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Special Issue

Green-Winged Teals (*Anas crecca*) inhabiting artificial wetlands are likely to ingest higher levels of microplastics compared to those in natural wetlands

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ABSTRACT: Wetland ecosystems underpin goods and services that are essential for the sustainability of human development. However, wetlands function as receivers of plastic debris from terrestrial and marine sources. To understand the effects of microplastic pollution in wetlands, investigating microplastic contamination in a representative bioindicator is crucial. In this study, we analyzed microplastic accumulation in the feces of green-winged teal (*Anas crecca*) in two major types of the species' habitat in Taiwan: a natural wetland (Guandu) and an artificial wetland (a rice paddy in Yuanshan), using Micro-FITR. The results revealed that a natural wetland ecosystem accumulated fewer microplastics in green-winged teals than a wetland ecosystem with high human activity. More importantly, we developed the methodology for microplastic analysis in a migratory bird at a high trophic level, with feeding habits that make it prone to ingesting microplastics in sludge. Our findings provide insights into developing a regional agreement for protecting wetland environments in countries along the East Asian-Australasian Flyway.

KEY WORDS: Biomonitoring, East Asian-Australasian Flyway, pollution solution, wetland management.

INTRODUCTION

Wetlands are among the most important ecosystems on Earth, providing habitats for diverse flora and fauna. The organisms serve as sources of food, potential energy, and other valuable resources for humans (De Groot *et al.*, 2018; Jisha and Puthur, 2021; Fang *et al.*, 2023a). In other words, wetlands offer vital ecosystem services, such as regulating values (e.g. flood mitigation), as well as cultural, recreational, and educational values (Zedler and Kercher, 2005; Lo *et al.*, 2019; Pedersen *et al.*, 2019). Wetlands function as buffer zones and pathways between freshwater systems and the ocean, playing a crucial role in water filtration and purification (Woodward and Wui, 2001). However, in the plastic era, plastic pollution has become a severe environmental issue (Barnes *et al.*, 2009). Consequently, wetlands are particularly vulnerable to accumulating pollutants from agricultural, urban, industrial, and plastic waste sources (Rasta *et al.*, 2020).

Among various pollutants, microplastic (< 5 mm) have emerged as a significant issue in wetland ecosystems (Lourenço *et al.*, 2017; Paduani, 2020; MacLeod *et al.*, 202; Qian *et al.*, 2021; Amini-Birami *et al.*, 2023). Notably, microplastic continuously break down into smaller particles, posing diverse ecological risks (Barnes *et al.*, 2009; Wilcox *et al.*, 2015; Galloway *et al.*, 2017), and adversely affecting numerous species, including amphipod, plankton, fish, and bird in wetland ecosystems (Teuten *et al.*, 2007; Bergami *et al.*, 2017; Lourenço *et al.*, 2017; Huang *et al.*, 2020; Amini-Birami *et al.*, 2023). As such, identifying a biological indicator to establish a standardized methodology is crucial for assessing microplastic pollution in a wetland ecosystem. Nevertheless, little research has focused on the impact of plastic debris on freshwater ecosystems (Holland *et al.*, 2016) and terrestrial-freshwater interfaces (Provencher *et al.*, 2018; Prokić *et al.*, 2021; Sherlock *et al.*, 2022).

Previous studies have revealed microplastic ingestion in freshwater birds (Holland *et al.*, 2016; D'Souza *et al.*, 2020; Jiang *et al.*, 2024). However, while necropsy and regurgitation methods were the mainstream approach for detection, they may not be suitable for all bird species and could bias the results (Provencher *et al.*, 2019; Jiang *et al.*, 2024). Avian tissue contamination is often regarded as a valuable and cost-effective bioindicator of environmental pollution (Bauerová *et al.*, 2017). On the other hand, fecal detection provides a consistent, less invasive, and non-lethal alternative (Lourenço *et al.*, 2017; Provencher *et al.*, 2018; Provencher *et al.*, 2019; Sherlock *et al.*, 2022), compared with necropsy and regurgitation. As such, microplastic detection of birds' fecal perhaps provides a useful bioindicator to assess the impact of plastic pollutants in a wetland.

To assess the impact of microplastic pollutants on a wetland, our study conducted a preliminary assessment of microplastic contamination in wetland environments using green-winged teal (*Anas crecca*) as a bioindicator. The green-winged teals immerse their bills in sludge during foraging, increasing the likelihood of ingesting

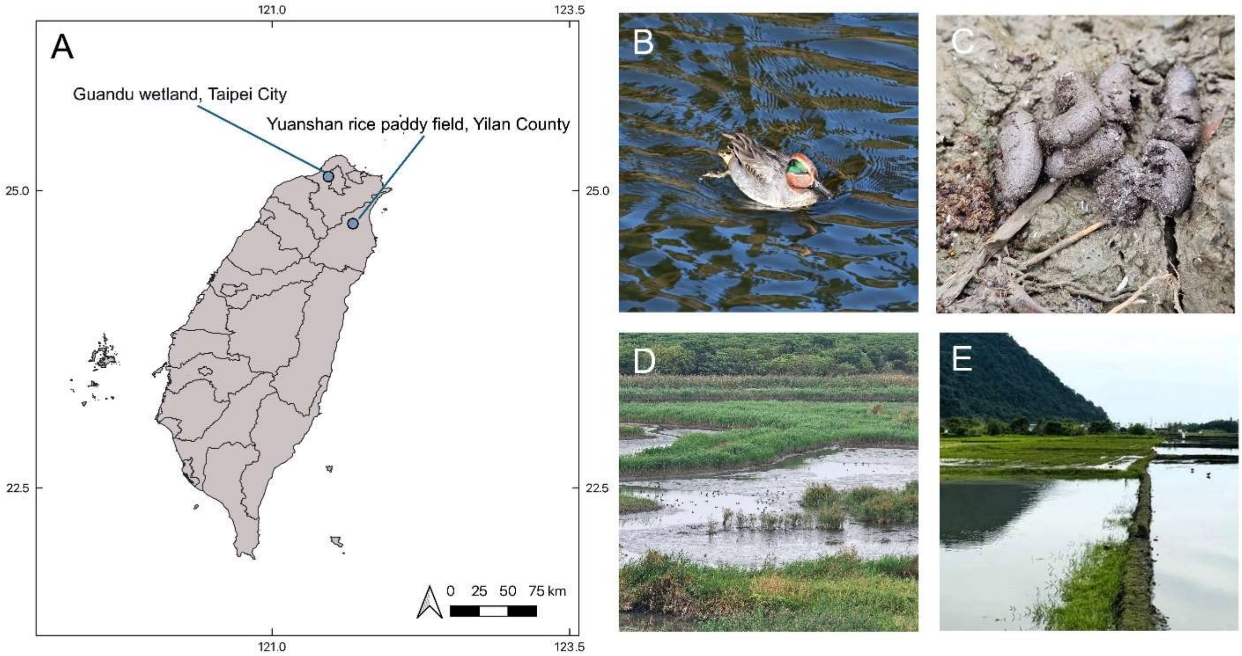

Fig. 1. The sampling site and in situ photos of green-winged teal. **A.** The sampling was conducted in the Guandu Wetland in Taipei City and the Yuanshan rice paddy in Yilan Country. **B.** green-winged teal. **C.** Feces of green-winged teal. **D.** The Guandu Wetland. **E.** The Yuanshan rice paddy.

microplastics. We collected and analyzed fecal samples of the green-winged teal at rice paddies in Yuanshan and a natural wetland in Guandu, Taiwan. In doing so, our study can showcase a useful bioindicator for comparing microplastic containment in a natural wetland and a constructed farm wetland for decision-makers to address strategies for managing wetlands.

MATERIALS AND METHODS

Study sites

The study sites chose the Guandu Wetland and Yuanshan rice paddies (Fig. 1). Guandu Wetland, a natural wetland along the Danshui River in Taipei City, hosts one of the largest populations of the mangrove species Kandelia candel (Fang *et al*., 2023b). It is recognized as a nationally important wetland and an internationally designated Important Bird and Biodiversity Area, with 283 recorded bird species. On the other hand, the Yuanshan rice paddies, an artificial wetland dominated by rice fields, provide seasonal habitats for waterbirds during winter when fields are left fallow and accumulate rainwater. Preliminary observations confirmed green-winged teal activity in both locations.

Sample collection

The green-winged teal is a winter migratory bird, it arrives in Taiwan around October and departs by February (Hsu *et al*., 2019). The collection work was carried out in February 2023. A total of 59 fecal samples were meticulously gathered from two sites, including Guandu Wetland (n = 30) and Yuanshan rice paddies (n = 29). Green-winged teals typically forage in wetland areas and come ashore to rest in groups. The collection of fresh fecal samples was conducted manually by our researchers. The shore where the teals rested was cleaned at 6:00 a.m., to avoid the collection of older samples. Our researchers then waited in the bunker for the teals to return after foraging. Once the green-winged teal group came to rest, the researchers picked up complete green-winged teal feces using metal tweezers and put them into glass tubes. All samples were stored at -20 °C at National Taiwan University before the experiment.

Microplastics separation

The fecal samples were freeze-dried for 8 hours to remove water. Then, an agate mortar was used to grind the fecal slightly, to be inserted into the $ZnCl_2$ solution (1.9 g/cm3), and mixed using a vortex mixer. Finally, the mixture is left to rest at room temperature for 72 hours for density separation. The $ZnCl_2$ solution, a proven and effective solution, was shown to recover both low- and high-density plastics (Pérez-Guevara *et al*., 2021). Most microplastic contained in the fecal sample floated to the surface during density separation.

Each sample underwent a meticulous filtration process using a vacuum glass filter device. This process, characterized by its care and precision, involved passing the solution through a stainless steel filter paper with a pore size of 0.1 μm of a 4 cm diameter. The emphasis is the stainless steel filter paper we make with a thickness

Table 1. Sample descriptions and microplastics analysis results for two sites. The former value of ± represents the mean, and the latter represents the standard error (SE).

Sampling site	Wetland type	N	Number of microplastics	Number of polymer compositions	Identified polymer compositions
Guandu	Wetland	29	5.30 ± 3.81	3.00 ± 1.89	24
Yuanshan	Rice paddy	30	7.55 ± 2.54	3.93 ± 1.28	22

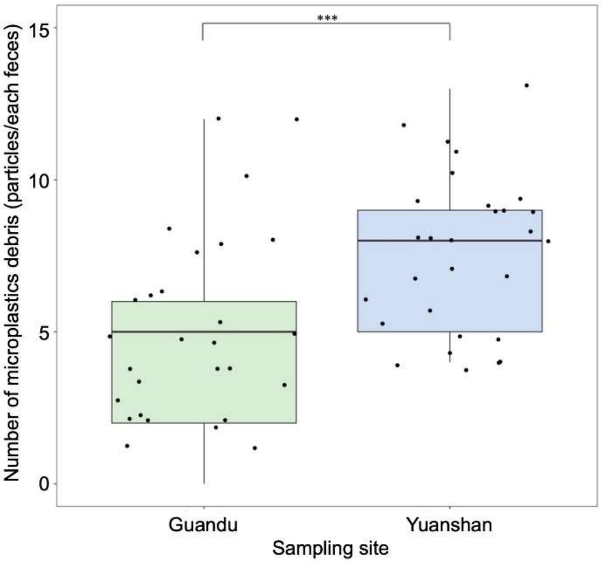

Fig. 2. The number of microplastics in two sampling sites. Box plots present the median, 1st, and 3rd quartiles, and dots represent outliers. Asterisks indicate significant differences. (*** $p < 0.001$).

of only 0.15 mm, significantly improving filtration efficiency and reducing sample loss. The stainless steel filter paper was then meticulously removed using a nipper, stored in a glass plate, and placed in an oven at 60 °C for a 48 hr drying process, ensuring complete removal of any residual water. Reducing water helps improve the spectrum quality during the Micro-FTIR analysis. Therefore, after drying the sample, the spectrum is immediately measured.

Identification chemical of microplastics

The Micro-FTIR instrument uses Thermo Scientific Nicolet iN10 MX. The instrument's spectral range was 400-4000 cm-1, and the optical mode used reflection. Second, place the stainless steel filter on the gold-plated surface to establish the spectral background value of the stainless steel filter, then focus on the surface of the particles and let Micro-FTIR scan for 12 s (64 times) to obtain the spectrum of the particles. OMNIC Picta Software was used for spectral modification (Correcting for Atmospheric Contaminants) and to compare the spectra of each debris with the Thermo Fisher library. This trusted resource ensures the validity of the polymer component analysis.

Quality assurance and quality control

To ensure the accuracy of our results, we included a crucial step in our procedure. During the sampling process, a blank sample was added for every 10 samples. The blank sample was opened and placed at the sampling position until sampling was finished. In the laboratory, a blank sample was treated with the same microplastics separation process, and we were able to detect any potential contamination during the experiment.

After experimental procedures, we conducted a thorough examination. If debris with a similar shape, color, and component was found in both the sample and blank sample, the sample was considered contaminated, and this debris type was also removed from the set of samples. As a result, no debris was found in the blank samples throughout the process, confirming that no contamination had occurred.

Statistical analysis

Building on previous research, this study employed the number of debris as the measurement (Lourenço *et al*., 2017; Provencher *et al*., 2017; Sherlock *et al*., 2022; Chen *et al*., 2025), it has allowed us to obtain the mean ± SE of the number of microplastics in the feces of green-winged teals. The data we collected did not follow a normal distribution under the Shapiro–Wilk test. Therefore, the Kruskal–Wallis test was used to investigate differences in the number and the kind of polymer component of microplastics in Guandu and Yuanshan. The data was compiled using EXCEL version 16.70, and R version 4.2.0 was used for statistical analysis and data visualization.

RESULTS

Prevalence of microplastics in feces

We found that the number of microplastics in Yuanshan (n = 29; mean ± SE = 7.55 ± 2.54; median = 5) is significantly higher than that in Guandu (n = 30; mean ± SE = 5.30 ± 3.81; median = 8) (Kruskal-Wallis test, $p < 0.001$; Fig. 2; Table 1).

Polymer component of microplastics in feces

We found 24 polymer components in Guandu and 22 in Yuanshan (Fig. 3). The polymer component of microplastics was determined at Guandu to be 68.6%, while at Yuanshan, the polymer component was 75.3% (Fig. 3). One of the most interesting findings was that cellophane constituted the highest proportion of microplastics at both sites (Fig. 4). In Guandu, polyester was the second highest component, and polyamide was the third most. In Yuanshan, the second-highest component is polyamide, and the third-highest was

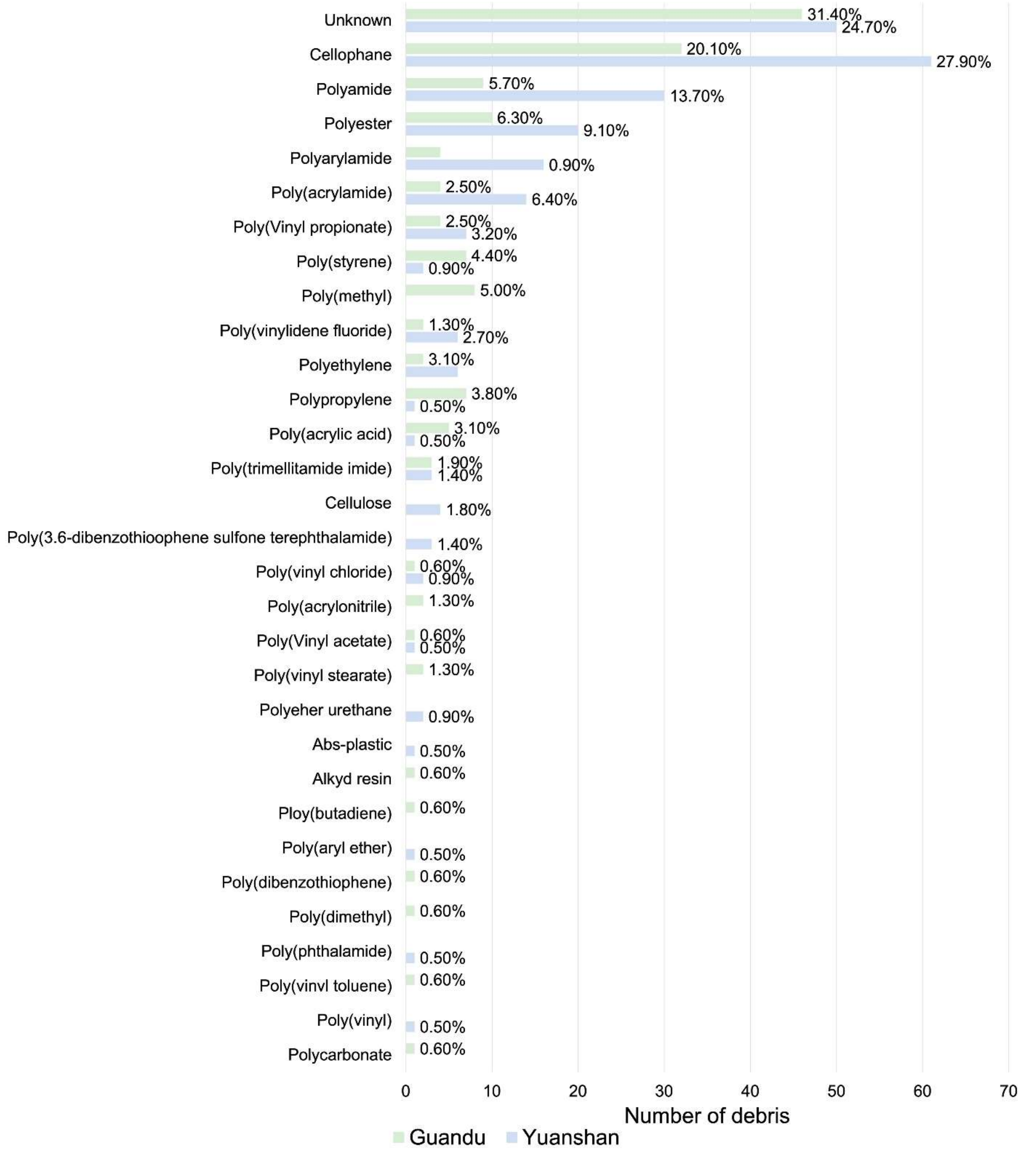

Fig. 3. Polymer components of microplastics in two sites. The vertical axis represents the polymer component of the debris, the horizontal axis represents the number of debris The proportion (in percentage) is shown after each bar.

polyester. In addition, many human-use plastics are found, such as polypropylene, polyethylene, polyester, and polyamide (Fig. 3). Finally, we found that the number of microplastics' polymer component in Yuanshan (n = 29; mean ± SE = 3.93 ± 1.28; median = 2.5) is significantly higher than that in Guandu (n = 30; mean ± SE = 3.00 ± 1.89; median = 4) (Kruskal-Wallis test, $p < 0.001$; Fig. 5; Table 1).

DISCUSSION

Taiwan's green-winged teal population has been steadily declining (Hsu *et al*., 2014; Lin *et al*., 2023). To minimize disturbance to the population, we used fecal samples as a noninvasive sampling method. Wild birds can excrete up to 75% of ingested plastic within a month (van Franeker and Law, 2015). Therefore, most of the

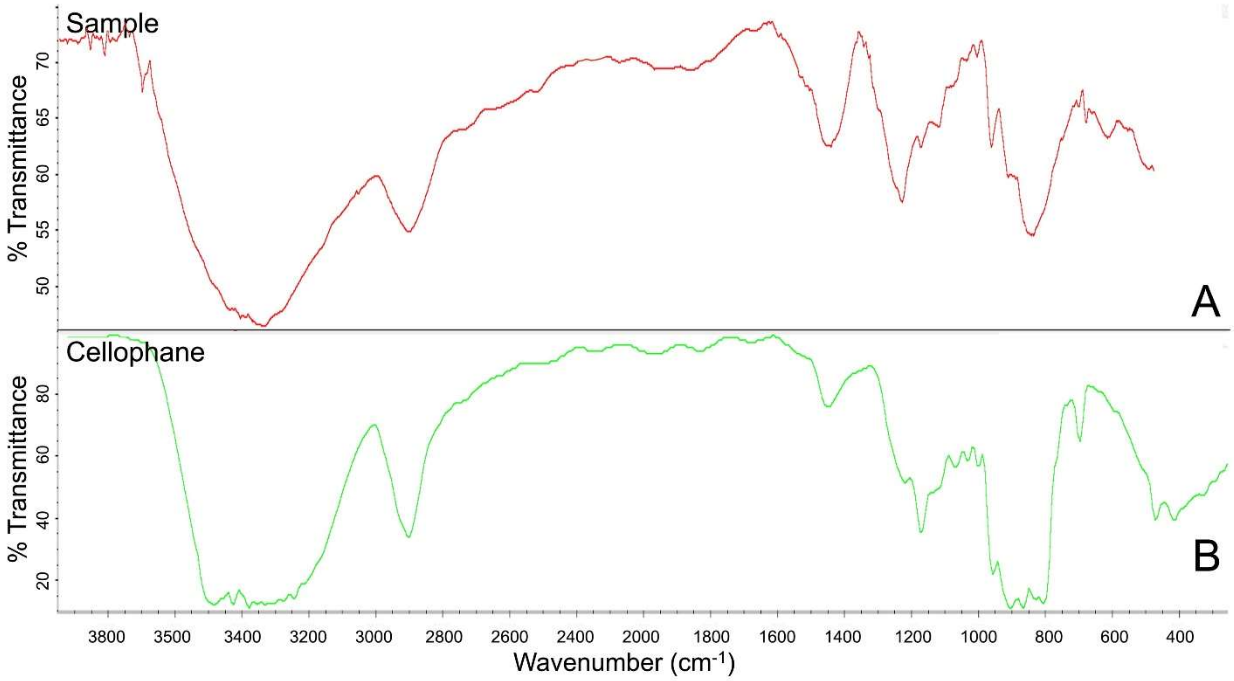

Fig. 4. Micro-FTIR spectra of cellophane found in green-winged teal. **A.** Spectra classified as cellophane in green-winged teal feces; **B.** Cellophane spectra from Thermo Fisher library.

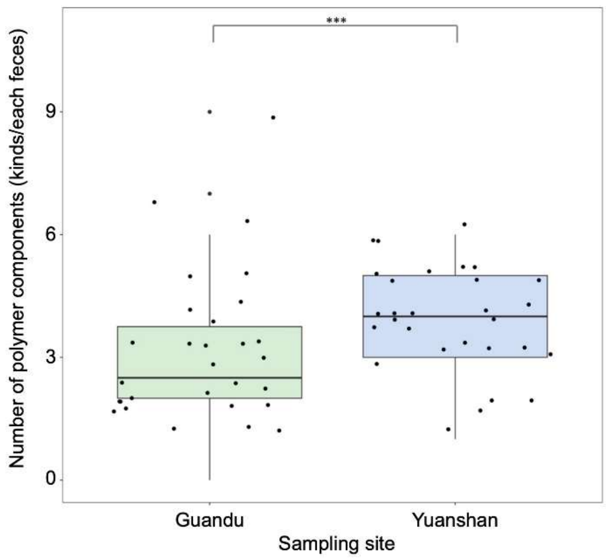

Fig. 5. The number of microplastics' polymer component in two sampling sites.

microplastics found in the fecal samples we collected likely originate from the birds' foraging habitats in Taiwan. This study demonstrates that fecal samples can be used as an effective tool to monitor microplastic ingestion in birds (Lourenço *et al*., 2017; Provencher *et al*., 2018; Provencher *et al*., 2019).

Our results indicate that fecal samples from green-winged teal in rice paddies contained higher mounts of microplastics, suggesting that these birds ingest more microplastics in this habitat. Rice paddies, a type of wetland, serve as key stopover sites along major migration corridors for many waterbird species (Remsen *et al*., 1991; Elphick and Oring, 1998; Huner *et al*., 2002). For instance, rice paddies in the southern USA, south-central South America, southern Europe, parts of Asia, and other regions are heavily utilized by migrating waterbirds (Acosta *et al*., 2010; Fujioka *et al*., 2010; Longoni, 2010). Our findings underscore the urgent need to address plastic pollution in rice fields and align with previous studies suggesting that agricultural wetlands may accumulate more microplastics than other wetland types (Lv *et al*., 2020; Ashjar *et al*., 2023; Amirhosseini *et al*., 2023).

Two main factors may contribute to this accumulation. First, in conventional rice farming, sediment is retained within the same paddy field over multiple farming cycles, allowing plastic pollutants to accumulate and increasing their concentration. Second, farmers commonly use plastic mulch to suppress weeds and facilitate movement in the fields (Amirhosseini *et al*., 2023). However, these plastic mulches quickly degrade into smaller plastic debris. Huang *et al*. (2020) demonstrated that continuous plastic mulching significantly increases soil microplastic concentrations. Over 5, 12, and 24 consecutive years of mulching, soil microplastic abundance increased from 81 to 308 and 1076 particles/kg, respectively. Polyethylene, the primary component of plastic mulch (Qiang *et al*., 2023), is significantly prevalent in Yuanshan (Fig. 6).

Previous research highlights the environment risks associated with polyethylene in soil, as it alters microbial

Fig. 6. Ridge of field covered with plastic mulch in rice paddy in Taiwan.

community composition and affects soil water availability (Cramer *et al.*, 2022). Additionally, polyethylene disrupts soil properties and nutrient cycling, ultimately affecting the availability of water and nutrients for plants (Boots *et al.*, 2019; Smettem *et al.*, 2021). Toxicological studies have shown that polyethylene will cause endocrine disruption, apoptosis in the liver and alterations in animal plasma components (Rochman *et al.*, 2014; Bobori *et al.*, 2022; Lee *et al.,* 2023). Furthermore, polyethylene exhibits greater toxicity at smaller particle sizes (Bobori *et al.*, 2022). To mitigate these environmental risks, improving the recycling efficiency of plastic mulch and transitioning to biodegradable mulching films would be significant and promising solutions (Yang *et al.*, 2023).

This study identified a significant percentage of cellophane at both sampling sites, in contrast to other studies that have reported PE/PP as the primary plastic in wetlands (Dalvand and Hamidian, 2023). Cellophane, commonly used as a breathable film for packaging bread, processed meat, cheese, fresh produce, and as ovenproof wrapping (Sid *et al.*, 2021), has been frequently detected in aquatic and terrestrial animal samples due to its widespread use by humans (Garcia *et al.*, 2021; Lestari *et al.*, 2023; Carrillo-Barragán *et al.*, 2024). The prevalence of cellophane in Yuanshan may be attributed to inadequate local waste disposal practices. During fecal sample collection, cellophane containing human waste was observed in the rice paddies. Meanwhile, the latest research shows that cellophane can persist in wastewater effluent even after treatment, highlighting challenges in its complete removal (Maw *et al.*, 2022). This situation explain the presence of cellophane in Guandu Wetland, despite the presence of numerous wastewater treatment plants upstream.

Polypropylene, one of the polymers we identified in feces, has been studied in animal experiments regarding the toxicological effects of microplastics on birds. Research indicates that polypropylene can delay sexual maturity in female Japanese quail (*Coturnix japonica*) and increase the likelihood of epididymal cysts in males (Roman *et al.*, 2019). Additionally, the presence of microplastics in bird feces is significant, as it facilitates the transport of plastic debris across various habitats (Bourdages *et al.*, 2021). Microplastics also alter the density of feces, reducing its bioavailability (Coppock *et al.*, 2019). Consequently, the microplastics found in green-winged teal feces may pose additional risks that require further investigation.

Plastics are essential in modern life, providing benefits but also posing significant environmental challenges. To mitigate plastic pollution, Taiwan's local government could subsidize subsidize farmers in essential wetland areas to replace plastic mulch with biodegradable alternatives regularly. Authorities should also engage with farmers through awareness campaigns to prevent agricultural waste dumping and enhance rural waste removal systems. In urban areas, the government should educate citizens on reducing plastic use and ensure wastewater treatment plants effectively filter plastic debris.

Most importantly, Taiwan currently lacks a large-scale investigation plan for microplastic pollution in wildlife habitats. Without such an initiative, it remains unclear which habitats and species are most at risk. Establishing an international convention to address microplastic pollution could provide a valuable opportunity for global mitigation efforts. A relevant example is the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention), which fosters international cooperation to protect the marine environment in the North-East Atlantic. This convention integrates the 1972 Oslo Convention, which focused on waste dumping at sea, and the 1974 Paris Convention on land-based marine pollution. The OSPAR Convention selected the Northern Fulmar (*Fulmarus glacialis*) as an Ecological Quality Objective (EcoQO) indicator species as part of its environmental objectives. The Objective is to achieve the accumulation of plastic in Northern Fulmar exceeding 0.1g, which must be less than 10%. Yearly observations indicate a decline in plastic ingestion (van Franeker, 2011). However, no similar consensus exists in Asia (Chen *et al.*, 2025).

Countries in the East Asian-Australasian Flyway could benefit from selecting key bird species as bio-indicators for long-term plastic pollution monitoring. Using green-winged teal feces for sampling presents a feasible method for rapid and cross-border comparisons. Although, the diversity of plastic types and characteristics introduces additional challenges (Rochman *et al.*, 2019). We acknowledge that our study cannot be directly compared with others, as different bird species exhibit varying food preferences and digestive functions (Zhao *et al.*, 2016; Carlin *et al.*, 2020). Effective monitoring can only be achieved through international consensus and a standardized methodology (Provencher *et al.*, 2020). Nonetheless, this study provides a quick and non-invasive approach to assessing the pollution in bird populations, particularly those experiencing fluctuations or declines (Lourenço *et al.*, 2017; Provencher *et al.*, 2018; Provencher *et al.*, 2019).

CONCLUSION

Our research confirms that different wetland environments influence the amount and type of microplastics ingested by green-winged teal. We found that the average quantity of microplastics and polymer composition in the feces of green-winged teal residing in rice paddies were significantly higher than those in natural wetland environments. This alarming trend is likely driven by farmers' use of plastic mulch and other plastic products, as evidenced by the higher proportion of polyethylene plastic. Additionally, the retention of sludge exacerbates the issue, leading to the accumulation of plastic pollution. Urgent measures are needed to change farming practices and establish uniform reduction targets for countries along the East Asian-Australasian Flyway. We propose designating certain widely distributed bird species—such as green-winged teal, sanderling, and gray-tailed tattler—as potential bioindicators for microplastic monitoring. These species frequently forage in environments where sludge accumulates, making them suitable for assessing microplastic pollution. Implementing a standardized monitoring methodology would provide policymakers with essential data to develop effective regulations aimed at reducing microplastic contamination.

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