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RESEARCH ARTICLE

Effect of Microplastic Exposure to the Reproductive Energy and Fecundity of Female Wami Tilapia (*Oreochromis urolepis*, Norman 1922) Fish

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ABSTRACT

There is mounting evidence indicating that microplastics (MPs, < 5 mm) cause reproductive dysfunction in fish, yet information on the long-term effects of MP exposure remains scarce. In this study, Wami tilapia fries were exposed to 38–45 μm polyethylene (PE) MPs for their first 2 months in treatment groups of control (0 PE MPs/mL), 1, 10, and 100 PE MPs/mL (with 60 individuals per group in triplicates), and subsequently maintained in a ratio of 3 females to 1 male for an additional 5 months. Reproductive proxies and parameters of female fish health were calculated, and models were developed according to the Akaike information criterion (AIC) and no significant differences in fecundity, relative fecundity, hepatosomatic index (HSI), weight, length, gonadosomatic index, and oocyte packing density were found between treatment groups (one-Way ANOVA, $p > 0.05$). However, condition factors varied significantly between treatment groups (Kruskal–Wallis Test, $p = 0.006$), with the control group differing significantly from the groups exposed to 1 PE MPs/mL ($p = 0.007$), 10 PE MPs/mL ($p = 0.03$), and 100 PE MPs/mL ($p = 0.001$). Fecundity showed strong correlations with weight and length in all treatment groups except the group exposed to 10 PE MPs/mL (weight: $r = 0.471$, $p = 0.346$; length: $r = 0.688$, $p = 0.131$) and showed insignificant correlations with condition factors and HSI. Multiple regression models revealed that weight significantly contributed to fecundity in all treatment groups except the group exposed to 10 PE MPs/mL. Histopathological analysis indicated damage to livers and small intestines proportional to the dose of PE MPs. This study demonstrates that long-term exposure of fish to MPs has no significant effect on fecundity but impairs fish health, which could potentially jeopardize the quality of fish eggs and recruitment.

1 | Introduction

The retrieval of microplastics (MPs), nominally defined as < 5 mm, from the gastrointestinal tracts of fish caught in various aquatic environments of the ocean (Akhbarizadeh, Moore, and Keshavarzi 2018; Felline et al. 2022; Hermsen et al. 2017; Klangnurak and Chunniyom 2020), rivers (Khan et al. 2020; Pegado et al. 2018), lakes, and brackish waters (Abbasi et al. 2018; Calderon et al. 2019), underscores the widespread environmental concern across aquatic ecosystems. Numerous studies have

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indicated that MPs can affect the well-being of fish, particularly organs such as the gills (Hamed et al. 2021), gastrointestinal tracts (Mbugani, Machiwa, Shilla, Kimaro, et al. 2022), and livers (Batel et al. 2016; Ding et al. 2018; Lu et al. 2016), leading to inflammation and/or physiological stress.

In the gastrointestinal tract, MPs are reported to induce pseudo-satiation, cause blockages of intestinal pathways, and result in histopathological damage (Ahrendt et al. 2020; Pedà et al. 2016). In the liver, MPs reportedly cause damage, induce oxidative stress, and disturb energy metabolism (Ahmadifar et al. 2021; Ding et al. 2018; Lu et al. 2016; Qiao et al. 2019). Accumulation of MPs in the gills results in histopathological inflammation (Hamed et al. 2021). These effects on organs can subtly impact oxygen availability, digestion, nutrient absorption and catabolism, potentially affecting other physiological traits such as growth and reproduction (Chisada, Yoshida, and Karita 2019; Pitt et al. 2018). For instance, dietary exposure to PVC MPs for 30 and 60 days reportedly led to stunted growth of *Cyprinus carpio* var. larvae (Xia et al. 2020). Similarly, exposure to environmentally relevant microplastic concentrations decreases the growth of Wami tilapia, *Oreochromis urolepis* (Mbugani, Machiwa, Shilla, Joseph, et al. 2022). However, reproductive endpoints have so far received little attention.

The understanding that exposure to MPs can affect the reproductive potential of aquatic organisms has garnered significant interest, yet there remains a lack of consensus on the subject, with studies showing a range of outcomes. For example, reproduction in zebrafish (*Danio rerio*) (Qiang and Cheng 2021), Nile tilapia (*Oreochromis niloticus*) (Ismail, Saleh, and Sayed 2021), and marine medaka (*Oryzias melastigma*) (Wang et al. 2019) was found to be affected following exposure to MPs of various concentrations, whereas other fish species such as Japanese medaka (*Oryzias latipes*) and damselfish (*Acanthochromis polyacanthus*) (McCormick et al. 2023) displayed no reproductive effects. Additionally, some findings suggest that the effect may be sex-specific (Ismail, Saleh, and Sayed 2021; Wang et al. 2021). Understanding the association of long-term exposure of MPs and their histological impairment to the fish reproductive performance and energy deficit is of paramount importance. However, the majority of these studies are short-term (Wang et al. 2019) and/or utilize adult stocks (Assas et al. 2020; McCormick et al. 2023), contrary to natural settings where exposure occurs throughout their lifetime (Felline et al. 2022; Merga et al. 2020; Pegado et al. 2018).

The reproductive success of fish depends on the quantity of energy reserves, meaning that fish with greater energy reserves are expected to have higher reproductive performance, which can be expressed in terms of fecundity, gonadosomatic index (GSI), relative fecundity, and oocyte packing density (Ismail, Saleh, and Sayed 2021; Zulfahmi et al. 2018). Fish fecundity, which is described as the number of eggs or oocytes of fish in a given spawning cycle, shows a strong association with reproductive energy proxies. The commonly regarded reproductive energy proxies are hepatosomatic index (HSI), condition factor, and weight (Kennedy, Gundersen, and Boje 2009; Rodgveller 2019). Unlike weight, the utility of HSI and condition factor is time-dependent and becomes useful before the spawning period (Rodgveller 2019). Since fecundity is strongly correlated with energy or food availability, any process that either hinders food supply, interferes with the digestive process (Arias et al. 2019; Hamed et al. 2021), or impairs organ function (Ahmadifar et al. 2021; Qiang and Cheng 2021) impacts energy supply and eventually fish reproduction. However, studies describing the influence of long-term exposure to MPs on fish reproductive energy and subsequent fecundity are limited.

Our previous studies, in which *O. urolepis* were exposed via water a to polyethylene MPs for 2 months, were long-term and revealed impairments in the gastrointestinal tract (Mbugani, Machiwa, Shilla, Kimaro, et al. 2022) that persisted even after a depuration period of a further 2 months. Similarly, our study elucidated the stunted growth of fish caused by MP-induced histopathological damage of gastrointestinal tract (Mbugani, Machiwa, Shilla, Joseph, et al. 2022). This fish species is characterized by high fecundity and is an omnivore, able to thrive in a wide range of aquatic environments, from freshwater to brackish habitats (Mapenzi and Mmochi 2016; Nyinondi et al. 2020). The present study aims to investigate the long-term effect of MPs on fish reproductive energy and fecundity.

2 | Methodology

2.1 | Fish Farming and Larvae Production

The fish were transported to the School of Aquatic Sciences and Fisheries Technology at the University of Dar es Salaam facility. After being fed for several months, the fertilized eggs in mouth broods were extracted and transferred to an indoor hatchery for spawning. The spawned larvae were then kept in a continuous water flow cleaning system for 5 days post-hatch until their food reserve bellies disappeared. Healthy larvae were carefully selected, and 20 individuals were stocked in each 100 L aquarium, containing 80 L of filtered freshwater. They were left to acclimate for 24 h without feeding.

The larvae had mean initial weights of 0.0153 ± 0.0031 g, 0.0159 ± 0.0026 g, 0.0153 ± 0.0028 g, and 0.0163 ± 0.0031 g, and mean initial lengths of 1.14 ± 0.11 cm, 1.12 ± 0.11 cm, 1.13 ± 0.11 cm, and 1.14 ± 0.09 cm for the control, 1, 10, and 100 PE MPs/mL groups, respectively. Water was oxygenated using cement-made air stones to prevent the possibility of MP contamination from commercially produced air stones.

2.2 | Ethical Approval

This experiment protocol involving fish was approved by the University of Dar es Salaam Office of the Deputy Chancellor for Research.

2.3 | MP Preparation and Sampling

Polyethylene microsphere microplastics (PE MPs) with a size range of 38–45 μm and a density of 1.00 g/cc were procured from Cospheric LLC, located at Santa Barbara, CA, USA. The preparation of PE MPs and their concentration confirmation were conducted following a similar procedure to Mbugani, Machiwa,

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Shilla, Kimaro, et al. (2022). In brief, a suspension of PE MPs was prepared by adding 0.01% Tween 80 surfactants (product number: P8074; Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO, USA) to 20 mL of water, followed by mixing for an hour with a mixer at 150 rpm to ensure dispersion.

Samples of PE MPs were taken from exposure media, waste media, and feces. Five milliliters of each exposure and waste media, as well as 5 g of feces, were collected. The feces were digested in 10 mL of 10% w/v KOH (Karami et al. 2017) for 4 days at room temperature. In all samples, 1 mL of 0.1% Tween 80 Biocompatible surfactant was added to ensure uniform distribution of PE MPs. Subsequently, 1 mL of each digested feces, exposure media, and waste media was placed onto a Sedgewick rafter for observation and counted under a fluorescent microscope (Olympus) at a magnification of × 16.

2.4 | Fish Exposure

The experimental setup remained consistent with our previous studies on Wami tilapia (Mbugani, Machiwa, Shilla, Joseph, et al. 2022), employing the same treatment groups: Control (no PE MPs/mL), 1, 10, and 100 PE MPs/mL, each replicated in triplicate aquaria. Fish were fed to satiation twice daily at 9:00 and 14:00, with the feed sizes adjusted based on the age class and size of the fish. Larvae were initially fed powder for the first 2 weeks, followed by pellets of 0.5 mm, then 0.8 mm at intervals of 3 weeks. From the 3rd to the 7th month of exposure, feed sizes were increased to 1, 2, and 3 mm, respectively. Commercial feeds (De Heus, Lot A4, Vinh Long province, Vietnam) were used throughout all feeding regimes.

Eighty percent of the water was renewed every 2 days, followed by reapplication of the respective doses of PE MPs in each treatment to prevent MP accumulation. To avoid MP accumulation, aquaria were completely emptied, cleaned with a sisal brush, and refilled to the working volume at the end of each week. After 2 months of exposure, the sex of juveniles was identified using a green food dye solution applied to the genital papilla of each individual (Popma and Masser 1999). In each aquarium, juvenile fish were then reduced to four individuals in a ratio of 3 females to 1 male to provide ample space for optimal growth. MP exposures continued for a further 5 months. Additionally, the tops of the aquaria were covered with wire mesh, and half-height paper boxes were used to prevent fish from jumping out and to reduce stress, respectively.

Temperature, pH, oxygen, and ammonia levels were regularly monitored. Temperature and pH were measured using a starter pen meter (ST 20 Ohaus), while oxygen levels were determined through the Winkler test. Temperature ranged from 25.9°C to 29.2°C (average 27.3 ± 0.82°C) for the control group, 26.1°C to 29.8°C (average 27.4 ± 0.88°C) for the 1 PE MPs/mL group, 25.9°C to 29.2°C (average 27.3 ± 0.89°C) for the 10 PE MPs/mL group, and 26°C to 29.5°C (average 27.4 ± 0.85°C) for the 100 PE MPs/mL group. pH levels ranged from 7.0 to 8.26 (average 7.60 ± 1.29) for the control group, 6.91 to 8.36 (average 7.50 ± 2.48) for the 1 PE MPs/mL group, 6.94 to 8.18 (average 7.50 ± 1.51) for the 10 PE MPs/mL group, and 6.94 to 8.23 (average 7.50 ± 1.89) for the 100 PE MPs/mL group. Oxygen levels ranged from 4.15 to 9.96 mg/L (average 6.60 ± 1.29 mg/L) for the control group, 3.32 to 18.26 mg/L (average 7.59 ± 2.57 mg/L) for the 1 PE MPs/mL group, 3.32 to 9.96 mg/L (average 6.45 ± 1.51 mg/L) for the 10 PE MPs/mL group, and 3.32 to 12.45 mg/L (average 6.80 ± 1.76 mg/L) for the 100 PE MPs/mL group. Ammonia levels in all treatments ranged from 0 to 0.18 mg/L, within acceptable limits.

2.5 | Fish Sampling and Tissues Collection

At the conclusion of the experiment period, all fish were transferred to aquaria containing MP-free media for 24 h, then rinsed and anesthetized using a few drops of clove oil. The fish were weighed using sensitive digital Sartorius balance and their length was measured by meter ruler. Ovaries containing eggs/oocytes and livers were removed and weighed from 30 female individuals (control, $N = 8$; 1 PE MPs/mL, $N = 8$; 10 PE MPs/mL, $N = 6$; 100 PE MPs/mL, $N = 8$). From each fish, a subsample of 40 eggs was taken from the anterior, middle, and posterior sections of the ovary and individually weighed using a microbalance. The entire livers, along with 3-cm pieces of small intestines (referred to as ilium sections), were immediately fixed in 20 mL of 10% buffered formaldehyde for 48 h for histopathological analysis.

2.6 | Histopathological Investigation of Liver and Small Intestine

Histopathological investigation of both the liver and small intestine was conducted following the protocol employed by Mbugani, Machiwa, Shilla, Kimaro, et al. (2022). In summary, small intestine and liver tissues fixed in 10% neutral buffered formalin for 48 h were dehydrated in a series of alcohol concentrations of 70%, 80%, 90%, and 100%, cleared with xylene, and then embedded in paraffin. The tissues were sectioned with a microtome into 5-μm slices, mounted on slides, hydrated in a reversal alcohol concentration, and stained with hematoxylin and eosin dyes. Finally, the tissues on slides were covered with coverslips for further observation using an Olympus microscope, and impairment investigation and evaluation were conducted according to the method outlined by Bernet et al. (1999). Pathological changes observed included circulatory disturbances, regressive changes, progressive changes, and inflammation of tissues.

2.7 | Reproductive Performance and Energy Proxies' Analysis

Determination of total fecundity was done by gravimetric methods according to Rodgveller (2019) with minor modification, as shown by Equation (1):

$$\text{Fecundity} = \frac{\text{GW} \times 40 \text{ eggs}}{\text{Weight of 40 eggs (g)}} \quad (1)$$

Relative fecundity, that is, the ability of fish to produce eggs concerning body weight, was calculated (Equation 2) according to Kennedy, Gundersen, and Boje (2009) and Zulfahmi et al. (2018):

$$\text{Relative fecundity} = \frac{\text{Fecundity}}{Wf} \quad (2)$$

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Oocyte packing density (Equation 3) indicates the developmental stage of the ovary, which is the number of oocytes per gram of ovary wet weight (Kennedy, Gundersen, and Boje 2009):

$$\text{Oocytes packing density} = \frac{\text{Fecundity}}{\text{GW}} \tag{3}$$

The gonadosomatic index (GSI) was computed using collected data (Equation 4). The GSI values were obtained using the following equation (Kennedy, Gundersen, and Boje 2009):

$$\text{GSI} = \frac{\text{GW} \times 100}{Wf} \tag{4}$$

In the determination of the influence of reproductive energy indices, the HSI (Equation 5) and condition factor (index) (Equation 6) were computed (Kennedy, Gundersen, and Boje 2009; Rodgveller 2019). The HSI was obtained by:

$$\text{HSI} = \frac{\text{LW} \times 100}{Wf} \tag{5}$$

While the condition factor was derived from the Fulton equation as employed by Mapenzi and Mmochi (2016):

$$\text{Condition factor} = \frac{Wf \times 100}{\text{TL}^b} \tag{6}$$

where GW = gonad (ovary) weight; W_f = total body weight; LW = liver weight; TL = total body length; and $b = 3$ (assuming isometric growth pattern).

The general multiple regression model (Equation 7) expressing fecundity as function of reproductive energy proxies, namely condition factor, HSI, weight, intercept and normal random error was given in the equation below according to Rodgveller (2019) with minor modification:

$$\text{Fecundity} = \text{CF} + \text{HSI} + Wf + \text{TL} + \text{I} + ei \tag{7}$$

Where, CF = condition factor (index); I = intercept, and ei = normal random error.

Length was omitted from the multiple linear regression model due to its lack of status as a reproductive energy proxy (Kennedy, Gundersen, and Boje 2009) and its potential to cause multicollinearity. Nonetheless, length is increasingly recognized as being correlated with fecundity and is considered one of the best estimates of population potential (Kennedy, Gundersen, and Boje 2009), a fecundity predictor (Rogers et al. 2019), and easy to collect (Rodgveller 2019).

Correlations between fecundity and length, weight, HSI, and condition factor were separately analyzed for each treatment group. The Akaike Information Criterion (AIC) values for the full and reduced models were compared, with the model exhibiting the smallest AIC being selected as the best-fitting model (Rodgveller 2019).

2.8 | Data Analysis

All values were presented as mean ± standard deviation. The normality of data was assessed using the Shapiro–Wilk test. Data for comparison between treatment group endpoints were transformed as follows: log transformation for length and relative fecundity and square root transformation for fecundity when necessary. Comparison of treatment groups was analyzed using one-way ANOVA with Tukey's Honestly Significant Difference (HSD) post hoc test for parametric data and Kruskal–Wallis test followed by pairwise comparison using Dunn's test for non-parametric data. Two-way ANOVA was conducted to determine the relationship between PE MPs in feces, new media, waste media, and treatment groups.

Pearson correlation and multiple linear regression analysis were employed to determine the relationship of fecundity to other fish reproductive performance and energy proxies, as well as the functional influence of the latter. Fisher's test was used to compare correlation coefficients (r) between treatment groups. Both outliers and normality of residual errors in models were checked. All statistical analyses were performed using SPSS version 23 and R-version 4.2.3 software. Values were considered statistically significant at $p \leq 0.05$.

3 | Results

3.1 | PE MPs Sampling

> *"The reproductive success of fish depends on the quantity of energy reserves, meaning that fish with greater energy reserves are expected to have higher reproductive performance, which can be expressed in terms of fecundity, gonadosomatic index (GSI), relative fecundity, and oocyte packing density."*

The average amounts of PE MPs in fresh exposure media, waste media, and feces for the 1 PE MPs/mL treatment were 1.0 ± 1.0, 6.7 ± 6.7, and 375.3 ± 233.3, respectively. For the 10 PE MPs/mL treatment, the averages were 24.3 ± 5.8, 80.3 ± 77.7, and 494.7 ± 279.9, respectively. For the 100 PE MPs/mL treatment, the averages were 128.7 ± 45.94, 485.3 ± 311.7, and 8360.0 ± 1103.0, respectively (**Exhibit 1A,B**). No PE MPs were found in the control treatment group. Feces contained substantial amounts of PE MPs compared to the media. The two-way ANOVA revealed significant differences between fresh media, waste media, and feces ($p = 6.51 \times 10^{-12}$), between PE MPs treatment groups ($p = 3.04 \times 10^{-12}$), and their interactions ($p = 9.67 \times 10^{-12}$).

3.2 | Effect of PE MPs to Fecundity and Reproductive Energy Proxies and Performances

The exposure of female *O. urolepis* to various doses of PE MPs yielded different results of reproductive performance parameters and energy proxies. The total length of female fish in the control, 1, 10, and 100 PE MPs/mL groups ranged from 11.5 to 21 cm, 13 to 22.1 cm, 11.8 to 23 cm, and 12.7 to 23 cm, with corresponding weights ranging from 27.2 to 185.4 g, 36.2 to 191.2 g, 31.6 to 205.9 g, and 37.3 to 214.7 g, respectively (**Exhibit 2A,B**). Similarly,

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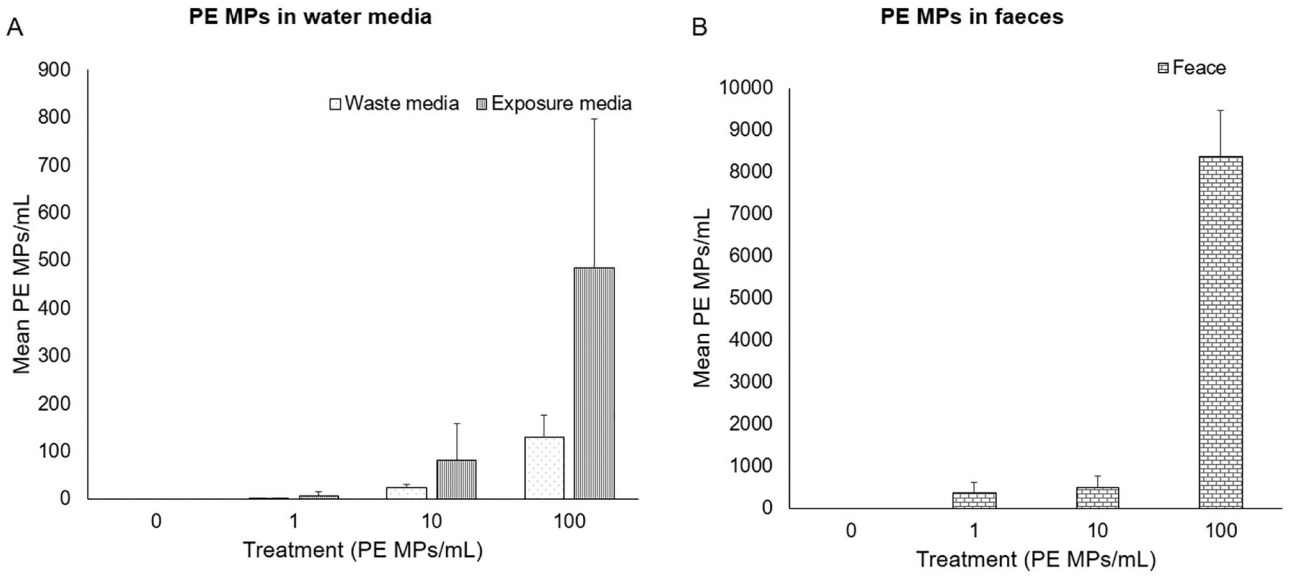

EXHIBIT 1 | The mean concentration (+SD) of PE MPs retrieved from 1, 10, and 100 PE MPs/mL treatment groups in fish feces (sampled before disposal of waste water), new media (soon after PE MPs addition to the media for exposure) and waste media (wastewater to be disposed after two days fish exposure). *Note:* No PE MPs were found in control treatment. Significant difference existed between treatments and compartments.

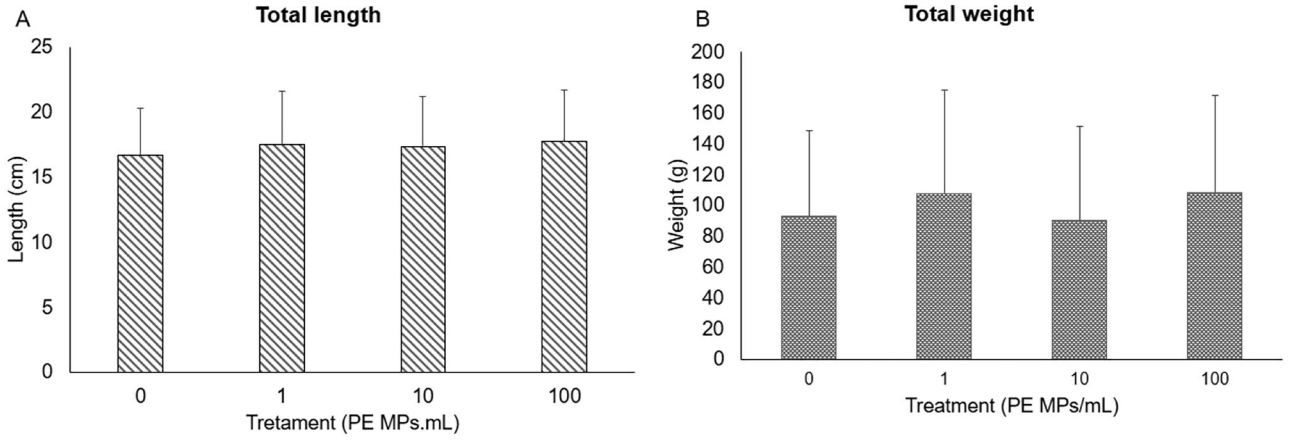

EXHIBIT 2 | Means (± SD) of *O. urolepis* total length and total weight of treatment groups after 7 months PE MPs exposure from larvae to sexual maturity.

fecundity ranged from 263 to 3626, 209 to 5334, 235 to 2592, and 182 to 2786 (**Exhibit 3A**). There were no significant differences in fecundity (one-way ANOVA, $p = 0.945$), relative fecundity (one-way ANOVA, $p = 0.91$), total length (one-way ANOVA, $p = 0.827$), and total weight (one-way ANOVA, $p = 0.921$) between treatment groups (Exhibits 2 and 3). With the exception of the 10 PE MPs/mL group, fecundity showed a strong positive significant correlation with either total weight or total length in the other treatment groups (**Exhibit 4**).

Fish in the control treatment showed the highest mean HSI value, which decreased as PE MPs exposure dose increased. However, there was no significant difference in HSI between treatment groups (one-way ANOVA, $p = 0.128$) (**Exhibit 5A**). The condition factors showed a significant difference between treatment groups (Kruskal–Wallis Test, $p = 0.006$). The post hoc test displayed a significant difference between the control and 1 PE MPs/mL ($p = 0.007$), 10 PE MPs/mL ($p = 0.03$), or 100 PE MPs/mL ($p = 0.001$) groups (Exhibit 5B). The fecundity of females fish in various PE MPs exposure groups displayed no significant correlation with either HSI or condition factors. Generally, the correlation of fecundity showed a trend of change from moderately strong negative to weak positive with HSI and conversely with condition factors as PE MPs dose increased (Exhibit 4). The significant difference in correlation was only shown between the control and 10 PE MPs/mL treatment groups for fecundity and condition factor ($z = 1.982$, $p = 0.047$). There was no significant difference in oocyte packing density (Kruskal–Wallis test, $p = 0.387$) and GSI (Kruskal–Wallis test, $p = 0.499$) between treatment groups (**Exhibit 6**).

3.3 | Fish Fecundity Regression Model

The multiple regression models illustrate the influence of reproductive energy proxies on fecundity, with the best models selected

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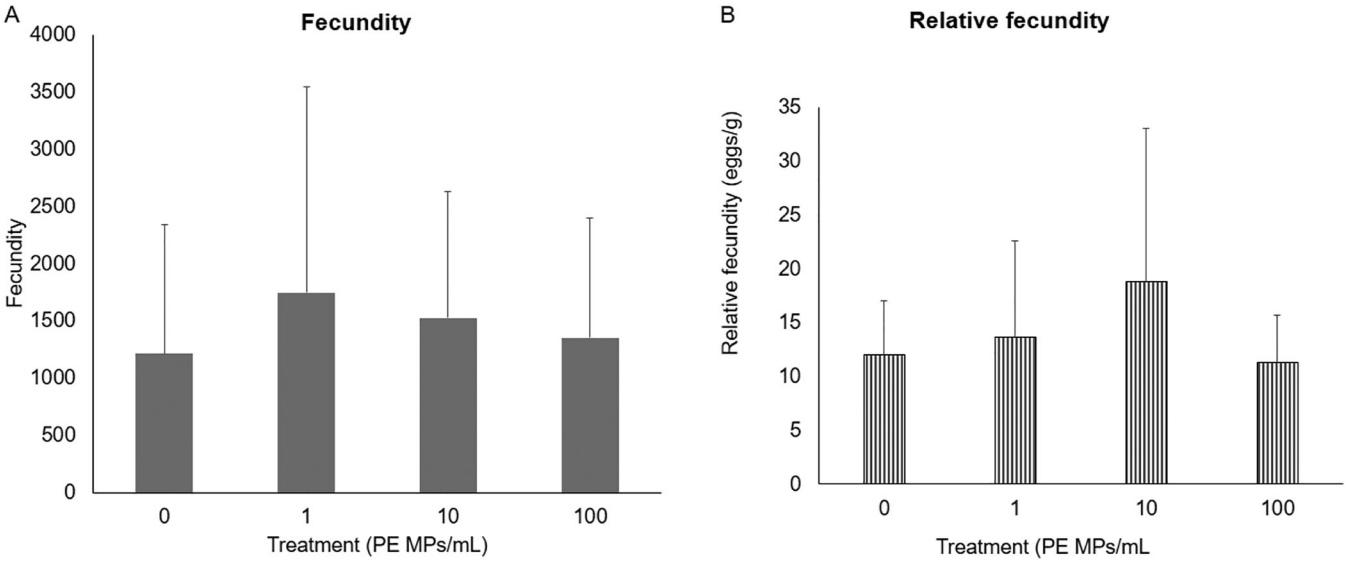

EXHIBIT 3 | Means (± SD) of *O. urolepis* fecundity and relative fecundity of treatment groups after 7 months PE MPs exposure from larvae to sexual maturity.

EXHIBIT 4 | Pearson correlation of fecundity versus reproductive energy proxies/parameter of *O. urolepis*.

Treatment	Fe vs. TW	Fe vs. TL	Fe vs. HSI	Fe vs. CF
Control	$r = 0.932^{a}$	$r = 0.907^{a}$	$r = -0.515^{a}$	$r = 0.323^{a}$
	$p = 0.0007$	$p = 0.0019$	$p = 0.192$	$p = 0.435$
1 PE MPs/mL	$r = 0.747$	$r = 0.771^{b}$	$r = -0.536$	$r = 0.047$
	$p = 0.033$	$p = 0.025$	$p = 0.183$	$p = 0.912$
10 PEMPs/mL	$r = 0.471$	$r = 0.688$	$r = 0.083$	$r = -0.805$
	$p = 0.346$	$p = 0.131$	$p = 0.876$	$p = 0.053$
100 PE MPs/mL	$r = 0.914$	$r = 0.908$	$r = 0.126$	$r = -0.298$
	$p = 0.0015$	$p = 0.0018$	$p = 0.767$	$p = 0.474$

Abbreviations: a, square root of fecundity versus individual reproductive energy proxy or parameter; b, log length.

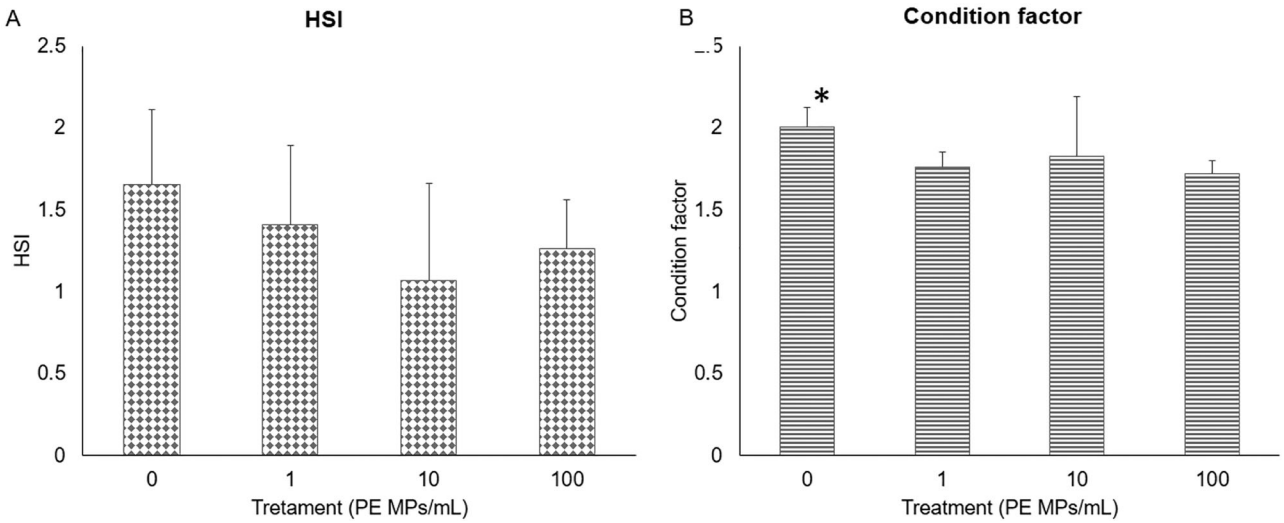

EXHIBIT 5 | Means (± SD) of reproductive HSI (A) and condition factors (B) of *O. urolepis* females exposed to varying PE MPs concentration from fries (larvae) to reproductive adult. Note that 0 PE MPs/mL is a control group; "*" means that control groups at $p < 0.05$.

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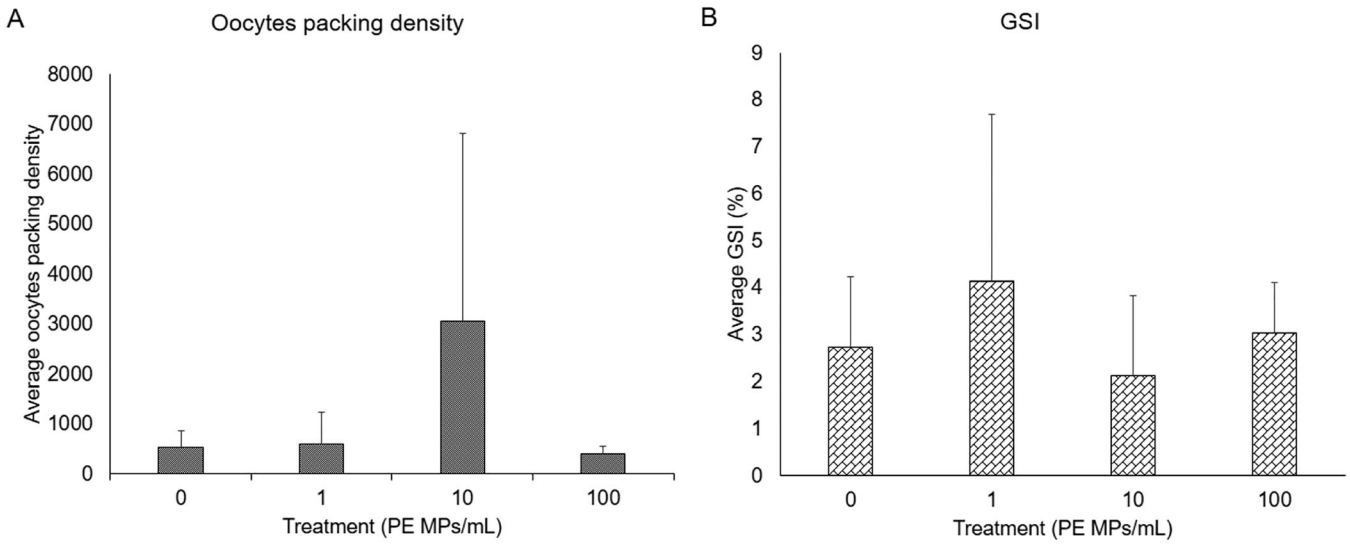

EXHIBIT 6 | Means (± SD) of reproductive performance parameters of *O. urolepis* females exposed to varying PE MPs concentration from fries (larvae) to reproductive adult. GSI = gonadosomatic index.

based on the AIC (**Exhibit 7**). The residual errors showed no outliers and followed a normal distribution. In these models, length was excluded due to multicollinearity. Weight was found to account for significant changes in fecundity in all treatment groups except for the 10 PE MPs/mL group, where the condition factor appeared to play a significant role. However, in the best-fit model, AIC included weight in the regression model for the 10 PE MPs/mL group despite showing an insignificant contribution.

3.4 | Histopathology

3.4.1 | Liver

In the control group, fish liver displayed normal lobes, classical tubules, capsules, and a central vein. The hepatocytes were arranged in proper order towards the central vein with normal apoptosis. However, the classical lobules were not as clear in this fish species compared to mammals (**Exhibit 8A**). In the 1 PE MPs/mL treatment group, inflammatory cells were clearly observed (Exhibit 8B1 and B2). Hepatocytes contained condensed dark chromatin in nuclei, which was relatively high compared to the control. The cytoplasm appeared abnormally enlarged and pale rather than pink. Blood congestion was observed in capillaries. Vacuolization of hepatocytes was clearly visible in the 10 PE MPs/mL treatment group fish. They exhibited pale cytoplasm and unusual dark nuclei, sometimes disoriented towards the central vein. Blood congestion and aggregation of leukocytes observed in the veins were relatively higher than in the 1 PE MP/mL group. Aggregation of inflammatory cells increased around the blood vessels (Exhibit 8C1 and C2). The vacuolization of hepatocytes was relatively pronounced in the 100 PE MPs/mL treatment group compared to the 10 PE MP/mL group. The architectural arrangement of tissues toward the central vein was highly distorted. Both lymphatic vessels and central veins were congested (Exhibit 8D1 and 8D2).

3.4.2 | Small Intestine

In the control treatment, the small intestines' villi were lined with simple columnar epithelia cells with few scattered goblet cells. Most of the time, lymphocytes aggregated in the propria submucosa, while leukocytic cells were rarely observed or absent altogether (**Exhibit 9A**). Unlike the control group, fish exposed to 1 PE MPs/mL showed some hyperemia in certain parts (Exhibit 9B). Vacuolation and degeneration of epithelial cells could be observed with little to moderate leukocytic infiltration. The goblet cells were more pronounced in this treatment group than in the control. Some blood congestion started to appear in the villi, which still showed good branching. Fish exposed to 10 PE MPs/mL revealed increased infiltration of leukocytic cells (Exhibit 9C), and blood congestion was comparatively higher than that in the 1 PE MPs/mL group. On the other hand, fish in the 100 PE MPs/mL group displayed high leukocytic infiltration in both the mucosa and submucosa (Exhibit 9D). The blood congestion was highest in the fish villi of this treatment group compared to others.

4 | Discussion

The present study demonstrates that chronic exposure of various MP doses to *O. urolepis* fish from larvae to reproductive adults had no significant effect on subsequent fecundity. Among the reproductive energy proxies, only the condition factors of PE MPs-exposed treatment groups were significantly affected compared to the control (Exhibit 5B). Recent studies have increasingly investigated the effect of MP exposure on fish reproduction, yielding variable results (Assas et al. 2020; McCormick et al. 2023; Wang et al. 2019, 2021). Consistent with our findings, no significant difference in fecundity was observed in Japanese medaka and Java medaka fish (Assas et al. 2020), zebrafish (*D. rerio*) (Marana et al. 2022), and damselfish (*A. polyacanthus*) (McCormick et al. 2023) exposed to MPs. Despite this similarity in fecundity endpoints, our study has, for the first time, presented

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EXHIBIT 7 | Best-fit models for maternal *O. urolepis* under various PE MPs treatment indicating functional dependence of fecundity over reproductive energy proxies.

Treatment	Response	Explanatory	Estimate	SE	*t*-value	*p*	R^2 Adj	AIC
Control	fecundity	Intercept	− 496.766	370.094	− 1.342	0.228	0.79	98.61
		Weight	18.351	3.468	5.292	0.00184		
1 PE MPs/Ml	fecundity	Intercept	− 384.820	898.803	− 0.428	0.683	0.49	116.30
		Weight	19.851	7.203	2.756	0.033		
10 PE MPs/mL	fecundity	Intercept	5150.68	1462.399	3.522	0.0389	0.70	78.54
		Weight	7.43	4.384	1.695	0.1886		
		Condition F	− 2349.45	742.292	− 3.165	0.0507		
100 PE MPs/mL	fecundity	Intercept	− 269.085	334.752	− 0.804	0.452	0.81	95.61
		Weight	15.01	2.713	5.533	0.00147		

Abbreviations: AIC, Akaike information criterion; Condition F, condition factor; SE, standard error; R^2 Adj, R^2 adjusted.

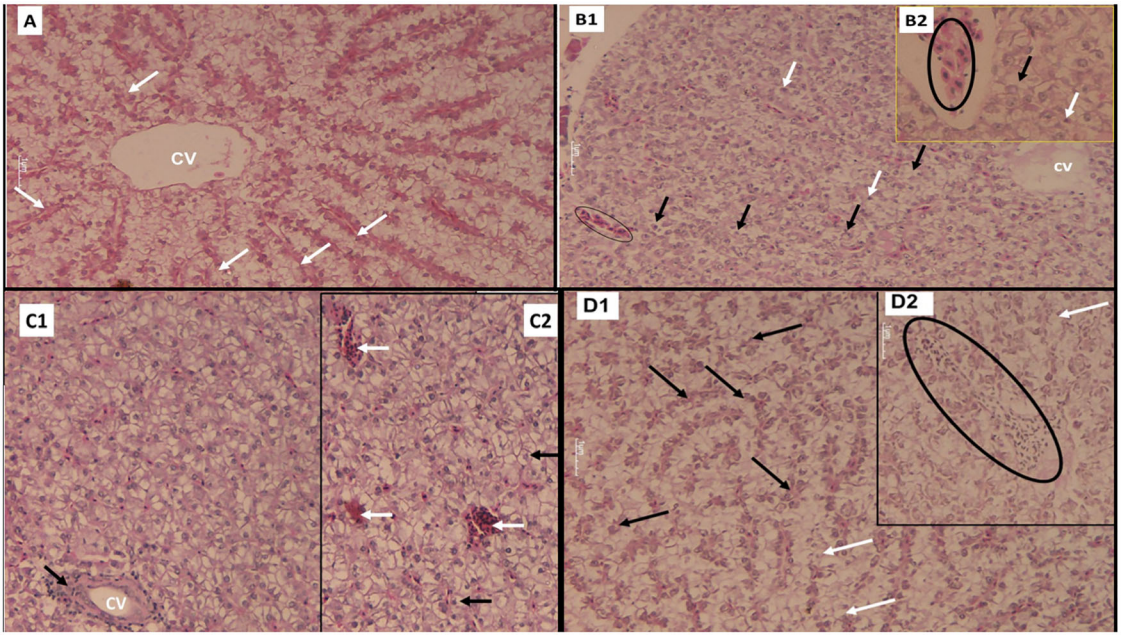

EXHIBIT 8 | Transverse section of liver of *O. urolepis* (H and E; Magnification 40 ×, 80 × and 160 ×). (A) Control group CV: central vein; white arrows: proper architectural arrangement of hepatocytes and Kupffer cells toward central vein; (B1 and B2) Treatment 1 PE MPs/mL CV: central vein; white arrows: dark chromatin; black arrows: cytoplasm; oval encircle: congested capillaries. (C1 and C2) CV: central vein; white arrows: blood congestion in vein; black thin arrows: dack nuclei and vacuolation; black thick arrow: blood congestion and aggregation of leucocytes around central vein. (D1 and D2) oval encircle: blood congestion in lymphatic vessels and veins; black arrows: disoriented hepatocytes and sinusoidal cells; white arrows: vacuolation of hepatocytes. [Color figures can be viewed at wileyonlinelibrary.com.]

the effect of MP exposure on the life cycle of *O. urolepis* over the long term. Conversely, MPs caused fecundity decline and reproductive disruption in ricefish (*O. melastigma*) (Wang et al. 2019) and fecundity decline in oysters (Sussarellu et al. 2016). This decline in fecundity could have resulted from the small particle size and high MPs dose used. Furthermore, variability in the effect of MPs on fecundity among various findings might be attributed to the variation in fish species, age class of fish, and exposure duration versus characteristics of MPs used. Relative fecundity was also not significantly different between treatment groups, similar to fish that were not under MP exposure (Jaspe and Caipang 2011).

The success of fish reproduction is a function of the amount of energy reserves, commonly weight, HSI, and condition factors (Rodgveller 2019; Skjæraasen et al. 2010). Weight is an important energy proxy in determining the fecundity of fish because this is where energy and protein are stored, thus useful during oocyte recruitment and early vitellogenesis (Kennedy, Gundersen, and Boje 2009; Rodgveller 2019). Under ideal conditions, weight correlates strongly with fecundity (Tadesse 1997). Bigger sized fish are asserted to have higher fecundity than smaller fish (Barneche et al. 2018). Our findings showed that fish final weights were insignificantly different between treatment groups. Within each treatment group, final weight correlated strongly

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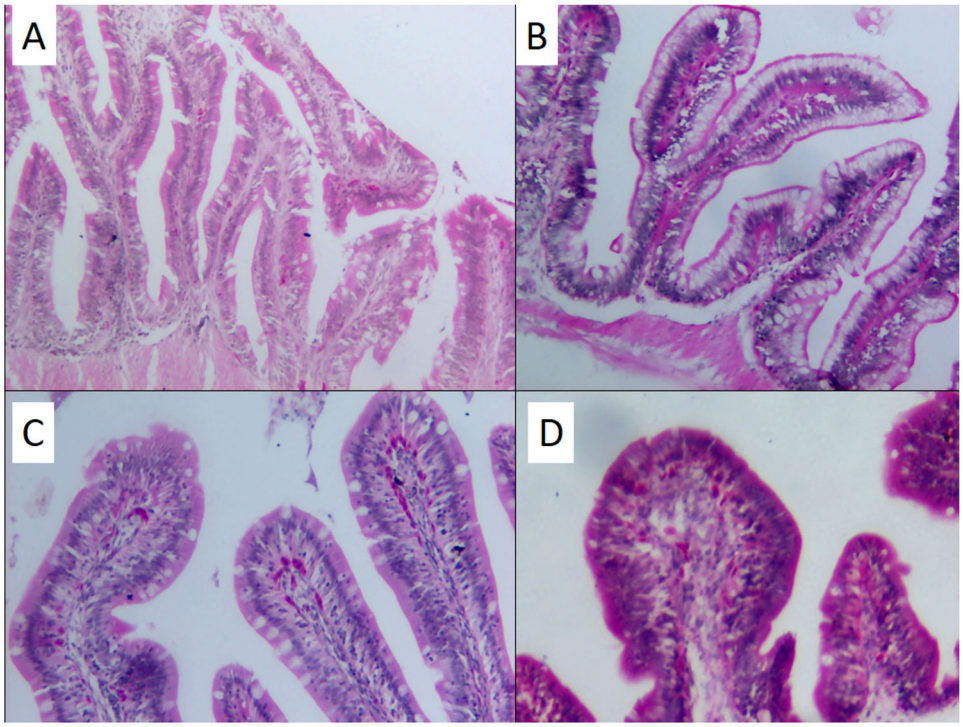

EXHIBIT 9 | Pathological effect in small intestine of *O. urolepis* exposed to various concentration of microplastics. (A) control, (B) 1 PE MPs/mL, (C) 10 PE MPs/mL, (D) 100 PE MPs/mL. [Color figures can be viewed at wileyonlinelibrary.com.]

with fecundity except in the 10 PE MPs/mL group, yet the correlation of their correlation coefficients was insignificant. The lack of difference in fecundity could therefore be attributed to the similarity in fish weight, which was unaffected by MP exposure across treatment groups. A similar trend was reported between fecundity and length in this study. Our findings are comparable to MP (McCormick et al. 2023) and non-MP (Jaspe and Caipang 2011; Shoko et al. 2014; Wagaw, Mengistou, and Getahun 2022) experiments in fish. Contrary to our findings, Wang et al. (2021) reported a strong variation in the final weight of marine medaka (*O. melastigma*) exposed to various concentrations of MPs higher than in our study and others that accounted for strong histopathological effect, oxidative stress, and reproductive impairment to fish.

HSI is a proxy of energy storage available for egg yolk formation in teleost fish through vitellogenin production (Rinchard and Kestemont 2003; Rodgveller 2019). Their changes are seasonal and normally reach a maximum prior to spawning. In our study, insignificant differences in HSI between treatment groups suggested that PE MPs-induced histopathological damage did not significantly affect energy storage in the liver, which had likely adapted to chronic stress. Our findings corroborated with those of McCormick et al. (2023), who exposed damselfish (*A. polyacanthus*) to MPs for 150 days. Furthermore, the lack of significant correlation between HSI and fecundity in all treatment groups, and between their correlation coefficients in our study, suggested that HSI is not a good indicator of fecundity. This is consistent with female fish in other experimental studies (Wang et al. 2019, 2021).

Prior to breeding, condition factors increase as fish accumulate energy reserves in preparation for egg production (Tadesse 1997). This increase is followed by a decline during spawning as energy is consumed for egg production and feeding activities decrease, especially in mouth brooders like *Oreochromis* spp. (Offem, Akegbejo-Samsons, and Omoniyi 2007; Tadesse 1997; Wagaw, Mengistou, and Getahun 2022). A high condition factor is associated with better fatty accumulation, a higher state of maturation, and gonad weight (Wagaw, Mengistou, and Getahun 2022). Fish with high condition factors are expected to have high fecundity (Offem, Akegbejo-Samsons, and Omoniyi 2007). The significant decline in condition factors observed in PE MPs treatment groups compared to the control indicates poor fish condition induced by MPs-induced stress, as also reported in Atlantic cod (*Gadus morhua*) (Mion et al. 2018) and planktivorous reef fish (*A. polyacanthus*) (Critchell and Hoogenboom 2018). The changes from a positive to a negative correlation shown between fecundity and condition factors in our findings, contrary to some studies (Rodgveller 2019), reveal the influence of PE MPs on this relationship.

The GSI and oocyte packing density are indicators of the developmental stage of oocytes, with high and low values indicating relatively matured eggs close to spawning (Dadzie and Wangila 1980; Kennedy, Gundersen, and Boje 2009). Tadesse (1997) reported a similar trend in the relationship between GSI and the maturity of *O. niloticus*, a close relative of *O. urolepis*. The lack of significant differences in GSI and oocyte packing density in our study suggests that oocytes were in a similar maturity stage and not influenced by PE MP exposure.

In all treatment groups, the best models explaining changes in fecundity were strongly influenced by the weight variable, except in the case of 10 PE MPs/mL. The model for 10 PE MPs/mL included condition factors in addition to weight, but neither

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variable significantly explained changes in fecundity. The magnitude of contribution of weight declined slightly with increase in MPs. The sudden increase and significant contribution of weight in the 100 PE MPs/mL group may be because other energy proxies (HSI and condition factor) were low, thus weight provided much energy for oocyte development due to the larger size of the fish. The inclusion of the condition factor and the insignificant contribution of weight in the 10 PE MPs/mL model may have been a compensation for the low weight of the fish. Overall, our models are comparable to those reported by Rideout and Morgan (2010) for four fish species collected from the wild. Kennedy, Gundersen, and Boje (2009) demonstrated that both HSI and condition factors were poor indicators of energy reserves which may explain lack of their contribution in the models.

The significant difference in PE MPs between new media and waste media in all PE MP treatment groups indicates that fish ingested PE MPs, reducing their concentration in the latter. The significant presence of the highest amount of PE MPs in feces suggests that fish ingested massive quantity of PE MPs. The ingested PE MPs were responsible for the observed histopathological damage and other direct or indirect malfunctions in fish as elucidated by changes in the composition of microbiota in the gut of fish, as found in zebrafish and common carp (Marana et al. 2022; Ouyang et al. 2021). Nonetheless, the possibility of MPs from water media embedding on the surface of floating feces cannot be ignored.

Our previous studies described histopathological damage in the gastrointestinal tracts of *O. urolepis* fish, with subsequent effects on their well-being and growth (Mbugani, Machiwa, Shilla, Joseph, et al. 2022; Mbugani, Machiwa, Shilla, Kimaro, et al. 2022). Similar findings have been reported in various fish species from the wild (Barboza et al. 2020) and in experimental setups (Critchell and Hoogenboom 2018; Ismail, Saleh, and Sayed 2021). Recent studies demonstrate that endocrine disruptions and oxidative stresses induce morphological damage to other organs, such as the liver and testes, which may not be in physical contact with MPs (Wang et al. 2021). These findings corroborate our results (Exhibit 8). Despite the reported histopathological impairments in the small intestines and livers, fecundity was not affected.

5 | Conclusion

This study has demonstrated that long-term exposure of *O. urolepis* fish to MPs from juvenile to adult has no significant effect on fecundity. However, the condition factors of PE MPs exposed treatment groups were significantly affected compared to the control group. Despite the lack of effect of MPs on fecundity, it is important to investigate other aspects such as the quality, hatchability, and delay of hatching of the offspring, as well as the quality of the fries. Additionally, the histology of eggs and hatchability need to be examined to determine the long-term influence of MPs on egg viability. Any impairment in eggs or fries could have subtle effects on fish development and eventual reproduction capability, which could have implications for fisheries resources management and the blue economy at large.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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