Surveillance of Hemolymph in Aplysia Californica as an Early Indicator of Aplysia Abyssovirus 1 (AAbV)

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Background

- *Aplysia californica*, marine gastropods, are model organisms for studies of the cellular basis of memory and learning.
- These marine invertebrates have a simple central nervous with giant neurons and live for approximately 12 months.
- Their diet consists primarily of red algae, both in their natural habitat, off the coasts of California, and in laboratory settings.
- *Aplysia abyssovirus* 1 (AAbV), a nidovirus, can be found in tissues as well as hemolymph of both wild and captive *A. californica*.
- Chronic monitoring of AAbV infections requires non-lethal methods.
- Implications of AAbV on Aplysia remain relatively unexplored.

Hypothesis

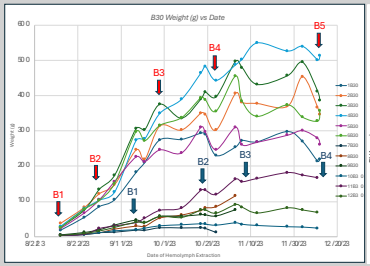
- AAbV RNA levels in hemolymph in *A. californica* are an early predictor of viral load in tissues.

Conclusions

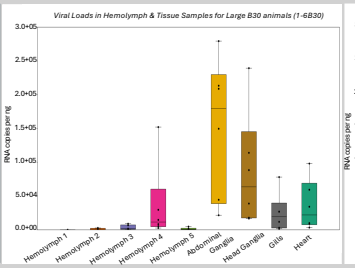
- AAbV RNA levels in hemolymph are highly variable, yet are consistently indicative of the individual being high or low virus, suggesting hemolymphs could be used as a general indicator of viral infection.
- Our results express a trend of high virus animals remaining high in the hemolymph throughout time and expressing high virus in tissues, with an emphasis on the abdominal and head ganglia whereas low virus animals remained low in both the hemolymph and four tissue samples.
- B30's egg mass started off positive and these animals exhibited high and increasing viral load over time.
- B36's egg mass started off negative and remained negative or relatively low over time.

Results

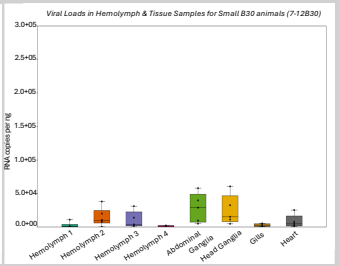
(F1)



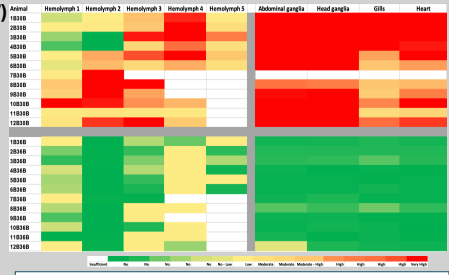
(F2)



(F3)

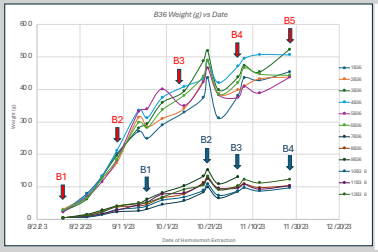


(F7)

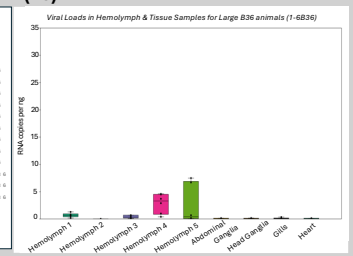


(F7): Heat map comparing the RNA viral load in the hemolymph and tissues of 1-12B30 & 1-12B36 *A. californica*.

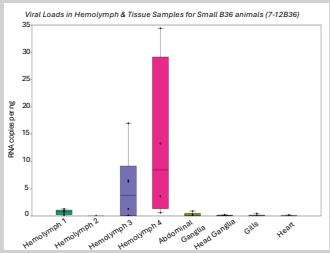
(F4)



(F5)



(F6)



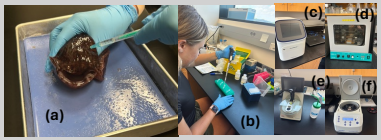
Figures: Growth by weight (g) over time showing hemolymph sample points for large (red) vs. small (blue) individuals for batches B30 **(F1)** and B36 **(F4)**. AAbV copy numbers determined from hemolymph samples indicated in **(F1)** and **(F4)** as well as terminal tissue samples for large B30 animals **(F2)**, small B30 animals **(F3)**, large B36 animals **(F5)** and small B36 animals **(F6)**.

Aplysia californica, commonly known as the California sea hare

Methods and Materials

- Hemolymph in *A. californica* was surveyed in two brood stock batches; B30 and B36. 12 from each batch, including 6 large and 6 small were selected. Each individual was weighed weekly and hemolymph samples were extracted monthly. At the end of the 4-month period, the individuals were anaesthetized using magnesium chloride and subsequently underwent nine tissue extractions, with focus on the abdominal ganglia, head ganglia, gills, and heart.
- RNA was extracted from hemolymph using Zymo-Quick- DNA/RNA MicroPrep Plus Kit and from the tissues using Thermofisher's standard protocol for Trizol RNA Isolation. RNA was converted to cDNA and used for qPCR with primers for a 5' region of AAbV. Virus levels were calculated as copies per ng of starting RNA based on a standard curve.

(F8)



(T1)

Batch	Date Laid	Date Hatched	Egg Viral Load (copies of viral RNA/ng)	Age Range for Experiment (months)
B30	4/6/2023	4/13/2023	8.7	4-8
B36	4/20/2023	4/27/2023	0.36	4-8

(F8): Hemolymph extraction from *A. californica* **(a)**. RNA isolation of hemolymph samples **(b)**. Machinery used to process samples **(c-f)**. **(T1)**: Table 1 demonstrates that day 7 eggs from B30 were slightly positive whereas B36 day 7 eggs were negative at the start of the study. **(T1)**

Future Directions

Further analysis on the effects of AAbV in *A. californica*, including growth and reproductive success is pending.

Acknowledgment

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