

Variability in microplastic color preference and intake among selected marine and freshwater fish and crustaceans

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Abstract

Plastic pollution is considered a pervasive global environmental challenge. Colored microplastics are prevalent in aquatic environments and the gastrointestinal tracts of wild-captured fish and crustaceans. Fish are thought to employ color vision to detect microplastics, prompting our investigation into microplastic ingestion patterns in three marine fish species, *Chrysiptera cyanea*, *Hypoatherina tsurugae*, and *Plotosus japonicus*, and three freshwater fish species, *Rhodeus ocellatus*, *Pseudorasbora parva*, and *Misgurnus anguillicaudatus*. Microplastics in red, blue, yellow, green, and gray were introduced to these species, and their ingestion was observed over a 4 h period under light conditions. Additionally, as crustaceans play an important role in connecting producers (phytoplankton) and consumers (fish) in natural ecosystems, we assessed a freshwater crustacean, *Neocaridina denticulata*. The crustacean did not ingest microplastics, and only 14% of *P. parva* individuals ingested microplastics (a proportion lower than that observed in the other fish species). Notably, *C. cyanea*, *P. japonicus*, and *R. ocellatus* exhibited color preferences in microplastic ingestion, with *C. cyanea* favoring red, *P. japonicus* preferring blue and gray, and *R. ocellatus* favoring red and yellow. Conversely, *H. tsurugae*, *P. parva*, and *M. anguillicaudatus* did not exhibit such color preferences. These findings suggest that certain fish use color vision to recognize and selectively ingest microplastics, with color preference being species-specific. Overall, this study provides new insights into microplastic ingestion by fish, potentially informing future toxicological assessments of microplastics in marine and freshwater fish and crustaceans intendant consumption.

Keywords Plastic pollution · Colored microplastic · Microplastic ingestion · Fish · Gastrointestinal tract

1 Introduction

Plastic pollution has become a severe global environmental issue [1, 2]. In particular, microplastics, defined as plastics < 5 mm in size, pollute diverse ecosystems, including rivers [3], seas [4], sediments [5], and the atmosphere [6]. In water environments, microplastics may absorb harmful chemicals, leading to their potential ingestion by and

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accumulation in aquatic organisms [7]. Consequently, these substances, along with microplastics, may ascend the food chain as their consumers are themselves consumed by fish and seabirds [8]. Therefore, investigating the ecotoxicity of microplastics in aquatic organisms has become a pivotal research focus.

Microplastics found in freshwater and marine environments exhibit transparency or a spectrum of colors, including black, blue, gray, green, red, white, purple, and yellow [9, 10]. The prevalence of residual colored microplastics varies widely across sampling sites. For example, Curren and Yew Leong [11] observed green (40%), white (40%), and blue (20%) fragments in the coastal regions of Singapore. Similarly, Yu et al. reported [12] blue (42.7%), black (25.9%), and transparent (12.8%) microplastics, as well as other colors (18.6%), in the Southwestern South China Sea. Additionally, colored microplastics have been found in wild-captured fish [13–15], indicating their potential mistaken ingestion as food. Investigating whether fish mistakenly ingest colored microplastics can provide crucial insights into the misuse of these particles by aquatic organisms in natural environments. Therefore, in our research, we hypothesized that color influences the unintentional ingestion of microplastics by fish. Notably, we found that the clown anemonefish, *Amphiprion ocellaris*, recognizes the color of microplastics, preferably ingesting red, yellow, and green particles [16]. Furthermore, Ríos et al. [17] found that *Psilododon eigenmanniorum* predominately consumed yellow and blue microplastics while avoiding white fragments. Hence, a microplastic color preference appears to exist in certain fish species. Moreover, the unintentional ingestion of colored microplastics varies among fish species; for instance, clown anemonefish and zebrafish (*Danio rerio*) frequently ingest microplastics, whereas Japanese (*Oryzias latipes*) and Indian (*Oryzias melastigma*) medaka do not [16]. Thus, unintentional ingestion of colored microplastics may also be species-specific.

In the present study, we hypothesized that aquatic organisms exhibit a species-specific tendency to consume colored microplastics. We tested this hypothesis using various aquatic species (Fig. S1): three marine fish species, *Chrysiptera cyanea*, *Hypoatherina tsurugae*, and *Plotosus japonicus*; three freshwater fish species, *Rhodeus ocellatus*, *Pseudorasbora parva*, and *Misgurnus anguillicaudatus*; and a freshwater crustacean, *Neocaridina denticulata*. These species are prevalent in Japanese rivers and coastal areas, with details on their habitats and diets provided in Table S1 in the supplementary section. Initially, we compared microplastic ingestion rates among the seven study species. Subsequently, we aimed to determine whether these organisms exhibited a color preference. Finally, we compared the present results with previous findings to delineate trends in fish species unintentionally ingesting microplastics of various colors. The findings of this study will be useful for further exploring microplastic ecotoxicity in specific species.

2 Materials and methods

2.1 Test animals and colored microplastics

All animal experiments adhered to the relevant national guidelines (Act on Welfare and Management of Animals, Ministry of the Environment, Japan), and fish handling followed the animal care and use guidelines of Kobe University. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Research Center for Inland Sea, Kobe University (permission number: 2021–04), and the research was performed in accordance with ARRIVE guidelines.

All test animals were wild-collected at the collection sites shown in Table S1. The aquatic organisms used in this study inhabit surface or shallow water environments. After collection, they were acclimatized in a laboratory at Kobe University, with feeding behavior observed, in a 65 L glass tank (width, 60 cm; depth, 30 cm; height, 30 cm) under the following conditions: $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, a 16:8 h light:dark photoperiod, and (for marine fish) salinity at 33 ± 1 PSU. During the acclimatization period, the organisms were fed freshly hatched brine shrimp nauplii (*Artemia* spp.) three times daily. The sex and age of the organisms were unknown owing to their wild-caught nature and lack of sexual dimorphism. Total body size, wet body weight, and number of organisms (n) were, respectively, as follows (mean $\pm$ standard error; Fig. S1): 32.0 ± 4.0 mm and 541.9 ± 260.3 mg for *C. cyanea* ($n = 90$); 38.3 ± 5.3 mm and 312.6 ± 108.0 mg for *H. tsurugae* ($n = 63$); 59.0 ± 6.0 mm and $1,004.5 \pm 380.6$ mg for *P. japonicus* ($n = 59$); 38.2 ± 4.4 mm and 547.2 ± 196.4 mg for *R. ocellatus* ($n = 90$); 32.7 ± 4.9 mm and 245.8 ± 109.8 mg for *P. parva* ($n = 89$); 82.2 ± 12.4 mm and $2,158.6 \pm 854.5$ mg for *M. anguillicaudatus* ($n = 76$); and 18.9 ± 0.9 mm and 64.5 ± 11.0 mg for *N. denticulata* ($n = 95$).

Five different colored polyethylene microspheres (red, blue, yellow, green, and gray) were obtained from Cospheric (Santa Barbara, CA, USA). As reported by Okamoto et al. [16], the mean ($\pm$ standard error) diameters and

Table 1 Number and percentage of aquatic organisms that ingested microplastics

	Species	Number (percentage) of individuals ingesting microplastics
Marine fish	<i>Chrysiptera cyanea</i>	49 of 90 (54%)
	<i>Hypoatherina tsurugae</i>	33 of 63 (52%)
	<i>Plotosus japonicus</i>	22 of 59 (37%)
	<i>Rhodeus ocellatus</i>	60 of 90 (66%)
	<i>Pseudorasbora parva</i>	12 of 89 (14%)
	<i>Misgurnus anguillicaudatus</i>	38 of 76 (50%)
Freshwater crustacean	<i>Neocaridina denticulata</i>	0 of 95 (0%)

particle densities of each colored microplastic were, respectively, as follows: red: $219.2 \pm 22.6 \mu\text{m}$ and 0.98 g/cc ; blue: $279.0 \pm 17.0 \mu\text{m}$ and 1.00 g/cc ; yellow: $256.9 \pm 21.2 \mu\text{m}$ and 1.00 g/cc ; green: $253.6 \pm 20.4 \mu\text{m}$ and 0.98 g/cc ; and gray: $257.7 \pm 21.7 \mu\text{m}$ and 1.00 g/cc .

2.2 Microplastic ingestion test

The microplastic ingestion test, previously described by Okamoto et al. [16], involved a microplastic exposure concentration of 17.5 mg/L for each color (total concentration, 87.5 mg/L), determined based on maximum microplastic ingestion observed in a previous study [16]. Typically, seven aquatic organisms (discrepancies described below) were placed in each 5 L glass tank (width, 20 cm; depth, 20 cm; height, 20 cm), containing 4 L of freshwater or artificial marine water, 24 h before microplastic exposure. Test conditions were as follows: $25^\circ\text{C} \pm 2^\circ\text{C}$, light photoperiod, and (for marine fish) salinity at $33 \pm 1 \text{ PSU}$. Artificial marine water was produced using Marine ART Hi (Osaka Yakken Co. Ltd., Osaka, Japan).

Given their wild-collected nature, there were variations in the numbers of test organisms. Specifically, each tank contained seven aquatic organisms, with some discrepancies: 13 replicate tanks (12 tanks containing 7 fish and 1 tank containing 6 fish) for *C. cyanea*; 9 replicate tanks (each containing 7 fish) for *H. tsurugae*; 9 replicate tanks (8 tanks containing 7 fish and 1 tank containing 3 fish) for *P. japonicus*; 13 replicate tanks (12 tanks containing 7 fish and 1 tank containing 6 fish) for *R. ocellatus*; 13 replicate tanks (12 tanks containing 7 fish and 1 tank containing 5 fish) for *P. parva*; 11 replicate tanks (10 tanks containing 7 fish and 1 tank containing 6 fish) for *M. anguillicaudatus*; and 14 replicate tanks (13 tanks containing 7 crustaceans and 1 tank containing 4 crustaceans) for *N. denticulate*.

To ensure gastrointestinal tract emptiness after transfer to the 5 L glass tanks, no feed was provided to the aquatic organisms for 24 h. Subsequently, they were exposed to a mixture of five different colored microplastics at a total concentration of 87.5 mg/L for 4 h (until feeding behavior ceased) under light conditions. Exposure time was determined based on the feeding behavior duration observed in a previous study (Okamoto et al. [16]). During exposure, mixture of five different colored microplastics were circulated by aeration. After 4 h of microplastic exposure, the aquatic organisms were anesthetized using 200 mg/L MS-222, and their gastrointestinal tracts were dissected. Microplastic color and number in the gastrointestinal tract were assessed under a stereomicroscope (SZ61, Olympus).

2.3 Statistical analysis

All data were analyzed in Microsoft Excel. To assess the color preference of each fish species, the open-source statistical software R (<http://www.R-project.org/>) and Steel–Dwass multiple comparison tests (5% significance level) were used.

3 Results and Discussion

3.1 Microplastic intake of the seven test species

Table 1 shows the percentage of individuals that ingested microplastics: *C. cyanea*, 54% (49 of 90) (Fig. 1B); *H. tsurugae*, 52% (33 of 63) (Fig. 2B); *P. japonicus*, 37% (22 of 59) (Fig. 3B); *R. ocellatus*, 66% (60 of 90) (Fig. 4B); *P. parva*, 14% (12 of 89) (Fig. 5B); *M. anguillicaudatus*, 50% (38 of 76) (Fig. 6B); and *N. denticulata*, 0% (0 of 95). Thus, *P. parva* and *N. denticulata* exhibited a low likelihood of microplastic ingestion compared with the other species.

The mean ($\pm$ standard deviation) and median number of microplastics ingested in each species were, respectively, as follows (Table 2): 99 ± 217 and 6 for *C. cyanea*; 39 ± 87 and 11 for *H. tsurugae*; 20 ± 42 and 4 for *P. japonicus*; 19 ± 53 and 3 for *R. ocellatus*; 11 ± 24 and 2.5 for *P. parva*; and 13 ± 52 and 3 for *M. anguillicaudatus*.

Fig. 1 **A** Colored microplastic particles observed in the gastrointestinal tract in *Chrysiptera cyanea*. **B** The number of microplastic particles observed in the gastrointestinal tract according to body length. **C** Box plot of the number of microplastic particles of various colors observed in the gastrointestinal tracts of *Chrysiptera cyanea*. *Values that are significantly different (Steel–Dwass multiple-comparison tests; * $P < 0.05$)

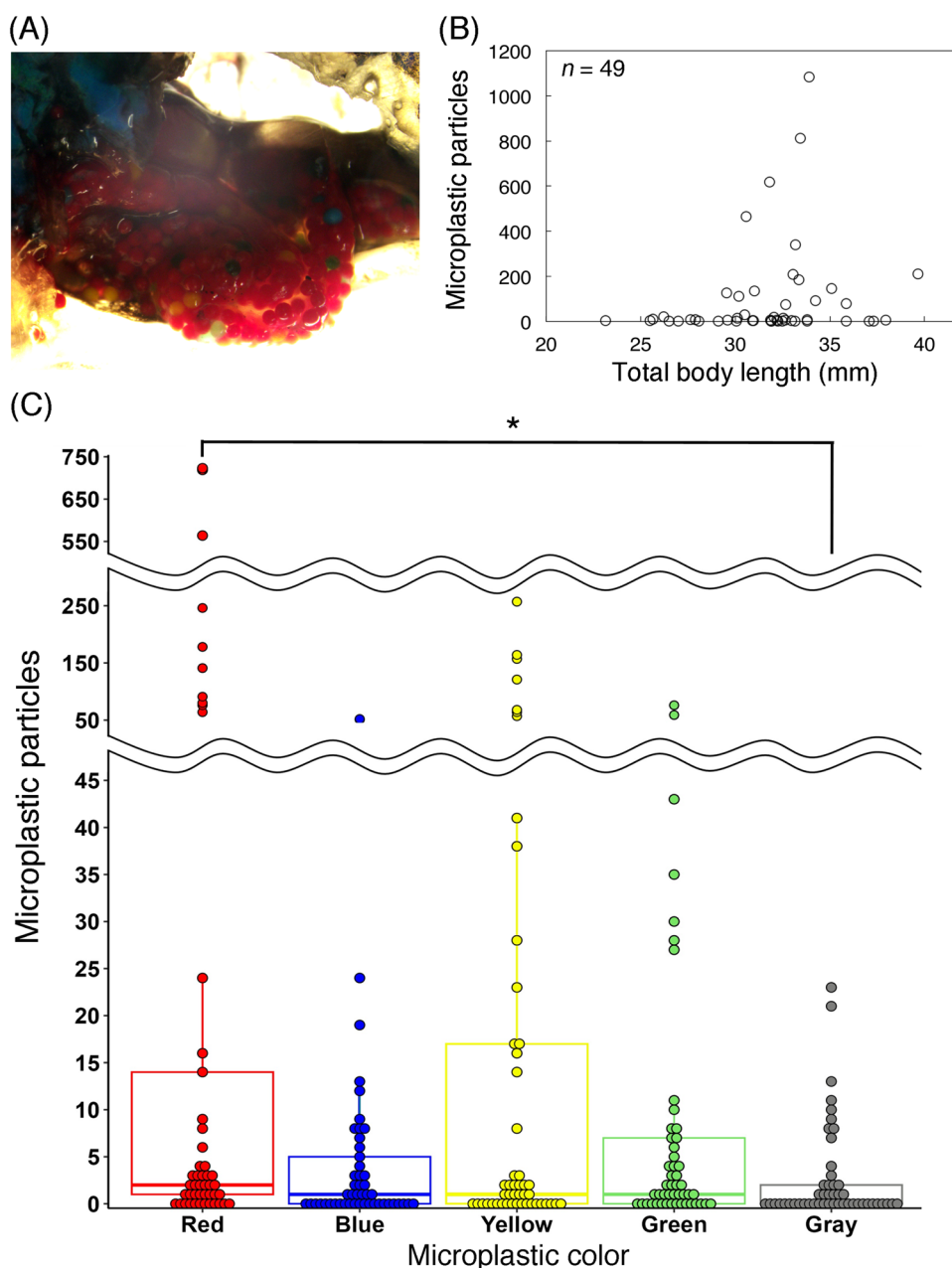
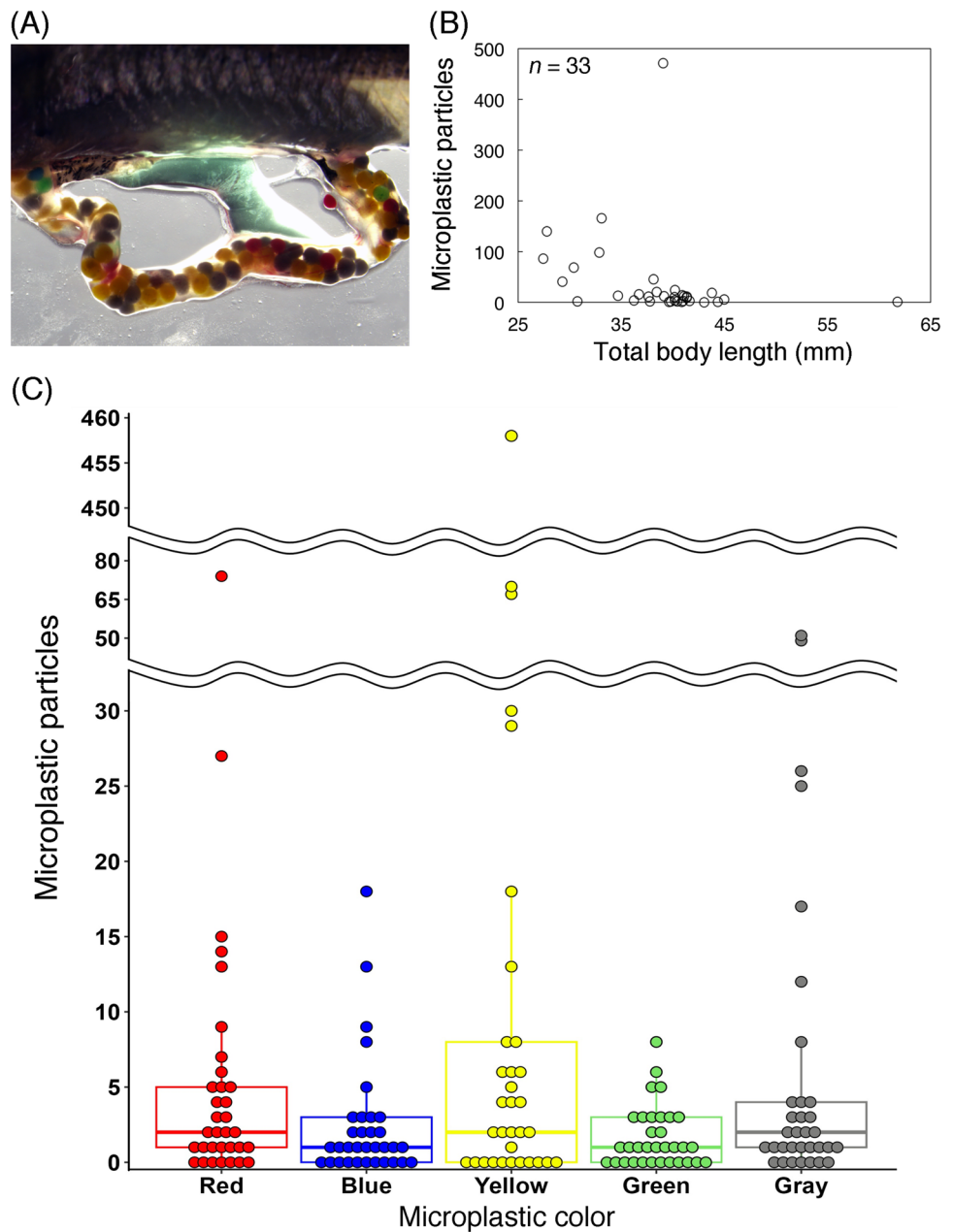


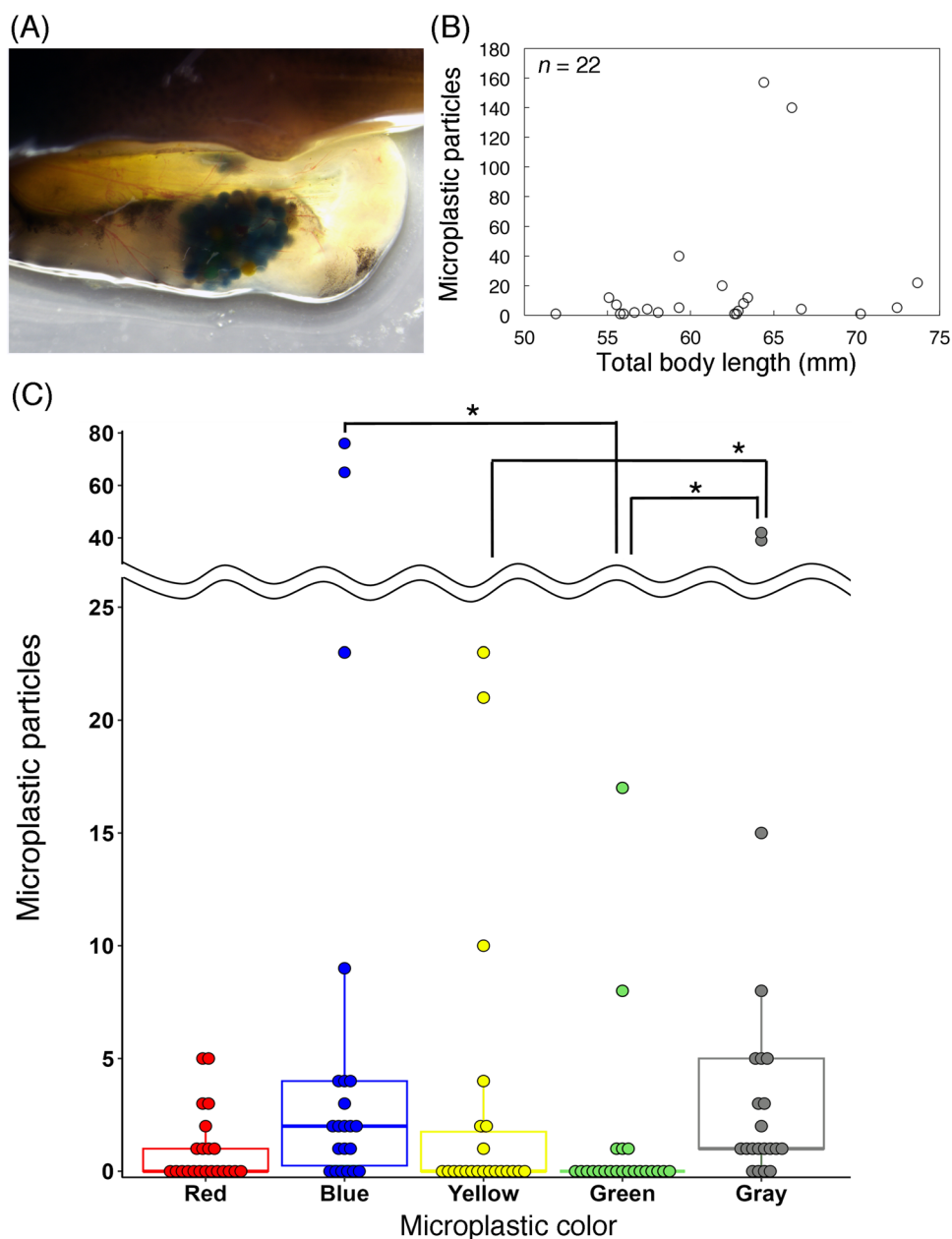
Fig. 2 **A** Colored microplastic particles observed in the gastrointestinal tract in *Hypoatherina tsurugae*. **B** The number of microplastic particles observed in the gastrointestinal tract according to body length. **C** Box plot of the number of microplastic particles of various colors observed in the gastrointestinal tracts of *Hypoatherina tsurugae*



Several studies have identified microplastics in the gastrointestinal tracts of wild fish. For example, the percentages of wild marine fish with microplastics in their gastrointestinal tracts were as follows: 47% of *Sardina pilchardus* (17 of 36 fish), 42% of *Pagellus erythrinus* (8 of 19 fish), and 32% of *Mullus barbatus* (8 of 25 fish) [18]; 30% of *Scomber scombrus* (3 of 10 fish) and 35% of *Merluccius merluccius* (7 of 20 fish) [19]; 21% of *Sardinella gibbosa* (68 of 321 fish) and 14% of *Leiognathus lineolatus* (50 of 356 fish) [20]; and 67% of *Chirocentrus dorab* (12 of 18 fish), 93% of *Drepane longimana* (13 of 14 fish), and 100% of *Drepane punctata* (3 of 3 fish) [21]. Under laboratory conditions, 100% of *Engraulis japonicus* (108 of 108 fish) individuals ingested microplastics [22], whereas 21% of *O. latipes* (15 of 70 fish), 12% of *O. melastigma* (9 of 70 fish), 87% of *D. rerio* (61 of 70 fish), and 88% of *A. ocellaris* (62 of 70 fish) [16] individuals ingested microplastics. These results and the present findings suggest that the rate of unintentional microplastic ingestion does not markedly differ between wild fish and those under laboratory conditions, although the rate varies by species.

In previous studies, the mean ($\pm$ standard deviation) and the median number of microplastics (diameter: 250–300 μ m) ingested by various species under laboratory conditions were, respectively, as follows: 19.8 ± 14.1 and 7.5 for *Fundulus heteroclitus* and 52.3 ± 23.1 and 32.0 for *Pagrus major* [23]; 31.2 ± 9.1 and 27.7 for *E. japonicus* [22]; and 13.0 ± 20.8 and

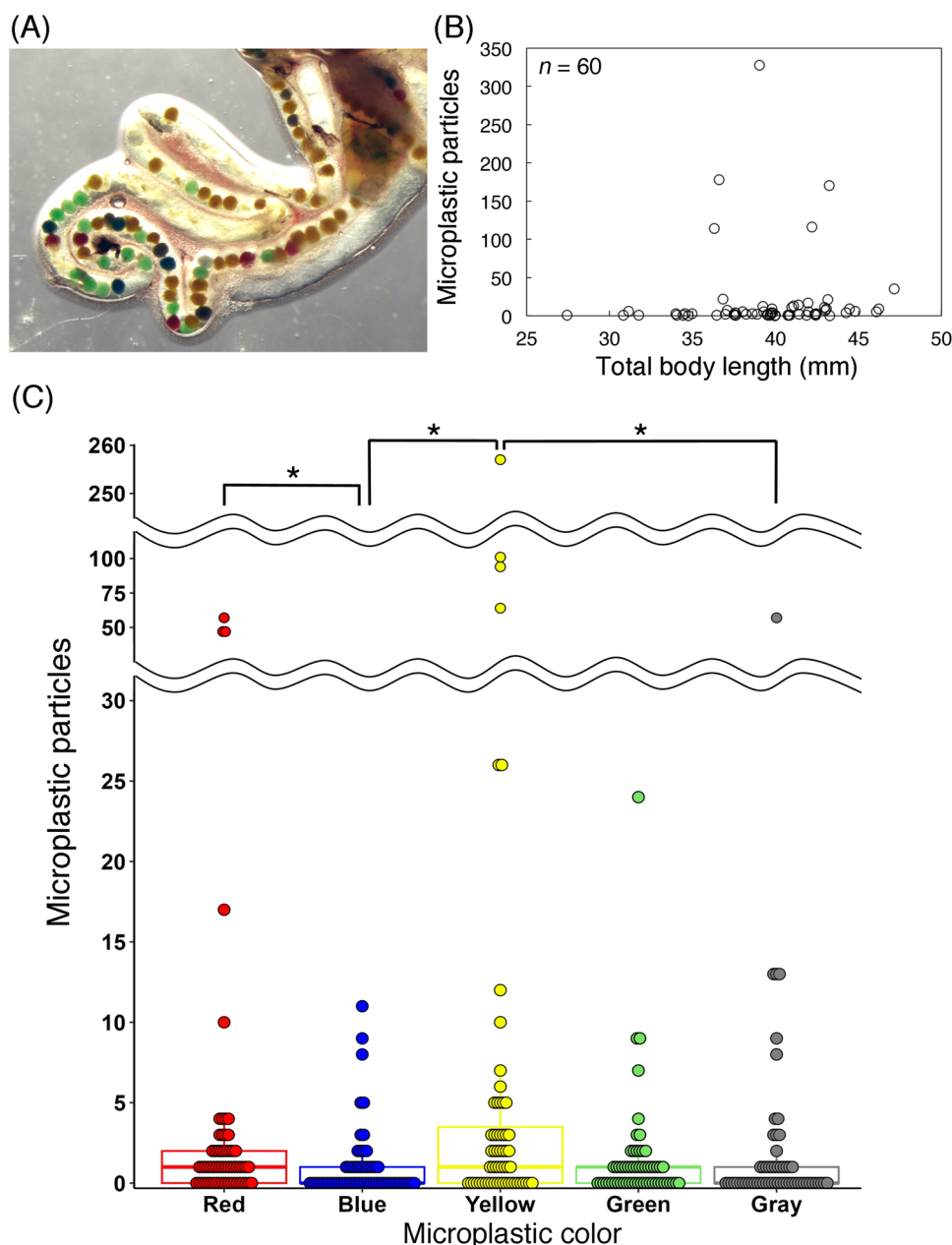
Fig. 3 **A** Colored microplastic particles observed in the gastrointestinal tract in *Plotosus japonicus*. **B** The number of microplastic particles observed in the gastrointestinal tract according to body length. **C** Box plot of the number of microplastic particles of various colors observed in the gastrointestinal tracts of *Plotosus japonicus*. *Values that are significantly different (Steel–Dwass multiple-comparison tests; * $P < 0.05$)



3.0 for *O. latipes*, 10.4 ± 12.1 and 6.0 for *O. melastigma*, 21.5 ± 14.8 and 23.5 for *D. rerio*, and 245 ± 196.4 and 208.5 for *A. ocellaris* [16]. Although microplastic exposure concentrations varied in each study, a comparison of median values in these studies and the present study suggests that *A. ocellaris* followed by *P. major*, *E. japonicus*, and *D. rerio* tend to readily ingest microplastics. However, whether species ingest microplastics does not appear to be taxonomically dependent (i.e., at the order or family level), as *A. ocellaris* and *C. cyanea*, closely related species, exhibited markedly different median microplastic ingestion numbers. Additionally, considering that *D. rerio* is a freshwater fish and *P. major* and *E. japonicus* are marine fish, microplastic ingestion does not seem to depend on habitat, i.e., marine vs. freshwater habitats. Therefore, further investigation is required to determine the factors influencing microplastic ingestion and avoidance.

Crustaceans play a crucial role in connecting producers (phytoplankton) and consumers (fish) in natural ecosystems. The shrimp used in the present study are also consumed by fish. Some species of crustaceans can fragment microplastics. For example, Mateos-Cárdenas et al. [24] found that the freshwater amphipod *Gammarus duebeni* rapidly fragments polyethylene microplastics, thereby forming nanoplastics. Cau et al. [25] revealed that the stomach of *Nephrops norvegicus* serves as a size bottleneck for ingested microplastics, promoting their fragmentation into smaller plastic debris, which is subsequently released into the intestine. In the present study, *N. denticulata* did not feed on microplastics.

Fig. 4 **A** Colored microplastic particles observed in the gastrointestinal tract in *Rhodeus ocellatus*. **B** The number of microplastic particles observed in the gastrointestinal tract according to body length. **C** Box plot of the number of microplastic particles of various colors observed in the gastrointestinal tracts of *Rhodeus ocellatus*. *Values that are significantly different (Steel–Dwass multiple-comparison tests; * $P < 0.05$)



3.2 Microplastic color preference in six aquatic species

The number of microplastics ingested by individual fish in each species are shown in Fig. S2, Fig. S3, Fig. S4, Fig. S5, Fig. S6 and Fig. S7 in the supplementary section. Results of Steel–Dwass multiple comparison tests (5% significance level) revealed that *C. cyanea*, *P. japonicus*, and *R. ocellatus* exhibited significant microplastic color preferences (Figs. 1C, 3C, and 4C). Contrastingly, *H. tsurugae*, *P. parva*, and *M. anguillicaudatus* showed no color preferences (Figs. 2C, 5C, and 6C). In *C. cyanea*, significantly more red microplastics were ingested compared with gray microplastics (Fig. 1C). In *P. japonicus*, significantly more blue and gray microplastics were ingested compared with green microplastics, and significantly more gray microplastics were ingested compared with yellow microplastics (Fig. 3C). In *R. ocellatus*, significantly more red and yellow microplastics were ingested compared with green and blue microplastics, and significantly more yellow microplastics were ingested compared with gray microplastics (Fig. 4C). Although not statistically significant, it was also found that more red microplastics were ingested compared with gray microplastics ($p = 0.07$; Fig. 4C).

Figure 1 consists of three panels. Panel (A) is a photograph of a juvenile rainbow trout, showing its internal organs and a cluster of colorful microplastic particles in its gut. Panel (B) is a scatter plot showing the number of microplastic particles (y-axis, 0 to 100) versus total body length in mm (x-axis, 25 to 45) for $n = 12$ fish. The data points are open circles. Panel (C) is a box plot showing the number of microplastic particles (y-axis, 0 to 40) by color (x-axis: Red, Blue, Yellow, Green, Gray). The boxes represent the interquartile range, the horizontal line inside the box is the median, and the whiskers extend to the minimum and maximum values. Outliers are shown as individual points.

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Fig. 6 **A** Colored microplastic particles observed in the gastrointestinal tract in *Misgurnus anguillicaudatus*. **B** The number of microplastic particles observed in the gastrointestinal tract according to body length. **C** Box plot of the number of microplastic particles of various colors observed in the gastrointestinal tracts of *Misgurnus anguillicaudatus*

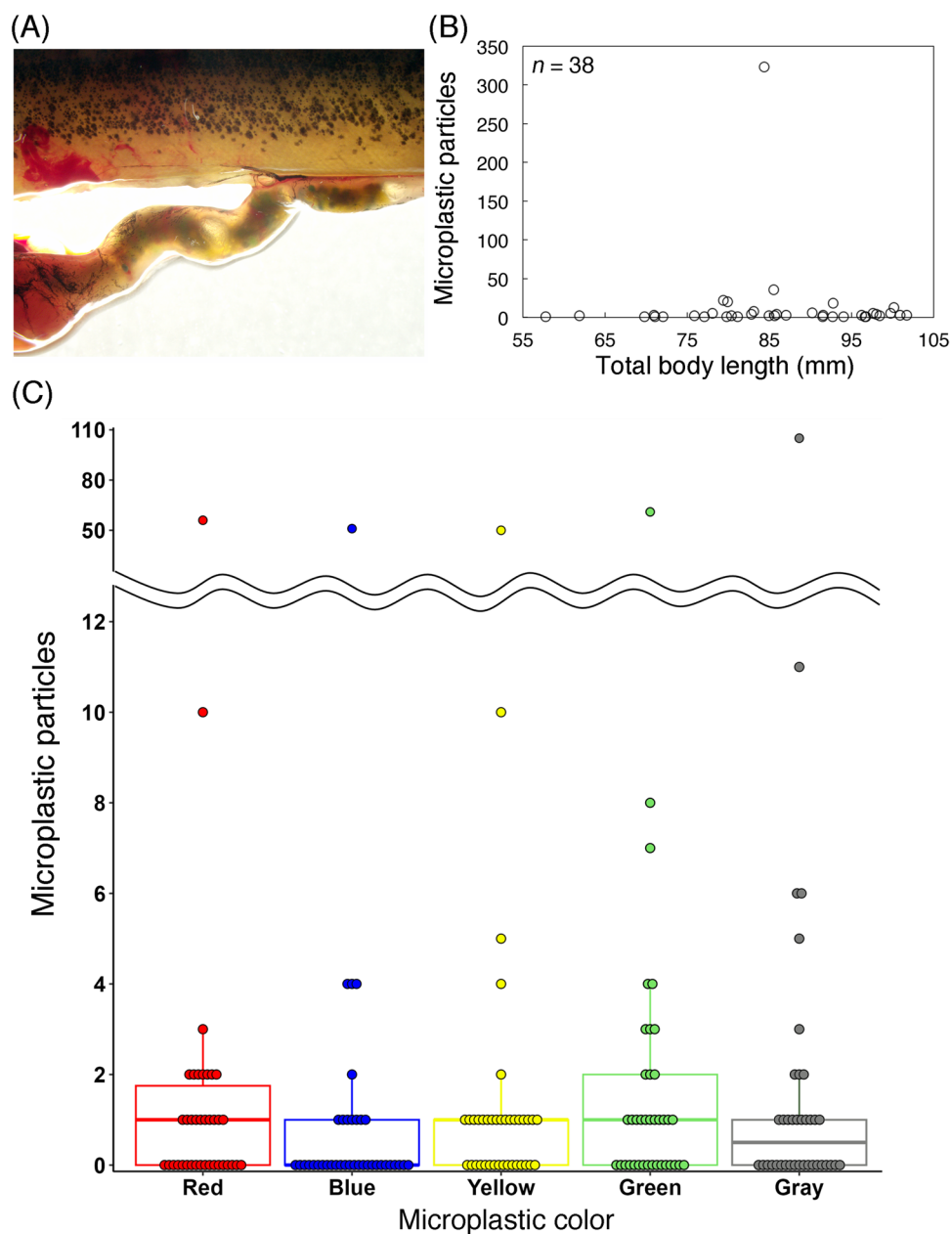


Table 2 The mean ($\pm$ standard deviation) and the median number of microplastics ingested in each species

	Species	The mean ($\pm$ standard deviation) of number of microplastics ingested	The median number of microplastics ingested
Marine fish	<i>Chrysiptera cyanea</i>	99 $\pm$ 217	6
	<i>Hypoatherina tsurugae</i>	39 $\pm$ 87	11
	<i>Plotosus japonicus</i>	20 $\pm$ 42	4
Freshwater fish	<i>Rhodeus ocellatus</i>	19 $\pm$ 53	3
	<i>Pseudorasbora parva</i>	11 $\pm$ 24	2.5
	<i>Misgurnus anguillicaudatus</i>	13 $\pm$ 52	3

color preferences, although *H. tsurugae*, *P. parva*, and *M. anguillicaudatus* showed no such preferences. These results indicate that microplastic color preference is species-specific.

Typically, vertebrates possess cone cells and rod cells, with the former responsible for color vision, functioning effectively under bright conditions, and the latter (e.g., rod opsin or RH1) responsible for scotopic vision. Bony fish possess four types of cone cells, namely LWS, RH2, SWS2, and SWS1, which sense red, green, blue, and ultraviolet light, respectively [32–34]. In the NCBI database (<https://www.ncbi.nlm.nih.gov/>), LWS (KX766132), RH1 (KX766117), RH2 (RH2A: KX766102; RH2B: KX766087), SWS2B (KX766057), and SWS1 (KX766072) are listed for *C. cyanea*; LWS (EU919549), RH2 (EU919550), SWS2 (EU919552), and SWS1 (EU919551) for *P. parva*; and RH1 (MT544361) for *M. anguillicaudatus*. Thus, *C. cyanea* and *P. parva* can visually distinguish colors. Although information on *P. japonicus* and *R. ocellatus* cone cells is lacking, their relatively high rates of blue and yellow microplastic ingestion, respectively, suggest possible color vision. *Amphiprion ocellaris* possesses four types of cone cells (LWS, RH2, SWS2, and SWS1) [35] and visually distinguishes red, yellow, and green microplastics [16]. Future studies should aim to elucidate the determinants of microplastic color preference in fish, which may include diet, habitat, or ecosystem roles. Furthermore, within species, individuals exhibiting substantial variation in microplastic ingestion (> 100 pieces) warrant further investigation. Such studies will contribute to understanding the factors influencing unintentional ingestion of microplastics by aquatic organisms.

4 Conclusion

Microplastics with diverse colors are present in natural environments, underscoring the importance of investigating the unintentional ingestion of colored microplastics by aquatic organisms. To the best of our knowledge, this study is the first to reveal that the color of microplastics ingested by fish under laboratory conditions varies by species. Specifically, *C. cyanea*, *P. japonicus*, and *R. ocellatus* exhibited significant color preferences regarding microplastic ingestion. Overall, these findings offer new insights into the unintentional ingestion of microplastics by fish. However, the factors contributing to color preferences among fish species and the substantial individual variations in the number of unintentional ingestions remain unclear. Consequently, further research is essential to fill these knowledge gaps.

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Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by [YH], [KM], and [DU]. Statistical analysis was performed by [KY]. Visualization was performed by [SH]. The first draft of the manuscript was written by [YH], [JMR], and [CKY]. Reviewing and Editing were performed by [HO] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The authors confirm that all data underlying the findings are fully available without restriction.

Code availability Not applicable.

Declarations

Ethics approval The fish which was used in the present study were handled according to guidelines of Kobe University.

Consent to participate This research did not involve human subjects, so clinical trial registration is not applicable.

Consent for publication The authors certify that this manuscript is our original unpublished work, has not been published elsewhere, and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission.

Competing interests The authors declare no competing interests.

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