



Article Impaired Growth Performance of Wami Tilapia Juveniles (Oreochromis urolepis) (Norman, 1922) Due to Microplastic Induced Degeneration of the Small Intestine

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Abstract: Microplastics-induced histopathological changes in gastrointestinal tracts of fish have been widely reported. However, the translation of adverse effects in the gut to impacts on growth are understudied. This study investigated the effect of MP-induced small intestinal histomorphological changes on growth performance of Oreochromis urolepis juveniles. Sixty larvae were exposed in control (0), 1, 10, and 100 polyethylene microplastic particles (PE MPs)/mL treatment groups. On day 65, juveniles were euthanized, dissected, and biometric data were taken. Small intestine histomorphological lesion index (HLI) was calculated following histological preparation using routine hematoxylin and eosin procedure. Results showed increase in HLI proportional to PE MPs exposure dose. These deteriorations equally reduced growth in final weight, weight gain and total length (One-Way ANOVA, p > 0.05), and Specific Growth Rate (SGR) (Kruskal–Wallis Test, p > 0.05), though there were insignificant differences between treatment groups. Condition factors of fishes in control and 1 PE MPs differed significantly and with other treatment groups (Tukey HSD, p < 0.05). Small intestines HLI correlated significantly with growth pattern (Spearman, r = 1.00, p = 0.01), condition factors (Pearson, r = -0.995, p < 0.05), final weight, weight gain, and total length (Spearman, r = -1.00, p = 0.01) but not with SGR. The allometric growth pattern changed towards isometric corresponding to increasing HLI. These findings suggest that MPs damaged small intestine structure and thus impaired digestion and nutrients absorption functions which disrupted growth. Such effects may impair juveniles' ability to escape enemies, find food, and eventually reproduce, and therefore require further study.

Keywords: microplastics; histomorphological lesion indices; growth performance; length-weight relationship; *Oreochromis urolepis*; physical parameters; ingestion

1. Introduction

The ubiquitous presence of plastic pollution in both marine and freshwater aquatic environments has been intensely researched with regards to their biotic interactions [1,2]. Water birds, invertebrates, gastropods, and fish are commonly found to contain microplastics (MPs, denoted as plastic < 5 mm) in their feces [3] and gastrointestinal tracts [4,5]. Such ubiquity reduces food accessibility and [6] and jeopardizes fish welfare [7]. Various



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). studies reveal that the ingestion of MPs by fish [8] causes not only false satiation but also histopathological impairment with subsequent effects on physiological status [9–13].

Fish in the wild are vulnerable to MP ingestion and retention at all life stages from larvae [14] to adulthood [5], regardless of whether they are pelagic or benthic. The majority of MPs are less dense than water, but may flocculate or biofoul to become denser and sink to the bottom, making them available in all habitats across pelagic and benthic profiles [15,16]. Most fish undergo a feeding shift from filter feeders [17] to herbivores, carnivores, or omnivores [5,18] as exhibited by *Oreochromis urolepis* [19], which increase their susceptibility to MP intake. Therefore, fish can unintentionally ingest MPs by mistaking it for food or through the food chain by feeding on individuals in lower trophic level that contain MPs [20–22].

Fish growth is an aspect of interest in aquaculture and fisheries management due to its importance to yield and population size structure. Growth is influenced by intrinsic and extrinsic factors (e.g., food, oxygen, temperature, pH, pollution). Of these factors, food availability and assimilation is the most imperative factor influencing fish growth [23]. Fish exhibit a high growth rate when changing from larvae to juveniles which last until they reach maturity [24,25]. Attainment of desired size and weight is essential in escaping predators, searching for food, and fecundity size to ensure recruitment of adults and reproduction performance [26]. To compensate, fish take up a substantial amount of food from their habitats through well-developed feeding modes to sustain their needs [20]. Such demand coupled with the availability of plastics facilitate MPs intake. Studies on retrieval of MPs from gastrointestinal tracts [5,8,27] and their damage are under broad scrutiny. However, the information of the effect of MPs ingestion on fish growth is limited both in Africa and globally.

The effects of MPs in fish guts are relatively well studied. Physical damage in gastrointestinal tracts have been widely reported on various fish species [13,28,29]. *O. niloticus* exposed to a various dose of MPs for 15 d revealed proportional degeneration of a number of intestinal cells [28]. *Girella laevifrons* treated with MPs for 45 d showed severe inflammation and regressive change to intestinal tissues. Like most histopathological studies, the aforementioned studies did not investigate growth as an endpoint. Moreover, the few studies elucidating the influence of MPs to growth have not been linked with a histopathological aspect. For example, *Cyprinus carpio* larvae exposed to polyvinyl chloride MPs for 60 days revealed inhibition of growth and oxidative stress [30].

Our previous study investigated the ingestion of fluorescent green polyethylene microsphere (denoted as 'PE MPs') by Wami Tilapia (*Oreochromis urolepis*) (Norman, 1922) and their histomorphological impact after exposure and depuration phases [13]. We found that small intestine histological tissues were damaged, and the effect persisted even after the depuration period. Owing to the digestive and absorption functions of small intestines, their damage may lead to malnutrition and stress that are likely to impair physiology of fish including growth. However, studies coupling histopathological effects of MP exposure to fish growth are scarce.

Following our previous research [13], the present study aimed to investigate the association between MPs-induced degeneration of small intestine with growth impairment of *Oreochromis urolepis* [31]. Fish were chronically exposed (65 days) to concentrations of 1, 10, and 100 PE MPs/mL and whilst the highest concentration exceeds environmental relevance, such high doses are commonly employed within experimental studies to determine the potential effects and mechanisms of MP exposure to aquatic biota [32–34]. Similarly, whilst it is also established that pristine microbeads lack environmental relevance, again the use of a standard MP type is well described in the ecotoxicological literature [32–34]. It is hypothesized that chronic exposure to PE MPs would cause intestinal damage of *Oreochromis urolepis* growing from larvae to juveniles, leading to nutritional deficit and an eventual effect on fish growth performance.

2. Materials and Methods

2.1. Fish Larvae (Fries) Production

Adult *O. urolepis* Eccles [31] fish were introduced into concrete tanks of approximately 6.7 m³ filled to 80% of their volume with freshwater at the University of Dar es Salaam Kunduchi campus. The 24 h acclimatized fish were fed twice a day with commercial feeds (De Heus, Lot A4, Vinh Long province, Vietnam) equal to 5% of body weight until fertilization. The fertilized eggs were then transferred into an indoor hatchery. Five days post-hatching larvae (fries) were randomly distributed between 12 aerated 100 L aquaria having 40 L filtered freshwater at a photoperiod cycle of 16: 8 h light and dark.

2.2. PE MPs Exposure Dose Preparation

The PE MPs with a density of 1.00 g/cc (product ID of UVPMS-BG) and size range 38–45 μ m were purchased from Cospheric LLC, Santa Barbara, CA 93160, USA, order # 117,025, and were similar to those used by Mbugani et al. [13]. Tween 80 surfactants (product number: P8074) were supplied by Sigma-Aldrich (Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA).

The 1, 10, and 100 PE MPs/mL treatments were prepared in triplicates equivalent to 0.0031 g, 0.0308 g, and 0.3077 g of PE MPs in 40 L of filtered and dechlorinated freshwater respectively. Each suspension was prepared first by adding a given concentration of PE MPs in 20 mL from stock solution made by mixing 0.5 mL (0.1%) Tween 80 Bio-compatible surfactant with 500 mL of hot distilled water, thoroughly mixed by shaking with WiseShaker Model SHR-2D at 150 rpm for a duration of an hour. The resulting suspension was poured into the aquaria of the respective treatment group when the fish larvae were introduced.

2.3. Experimental Exposure Set Up

Twenty fish larvae (fries) weighing approximately 0.015 ± 0.004 g to 0.017 ± 0.003 g were stocked in each triplicate aquaria of control (no MPs), 1, 10, and 100 PE MPs/mL treatment groups. Initially, fries were fed with powdered feed equal to 10% of body weight in three portions per day which changed according to fish growth size, similar to Mbugani et al. [13]. In the last month, fish were fed 5% of body weight juvenile pellets (protein content 40%). Eighty percent of water was renewed and, except control, immediately spiked by the respective dose of PE MPs in every two days. All aquaria were aerated to enhance oxygen concentration in water. Water temperature and pH were measured twice a day while oxygen twice a week. Ammonia concentrations were measured once a week.

2.4. Examination of PE MPs Ingestion and Determination of Growth Performance

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On day 65 of exposure, 12 h past the last feeding, juvenile fish were introduced into a 2 L beaker filled three-quarters with distilled water, euthanized with four drops of clove oil, weighed, and their total length measured. Some growth performances were determined from the following equations:

The length–weight relationship equation (Equation (1)) as used by Maganira et al. [25]:

$$V = aL^b \tag{1}$$

when log transformed is expressed as (Equation (2))

$$LogW = Loga + bLogL$$
(2)

where the value of 'b' is 3 for isometric growth pattern fish and <3 or >3 for those exhibiting allometric growth patterns. It is advised to calculate the value of 'b' because 3 is not uniform across fish age class and for the majority of fish species [25]. The 'b' value of each treatment group was substituted to calculate condition factors (indices) of fish according to Fulton's equation (Equation (3)):

$$K = \frac{100Wf}{L^b}$$
(3)

Specific Growth Rate (SGR) is calculated according to Crane et al. [35] (Equation (4)):

$$SGR\left(\% \, day^{-1}\right) = (e^g - 1) \times 100 \tag{4}$$

where: Wf = Final weight of fish (g); Wi = initial weight of fish; L = Total final length of fish (cm); a = Rate of change of weight with length (intercept); b = Weight per unit length (slope) or growth pattern; K = Condition factor; T = duration of PEs exposure period; and g = instantaneous growth rate (Equation (5)).

$$e^{g} = \left(\frac{Wf}{Wi}\right)^{\frac{1}{T}}$$
(5)

Weight gain = Wf - Wi (6)

For PE MPs ingestion examination, half of the small intestine (estimated duodenum to jujenum) were introduced into a Sedgewick rafter and observed under fluorescent microscope using 485 nm light filter. Another section of small intestine (estimated part of jujenum and ileum) of approximately 3 cm was digested in 10 mL of KOH in a water bath according to Karami et al. [36] with modifications on the incubation period of 72 h. The average amount of MPs were determined by counting from triplicate volumes of digest.

2.5. Small Intestine Histomorphological Lesion Indices Diagnosis

The small intestine tissue sections from jujenum and ileum were collected and immediately fixed in 20 mL of 10% buffered formaldehyde for 48 h. Thereafter, the tissues were processed and stained with hematoxylin and eosin following a standard procedure [13]. The small intestine histomorphological lesions indices were obtained after evaluation of villi, epithelial cells, cryptic gland and goblets cells damages, leucocyte infiltration, and blood congestion according to Bernet et al. [37]. Each lesion was assigned an importance factor and the degree of observed damage was scored. The reaction indices were obtained by multiplying the importance factors and score values. The degree of damage of an organ (small intestine) was represented by the organ index, which was the sum of its reaction indices.

2.6. Data Analysis

Data were checked for normality using Shapiro–Wilk Test and homoscedacity with Levene Test, and non-parametric data were log transformed. One-Way ANOVA was used to compare means between treatments, followed by Tukey HSD post-hoc Test. Where parametric conditions were unfulfilled even after log-transformation of data, Kruskal–Wallis test was applied followed by Dunn test. Chi-square Goodness-of-fit test tested whether the occurrence of histomorphological lesion indices between treatment groups was significantly different. The association of small intestinal histomorphological lesion indices with growth performance were determined by Correlation test. In all cases, data were considered significant different when *p*-value ≤ 0.05 . All statistical data analyses were performed using IBM SPSS Statistics version 23 for Windows. The graphs were drawn using Python version 3.10.

3. Results

3.1. Water Quality Parameters

All water parameters remained within the optimal range for *O. urolepis* growth. The means of dissolved oxygen and temperature between treatment groups were not significant different (Table 1). The mean pH values varied significantly between control and 10 MP PE/mL, as well as control and 100 MP PE/mL treatment groups (One-Way ANOVA, Tukey HSD, p < 0.05). Dissolved ammonia was fluctuating but did not exceed 0.176 mg/L as prevented by regular two-day interval water renewal.

Water	Treatment Groups					
Parameters	Control	1 PE MPs/mL	10 PE MPs/mL	100 PE MPs/mL	<i>p</i> –Value	
Temperature	29.92 ± 1.41	28.66 ± 1.54	28.90 ± 1.25	28.84 ± 1.25	0.75	
Dissolved	5.53 ± 2.66	6.64 ± 2.83	4.56 ± 2.81	5.12 ± 2.31	0.59 ^a	
pH	7.78 ± 0.23	7.70 ± 0.22	7.66 ± 0.20	7.67 ± 0.19	0.016 ^a	

Table 1. One–Way ANOVA (*p*–value with superscript "a") and Kruskal–Wallis test for water quality parameters between treatment groups.

3.2. PE MPs Ingestion and Small Intestines Histomorphological Alteration

No PE MPs were found in small intestines of *O. urolepis* juveniles in the control group. A progressive increase was observed in fish juveniles proportional to PE MPs exposure dose (Figure 1).



Figure 1. PE MPs particles in the small intestines of *O. urolepis* juveniles. (**A**): Control (no PE MPs); (**B**): 1 PE MPs/mL; (**C**): 10 PE MPs/mL; (**D**): 100 PE MPs/mL. Scale bar = 100 μm at magnification of 40.

The occurrence of histomorphological lesion indices in the small intestine of fish significantly increased with PE MPs dose ($\chi^2 = 44.38$; df = 2; *p* < 0.05). The detailed lesions are summarized in Table 2 and Figure 2.

Table 2. Evaluation of histomorphological changes in small intestines of *O. urolepis*. The organ indices indicate sum of all reaction/alteration indices in a particular treatment.

Histomorphological	Reaction/Alteration Pattern Index				
Alteration	Control	1 PE MPS/mL	10 PE MPs/mL	100 PE MPs/mL	
Villi (height and width)	0	1	4	6	
Epithelial cells (height)	0	2	4	6	
Cryptic and goblet cells	0	1	4	6	
Leucocytic infiltration	2	4	8	12	
Blood congestion	0	4	8	12	
Organ indices	2	12	28	42	



Figure 2. Histomorphological lesion indices of small intestine of *O. urolepis* juveniles at different PE MPs concentration. Note an increased dose dependent damage.

3.3. Effect of Histomorphological Damage on Growth Performance

All data for total length, final weight, weight gain, and condition factors conformed to normality and homoscedasticity conditions (p > 0.05) except SGR. The R² values indicated a strong relationship between fish weight and length which increased with PE MP exposure dose (Figure 3 and Table 3). In comparison, growth patterns and the means of final weights, weight gain, total lengths, SGR, and condition factors of fish juveniles varied between treatment groups (Table 3). The measured means of total lengths size, final weights, and weight gain of fishes declined with increased PE MPs exposure dose, although not significantly (One-Way ANOVA, p > 0.05). Fish condition factors which entail information about fish health status differed significantly between treatment groups (one-Way ANOVA, p < 0.05). The highest means value of condition factor was computed for the control group and declined significantly with the addition of PE MPs exposure dose in treatment groups (Figure 4). The post-hoc test revealed significant variation of control and 1 PE MPs/mL groups, and with the rest of treatment groups (Tukey HSD, p < 0.05) but an insignificant variation between 10 and 100 PE MPs/mL (Tukey HSD, p > 0.05). The SGR varied between treatment groups relatively similar in trend as condition factors, though insignificant (Kruskal–Wallis Test, p > 0.05; Table 3).

Fish small intestinal histomorphological lesion indices correlated strongly significantly with growth pattern (Spearman, r = 1.00, p = 0.01), and means of condition factors (Pearson, r = -0.995, p < 0.05), final weight, weight gain, and total length (Spearman, r = -1.00, p = 0.01) but not with SGR (Spearman, r = -0.80, p > 0.05). All treatment groups displayed negative allometric growth patterns (Table 3).

Fable 3. Growth performance of	f <i>O. urolepis</i> juveniles exp	posed to different MPs doses. S	D = Standard deviation.
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Parameters	Treatment Group				
Turumetero	Control	1 PE MPs/mL	10 PE MPs/mL	100 PE MPs/mL	
Total length (cm) \pm SD	6.24 ± 1.10	6.21 ± 0.80	6.15 ± 1.07	6.01 ± 1.21	
Initial weight (g) \pm SD	0.015 ± 0.004	0.017 ± 0.003	0.016 ± 0.004	0.015 ± 0.004	
Final weight (g) \pm SD	4.626 ± 1.987	4.530 ± 1.518	4.411 ± 2.055	4.246 ± 2.430	
Weight gain (g) \pm SD	4.611 ± 1.987	4.513 ± 1.518	4.396 ± 2.055	4.231 ± 2.430	
SGR (% day^{-1})	8.80 ± 0.78	8.42 ± 0.62	8.33 ± 0.85	8.42 ± 0.95	
Condition factor	3.276 ± 0.379	2.424 ± 0.210	1.946 ± 0.144	1.796 ± 0.170	
Growth pattern (b)	2.671	2.842	2.943	2.989	
Coefficient of determination (R ²)	0.947	0.954	0.982	0.977	



Figure 3. Length–weight relationship graphs of *O. urolepis* after exposing larvae to the outset of feeding for 65 d to juveniles. (**A**): Control (no PE MPs/mL); (**B**): 1 PE MPs/mL; (**C**): 10 PE MPs/mL; and (**D**): 100 PE MPs/mL.



Figure 4. Mean condition factors of *O. urolepis* juveniles (One-Way ANOVA, post hoc Tukey HSD, p < 0.05) exposed to various PE MPs/mL. The "*" represents significant difference of a group from the rest of treatment groups.

4. Discussion

4.1. Ingestion of MPs and Associated Histomorphological Changes

The results of the current study showed that the ingested PE MPs significantly inflicted histomorphological alterations in small intestines of *O. urolepis* juveniles in PE MPs in a dose-dependent manner. Lei et al. [38] observed cracking of villi and splitting of enterocytes upon exposure of adult zebrafish (*Danio rerio*) to a mixture of MPs types with size of 70 μ m for 10 days. The 90 days chronic exposure of European sea bass (*Dicentrarchus labrax*) to polyvinyl chloride resulted in the detachment of mucosal epithelium from the lamina propria, fusion and beheading of villi, and hyperplasia of goblet cells at the top of the villi on the 60th day [39]. Apart from Ahrendt et al. [29], who applied a pathological lesion

indices evaluation tool, corroborating our study, the majority of studies did not reach that length of observation. It was concluded that presence of MPs in the small intestine causes histopathological lesions and compromises nutrients availability and energy metabolism, leading to significant deterioration of fish welfare [9].

4.2. Effect of Histomorphological Alteration on Growth Performance

Under normal environmental conditions and good nutritional status, tilapians length increases exponentially from larvae to juveniles and declines towards adulthood, unlike their weight [25,40]. To achieve this, younger fish consume a substantial amount of food if available to extract sufficient nutrients using numerous villi covered with microvilli to enhance surface area for absorptive function of the small intestines [41]. As a result, juvenile fish exhibit a more negative allometric pattern than adult fish [42]. In our study, despite supplying a relatively similar quantity of feed to all treatment groups, a variation in growth occurred. It is regarded that small intestines PE MPs-induced damage impaired digestion and absorption processes in fish. This led to malnutrition and subsequent energy and nutrients deficit, resulting in retardation in length and decline in final weight and weight gain (Table 2) of the exposed fish. The growth patterns of our juvenile fish exposed to the highest dose of PE MPs were relatively close to adult O. urolepis [25,43], reflecting the effect of MP-induced small intestine malfunction. The similar results were obtained after exposing planktivorous reef fish (*Acanthochromis polyacanthus*) [44] and glassfish (*Ambassis*) dussumieri) [45] to MPs particles, besides lacking a histopathological explanation. Our recent study showed that ingestion does lead to intestinal damage [13], and here we demonstrated the impact of that damage as being a departure from the conventional growth of tilapia juveniles as further affirmed by coefficient of determination (Table 3).

Condition factors convey information on fish health status and whether a fish can make good use of its food source [40,46], leading to growth. In a given fish species, condition factors normally change due to age class [25], season, sex, environmental conditions [47], and slightly due to population density [40]. Evidence of decline in condition factors were reported in O. urolepis [25,48], O. niloticus [47], and Anguilla Anguilla [49] due to hypersalinity, season, and pollution respectively. Salinity, season, and age, however, could not be regarded as the main source of variation in our study as water and fish larvae came from a single source and almost all water quality parameters (temperature, oxygen, ammonia, salinity) were relatively constant. The recorded significant variation of pH was largely due to possible dissolution of carbon dioxide formed by respiration, but nonetheless lied within the optimal growth range for tilapia [50]. Tran-Duy et al. [23] revealed that smallweight fish consume a substantial amount of oxygen and release a corresponding amount of carbon dioxide, as exhibited by our fish, resulting in the decline of pH converse to the increase in MPs dose. PE MPs-induced damage to O. urolepis small intestine, as supported by Qiao et al. [10] and Lei et al. [38], might have caused oxidative stress and insufficient nutrient assimilation that impaired condition factors. The condition factor in our control group fish was high compared with those of hybrid of O. niloticus and O. urolepis juveniles stocked in varying stocking density for 63 d [40]. In our study, condition factor decreased with the rise in PE MPs-induced histomorphological lesion indices (Figure 2). Girella *laevifrons* [20] collected from MPs prone intertidal pools showed a similar trend despite lacking information on intestinal histormophological status, which was later demonstrated by Ahrendt et al. [29] using poly (styrene-co-divinylbenzene) MPs.

In most fish species, SGR typically declines with increasing in fish body weight [51]. Conversely, other studies reveal that SGR of *O. urolepis* is directly proportional to the weight gain [40,48], which is similar to our study. Within fish species, however, the variation in SGR is influenced by age and extrinsic factors such as salinity [48], temperature, ammonia, pH, and oxygen [23,52], which in our study all lied within the optimal range of fish growth. Studies reported high SGR in low stocking density [40], with salinity of 25 and high oxygen concentration in *O. nilotius* sub-adults [23]. In our study, fish abundance was relatively similar in all treatment groups, and hence stocking density did not account for variation

in growth performance. These means oxygen concentrations were similar, despite being below the recommended value of 5 mg/L for tilapia in the 10 PE MPs/mL treatment group [25]. However Shoko et al. [50] described that omnivorous fish including *O. urolepis* have low metabolic rates and require relatively low quantities of oxygen. Therefore, PE MPs-induced histomorphological impairment of fish small intestine remains the sole reason for the variation of SGR between treatment groups, although insignificant.

In the natural setting, climate and fishing have been influencing fish growth at different scales [53]. Climate change heavily affects the nutrient supply, habitat, fishing, and adaptation [53]. The recent recognition of plastics as an emerging pollutant have worsened the already existing problem [54]. Their ubiquity in pelagic zones may smoothen phytoplankton, which comprise the foundation of food chain, impairing primary production and eventual food availability to fish. On the other hand, ingestion of plastics induces false satiation, and may cause gastrointestinal blockage and histopathological damage to fish [13]. This may lead to starvation or reduced growth [55].

Research on MPs interaction with freshwater fish including *Oreochromis* spp. is scarce [1] and in most cases uses juveniles or adults [45]. Attempting to correlate histomorphological damage to growth is ecologically vital, but it is important to note that the concentrations, type, and size of MPs employed in our study are not typical of those encountered in freshwater bodies studies in Africa or globally [6,56,57]. Such high exposures with a standard homogenous bead particle are commonly found in literature with the justification of trying to understand the effects and mechanisms of MP exposure [13,29,34,58,59]. Extending MP research to include greater environmental realism with respect to a diversity of shapes, sizes, morphologies, and aging is a current concern [32–34,59,60]. Nevertheless, the present study elucidates an important insight into the effects on growth and threat may extend to recruitment of larvae and juvenile fish in nursery grounds [45] and have an impact at the population level.

5. Conclusions

Freshwater ecology supports a multitude of organisms and provides numerous sources of proteins, particularly to land-locked countries in Africa and across the globe. As a result, they are under huge pressure for resources and from indiscriminate pollution including by plastics. This study showed how MP exposure to the newborn *O. urolepis* fish may affect digestive and absorptive function of the small intestine, leading to nutrient deficit and subsequent impairment on growth and fish health.

Therefore, the existing trend of plastic, both macro and micro, production and its input into aquatic environments may interfere with energy uptake and the process of larvae recruitment to adult fish. The decline in fish size has a great impact on fecundity as small-bodied fish produce few eggs. Moreover, the resulting malnutrition may impair the reproductive capability of fish, which requires further investigation. Moreover, the stunted growth in fish may result in small-sized fish that jeopardize the human economy and food security. Future studies on the effect of MPs on reproduction are required.

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