

## Microplastics Reduce the Growth of Exposed Marine Invertebrates: A Meta-Analysis

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### ABSTRACT

There is a need for a quantitative approach to ascertain whether microplastic pollutants functionally affect exposed organisms. This meta-analysis aimed to determine if microplastics reduce the mean growth of exposed aquatic invertebrates in the marine environment. Twelve studies investigating microplastic exposure were submitted to meta-analytic techniques to obtain the overall combined effect. The random effects model was used. Standardized mean difference in growth was reported as Hedges' *g* value. The robustness of the data was confirmed through a leave-one-out method of sensitivity analysis. Results shown by forest plot suggested that overall, the samples exposed to microplastics had lesser mean length after the exposure period as compared to samples not treated with microplastics. Mean difference in growth was equal to -1.324, as given by standardized Hedges' *g*. A sensitivity analysis using leave-one-out method further showed that the data were robust, confirming overall reduction in growth. Growth reduction could be attributed to the size-selective ingestion of microplastics by the organisms, whereby they ingest smaller microplastic particles more readily than larger ones. Ingestion and egestion of microplastic particles entail an energetic cost for organisms, consequently reducing energy devoted to growth. The findings showed that microplastics can negatively influence the growth and eventually the overall well-being of marine organisms.

**Keywords:** Forest plot, Growth, Meta-analysis, Microplastics

### INTRODUCTION

First reports of plastic litter in the oceans emerged in the 1970s (Andrady, 2011). About 10 % of plastics worldwide persist and accumulate in ocean gyres and sediments (Thompson *et al.*, 2004). Moreover, the marine environment is estimated to have > 5 trillion pieces of plastic debris afloat (Eriksen *et al.*, 2014). However, the plastic debris can be transformed into smaller fragments known as microplastics. Weathering-related fracturing and surface-embrittlement of plastic debris due to physical, biological and chemical processes lead to formation of microplastic particles (Andrady, 2011). Microplastics have already been found to

invade the aquatic environment, posing risks to susceptible organisms.

Eighty percent of plastics found in the marine litter are contributed by terrestrial sources (Cole *et al.*, 2011). The indiscriminate disposal of plastic waste items that are directly or indirectly transferred to the aquatic environment poses concern of microplastic pollution. Samples from surface waters and beach sand contain litter that includes resin pellets and smaller fragments of plastics derived from larger plastic debris (Andrady, 2011). This shows that marine microplastics have been introduced from land sources via runoff and through degradation of larger plastics afloat on the ocean.

Increased research activity has aimed to understand the mechanism of microplastic ingestion. The uptake of microplastics by marine organisms may occur through passive filtration and deposit-feeding (Barboza *et al.*, 2018). For instance, bivalves such as blue mussels are suspension-feeders that can filter around two liters of seawater every hour; therefore, they could ingest microplastics from the surrounding waters (Nerland *et al.*, 2014). These suspension- and filter-feeding organisms at the lower trophic level are particularly susceptible to microplastic ingestion due to their limited ability to differentiate between plastic particles and food (Cole *et al.*, 2011).

From an array of studies investigating filter-feeding organisms, samples taken from various locations often contained plastic debris. For instance, edible mussels in the Philippines (Argamino and Janairo, 2016) were found to possess microplastics. Even oysters grown in aquaculture for human consumption can contain microfibers (Jauregui, 2017). These findings raise possible health concerns for the consuming public.

Due to their potentially negative effects (Cole *et al.*, 2013), studies on the impacts of microplastics have been extensively conducted. An array of impacts received emphasis as scientists claimed that microplastics have debilitating consequences, one of which is the reduction in growth of organisms. Laboratory observations of bivalves and crustaceans have shown that growth reduction is one of the adverse effects of exposure to concentrations of microplastics (Welden and Cowie, 2016; Straub *et al.*, 2017).

The growing number of published studies on the effect of microplastic exposure on growth of organisms seem to present conflicting results, with some studies showing negative effects and others showing no effect. In addition, if there is indeed a negative effect on growth, one has to determine if the size of such effect is sufficient to justify the concern of negative impacts. Aquatic invertebrates, including mollusks and arthropods (crustaceans), play important roles in the aquatic food chain and therefore need to be considered in studying the effects of microplastics (Green *et al.*, 2016; Green, 2016).

The basis as to whether microplastics have a measurable negative effect on the growth of exposed organisms is limited and scattered, and many scientists are uncertain if such effect is functionally significant. Widely acclaimed effects have been shown in marine and freshwater mollusks (Green, 2016; Imhof and Laforsh, 2016), crustaceans (*Gammarus fossarum*, *Gammarus pulex* and *Hyaella azteca*, among others), and marine worms (Green *et al.*, 2016; Welden and Cowie, 2016; Vasilakis, 2017). Several studies however, claimed that the growth of organisms exposed to microplastics was not at all affected (Bruck *et al.*, 2018). Determinants used to measure the effect on growth are the changes in either the body length or body mass of the organism after exposure. Indeed, there is a need to better understand and quantify the effect of microplastic uptake on the growth of aquatic organisms.

In this study, a systematic review of the most relevant scientific literature that meets the inclusion criteria was achieved through a meta-analysis. Specifically, it determined if the growth of aquatic invertebrates is negatively affected by exposure to microplastics; it explored the extent of the growth effect through analysis of effect sizes to verify if there is a significant reduction in growth; and, it identified the factors that could have contributed to the observed effect of microplastics on the growth of organisms under study.

## MATERIALS AND METHODS

### *Selection criteria*

Searches using the Web of Science website and Google Scholar search engine consisted of the keywords “Microplastic and growth,” “Microplastic and effect,” and “Microplastic and impact.” The techniques known as forward chaining and backward chaining of references were also performed to obtain studies of similar nature. The researchers looked for studies conducted in line with measuring the effect of microplastic on the growth of different aquatic organisms that were conducted from the year 2010 to the present (July 2019). In addition to the nature of study and date of publication,

consideration was given to the type of subjects in the study. Since the concern is on aquatic organisms, the researchers specifically focused on those, such as aquatic invertebrates. They were categorized by phylum, namely arthropods (crustaceans, brine shrimp, isopods, amphipods) and mollusks (gastropods, bivalves). All of these animals are essential players in the aquatic food chain, which was also part of the consideration for the researchers.

The criteria for choosing studies to be included in the meta-analysis are the following: (1) the study must involve the growth of the exposed organism; (2) the measure of growth is in terms of the difference in mean body length after and before the experiment; (3) the subject organisms are aquatic invertebrates that are commercially important species; (4) organisms are subjected to control (no microplastics) and treatment (with microplastics) conditions; (5) other intervening factors such as pH, temperature, and oxygen content are kept constant in both treatment and control groups to ascertain that any effect can be attributed solely to microplastic exposure; (6) experiments are done in laboratory through assays or microcosms mimicking the actual environmental conditions where the organisms inhabit; (7) mean growth and standard deviation are obtained after the experimental procedure; (8) microplastics used in the treatment group are those that represent the most plausible type of microplastic ingested by exposed organisms in the aquatic environment where they are found.

There were 43 studies initially identified based on the input operators and search terms; however, only 12 studies satisfied the criteria set above for the research. They were outlined in Table 1 (see Results and Discussions). These were used as the final source of literature for analysis of microplastic effect on growth.

#### *Data analysis of effect sizes*

The published studies included in this meta-analysis reported mean, standard deviation and sample size for both control and treatment groups. Our goal was to determine to what extent there was a reduction in growth, or in other words, the magnitude of the effect of microplastic exposure

on the samples. This is known as effect size. The null hypothesis for this meta-analysis was that there was no difference in the growth of organisms between control and treatment groups and this had to be confirmed by determining the combined effect from different studies. The authors did not report effect sizes, so the next step was to identify the effect size for each of the studies by analyzing their data points. Thereafter, we determined the effect size in the population by combining the effect sizes obtained from the individual studies (Field, 2005). This population effect size is known as the combined effect.

The measure of effect size in this paper is represented in terms of standard mean difference, known as Hedges' *g*. This was adopted since not all studies in this meta-analysis used the same scale (Borenstein *et al.*, 2007). In this case, the scale for each study is different primarily because the investigators did not use the same instruments to obtain data on the growth of organisms. Thus, there was a need to standardize the effect size in terms of Hedges' *g*. Using the software application Comprehensive Meta-Analysis (CMA), data points for the 12 studies were entered to obtain the standardized mean difference. This software automatically calculated the value of Hedges' *g* for each individual study given the mean, standard deviation and sample size of the control (no microplastics) and treatment (with microplastics) groups.

To estimate the overall or combined effect, two models can be used: fixed effect (general linear model) or random effects model (Ellis and Kong, 2009; Ellis, 2010). These both show analysis for weighted effect sizes; however, there are considerations for their use. The fixed effect model is sometimes disadvantageous and is criticized for giving too little weight for studies with small sample sizes and giving too much weight for those with large samples. In the random effects model, the assumption is that large studies may yield more precise estimates than small studies; however, each of the studies included here estimated a different effect size (Borenstein *et al.*, 2007). Therefore, in the random effects model, the weights assigned for each study are more balanced; in the fixed effect

model, in contrast, large studies are likely to be given more weight or dominate the analysis, and small studies are likely to be underestimated (Borenstein *et al.*, 2007).

In this meta-analysis, we examined studies that compared the effects of microplastics on the growth of marine invertebrates (exposed to microplastics versus not exposed to microplastics or control). If growth was negatively affected by the presence of microplastics, we should expect the effect size to be similar but not identical across the studies included. The impact of microplastic exposure on the growth of samples might have been more pronounced in some studies where organisms used are naturally more susceptible to microplastic ingestion. Therefore, in this analysis, random effect weights were assigned to each study.

The effect size of each individual study and the overall combined effect were plotted in Microsoft Excel 2016 version to create a forest plot. The plot was visually analyzed to determine if there was a significant decrease in the growth of exposed organisms. The forest plot shows a vertical “line of no effect,” and if the effect size is plotted at the right side of this line, there is a higher growth in the treatment group. If plotted at the left side, it denotes the opposite scenario, where there is more growth in the control, given the same environmental parameters as the treated samples. The latter scenario would mean that microplastic exposure reduced the growth of organisms in the microplastic-treated samples as compared to control samples.

To ensure that the data shown in the meta-analysis were robust, and to test for the presence of outliers, a sensitivity analysis was conducted using the “leave-one-out” method (Bruno *et al.*, 2017; Penn State Eberly College of Science, 2018). Four studies deviated markedly from the other studies and were considered as outliers. Using this method, each of the four studies was removed individually, and a meta-analysis was conducted on the remaining studies. If the combined effect size for the remaining studies is consistent with the combined effect considering all twelve studies, then there is confidence that the overall meta-analysis is robust.

## RESULTS AND DISCUSSION

Twelve scientific studies recording the effect of microplastics on growth of aquatic organisms, specifically mollusks (e.g. oysters, mud snails, clams) and aquatic arthropods (e.g. water fleas, amphipods, brine shrimp) were reviewed (Table 1). Each study compared the growth of organisms in control (no microplastics) and treatment (with microplastics) groups. The combined effect size using the random effects model was -1.324 (not shown), as given by standardized Hedges'  $g$ . This value represents the difference in growth between the two groups (no microplastics versus treated with microplastics).

A forest plot was used to represent the 12 studies with the combined effect size of -1.3241 (Figure 1). There was considerable heterogeneity in the effect sizes of the study results with I-squared ( $I^2$ ) value of 96.6 %. The vertical “line of no effect” of microplastic exposure (Hedges'  $g=0$ ) in the figure can be interpreted as no difference in growth of the two groups. In this meta-analysis, the solid dots/shapes and the associated horizontal line represent an effect size for the individual study and its 95 % confidence interval (Figure 1).

It was shown from the combined effect size that there was a significant reduction in the growth of organisms when treated with microplastics (Hedges'  $g=-1.3241$ ), with the confidence interval not reaching the zero value (Figure 1). Looking at the individual studies, four of them (Ziajahromi *et al.*, 2017; Redondo-Hasselerharm *et al.*, 2018; Ziajahromi *et al.*, 2018) markedly deviated from the effect size values of the rest of the studies. The others showed lower growth in the treatment groups, but this reduced growth was not significant, as indicated by the confidence interval overlapping the vertical “line of no effect.” It was assumed that the four studies had enough influence on the combined effect size to pull it towards the negative side. Therefore, the strength of data was tested to establish how robust the analysis was. With the use of the leave-one-out method of sensitivity analysis for the strength of data, it was found that when omitting each of these four studies from the analysis, the overall effect size remained consistent.

Table 1. List of studies alphabetically arranged based on author name showing the range of microplastic sizes used and the average mean growth (in millimeters) between control and treatment groups. Studies denoted with letters (a, b and c; d and e) are part of one published paper investigating different species.

Study	Microplastic size range ( $\mu\text{m}$ )	Average mean growth (mm)	
		Control	Treatment
Green (2016)	65.6	5.50	5.00
Imhof and Laforsch (2016)	4.64-602	3.84	3.84
Kokalj <i>et al.</i> (2018) <sup>d</sup>	20-500	0.99	0.798
Kokalj <i>et al.</i> (2018) <sup>c</sup>	20-500	0.85	0.856
Lo and Chan (2017)	20-500	0.624	0.604
Redondo-Hasselerharm <i>et al.</i> (2018) <sup>a</sup>	20-500	2.19	2.10
Redondo-Hasselerharm <i>et al.</i> (2018) <sup>b</sup>	20-500	6.30	5.45
Redondo-Hasselerharm <i>et al.</i> (2018) <sup>c</sup>	20-500	5.65	5.50
Redondo-Hasselerharm <i>et al.</i> (2016)	20-500	5.60	5.50
Ziajahromi <i>et al.</i> (2017)	1-4	0.95	0.65
Ziajahromi <i>et al.</i> (2018)	1-126	12.9	7.60
Ziajahromi (2018)	10-126	0.0105	0.103

Note: a: *Hyalella azteca*; b: *Gammarus pulex*; c: *Sphaerium corneum*; d: *Artemia franciscana*; e: *Daphnia magna*

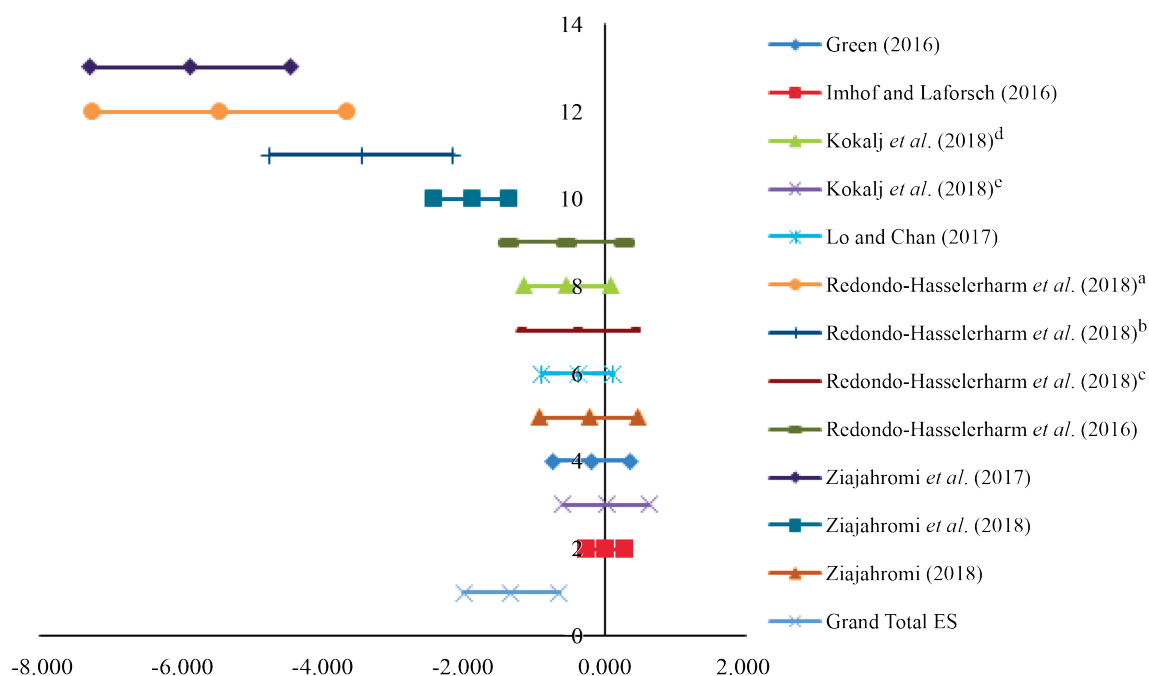


Figure 1. Horizontal lines representing the effect size and 95 % confidence interval of 12 studies investigating the effect of microplastics on growth of aquatic invertebrates.

The combined effect when the four studies were removed individually from the analysis is shown in Figure 2. From the first plot, there was still a significant effect when the study of Ziajahromi *et al.* (2017) was removed. This was the same for the second plot when Study 2 was removed, and so on through the fourth study. Compared to the overall effect size when all studies were considered, the removal of each of these studies that were detected as potential outliers provided no change in the overall effect. The effect remained significant, showing that the data are robust.

The findings suggest that microplastics have the potential to negatively affect the growth of exposed organisms by reducing their mean length after days of exposure (Ziajahromi *et al.*, 2017; Redondo-Hasselerharm *et al.*, 2018; Ziajahromi *et al.*, 2018). Microplastics used in the studies had particle sizes ranging from 1-602  $\mu\text{m}$ .

The study by Ziajahromi *et al.* (2017) recorded the highest difference in mean growth between treatment and control groups. This can be attributed to the acute exposure to organisms that the researchers applied in their experimental procedure. In addition, the microplastics used were of relatively small size, with a range of 1-4  $\mu\text{m}$  (Ziajahromi *et al.*, 2017). These smaller particles are ingested more frequently than larger microplastics. This is consistent with the same authors' findings in another paper (Ziajahromi *et al.*, 2018), which showed that larger microplastics are less ingested and therefore have less effect on the physiological activities of organisms. The reduced growth in the organisms can be attributed to the ingestion and egestion of microplastics that make use of available energy derived from food. This energy would have otherwise been spent on other metabolic activities essential for the growth of the organisms. The egestion of these particles that are foreign to their

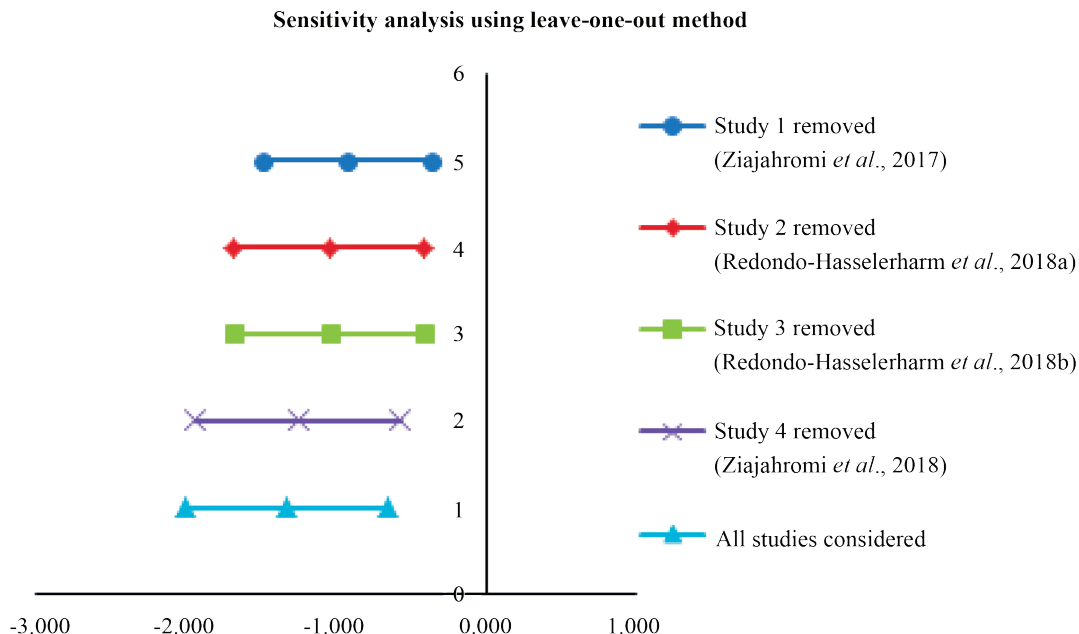


Figure 2. Combined effect size of the remaining studies with studies 1, 2, 3 and 4 individually removed compared to combined effect size with all studies included.



natural habit underwater incurs additional energetic cost, and in return, affects growth (Ziajahromi *et al.*, 2018). This is further supported by the findings from Redondo-Hasselerharm *et al.* (2018), in which the reduction in growth was attributed to the size-sensitive uptake of microplastics in the exposed organisms, leading to depletion of energy. Those studies that did not record a significant difference in growth between treatment and control groups used microplastics of a larger size range (Imhof and Laforsch, 2016; Kokalj *et al.*, 2018, among others), from 20-602  $\mu\text{m}$ , which therefore support that uptake is based on size. Small filter-feeding organisms do not readily ingest these much larger microplastics. In the process of active filtration of water (e.g., mollusks), particles of smaller sizes (1-7  $\mu\text{m}$ ) are the only ones sorted by the labial palp for ingestion (Beecham, 2008), although some species of mussels can ingest particles up to 200  $\mu\text{m}$ .

The duration of exposure differ among the studies examined, ranging from 2 to 95 days. However, the exposure time may not be a factor in the reduction in the growth of organisms in the treatment groups. In their study, Lo and Chan (2017) exposed the sample organisms for 95 days. Although there was a reduction in growth, this was not significant as compared to the studies with shorter exposure time (e.g. 48 h; Ziajahromi *et al.*, 2017). The four studies showing a significant reduction in growth all employed different durations of exposure from each other and from the other studies. However, due to the small particle sizes used in the experimental treatments, the microplastics were readily ingested within a short time period, and thus resulted in an energetic cost to the organism.

Indeed, microplastic exposure of filter-feeding and suspension-feeding organisms such as aquatic crustaceans and mollusks resulted in growth reduction. This can be attributed to the factor of size-selective consumption. Bivalves can sort particles prior to ingestion. They can discriminate between particle qualities, and unfavorable particles are rejected as pseudofeces (Gosling, 2003). We may therefore assume that they can discriminate microplastics and reject them, as they are not food

particles. However, fluorescence microscopy of the gut cavity from samples of bivalves revealed the presence of 2  $\mu\text{m}$  and 4-16  $\mu\text{m}$  microplastics, which means that they did not select based on quality but rather based on size (Wright *et al.*, 2013). These sizes conform to the optimum size range of foods that bivalve groups assimilate. Due to their inherent feeding strategy, this apparent inability to sort microplastics before ingestion can be applied to all other groups with similar feeding mechanisms (Wright *et al.*, 2013).

## CONCLUSION

The exposure of aquatic invertebrates to microplastics leads to a reduction in their growth as exemplified by the effect on samples treated with microplastics. The combined effect size based on results from the 12 studies included in our meta-analysis is significant. Size-selective ingestion of filter-feeding organisms is one factor that leads to the negative effect on mean growth. Although some studies exposed sample organisms for a minimal amount of time, the treatment with small microplastic particles contributed to a higher ingestion rate, which entails an energetic cost to the organisms exposed. This eventually can lead to reduced growth.

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