

Sex Specific Effects of PET-MPs On Drosophila Lifespan

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Abstract

In recent years, as an emerging pollutant, microplastic pollution is gradually becoming a research hotspot. Microplastics are ubiquitous in the entire ecological environment. Organisms can be exposed to microplastics via inhalation or ingestion. In view of the widespread of microplastics pollution, the impact of microplastics on biology should be further investigation. In previous experiments, we have conducted research on the physiology of *Drosophila* exposed to PET Microplastics (PET-MPs). However, under long-term exposure, will PET-MPs affect the lifespan of *Drosophila*? Our experimental results indicate that for ANOVA analysis, there are significant differences between males and females ($F(1, 895) = 68.19$, $p < 0.001$), between PET-MPs concentration ($F(3, 895) = 8.11$, $p < 0.001$). There are also significant interactions between sex and Microplastic concentration ($F(3, 895) = 4.00$, $p < 0.01$). For Cox and log rank test, 1g/L of PET-MPs prolongs the lifespan of male flies. The reason of this phenomenon may be the hormesis effect.

1 Introduction

Microplastics are plastic fragments with a diameter of less than 5 mm. Since 2004, British scientists first proposed the concept of microplastic (Thompson et al., 2004), the researchers on microplastic pollution are gradually deepening. Previous studies have illustrated the observations of microplastic deposition in the atmosphere (Chen et al., 2020), water (Sharma and Chatterjee, 2017), soil (Rillig and Lehmann, 2020), as well as common foods such as oysters, mussels, fish, sea salt, honey (De-la-Torre, 2020).

Microplastics even exist in the atmospheric sediments of remote mountainous (French Pyrenees) (Allen et al., 2019) and in the frozen layers of the Antarctic (Kelly et al., 2020). These researches are conveying an important message to us: microplastics pollution are ubiquitous around the world, and we must pay attention to the potential effects of microplastics pollution.

Humans can be exposed to microplastics via inhalation and ingestion. Will microplastics affect the human body? It has been reported that nylon flock workers are much higher exposed to harmful fibers than the general person. Microplastic fibers may irritate the lungs, causing lung volume decreased (van Dijk et al., 2021). Kelly and Wright predicted that the intake of microplastics may wreck immune cells, induce cell necrosis and tissue inflammation (Wright and Kelly, 2017). And nanoplastics may cause the more serious impact. The potential health risks of nanoplastic can be assessed the same as engineered nanoparticles (Chain, 2016). Research studies revealed that the size and hydrophobicity of nanoparticles enable them to pass through the blood-brain barrier and placenta into the lungs and intestines (Seltenrich, 2015), and cause changes in endogenous metabolites and the intestinal microbial community (Bergin and Witzmann, 2013; Cui et al., 2019). The impact of microplastics on human health has not yet been determined. In this stage, continuous experiment is required to have a comprehensive understanding on the potential hazards of microplastics.

We previously explored the effects of polyethylene terephthalate microplastics (PET-MPs) on the physiology of *Drosophila*. Our findings demonstrated that PET-MPs can affect the number of eggs laid by

female flies and the TG content of male flies (Shen et al., 2021). Under the long-term exposure, can animals regulate immune system and offset the impact of PET-MPs. Based on the question, we investigated the effect of PET-MPs on the flies' lifespan. The results showed that microplastics had no significant negative effect on the lifespan of female flies. To our surprise, the lifespan of male flies increased at the concentration of 1g/L. This is an interesting experimental phenomenon.

2 Materials And Methods

Drosophila Culture and Life Span Assays

The *Drosophila melanogaster* wild-type stock Canton-S (Bloomington Stock Center) was maintained on a 12 h:12 h light: dark cycle at the temperature of 25°C, humidity of 60% and light intensity of 500 Lux. Enclosing adult flies were collected over a 24-hour period and mated for 48 hours before sorting into male vials or female vials. Life span experiments were conducted with a density of 40 flies per vial on sugar – yeast – agar (SYA) medium. Flies were transferred to new vials every other day and the number of deaths was counted.

Preparation of PET-MPs suspensions

PET-MPs of 2µm were used in this experiment. The ethanol and water were prepared to facilitate particle distribution in the 1:1 ratio. That is, suspend 1 g, 10 g and 20 g PET-MPs into 60 ul ethanol and 60 ul H₂O. So as to make it evenly mixed in the food, magnetic stirring was conducted for 2 hours at first, and before the food was finished, ultrasonic vibration was executed for 30 minutes to make it evenly dispersed. When the food temperature is cooled to 60°C, 1 g/L, 10 g/L or 20 g/L PET-MPs was added to 1 L of food respectively. The 60ul ethanol and 60ul H₂O was add for the control group.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v. 6 and SPSS 25.0. 2-way ANOVA was conducted on gender and microplastic concentration analysis. The mean, median, maximum, minimum, Log rank tests and COX proportional hazards analysis were performed on survival curves.

3 Results

In our experiment, we investigated the effect of PET-MPs of different concentration on the flies' life span. The 2-way ANOVA test reveals that there are significant differences between males and females ($F(1, 895) = 68.19, p < 0.001$), between PET-MPs concentration ($F(3, 895) = 8.11, p < 0.001$). Moreover, significant interactions were observed between sex and PET-MPs concentration ($F(3, 895) = 4.00, p < 0.01$) (Table 1). To understand how the interaction affects survival, we further investigated simple effect.

Table 1
Statistical summary for 2-way ANOVA.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	24295.098 ^a	7	3470.728	14.578	.000	.102
Intercept	951623.922	1	951623.922	3997.020	.000	.817
food	5794.004	3	1931.335	8.112	.000	.026
sex	16235.863	1	16235.863	68.194	.000	.071
food * sex	2855.498	3	951.833	3.998	.008	.013
Error	213084.605	895	238.083			
Total	1191596.000	903				
Corrected Total	237379.703	902				
a. R Squared = .102(Adjusted R Squared = .095)						
Food indicates the concentration of PET-MPs. 1 is control, 2 is 1g/L, 3 is 10 g/l, 4 is 20 g/L.						

Simple effect result presented that for Control, 1g/L, 10g/L group, at the same concentration, the mean lifespan between female and male presence significant difference (Table 2). Under the same sex, 10 g/L PET-MPs caused 20.41 % decrease in female mean life span, and 20 g/L PET-MPs caused 16.0 % decrease in male mean life span (Table 3). But ANOVA is a significant test based on the mean of the sample (Fisher, 1992). Therefore, we conducted Cox analysis and log rank test to analyze under the same sex, the significance of microplastics on the lifespan of flies.

Table 2
Simple Analysis of sex on the 4 Levels of PET-MPs Concentration

Dependent Variable: lifespan							
sex	(I) food	(J) food	Mean Difference(I-J)	Std Error	Sig. ^b	95% Confidence interval for Difference ^b	
						Lower Bound	Under Bound
0	1	2	1.910	2.031	.923	-3.444	7.264
		3	6.362*	2.098	.015	.831	11.893
		4	3.325	2.018	.468	-1.997	8.646
	2	1	-1.910	2.031	.923	-7.264	3.444
		3	4.452	2.085	.182	-1.046	9.949
		4	1.415	2.005	.980	-3.872	6.701
	3	1	-6.362*	2.098	.015	-11.893	-.831
		2	-4.452	2.085	.182	-9.949	1.046
		4	-3.037	2.073	.604	-8.502	2.428
	4	1	-3.325	2.018	.468	-8.646	1.997
		2	-1.415	2.005	.980	-6.701	3.872
		3	3.037	2.073	.604	-2.428	8.502
1	1	2	-4.265	2.045	.204	-9.657	1.127
		3	1.814	2.065	.943	-3.631	7.259
		4	6.027*	2.022	.018	.695	11.358
	2	1	4.265	2.045	.204	-1.127	9.657
		3	6.079*	2.100	.023	.541	11.617
		4	10.292*	2.058	.000	4.865	15.718
	3	1	-1.814	2.065	.943	-7.259	3.631
		2	-6.079*	2.100	.023	-11.617	-.541
		4	4.213	2.078	.231	-1.266	9.691

b. Adjustment for multiple comparisons: Sidak.

"sex1" indicates female ,2 is male. Food indicates the concentration of PET-MPs. 1 is control, 2 is 1g/L, 3 is10 g/l, 4 is 20 g/L.

Dependent Variable: lifespan						
4	1	-6.027*	2.022	.018	-11.358	-.695
	2	-10.292*	2.058	.000	-15.718	-4.865
	3	-4.213	2.078	.231	-9.691	1.266
Based on estimated marginal means						
*. The mean difference is significant at the .05 level.						
b. Adjustment for multiple comparisons: Sidak.						
"sex1" indicates female ,2 is male. Food indicates the concentration of PET-MPs. 1 is control, 2 is 1g/L, 3 is10 g/l, 4 is 20 g/L.						

Table 3
Simple Analysis of PET-MPs Concentration on the 2 Levels of sex

Dependent Variable: lifespan							
food	(I) sex	(J) sex	Mean Difference(I-J)	Std Error	Sig.b	95% Confidence interval for Difference b	
						Lower	Under
1	0	1	-6.486*	2.026	.001	-10.463	-2.509
	1	0	6.486*	2.026	.001	2.509	10.463
2	0	1	-12.661*	2.049	.000	-16.683	-8.639
	1	0	12.661*	2.049	.000	8.639	16.683
3	0	1	-11.034*	2.135	.000	-15.224	-6.844
	1	0	11.034*	2.135	.000	6.844	15.224
4	0	1	-3.784	2.014	.061	-7.736	.168
	1	0	3.784	2.014	.061	-.168	7.736
Based on estimated marginal means							
*. The mean difference is significant at the .05 level.							
b. Adjustment for multiple comparisons: Sidak.							
"sex1" indicates female ,2 is male. Food indicates the concentration of PET-MPs. 1 is control, 2 is 1g/L, 3 is10 g/l, 4 is 20 g/L.							

For log rank test, our result indicated that PET-MPs had no effect on the lifespan of female flies (Fig. 1A). However, it is interesting that 1g/L PET-MPs prolongs the lifespan of male flies ($P < 0.05$) (Fig. 1B) (Table 4). The media life span of male flies was augmented by 9.52 %. At the concentration of 20 g/L, the average life span of male flies was reduced by 17.39 %, but the $p > 0.05$. KM is a non-parametric test while Cox is a semi-parametric test with higher test effect. Therefore, we choose Cox to verify the experimental conclusions (David, 1972). For Cox analysis. the results were consistent with log rank test (Table 5, Table 6).

Table 4
Statistics of life span with Log rank test assay

Drug	Sex	N	Mean	Median	Min	Max	Δ Mean	Δ Median	<i>p</i> value
con	F	113	31.16	38	2	50	-	-	-
1 g/L	F	117	29.25	36	2	52	-6.13	-5.26	0.24
10 g/L	F	103	24.80	32	2	52	-20.41	-15.80	0.69
20 g/L	F	120	27.63	34	4	52	-10.69	-10.53	0.65
con	M	118	37.64	42	2	60	-	-	-
1 g/L	M	110	41.91	46	2	66	11.34	9.52	0.000026*
10 g/L	M	106	35.83	38	2	66	-4.81	-9.52	0.76
20 g/L	M	115	31.62	38	2	62	-16.0	-17.39	0.5

Table 5
Statistics of female life span with Cox assay

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
Food			.887	3	.829			
Food (1)	.123	.132	.878	1	.349	1.131	.874	1.465
Food (2)	.060	.137	.193	1	.661	1.062	.811	1.391
Food (3)	.052	.131	.157	1	.692	1.053	.815	1.362

Table 6
Statistics of male life span with Cox assay

	B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
							Lower	Upper
food			18.669	3	.000			
Food (1)	-.457	.135	11.442	1	.001*	.633	.486	.825
Food (2)	-.062	.135	.211	1	.646	.940	.721	1.225
Food (3)	.084	.132	.409	1	.522	1.088	.840	1.408

4 Discussion

Before the experiment, we surmised the PET-MPs to increase mortality. But the experimental results, PET-MPs did not cause high mortality, which is different from our envision. One explanation of the result could be that the chemical properties of PET is highly inert and it has been designed for food packaging purposes (Zimmermann et al., 2019). The previous reports highlighted PET-MPs do not negatively affect the survival, metabolism and feeding activity of *Gammarus pulex* (Weber et al., 2018). There was no significant effect on the head capsule lengths, weight, HSP70 level in *Chironomus riparius* exposed to PET microfibers (Setyorini et al., 2021). Another potential explanation is related to peritrophic matrix (PM). PM, mainly composed of chitin and protein (Lehane, 1997), is a four-layered membrane secreted by *Drosophila cardia* (King, 1988). PM acts as physical barrier between innergut epithelial cell and food, and can protect innergut epithelial cells against mechanical damage caused by granular food (Hegedus et al., 2009). It has been reported that PET-MPs are densely packaged in the digestive tract of *Gammarus pulex*, indicating they may be covered in the PM and subsequently processed (Weber et al., 2018). According to this hypothesis, we speculated that the PM protects the flies from the damage of PET-MPs to the digestive system. Thus, no negatively effect was observed on flies exposed to PET-MPs. The possible explanation for the result could be due to the purification effect of microplastics. *Drosophila* exposed to 1µm of Polystyrene fluorescent microplastics, cleared them in 24 h (Matthews et al., 2021). The diameter of flies' gut is 160µm (Buchon et al., 2013), and the larger particles are easy to form intestinal obstructions. The size of the microplastics used in our experiment is 2µm. We surmised that the microplastics may be excreted smoothly from flies' intestines.

An interesting phenomenon was found in our experiment. The exposure to 1 g/L PET-MPs increased the lifespan of the flies. The possible reason for this phenomenon is hormetic effects. Slight stress would disturb the homeostasis of the organism, forcing the animal to make adaptive response. Slight stress can improve functional capacity of the organism and even prolong longevity: this phenomenon is called hormesis (Minois and Rattan, 2003). Exposure to mild stress, such as hypergravity (Le Bourg and Minois, 1999), heat shock (Sørensen et al., 2007), low dose radiation (TG, 2003), cold shocks (Le Bourg, 2007), can slightly extend the life of flies. We speculated microplastics can also be seen as a mild stress.

Exposure to PET-MPs induces faster growth in the giant snail *Achatina reticulata* (De Felice et al., 2021). Low concentration of MPs can stimulate the activity of SOD, and activate the antioxidant system, while SOD activity and the destruction of oxidant system at high concentrations (Trestrail et al., 2020). In our previous study, PET-MPs enhanced the spontaneous activity of flies. We speculated that microplastics can induce the adaptive responses of flies and enhance biological performance to extend the lifespan of flies.

Interestingly, the phenomenon of prolonging life only appeared in male flies. Sex specific hormetic in *Drosophila* is very common. The beneficial effects of mild stress often occur in male flies. For example, hypergravity (Le Bourg et al., 2000), irradiation (TG, 2003) and heat shock (Sørensen et al., 2007), induced hormesis for life span restricted to male. The reason could be that male flies and female flies respond differently to environmental intervention. The same genes in different sexes act in various environments. For example: *mei-41* is a gene encoding the protein of DNA repair (Sekelsky et al., 2000). Furthermore, *mei-41* gene is necessary for hormesis, and the lack of hormesis is shown in mutants with inactivated *mei-41* (Moskalev et al., 2011). After radiation exposure 72 hours, *mei-41* is overexpressed in male flies. Whereas there are no expression changes of *mei-41* in female flies (Zhikrevetskaya et al., 2015). Another reason may be related to the dissimilar metabolic levels on flies. Recently reported intestinal sex differences in metabolic gene expression (Hudry et al., 2016). sex-biased intestinal metabolism might contribute to sex differences in whole-body. In previous experiment, our results found that microplastics have a significant effect on male lipid and glucose content, but no obvious effect was observed on female flies. There is no doubt that many differences in development and physiology exist between male and female flies. Hence, in future experiments, the issue of sex specific deserves further investigation.

Despite no negative effects of PET-MPs on the flies' lifespan in this experiment, we still cannot ignore microplastics potential risks. Plastic products contain additives such as antimicrobial agents, heat stabilizers, plasticizers, flameretardants, flame retardants, UV stabilizers, pigments, fillers, and to provide specific properties (Lithner, 2011), while pure plastic powder was chosen in our experiment. Studies have shown that at the highest leachate of recyclable plastics, the survival of barnacle *Amphibalanus amphitrite* larvae was significantly lowered (Li et al., 2016). In addition, due to higher surface area, microplastics are easier to accumulate persistent organic pollutants (POPs) such as fluorobenzenes (CBS), perfluorochemicals (PFCS), polyfluorobiphenyls (PCB), heavy metals, viruses, bacteria, and so on (Mato et al., 2001). A recent study indicated that MPs can aggravate the toxicity of Cadmium and induce an enhancement of gene silencing in somatic tissues of *Drosophila* (Zhang et al., 2020). Therefore, evaluating the synergistic effects of MPs interaction with additives and environmental pollutants is critical in future studies.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

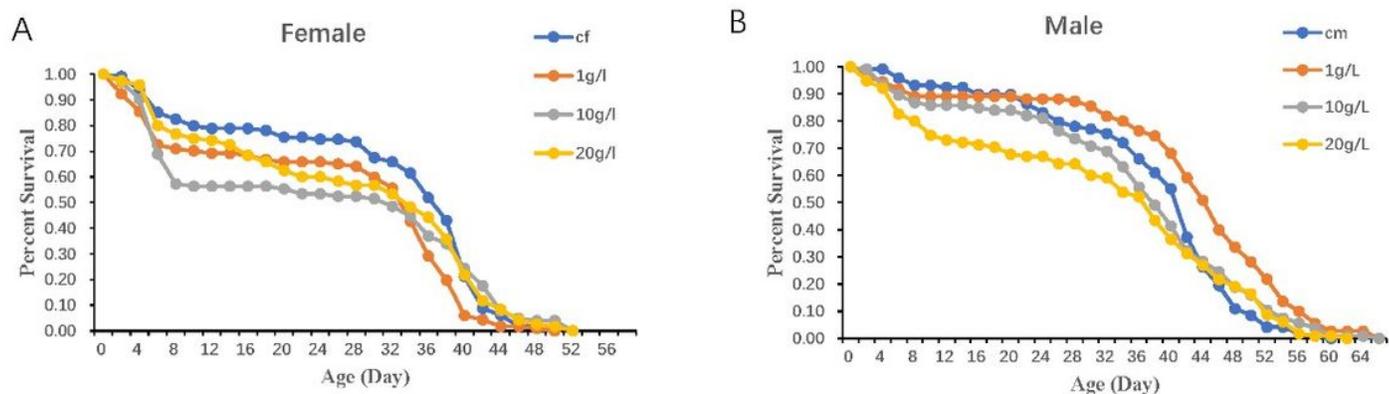


Figure 1

The effect of PET-MPs on *Drosophila melanogaster* survival at Control, 1 g/L, 10 g/L and 20 g/L. (A) Female flies. (B) Male flies.