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Sandia National Laboratories

Save the Algae, Save the World

Sequencing Bacteria that Protect Algae from Microscopic Grazers

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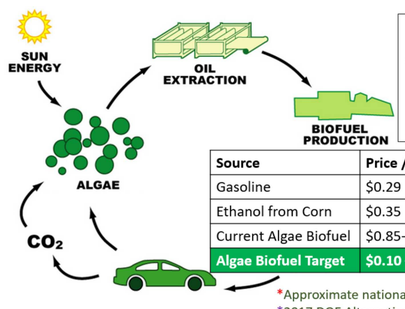
Biofuel from algae is sustainable, but not yet economical.

In order to achieve usable algal biofuels, we must overcome the market's barrier to entry.



Target selling prices for algae biomass in 2022 is $494/ton AFDW (ash-free dry weigh; 2014$) to achieve $5/GGE (gasoline gallon equivalent). - BETO MYPP, 2014

Source	Price /lb	Price /gal
Gasoline	$0.29	$2.49*
Ethanol from Corn	$0.35	$2.92*
Current Algae Biofuel	$0.85- $3.67*	$7.06 - $30.46
Algae Biofuel Target	$0.10 – 0.25*	$1.50 - $2.10

*Approximate national average for 2017
*2017 DOE Alternative Fuel Price Report
*Sun et al, *Energy*, 36 (2011) 5169-51795.
*BETO Multi-Year Program Plan, March 2016

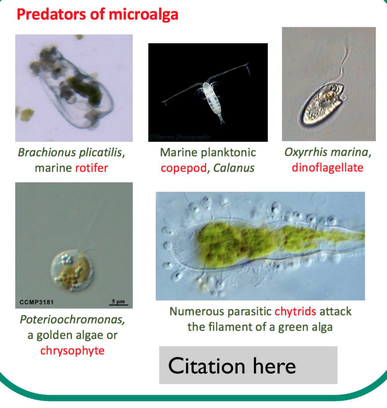
The first step towards biofuel reality is maintaining a substantial crop production. Algae is grown in **large, open-air ponds** which are **unprotected against microscopic predators**, leading to frequent "**crashes**".

Algal population crashes cause **losses of up to 30% of annual crop production** from the typical open raceway system.

Citation here

Tiny predators eat into profits.

Predators of microalga



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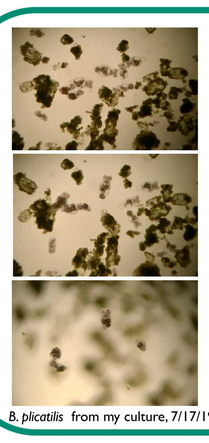
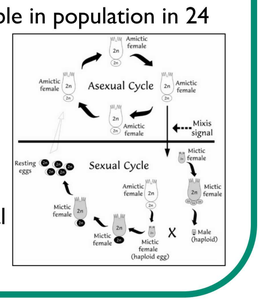
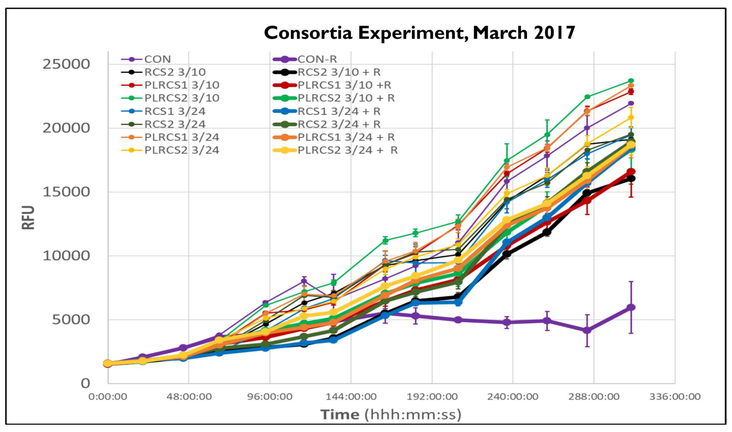


B. plicatilis from my culture, 7/17/19

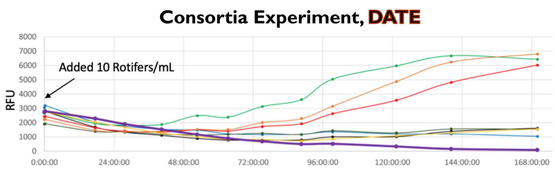
- Rotifers are considered the smallest animals on earth.
- A single rotifer can eat 200 algae cells per minute.[1]
- They can double in population in 24 hours.[2]
- Rotifers can reproduce sexually or asexually, depending on environmental conditions.[3]



Our lab found a consortium of bacteria that protected the alga *M. salina* from the rotifer *B. plicatilis*.



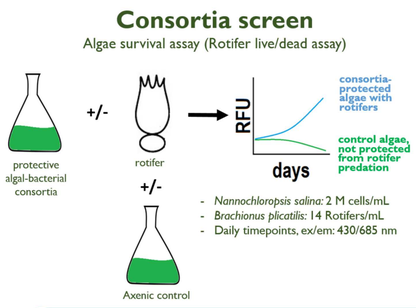
# rotifers in each well		sample name
~10	4	Consortia 1
0	2	Consortia 2
0	9	Consortia 3
>20, fast	1	Consortia 4
3	5	Consortia 5
0	3	Consortia 6
>20, fast	4	Consortia 7
>50, fast	>50, fast	Ns control



Previous Experimental Results, March 2017 and DATE

After rotifers were added (Day 4), the control culture crashed, but cultures with a mixture of bacteria continued to grow.

Final rotifer counts were lower in the consortia samples than in the control.

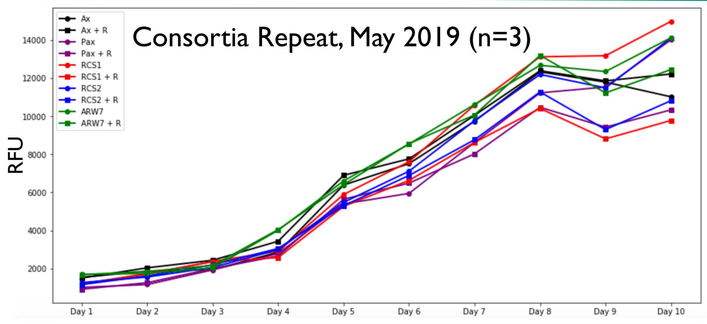


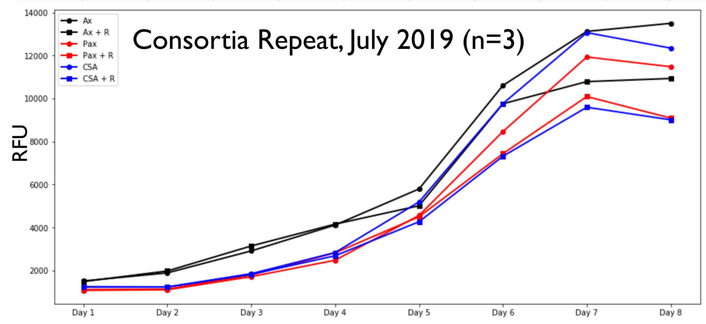
Representative flasks from 2017 experiment, showing differing concentrations of algae

My Experimental Results, May 2019 and July 2019

I attempted to replicate phenomena observed in multiple experiments in 2017. None of my algae cultures crashed, including the control. This result was unexpected; algae grazed by rotifers normally crash.

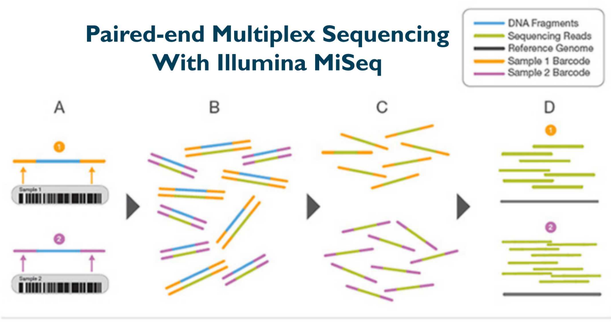
Future experiments: Possible solutions include increasing rotifer density or repeating the procedure with a more carefully maintained axenic control.





We are analyzing the protective bacterial consortia with16S RNA sequencing.

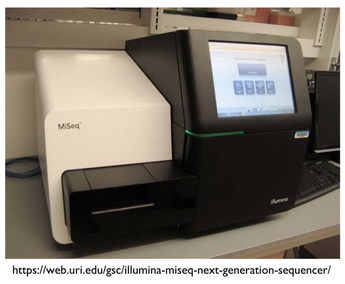
Paired-end Multiplex Sequencing With Illumina MiSeq



A. Two representative DNA fragments from two unique samples, each attached to a specific barcode sequence that identifies the sample from which it originated.
B. Libraries for each sample are pooled and sequenced in parallel. Each new read contains both the fragment sequence and its sample-identifying barcode.
C. Barcode sequences are used to de-multiplex, or differentiate reads from each sample.
D. Each set of reads is aligned to the reference sequence.

Source: www.illumina.com

Multiplex sequencing reads DNA from multiple samples at once, saving time and reducing cost. It uses nucleotide sequences as barcodes to "demultiplex" (sort) the data after reading.

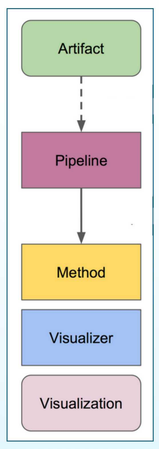


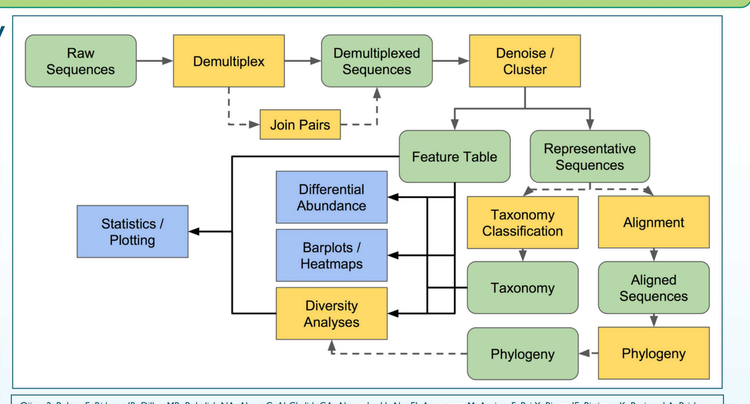
https://web.uri.edu/gsc/illumina-miseq-next-generation-sequencer/

MiSeq's output files must be processed to be useful.

I am using Qiime2 to analyze our data.

Qiime Workflow





Qiime 2: Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet C, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope E, Da Silva R, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley G, Janssen S, Jarmusch AK, Jiang L, Kaehler B, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MG, Lee J, Ley R, Liu Y, Lofffield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton J, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson, II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CH, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. 2018. QIIME 2: Reproducible, interactive, scalable, and extensible microbiome data science. PeerJ Preprints 6:e27295v2 https://doi.org/10.7287/peerj.preprints.27295v2

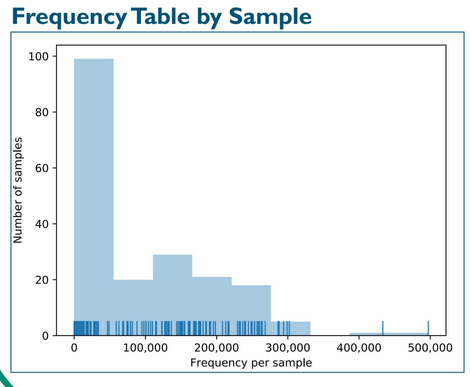
My Qiime Data So Far (in progress!)

Feature Table

Feature ID	Sequence Length	Sequence
0d7d8d7c1fce38d88457042d016e0298	252	CTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGGGGAAACCCTGATCTAGCCATGCCGCGTGAGTGACGAAGGCC
806c99b199101144bc2dfa667c10fef9	252	CTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAATGGGCGCAAGCCTGATCTAGCCATGCCGCGTGAGTGATGAAGGCC
0fdc0f60ce2bd6d95bd0d82cfdaa7729	252	CTCCTACGGGAGGCAGCAGTGGGGAATCTTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCC
067835b2d2d7bd7e262643e28c225278	252	CTCCTACGGGAGGCAGCAGTGAGGAATTTTCTGCAATGGGCGAAAGCCTGACAGAGCAATACCGCGTGGGGGATGAAGACC
ecab246ac2aa2ee763f4748ae54582dd	252	CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCC
87424f17d620a107322537b79a26de98	252	CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCC

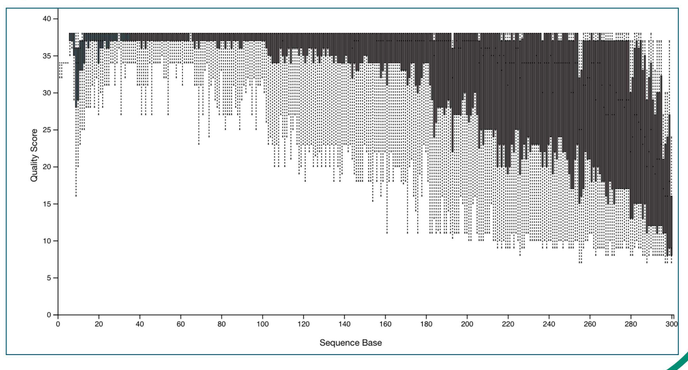
Sequences from the raw data are mapped to known DNA sequences called Operational Taxonomic Units (OTUs). These can be used with existing databases for taxonomic identification.

Frequency Table by Sample

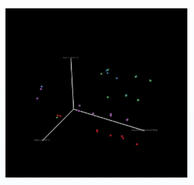


Metrics like these reflect the quality & quantity of the data, helping us choose a suitable depth of analysis.

Quality Table for Forward Reads

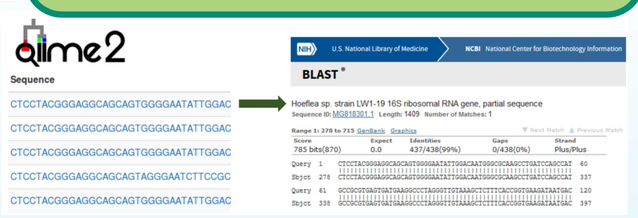


Next Steps

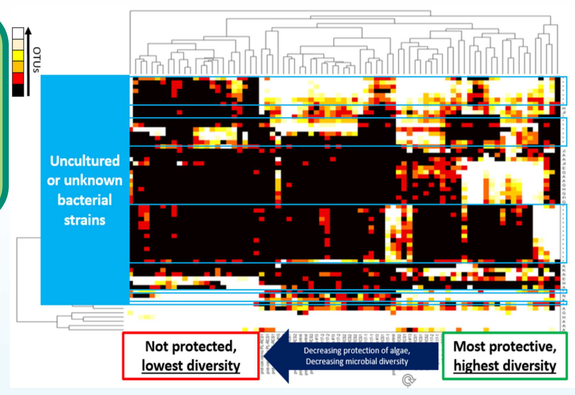


Qiime2 plugins can generate 3D graphs of alpha and beta diversity. (Sample image, "3D PCoA with Emperor," from Qiime2 website)

I will continue working with this data to run statistical analyses and generate interpretive visualizations. We will compare these results with those of previous Consortia experiments. The main goal is to identify how the microbiota present in the protected algae cultures differed from those that were not protective.



Sequences mapped to BLAST genome library (Fisher, Lane, et. al., 2017)



Heatmap of previous Consortia data (Fisher, Lane, et. al., 2017)

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SAND No. _____

1, 2: K. Hirayama, S. Ogawa, "Fundamental studies on physiology of rotifer for its mass culture – I: filter feeding of rotifer," *Bul Japan Soc Sci Fish* 38 (11) (1972), pp. 1207-1214.
3: Denekamp, Nadav & A S Thorne, Michael & Clark, Melody & Kube, Michael & Reinhardt, Richard & Lubzens, Esther (2009), "Discovering genes associated with dormancy in the monogonont rotifer *Brachionus plicatilis*." *BMC genomics*. 10. 108. 10.1186/1471-2164-10-108.

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