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A Novel Approach to Bio-Friendly Microplastic Extraction with Ascidians

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ABSTRACT

Microplastic pollution in water is now recognized as a devastating problem by many organizations, such as the National Oceanic and Atmospheric Administration, with recent studies estimating that the average American consumes around 52,000 of these plastic, toxic particles a year. A successful solution for the extraction of microplastics from oceans must be feasible to be implemented on a large scale and bio-friendly to not further disrupt the environment. To this end, the efficacy of using filter feeders (Ascidians) as biofilters to reduce microplastic pollution was explored. The efficacy of this filtration method was evaluated by adding ascidians to saltwater tanks contaminated with microplastics (experimental group) and comparing the water's plastic concentration over time against a control. Water samples were then systematically tested with a fluorescence-activating microscope and fluorescent scanner. Fluorescent microplastics were used which allowed for the collection of both quantitative and qualitative data. The samples from the experimental group demonstrated a 24.7% (29.64mg) reduction in microplastics within the first day and a 94.7% (113.64mg) decrease by day 4. The control group showed negligible deviation in microplastic concentration. It is concluded that the Ascidians filtered microplastics from water through their natural feeding and respiratory process. We extrapolate that a 1m x 1m x 1m cage of Ascidians would filter approximately 300g of microplastics every day. This research demonstrates that microplastic filtration with invertebrate filter feeders is an effective and feasible option for extracting microplastics from polluted water.

Introduction

Ascidians are marine invertebrate suspension filter feeders that are found all over the world. There are over 2,300 species of Ascidians that are categorized into 3 main types: solitary, social, and clumped. All three of these types are immobile creatures as Ascidians remain firmly attached to their substrata, such as rocks and shells. Ascidians feed by filtering water. They take in water through their oral siphon, which then flows down their mucus-covered gill slits, into a water chamber called the atrium. Inside this water chamber, various enzymes will extract the nutrients from the water which then exits through the atrial siphon of the Ascidian.

In our experiment, we tested to see if the natural feeding process of the Ascidian could be utilized to filter out microplastics from water. Microplastics are small pieces of plastic that pollute the environment. By definition, "microplastics" refers to any type of plastic fragment less than 5mm in diameter and in length. Microplastic pollution is so widespread that microplastic pollution is now regarded as a problem from the Northeast Pacific to the Antarctic oceans. Microplastics enter the ocean through a variety of different sources. The main sources of microplastic include tiny microbeads that wash down our drains from personal care products, fragments of single-use plastic items, such as coffee cup lids and plastic straws, and plastic microfibers that shed from textiles in laundry and other processes. There is still research needed to be done regarding the entire cycle of microplastics in the environment; however, it is well-agreed upon that microplastics end up ingested and incorporated into the bodies and tissues of many organisms due to bioaccumulation and natural marine processes.

While we were designing our experiment, we asked ourselves why ocean plastic pollution was such a prevalent issue. With research, we realized that seafood is an important source of food for the human population. The microplastics that are ingested by fish and other seafood are inevitably consumed by humans. Many toxic and carcinogenic chemicals make up plastic, some of which are extremely harmful to the human body. Consequently, microplastic accumulation in humans has a wide range of health problems attached to it. Even among the marine population itself, microplastics are now a major concern to the marine food chain. Marine wildlife is accidentally ingesting microplastics as food. This is extremely harmful to living organisms because once ingested, microplastics can cause digestive system blockage, physical injury, altered feeding behavior, and changes to their cells. This leads to issues that affect growth and reproduction, often leading to the disruption of an entire food chain. With this in mind, we sought to explore novel methods of microplastic extraction from the ocean. An effective method to remove microplastics from the ocean would be meaningful to the scientific community and could potentially provide a global solution to the devastating problem of microplastic pollution.

Research Question

Can Ascidian class Tunicates act as a natural and Bio-Friendly option to filter out microplastics from contaminated water?

Hypothesis

During Ascidians' respiration and feeding, they take in water through the incurrent (inhalant) siphon and expel the filtered water through the excurrent (exhalant) siphon. It is expected that microplastics may also be filtered out of the water during the natural feeding process of the Ascidians. We hypothesize that due to the filter-feeding attributes of Ascidians, Ascidians placed in microplastic contaminated water will filter the microplastics from the water.

Procedure

High-level Overview

Tanks containing equal amounts of fluorescent microplastics (2.5mL solution of microbeads) were set up. Three tanks were used as negative control groups and only contained the microplastics, while the other three tanks contained three Ascidians in each tank. Every day three 1mL samples were collected from each tank. This process was conducted over 4 days. After this, the samples were viewed under a fluorescent plate reader and under a fluorescent activating microscope to measure microplastic concentrations.

A. Water tanks set up containing living Ascidians:

1. The tanks in which the Ascidians were housed were completely sterilized. At first, they were washed with soap and hot water, followed by a rinse and wash with boiling water to rinse off any residual soap and kill off any lasting bacteria.
2. 12L of compliments brand distilled water was poured into each tank. Following that, 343 g of Aquavitro salinity mix was added to each tank to reach a salinity of 31 ppt.
3. The Aquavitro salinity solution was then completely dissolved into each tank using a stirring rod which had been sterilized with boiling water. Each tank received 2 minutes of stirring to ensure that the salinity was

controlled. Following that, a hydrometer was used to ensure that the salinity was accurate and controlled amongst each experimental group.

4. Before adding the Ascidians to the tanks, the Ascidians were given 30 minutes to acclimate to the new tank temperature so that there would be no sudden temperature shock, which could have resulted in almost immediate death.
5. The tanks were kept at a controlled temperature of 20-21 degrees C.
6. A total of 9 Ascidians from "Gulf Marine Specimen" (Florida, USA) were carefully placed into each of the 3 experimental tanks, labelled Group A, Group B, and Group C.

B. Fluorescent microplastics are added to each tank:

1. The tanks were kept in a light-controlled environment (dark) to minimize fluorescence degradation over time.
2. The Ascidians were fed twice a day at 7 am and 7 pm to keep them alive. The Ascidian feeding process consisted of 1mL of MarineSnow per feeding per tank. The food was added to all the tanks to maintain consistency.
3. The first set of water samples were collected before the microplastics being added to be used as a control.
4. A microplastic suspension of 2.5mL (10µm diameter, 2.5% w/v, Polystyrene Fluorescent beads from Magsphere) per 12L of water was prepared by extracting 2.5mL of the aqueous solution. This was done by using a pipette accurate to 1 µL.
5. The microplastic suspension was then added to the tanks and stirred for 1 minute for each tank repeating the process of sterilizing the stirring rod before and after each tank using boiling water.

C. Water samples were collected from the tanks each day to monitor the levels of microplastics

1. A set of water samples were then collected from each tank every day at 7 pm. The samples consisted of 1mL of water from its corresponding tank and were placed in glass vials. The samples were collected via a pipette accurate to 1µL and new sterile pipette tips were used for each collection of each tank.
2. All samples collected during the entirety of the experiment were kept in a climate-controlled (4 degrees Celsius) and light-controlled dark environment to ensure no bacteria grew and that the fluorescence of the microplastics was not affected.

D. The levels of fluorescent microplastics were measured using a Fluorescence Plate Reader

1. The samples were brought to UBC to the laboratory of Dr. Abby Collier (UBC) and measured with the assistance of Dr. Alexander Smith of the Collier Lab to measure the levels of fluorescence in the samples, as a method to measure the amount of microplastics remaining in the water.
2. The samples were first shaken for 15 seconds to ensure the microplastics were distributed equally.
3. Each sample of water was then divided into three separate samples, each having a volume of 250µL. These samples were placed on a 96 well plate to be assayed by the plate reader.
   The 96 well plate was then inserted into the plate reader (FlexStation® 3 Multi-Mode Microplate Reader). The settings of which were placed at Emission: 480 nm, Excitation: 538 nm.

E. The levels and localization of fluorescent microplastics were examined using a Fluorescence Microscope

1. The remaining 250µL from each sample was then used to analyze the water samples under the microscope. The microscope was both used under a Fluorescent and a light setting to observe both abnormalities and the levels of fluorescence microplastics. The microscope was also used to capture photos of the water samples.
2. Once all the data had been collected, the data was inputted into a spreadsheet.
3. The Ascidians that were used during the study were frozen for later research and in part to follow the agreement we had signed when purchasing these Ascidians.
4. At the lab, the Ascidians were dissected into three sections inside a biosafety cabinet. These sections of the Ascidians were placed into transparent plates and viewed under the microscope.
5. Photos were taken at different parts of each section and Ascidian.

F. Disposal of Microplastics

1. The remaining microplastics were filtered out of the water using a 5 µm filter. The water was then poured out and then the microplastics were recycled.

Materials

1. 9 Polycarpa Circumarata Ascidians
2. 72L of Compliments Distilled water
3. 15 mL of MAGSPHERE® 10 µm Fluorescent Polystyrene Latex Particles
4. 500mL of MarineSnow® Planktonic Food
5. 1 METLERTOLEDO pipette (1 µl-1 mL)
6. 6 32L type 5 plastic bins
7. 96 well plate, transparent & oblique
8. 10 cm transparent plate
9. Fisherbrand™ box of Latex Gloves
10. FlexStation® 3 Multi-Mode Microplate Reader (Fluorescence Plate Reader)
11. Zoe™ Fluorescent Cell Imager (Fluorescence activating Microscope

Variables

Independent Variable: The presence of Ascidians
Dependent Variable: The concentration of Microplastics
Controlled Variables:

1. Sanitization of the tanks- all tanks were sanitized before use
2. Number of Ascidians in each experimental group
3. Water temperature- all tanks were kept in a climate-controlled room
4. Amount of food given
5. Light exposure- all tanks were covered with reflective blankets
6. Water purity- distilled water was used
7. Salinity of water- a hydrometer was used to maintain constant salinity of 31ppt
8. The initial starting concentration of microplastics added to each tank
9. Time- all samples were collected at defined times

Control Group: For every experiment, we included a control that we treated the same as the rest of the experiment, but without the independent variable. The negative control groups were fed the same amount and at the same time as the experimental group to ensure that the food's possible fluorescence did not interfere with the experiment's results. There was one control group for every experimental group.

Results

The fluorescent microplastics in the water of each tank were monitored over time by measuring the amount of fluorescence of the fluorescent microbead **(Table 1)**. The microbead concentrations were significantly reduced in the three tanks that contained Ascidians compared to the controls **(Figure 2)**. The average microbead concentration in the Ascidian tanks was reduced by 24.7% after 1 day ($p = 0.04$). By day 4, the average microbead concentration had decreased by 94.7% ($p < 0.0001$) in the tanks containing the Ascidians. The negative control groups showed no significant change in microbead concentrations throughout the study, varying an average amount of 6% from start to end. Table 1 is comprised of the averages of the three samples collected from each day and each tank. As seen in the color-coded data, the control groups did not show changes in plastic concentration while the experimental group did.

Table 1. The Mass of Microplastics in Each Water Sample (mg/tank)

Day	Tank A	Tank B	Tank C	Control D	Control E	Control F
0	118	126	116	124	115	122
1	78	100	93	107	112	123
2	71	33	67	117	110	126
3	34	32	32	124	107	124
4	11	7	1	120	106	111

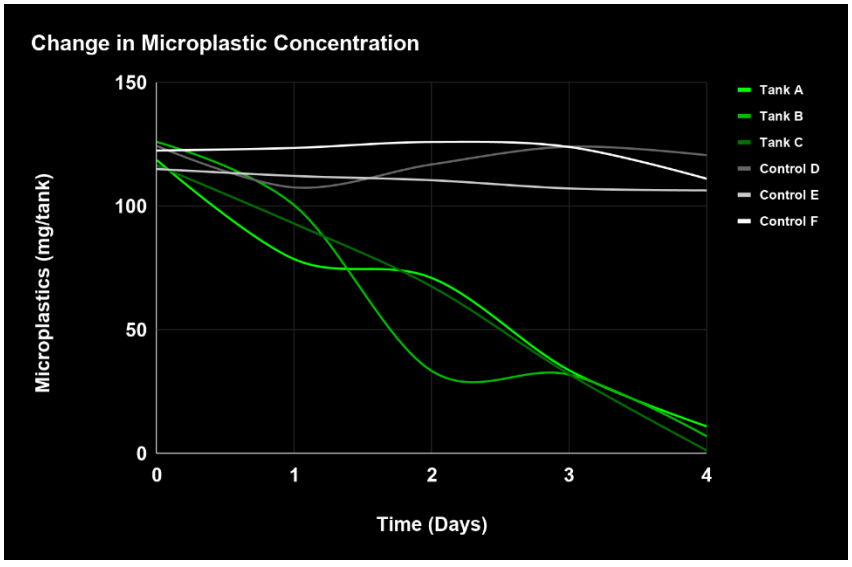

Figure 1. Change in Microplastics Concentration

As seen in Figure 1, the controls (D, E, F) showed very little decrease in change of microplastic concentration throughout the experiment, while the experimental group showed a steady decrease in microplastic concentration each day, resulting in a significant 94.7% overall decrease in microplastic concentration by the end of the experiment. The linear graph supports our hypothesis because ascidians have set times for their biological respiration and feeding processes,

like how humans eat meals at defined times throughout the day. From this, we reason that the filtration speed will also be constant which is why the graph should be linear.

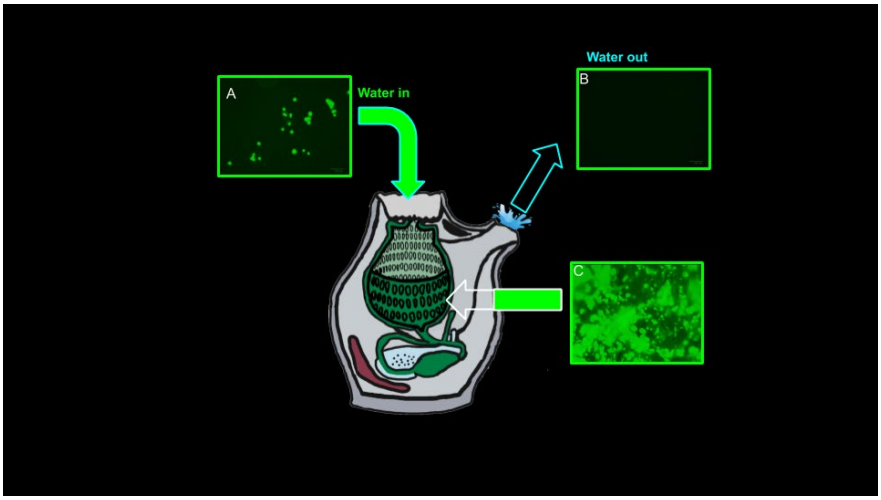

Figure 2. Graphic Representation of Microplastic-Flow Through the Ascidian

Figure 2 demonstrates the filtration through the Ascidian and where the microplastics are held. This information was gathered through dissecting the Ascidians and viewing them through a Fluorescence activating microscope.

1. Section A represents the water that is being taken in by the Oral Siphon. This water is contaminated with Microplastics.
2. Section **B** represents the water that is ejected from the Ascidian once the extraction process is complete. The microplastics that once inhabited the water are now gone and the water is clean.
3. Section **C** represents the various parts in which the microplastics are held following the extraction. Based on our results we hypothesize that the plastic particles are absorbed by the gut into the blood or other tissues of the Ascidian.

Discussion and Conclusions

Live Ascidians can be used as Efficient and Bio-Friendly Filters for Microplastics

We conclude that ascidians can efficiently filter microplastics from water through their natural feeding process. Our research demonstrates that this method of bio-filtration is an effective and feasible option for the extraction of microplastics in the ocean. These findings could lay the path for future research in efficient microplastic extraction from the ocean using invertebrate filter feeders. Based on our results, we extrapolate that a 1mx1mx1m cage of Ascidians would filter out approximately 300g of microplastics every day. This newfound knowledge can lead to many practical applications in the field of ocean pollution for instance mass-producing Ascidians for microplastic extraction.

Bio-Friendly Considerations

We define ascidians to be bio-friendly because current extraction methods mostly involve utilizing chemicals (such as ferrofluids) to remove plastics. These methods introduce foreign chemicals into an ecosystem which can negatively impact it (such as pH shifts as a result of adding chemicals), limiting the feasibility of those solutions. Our method with ascidians does not pollute the environment any further with any external chemicals or other synthetic materials.

Study Limitations

Since we did not have access to a saltwater lab, we could only test our hypothesis in still water. Our results may have been more accurate if we had a larger tank to better simulate ocean conditions, such as varying pressure and current flow. These dynamic variables were not incorporated in our original design because we needed to control the conditions to have a valid scientific experiment. Additionally, if we had more microplastics to work with, we would have created multiple microplastic suspensions to examine the effect of varying levels of plastic concentrations, which would be more representative of the real world.

Variation in Current Data

Our graphed data points show slight deviation from each other, and a possible explanation for this is that the Ascidians' physical sizes were different to begin with which may have affected their extraction ability. Additionally, we believe that taking measurements from data produced by live organisms is bound to have some variation that we, as scientists, cannot control.

Study Extensions

We now believe that the possibility of extracting microplastics from water may not only be limited to Ascidians. Ascidians are part of the Tunicate subphylum of filter feeders, which contains a broad range of filter feeders. We chose Ascidians for this project because the anatomy of the Ascidian appeared well-suited to the task of microplastic filtration. Indeed, we observed that the anatomy of Ascidians allowed the microplastics to be absorbed into their tissue and removed from the external environment. We suspect that other filter feeders, such as sea sponges, could also be used to filter microplastics due to the similarity in feeding mechanics.

Another path for extending our project would be to begin considering the engineering aspect of this filtration method. A moving cage system must be designed to protect the ascidians from any predators. Currently, we envision that after the Ascidians have absorbed all the microplastics, the Ascidian would be collected, decomposed and the plastics safely recycled.

Relevance and Implications

Scalability and Feasibility

Ascidians are found all over the world in shallow ocean waters, meaning that nearly all regions on Earth would have easy access to Ascidians. In addition to ease of access, Ascidians can reproduce both sexually and asexually, which contributes to them having a very fast reproductive cycle. Ascidians can produce a larva within 24 hours under prime temperature, and once in the larva stage, it takes Ascidians approximately 2 days to complete metamorphosis into a juvenile Ascidian, complete with incurrent and excurrent siphons to filter out microplastics. This means that there would be opportunities in setting up mass-scale ascidian farming operations.

Real-world Relevance

We decided to pursue a project on microplastic extraction because water pollution is a major worldwide problem that desperately requires innovative solutions. Our findings contribute to the current scientific understanding of

microplastic extraction by proving that bio-friendly ways to remove microplastics are feasible. With our research, there is now evidence that (1) Ascidians rapidly take up and hold onto microplastics from the water, and (2) extracting microplastics from water using invertebrate filter feeders as biofilters is feasible and effective.

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