

# Micro Plastic Effects on Fresh Water Fish-Take Micro Plastic Polypropylene's Effects on Medaka Fish as an Example

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Microplastic with size less than 5 mm is very extensively used in the daily life. The microplastic are broadly release to the nature environment, such as the land, mountain, sea and river, and lots of the researches have showed microplastics growing pollution already impact the health of the plants, animals and human being. This paper reviewed the definition of microplastics, the research progress of the microplastics pollution to the environment, and effects of microplastics to the animal and human being. This research is to study micro polypropylene (PP) effects on body length, body weight and mortality of medaka, using the outcome of the experiment as an example to verify the effects of microplastics on freshwater fish. And provide theoretical basis for the government, enterprises, and society to better reduce the generation and emission of microplastics. Research approach: Case study, set 2 groups of Medaka, one group is control group the fish was growing in the pure water without the microplastic (PP), and another group is experiment group the medaka fish was growing in the pure water without microplastic (PP). At starting date and ending date of experiment I checked the length, weight and mortality of the medaka. I use the microscope to check the exiting of micro plastic in the fishes for both groups during the research. Research result, the microplastic (PP) has negative effect on length, weight, and mortality of Medaka. this research gives strong evidence to government, enterprise and society to reduce the plastic pollution to the environment through reduce, repeat or eliminate use of plastic. The research has the limitations: 1) The sample quantity is small. 2) there is no research on the mechanism of the effect of micro plastic (PP) on Medaka. So next research direction will be big quantity testing and more research on the mechanism of the effect of microplastic on fish.

**Keywords:** Micro plastic (polypropylene), Medaka, Microplastic distribution

## Introduction

Plastic products are extremely widely used worldwide and in all aspects of human life<sup>1</sup>. The use of plastics has reached 335 million tons from 1950 to 2016<sup>2</sup>.

Microplastics are plastic fibers, fragments, or particles with a diameter of less than 5 millimeters<sup>1</sup>, it is a major carrier of pollution. Compared to "white polluting" plastics, microplastics can easily affect aquatic organisms and cause environmental disasters, and they can remain in water for thousands of years without disappearing, and even if they are biodegradable, they are difficult to degrade in a short period of time, Thomson first proposed the concept of "microplastics" in 2004. Later, most of the studies found that most microplastics can be included in the diameter range of 5 mm, and the microplastics with a diameter of less than 5 mm are more abundant compared with the large particles of microplastics and can be absorbed by the organisms more easily, and they have a larger surface area and the ability to adsorb pollutants, thus they have a greater impact on the ecosystems.

The effect of microplastics on bio-toxicity currently mainly focuses on the effect of microplastics on survival, growth, repro-

duction, feeding activity, physiological and biochemical parameters and genetic changes of organisms.

There is no research on Effect of the microplastics (PP) on the freshwater fish.

The main purpose of this paper is to study the effect of micro plastic (polypropylene) on the length, weight, and mortality of medaka as an aspect to verify the effect of microplastics on freshwater fish. It also provides theoretical basis for the government, enterprises, and society to better reduce the generation and emission of microplastics.

## Literature Review

### Definitions of Plastic

Plastics are polymer compounds made from monomers that are polymerized through polymerization or condensation reactions. Various additives such as stabilizers, plasticizers, flame retardants, dyes and fillers are usually added to optimize the properties of the plastic. Currently, plastic products are extremely widely used worldwide and in all aspects of human life<sup>1</sup>. The use of plastics has reached 335 million tons from 1950 to 2016<sup>2</sup>.

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## Definition of Microplastics

Microplastics are plastic fibers, fragments, or particles with a diameter of less than 5 millimeters<sup>1</sup>, and are a major carrier of pollution. Compared to "white polluting" plastics, microplastics can easily affect aquatic organisms and cause environmental disasters, and they can remain in water for thousands of years without disappearing, and even if they are biodegradable, they are difficult to degrade in a short period of time.

## Microplastics exposure to the Environment, Animal, People

The microplastics with less than 5 mm diameter are more abundant compared with the large particles of microplastics and can be absorbed by the organisms more easily, and they have a larger surface area and the ability to adsorb pollutants, thus they have a greater impact on the ecosystems. Currently microplastics have been found in plankton<sup>3</sup>, bivalves<sup>4</sup>, birds<sup>4</sup>, fish<sup>2</sup>, and even in humans, microplastic abundance in freshwater fish such as porpoise ranged from 0-168.48 units/g<sup>2</sup>.

## Microplastics Exposure in the Sea and Freshwater

Eriksen evaluate that there are 5.25 trillion units of plastic exist in the sea<sup>5</sup>. Hulingling's research analyzed the distribution and characteristics of microplastics in 25 small water-bodies from the Shanghai city and Zhejiang provinces, which are the most populous and rapidly developing areas in China, and where production and use of plastics are significant. Microplastics were detected in surface water and sediment with abundances ranging from 0.5 to 21.5 units/L and 35.8 to 3185.3 units/L, respectively.<sup>1</sup>

## Biotoxic Effects of Microplastics

Such as polypropylene vinyl (PS), polyamide (PA) microspheres have no effect on the survival of spiny fish, gastropods, and pedipodal fishes<sup>2</sup>, and 0.05um PS has a significant effect on the survival of algae and copepods<sup>6</sup>, 0.05um PS affects clawed jenny with deformation of head and tail, shortening of body length and even embryonic death<sup>7</sup>, polyvinyl chloride (PVC) causes increased phagocytosis activity of immune cells of marine earthworms, and exposure of polypropylene (PP) crabs leads to decreased feeding and growth energy<sup>2</sup>, PS microplastics exposure resulted in decreased oyster oocyte number, diameter and sperm velocity, and reduced growth<sup>2</sup>, PS ion exposure resulted in enhanced antioxidant enzyme activity and hepatocyte necrosis in zebrafish<sup>8</sup>.

## Purpose of the Study

1. According to above research in sector 1.3.2 that microplastics is extensively existing in the fresh water. Poly propy-

lene account for 76% among the polymer plastic, and most of them are less than 0.5mm<sup>1</sup>. So far there is no research regarding the effect of the micro plastic (PP) on fish in freshwater in terms of length, weight, and mortality. The research regarding effect on microplastics (PP) on freshwater fish become very important.

2. The Medaka is a small fish native to East Asia that grows widely in small bodies of freshwater. The Medaka is now widely used in developmental biology, ecopathology, and cancer research<sup>9</sup>. It is a well-established experimental fish and is well suited for microplastics research. So, I choose Medaka to represent the freshwater fish for the research.
3. The main purpose of this study is to investigate the effect of micro polypropylene (PP) on the length, weight, and mortality of medaka. It can provide a theoretical basis for the government, enterprises, and the society to better reduce the production and elimination of microplastics.

## Research Hypothesis and Design

### Research Hypothesis

- *Micro plastic (PP) has effect on the length of medaka.*
- *Micro plastic (PP) has effect on the weight of medaka.*
- *Micro plastic PP has effect on the mortality of medaka.*

Note: PP refers to Polypropylene in this paper.

### Small Sample Testing-Experimental Preparation

#### Main Instruments:

- Electronic scale ( $\pm 0.001$ g) from Yingheng
- Beakers, Petri dishes, Filter funnels from 91 Labs
- Laboratory Microporous Filter Membrane Nitrocellulose (50mm 0.45um) from Chengde Laboratory Company
- Ruller

#### Chemical:

- 35% hydrogen peroxide from the lab
- Polypropylene particles (10um) from Xingyuan Fireproof Material Factory
- Purified water
- 70% alcohol

Medaka fish, fish food, and fish nets from the Experimental Zebrafish Model Center.

### Microplastics (PP) Solution Preparation Methods:

Take 0.19g particles dissolved in 10g 70% alcohol dispersion, and then diluted to 1L of pure water for backup, take 15ml of the prepared solution, add 300ml of pure water: The mass concentration of PP in water is  $0.19 \times 1000 \times 1.5\% / 300 \times 1000 = 9.5 \text{ mg/l}$ . This mass concentration equal to 15,262 Units/L micro-PP. According to research in 1.3.2 section, the concentration in Sediment is 3185.3 units/L of microplastics. So, we chose 15,262 Units/L concentrations, which is 5 times as natural concentration to do the reinforcement experiment. Since the testing time is 21days, much shorter than the natural environment.

**Table 1** The mass concentration (mg/L) of polypropylene at the concentration of 10,000 units/L (Hulingling, 2019)

	Units concentration	Mass concentration
Micro PP	10,000units/L	6.29g/L

## Experimental Procedures

The experiment set two medaka groups including the control group and experiment group, make sure the environment is same to except the experiment group grow in the water with PP while control group grow in the water without PP. This way to eliminate the confounding variables. At the starting date and ending date of experiment, each fish was checked the weight, the scale accuracy is  $\pm 0.001 \text{ g}$ . and each fish were checked the length, the ruler precise is 0.1mm. the average weight and length of 4 fishes in both groups were calculated at starting date and ending date. Then the average difference between starting date and ending data were calculated. Compare the average change between control group and experiment group to validate if there are effects of Microplastics (PP) on length and weight of Medaka. The mortality number of fish was calculated for control group and experiment group to see the effect of microplastics (PP) on mortality of Medaka. I also checked the filter paper weight that used to filter dissolved fish solution with PP and without PP to check the residue of microplastics (PP) in the body of medaka.

- Control group:** 4 medaka fishes grow in 4 beakers with 300 ml of pure water, which was changed every 2 days and fed with enough fish food every day.
- Testing group:** 4 medaka fish in 4 beakers with 300 ml of pure water, changed every 2 days and fed with enough fish food every day. Meanwhile, 15 ml of microplastics suspension was add in each beaker in the first week, second week, and third week.

- Each fish is measured by length and weight at the beginning and the ending date of the experiment. And died number also been checked.
- At the end of 3 weeks, medaka fish were dissolved through 33% hydrogen peroxide and filtered through filter paper to obtain the microplastics content in the body as follows steps:
  - Filter 2L of purified water through a 0.45um filter paper to avoid the confounding variables.
  - Filter 500 ML of 35% H<sub>2</sub>O<sub>2</sub> through 0.45um filter paper and set aside with the same re-season.
  - Wash each fish from experiment group for 3 times in 30 ml of filtered purified water and cut into 3 separate pieces and place them in a flask.
  - Add 30 ml of 35% H<sub>2</sub>O<sub>2</sub> to each flask with sliced fish and let it stand for 24 hours and then adding another 30 ml of hydrogen peroxide to dissolve it. During this time the flask is placed on a pan and heated with hot water for about 2 hours.
- Weighing the filter paper.
- After thorough dissolve the fish. the solution was filtered through filter paper and placed in a petri dish to dry naturally.
- Measure the weight of filter paper before and after filtration. the difference is the residual of microplastics (PP) in the Medaka.

## Analysis of Experimental Data and Conclusions of The Study

### Summary of Length and Weight Data

Four medaka fishes were into experimental group and another four medaka was put into control group, each fish was put into one beaker, and a prepared microplastics solution was added to the experimental group. The water was changed, and food was fed according to the plan. Body weight and length were measured at the beginning date and ending of three weeks. All condition keeps same except put put microplastics in experiment group. Fig. 1, Fig. 2 shows the experimental conditions.

Table 2 shows the experimental data. Each fish was measured the length and weight at the beginning and ending of the experiment for both control groups and experiment groups. The average weight and length at the starting and ending of the experiment was calculated and the difference of the average weight and length was calculated both in control group and experiment group.



**Fig. 1** Experimental and control group



**Fig. 2** Weighting process

### Recordings of PP Residues in medaka fish

According to the procedure as mentioned above, the fish was dissolved in the H<sub>2</sub>O<sub>2</sub> solution and filtered through the filter paper, the microplastics (PP) residue filtered on the paper. Put the filtered paper for natural drying. Then weight it on the scale to know the weight of the filter paper before filtration and after filtration, the weight difference is the microplastics (PP) residual (Fig: 03).

The weight difference is 0.023g, this indicate that microplastics (PP) exist in medaka in experiment group. at the same time the filter paper weight may impact the filter paper itself, so it is necessary to find another way to justify that micro plastic (PP) exist in body of madaka.

**Table 2** Microplastic residues in medaka

Filter paper weight before filter (g)	Filter paper weight after filter (g)	Micro PP residue in body of fish (g)
41.697	41.720	0.023

### Research Data Analysis

#### Analysis of length, weight data of medaka:

The average weight of control and experiment group at the beginning and ending date of experiment are calculated. The difference between average weight and length of starting date and ending date for both groups are calculated.

**Table 3** Length and weight change analysis

	Start date Oct 18th	End date Nov.7th
Average weight (g)	Control group without PP group 0.434	0.434
0.526	0.092	
	Experiment group with PP group 0.470	0.470
0.510	0.040	
Average length (mm)	Control group without PP 3.44	3.44
3.43	-0.01	
	Experiment group with PP group 3.58	3.58
3.55	-0.03	

Weight, for control group the start date average weight is 0.434 g and end date is 0.526 g, the weight increased 0.092g. for experiment group the weight increased 0.040g which is lower than the control group. The micro plastic(pp) has effect on the weight of the medaka (Fig 04).

Length, for the control group the start date average length for 4 fishes is 3.44 mm and end date is 3.43mm, the average length decreased 0.01 mm. for experiment group the length decreased 0.03 mm which is decreased quicker than the control group. Microplastics (PP) has effect on the length of madaka (Fig 05).

#### Mortality data analysis:

At starting date of the experiment there are 4 alive madaka in control group and 4 alive madaka in experiment group.

**Table 4** Mortality analysis

	Starting date Nov.18th	End date Nov.7th
Control group without PP	4	4
Experiment group with PP	4	4

There is no fish died on control group and experiment. From this test there is no effect of microplastics (PP) on mortality of madaka. Need to check further with big quantity of sample.

**Table 2:** Starting date and ending date body weight, body length

		Control group (Without PP)				Average	Experiment group (With PP)				Average
		1	2	3	4		1	2	3	4	
Weight (g)	Start date	0.369	0.502	0.453	0.413	0.434	0.524	0.413	0.419	0.524	0.470
	Nov 7 <sup>th</sup>										
	End date	0.553	0.524	0.54	0.488	0.526	0.591	0.416	0.618	0.416	0.510
	Oct 18 <sup>th</sup>										
Length (mm)	Start date	3.40	3.45	3.50	3.40	3.44	3.60	3.65	3.50	3.55	3.58
	Nov 7 <sup>th</sup>										
	End date	3.50	3.50	3.30	3.40	3.43	3.80	3.60	3.50	3.30	3.55
	Oct 18 <sup>th</sup>										
Weight (g)	Average difference					0.092					0.040
Length (mm)	Average difference					-0.01					-0.03



**Fig. 3** Dissolution filtration and weighing of microplastics in clear killifish (*Oryzias latipes*).

### Large Sample Testing - Testing with Fluorescent Microplastics (PP)

The quantity of medaka in each group were increased from 4 to 12 to better reflect the real situation and make the research more accountable. In order to verify that microplastics (PP) are indeed present in the body of medaka in the experimental group, we will use the fluorescent microplastics (PP) to conduct the test. And the check the existing of microplastics (PP) in medaka through microscope.

#### Main Instruments

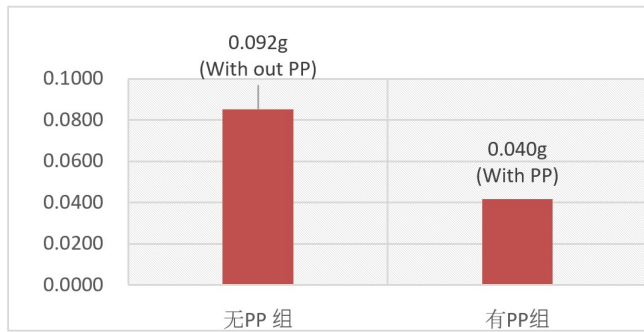
The main apparatus used in this experiment: Yingheng high-precision electronic scale (0.001g), beakers, ruler, fluorescent microplastics (PP) (10nm, maximum excitation wavelength: 470 nm, maximum emission wavelength: 526 nm) from Tianjin

Bexler Co. Microscope was Nikon SMZ25 with Nikon Ds-Ri2camera (Nikon, Tokyo, Japan). Medaka fish, fish food, fish nets, etc. from the Experimental Zebrafish Model Center

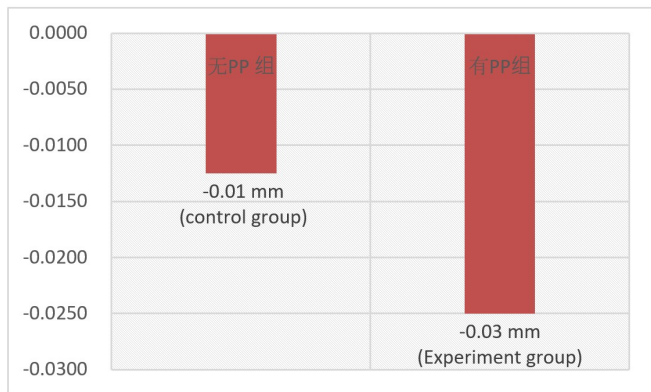
#### Experimental Design

There were two sets of groups:

1. Control group: 12 medaka fish were grow in 12 beakers with 300 ml of pure water which were changed every 2 days and fed with enough fish food every day.
2. Experimental group: 12 medaka fish were grow in 12 beakers with 300 ml of pure water, which were changed every 2 days and fed with enough fish food every day. At the same time, 2 ml/300 ml of fluorescent microplastics (PP) were placed in each beaker every week. The grow



**Fig. 4** average weight difference between control group and experiment group



**Fig. 5** average length changes between control group and experiment group

conditions remain same to avoid the confounding variables except to add the microplastics into the experiment group.

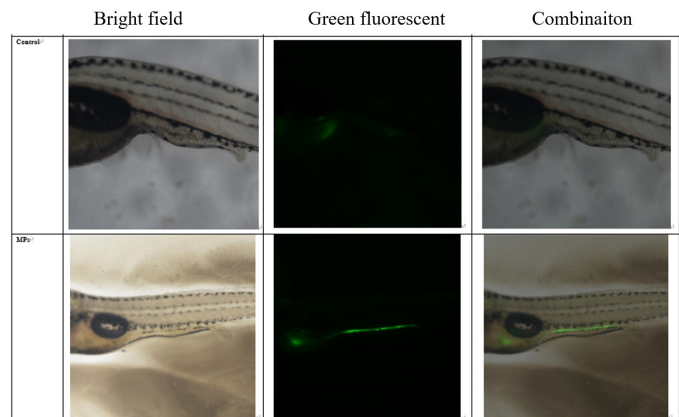
3. Measure the weight and length of each fish at the beginning and ending date of the experiment for both control group and experiment group.
4. Three weeks later I take one medaka from both the experimental and control groups to check the existing of microplastics residues in the medaka fish through microscope.

### Analysis and Conclusions

1. Residues of fluorescent microplastics (PP) in medaka. Below are the residues of fluorescent PP microplastics in medaka fish, the top row is the control group, and the bottom row is the experimental group, from the photos we can see the presence of microplastics in medaka fish in experiment group with fluorescent PP microplastics. While there is no PP microplastics in the control group.
2. Effect of microplastics (PP) on body weight and length. The average weight of 11 medaka at the beginning and

ending of experiment date was measured for both control and experiment group. the control group average weight difference is 0.042g, the average weight increased 0.042g. the experiment group average weight difference is -0.005g, the average weight decreased 0.005g. The microplastics (PP) has effect on the weight of Mekada. The average length of 11 medaka at the beginning and ending of experiment date was measured for both control and experiment group. the control group average length difference is 0.13mm, the average length increased 0.13mm. the experiment group average length difference is -0.02g, the average length decreased 0.02mm. The microplastics (PP) has effect on the length of Mekada.

3. Effect of microplastics (PP) on mortality The mortality number of medaka in control group is 7 and in experiment group is 9. The mortality number of fish in control group is higher than experiment group. they concentration of PP and testing quantity of Medaka may affect the result of the mortality. The microplastics (PP) has effect on the mortality of medaka.



**Fig. 6** Fluorescence microscopy of microplastic residues in madaka

### Hypothesis Validation

Micro plastic (PP) has effect on the length of medaka.	<b>Accepted</b>
Micro plastic (PP) has effect on the weight of medaka.	<b>Accepted</b>
Micro plastic (PP) has effect on the mortality of medaka.	<b>Accepted</b>

Medaka exposed to fluorescent micro plastics (PP), which are ingested into the body of the fish and can be visualized by microscope. The presence of this microplastics leads to a decrease in body weight, decrease in body length, and has effect on the mortality of medaka.

**Table 5** Effect of microplastics (PP) on body weight and length

		Start date April 19	End date May 1	Difference
Average weight (g)	Control group without PP group	0.262	0.304	0.042
	Experiment with PP group	0.314	0.309	-0.005
Average length (mm)	Without PP group	3.31	3.45	0.13
	With PP group	3.35	3.33	-0.02

**Table 6** Microplastic (PP) mortality effects on medaka

Mortality	Start date May 1	Ending date June 3	Died fish
Control group without PP group	9	2	7
Experiment group with PP group	9	0	9

The micro plastic (PP) effect on the length, weight and mortality of fish may be because of the micro plastic (PP) impact on the digest physiology, metabolic physiology, respiratory system, and reproductive effect. Micro plastic deposit in body also impacts the ingest behavior and motor behavior<sup>10</sup>. and the previous research shows micro plastic (PES) will damage the gill and intestinal canal. Above result is aligned with the previous research on the effect of micro plastic to ocean fish. While some other research indicates that micro plastic has no effect on length of fish<sup>1</sup>.

## Summary and Outlook

### Implication of the Study

#### Innovations and theoretical implications of this study:

Micro plastic (PP) has a huge pollution in fresh water. The effects of microplastic (PP) on Mekada in fresh water has not been studied in the same way before, and this research revealed that Mekada were exposed to microplastics (PP), residues could be observed. Micro plastic (PP) has negative impact on body weight and length growth of medaka. It also increased mortality of medaka.

#### Social implication of the study:

The experimental demonstration of the effects of microplastics on medaka can prompt the government, society, and the media to recognize the effects of microplastics on fish, which in turn may have an impact on the health of humans. and provide evidence for the government to designate a future microplastics control policy provide evidence for the community to reduce microplastics use and emissions.

#### Economic implication of the study:

Provide evidence for companies to reduce their use of microplastics and develop new material to replace plastic material for better innovation and sustainability.

### Research Limitation

The sample number is not big enough so it may not represent all situations. Moreover, this paper did not conduct the research on the mechanism of the impact of microplastics on fishes.

### Future Research Direction

Further study the mechanism of the impact of microplastics on fishes, Toxicological studies and larger sample size studies to ensure the principle of universality of research.

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