

Fall 12-1-2012

Optimizing *Aplysia californica*'s Living Conditions

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Recommended Citation

Xu, Ruiying, "Optimizing *Aplysia californica*'s Living Conditions" (2012). *Intersections Fall 2020*. 8.
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Optimizing *Aplysia californica*'s Living Conditions

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Mentor: Dr. Hillel Chiel



Outline

Introduction

Methods

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Future Work

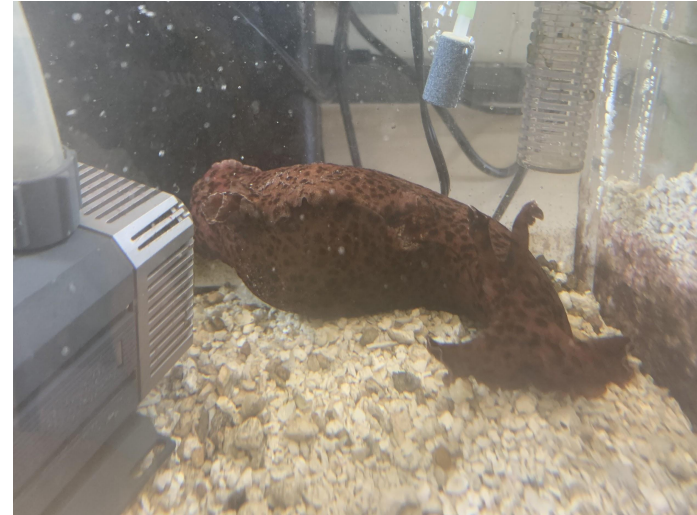
Introduction

Aplysia californica

- Excellent model for studying the relationship between neuron circuitry and behavior (Kandel, 1976)
- Can determine the health of animals by looking at the body and behavior:
Inking response (Carew & Kandel, 1977), feeding responses (Kupfermann, 1974), skin lesions

Prior Work in Maintaining *Aplysia californica*

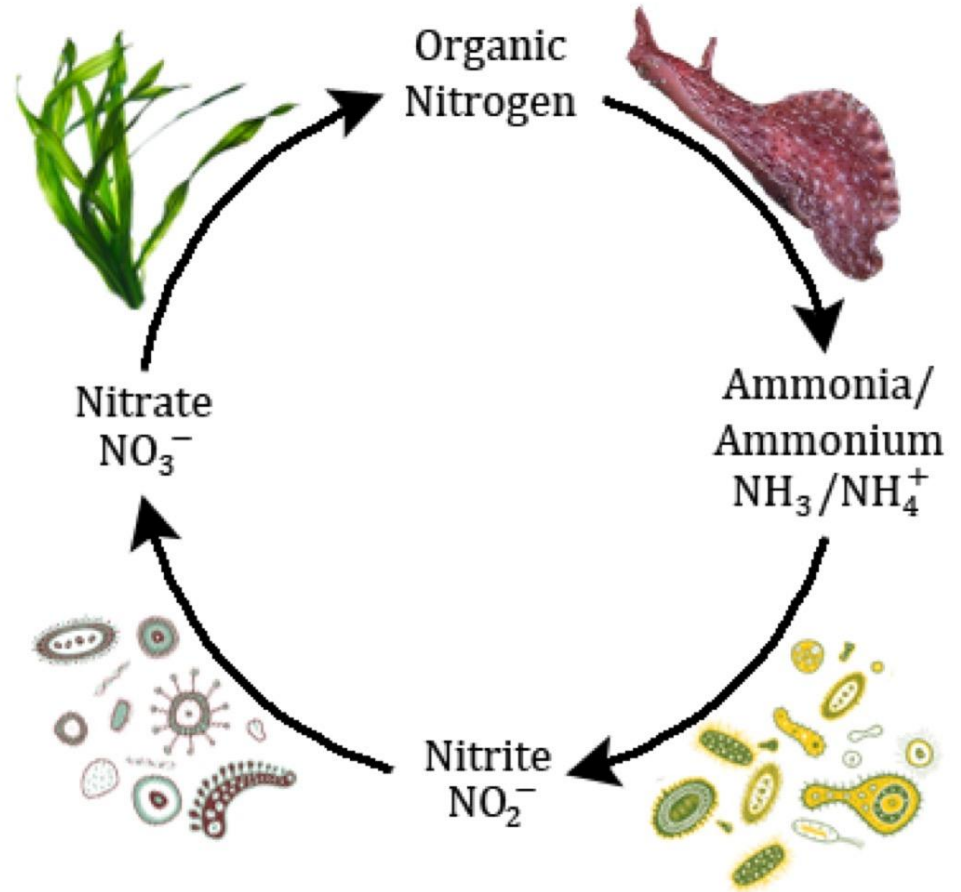
Frequent replacement of tank sea water (Smith, 2011)



Nitrogen Cycle

Introduction

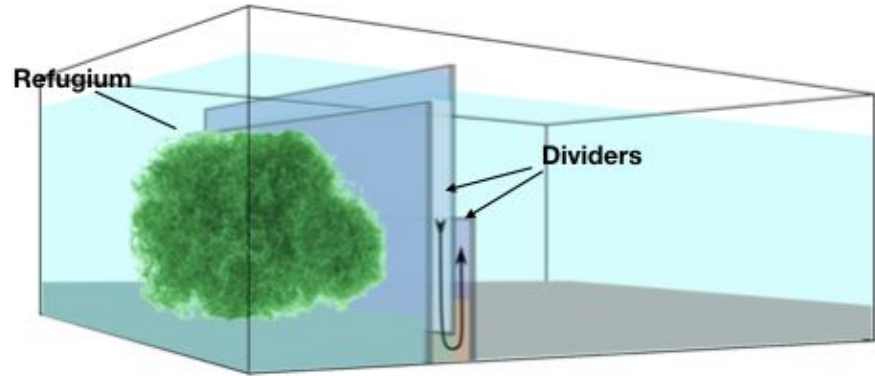
- Nitrogen Cycle
(Kalvelage *et al.*, 2013)
Ammonia, nitrite and high concentration of nitrate are toxic
(Camargo, Alonso, & Salamanca, 2005)



Methods

Tank Set-up (Kehl, 2019)

- Gravel, Sand, Dividers (filter floss and gravel),
Sponge Rock
Bacteria attach to their surface
- 2 Compartments: Refugium & Main Tank
Chaetomorpha in the refugium
- Filters
- Chiller & Pump
- Air pump
- Protein skimmer
- Lamp (Set to 12-hour/12-hour on-off cycle)



Introduction

Goals:

- To test if a refugium can control nitrate and ammonia levels and thus maintain *Aplysia's* health
- To test the impacts of accumulation of nitrate on *Aplysia*

Hypothesis 1: *Chaetomorpha* in the refugium sequesters nitrates

Hypothesis 2: Nitrate toxicity can cause long-term negative impacts on both *Aplysia* and the environment

Prediction: The removal of macroalgae from the refugium will cause nitrate accumulation in the system and cause long-term harm to *Aplysia*.

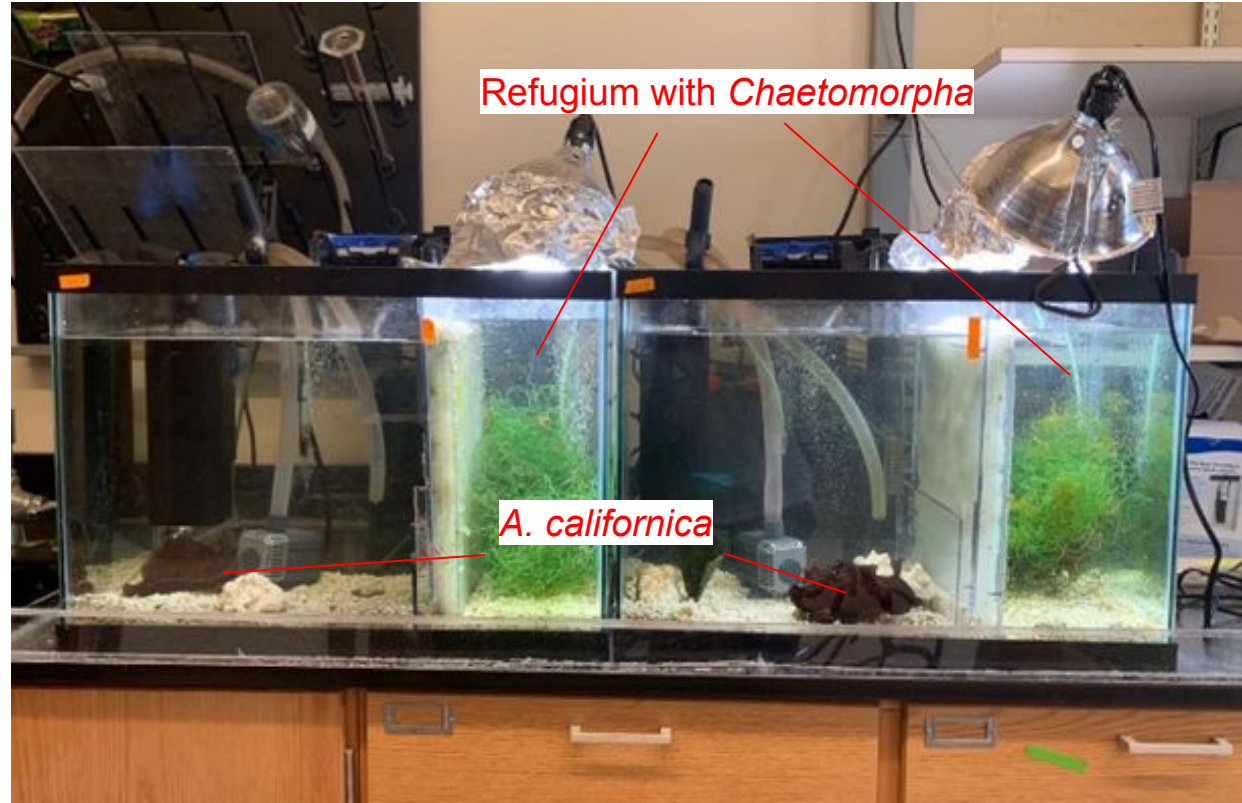
Methods

Two tanks (control and experimental groups); two *Aplysia* from the same shipment within a similar weight range

Three stages:

Stage One. Observation Period

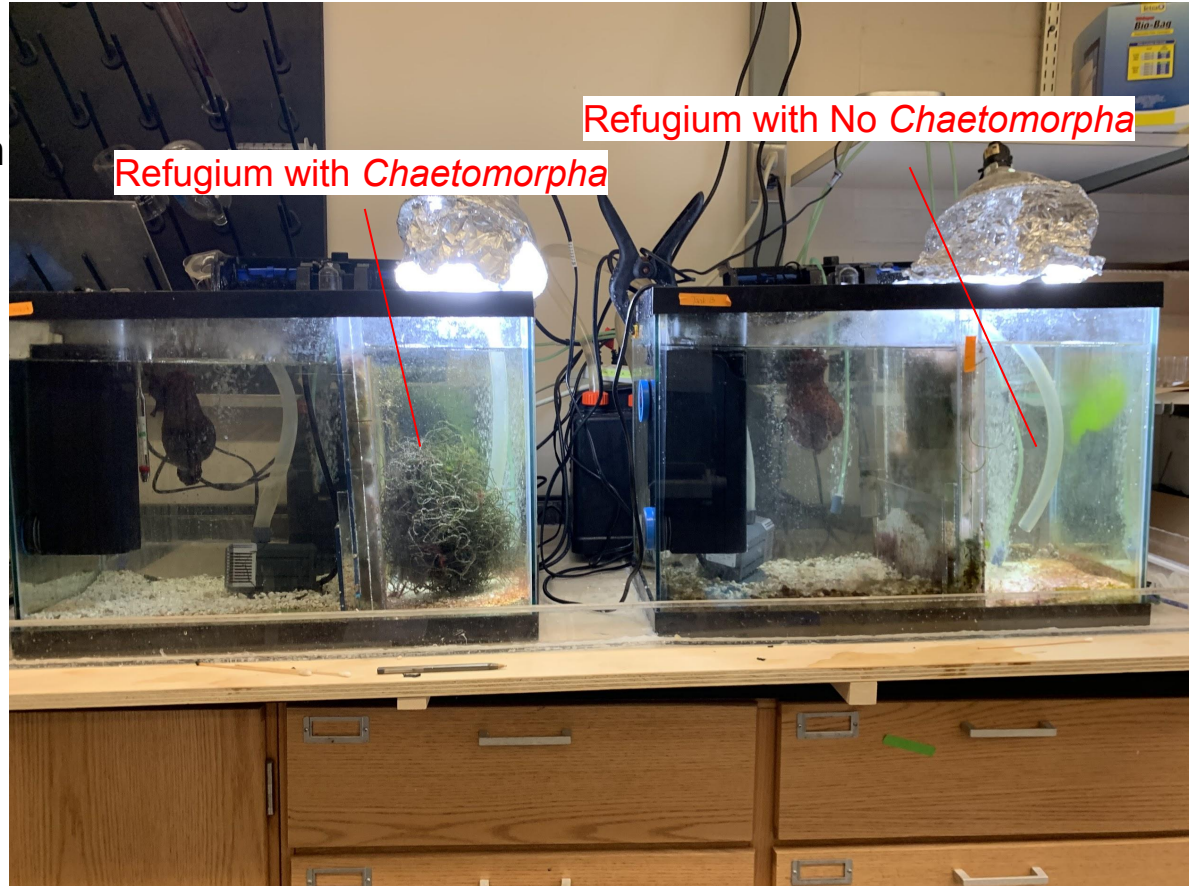
Observed healthy *Aplysia californica* with normal nitrate and ammonia levels with *Chaetomorpha* in both refugia.



Methods

Stage Two. Nitrogen Accumulation

Removed *Chaetomorpha* and observed the health conditions of *Aplysia californica*'s and environmental changes. *Chaetomorpha* was moved to an unrelated tank.



Methods

Stage Three. Return of *Chaetomorpha*

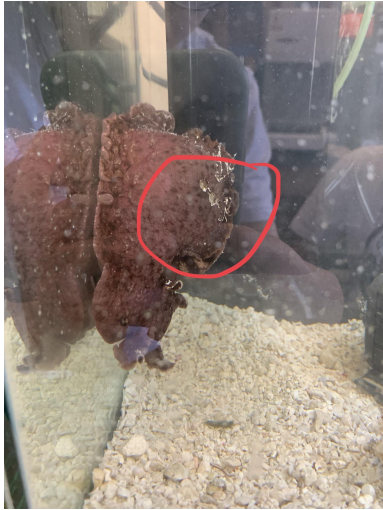
Returned of *Chaetomorpha* to the empty refugium, and observed the health of *Aplysia californica*.

Methods

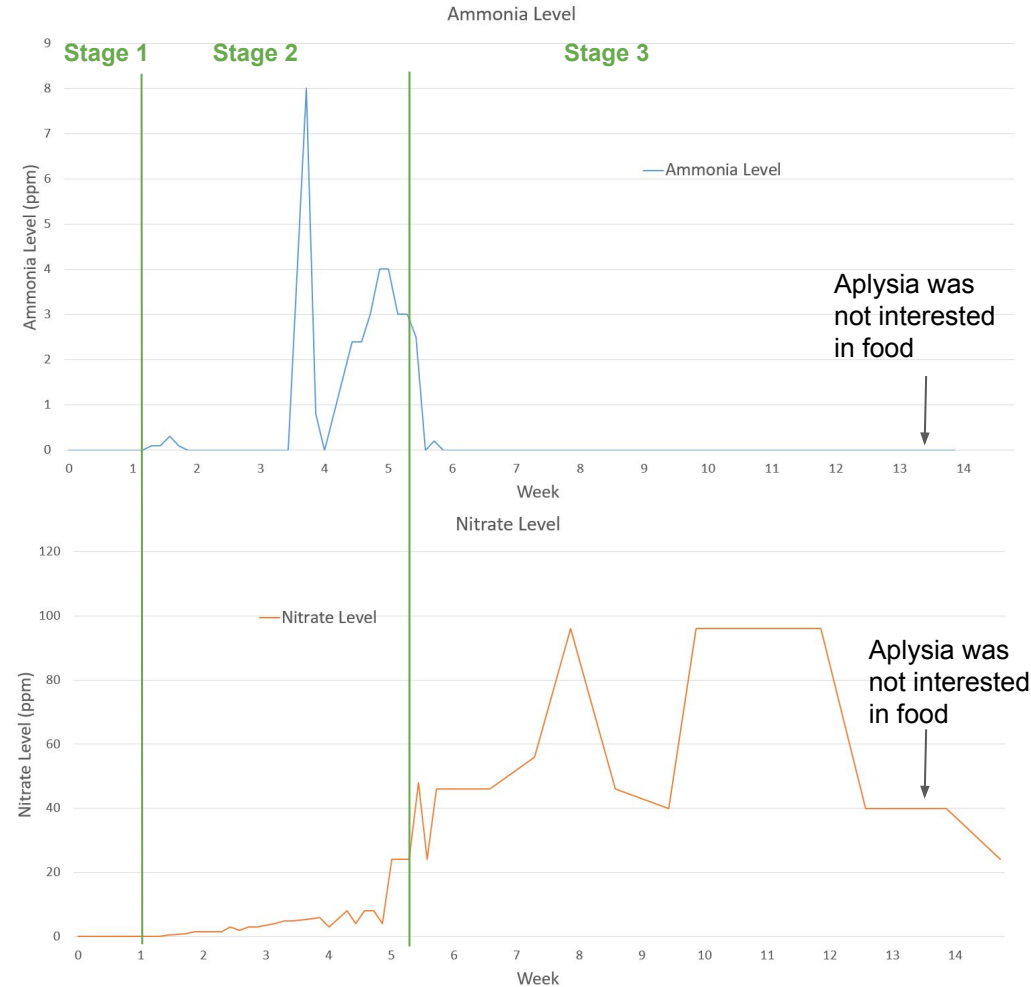
- Took measurements:
 1. Nitrate and ammonia levels: nitrate and ammonia kits (Red Sea);
 2. *Aplysia's* health conditions: weight, biting intervals (Kupfermann, 1974), swallowing intervals (Kupfermann, 1974), amount eaten, inking frequency, skin lesions;
 3. Mass of *Chaetomorpha*
- Fed 1.8-1.9 grams of dry Nori (9-11 grams of when wet) as the only food every two days.
- Biting intervals were measured using one Nori piece;
Swallowing intervals were measured using 0.5cm x 19cm Nori strips with lines marking one centimeter intervals;
5 bites and swallows were taken to obtain average responses.

Results

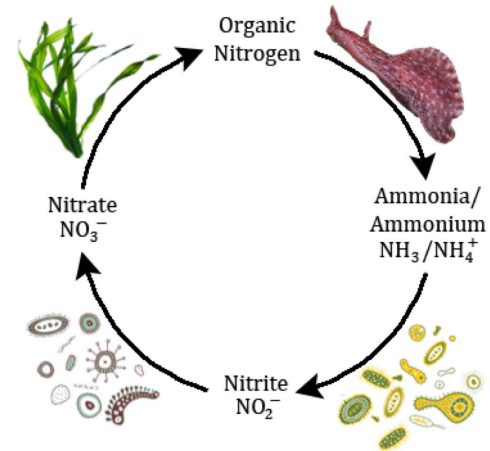
- *Aplysia* from the control group became infected and died before stage two.



Nitrate and Ammonia Levels

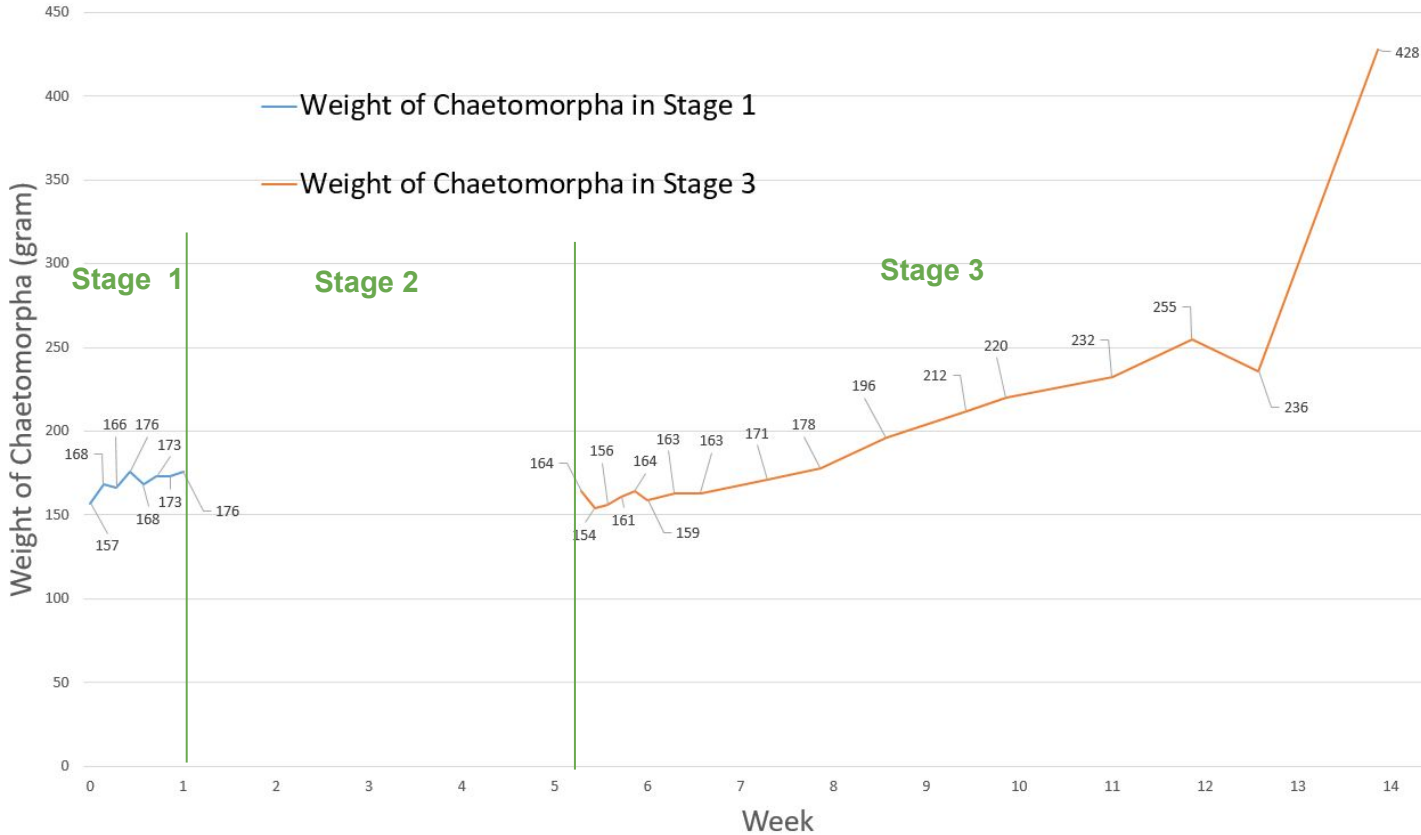


We can see a steady increase of ammonia and nitrate during stage two, after *Chaetomorpha* removal, and a decrease in ammonia level and a fluctuation in nitrate level in stage three after returning of the *Chaetomorpha*.



Weight of *Chaetomorpha* Over Time

Weight of *Chaetomorpha*

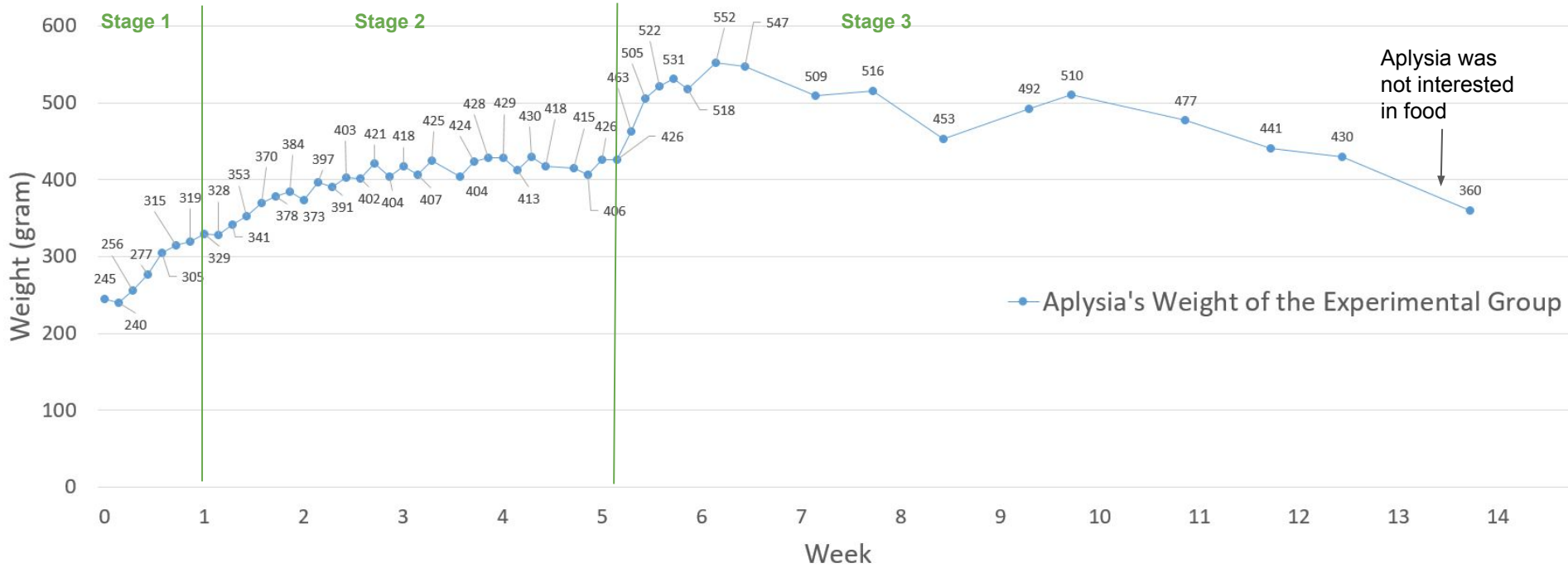


The weight of *Chaetomorpha* steadily increased in the refugium.

We interpreted that *Chaetomorpha* is consuming nitrate to gain weight.

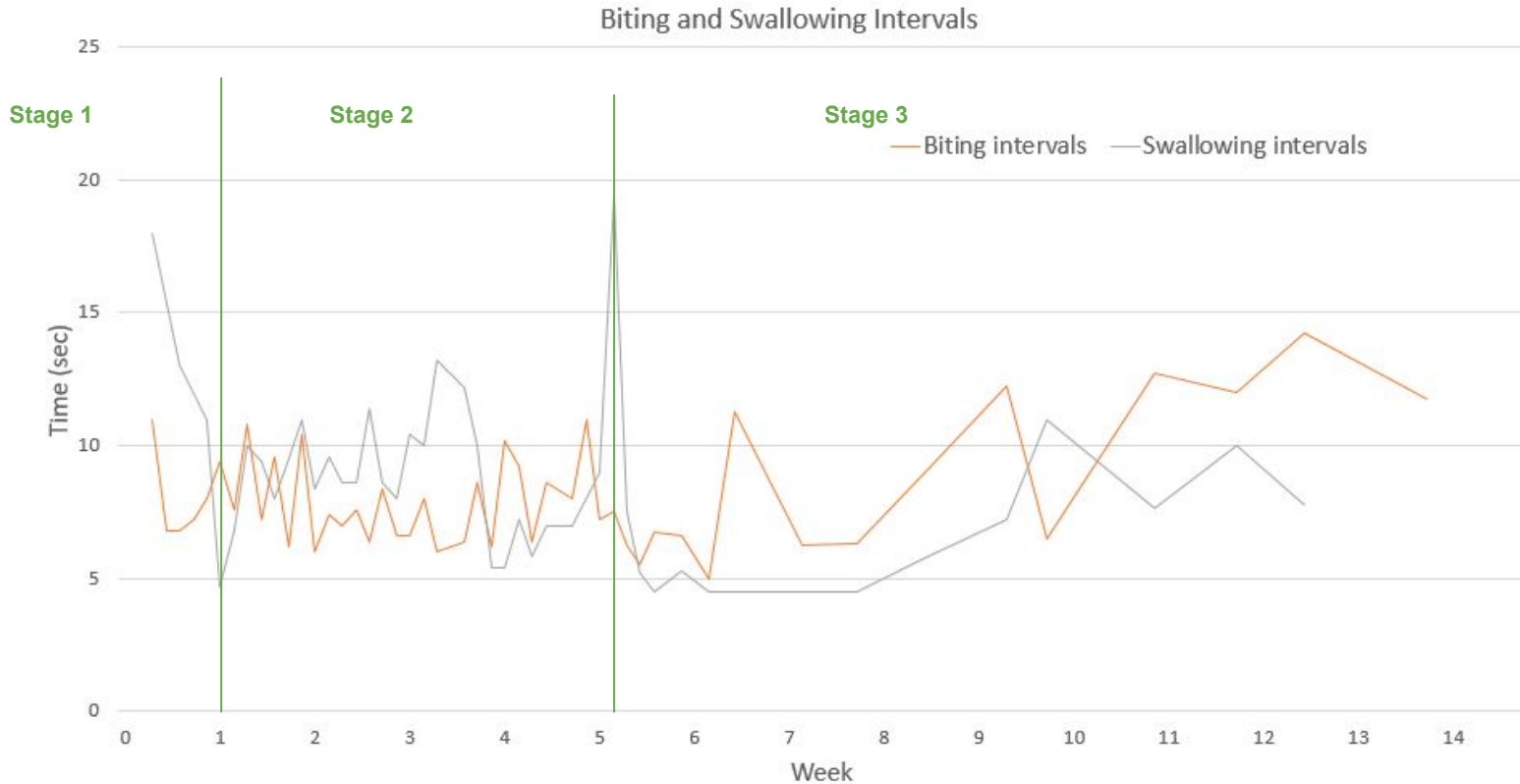
Weight of *Aplysia californica* Over Time

Aplysia's Weight of the Experimental Group



Weight of *Aplysia* increased in stage 1, fluctuated in stage 2, boosted and drastically decreased in stage 3.

Changes of Biting and Swallowing Intervals Over Time



Preliminary results suggest that the environmental stress caused by a high nitrate level may not affect *Aplysia's* biting and swallowing intervals.

Discussion

- The removal of *Chaetomorpha* was associated with increase in both nitrate and ammonia;
- The *Chaetomorpha* may sequester nitrates since the weight of the *Chaetomorpha* increased with nitrate level in stage 3;
- The *Aplysia*'s appetite decreased and it lost weight with an increase in nitrate and ammonia levels after the removal of *Chaetomorpha*;
- The environmental stress due to the accumulation of nitrates does not cause obvious changes in biting and swallowing intervals.

Future Application & Value of the Research

1. A successful control group to compare nitrate levels and *Aplysia*'s health with *Chaetomorpha* inside the refugium through all stages;
2. Replications are needed to provide further evidence for the function of the refugium and the impact of a high nitrate level on the system and *Aplysia*. A second trial which started in Sept. 2020 is still in progress.
3. Further changes to the tank environment might include adding snails, shrimps or different kinds of macroalgae to improve the capacity of the tanks to house *Aplysia*.

Acknowledgements

- Dr. Hillel Chiel, Department of Biology, CWRU
- Dr. Catherine Kehl, Department of Biology, UNC-CH
- Dr. Jeffrey Gill, Department of Biology, CWRU
- Members of Dr. Chiel's lab