Dunaliella salina and Iodine in search of Thyroxine

Purpose:

Certain sea-water invertebrates are affected by Thyroid-like Hormones, most of which are provided for by their food. One of the discovered providers was *Dunaliella tertiolecta*, so we set out to test whether this trait extended to other *Dunaliella* species, in this case *Dunaliella Salina* (*D. salina*), and if so, whether it played an active role in the lives of *D. Salina's* predators. *Dunaliella Salina* is a species already used in producing Carotenoid, so being able to harvest Thyroxine (T4) would increase productivity and make T4 more readily available.

Materials Used:

The Usual:

- Micropipettes and tips: p1000, p200, p20
- Pipettes: 5mL, 10mL, 25 mL
- Microtubes
- Microscope: 40x
- J6-HC Beckman Centrifuge with rotor JS-4.2
- Vortex
- Vacuum Centrifuge
- Hood

Incubation:

- *Dunaliella Salina*, colony 19/18
- 1 L 1.0M MM_3
- C-Chips
- Potassium Iodide: 1 µM, 100 µM
- 6 50 ml bottles
- 6 200 ml bottles

ELISA:

- Total T4 ELISA KIT
- innOva 3100
- 6 mL 1 M Calcium Chloride Dihydrate
- 10 mL 100% Methanol
- 20 mL 100% Chloroform
- MM_3
- SpectraMax M5
- 18 50 mL tubes

Procedure used:

Only July 7th, we started the experiment. We incubated the *D. Salina* in 30.5 µL solution of 1.0 MM_3 mixed with varying molarities of KI: 0, 1, and 100 µM, with two containers per molarity. We added 5.5 µL of *D. Salina* at 1.81 cells per mL to 25 mL of solution. We incubated the *D. Salina* in a moving container, innOva 3100 at a regulated light intensity of 90-110 µE/(m^2•s) and a regulated temperature of 28.0˚C.

Every day, we took 90 µL out of each container to measure density.

On July 14, we separated the cells and the medium via transferring each culture into three tubes and centrifuging them in the Beckman Centrifuge at 4544 g for fifteen minutes. We removed the supernatant and then combined the cells via resuspension with MM_3. We centrifuged the cells again and removed the medium, adding 3 mL to each tube of lysing mixture composed of two parts Chloroform and one part Methanol. We mixed the contents via vortex, to mix the Methanol in, allowing the Methanol to allow the Methanol to lyse the cell membrane and proteins. We now put the tubes on ice in between processes. We centrifuged the tubes for five minutes at 4544 g in order to isolate most of the cell debris into a pellet, and transferred the supernatant into new tubes. We added 1 mL of 5mM Calcium chloride dihydrate to each tube to make the Chloroform insoluble with the Methanol. We centrifuged the tubes for an hour to strengthen the separation and separate the debris from the upper layer of Chloroform. We extracted the Chloroform, which would contain the T4. We dried the solutions by putting them into a vacuum centrifuge with their tubes' caps left open.

On July 15, we loaded 25 µL of standards and samples and 100 µL of enzyme conjugate into wells of a Total Thyroxine ELISA KIT. We then incubated the plates at room temperature for 60 minutes with an aluminum cover, before washing out the samples with 300 µL of wash buffer five times (pipetting up and down once and tapping the plate upside-down firmly twice per wash). We added 100 µL of substrate per well. After incubating the samples at room temperature for 30 minutes, we mixed in 50 µL of stop solution per well and measured the optical densities with a SpectraMax M5. The stronger the color, the less T4 would be in the sample. In our ELISA KIT, Antibodies, connected to the wells, bind with Thyroxine. The sample's T4 and the enzyme-linked T4 compete to bind to the antibodies lining the well. With more competition, less enzyme-linked T4 can bind. Therefore the substrate, which turns blue when reacting with the Enzyme, would cause a paler color when reacting with a sample with lots of Thyroxine.

We repeated the experiment with more variations, starting July 15. We placed three cultures with Potassium Iodide by the window sill to simulate day and night light exposure. We did the same with four cultures without Potassium Iodide. We put three cultures with Potassium Iodide in constant light exposure. We did not record the growth rate, as prior results showed that Iodine did not affect growth, and the varying exposure to light would make growth rate results unreliable.

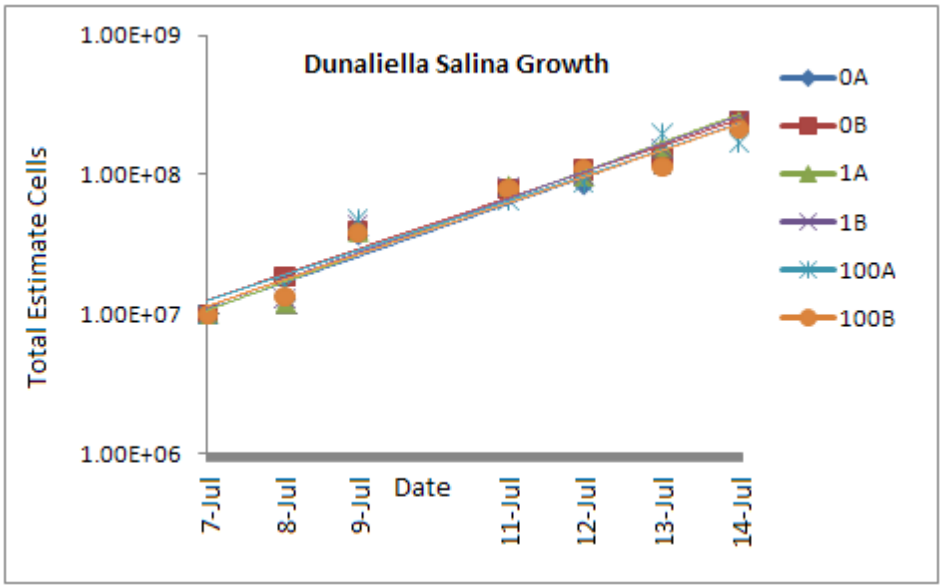
On July 28, we repeated the process with 250 mL containers. Because of centrifuge loading constraints, we loaded culture into three tubes per sample when first removing the supernatant. We reloaded the tubes with more culture and re-centrifuged the samples until the containers were empty. The rest of the process remained the same.

Data and Conclusions:

Trial One Total Estimate Cells

Total Estimate Cells		0A	0B	1A	1B	100A	100B
7-Jul	14:00	9.95E+06	9.95E+06	9.95E+06	9.95E+06	9.95E+06	9.95E+06
8-Jul	13:45	1.27E+07	1.86E+07	1.20E+07	1.30E+07	1.57E+07	1.34E+07
9-Jul		3.69E+07	3.97E+07	3.93E+07	4.33E+07	4.73E+07	3.81E+07
11-Jul	10:00	7.38E+07	7.84E+07	8.42E+07	8.14E+07	6.62E+07	7.99E+07
12-Jul	9:00	8.51E+07	1.08E+08	9.82E+07	9.88E+07	8.81E+07	1.10E+08
13-Jul	9:00	1.48E+08	1.34E+08	1.60E+08	1.54E+08	1.97E+08	1.15E+08
14-Jul	8:30	2.14E+08	2.39E+08	2.46E+08	2.39E+08	1.71E+08	2.12E+08

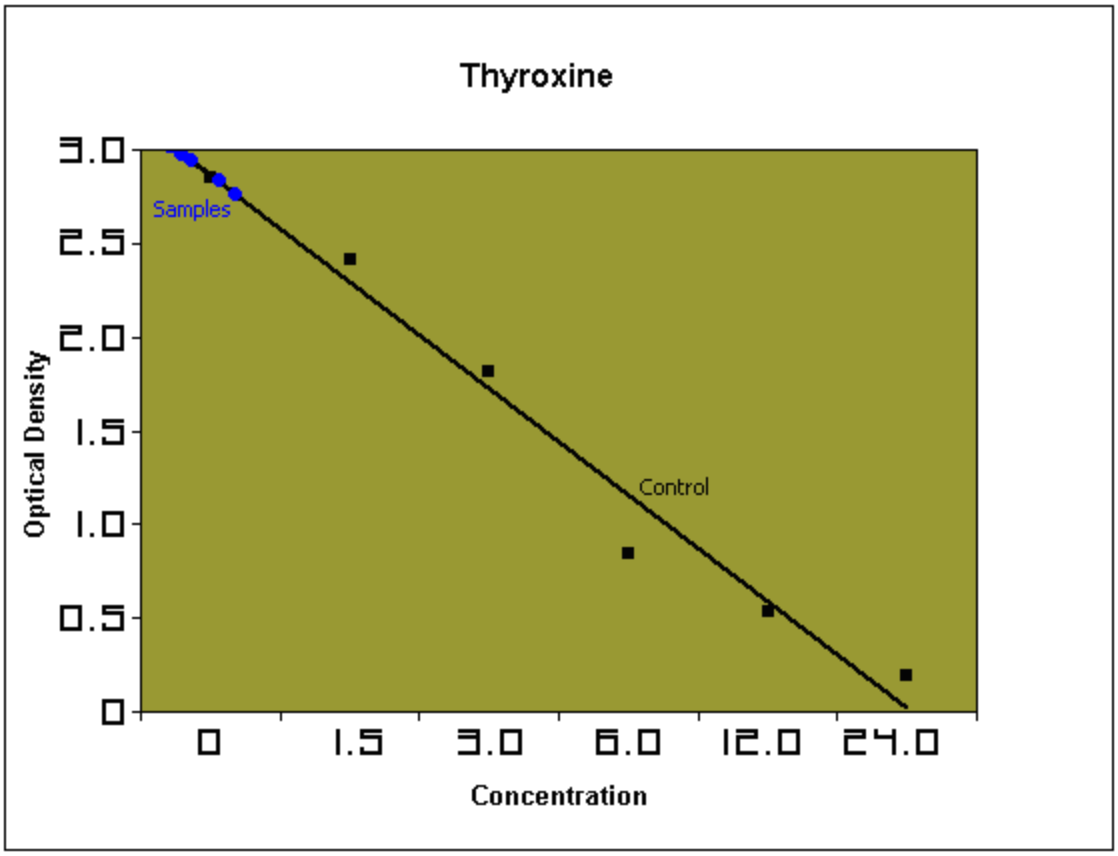
*On July 12th, we changed the containers to a bigger size and added medium to increase the volume to 150 mL, approximately five times the original volume.



The cells seemed to not vary too strongly in growth rates, with each culture staying roughly within the same exponential 10th indicating that Iodine is not a strong inhibitor or promoter of growth.

Trial One ELISA Results:

Concentration	Optical Density		Sample	Optical Density
0	2.8533		0A	2.9489
1.5	2.4086		0B	2.7819
3	1.8183		1A	3.0647
6	0.8481		1B	3.0448
12	0.5322		100A	2.955
24	0.1957		100B	2.6973

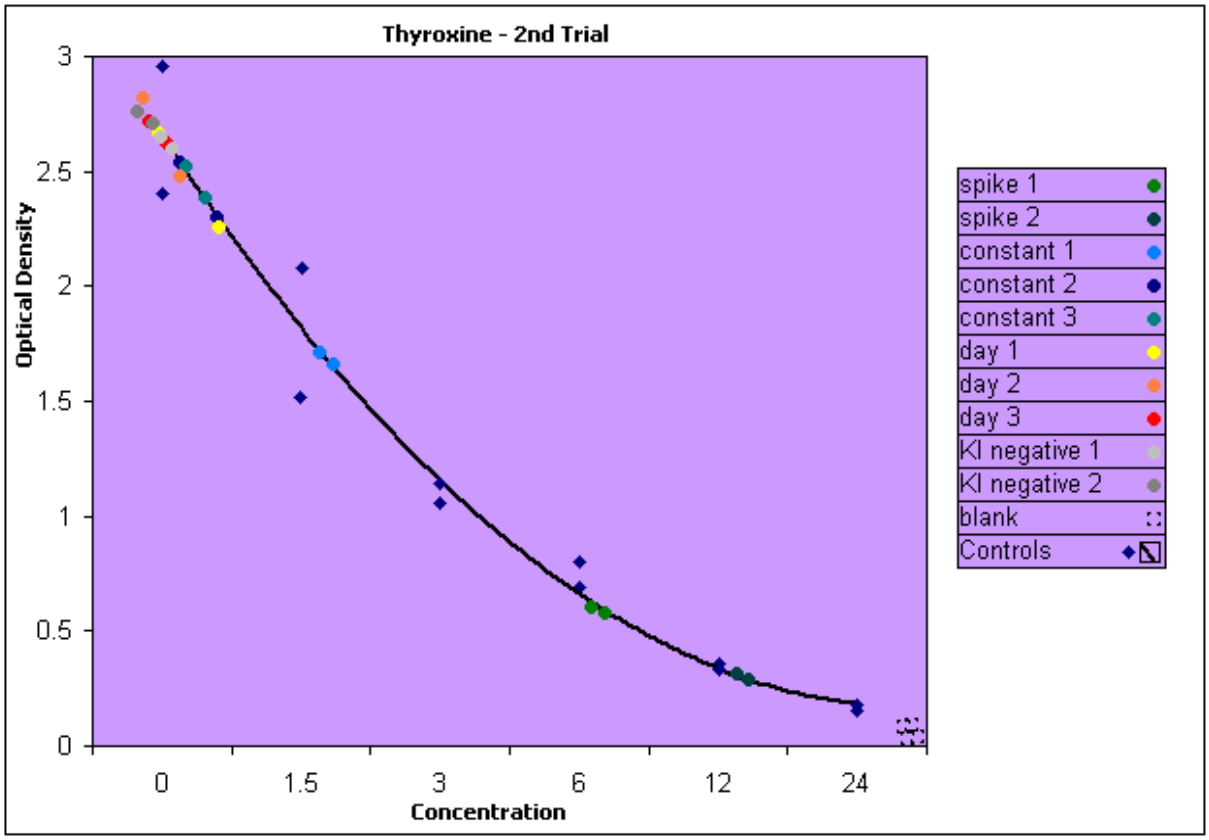


The closeness of the optical density of the experiment samples to the value of the optical density of the control's zero Thyroxine sample indicate that *Dunaliella salina* did not produce Iodine. We failed to remember to spike one of the cultures with Thyroxine as an added control on this experiment, however, and we did not vary the conditions of the *D. salina* outside of the amount of Iodine. Therefore we cannot say that *D. salina* does not produced iodine. It's just more unlikely now.

Trial Two ELISA Results:

Concentration	Controls A	Controls B
0	2.4021	2.9607
1.5	2.0823	1.5091
3	1.0529	1.1337
6	0.691	0.7943
12	0.3556	0.3287
24	0.1556	0.173

	Trial A	Trial B	Trial C	Trial D
spike 1	0.6087	0.6505		
spike 2	0.3251	0.3018		
constant 1	1.6965	1.6335		
constant 2	2.2726	2.5766		
constant 3	2.3627	2.5165		
day 1	2.2428	2.6225		
day 2	2.4587	2.8483		
day 3	2.6066	2.7129		
KI negative 1	2.6906	2.7001		
KI negative 2	2.7324	2.8694		
blank	0.0534	0.0562	0.048	0.048



One of the cultures with constant exposure to Potassium Iodine tested positive for Thyroxine, so *Dunaliella salina* may produce T4 if subjected to continuous exposure to intense light. However, only one culture subjected to constant exposure tested positive, so more experiments would be required to provide definitive results.