At what rate will algae reproduce in a micro-gravitational setting versus on Earth?

Published by **Admin** on June 21, 2019

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ABSTRACT

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This study investigates the viability of growing microalgal biomass in a microgravity environment. Microalgal biomass has numerous useful applications; it can be used to produce biofuel, animal feed, and even dietary supplements for human consumption (Yaakob, Zahira, et. al. 2014). Moreover, the primary purpose of this experiment is to determine if algae can be a reliable and renewable source of biofuel. Generally, microalgae produce an average of 5000-15000 gallons of oil per acre per year ("Algal Oil Yields." n.d. Web. 14 Dec. 2014), which is almost seven times more productive than the next productive oilseed yield (oil palm). In fact, previously tested algae samples have shown that certain types of green algae can produce up to 77% oil content as compared to oil seeds ("Algal Oil Yields." n.d. Web. 14 Dec. 2014; Zhao, Chen, et. al. 2013). However, these values may not hold under microgravitational conditions. As participants in the Student Spaceflight Experiments Program (SSEP) we proposed to test growth under microgravity conditions, we used a heterotrophic strain of algae, *Scenedesmus*, that is able to reproduce in the absence of sunlight. *Scenedesmus*is frequently used in biofuel production due to its durability, 40% lipid content, and its ability to survive solely on glucose in order to reproduce (Hannon,

Michael, et. al. 2010; Radler, Don. 15 June 2001; Rogers, Rob. 11 Aug. 2013). Our results show that the algae survived a period of 29 days of microgravity, and were able to reproduce when sent to the International Space Station. Further, the viability in the micro-gravitational environment was comparable to the viability of algae grown on Earth. This experiment did not measure oil production, so further studies would be needed to determine whether microgravity impacts oil production by this algae.

INTRODUCTION

Fuel is a key determinant of the cost of space expeditions; if fuel could be produced in space, the cost of space exploration would be significantly reduced. The algal biofuel industry is capable of producing billions of gallons of renewable fuels, which could be more efficient and environmentally sound than traditional fuel sources (“Algal Oil Yields.” n.d. Web. 14 Dec. 2014). In this experiment, empirical evidence describing the rate of microalgal biomass reproduction in microgravity will be gathered in order to compare it to the rate of reproduction on Earth. Microgravity, as defined on NASA’s website, “comes about whenever an object is in free fall.” In the case of an orbiting spacecraft the object is “falling” around the

Earth, thus experiencing only 89% of the gravity felt on the surface of Earth ("What is Microgravity." Feb 13, 2009). If the algae in a micro-gravitational environment reproduce at a rate comparable to its rate of reproduction on Earth, it could be concluded that algae is a viable source of biofuel in a micro-gravitational setting. In the experiment, two closed samples of the *Scenedesmus*strain of algae will be compared. For each sample, algae will be placed in an FME (fluids mixing enclosure) tube and given only a glucose solution as a food source. All confounding variables will be eliminated so that only the effects of the independent variable (the environment the algae was exposed to) are evaluated. If the micro-gravitational sample produces a higher yield of extractable and usable oil (hypothetically based on prior studies of algal growth in the absence of gravity), there would be evidence to indicate that biofuel production is even *more* efficient in space (Wiltberger, 1987). It is beneficial to study different settings in which algae could grow in order to take advantage of the setting with the highest reproduction rate and highest efficiency. With the increasing demand for biofuel for multiple industries on Earth, the need for rapid production of oil will need to be utilized. Experiments pertaining to the reproduction of algae in microgravity

have been performed prior to this experiment, however, current knowledge is limited to the measurement of carbon dioxide emissions of the algae rather than its functionality as a biofuel source. With the analysis of the reproduction of microalgal biomass that was exposed to a microgravity environment, the feasibility of using algae as a biofuel source in outer space can be determined.

METHODS

A. Growing the Algae Culture

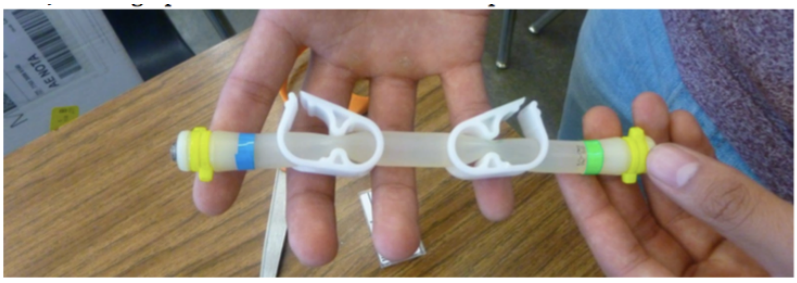
Algae was purchased from Carolina Biological Supply Company (2700 York Rd, Burlington, NC 27215) and shipped to Petaluma High School (Petaluma, CA). Algae was transferred within 3 days time to an acid-washed 500 ml Erlenmeyer flask with aeration provided by stirring (on a stir plate) and covered with sterile foil. With some initial optimization of cell culture maintenance based on procedural suggestions from the Wilkerson-Dugdale laboratories (Wilkerson, Frances. February 2015; “Australian National Algae Culture Collection – Methods.” n.d. Web. 14 Dec. 2014.https://www.csiro.au/en/Research/Collections/ANACC) we settled on maintaining culture in premade “Carolina” Alga-Gro freshwater medium recommended for*Scenedesmus*by Carolina for growth. The

solution was maintained at room temperature and at a pH of 7 (+/- 0.3), with additions of potassium hydroxide added as a buffering agent. Cell culture was periodically checked for cell growth and condition (checking for contamination). While some algae collected on the walls of the flasks the sample remained primarily in suspension. As the culture grew, aliquots of 10 ml were transferred to 200 ml of Alga-Grow Solution (Carolina Biological) periodically to reduce the likelihood of the sample crashing due to overcrowding. Aseptic technique was used during transfer to prevent contamination of the samples. Surfaces were wiped down with 10% bleach solution, gloves were worn, and only sterile pipettes were used for transfer to acid-washed flasks.

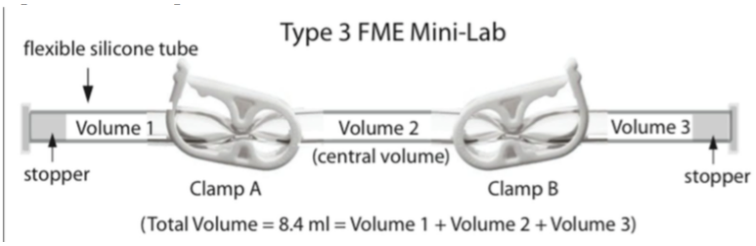
B. Preparing Samples for Launch
In order to be launched into a microgravity environment, the algae cells were prepared in a two-experimental volume fluid mixing enclosures (FME), one to be sent to the ISS and one to remain on Earth as the control.

Figure 1: Fluid mixing enclosure (FME) Mini-lab used to culture algae under microgravity and Earth-gravity conditions.

a) Photograph of actual Mini-lab used in experiments.



Minilab image taken at Petaluma High School b) Diagram of Mini-lab showing the three chambers and two clamps used in the experimental set-up.



Volume 1 consisted of the algae culture. Volume 2 consisted of the 1% glucose solution used to feed the algae culture at a specified time point. Volume 3 consisted of glutaraldehyde used to fix the sample at a specified time point. Minilab design diagram courtesy of NCESSE (National Center for Earth and Space Science Education).

The FME mini-lab was a flexible silicone tube with three compartments for fluids, separated by clamps that could be unclamped upon reaching microgravity (Figure 1A, 1B). The total volume of the silicone tube was 8.4 mL, divided evenly into three compartments. One compartment was prepared to hold one hundred thousand cells of microalgal

biomass, one held 1% glucose solution to provide the algae with a food source in low light situations (Zheng, Yuting, et. al., 14 Nov. 2013)and a third held a fixative solution of 0.5% glutaraldehyde. Two identical FME tubes were prepared: one as a ground sample, and one to go into a microgravity environment. A hemocytometer was used to count out approximately one hundred thousand algae cells (for each FME). Once inserted in the FME (with a stopper on one end), the clamp was inserted on the FME to close off the volume of algae cells. The second volume was then filled with glucose solution and the second clamp was inserted on the FME to close off the middle chamber. Finally, the third volume was filled with glutaraldehyde solution and a stopper was placed on that end of the tube. Both FMEs were then packaged and refrigerated. Control (earth) FME remained in refrigeration at Petaluma High while the microgravity FME was shipped to NanoRacks (555 Forge River Rd Ste 120, Webster, TX 77598)for pre-flight preparation. NanoRacks is a private company that offers commercial services payload preparation for spaceflight. Shipping of samples occurred on March 13, 2016 and samples were received on March 16th, 2016. Our experimental treatment FME was taken out of shipping packages and refrigerated at Nanoracks prior

to launch. NanoRacks logged receipt of shipment, heat sealed level 2 and 3 containment bags around the mini-laboratory and loaded the mini-lab into the SSEP Mission 7 Payload. Control (ground) samples were removed from refrigeration on the same day that SSEP Mission 7 Payload was loaded to the Dragon capsule. Our Earth-based FME was then placed in a darkened room at room temperature incubation, similar to conditions on the International Space Station (ISS).

C. Microgravity Procedure
On March 30, 2016, SSEP Mission 7 Payload was loaded into Ferry Vehicle. The samples were launched aboard a Falcon 9 rocket with Dragon capsule on April 8, 2016. Following is a record of Mission 7 from the Experimental Log: “At 6:44 pm ET NanoRacks reported that ISS crew member Tim Peake performed the A=0 crew interactions for the Odyssey II payload on-orbit on April 11, 2016.” Following is a record of Mission 7 from the Experimental Log:

- “At 6:44 pm ET NanoRacks reported that ISS crew member Tim Peake performed the A=0 crew interactions for the Odyssey II payload on-orbit on April 11, 2016.”
- “At 1:42 pm ET NanoRacks reported that ISS crew member Tim Peake performed

the A+2 crew interactions for the Odyssey II payload on-orbit April 13, 2016. The interactions began at approximately 10:00 AM ET."

- "At 4:48 pm ET NanoRacks reported that ISS crew member Tim Peake performed the U-14 crew interactions for the Odyssey II payload on-orbit on April 27, 2016. The interactions began at approximately 5:45 AM ET (see Table 1)"

Table 1: Handling instructions to ISS astronaut staff. Where "A" represents arrival to the ISS, and "U" represents undocking from the ISS for the return to Earth. Each number is one day.

Allowed Crew Interaction Day	Our Requested Interaction
A=0	
A+2	
U-14	Open Clamp A and shake vigorously for 10 seconds
U-5	Open Clamp B and shake vigorously for 10 seconds
U-2	

- "At 4:46 pm ET NanoRacks reported that ISS crew member Jeff Williams performed the U-5 crew interactions for the Odyssey II payload on-orbit on May 7, 2016. The interactions began at approximately 4:40 AM ET."
- "At 4:18 pm ET NanoRacks reported that the U-2 crew interactions for the Odyssey II payload were performed on-orbit on

May 9, 2017. The interactions began at approximately 4:00 AM EDT. (see Table 1)"

- "On May 12, 2017, the samples splashed down off Long Beach, CA." "The SSEP Mission 7 Odyssey II payload of experiments will be in-hand at NanoRacks in Houston on May 13, 2016. Our mini-lab was held at NanoRacks through the weekend and will be shipped overnight on Monday with delivery on Tuesday, May 17, 2016"((Hamel, Stacy. *SSEP Mission 7 Experimental Log*. 2016. NCESSE. Web.http://ssep.ncesse.org/current-flight-opportunities/ssep-mission-7-to-the-international-space-station-iss/ssep-mission-7-to-iss-flight-operatio ns/ssep-mission-7-to-iss-experiment-log)).

Microgravity Sample:

Please refer to Table 1 and Appendix I for the Experiment Details Confirmation Form which details the exact handling for the samples during flight. At U-14 the clamp that was between the volumes of the algae and the glucose solution was removed, and the tube was gently shaken so that the two volumes mixed. At U-2 the second clamp was released and the tube was gently shaken so that the two volumes mixed. This in effect fixed any

living algae at that point in time, so that when we analyzed the samples later we would be able to determine the state of the algae after 2 weeks in microgravity. Upon return to Earth, the experimental apparatus was repackaged (which included the addition of the cold pack), and shipped back to Petaluma High School.

Ground Sample:

In order to eliminate the possibility of confounding variables, the ground sample was exposed to the same experimental procedure as the microgravity sample. At approximately the same time as the microgravity sample, the cold pack of the ground sample was removed and the FME was allowed to thaw and acclimate to the temperature of the environment in Petaluma. The algae sample continued to settle and reach room temperature. On April 27th team members Alana Roberts and Liza Strong removed clamp A and the experiment was gently shaken so that the volumes of the algae and the glucose solution could combine (equivalent to U-14, see Table 1). The algae of the ground sample was then allowed to undergo reproduction, undisturbed. On May 7th team member Miranna Lindberg performed the U-2 interaction for ground samples (see Table 1). In the end, both

samples were analyzed as described below and the results were compared.

D. Analysis of Samples

Following the sample removal from the protective shipping material, the FME tubes were cut open and the algae samples were placed into clean glass vials. SSEP members later placed small portions of the algae samples into individual centrifuge tubes. Trypan blue was added to each centrifuge tube at a 1 to 1 ratio. The trypan blue was used in order to distinguish the dead algae apart from the live, as the dead is permeable to the blue coloring. One drop of sample with trypan blue was added to the hemocytometer via pipette, with a coverslip placed over it.

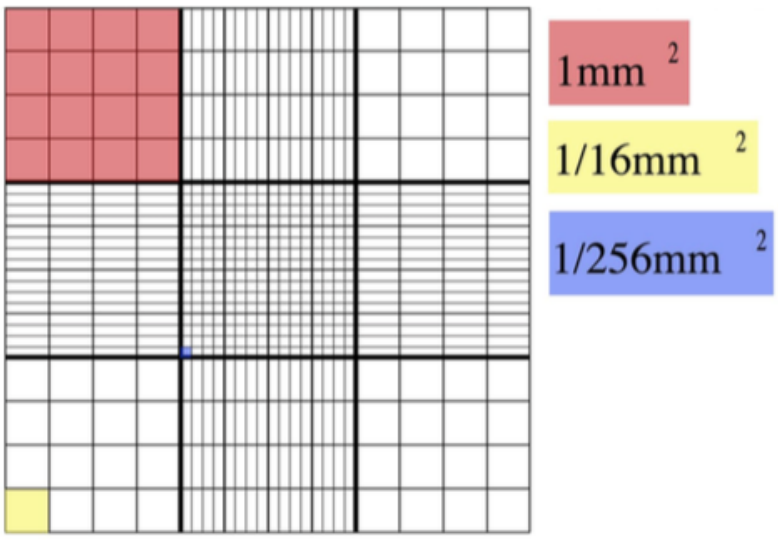
The hemocytometer was placed under a microscope, where SSEP members counted live algae cells as well as the dead. Methods for using the hemocytometer were found on "Introduction to Counting Cells – How to Use a Hemocytometer"1. Basically, the cells were counted in the four corner squares and the center square of the hemocytometer (Figure 2). Cells/ml were calculated as follows:

cells/ml = (number of cells counted x 2)/5 x 10000

Where 2 = the dilution factor in trypan blue and 5 = the number of cells counted

In between sample countings, the glass vials of algae samples were sealed with parafilm wrap and covered in foil in order to protect against contamination and degradation of samples. Our student authors organized themselves into analysis partners and repeated the steps at least three times in order to calibrate our technique across analysis teams. Then teams performed analysis according to their availability, and data from all teams were added to a shared data sheet, to be analyzed at a later date.

Figure 2: Hemocytometer grid, ((“Haemocytometer grid.png.”, 17 March 2007)



ANALYSIS & DISCUSSION

Each sample returned had a volume of 8.4 ml. Because of the limited time we had to complete our analysis, we used a power analysis to determine the minimum size of

each of our algae samples needed in order to have a sufficiently representative sample of each (Zarr, Jerrold H., 1999). With the time and personnel available, we determined that we would analyze enough sample to have greater than 60% confidence of adequate statistical power. While more samples would have given us greater confidence in our statistical result, we worked within the constraints of the SSEP protocol and our own time limitations to the best of our ability. The data are recorded in Table 2.

Table 2: Data from hemocytometer counts

		Ground Data				
	Quadrants	Top Left	Top Right	Middle	Bottom Left	Bottom Right
9/12/2016	# of Live Cells	19	29	7	19	20
	# Dead Cells	3	2	2	1	3
10/11/2016	# of Live Cells	6	14	30	13	5
	# Dead Cells	3	9	4	4	3
10/26/2016	# of Live Cells	8	10	13	14	13
	# Dead Cells	20	8	6	7	16
10/27/2016	# of Live	0	1	0	1	1
	Cells					
	# Dead Cells	1	1	0	0	0
10/27/2016	# of Live Cells	7	12	7	0	9
	# Dead Cells	5	11	8	0	29
11/4/2016	# of Live Cells	11	16	12	21	10
	# Dead Cells	6	10	7	3	3
1/17/2017	# of Live Cells	22	19	9	13	9
	# Dead Cells	4	19	12	12	3

		ISS Data				
	Quadrants	Top Left	Top Right	Middle	Bottom Left	Bottom Right
10/05/2016	# of Live Cells	10	4	11	8	10
	# Dead Cells	0	5	2	1	1
10/31/2016	# of Live Cells	15	18	17	12	9
	# Dead Cells	5	8	4	3	5

We performed a chi-squared analysis of our data to determine whether or not there is a measurable difference in survival of *Scenedesmus*between microgravity and Earth-gravity samples. In this instance, our null hypothesis was that there should be no difference in percent viability between the control (Earth-gravity) samples and the experimental (microgravity) samples. In other words, the null hypothesis stated that the algae should have an equal chance of survival in either microgravity or Earth gravity. For the observed values, we used the ratios of live and dead cells counted from both treatment and control samples. Chi squared analysis is sometimes used in biological analysis where samples are assumed to be “normal” or as expected. In this test, the measured values are compared to the expected values and tested to see whether they fall within one degree of freedom of the expectation. In our samples we used the average cells/ml of all counts as the expected value. Under the null hypothesis the counted values should fall within a degree

of freedom of the average. If they do not then they are unusual and suggest a difference between the treatment and control. See calculations presented in Table 3 and Table 4.

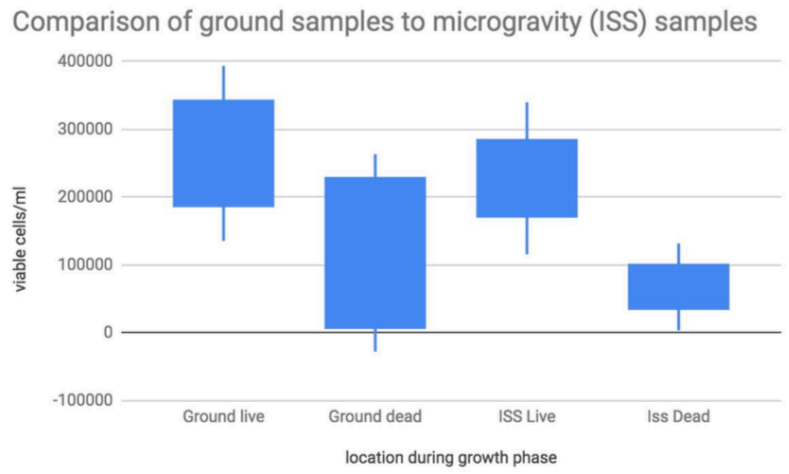
Table 3: Calculating the % Viability of Samples

	Ground Sample analysis		Microgravity Samples	
	# of viable cells/mL	# of non-viable cells/mL	# of viable cells/mL	# of non-viable cells/mL
	number counted x 20,000	number counted x 20,000	number counted x 20,000	number counted x 20,000
Average observed	264,666.67	128,571.43	228,000.00	68,000.00
Median	276,000.00	116,000.00	228,000.00	68,000.00
Standard Deviation	77,249.38	86,731.11	79,195.96	45,254.83
Standard Error	51,780.00	35,407.83	56,000.00	32,000.00
% Viability Observed	**67.30**		**77.03**	

Table 4: Chi^2 Analysis of Viability

	% Viability Observed	% Viability expected	(O-E)2/E
Microgravity samples	77.03	70.51	0.33
Ground samples	76.30	70.50	0.14
X2			**0.47**

Figure 3: Graph of viable & non-viable (dead) cells in ground samples and microgravity samples.

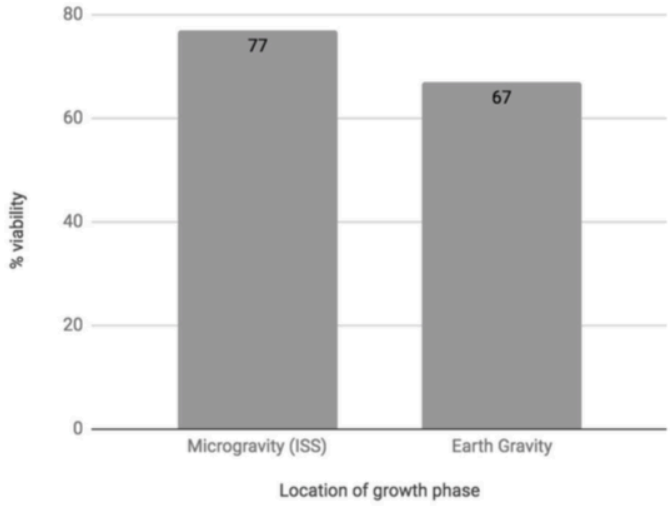


This range graph shows that there is a lot of overlap in terms of the numbers of live & dead cells for both the Earth-gravity samples and ISS samples. There is also overlap between live & dead cells (within standard error) for both treatments.

We report a value of 67% viability in the ground sample and a value of 77% viability in the microgravity sample. For the expected values, we averaged the observed ratio values for both treatments (72.1% viability; Table 4 and Figure 4). We calculated a Chi^2 value of 0.65. Comparing this to a published table of critical Chi^2 values we found that there is a 50-25% chance of observing a result such as ours (Zar, 1999). As a result, we accept our null hypothesis; there is no statistical difference between our samples. Microgravity has no measurable effect on survival of *Scenedesmus*, as the algae grew equally well in both environments. While our data showed no statistically significant difference, it does suggest that the algae may actually grow a little better in microgravity, but further studies should be performed to confirm this trend.

Figure 4

Comparison of algal cell viability between Earth gravity and microgravity.



This graph shows that percent viability was very similar between the two treatments. The Chi^2 analysis supports the assertion that there is no significant difference between control (Earth-based ground samples) and treatment (microgravity) samples. Scenedesmus is hearty enough to be a candidate algae for growth in microgravity environments.

The results of our experiment have important ramifications for the future of space travel. The purpose of this investigation was to determine if algae would successfully reproduce in a micro-gravitational setting, which would enable space stations and explorers to use algal biofuel to sustain longer and less expensive flights. Although more experiments should be completed to reinforce the validity of the data we collected, our results show that algae is indeed a viable source of biofuel in a micro-gravitational

environment. This information could be used to drive new research into the use of algae as a biofuel, thereby reducing the amount of fuel needed for a space mission, which could reduce the costs and hazards associated with traditional fuel sources. Simply bringing a sufficient starting stock of algae and growing the algae for fuel on the mission would significantly reduce the volume and weight of items needed at launch.
Growing algae on a space mission could be a source of oxygen and nutrients for astronauts (Wiltberger, 1987). Our result could inspire new research into oxygen production and carbon dioxide consumption of algae in a space station and/or interplanetary shuttle type environment.

Solving complex engineering problems such as these will be instrumental if we are to expand the scope, utility and frequency of space exploration in the future. Although our experiment produced clear results supporting the viability of algae as a fuel source in a micro-gravitational environment, more trials of our experiment should be performed to eliminate any sources of uncertainty. After this is complete, methods for transporting sufficient amounts of algae into space for fuel could be developed. An efficient method for refining the fuel in space should also be developed. Over the past few decades there

has been a lot of interest and study of the potential for biofuels and the processing of biofuels but most of that has been focused on plant-based material and less on algae (Umakanta Jena, and S. Kent Hoekman, 2017). One consideration would be the need for a water source once in space since water is required for growing and converting algae to a fuel source (Wiltberger 1987). For example, a base on the Moon or on an asteroid where ice is present could ensure a source of water for biofuel production. Once these methods are created, algae could actually be used as fuel in space missions.

ACKNOWLEDGEMENTS
We would like to thank all those who sponsored our work financially as well as all those who supported and encouraged us to reach for the stars!

Specials thanks to the NCESSE and their SSEP with Stacy & John Hamel, Jeff Goldstein and their partners at Nanorocks, particularly Hannah Gorse.

Major Funders include Petaluma City Schools, Environmental Protection Service (EPS), Tamura Environmental, Stu Clark, General Dynamic, EBA Engineering, Beyers Costin Simon law firm, and Advanced Stormwater Protection.

Lastly, we would like to thank the many individual contributors, our families and community members that made our work possible. And we would certainly be remiss if we did not acknowledge the major assistance from Petaluma High Principal David Stirrat, Vice Principal Debbie Richardson, and the Petaluma High Science Department. Thank you for believing in us!

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1. "Introduction to Counting Cells – How to Use a Hemocytometer." 23 July 2013) and demonstration from staff at the Wilkerson Lab of the Romberg Tiburon Center (1mm2, See Figure 2; (Wilkerson, Frances, February 2015. [↩]

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