Variables that effect why larval *Pimephales promelas* can discriminate between microplastics and zooplankton

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Abstract

Microplastics are plastics less than 5 mm and are abundant in the world's oceans and have been shown to cause negative effects in many marine species (Fonte et al., 2016; Ivleva et al., 2017). However, studies have been done to show that some larval species are able to identify and avoid microplastics (Kaposi et al., 2013; Malinich et al., 2018). The reason as to why this is is unclear, but Malinich et al. (2018) provided some suggestions: the plastic was too large to consume, they are able to distinguish plastic from their prey by use of visual cues, olfactory cues, and movement, or that plastics are so new to them so they don't try to consume it. This study will look at four variables to see if these factors have an affect on consumption: size, smell, movement, and color. This study will use *Pimephales promelas* to test these effects. In addition, it is unknown if these fish can distinguish between zooplankton that have consumed plastics with those who have not. It has been determined that some species of zooplankton do ingest plastic whereas others do not (Cole et al., 2013). The expected results are that each variable will influence the amount of plastic consumed by larval fishes, but a combination of them all is why they are able to avoid these plastics. The other expected result is that larval fishes will not be able to distinguish between zooplankton with or without plastics because the variables tested cannot be used when the plastic has been ingested. This is important to study because plastics affect all aspects of the food chain and the higher up the food chain you go, the more plastic is found in the organism (Boerger et al., 2010; Ivleva et al., 2017). The reason larval fishes are unharmed by plastics is also unknown, as juvenile fish are harmed (Fonte et al., 2016). This study will allow for suggestions as to why this is, such that if larval fishes cannot distinguish the zooplankton, it is beneficial to be able to eat whatever is available without harm. A problem with this is that the fish may consume plastic as it ages and causes problems in the future for the fish and its predators. It is important to study this in order to find a solution to better protect the marine organisms.

Introduction

Microplastics are plastics that are smaller than 5 mm (Ivleva et al., 2017). They can come in many shapes such as beads, fibers, and fragments. These plastics break off a larger product, such as plastic bags, and make their way into the water column. Since they are so small, they are not captured by wastewater treatment plants and enter the oceans. Their small size makes them easily ingestible by many marine organisms, especially the smaller ones such as zooplankton or larval organisms (Cole et al., 2013; Kaposi et al., 2013). In addition, the most common colored plastics to be consumed by larval fishes are blue, white, and clear, as they look like zooplankton (Boerger et al., 2010). These plastics can cause harm not only to the organism that digests them, but also to their predators. If a predator consumes zooplankton that ingested microplastic, that predator will also consume the plastic, and this will continue to move up the food chain. This can also result in accumulation of plastic in a predator the more organisms with plastic it consumes (Ivleva et al., 2017). Studies have shown that the larger the organism, the more plastic that has been accumulated. More than one third of fish species have been shown to have ingested these plastics (Boerger et al., 2010).

Microplastics have been found to cause many health problems, especially in larger organisms such as releasing dangerous toxins in the body and blocking the intestines (McCormick et al., 2016). However, microplastics have also been found in larval organism and zooplankton and have been found to have no negative effect on the organism. Kaposi et al. (2013) looked at sea urchin larva, T*ripneustes gratilla*, and found the fitness of the organism was not affected by ingesting microplastics. Malinich et al. (2018) looked at larval fathead minnow, *Pimephales promelas*, and found that there was no effect on their growth after ingesting these plastics. They also found that these organisms can avoid eating these plastics when they are present. They didn't understand the reason behind this but gave suggestions as to why: the plastic was too large to consume, they are able to distinguish plastic from their prey by use of visual cues, olfactory cues and movement, or that plastics are new to them so they don't try to consume it. Another gap in their research was to determine if these larval fish could still discriminate microplastics if they were found in zooplankton. Cole et al. (2013) found that zooplankton do ingest microplastics but also found that some species, such as *Parasagitta* sp., do not. This allows larval fish to be able to choose zooplankton that have not ingested plastic.

This study will focus on why these larval fish do not ingest microplastic as well as if they are still able to discriminate between zooplankton that have or have not ingested the microplastic. To keep this study consistent *Pimephales promelas* will be used to test these effects. These fish species exhibit a larval stage similar to other larval fish species and are known to come across microplastics in their natural habitat. In addition, they also encounter prey items that look like microplastics, meaning they can discriminate between the two (Malinich et al., 2018). This study aims to understand why no harm is done to larval organisms when consuming microplastics. A hypothesize is that all these factors (size, smell, movement, and color) play a part as to why fathead minnow larvae can avoid microplastics and that they are not able to distinguish between zooplankton.

Methods

Larval fathead minnows will be collected from Purdue University's Baker Aquaculture Research Lab where they breed adult minnows and hatch them from eggs. Plastic microbeads will also be obtained. 20, 8-liter tanks will be set up each containing 10 minnows. Each variable will be replicated 10 times. Four variables will be tested in each group: size, smell, color, and movement of the plastic. Each tank will contain food for the minnows, in this case *Artemia* nauplii will be used, as well as the microplastic to determine if they can discriminate between the two with different variables. Each test will be done for three hours. Each tank will contain 100 3 mm scentless and clear plastics unless otherwise stated in order to avoid testing more than one variable.

Size: Three different sized microbeads will be placed in each tank along with brine shrimp: 5 mm, 3 mm, and 1 mm. Each size will contain 100 particles. The number of particles left of each size will be counted and recorded for all 10 trials.

Smell: Plastic will be obtained and covered with scent given off by zooplankton. 100 plastic particles with and 100 particles without the scent will be placed in the tank and the amount of each plastic left in the tank will be recorded.

Movement: Particles will be placed in a tank with brine shrimp. To mimic movement, a current will be generated around the tank, so it can carry the plastic around. The number of particles that remain will be recorded. Another experiment will be done where no current is generated.

Color: Plastics of different colors will be placed in a tank. Each color will contain 100 particles. The colors to be tested are blue, clear, white. These colors will be chosen to mimic zooplankton colors to see if they can discriminate based on color. 100 red beads will also be introduced to see if the larval fish will avoid them more because it is not a natural color. The number of each colored bead will be recorded.

Another experiment will be done to determine if these larval fishes can discriminate between zooplankton that have ingested microplastic versus those without. *Acartia clausi* will be used because it has been known to eat small microplastics (Cole et al., 2013). These will be split into two tanks (15,000 in each) and one group will be given 1 mm microplastics where the other will not be exposed to microplastics. The zooplankton will be looked at to see which ones ingested the plastic. The same procedure will be followed as above for the fathead minnows but instead of adding microplastics, 300 zooplankton which had ingested microplastics and 300 that had not will be introduced into each tank. The number of zooplankton from each group will be recorded by examining the zooplankton for plastic.

Discussion

When looking at size, the expected outcome is that the smaller particles (1 and 3 mm) will be consumed more often than the larger ones (5 mm) because the minnows are not able to fit the larger particles in their mouths and the smaller particles resemble zooplankton size. For smell, it is expected that the larval fishes will consume more scented particles than not because they will recognize it as a prey item and the smell will help confirm it. If the particles are moving, more particles will be ingested than those that are floating in the tank. For color, the red plastics will not be consumed as often. Although all these factors may have a result on consumption, there is a strong indication that all these factors together result in the larval fish avoiding these plastics. For example, if a particle is moving but does not smell like prey, they may avoid it. The expected result of the zooplankton study is that larval *Pimephales promelas* will not be able to discriminate between zooplankton that consumed the plastic versus those who have not. This is because the variables tested cannot be seen when the plastic is already consumed. They cannot use visual or olfactory cues, the size doesn't paly a role if it is already consumed, and movement of the particle cannot be detected.

Kaposi et al. (2013) and Malinich et al. (2018) found that there was no effect on growth and fitness on larval organisms specifically looking at *Pimephales promelas* and T*ripneustes* *gratilla*. The reason behind this is unclear and this study can further help to address this. One suggestion is that if larval fish cannot discriminate zooplankton with and without microplastic, it is beneficial to be able to digest the plastic so the fish won't be dying from eating contaminated prey. Kaposi et al. (2013) also found that larval sea urchins only eat plastics in absence of food and can possibly recognize that these plastics don't have the necessary nutrients needed to survive. This can suggest that in the absence of food, the larvae can ingest plastic and may receive small amounts of nutrition, but this would need to be further studied. In addition, if larvae are unharmed, it could act as a defense mechanism, as predators of larval fishes are affected by the plastics and may possible avoid these fishes if they are contaminated, although further study is need to determine if larger fishes can discriminate between this. This is important to understand because there may be problems with this. Even though larvae can discriminate between microplastics and food, there is still some plastic that has been digested. In juvenile fishes this does course harm and sometimes death to the fish because they can no longer ingest it (Fonte et al., 2016). Because there is no harm done to larval fish, the fish may continue to consume these plastics as juveniles, which can cause negative effects.

It is also important to study the long-term effects of this issue. One variable not tested in this study is that larval fish don't consume microplastics because they are fairly new in their environment and so they ignore them. The effect of this should be tested overtime because eventually these plastics will become a normal occurrence in these habitats. If this is the case, these larvae fishes may start to consume them because they are small and readily available. This will not only hurt the fish as it ages but will continue to affect the larger predators even more if more fish are consuming the plastics. This study is also significant because it is important to understand why these fishes aren't harmed by these plastics. If we can understand this, we can try and help other organisms that cannot digest these plastics. In addition, this is important in order to understand the effect plastics have on the organisms in the ocean in order to try and use a more sustainable material that other organisms can digest. If we cannot find a better substitute, another solution is to change its property; for example, changing the color of plastics to red because fishes are less likely to consume it or creating a scent that fishes avoid in order to deter them from eating it. This is important, overall, to be able to understand and further protect marine species affected by microplastics.

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