Aurelia Aurita Mucus's Percent Uptake of Polyethylene, Capturing Microplastics with Jellyfish Mucus

Ellie Sohn

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Approximately 51 trillion microplastics (MPs) are floating alongside marine organisms in the ocean (Florida Atlantic University). Previous studies successfully proved that mucus from Medusa (mature jellyfish) successfully accumulated nanoparticles and MPs. In my 3-part experiment, using Fluorescent Green Polyethylene Microspheres, I tracked the accumulation of MPs in mucus from *Aurelia Aurita*. First, mucus was extracted from medium-sized Medusa and was mixed in a 3:2 ratio of MP stock solution to mucus. Next, to examine varying stimulus for mucus secretion, Medusa were placed in two varying conditions of non-scented water and brine shrimp scented water. Mucus was collected from both tanks 20 minutes later. Lastly, to pinpoint the most effective way of MP collection, three batches of mucus were extracted from Medusa stimulated with brine shrimp (*Artemia*) scented water. One batch was refrigerated for 48 hours, one fresh batch was placed on a mesh filter, and one batch was put in a container. The MP stock solution was run through all three batches. All solutions were photographed with a UV microscope and analyzed with ImageJ. The results indicate that mucus does successfully accumulate MPs and significantly more mucus is secreted when *Aurelia Aurita* are stimulated with brine shrimp (*Artemia*) scented water. In addition, mucus on top of a mesh filter better collects mucus than without a filter, and refrigerated mucus is ineffective in microplastic accumulation. These findings demonstrate that jellyfish mucus is capable of accumulating microplastics and in controlled conditions, Medusa can be stimulated to secrete greater amounts of mucus than usual. However, mucus must be used within 1 hour and cannot be stored as it turns ineffective to MP collection. Jellyfish mucus can be used to clean MP-filled ocean waters.

Keywords: Microplastics, Jellyfish, *Aurelia Aurita* Mucus, Microplastic pollution.

Abbreviations and Acronyms: Microplastics - MP

Introduction

Aurelia Aurita is a species of transparent jellyfish primarily found in the Pacific, Atlantic and Indian oceans also known as 'moon jellyfish'. Moon jellyfish begin their life cycle as planulae, fertilized eggs, and eventually hatch into polyps as they settle. These polyps eventually bud and become strobilating polyps that eventually release ephyra. Under the right conditions of salinity, temperature, and food, ephyra mature and become medusa – commonly seen in aquarium displays [1]. In the wild, moon jellyfish thrive in both oceanic coastal waters and warm tropical waters ranging in temperature from 6- 19 degrees Celsius [2]. They are carnivores and feed on zooplankton in the ocean. In lab settings, they are often kept in an Imhoff cone or Kreisel tank, varying by their stage in the life cycle. Moon jellyfish are kept at room temperature in 35 ppt salinity (can vary from 32-36 ppt). A bubbler is placed in the cones to mimic the tidal waves and brine shrimp (*Artemia*) are fed to the moon jellyfish. These *Artemia* can also be hatched in Imhoff cones as was done in my lab setting.

Jellyfish are known to produce mucus at varying amounts depending on their size. Their epidermis, outer layer of skin, and gastrodermis, inner lining of membrane, contain gland cells that produce mucus [3]. Jellyfish release up to 400mL/kg of mucus during digestion, reproduction, and under stress. They release this substance in order to clean, feed, and defend themselves from predators.

Jellyfish mucus is a hydrogel that consists of 95% water, 3% mucins, and 2% foreign substances [4]. Because jellyfish is an abundant raw biomaterial, its effective usage could be expanded across all oceans.

Polyethylene is also commonly found in oceans as it is the most common plastic used in the world at a demand of 53 million tons [5]. Polyethylene is used in production of plastic bags, containers, toys and household items. Although certain forms of polyethylene are recyclable, a significant amount of polyethylene ends up in oceans, approximately 51 trillion microplastics. The current methods for collecting and filtering microplastics out of the ocean rotates around Wastewater Treatment Plants (WWP). The primary WWPs are "discfilter (DF), rapid sand

filtration (RSF) and dissolved air flotation (DAF) and membrane bioreactor (MBR)"[6]. However existing methods have proved to have possible counter effects with WWPs acting as entryways to MP, or having size restrictions, filtering bigger size MPs. Other methods include using natural organic materials and forming biological "sponges" by intertwining plant material within the ocean[7]. Despite its natural roots, the sponge must be manually created by researchers, compared to the mucus which jellyfish naturally reproduce.

In previous experiments done by the University of Ljubljana, National Institute of Biology, and researchers across France, jellyfish mucus was found to be capable of capturing nanoparticles and microplastics[8]. Prior experiments focused on collecting mucus from multiple species of jellyfish and proving whether or not mucus can collect the MP, and nanoparticles. In my experiment, I went further and measured the percent uptake of MP in mucus produced from *Aurelia Aurita* medusa in multiple settings and stimuli, testing their percent capacity. Fluorescent Green Polyethylene Microspheres (1-5 microns in size) were used throughout the experiment to simulate MP in the ocean and to be easily trackable using UV light that illuminates the fluorescence. A control group was analyzed to ensure that there were no Fluorescent MP in the original lab water before its addition. My 3-part experiment seeks to confirm that mucus does accumulate MP, analyze if jellyfish can be stimulated to create more mucus, and what method is most effective in collecting MP. A study previously conducted found that jellyfish mucus was capable of accumulating MP and nanoparticles. Therefore, my hypothesis is that mucus can collect MP, and jellyfish can be further stimulated. Specifically, to my experiment, I predict that there will be significant uptake of MP by mucus with a mesh filter and jellyfish will secrete the most mucus under the stimulus of food. Jellyfish naturally secrete mucus in response to food, so their sensory recognition of that stimulus will likely trigger greater quantities of secretion. Further, the mesh filter will likely catch any microplastics that may accidentally slip through the mucus, helping increase MP collection in general. The results of the experiment will further our knowledge of effective ways to clean the MP- filled oceans using biomaterials such as mucus, answering: Can we clean our oceans non-invasively using jellyfish? And, what's the most effective way to do so?

Methods and Materials

I used Fluorescent Green Polyethylene Microspheres, brine shrimp, and *Aurelia Aurita* medusae. The organisms were each bred individually in an Imhoff cone. I stored the brine shrimp in 25 ppt water and medusa in 35 ppt water (Fig. 1). The salinity was measured weekly with a salinometer. Further, I used an ultraviolet (UV) light and microscope to examine the organisms and the microplastics in them. To prevent any safety risks, I wore gloves and UV filter glasses. I also put the medusa on top of lightboards to better examine them and identify mucus secretion to collect. Micropipettes were used to measure quantities to ensure accuracy.

The experiment was run in three separate parts. First, I extracted mucus from a medium-sized medusa grown in a Kreisel tank. In order to extract the mucus, one medusa was isolated in a 1L plastic container with 50mL of 35 ppt water and stimulated using physical stressors. I used the micropipette, pushing the water up and down to change the water current. Then with gloved hands, the medusa was aggravated by massaging the bulb of the jellyfish for 30 seconds. After stimulation, the jellyfish was left to secrete mucus for 5 minutes, and then the mucus was pipetted using a micropipette with its tip cut to make the opening wider for easier collection. The mucus was pipetted into two smaller containers of 10mL with two separate batches of 2.380 mL and 2.378 mL respectively. Then I created a stock solution of 32 mL of Fluorescent Green Polyethylene Microspheres (MP) from 20 mg/L. This solution was photographed under a UV light and microscope (Fig. 2, 3B). Then I pipetted 3mL of MP stock solution and 2mL of mucus twice to create two containers of 5mL MP-mucus solution. One container was spun on the Vortex for 10 seconds, the other container was spun on the Vortex for 30 seconds. The differing times were a cautionary step taken to make sure we could find the best conditions for equal & accurate mixing of MP and mucus. 10 seconds and 30 seconds were chosen as short enough times that would not harm the mucus viscosity with enough of a gap to have significant difference. In the first 5 attempts of part 1, the storage of MP-mucus solution past 12 hours proved to be ineffective. In the 6th, successful attempt, I photographed the MP-mucus solution right after spinning it on the Vortex, putting it under a UV light and using a microscope (Fig 3A, 3B).

In the second part, I isolated two medium-sized medusa in separate 1L tanks with 50mL of 35 ppt water. One container was identified as the control with no additional stimuli, the other container was stimulated with *Artemia* (Brine Shrimp) scented water. The Brine Shrimp were harvested in Imhoff cones in 25 ppt water and hatched for 48 hours. I extracted 20 mL of Brine Shrimp and ran it through a mesh filter to allow the actual Brine Shrimp to be filtered out. Then I pipetted 3mL of Brine Shrimp scented water 5 times into the experimental container. I then aggravated the medusa in both tanks by massaging the bulb of the jellyfish and let the jellyfish secrete mucus for 5 minutes. I then pipetted mucus from the control container and experimental container separately into 10 mL containers one with 5mL and the other with 8mL, respectively. I photographed the control under a UV light and microscope (Fig. 4A). And also photographed the experimental (Fig. 4B). I then refrigerated the 8mL batch of mucus from the experimental container in a 10mL container. The refrigerated batch was kept at approximately 40 degrees Fahrenheit, and placed on a container rack to prevent

degradation due to mishandling.

In the third part, I isolated one medium-sized medusa in a 1L container with 50 mL of 35 ppt water. The medusa was stimulated by aggravation through massaging of its bulb and 15mL of brine shrimp scented water. The secreted mucus was collected using a pipette and was separated into two 10mL containers each containing 2mL of mucus. One container was labeled as the control and the other as experimental. The experimental container had 2mL of mucus spread over a mesh filter and a 50mL container below the mesh (Fig. 5A). 2mL of the mucus solution refrigerated for 48 hours was also pipetted into a 10mL container. Then, 3mL of MP stock solution was pipetted into every container of mucus. The control had the MP stock solution mixed with the mucus using the Vortex for 30 seconds. The experimental container had MP stock solution run through the mucus-mesh filter (Fig. 5B). The last container of refrigerated mucus had MP stock solution added and was mixed using the Vortex for 30 seconds. Then each sample was photographed under a UV light and microscope. Finally, using Fiji (ImageJ) to analyze the data, I first used color thresholding, then converted the image to a binary mask, then filled the holes of the MPs, and lastly, used the analyze particles function at size 2-infinity. ImageJ was utilized to calculate the MP uptake by measuring the ratio of the areas in the UV light microscope images.

Results

Fig. 1 *Aurelia Aurita* Medusa in 1L container of 35 ppt water

Part 3 shows refrigerated mucus has deteriorated in size compared to its original status in the left photo of Part 2 and it's largely unsuccessful in MP collection (less fluorescence). Fresh mucus with mesh filter shows the clean filtered MP solution and the control shows successful accumulation.

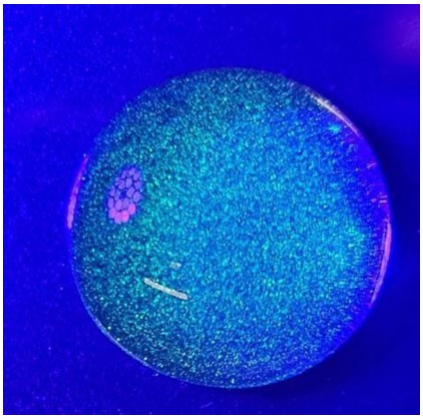

Fig. 2 Stock solution of 32 mL of Fluorescent Green Polyethylene Microspheres (MP) from 20 mg/L under UV light

Fig. 3A MP mixed with Mucus using vortex in Part 1 of experiment under UV light

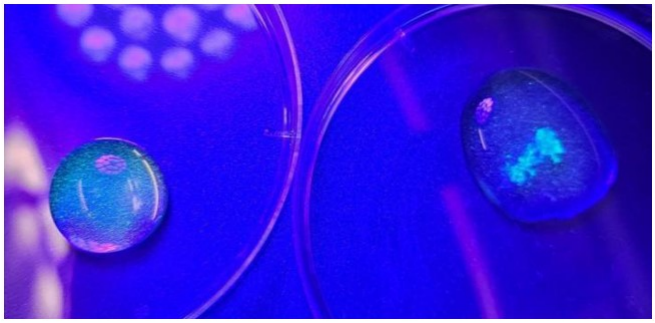

Fig. 3B Figure 2 and Figure 3A photographed side to side showing the mucus accumulation of MP stock solution under UV light

Discussion

I hypothesized that mucus could collect MP, and jellyfish could be further stimulated to create greater quantities of mucus. Further, in my experiment, I predicted that there will be significant

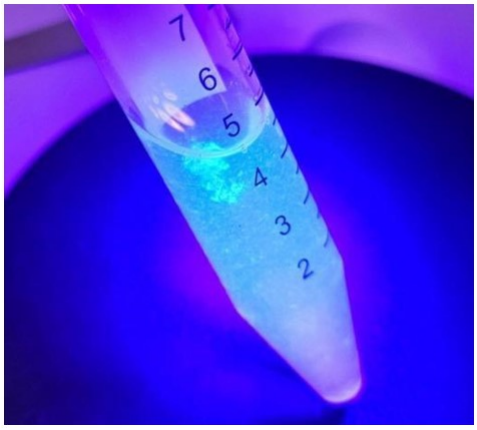

Fig. 4A Control of Part 2 of experiment, a MP-mucus mix with no additional stimuli under UV light

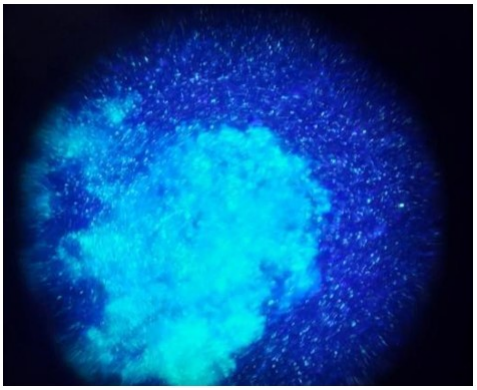

Fig. 4B Experimental of Part 2 of experiment, mucus from Medusa stimulated with Brine Shrimp scented water mixed with MP stock solution under UV light

Fig. 5A Experimental from Part 3, fresh mucus spread over a mesh filter with a 50mL container underneath from

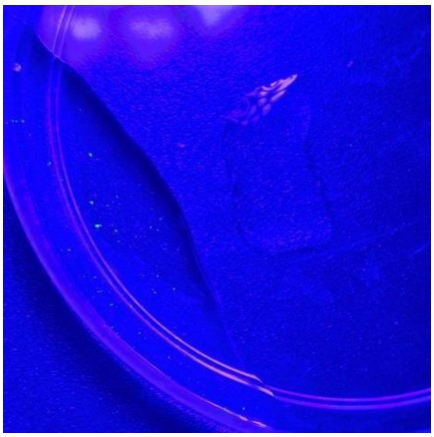

Fig. 5B Experimental result from Part 3, MP stock solution after being filtered through mucus-mesh filter

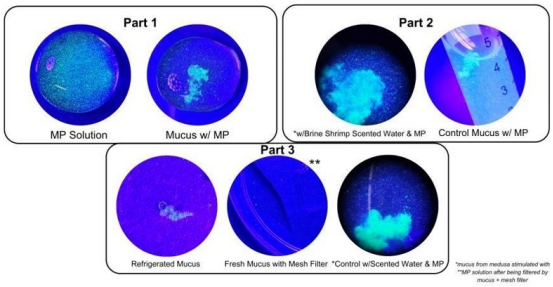

Fig. 6 Summary of findings, Part 1 shows mucus successfully accumulated MP from stock solution. The mucus is concentrated in the middle. Part 2 shows Medusa produced more mucus upon stimulation with Brine Shrimp scented water compared to the control on the right. Both successfully uptook MP.

Part 1		
Status	Percent Uptake of MP by Mucus	2SEM
Fresh Mucus	85.207	4.307
Part 2		
Status	**Mucus Secretion (mL)**	**2SEM**
Control (w/o Scented Water)	5.234	1.134
*w/ Brine Shrimp Scented Water	8.453	1.432
Part 3		
Status	**Percent Uptake of MP by Mucus**	**2SEM**
*Control (w/ Scented Water)	89.642	3.721
Refrigerated Mucus	5.921	1.632
Fresh Mucus with Mesh Filter	87.341	4.235
*=medusa stimulated with		

Fig. 7 Summary data table of all parts of the experiment with successful percent uptake of MP for Part 1 and 3 and comparable mucus secretion in Part 2. The data table does not reflect differences in MP collection of mucus depending on the time the container was put on the Vortex (10 sec, 30 sec), because data did not reflect statistical difference,

uptake of MP by mucus with a mesh filter and jellyfish will secrete the most mucus under the stimulus of food. My hypothesis

Fig. 8A There is a significant difference in mucus secretion between the control and experimental, Medusa stimulated with Brine Shrimp scented water. There was a near 3mL difference of production. Results were gathered by exposing species to microplastics. Sample size was n=1 for all data points. Sample size indicates the singular jellyfish used to extract mucus but does not reflect the 3 batches of mucus that were collected to calculate error bars. Error bars represent +/- 2SEM (2 x Standard Error of the Mean).

Fig. 8B There is no significant difference between the control and mucus with a mesh filter in percent uptake of MP by mucus. However, refrigerated mucus was significantly ineffective in MP accumulation at only 5 percent uptake. Results were gathered by exposing species to microplastics. Sample size was n=1 for all data points, error bars represent +/- 2SEM.

was fully supported by the data, as the mucus had an 88.132% average percent uptake of MP available and Medusa could be stimulated to produce 3.309mL more mucus using Brine Shrimp scented water. However, my prediction was slightly off because while food (Brine Shrimp) did stimulate more mucus secretion, mucus with a mesh filter did not have a significant difference compared to its control. The control with no mesh filter and only mucus had an 89.642% average percent uptake with 2SEM of 3.721. The experimental batch with the mesh filter and mucus had an 87.341% average percent uptake with 2SEM of 4.235. On the other hand, the refrigerated mucus had a mere 5.921% average percent uptake of MP showing a significant decrease in functionality compared to fresh mucus. These findings reflect significant uptake, and demonstrates the *Aurelia Aurita* mucuses' capacity to further be utilized to collect MP. This data can be applied for other experiments, and even functional engineering experiments looking to build a feasible prototype for the ocean.

The main findings in the experiment, mucus can successfully and effectively collect MP was not surprising, given the past studies that have shown jellyfish mucus can collect MP and nanoparticles. However, it was surprising to see the significant difference food stimulus made to the secretion of mucus by jellyfish. Whereas jellyfish do secrete mucus as a bodily function in response to food, the significantly large quantities of mucus secretion were unexpected. Furthermore, the functional deficiency of refrigerated mucus shows that mucus can best collect MP when in its freshly produced form. An indirect finding of this experiment was surprising, which was the fact that smaller medusa was unable to secrete large amounts of mucus and that mucus cannot be stored over long periods of time. In the first few trials of Part proved unsuccessful due to the lack of mucus secretion by smaller medusa. In addition, the mucus's self-dissolving properties when placed with small amounts of 35 ppt water after 24 hours proved to be a difficulty. However, when using medium sized medusa and fresh mucus, the experiment proved to be successful. A point to note is that the storage of absolute mucus with minimal to no water could decrease the rate of dissolving of the mucus. This goes to show that if medium sized or larger medusa can be collected from the sea and their mucus freshly collected, microplastics could be effectively cleaned from the sea waters.

I trust that my overall findings are correct, but I do not fully trust the specific data points in Part 3. This is because while quantifying the microplastics in Fiji (Image J) a few errors may have occurred: particles may have been counted multiple times, and particles may have been counted as one, when in a clump, instead of as individual MP beads. These errors were challenging to completely eliminate given the quality of pictures taken through the microscope. However, I have full confidence in my main results that mucus does successfully uptake MP, food stimulus is effective, there is no significant difference in between the presence and absence of a mesh filter with mucus, and mucus cannot be stored through refrigeration. This is because there was a visible accumulation of most MP in the mucus in Part 1 and multiple trials were run to confirm this uptake. In addition, food stimulus is effective because Brine Shrimp scented water did significantly increase the mucus secretion both quantitatively and visibly. The mucus was pipetted and accurately measured with minimal error. Despite the possible errors in Fiji for Part

3, there was still a visible accumulation of MP in the mucus and a visible filtration of MP through the mucus-mesh filter. In addition, the slight quantitative errors do not change the findings that mucus with or without a mesh filter does uptake MP successfully. The ineffectiveness of refrigerated MP in uptake was noticeable in the pipetting of the mucus as it was flaky rather than fluid, and there was no fluorescence in the refrigerated MP after mixing it with MP stock solution. Due to the study's focus set on comparing effectiveness of mucus in different forms, the refrigerated mucus samples were largely controlled in temperature and handling. The refrigerated mucus may have been degraded due to other variables such as change in water concentration, or length of storage. This experiment could be further expanded, but we trust that the functionality of refrigerated mucus stored for 48 hours is largely ineffective, due to the elimination of central exterior factors. Therefore, this shows despite possible quantitative errors, the main findings hold true.

On a broader scale, these findings help scientists and environmentalists devise ways to clean oceans filled with microplastics non-intrusively using biomaterials like jellyfish mucus. The microplastic used in this experiment, Polyethylene, is the most commonly used and could lead to the death of primary and secondary consumers, leading to a disruption in trophic levels that could be catastrophic for organisms higher up in the pyramid and organisms as a whole. Especially because humans consume nearly 5 grams of microplastics every week, it is essential to focus on cleaning our contaminated ocean waters[9]. Furthermore, the mass reproduction of jellyfish is straining the ecosystem, illuminating the need for jellyfish to be removed from water and to play a bigger role in cleaning ocean waters. When water temperatures become abnormally warm, jellyfish typically reproduce at faster rates, causing 'jellyfish blooms'. Jellyfish blooms are detrimental to the food web structure across trophic levels because the jellyfish's mass consumption of plankton disrupt and invade into the food supply of other marine organisms causing a ripple effect across the food web[10]. *Aurelia Aurita* were used within this experiment due to resource limitations but also to reflect the functionality of an organism that has a large population and presence within the ocean, with no known practicality. The findings of this experiment shows that it's possible to tackle two problems at once, of both MP contamination and jellyfish blooms. In effectively removing large quantities of jellyfish, collecting their mucus, and running it through the oceans water using forms of mesh filter contraptions, we could clean our oceans while helping alleviate the disruption of jellyfish blooms. The decrease in jellyfish blooms, if successful, could alleviate the current ecosystem from growing pressure.

Further, the feasibility of real-world application can be explored. Although it is not clear through this experiment the necessary quantity of mucus to clean each mL of the ocean, accounting the widespread water pollution, it's likely giant batches of mucus will be necessary. As this experiment and previous experiment[8] have demonstrated, *Aurelia Aurita* and other species of jellyfish are capable of secreting mucus that can be used effectively. Further, jellyfish can secrete greater amounts of mucus depending on size, so the older jellyfish that have greater bell diameters will likely provide large quantities. Although quantitatively unclear, based on experimentation, current jellyfish population, and precedents, jellyfish mucus can be collected in greater amounts to cover large areas within the ocean. The mucus would likely have to be deployed in a mesh filter matrix or similar contraption that can keep the mucus within the contraption, whilst filtering and collecting the MP. An engineering based experiment to develop such a prototype would make real-world applications more feasible. Limitations exist currently due to the little research and experimentation on utilizing jellyfish mucus. Questions such as, "how do we store the mucus?" or "how can we cross-apply this solution across the world?", are presented. However, continued research, as demonstrated in the paragraph below, can help expand the possibility of jellyfish mucus in collecting MP within large bodies of water.

In the future, we need to learn if mucus can be stored for longer periods of time and in what ways that could be done. The longer storage and effectiveness of mucus could make it easier to use over long periods of time. An experiment to test this would take a similar route to Part 3 of my experiment but could involve multiple batches that are frozen, refrigerated, stored with extremely minimal concentrations of excess water, or spun on the rotisserie. This experiment would let us see if mucus could be mass produced at once and used over intervals of time. Another experiment would be to test different contraptions that could be run through large bodies of water that would simultaneously trap the mucus while filtering out the microplastics. For example, a larger form of the mesh filter + mucus made in Part 3 could be created and run through a larger body of water, to see if similar methods would work. It's essential to continue the research and experimental work towards this sector of environment conservation because microplastics could prove fatal to the long chain of organisms, and finding a way to filter out microplastics, especially using biomaterials like mucus, from the ocean could be the first step towards a healthy and sustainable environment.

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Biography

Ellie Sohn is a 4-year student researcher focusing on examining microplastic pollution in the ocean correlated with marine organisms like *Aurelia Aurita*. She has competed in the LA

County Science Fair, California Science Fair, and Stockholm Regional Fair. She hopes to study environmental science or animal biology in the future.

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