Physiological effects of kainic acid & the potential ties to sea star wasting

Dennie Truong[1], Reyn Yoshioka[2], Maya Groner[2]

[1] Colby College Waterville, ME

[2] Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, ME

INTRODUCTION

Research Question

Does domoic acid (DA) have any physiological effects on sea stars?
Is there a connection between DA and sea star wasting (SSW)?

Background

- In 2013: billions of sea stars across 20+ asteroid species perished due to a marine epizootic known as sea star wasting (SSW) on both Pacific and Atlantic coasts (Bucci et al., 2017; Oulhen et al., 2022).

Signs associated with SSW

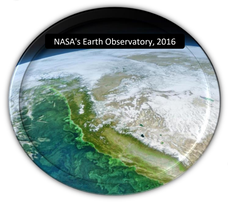
White lesions (1), Abnormally twisted arms (2), Autotomy (3), Disinterest in nearby prey, Turgor loss, Decaying of tissues (Kohl et al., 2016; Jaffe et al., 2019).

About domoic acid:

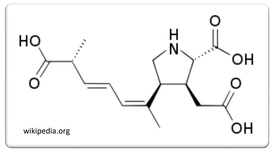
- DA = excitatory neurotransmitter
  = binds to glutamate and kainate receptors
- When DA binds to glutamate receptors in the neural tissues of vertebrates, it causes an uncontrolled intracellular influx of Ca^{2+} into the neuron which can lead to neuronal swelling, neurological dysfunction, DNA damage, and cell death (Zabaglo et al., 2016).
- In crinoids, glutamate binding can trigger an irreversible "destiffening response" that then can result in autotomy (Wilkie et al., 2021).
- Due to supply chain bottleneck, kainic acid (an agonist) was used as a proxy for domoic acid.

Organic matter loading & depleted oxygen conditions induced lesions (Aquino et al., 2021). Suggests high primary productivity → SSW

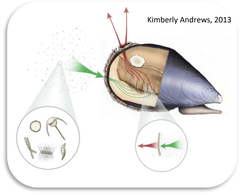
HYPOTHESIS



Pseudo-nitzschia dominated harmful algal bloom



Large production of harmful algal bloom neurotoxin called domoic acid in surrounding water bodies



Filter feeders (i.e. mussels) absorb domoic acid through filtering for algae

Sea stars accumulate domoic acid through consumption of mussels

High levels of domoic acid bioaccumulation may lead to signs of diseased/SSW

METHODS

Collection & Study Site:

Asterias forbesi were collected by David & Kipp Quinsby from Lamoine State Park (Maine)

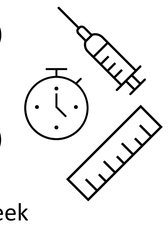
Experiment was conducted in a seawater suite at Bigelow Laboratory for Ocean Sciences

Explanatory variables/Treatments:

- Control/Phosphate buffer saline (PBS)
- Kainic acid @ 3 ppm (1X)
- Kainic acid @ 30 ppm (10X)

Response variables:

- Righting Time (in sec)
- Arm circumference (in cm)



Logistics:

- Stars were fed 3 times a week
- Stars were kept between 13°C to 16°C
- Flow rate: ~50mL per min for each tank

Data Collection:

- Baseline data collection on 4/2/23
- Experiment lasted 7 days (4/6 to 4/13/2023)
- Sampled 6 times (t = 0, 1, 2, 3, 5, 7)

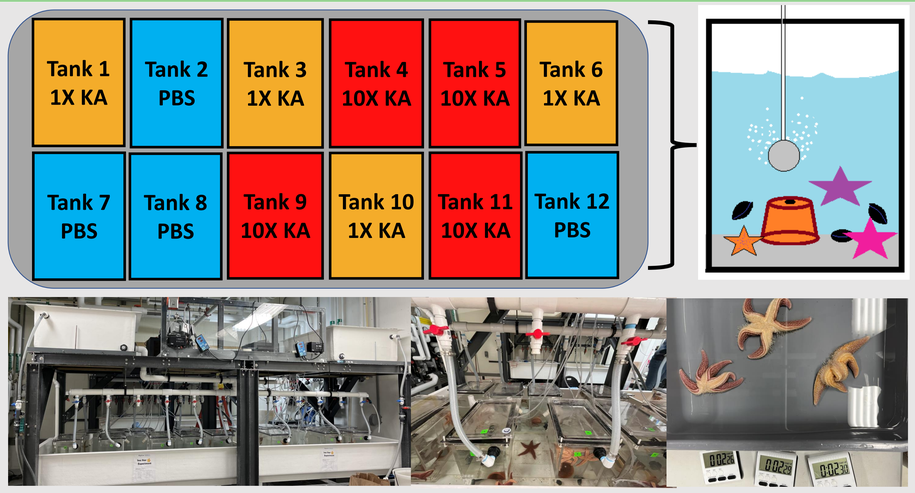


Figure 1. Experimental design: Each treatment (PBS, 1X KA, 10X KA) are replicated 4 times with a total of 12 tanks used. Each tank contain three stars (aimed to be between 80 to 120 grams), a flowerpot, an air stone, and a mussel for each star. A total of 36 sea stars were part of the experiment.

Identification: Sea stars are identified using body mass and color (unique for distinguishment between stars)

Injection:

- Treatments were applied using a 1mL syringe into each of the 5 arms (aimed to inject into the pyloric caeca)
- Dosage of treatments are based on baseline sea star's mass to the nearest tens

Data Analysis:

- Candidate models fit using Gamma and linear mixed-effects models
- Fixed effects: treatment and time (with cubic spline), random effects: individual nested in tank
- Treatment models compared against null (time + random effects) models using likelihood ratio test

RESULTS

Before Injection

Figure 2. Scatterplots depicting response variable plotted against body mass prior to the exposure of the 3 treatments.

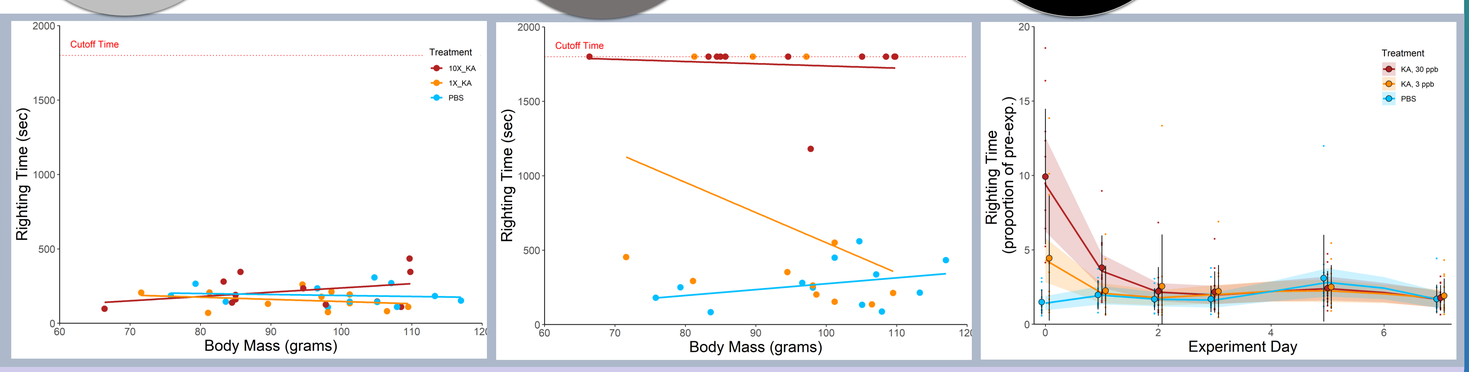
After Injection

Figure 3. Scatterplots depicting response variable plotted against body mass immediately after the exposure of the 3 treatments (t=0).

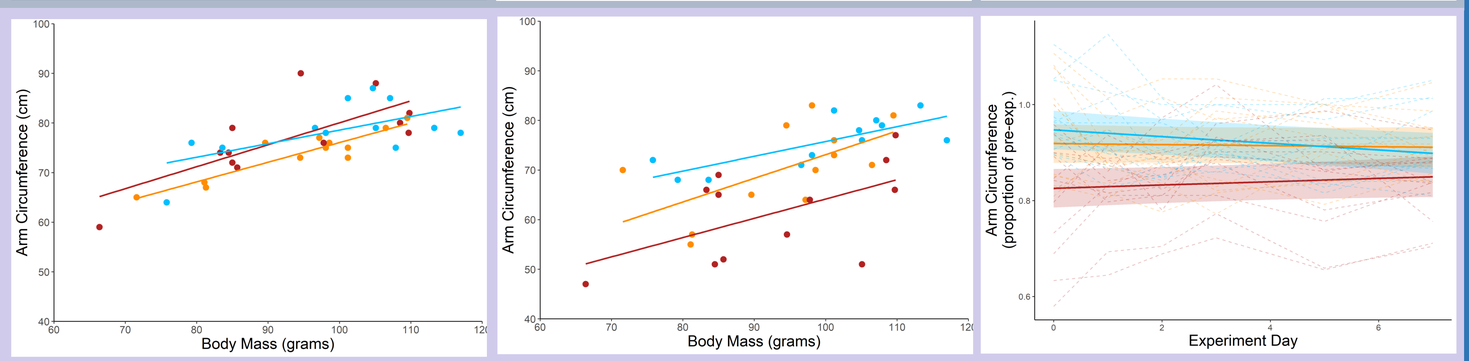
Over Time

Figure 4. Model predictions of (A) Righting time (B) Arm circumference over the course of the 7 experimental days.

A. Righting Time



B. Arm Circumference



- Stars dosed with 30 ppb KA took 9.9 $\pm$ 4.6 times longer than their pre-treatment times to right themselves
- Stars dosed with 30 ppb KA had a 0.82 $\pm$ 0.040 times lower arm circumference than their pre-treatment measures
- The righting time model is significantly different from the null (Likelihood ratio test, χ^2 = 71.5, df = 10, p-value < 0.001)
- The arm circumference model is significantly different from the null (Likelihood ratio test, χ^2 = 18.6, df = 4, p-value < 0.001)

ELISA TESTS

Figure 5. Image of dissected *Asteria forbesi* for their 4 tissues.

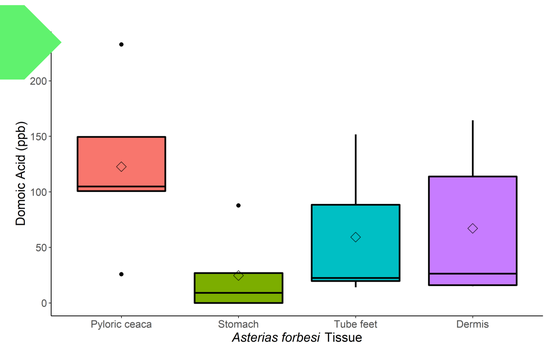


Figure 6. Boxplots comparing baseline domoic acid across 4 tissues from 5 randomly selected sea stars (Mass range from 50.9 to 89.5 grams).

DISCUSSION

- Kainic acid negatively affects sea stars physiologically & decrease turgor
- Molecularly: Binding of kainic acid to glutamate and kainate receptors caused large influx of Ca^{2+} into the cell and probably resulted in overactivation of the receptors → paralysis to the mutable collagenous tissue
- DA may have contributed to wasting by making stars more susceptible to other stressors; however, the doses tested did not show kainic acid alone causes wasting

Future Directions

- Analyze dermis tissue for gene expression
- Using domoic acid in future experiment
- Test the viability of domoic acid uptake through mussels
- Test sea star's ability to bioaccumulate domoic acid through consumption of mussels

References

SCAN ME

Acknowledgements

We would like to thank and recognize the following people for their contributions to our experiment! Also thank you to Colby College and Bigelow Laboratory for the opportunity and funding of this project.

Christopher Salatino (Bigelow)
Alexandre Lépeule (Bigelow)
Kathi Lefebvre (NOAA/NWFSC)
Justin Becknell (Colby College)
Price & Rasher Labs (Bigelow)