may cause injury to the captured individuals. In contrast, the insect net and hand capturing methods are less likely to cause such damage, which may explain the higher survival rate observed in those groups. Even if individuals can eventually recover from injuries caused by the catch–and–release method, the regeneration process incurs physiological and energetic costs that can significantly affect their survival and growth. Injury and regeneration in marine invertebrates have been shown to impact individual reproduction, behavior, and physiological condition, with potential consequences at the population and ecosystem levels (Lindsay, 2010). When aiming for non–invasive environmental monitoring, it is important to consider not only the capture efficiency but also the impact that the method could have on the captured organisms. Since the goal of this study is non–invasive sampling, we chose hand capturing for wharf roach capture. As the experiments progressed, although it was not as effective as the custom–made collection tool, we found that a two–person method (where one person gently guides the wharf roaches with a stick and the other captures them by hand) was more efficient than hand capturing by a single person.

Microplastics Concentrations in Wharf Roach and Seawater Samples

From May to October, microplastics were detected in the digestive tracts of wharf roaches at a detection rate of 75–100% (3/4–4/4), with a mean concentration of 4.15 ± 3.05 items/individual (0.47 ± 0.44 μg/individual, 5.44 ± 4.53 items/g–body weight, 0.52 ± 0.47 μg/g–body weight) (Fig. 2A, B). In November, the detection rate dropped to 25% (1/4), and the mean concentration showed a decreasing trend to 0.50 ± 1.00 items/individual (0.02 ± 0.04 μg/individual, 0.65 ± 1.30 items/g–body weight, 0.03 ± 0.06 μg/g–body weight) (P = 0.07, between October and November).

The decline in microplastic concentration in November likely reflects the reduced activity (feeding rate) of wharf roaches during winter dormancy. Therefore, it is suggested that wharf roaches ingest microplastics primarily through feeding. Additionally, Lee *et al*. (2025) analyzed wharf roaches collected from the Japanese coast in September using μFT–IR and reported a microplastic concentration of 9.70 ± 4.47 items/individual. This value is comparable to the results of this study, supporting the reliability of the microplastic concentrations obtained here.

In this study, microplastics were detected in 100% of the fecal samples throughout the study period. Although the detection rate in the digestive tract decreased in November, the fecal samples were pooled from 10 individuals, which likely maintained the detection rate. The concentration of microplastics in feces averaged 0.085 ± 0.043 item/mg–feces (0.012 ± 0.008 μg/mg–feces) from May to October but dropped to 0.037 items/mg–feces (0.003 μg/mg–feces) in November (an insufficient number of wharf roaches were collected

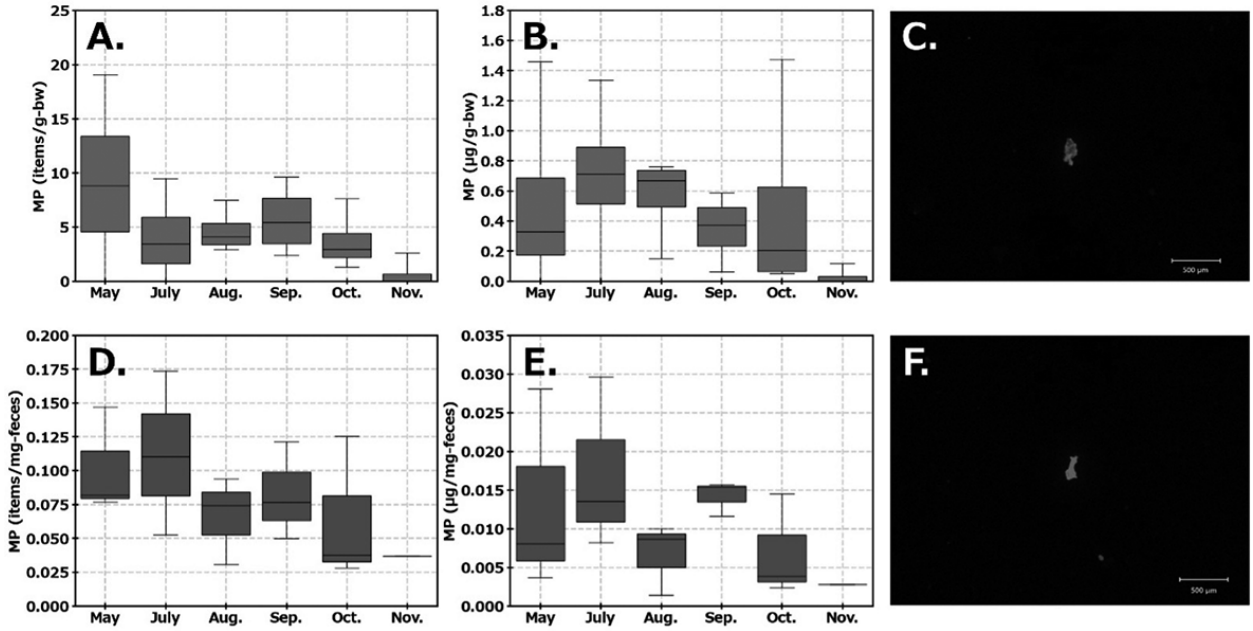

Fig. 2. Microplastics in wharf roach samples.
The boxplots show microplastic concentrations in the digestive tracts (A, items/g–body weight; B, μg/g–body weight) and feces (D, items/mg–feces; E, μg/mg–feces) of wharf roaches, along with their Nile Red fluorescence images (C, digestive tract; F, feces). The boxplots represent the interquartile range, displaying the minimum, 25th percentile, median, 75th percentile, and maximum values for each dataset. C and F show grayscale fluorescence images of microplastics, in which gray areas indicate the presence of microplastics. The scale bar indicates 500 μm.

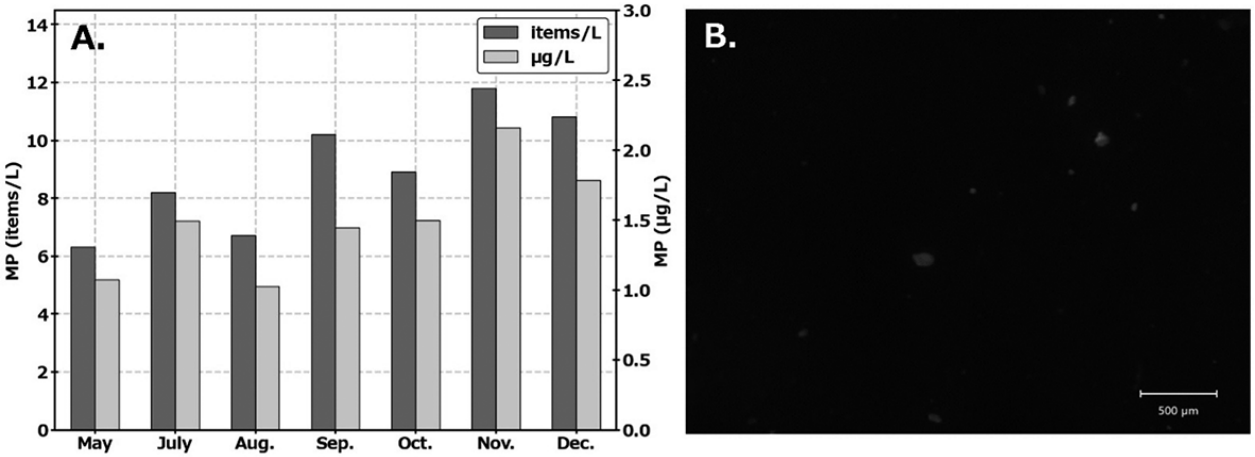

Fig. 3. Microplastics in seawater samples.
Microplastic concentration in seawater (A) and its Nile Red fluorescence image (B). B shows a grayscale fluorescence image of microplastics, in which gray areas indicate the presence of microplastics. The scale bar indicates 500 μm.

in November, resulting in data from only a single tank, n=1) (Fig. 2D, E). This trend was consistent with the seasonal variation in microplastic concentrations observed in the digestive tracts, further supporting the hypothesis that the decline is due to decreased feeding activity in winter. The similarity in seasonal trend between digestive tract and fecal microplastic concentrations suggests that monitoring wharf roach feces can be an effective non–invasive approach to assessing microplastic accumulation in these organisms. Additionally, as a non–invasive sampling method using feces, Hano *et al*. (2018; 2021) demonstrated that changes in metabolites and microbiota in fish feces reflect the physiological condition of the fish. Therefore, if the physiological condition (health) of wharf roaches can be assessed based on their feces, the investigation of wharf roach feces may also serve as a method for evaluating the ecological health of coastal environment.

The concentration of microplastics in seawater during the study period ranged from approximately 6–12 items/L (1–2 μg/L), with a slight increase observed in November and December (Fig. 3A). However, this trend differed from that observed in wharf roach samples, where microplastic concentrations decreased in November. In addition to the above discussion, this discrepancy suggests that microplastics found in the digestive tract of wharf roaches are not primarily derived from seawater but are instead ingested through feeding.

Microplastics Shape Characteristics in Wharf Roach and Seawater Samples

No significant difference was observed in the size of microplastics detected in the digestive tract, feces, and seawater. The median particle diameter of microplastics was 83.2 μm (mean ± SD, 93.6 ± 36.6 μm) in digestive tract samples, 83.1 μm (100.2 ± 46.1 μm) in fecal samples, and 78.1 μm (92.7 ± 37.5 μm) in seawater samples.

Simon *et al*. (2018) reported that the ratio of minor to major diameters of microplastics detected in wastewater was 0.67 (median), with similar values reported in other studies: 0.67 in a meta–analysis of environmental microplastics (Kooi *et al*., 2021), 0.70 in wastewater from Germany (Primpke *et al*., 2020), and 0.73 in Tokyo bay, Japan (Ueda *et al*., 2024). In this study, the median ratio of particle diameters for microplastics detected in seawater from Nishinoura Fishing Port (Fig. 3B) was 0.76, which remained relatively stable throughout the sampling period. However, the ratios for microplastics detected in the digestive tract and feces of wharf roaches were 0.51 and 0.52, respectively (Fig. 2C, F), indicating a potential difference in shape compared to environmental microplastics. The ingestion of microplastics by wharf roaches may have been influenced by the morphology and size of their mouthparts, potentially leading to a size and shape bias in the detected microplastics. However, Lee *et al*. (2025) demonstrated that wharf roaches fragmented and ingested expanded–polystyrene through accidental ingestion. Thus, the differences in microplastic diameter ratios between seawater and wharf roach samples may have resulted from mechanical breakdown during ingestion. These results suggest that when wharf roaches ingest organic substances, such as biofilms attached to plastic waste, they may accidentally fragment the plastic and uptake it into their bodies. While the extent of adverse effects on wharf roaches remains unclear, Choi *et al*. (2023) have reported that plastic ingestion can alter expression of genes associated with metabolic and immune systems in wharf roaches, which may be attributed to the accumulation of additives present in microplastics. Therefore, to protect wharf roaches and the broader coastal ecosystem, more proactive policies and initiatives aimed at reducing plastic waste are necessary.

AUTHOR CONTRIBUTIONS

Yuki Takai: Conceptualization, Formal analysis, Funding acquisition, Investigation, Visualization, Writing – original draft. Miharu Tokunaga: Conceptualization, Investigation. SeokHyun Lee: Investigation. Jeffrey Lebepe: Writing – review & editing. Yohei Shimasaki: Writing – review & editing. Yuji Oshima: Writing – review & editing.

ACKNOWLEDGEMENT

This work was supported by the Sasakawa Scientific Research Grant from The Japan Science Society (2024–6010). We would like to thank Takeshi Hano and Mana Ito for their valuable comments on this study during the 2025 Spring Meeting of the Japanese Society of Fisheries Science.

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