วารสารวิจัยนิเวศวิทยาป่าไม้เมืองไทย 8(1): 305-316 (2567)

นิพนธ์ต้นฉบับ

การปนเปื้อนไมโครพลาสติกในลูกอ๊อดสัตว์สะเทินน้ำสะเทินบกสามชนิด: ผลกระทบต่อระบบนิเวศของสัตว์สะเทินน้ำสะเทินบก

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รับต้นฉบับ: 24 มีนาคม 2567 ฉบับแก้ไข: 10 มิถุนายน 2567 รับลงพิมพ์: 13 มิถุนายน 2567

บทคัดย่อ

ความเป็นมาและวัตถุประสงค์: การศึกษานี้มุ่งศึกษาการปรากฏและลักษณะของไมโครพลาสติกในลูกอ๊อดสัตว์สะเทินน้ำสะเทินบก 3 ชนิด คือ กบนา เขียดบัว และอึ่งอ่างบ้าน

วิธีการ: เก็บตัวอย่างลูกอ๊อดจากพื้นที่ศึกษา 2 จังหวัด คือ บึงกาฬและขอนแก่น สลบลูกอ๊อดด้วยสารละลายคลอรีโทนและเก็บรักษาสภาพด้วยแอลกอฮอล 70% จำแนกชนิดลูกอ๊อดโดยการศึกษาลักษณะสัณฐานวิทยาภายนอกเปรียบเทียบกับคำบรรยายลักษณะที่เกี่ยวข้อง ดำเนินการย่อยลูกอ๊อดด้วยไฮโดรเจนเพอร์ออกไซด์ความเข้มข้น 30% โดยบ่มที่อุณหภูมิ 60 องศาเซลเซียส แล้วปล่อยให้ตกตะกอนที่อุณหภูมิห้อง จากนั้นกรองเศษเหลือจากการย่อยด้วยกระดาษกรอง Whatman เบอร์ 1 (110 มม.) และนำกระดาษกรองมาตรวจหาชิ้นส่วนไมโครพลาสติกด้วยกล้องสเตอริโอ Nikon SMZ-745T ถ่ายภาพและวัดขนาดไมโครพลาสติกด้วยโปรแกรม NIS Elements

ผลการศึกษา: การศึกษานี้ใช้ตัวอย่างลูกอ๊อด 12 ตัวอย่าง ประกอบด้วย ลูกอ๊อดกบนา 3 ตัวอย่าง (TL เฉลี่ย 17.5±1.03; ระยะ 29) เขียดบัว 3 ตัวอย่าง (TL เฉลี่ย 38.5±5.69; ระยะ 43) และอึ่งอ่างบ้าน 6 ตัวอย่าง (TL เฉลี่ย 26.8±1.23; ระยะ 38) ผลการศึกษาพบชิ้นส่วนไมโครพลาสติกจำนวน 26 ชิ้น ประกอบด้วยชิ้นพลาสติกที่เป็นแผ่นและเส้นใย อึ่งอ่างบ้านพบจำนวนไมโครพลาสติกมากที่สุด (15 ชิ้น) ซึ่งทั้งหมดเป็นเส้นใย ลูกอ๊อดทั้ง 3 ชนิด พบไมโครพลาสติกที่มีขนาดแตกต่างกัน โดยกบนาพบไมโครพลาสติกแบบแผ่นจำนวนมากกว่า

สรุป: การศึกษานี้รายงานการพบไมโครพลาสติกในลูกอ๊อดสัตว์สะเทินน้ำสะเทินบกจากประเทศไทย และย้ำถึงความสำคัญของการศึกษาเพิ่มเติมในอนาคต

คำสำคัญ: ไมโครพลาสติก, สัตว์สะเทินน้ำสะเทินบก, ลูกอ๊อด, การปนเปื้อน, นิเวศวิทยา

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https://doi.org/10.34044/j.tferj.2024.8.1.03

ORIGINAL ARTICLE

Microplastic Contamination in Three Amphibian Species: Implications for Amphibian Ecosystems

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Received: 24 March 2024 Revised: 10 June 2024 Accepted: 13 June 2024

ABSTRACT

Background and Objectives: This study aims to investigate the presence and characteristics of microplastics in the tadpoles of three amphibian species: *Hoplobatrachus chinensis, Hylarana erythraea*, and *Kaloula pulchra*.

Methodology: Tadpole samples were collected from two study areas, Bueng Kan and Khon Kaen provinces. The tadpoles were euthanized with chlorotone solution and preserved in 70% alcohol. The species were identified by examining external morphological characteristics and comparing them with relevant descriptions. The tadpoles were digested using 30% hydrogen peroxide at 60°C, then allowed to settle at room temperature. The remaining residue was filtered using Whatman No. 1 filter paper (110 mm), and the filter paper was examined for microplastic particles using a Nikon SMZ-745T stereo microscope. Images and measurements of the microplastics were taken using the NIS Elements software.

Main Results: This study used 12 tadpole samples, consisting of 3 tadpoles of *H. chinensis* (average TL 17.5±1.03; stage 29), 3 *Hy. erythraea* (average TL 38.5±5.69; stage 43), and 6 *K. pulchra* (average TL 26.8±1.23; stage 38). The results revealed a total of 26 microplastic particles, comprising both fragments and fibers. The banded bullfrog had the highest number of microplastics (15 particles), all of which were fibers. Microplastics of varying sizes were found in all three species, with the rice frog showing a higher number of fragment-type microplastics.

Conclusion: This study reports the presence of microplastics in tadpoles of amphibians from Thailand and emphasizes the importance of further research in the future.

Key words: Microplastics, amphibians, tadpoles, contamination, ecology

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https://doi.org/10.34044/j.tferj.2024.8.1.03

Introduction

Microplastic pollution has emerged as a significant environmental concern globally, with its detrimental impacts extending across various ecosystems (Barnes *et al*., 2009; Wagner *et al*., 2014; Wu *et al*., 2017; Munno *et al*., 2018; Singh *et al*., 2021). Anuran tadpoles, as integral components of aquatic ecosystems, are susceptible to the effects of microplastic contamination due to their reliance on aquatic habitats during their early life stages (Hu *et al*., 2018; Araújo & Malafaia, 2020; Hou & Rao, 2022). Despite growing awareness of microplastic pollution (Wu *et al*., 2017), studies investigating its presence and potential impacts on amphibians, particularly tadpoles, remain limited.

Northeastern Thailand, characterized by its rich biodiversity and extensive freshwater habitats, serves as an ideal region to assess microplastic contamination in anuran tadpoles. This preliminary study aims to assess the prevalence of microplastic pollution in tadpoles belonging to three common species: *Hoplobatrachus chinensis* (family Dicroglossidae, *Kaloula pulchra* (family Microhylidae), and *Hylarana erythraea* (family Ranidae), inhabiting in freshwater environments across in northeastern Thailand. These species *H. chinensis*, *K. pulchra*, and *Hy. erythraea* are widespread species found in various aquatic habitats, including ponds, marshes, and rice fields throughout the country (Chuaynkern & Chuaynkern, 2012; Chuaynkern & Duengkae, 2014; Frost, 2024). Despite their ecological importance, tadpoles of these species (along with other Thai species) have remained not investigated concerning microplastic contamination. Through this preliminary assessment, the result reveal to enhance understanding of the potential threat posed by microplastics to anuran tadpoles in northeastern Thailand. This study contributes valuable insights into the extent of microplastic contamination in freshwater ecosystems and underscores the urgency of further research to mitigate its adverse effects on amphibian populations and ecosystem health.

Materials and Methods

Tadpole specimens (Figure 1) were collected from field sites situated in two provinces. *H. chinensis* tadpoles were sourced from Bueng Karn province, while those belonging to *K. pulchra* and *H. erythraea* were collected from Khon Kaen province, specifically obtained from Khon Kaen University. Animal ethics approval, under reference number ACKU66-SCI-019, was granted by the Kasetsart University's Institutional Animal Care and Use Committee, Thailand. Subsequently, the tadpoles were humanely euthanized by immersion in chloretone and then preserved in 70% ethanol. Upon arrival at the laboratory, located within the Department of Biology, Faculty of Science, Khon

Kaen University, the tadpoles underwent detailed examination of their external morphology to facilitate species identification.

The developmental stage of each tadpole was determined following the classification system outlined by Gosner (1960). The measurements of 11 morphological characteristics followed the methods described by Chuaynkern *et al*. (2019). These characteristics include: SVL (snout to vent length), TAL (tail length), TL (total length), MTH (maximum tail height), BH (body height), BW (body width), PP (interpupillary distance), ID (internarial distance), RN (rostro-narial distance), NP (nanopillar distance), and ED (maximum eye diameter). A digital caliper was utilized for conducting these measurements. For characters smaller than 1 mm, measurements were taken using an ocular scale attached to a stereomicroscope. The identification process was carried out in accordance with established taxonomic literature, as referenced in works by Aran *et al.* (2012) and Chuaynkern *et al*. (2023).

To prepare tadpole specimens for the study of microplastics, the tadpoles underwent a series of steps. Initially, they were washed with tap water to remove any traces of ethanol. Subsequently, each tadpole was immersed in 30% hydrogen peroxide and subjected to heat at 60 degrees Celsius for 24 hours or until the tadpole completely digested. The remaining materials were then collected and filled with saturated NaCl solution (prepared by dissolving 250 grams of NaCl per liter of water). The mixture was stirred until the NaCl was fully dissolved and left overnight for sedimentation. The clear solution was then filtered using 11-micron filter paper. The filtered solution was incubated at 60 degrees Celsius for 24 hours or until it was completely dry. Finally, the filter paper was examined under a stereomicroscope to identify microplastics. The type of plastics present was analyzed using Fourier-transform infrared spectroscopy.

Results

This study employed three *H. chinensis* tadpoles (average TL 17.5±1.03, Gosner stage 29), six *K. pulchra* tadpoles (average TL 26.8±1.23, Gosner stage 38), and three *Hy. erythraea* tadpoles (average TL 38.8±5.69, Gosner stage 43) for the assessment of microplastic contamination (Table 1). The identification of these tadpoles (Figure 1) relied on previous descriptions. Specifically, the *H. chinensis* tadpoles were identified according to the descriptions provided by Grosjean *et al*. (2004), Aran *et al.* (2012), and Chuaynkern *et al.* (2023), The *K. pulchra* and *Hy. erythraea* tadpoles were identified based on the descriptions by Chou & Lin (1997) and Inthara *et al*. (2005).

Table 1 Morphometric measurements (in mm) of tadpoles from three species: *Hoplobatrachus chinensis*, *Hylarana erythraea*, and *Kaloula pulchra*.

Species	*H. chinensis*	*Hy. erythraea*	*K. pulchra*		Species	*H. chinensis*	*Hy. erythraea*	*K. pulchra*
Gosner stage	29	43	38		BW	3.6±0.36	5.5±0.76	5.6±0.10
No. specimens	3	3	6		PP	2.4±0.34	7.0±1.26	5.2±0.17
SVL	6.6±0.55	10.1±1.26	6.0±0.34		NN	1.0±0.16	6.6±0.19	0.7±0.01
TAL	11.0±0.68	29.4±6.46	20.8±0.95		ID	0.6±0.13	2.4±0.21	1.4±0.10
TL	17.5±1.03	38.8±5.69	26.8±1.23		NP	0.8±0.25	0.9±0.08	1.5±0.03
MTH	1.9±0.23	4.1±0.23	4.3±0.42		ED	0.9±0.10	2.4±0.25	1.6±0.06
BH	2.8±0.40	5.5±0.76	4.6±0.10					

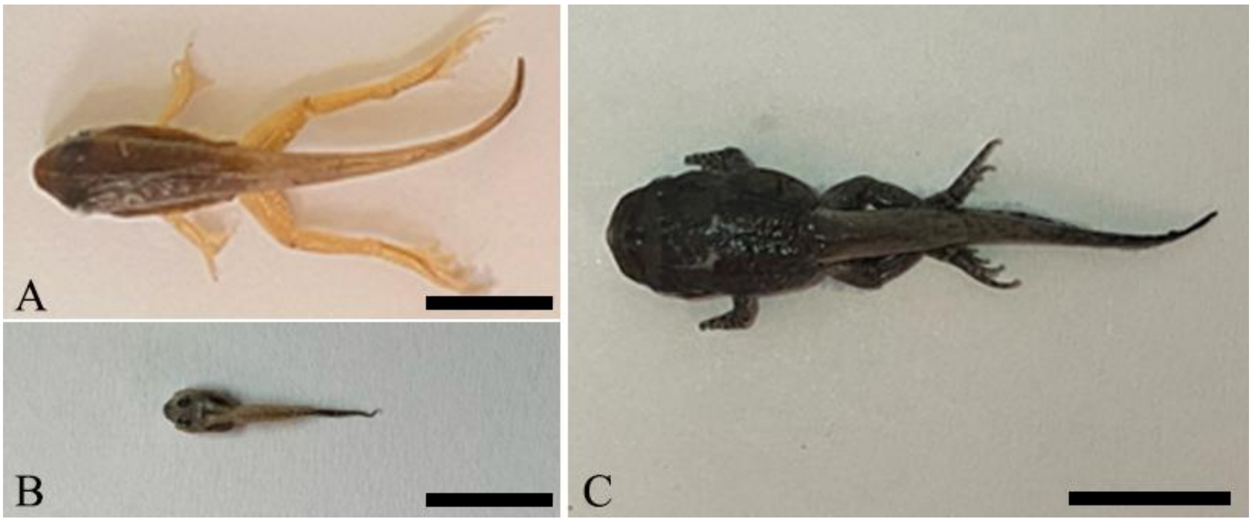

Figure 1 Photographs of preserved three tadpole specimens. A, *Hylarana erythraea*; B, *Hoplobatrachus chinensis*; C, *Kaloula pulchra*. Scale bar equals 10 mm.

Our analysis has revealed the presence of microplastics in tadpoles across all three investigated species: *H. chinensis* (3 individuals), *Hy. erythraea* (3 individuals), and *K. pulchra* (6 individuals) (Table 2, and Figure 2). A total of 26 microplastic pieces, identified as small plates (3 plates) and fibers (23 fibers), were detected among 12 tadpoles of these species. The abundance of microplastics varied among the species, with *K. pulchra* exhibiting the highest count of microplastic pieces (15 fibers), followed by *Hy. erythraea* with 7 microplastic pieces (comprising 2 plates and 5 fibers), and the lowest count observed in *H. chinensis* with 4 microplastic pieces (consisting of 1 plate and 3 fibers). Small microplastic plates were observed in *Hy. erythraea* and *H. chinensis* but not in *K. pulchra* (Table 3). Furthermore, three colors of small microplastic plates were identified: pink, red, and yellow plates. Small microplastic fibers were prevalent and found in all three tadpole species. Additionally, five colors of small microplastic fibers were detected: black, blue, green, orange, and red fibers. *K. pulchra* exhibited the highest count of small microplastic fibers (15 fibers).

Table 2 Summarizes the presence of microplastics observed in tadpoles of *Hoplobatrachus chinensis, Hylarana erythraea*, and *Kaloula pulchra*.

	Microplastic types (pieces)								**Average (pieces/ individual)**
	Plates			**Fibers**					
	Pink	**Red**	**Yellow**	**Black**	**Blue**	**Green**	**Orange**	**Red**	
H. chinensis	-	-	1	2	1	-	-	-	1.3
Hy. erythraea	1	1	-	3	-	1	-	1	2.3
K. pulchra	-	-	-	4	7	-	2	2	2.5

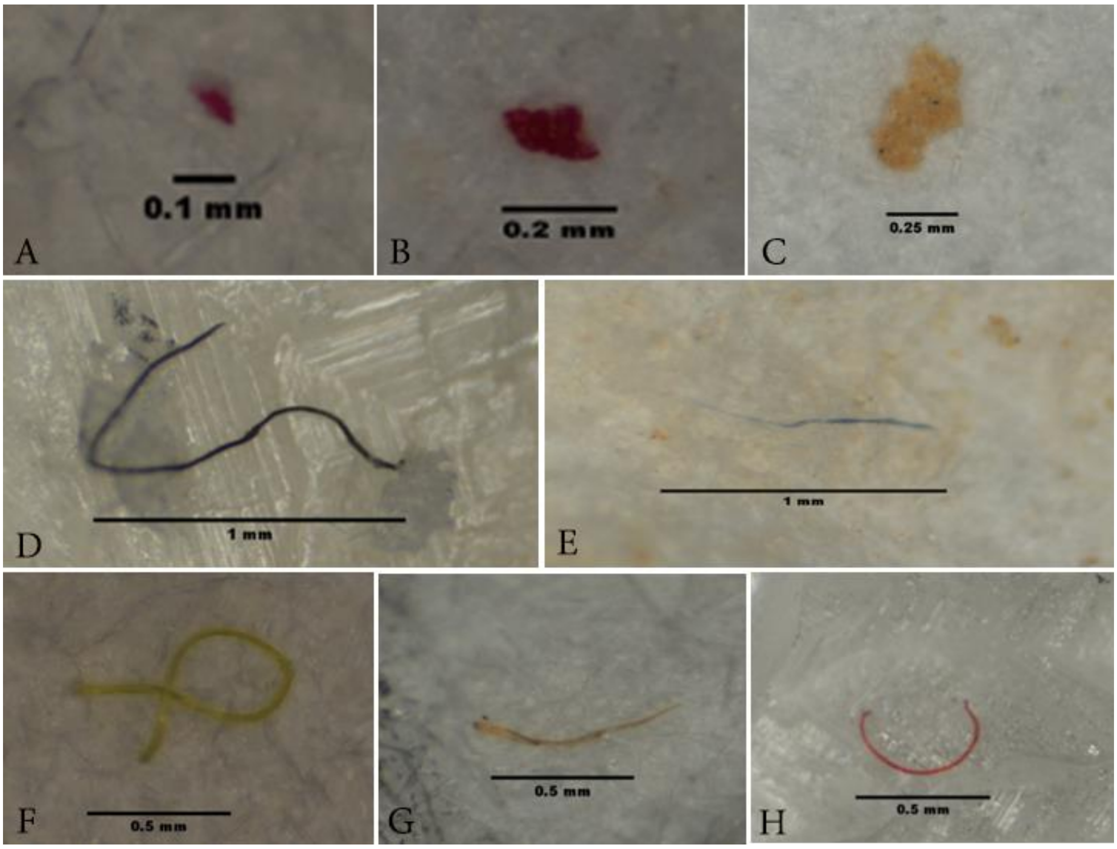

Figure 2 Selected photographs of microplastics found in three tadpole specimens: *Hoplobatrachus chinensis*, *Hylarana erythraea*, and *Kaloula pulchra*. A, Pink plate; B, Red plate; C, Yellow plate; D, Black fiber; E, Blue fiber; F, Green fiber; G, Orange fiber; H, Red fiber

The analysis of microplastics found in three amphibian species, namely *Hy. erythraea*, *H. chinensis*, and *K. pulchra*, revealed intriguing variations in their dimensions (Table 3). *Hy. erythraea* exhibited fiber microplastics with an average width of 0.021 ± 0.005 mm and a length of 1.713 ± 0.627 mm, while also detecting plate microplastics measuring 0.081 ± 0.070 mm in width and 0.104 ± 0.037 mm in length. Conversely, *H. chinensis* showed slightly larger dimensions for fiber microplastics, with an average width of 0.022 ± 0.005 mm and a length of 1.673 ± 0.926 mm. Notably, this species also found plate microplastics significantly larger than those of *Hy. erythraea*, measuring 0.269 mm in width and 0.445 mm in length. On the other hand, *K. pulchra* displayed fiber microplastics with a narrower width of 0.018 ± 0.004 mm but comparable length of 1.453 ± 0.823 mm. These findings suggest species-specific variations in microplastic dimensions among the studied amphibians, with *Hy. erythraea* and *H. chinensis* exhibiting similar sizes in fiber microplastics but differing significantly in plate microplastics dimensions, highlighting the complexity of microplastic interactions within amphibian ecosystems.

Table 3. Dimensions (in mm) of microplastics found in three tadpole species: *Hoplobabrachus chinensis, Hylarana erythraea*, and *Kaloula pulchra*.

Microplastic type	*H. chinensis*		*Hy. erythraea*		*K. pulchra*	
	Width	Length	Width	Length	Width	Length
Fiber	0.022±0.005 (0.019-0.027), *n*=3	1.673±0.926 (0.915-2.706), *n*=3	0.021±0.005 (0.016-0.030), *n*=5	1.713±0.627 (0.751-2.506), *n*=5	0.018±0.004 (0.012-0.025), *n*=15	1.453±0.823 (0.517-3.198), *n*=15
Plate	0.269 *n*=1	0.445 *n*=1	0.081±0.070 (0.031-0.130), *n*=2	0.104±0.037 (0.077-0.130), *n*=2	-	-

Discussion

Tadpoles serve as a significant food source in Thailand and elsewhere (Chuaynkern & Duengkae, 1014; Thomas & Buju, 2015). Various species of tadpoles have been consumed in northern and northeastern Thailand for a considerable period (Chuaynkern & Duengkae, 2014). Given the critical role of water environments for amphibians and their susceptibility to environmental changes, numerous studies have indicated the widespread contamination of tadpoles by microplastics (Hu *et al*., 2018; Bibfanti *et al*., 2020, 2021). Our findings represent the initial documentation of microplastic presence in tadpoles of three anuran species from Thailand (*H. chinensis*, *Hy. erythraea*, and *K. pulchra*). These results suggest the potential transfer of microplastics to subsequent trophic levels in ecosystems, including humans.

Differences in the types of microplastics consumed by three tadpole species (*H. chinensis*, *Hy. erythraea*, and *K. pulchra*) suggest potential variations in microplastic exposure or ingestion among the studied populations, which may be influenced by habitat characteristics, feeding habits, or other ecological variables. Tadpole feeding behavior is affected by various factors, including mouthpart structure and ecological niche. *H. chinensis* and *Hy. erythraea* tadpoles possess distinct mouthpart structures characterized by keratodonts, facilitating grazing on algae and detritus in aquatic habitats (Chou & Lin, 1997; Inthara *et al*., 2005; Aran *et al*., 2012; Moonasa *et al*., 2018; Chuaynkern *et al*., 2023). These tadpoles can scrape and rasp algae efficiently, aiding in their foraging. In contrast, *K. pulchra* tadpoles lack keratodonts and likely exhibit different feeding behaviors. Without specialized structures for grazing, they may rely more on filter or suspension feeding mechanisms to obtain nutrients. They may feed on suspended particles or planktonic organisms in the water column, using their oral discs or other mouthpart adaptations. The absence of keratodonts in *K. pulchra* tadpoles indicates a deviation in their feeding strategy compared to *H. chinensis* and *Hy. erythraea*. While the latter species are adapted for substrate grazing, *K. pulchra* tadpoles may exploit alternative food resources in their environment. These differences in feeding behavior could be attributed to ecological factors such as habitat characteristics, resource availability, and interspecific competition (Altig *et al*., 2007). Moreover, variations in foraging behavior among these tadpole species could affect their growth and development rates, as well as overall fitness and survival. Future studies investigating the specific dietary preferences and foraging efficiencies of these tadpoles could provide valuable insights into their ecological roles and interactions within aquatic ecosystems.

The morphological analysis of microplastics highlights species-specific variations in dimensions, particularly evident in plate microplastics. *H. chinensis* tadpoles displayed larger plate microplastics compared to *H. erythraea*, indicative of potential differences in microplastic sources or accumulation mechanisms between these species. Such variations underscore the complexity of microplastic interactions within amphibian ecosystems and emphasize the importance of considering species-specific responses when assessing the impacts of microplastic contamination.

The identification of multiple colors of microplastic plates and fibers further adds to the diversity of microplastic pollution observed in tadpole habitats. This diversity in color may reflect differences in polymer composition or weathering processes, highlighting the dynamic nature of microplastic pollution in aquatic environments. Additionally, the prevalence of small microplastic fibers across all tadpole species underscores the ubiquitous nature of microplastic contamination in freshwater ecosystems.

Finally, our study contributes to the growing body of research on microplastic pollution in amphibian habitats, providing insights into the distribution, characteristics, and potential ecological implications of microplastics in tadpole populations. Further investigations are warranted to elucidate the pathways of microplastic exposure, potential effects on tadpole health and development, and broader ecological consequences within amphibian ecosystems.

Conclusion

Our study provides valuable insights into the presence and characteristics of microplastics in tadpoles of three amphibian species: *H. chinensis, Hy. erythraea*, and *K. pulchra*. The investigation revealed a widespread occurrence of microplastics across all studied species, with *K. pulchra* exhibiting the highest microplastic count, primarily comprising fibers. Species-specific variations in microplastic dimensions, particularly in plate microplastics, highlight the complexity of microplastic interactions within amphibian ecosystems. The diversity of microplastic colors observed underscores the dynamic nature of microplastic pollution in freshwater habitats, with potential implications for polymer composition and weathering processes. The prevalence of small microplastic fibers across all tadpole species further emphasizes the ubiquitous nature of microplastic contamination in aquatic environments. These findings underscore the urgent need for continued research to better understand the sources, pathways, and ecological impacts of microplastic pollution in amphibian habitats. Efforts to mitigate microplastic contamination should prioritize species-specific responses and consider the broader implications for amphibian health and ecosystem functioning. Overall, this study contributes to the growing awareness of microplastic pollution in freshwater ecosystems and underscores the importance of addressing this issue to safeguard amphibian biodiversity and ecosystem integrity.

Acknowledgements

The authors would like to acknowledge the following individuals and organizations for their contributions to this research. This research and innovation activity is funded by National Research Council of Thailand (NRCT) (N34E670115), and Centre of Excellence on Biodiversity (MHESI) (BDC-PG1-167003). We would like to thank S. Sirila for assistance in the field. We would like to acknowledge the support of Khon Kaen University (Faculty of Science) in providing us with resources and facilities necessary to conduct this research. We would also like to thank anonymous reviewers for their constructive feedback and critical review of the manuscript. We would also like to acknowledge the contribution of all the study participants who made this research possible. Finally, we would like to express our gratitude to all those who have supported us throughout the course of this research.

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