The Toxic Effects of Microplastic and Nanoplastic Bioaccumulation in Aquatic Species

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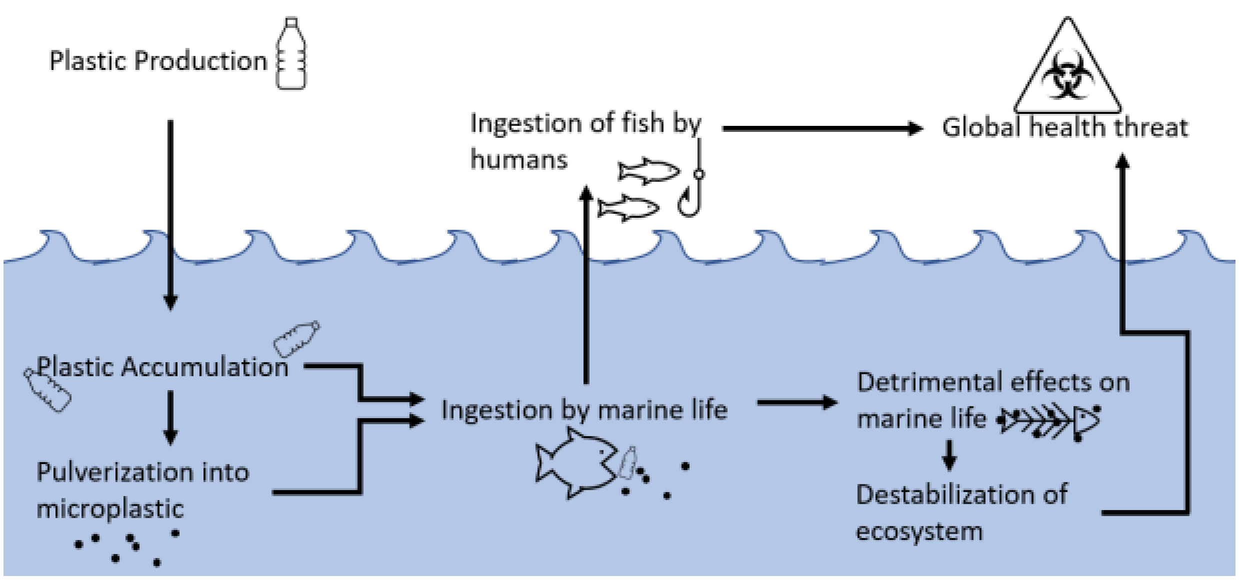
Abstract

Microplastics cause a series of health problems when ingested by living organisms, but the exact nature of these effects is not yet well known. As microplastic concentrations rise in bodies of water across the world, these health risks will become more prevalent, and therefore must be studied. Previous studies documenting the effects of microplastic exposure to six different aquatic species, including *E. sinensis* and *D. rerio,* were reviewed and common trends are noted. Microplastic exposure at concentrations as low as 10ppm was found to cause slowed feeding, slowed growth, and oxidative stress. As concentrations increased up to 50,000ppm, single-cell necrosis, tumors and death rates all increased. The severity of each effect increased with microplastic concentration in the environment. Many species were affected by exposure in the same ways, suggesting that these symptoms may be common among all organisms exposed to plastic. The findings provided a baseline for a potential safety threshold for microplastic concentration in the environment, which could be used for ocean cleanup efforts in the future.

Keywords: ocean, toxicity, health impacts, contamination, pollution



1.0 Introduction

Worldwide plastic production leads to pollution of the oceans and exposure of microplastics to aquatic life all over the planet, which leads to a variety of negative health effects. Plastic toxicity therefore poses a substantial risk to both specific species and the ecosystem; however, the exact effects microplastic exposure are not well understood. This means that finding ways to combat microplastic poisoning in the environment, and potentially in humanity, is difficult as the symptoms are unclear. Because of this, the study collects data on the effects of plastic on a variety of aquatic species.

Plastic has universal use across the planet, and as the use of plastic continues, old and used plastic continues to build up. Plastics do not biodegrade, leading to them having incredibly long lifespans.[1] This causes the amount of used plastic in the world to be ever increasing, as manufactured plastic is used and discarded. Eventually, this discarded plastic makes its way into the soil and waterways around the planet. While there is plastic in nearly every major body of water, the biggest sink for waterborne plastics is the oceans.[2]

Some plastics, such as polyethylene (PE), are light enough to stay suspended in the ocean water; other plastics, such as polyvinylchloride (PVC), end up accumulating in ocean sediments. Consumer plastics are already known to be a threat to marine life, either through acting as choking hazards or being mistaken for food. However, as plastic is worn down into microplastic, or plastic less than 5mm in any dimension, new threats to marine health are predicted.[3] Sources of microplastic vary. Some plastic is manufactured at micro size, and are called primary microplastics. Some of the most common sources for primary microplastics include plastic fibers used in clothing, or plastic beads included in hygiene products.[2] Other plastics are manufactured larger, but as they stay in the ocean, they're slowly pulverized by sun damage, wind erosion, and wave force into micro size; these are called secondary microplastics. Some of the most common sources for secondary microplastics include single-use water bottles and cigarette butts.[2] As time passes, these small plastics can be further broken up into nanoplastics, which are less than 1000nm in any dimension.

Once a piece of plastic is small enough to be considered a micro- or nanoplastic, it may be taken up accidentally with other food or inhaled through the gills. Several studies have already shown that microplastic is already taken up by fish in the wild, with some studies showing a percentage of plastic-containing individuals ranging from about 3% to as high as 83% (Table 1). While most studies found that less than half of all sampled fish contained microplastic, no studies reported 0% microplastic intake.

Table 1. Percentages of individual organisms containing plastic somewhere in the body.

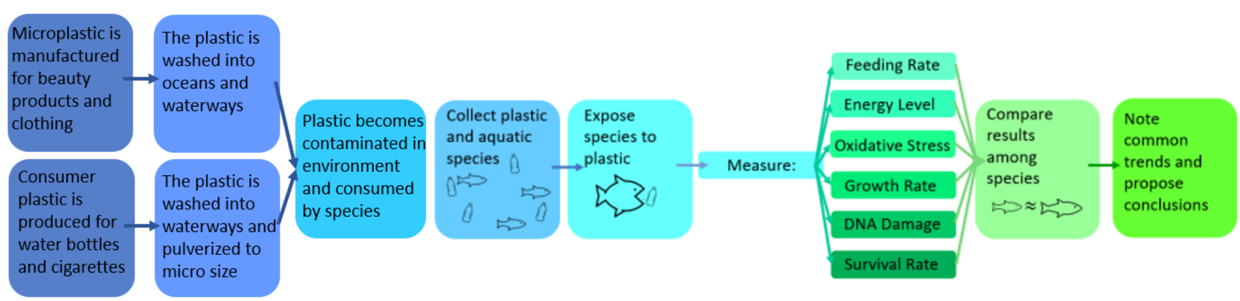
Microplastic Uptake in Wild Organisms

Organism	Percentage that Contained Plastic	Citation
Commercial Fish	2.60%	Lusher, A.L.[4]
Stellifer brasiliensis	6.90%	Dantas, D.[5]
Stellifer stellifer	9.20%	Dantas, D.[6]
Nephrops norvegicus	83.00%	Murray, F.[6]
Gobio gobio	12.00%	Sanchez, W.[7]
Lepas spp.	33.50%	Goldstein, M.S.[8]

The concentration of plastic particles in ocean waters and sediments is already high, and the plastic industry has no plans to slow or stop production; because of this, trends predict that the concentration will only continue to rise, and the threat of microplastics along with it.[9] This accumulation of plastic in the bodies of living things, or bioaccumulation, has been hypothesized to lead to a range of detrimental effects.[2] Additionally, the toxicity of microplastics is not the only health concern; other environmental pollutants tend to gather on the surfaces of microplastics, making it easier for these pollutants to be ingested. These pollutants can include polybrominated diphenyls (PBDs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), which have all been shown to cause a variety of organ toxicity, weight loss and cancer.[10,11,12] For these reasons, the use of these chemicals is discouraged in America; however, many still exist in costal ocean waters. Finding solutions to the issue of microplastic poisoning is confounded by the fact that the symptoms are not yet well understood.

A range of experiments have been run on several different marine species, in order to find which toxic effects presented commonly among the species. Each species was exposed to various concentrations of microplastics, either contaminated with environmental pollutants or uncontaminated 'virgin' plastic, and the consequential toxic effects were documented.

The flow of the experiment is illustrated below. Selecting a variety of species provided the broadest insight into trends that exist across the species, suggesting ways in which the ingestion of plastic affects most creatures. This then afforded a more comprehensive look at how the ocean ecosystem as a whole might be affected by plastic. The results document the various ways in which the toxicity of the microplastics affects each of the species. Common trends among the studies are noted, specifically which effects are most common and how the severity changes with plastic concentration. Potential safety thresholds for concentrations of microplastics are suggested based on the results, which could help solidify cleanup efforts.



Scheme 1. Illustrative scheme for the flow of the project.

Several studies were gathered for the purposes of the research, each study detailing the effects of microplastic on a particular species: in total, six species including *E. sinensis, D, rerio, O. latipes, P. viridis, M. galloprovinicalis,* and *D. magna* were studied.

2.0 Experimental

2.1 Materials and Methods: Overview

Each species underwent slightly different methodology in order to obtain results, but they all fit into the same overarching form. The species and the specific type of micro- or nanoplastic were obtained, the species was prepared for treatment and in some cases, the plastic was split into virgin and contaminated groups. The species was then exposed to the plastic for a set amount of time, usually in medium, and then afterwards, biomarkers and fluorescent particles were used to track changes in the organism bodies. The most tracked health effects included feeding rate, oxidative stress and survival rate, along with how they changed in regard to microplastic concentration.

2.1.1 Toxicity Effects

2.1.1.1 Mytilus galloprovincialis: Oxidative Stress

The species *Mytilus galloprovincialis*, more commonly the marine mussel, was used to study the correlation between contaminated microplastics and the toxic effects on the species.[17] Polyethylene and polystyrene microplastic beads were contaminated with pyrene, and then exposed to the mussels. Pyrene retention was measured by extraction with potassium hydroxide, and microplastic retention was measured by staining with dyes and subsequent microscopy. Pyrene appeared to cause health issues that virgin plastic did not.

2.1.2 Concentration Dependence

2.1.2.1 Eriocheir sinensis: Weight and Oxidative Stress

The species *E. sinensis*, commonly called the Chinese river crab, was the subject of a study that exposed the crab to varying concentrations of virgin polystyrene (PS) microbeads suspended in water.[13] The crabs were then monitored for oxidative damage and weight gain. Oxidative damage was measured by levels of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase. The wet weights of each crab before and after exposure were documented, as well as feeding frequency. The weights and feeding frequencies of crabs exposed to more microplastic appeared lower than the control crabs.

2.1.2.2 Danio rerio: Behavior and Microplastic Uptake

The embryos of the species *D. rerio,* commonly known as the zebrafish, were exposed to PS nanoplastics at concentrations of 0.1, 1 and 10 ppm in egg water medium for up to 120 hours post-fertilization.[14] Afterwards, larvae were transferred to an observation chamber to quantify behavioral changes through heart rate and imaged using fluorescence images with a 75W/2 xenon short-arc lamp to measure plastic uptake and distribution, showing that the embryos that had higher microplastic intake appeared less energetic.

2.1.2.3 Daphnia magna: Behavior and Survival Rate

The species *Daphnia magna*, a type of plankton, was cultured and used to test the toxic effects of microplastic bioaccumulation.[18, 19] *D. magna* was exposed to concentrations of PS nanoplastics of between 0.0001 and 1 mg/mL to determine dose response, and then 10 µg/mL for follow up experiments. Fluorescently labeled nanoparticles were used to determine buildup of the plastics in the organisms. Proteins released by the plankton created a corona around the plankton; these were isolated and then run through gel electrophoresis to be analyzed. Individuals with more microplastic retention were shown to have lower survival rates.

2.1.3 Contamination of Plastic

2.1.3.1 Oryzias latipes: Stress and Oxidation

The species *O. latipes* is widely accepted as a model fish specimen, and thus was chosen for a study examining the toxic effects of plastic and plastic contaminants.[15] Virgin plastic pellets were prepared alongside plastic pellets that were submerged in ocean tides for 3 months to absorb environmental toxins. The fish were fed a diet of 10% plastic by weight. Afterwards, RNA was isolated using TRIZOL reagent and treated with DNase to isolate the reference gene CYP1A. This was used to quantify the stress levels and oxidative damage to each fish in the liver.

2.1.3.2 Perna viridis: Respiration and Survival Rate

The species *Perna viridis*, or the green mussel, was exposed to varying concentrations of PVC microplastics.[16] The health effects monitored included respiration rate and death rate. PVC microbeads were split into two groups, one exposed to sea water and contaminated with fluoranthene; the other group acted as a control. The mussels were then exposed to the two sets of PVC beads at varying concentration in sediment. Respiration rate was measured by the change in the dissolved oxygen present in the water.

2.2 Present Analysis

The varying values of plastic concentration for all the above studies were all converted by the present author into a single unit, ppm, and then aggregated into a single data set. This allowed the increases in noted detrimental effects of the species to be compared to each other, and to the concentrations of plastics directly. The increasing death rates of species were compared at roughly the same plastic concentrations, and the common health effects of virgin and contaminated microplastics at the same plastic concentration were also compared.

Trends were drawn from the most commonly tested health effects; many of these effects were also shown to be dependent on the concentration of microplastic that the species were exposed to. These trends were also shown to be dependent on the state of the plastic, specifically whether or not the plastic had been contaminated. The confluence of evidence suggests a causal relationship between the consumed microplastic and the health effects tested for in each study.

3.0 Results

The exact nature of how micro- and nanoplastics can negatively affect the health of the organisms that bioaccumulate them, either through inhalation, ingestion or simply sticking to them, was studied by a variety of sources. An overview of the which species presented which toxic effects is displayed. Correlations were found between concentration of microplastics the species were exposed to and the severity of the presented effects, especially death rate.

3.1 Common Effects of Microplastic on Sea Life

Negative effects of plastic exposure varied by species, but common trends among the species were noted and recorded (Table 2). The negative effects of each species were confirmed as statistically significant from each of the papers the data is sourced from. The most common confirmed negative effects of microplastics across studies of different species include slowed feeding, slowed growth, decreased energy, and death. Rarer negative effects included DNA Damage and tumor formation.

Table 2. Overview of the negative effects of microplastics on various marine creatures.

Observed Negative Effects of Micro- or Nanoplastics

Organism	Slowed Feeding/ Respiration	Slowed Growth Rate	Decreased Energy	Oxidative Stress	DNA Damage, Tumors	Death	Source
E. sinensis	Yes	Yes	Untested	Yes	Untested	Yes	Yu, P.[13]
D. rerio	Untested	Untested	Yes	Yes	Untested	Untested	Pitt, J.[14]
O. latipes	Untested	Untested	Yes	Yes	Yes	Yes	Rochman, C.[15]
P. viridis	Yes	Untested	Untested	Untested	Untested	Yes	Rist, S.[16]
M. galloprovincialis	Untested	Untested	Untested	Yes	Yes	Untested	Avio, C.[17]
D. magna	Yes	Yes	Yes	Untested	Untested	Yes	Nasser, F.[18]; Ma, Y.[19]

The effects that were tested for between studies were not consistent, so it is possible that some species may exhibit more negative effects than are noted. Microplastic concentration also varied across studies, with ranges from 0.04-40ppm to 5,000-50,000ppm. Each of the observed negative effects increase in severity with respect to the microplastic concentration that each organism was exposed to.

3.2 Effects of Changing Microplastic Concentration

The average body weights of *E. sinensis* are plotted against the microplastic concentrations that the crabs were exposed to (Figure 2.) The figure compares the initial weight to the final weights of the crabs after exposure. The weights of the crabs dropped as more microplastics were introduced. The group of crabs exposed to the highest concentration of environmental microplastic, at 40,000 µg/mL, lost weight on average. The trend follows a negative correlation with respect to the increasing concentration of microplastics, showing that as microplastic concentration in the environment increases, the growth rate of the crabs drops.

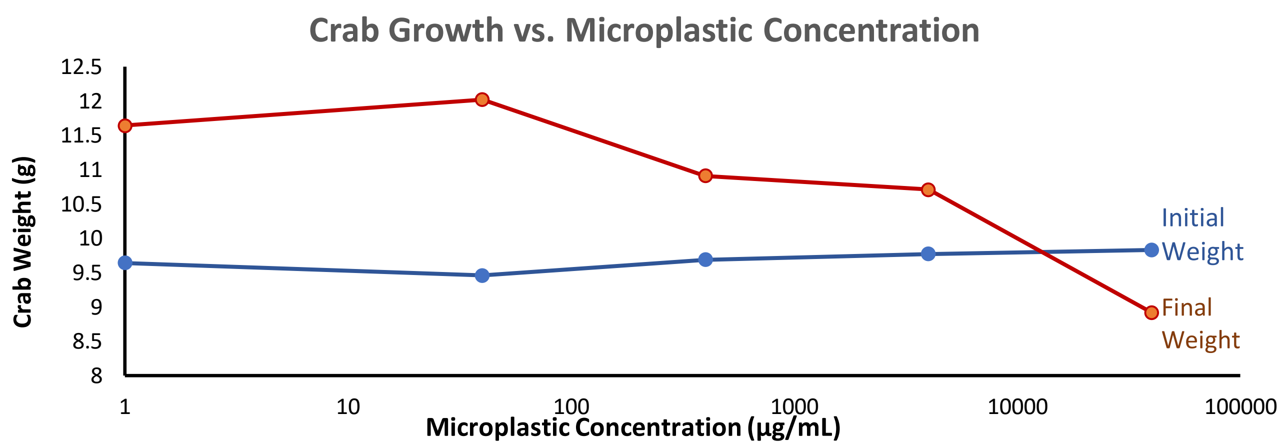


Figure 1. Initial and final weights of E. sinensis in g vs. microplastic concentration in environment in µg/mL.[13]

The same trend of the effects being dependent on microplastic concentration is shown in the relationship between *P. viridis* survival time and microplastic concentration (Figure 3). As the concentration of microplastics in the mussel medium increases, the survival time of the mussels exposed decreases significantly. The survival time therefore has a negative correlation with microplastic concentration.

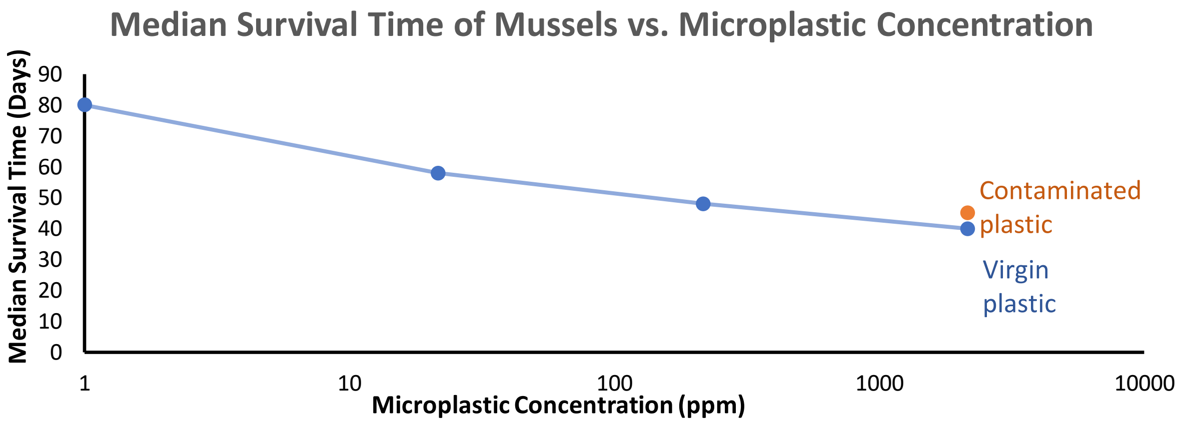


Figure 2. Median survival time of *P. viridis* after being continually exposed to microplastic vs. concentration of microplastic exposure.[16]

Only one data point is provided for the survival time with virgin microplastics but given that it is higher than the contaminated microplastic data point of the same concentration, it can be suggested that the virgin microplastic would present slightly longer median survival times. Survival rate as a percentage was also found to be affected by microplastic concentration in many species.

Survival rates of four species are compared by the concentration of microplastics that each species was exposed to (Figure 3). While data for every concentration was not available due to the inconsistency between studies, the visible trend with every species depicted is that as microplastic concentration increases, survival rate decreases. For the three more complex creatures *E. sinensis, O. latipes*, and *P. viridis*, the change in survival rate was fairly low, dropping to about 90% at the highest concentrations. The most extreme change appears in the species *D. magna,* or plankton, which reached 0% survival rate at 10,000ppm. Further, the adverse health effects from plastic consumption may not be purely from the plastic itself, but also from exposure to pollutants that stick to the plastic surface area.

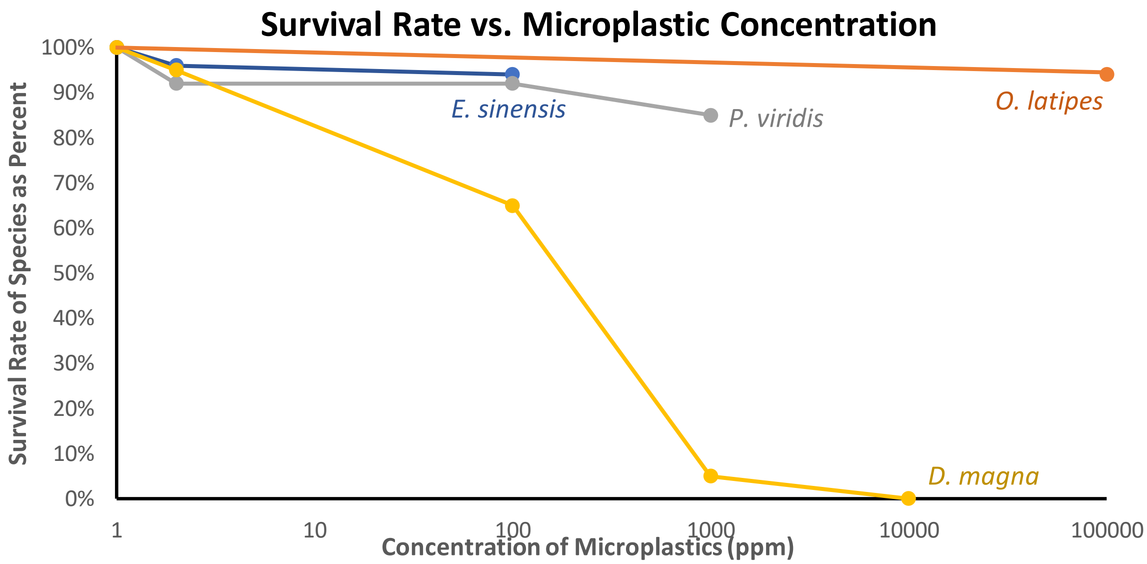


Figure 3. Survival rate of four species, *E. sinensis, O. latipes, P. viridis,* and *D. magna.*[13, 15, 16, 18] The survival rates of *P. viridis* were selected at a time of 30 days of ongoing exposure, in order to best fit the timelines of the other three studies.

3.3 Effects of Contaminated Microplastic

The health effects of contaminated plastic ingestion are compared to the effects of virgin plastic ingestion (Table 3). Plastic was contaminated by soaking in ocean water for 3 months prior to experiment; noted pollutants included PAHs, PCBs and PBDs. The contaminated plastic leads to more extreme adverse health effects for every single effect tracked. The percentage of fatty vacuolation in particular nearly doubles from virgin to contaminated plastic, compared to a much less extreme change between virgin plastic exposure and the control group. Contaminated plastic also appears to cause the issue of single-cell necrosis, which did not appear at all from virgin microplastic exposure.

Table 3. Comparison of the health effects of *O. latipes* by contaminated plastic, non-contaminated plastic, and no plastic.[15]

Health Effects of Plastic Contamination

	Contaminated	Virgin	Control
Glycogen Depletion	74%	46%	0%
Fatty Vacuolation	47%	29%	21%
Single Cell Necrosis	11%	0%	0%

In summary, common toxic effects of microplastic bioaccumulation among species included slowed growth, oxidative stress, and death. Each effect was shown to become worse as the concentration of microplastics the species was exposed to was increased, which further proves that microplastic is the cause. Contamination of the microplastics with environmental pollutants, specifically PAHs, PCBs and PBDs, caused more extreme health effects in every case observed. The implications of these trends and health effects for aquatic environments as a whole are discussed next.

4.0 Discussion

The presence of micro- and nanoplastics in the environment has toxic effects on *E. sinensis, D, rerio, O. latipes, P. viridis, M. galloprovinicalis,* and *D. magna*. These effects vary, but several common effects are noted. Death rates, survival time and slowed growth are shown to get worse as microplastic concentration increases, suggesting a dependence of the negative effects on the concentrations in the environment. Furthermore, the toxicity of virgin and contaminated microplastics is compared. Using all of the gathered information, a potentially safe concentration limit is proposed.

4.1 Common Trends of Microplastic Toxicity

The most common effects of microplastic ingestion included slowed feeding, decreased energy, oxidative stress, and death (Table 2). Rarer effects included slowed growth rate and tumor formation. Some combinations of effects may be causally linked and therefore more likely to appear together; for example, slowed feeding could lead directly to slowed growth and lower energy levels. Death is likely one of the most common side effects due to combinations of effects. This reinforces the previous hypothesis that increasing exposure to microplastic in the environment is having negative effects on aquatic life.[3] This is further supported by the fact that as the concentration of microplastic increases, the severity of the health effects increases as well.

4.2 Effective Dependence on Microplastic Concentration

The growth rate of the crabs was shown to slow significantly as microplastic concentration in the environment increased (Figure 2). This illustrates a dependence of the negative effects on the microplastic concentration, further confirming the hypothesis that as microplastic collects in the environment, the threat to wildlife will grow[3]. Death was also a common effect of microplastic uptake; four of the six studied species exhibited a trend of lowered survival rates after being exposed to increasing concentrations of microplastics (Figure 3). This again illustrates that the severity of the negative symptoms noted in species increases as microplastic concentration increases. The fact that this is true for all species studied suggests it may be true for similar organisms that are exposed to microplastics, which no previous paper has been able to provide evidence for.

E. sinensis, O. latipes and *P. viridis* are all complex and large, relative to the size of the microplastic pollutants; in contrast, *D. magna* is a small unicellular creature. Due to its body size, the same concentrations of plastic that may not affect larger creatures would likely have been overwhelming to *D. magna,* leading to much higher death rates in comparison. Microplastic toxicity is further amplified by environmental pollutants, which can adhere to the surfaces of the plastic and become ingested, creating a range of serious health risks.

4.3 Toxicity of Polluted Microplastic

There was a difference found between the toxic effects of virgin microplastic and those of microplastic contaminated by the environment. The study on *O. latipes* compared the adverse health effects of contaminated and virgin microplastics on the fish and shows that the pollutants made existing problems worse (Table 3). This builds on the hypotheses of the studies on *D. magna* and *P. viridis* that polluted plastic has a significant effect on the health of the exposed species.[13, 15] Furthermore, most species that exhibited higher rates of death – specifically *D. magna, P. viridis,* and *O. latipes* – were included in studies that tested the toxicity of microplastics exposed to contaminants. This shows that while microplastic itself can be deadly, the pollutants in the environment make the plastic more toxic.

The contaminated plastic used in the *O. latipes* study was prepared by leaving it to soak in ocean water.[12] That illustrates that the pollutants are already present in the ocean, and plastic may be more toxic than the previous studies have shown. Removing plastic from the ocean and reaching a safe concentration is of the utmost necessity, and ideally, concentrations of plastic should be kept to a certain minimum.

4.4 Concentration Safety Limit

The severity of the negative effects experienced by all species was directly correlated with the amount of microplastic that was allowed to bioaccumulate. Therefore, to protect the environment, there should be a maximum safe concentration of microplastics in the species environments at any given time.

Smaller species seem to be more seriously impacted by plastic pollution than larger ones (Figure 3). However, these species do not exist in a vacuum; they are part of the same environments and ecosystems. Therefore, a safe concentration of microplastics must depend on what the most sensitive species can survive. *D. magna* was most extremely affected by the rise in plastic concentration and is noted as an important central species in the ocean ecosystems, due to feeding on algae and in turn acting as food to several other species; this makes it a viable species to use as a gauge of the health of an ecosystem.[15] Therefore, the suggested safe level is based on the response of *D. magna*. Ideally, a safe maximum concentration of plastic in ocean water would be around 5ppm. This concentration is low enough that the more complex species displayed minimal health effects, if any, as seen in Figures 2 through 4. *D. magna* specifically was shown to have a survival rate of above 90%. This proposed safe maximum agrees with previously proposed safe maximums, specifically of around 6600 particles per cubic meter, or roughly 6.6ppm.[20]

In summary, exposure to microplastics is shown to lead to a series of toxic effects in aquatic life. These effects are shown to get worse as the microplastic concentration in the environment increases, and pollutants adhering to the surfaces of the microplastic can further worsen the effects. Using the species *D. magna* as a benchmark, a concentration of 5ppm is proposed as a safe environmental limit. For this safe level to be established, however, work to restore the oceans must begin immediately.

5.0 Conclusion

Ingestion of plastics has been shown to have a wide array of detrimental effects on sea life, from oxidative stress to slowed growth and feeding rates and even death. These symptoms increase in severity as the concentration of microplastic increases. Furthermore, a variety of species are affected by microplastics in largely the same ways, suggesting that some of the trends could be universal. Most fish are exposed to some amount of microplastic in the environment, meaning that the detrimental effects noted will be a threat to most aquatic populations. Further, the health risks could extend to non-aquatic life, such as humans.

Now that there are some baseline expectations of how microplastic ingestion affects species, there can be work done to mitigate these negative effects. Next works could include research into ways to alleviate symptoms of microplastic poisoning, or studies on how best to accelerate flushing of microplastic from the body. Smaller-scale research could include studying a variety of organisms at exposure to consistent microplastic concentrations, in order to more conclusively back up what is hypothesized here. This work also suggests new routes of research in medical fields as the threat of microplastics to humans is hypothesized. There are biological and ecological implications to further research based on reducing microplastic ingestion and concentration in the wild.

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Suggested Revisions

1. Abstract, Develop sections of the abstract more, esp. experimental. Cap with a future thought

This was completed, as it would result in a stronger abstract overall. The exact changes in the abstract are highlighted below.

"Studies documenting the effects of microplastic exposure to six different aquatic species, including *E. sinensis* and *D. rerio*, were reviewed and common trends are noted."

"The findings provided a baseline for a potential safety threshold for microplastic concentration in the environment, which could be used for ocean cleanup efforts in the future."

2. Intro, Cast the problem more effectively. Develop in a cause and effect manner, rewrite first paragraph. Rationalize choosing the given animals.

The first paragraph of the introduction was edited slightly, but not removed or changed outright. The purpose of the paragraph is to act as an overview of the introduction before diving directly into the specifics of the problem; removing it would lead to this section lacking an overview, which every other section has and feeds into the overall flow of the paper. The paragraph was, however, edited to be less wordy and leaves out previous mentions of the experimental, to flow more naturally into the following paragraphs.

"Worldwide plastic production leads to pollution of the oceans and exposure of microplastics to aquatic life all over the planet, which leads to a variety of negative health effects. Plastic toxicity therefore poses a substantial risk to both specific species and the ecosystem; however, the exact effects microplastic exposure are not well understood. Because of this, the study collects data on the effects of plastic on a variety of aquatic species."

3. Experimental, specify materials and chemicals

Small changes were made to the experimental section to specify the exact materials and chemicals used.

"Afterwards, larvae were transferred to an observation chamber to quantify behavioral changes through heart rate and imaged using fluorescence images with a 75W/2 xenon short-arc lamp to measure plastic uptake and distribution, showing that the embryos that had higher microplastic intake appeared less energetic."

"Afterwards, RNA was isolated using TRIZOL reagent and treated with DNase to isolate the reference gene CYP1A. This was used to quantify the stress levels and oxidative damage to each fish in the liver."

4. Results and Discussion, emphasize state of the art findings

Small changes were made to the Discussion section to emphasize the state of the art findings of the paper.

"The fact that this is true for all species studied suggests it may be true for similar organisms that are exposed to microplastics, which no previous paper has been able to provide evidence for."

"That illustrates that the pollutants are already present in the ocean, and plastic may be more toxic than the previous studies have shown."

5. References Italicize volume number, no comma after journal name

The references were fixed by italicizing the volume number and removing the commas included after the journal names, in accordance with proper JACS format. Though only one reference is shown here, the change was repeated for every reference in the paper.

"[20]. Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J.; Abu-Omar, M.; Scott, S.; Suh, S. Degradation Rates of Plastics in the Environment. *ACS Sustainable Chem* **2020**, *8*, 3494-3511."

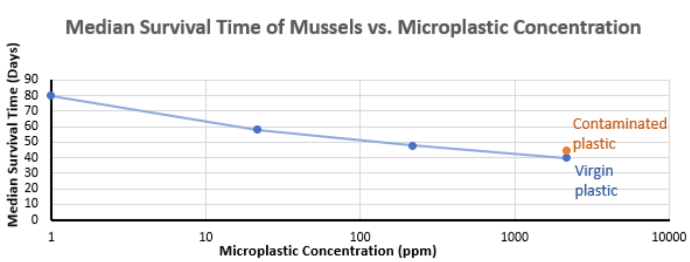
6. Scheme, label as a scheme not a figure

The caption of the scheme was changed from Figure 1 to Scheme 1 in accordance with proper format.

"Scheme 1. Illustrative scheme for the flow of the project."

7. Figures, label each line directly and make axes black instead of grey

The legends for the figures were deleted in favour of direct labelling of each line, and the labels of the axes were darkened from grey to black in order to show better against the page. Though only one figure is shown, the same revision was made to all figures.



8. Captions, table captions go above table not below

The captions of all tables were moved so that they were above each corresponding Table, not below, in accordance with proper format. Though only one table is shown below, the same revision was made to all tables.

Table 1. Percentages of individual organisms containing plastic somewhere in the body.

Microplastic Uptake in Wild Organisms

Organism	Percentage that Contained Plastic	Citation
Commercial Fish	2.60%	Lusher, A.L.[4]
Stellifer brasiliensis	6.90%	Dantas, D.[5]
Stellifer stellifer	9.20%	Dantas, D.[6]
Nephrops norvegicus	83.00%	Murray, F.[6]
Gobio gobio	12.00%	Sanchez, W.[7]
Lepas spp.	33.50%	Goldstein, M.S.[8]

9. Clarity of science, stress novelty of work

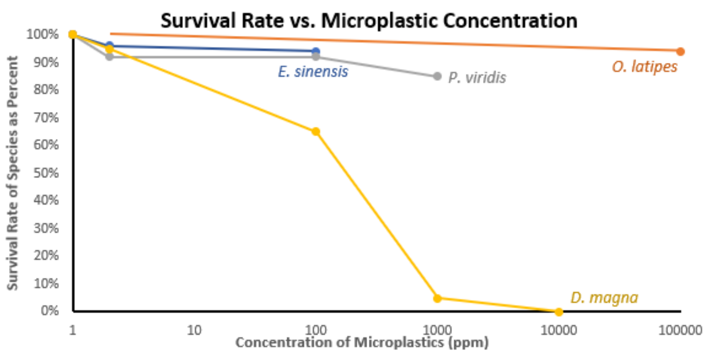
The same changes that were made to the Discussion section in accordance with suggestion number 4 apply here as well. Additionally, small changes were made to the introduction for the same effect.

“Common trends among the studies are noted, specifically which effects are most common and how the severity changes with plastic concentration.”

“Potential safety thresholds for concentrations of microplastics are suggested based on the results, which could help solidify cleanup efforts.”

10. Proper style, break down longer sentences for clarity, get rid of gridlines

Gridlines were removed from graphs in accordance with proper scientific format. Though only one figure is shown below, the same revision was made to all figures.



11. Format, remove subheadings from introduction

Subheadings were removed from the introduction in accordance with proper scientific format.

12. Word usage, adhere to scientific nomenclature for things like danio rerio

Any phrases that used scientific nomenclature incorrectly (most often incorrectly having the species name uppercase instead of just the genus name) were fixed.

“E. sinensis, O. latipes and *P. viridis* are all complex and large, relative to the size of the microplastic pollutants; in contrast, *D. magna* is a small unicellular creature.”

13. Subheadings should be more illustrative

The example the reviewer uses was already removed from the paper due to earlier suggested revisions, specifically the suggestion that the subheadings of the introduction be removed. However, this type of change was applied to the subheadings of the other sections of the paper, and small edits were made to the subheadings of the experimental and results sections.

14. Bridge the intro and experimental more effectively

A more effective preview of the experimental was included at the end of the introduction, after the scheme, in order to more effectively bridge the two sections of the paper.

"Several studies were gathered for the purposes of the research, each study detailing the effects of microplastic on a particular species: in total, six species including *E. sinensis, D, rerio, O. latipes, P. viridis, M. galloprovinicalis,* and *D. magna* were studied."

15. Align the experimental with the results and discussion as well.

A similar preview was added to the end of the experimental, which previews the results section in the same sequence the results are written in, and bridges the two sections of the paper.

"Trends were drawn from the most commonly tested health effects; many of these effects were also shown to be dependent on the concentration of microplastic that the species were exposed to. These trends were also shown to be dependent on the state of the plastic, specifically whether or not the plastic had been contaminated. The confluence of evidence suggests a causal relationship between the consumed microplastic and the health effects tested for in each study."

16. Delete unnecessary words and check for possible multiple meanings.

The specific example used by the reviewer was edited out of the document during other revisions, specifically the suggestion to rewrite the abstract.

17. In the abstract, start by saying the goal of the study and why it matters before describing the rest of the document.

The abstract was rewritten to more effectively communicate the point of the paper off the bat.

"Microplastics cause a series of health problems when ingested by living organisms, but the exact nature of these effects are not yet well known. As microplastic concentrations rise in bodies of water across the world, these health risks will become more prevalent, and therefore must be studied. Previous studies documenting the effects...”

18. Number the headings and subheadings of the document.

The headings of the document were numbered in order to better guide the reader during the reading process. Though only a section of the table of contents is shown here, the same change was applied to all headings.

19. Articulate the problem clearly.

There was some confusion in the introduction about what exactly the nature of the problem is, so it was rewritten in order to clarify.

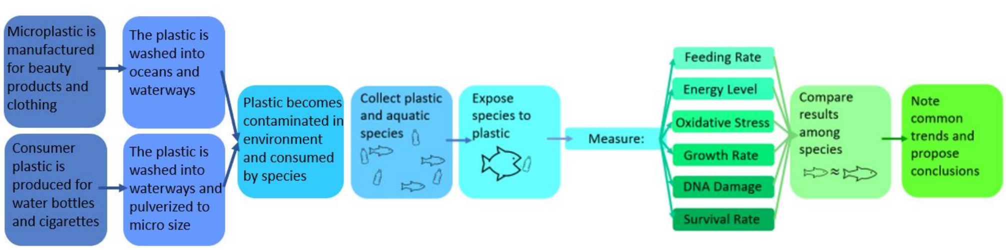
“This means that finding ways to combat microplastic poisoning in the environment, and potentially in humanity, is difficult as the symptoms are unclear.”

“These plastics become very easy to ingest, but the effects of this ingestions are not yet understood.”

“Finding solutions to the issue of microplastic poisoning is confounded by the fact that the symptoms are not yet well understood.”

20. Create a superscheme and combine with the contents of figure 1

It was suggested that a brief explanation of how plastic becomes microplastic in the oceans be made into a scheme and combined with the extant scheme. As such, a new scheme was built from the old scheme, incorporating the path plastic takes into the ocean.



21. Reorganize the experimental

The flow of the experimental is designed to go through each study one at a time, since each study has a slightly different methodology. Trying to separate the studies into steps and rearranging by those steps would only confound the paper, as not every paper follows the exact same set of steps. For example, some papers use contaminated plastic and others don't. However, the order of species were changed so that the studies focused mainly on microplastic uptake and confirming toxic effects were presented first, then the studies that focused on variance by concentration dependence, then the studies that focused on contaminated and virgin plastics. This helps the experimental follow the flow of the rest of the paper more closely. Though only three levels of subheadings are shown, there is a final level of subheading, one for each studies species.

22. Explain the tables and figures in text

The tables and figures are already explained in the text. Small notes were added among the paragraphs in order to explain details that may have been easily missed or not explicitly stated aloud.

"The figure compares the initial weight to the final weights of the crabs after exposure."

"For the three more complex creatures *E. sinensis, O. latipes*, and *P. viridis*, the change in survival rate was fairly low, dropping to about 90% at the highest concentrations. The most extreme change appears in the species *D. magna,* or plankton, which reached 0% survival rate at 10,000ppm."

"The percentage of fatty vacuolation in particular nearly doubles from virgin to contaminated plastic, compared to a much less extreme change between virgin plastic exposure and the control group."

23. In discussion, state which species are used instead of 'all studied species'.

The specified sentence was changed in accordance with the suggestion, to increase clarity.

"The presence of micro- and nanoplastics in the environment has toxic effects on *E. sinensis, D, rerio, O. latipes, P. viridis, M. galloprovinicalis,* and *D. magna*."