UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE & ATMOSPHERIC SCIENCE

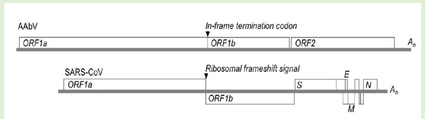
The Relationship Between Viral Load and Growth in *Aplysia californica*

Andrew M. Gelsomini, Dayanna Vidal & Michael C. Schmale
Department of Marine Science
Rosenstiel School of Marine and Atmospheric Science
University of Miami, Miami, FL, USA
amg545@miami.edu

Background

Aplysia californica, more commonly known as Californian Sea Hares, are large gastropods that inhabit the California coast. These organisms have been widely used as models for studies of the nervous system (neurophysiology) based on their simple nervous system, containing some of the largest neurons in the animal kingdom (up to 1mm in length). These features provide the perfect model for analyzing molecular and cellular changes in the nervous system that lead to overall changes in the organism's cognitive function (Kandel 1979).

Recently, a virus was discovered, Aplysia Abyssovirus (AAbV), that infects both hatchery raised and wild Aplysia. It is a member of the Nidovirales order, the same order as coronaviridae, and has one of the longest viral RNA genomes recorded at 35.9 kb. This virus was discovered by transcriptomic analysis of NCBI databases. AAbV was noted to have a similarly structured genome to SARS-CoV (Bukhari et al, 2018).



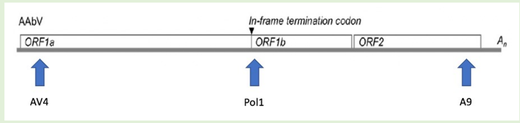
The effects of AAbV on Aplysia have not been determined. This study aimed to characterize the effect of viral load on growth in Aplysia and show transcription trends at different parts of the genome.

Methods

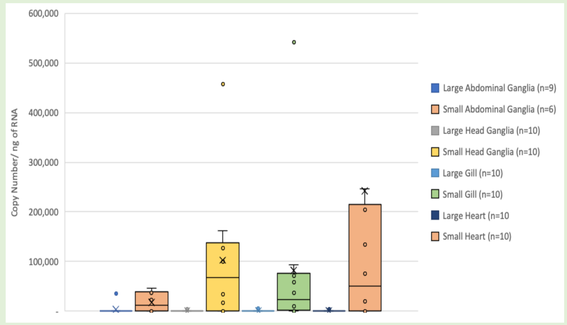
A. californica from two egg batch cohorts (Batch 56 and Batch 73) at the National Resource for Aplysia were evaluated to identify same-aged animals which differed conspicuously in size. Animals were euthanized and dissected for the desired tissues: abdominal ganglia (A), the head ganglia (G), the gill (L), and the heart (H). It is important to note that batch 56 was dissected at 5 months of age and Batch 73 was dissected at 7 and 8 months. RNA was then extracted using TRIzol. Qiagen protocols were then used to produce cDNA from random hexamers for qPCR reactions

Two A. californica from the same cohort at the same age. They represent the two size groups tested.

Real-Time Quantitative PCR was used with AV4, pol 1, and A9 primers to assess viral load in Aplysia. Different size cohorts were analyzed in batch 56 and 73 to compare viral load in small vs large animals of the same age. In addition, transcript ratios of AV4:Pol1:A9 were assessed in animals 14B73 and 15B73 to determine relative expression levels at different points in the genome. For all experiments, copy numbers were calculated as copies per ng of RNA within the sample using plasmid standards for each PCR product.

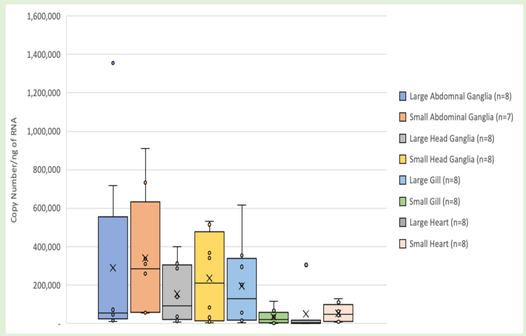


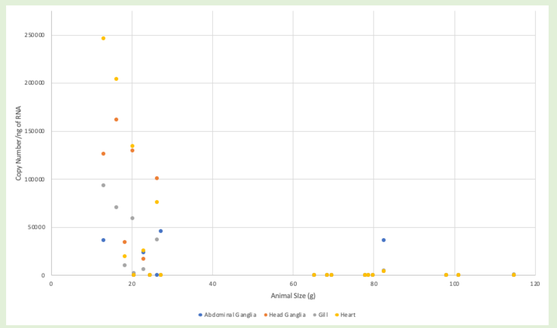
Results



Batch 56 Viral Load in Small Vs Large Aplysia

- Smaller Animals in Batch 56. showed higher viral loads in all tissues than larger animals ($p \leq 0.05$, Mann-Whitney U Test)
- Largest Differences were in the head ganglia and Heart
- Colored rectangle represents the upper and lower quartiles, bar through the middle is the median, X is the mean, bars outside rectangles are the range, and points outside range are outliers (Note: Figure was truncated so one outlier 1,700,000 copies in the small heart not shown)



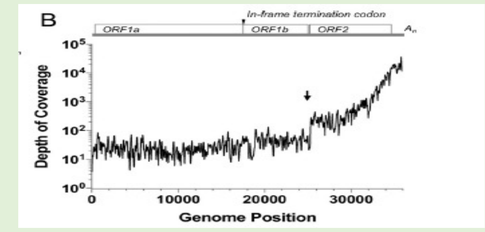


Batch 56 Viral Load Vs Size

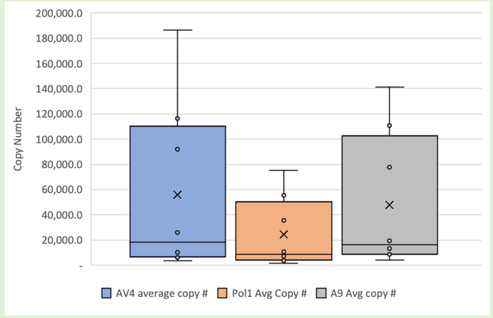
- Shows individual samples by weight plotted against copy number
- All animals with high viral load were small, but not all small animals had high viral load
- Evidence that viral load is not the only factor affecting Aplysia growth
- In this batch at this age most normally growing animals showed no evidence of infection.

Batch 73 Viral Load in Small Vs Large Aplysia

- Only data for the head ganglia were significantly different ($p \leq 0.05$, Mann-Whitney U Test)
- Other tissues showed the same trend where smaller animals had higher viral load (except in the gill)
- Both largest and smallest animals had a wide range of viral loads
- Batch 73 larvae and juveniles had high mortality and poor growth, such that all animals may have been impacted by the virus from an early age
- Animals were tested at older age than batch 56 which may have led to higher viral loads



RNAseq data showing transcript levels at different points of the genome (Bukhari et al, 2018)



Expression Ratios with Different Primers

- Previous study based on RNAseq data showed much greater number of transcripts in the ORF2, 3' region of the genome
- Our data shows 1:1 ratio of AV4:A9 and a 2:1 ratio of AV4 and A9 to pol1
- This data shows little evidence of sub-genomic transcripts from the ORF2 domain (3' prime end)
- 5' leader splicing may increase relative AV4 counts?

Conclusions

- AAbV hinders growth in young animals and/or smaller animals are more susceptible to AAbV infection
- This effect may be less apparent in cohorts where AAbV infection is more widespread
- Factors other than viral load are also involved in determining growth rates (i.e., genetics, early nutrition, etc.)
- 3' amplification in previous studies was likely due to poly A primer usage during cDNA synthesis which created a 3' bias
- AAbV genome may create a single polycistronic transcripts instead of creating subgenomic transcripts like SARS-CoV

References

Bukhari, K., Mulley, G., Gulyaeva, A. A., Zhao, L., Shu, G., Jiang, J., & Neuman, B. W. (2018). Description and initial characterization of metatranscriptomic nidovirus-like genomes from the proposed new family Abyssoviridae, and from a sister group to the Coronavirinae, the proposed genus Alphaletovirus. *Virology*, *524*, 160–171. https://doi.org/10.1016/j.virol.2018.08.010

Debat, H. J. (2018). Expanding the size limit of RNA viruses: Evidence of a novel divergent nidovirus in California Sea Hare, with a ~35.9 KB virus genome. https://doi.org/10.1101/307678

Kandel, E. R. (1979). *Behavioral Biology of aplysia: A contribution to the comparative study of Opisthobranch Molluscs*. W.H. Freeman.

Saberi, A., Gulyaeva, A. A., Brubacher, J. L., Newmark, P. A., & Gorbalenya, A. E. (2018). A Planarian Nidovirus expands the limits of RNA genome size. *PLOS Pathogens*, *14*(11). https://doi.org/10.1371/journal.ppat.1007314

ROSENSTIEL SCHOOL NATIONAL RESOURCE for APLYSIA

Supported by PHS grant P40 OD010952