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Distribution by Age and Tissue of a Novel Virus Identified in *Aplysia californica*

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Introduction

- *Aplysia* are used as models of basic neurophysiology and the cellular basis of memory and learning.[1]
- Using genomic databases, two labs have identified a novel RNA virus in *Aplysia* samples.[2,3]
- At ~35.9 kb, it is the largest RNA virus genome ever recorded.[2]
- This virus is likely in the order Nidovirales, closely related to invertebrate nidoviruses and also related to the family *Coronaviridae.*[2,3]
- It is likely neurotropic with a broad tissue distribution.[2]
- Samples as old as 10 years contain the virus.[2]
- To date, no pathology associated with the virus has been discovered despite widespread infection.
- I examined the tissue distribution of the virus and how that distribution changes with age.

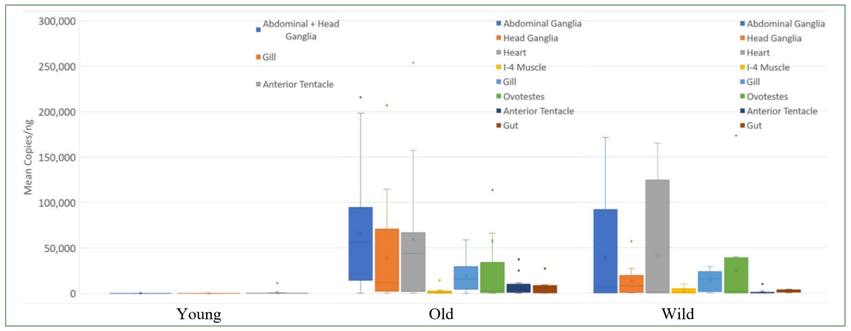
An Aplysia being weighed prior to dissection

Methods

- Animals were reared at the National Resource for Aplysia, unless otherwise noted. Young animals (<5 months), old animals (>11 months), wild collected animals, and eggs were sampled.
- Animals were anaesthetized using $MgCl_2$ prior to dissection.
- Tissues collected: Abdominal Ganglia (A), Head Ganglia (G), Anterior Tentacle (T), Gills (L), Heart (H), Hepatopancreas (P), I-4 Muscle (I), Ovotestes (O), and Gut (U). In young animals, T, L, and combined A+G were collected because other tissues were too small.
- RNA extractions using Trizol™ were performed on all samples.
- RNA was converted to cDNA for use in qPCR with SYBR Green dye; samples were run in replicates.
- The copy number and the concentration of RNA were used to find the copies/ng of input RNA.

Results

Figure 1: *Distribution of the viral load in tissues from young (<5 months old), old (>10 months old), and wild-collected animals. Copy numbers are shown as mean copies/ng RNA (the mean of the two qPCR replicates) for each sample in each group, sorted by tissue. N=63 for young animals, N=122 for old animals, and N=68 for wild animals.*



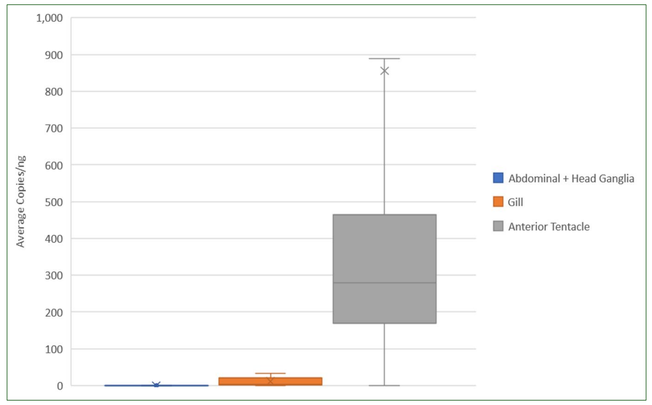


Figure 2: *Mean copies/ng RNA of tissues collected from young animals. N=63. Y-axis is scaled to fit the whiskers, excluding outliers.*

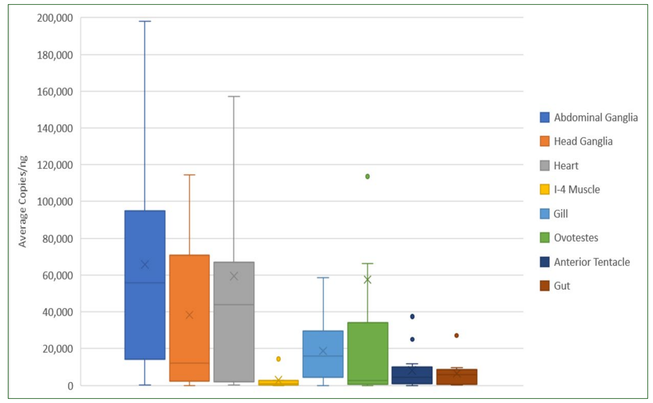


Figure 3: *Mean copies/ng RNA of old animal tissues. Scaled to fit the box and whiskers, excluding outlier points in the abdominal ganglia, head ganglia, heart, and ovotestes. N=122.*

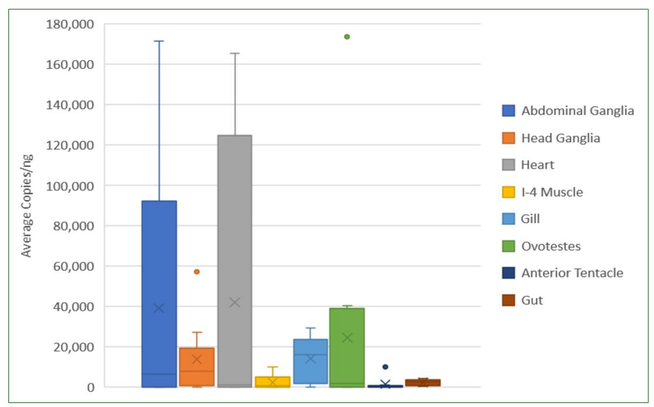


Figure 4: *Mean copies/ng RNA of the tissues collected from wild animals. Y-axis scaled to fit all data. N=68.*

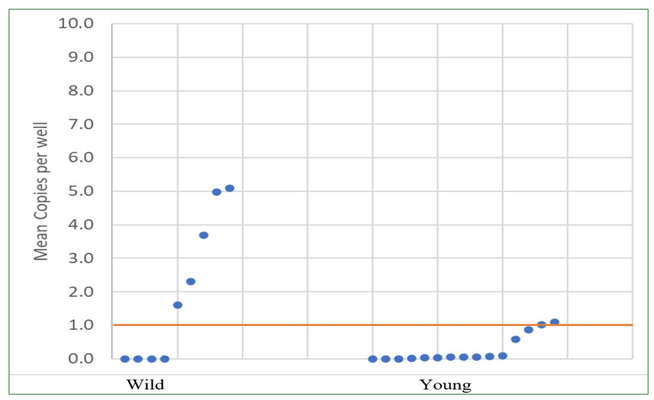


Figure 5: *Mean copy number (copies per well) for the lowest few samples from young and wild animals, arranged arbitrarily in increasing order. Wild N=9, young N=15. Note that I defined the virus negative samples as <1 copy/well and this value is denoted with an orange line. At this scale, the old animals do not appear because the lowest copy number for that group is nearly 25.*

Discussion

- Young animal tissues were significantly lower in mean copies/ng RNA than the old tissues (Mann-Whitney U, $p<0.001$). Old animals also had significantly higher mean copies/ng RNA compared to wild animals (Mann-Whitney U, $p<0.001$). Wild animals had significantly higher mean copies/ng RNA than young animals (Mann-Whitney U, $p<0.001$), so they placed in between young and old animals.
- 19 samples qualified as virus negative. 3 young animals and two eggs were entirely virus negative. There were no virus negative samples among the old animals.
- My results indicate the viral load increases with age. This could contribute to declines in neuronal function that are observed as *Aplysia* age,[6,7] but further research is needed to confirm this relationship.
- All tissues sampled had the virus; the tissue distribution is broad, as seen in related viruses.[8,9,10]
- In young animals, the tissues did not vary significantly. In old animals, the abdominal ganglia, the mean highest tissue, was significantly higher than the I-4 muscle, the mean lowest tissue (Mann-Whitney U, $p<0.001$). The head ganglia was also high and did not vary significantly from the abdominal ganglia (Mann-Whitney U, $p>0.1$).
- In wild animals, the abdominal ganglia and heart were the highest and the anterior tentacle was the lowest, but the tissues did not differ significantly.
- In old and wild animals, the virus is neurotropic. The high levels in the heart are surprising and warrant further study.
- Vertical transmission is possible, due to the presence of virus in nearly all egg samples, but more research is needed to explore this and other modes of transmission.

References

1. Moroz, 2011 2. Debat, 2018 3. Bukhari, 2018 4. Dhar et al., 2001 5. Bennett et al., 2015
6. Kempsell & Fieber, 2014 7. Kempsell & Fieber, 2015 8. Lu et al., 1995 9. Stenglein et al., 2014 10. Vega et al., 2004

Acknowledgments

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