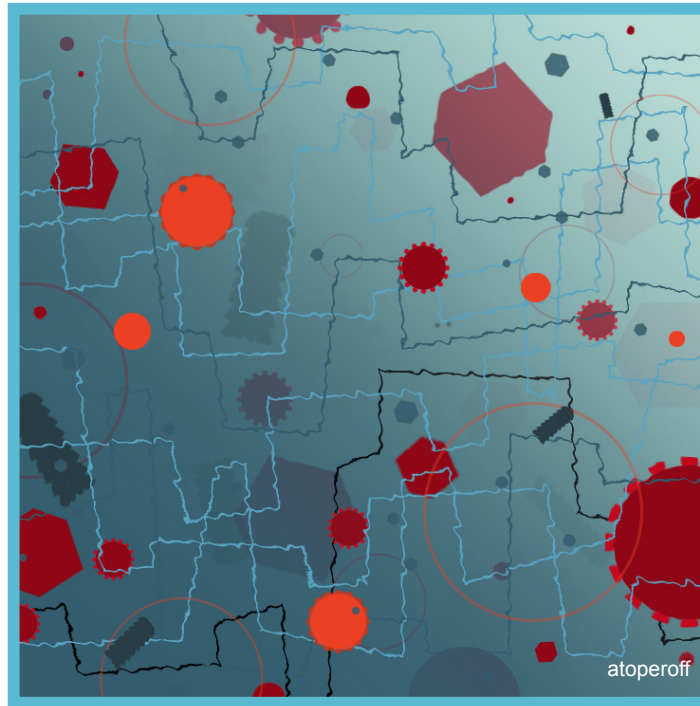


## The puniest of predators: RNA viruses in the sea



Alexander Culley  
Research Affiliate  
Marine Viral Ecology Lab



- Introduction to marine viruses

- RNA viruses in the sea

- RNA virus isolates that infect marine animals

- RNA virus isolates that infect protists

Heterosigma akashiwo RNA virus

- Characterization of the RNA virus community

Marine picornavirad diversity

Metagenomic analysis

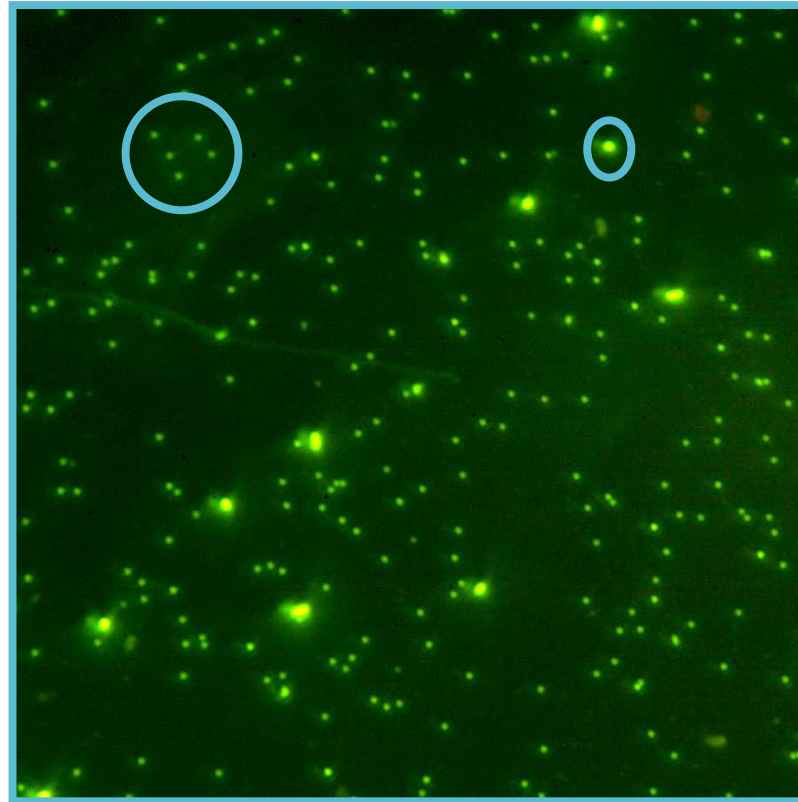
Estimating RNA virus Abundance

- Conclusions

- Upcoming research in the MarVEL

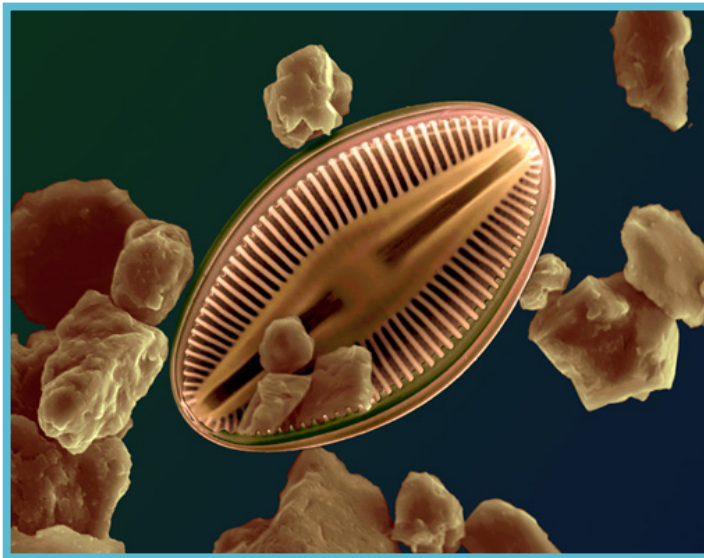
- Viruses in Antarctica

- Marine viruses are abundant ( >1 billion/ L)



- The viroplankton is morphologically & genetically diverse

- Viruses infect a diversity of marine organisms

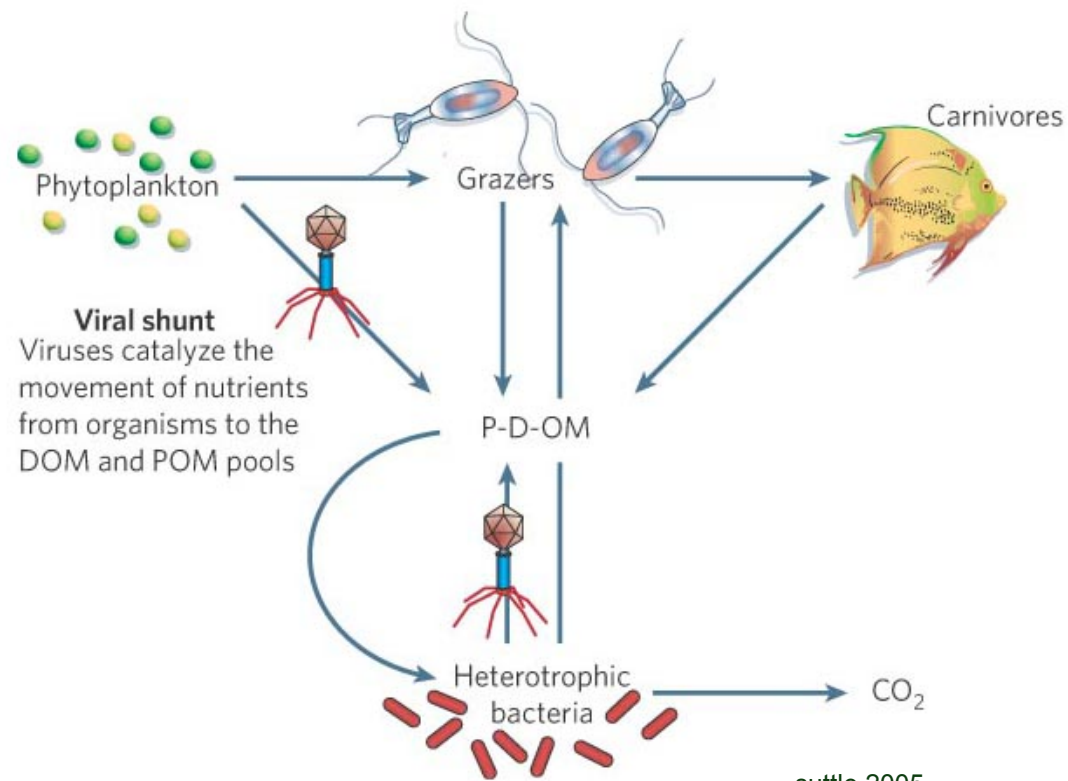


[ideo.columbia.edu](http://ideo.columbia.edu)

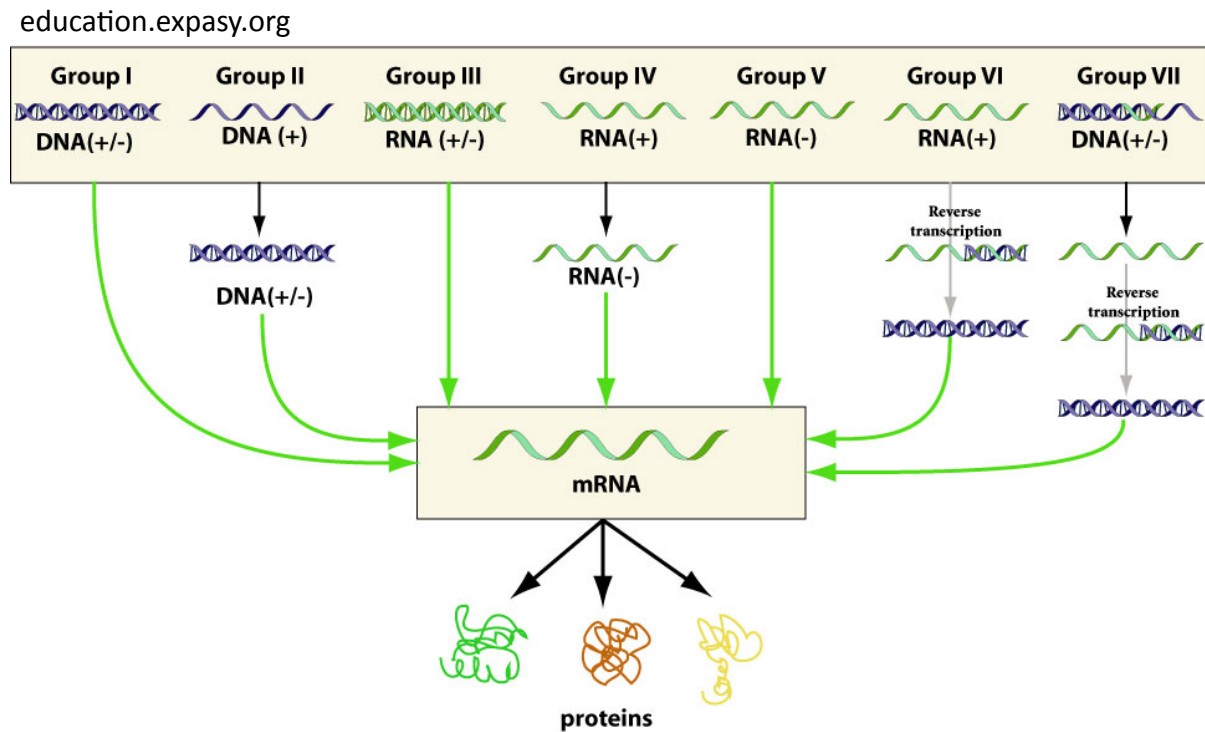


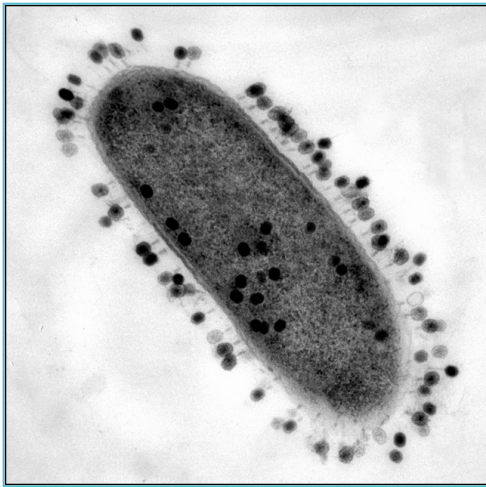
[yoto98.noaa.gov](http://yoto98.noaa.gov)

- Viral lysis results in the transfer of most of the carbon to the dissolved and colloidal pools

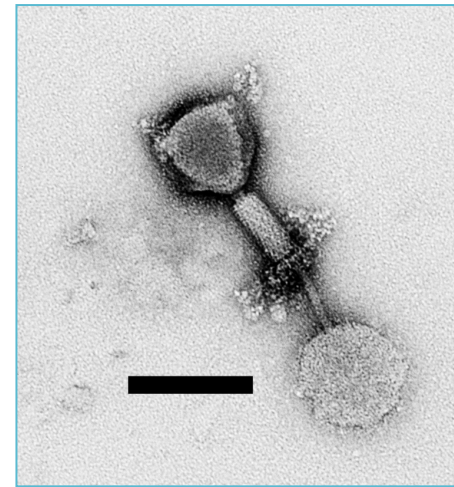
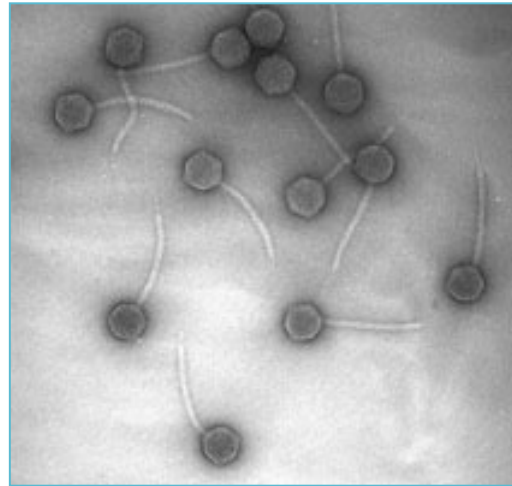


- The viral genome can be composed of RNA or DNA and may be either single or double stranded





kerrlab



proportal.mit.edu

- The majority of marine viruses is presumed to be phage
- However, marine RNA viruses may be important as well...



[afsc.noaa.gov](http://afsc.noaa.gov)



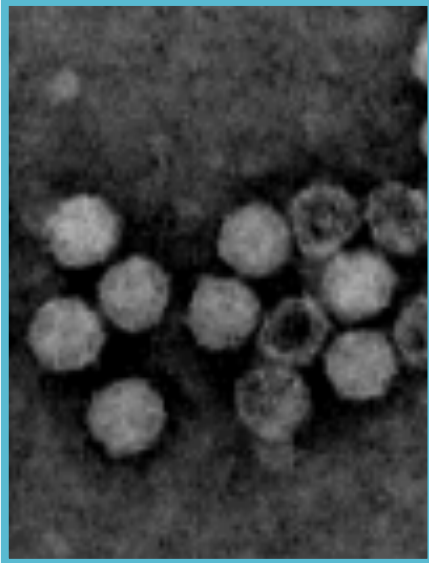
[fish-net.co.jp](http://fish-net.co.jp)



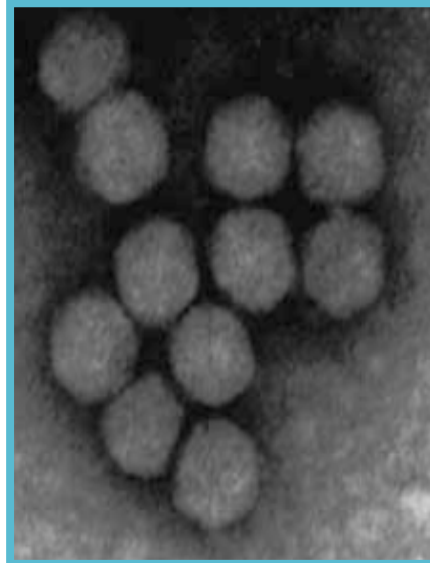
© 2003 Nick Kontonikolas/1000birds.com

- RNA viruses infect a diversity of marine animals

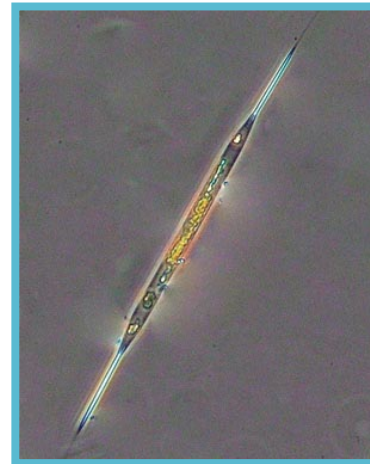




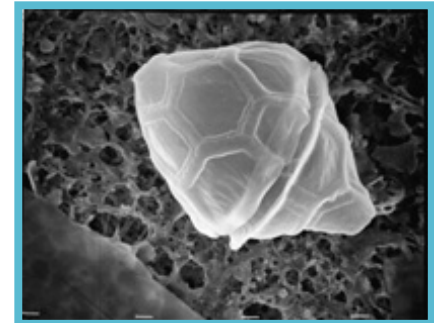
nagasaki et al. 2004



tomura et al. 2004



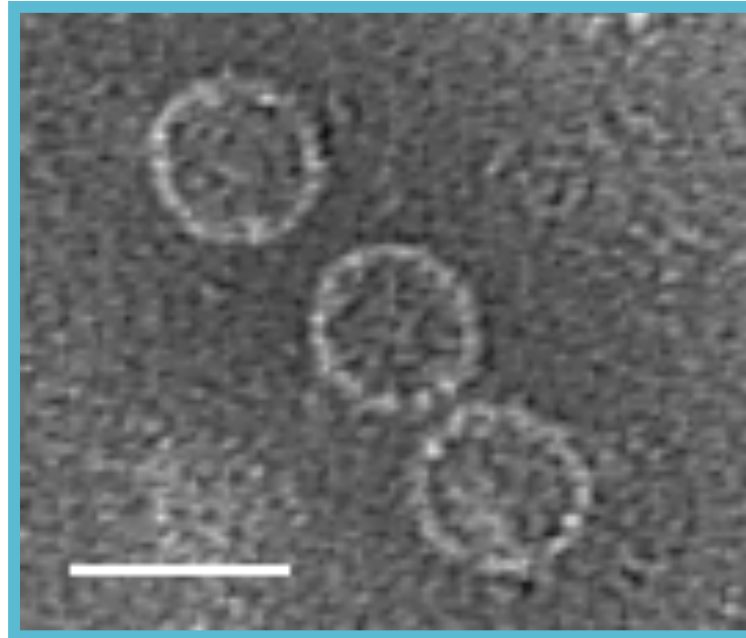
[www.serc.si.edu](http://www.serc.si.edu)

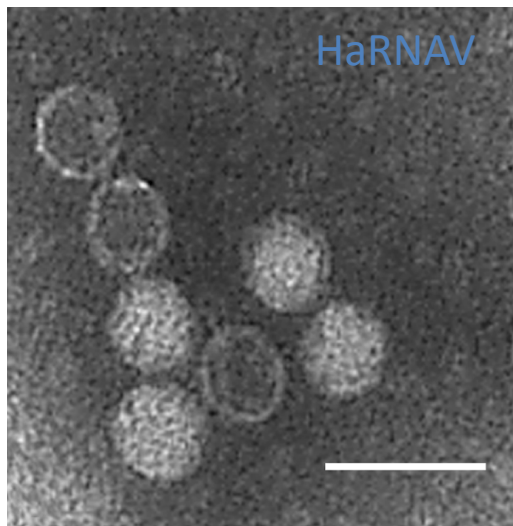


[www.univ-rouen.fr](http://www.univ-rouen.fr)

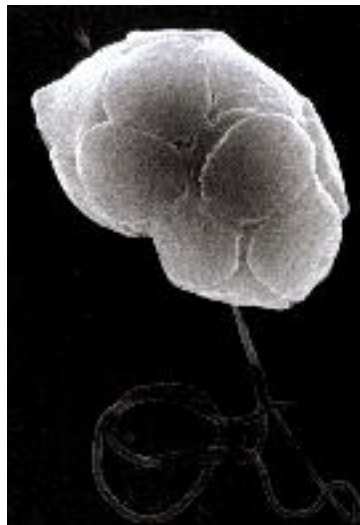
- The RNA viruses RsRNAV & HcRNAV infect two widespread species of phytoplankton, a diatom and a dinoflagellate
- Phytoplankton form the base of the marine food web and therefore the activity of these viruses can have important ecological consequences

## Characterization and genome sequence of Heterosigma akashiwo RNA virus (HaRNAV)





tai et al 2003



kagosima.suigi.jp



usa.com

- HaRNAV infects & lyses *Heterosigma akashiwo*
- *H. akashiwo* harmful algal blooms are ichthyotoxic



- RNA molecule 8587 nt long and encodes a single polyprotein
- HaRNAV has significant similarities to viruses in the order *Picornavirales*
- Picornavirads are diverse and are of economic and public health concern because they cause disease in animals, plants and insects



■ Characteristics shared between HaRNAV and picornavirads include:

ss + sense RNA genome

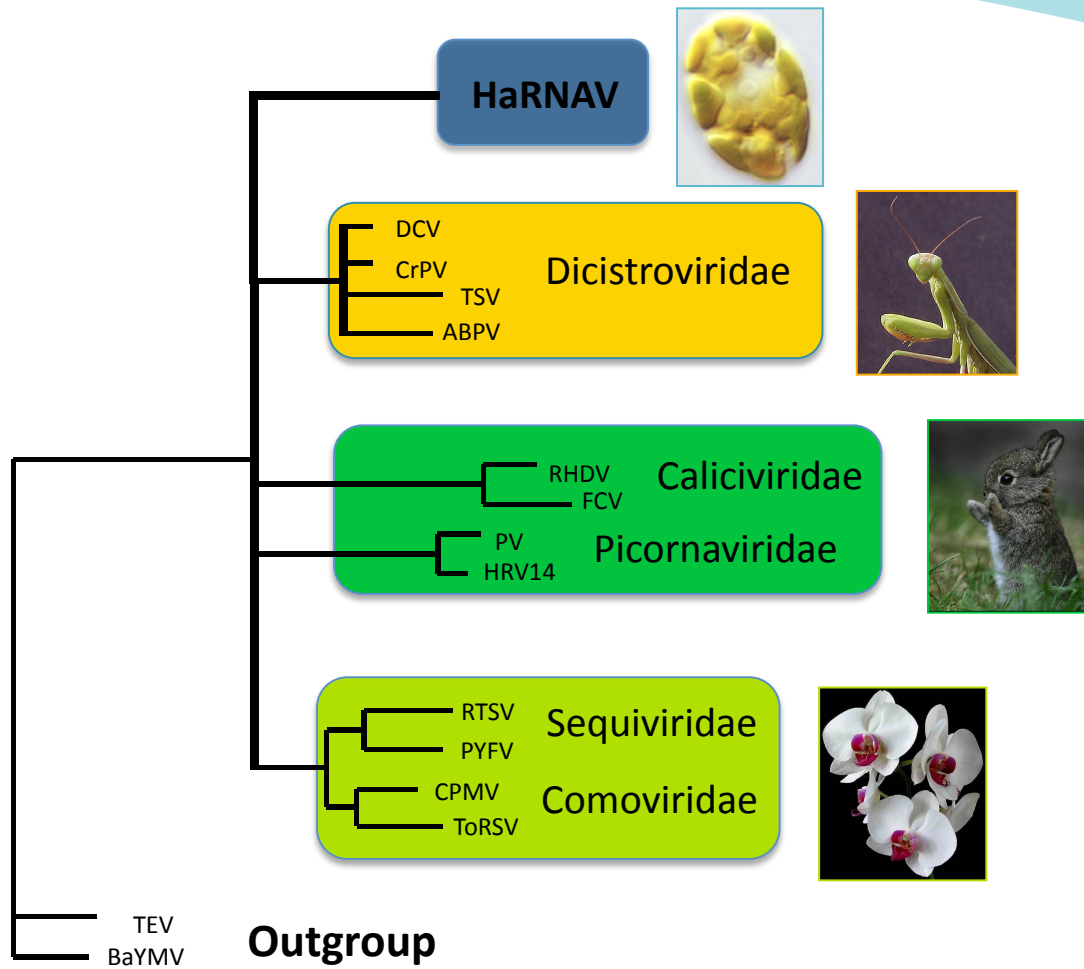
eukaryotic host

icosahedral morphology

3' poly A tail

conserved non-structural gene order

RNA-dependent RNA Polymerase sequence similarity



■ HaRNAV falls outside established families of picornavirads



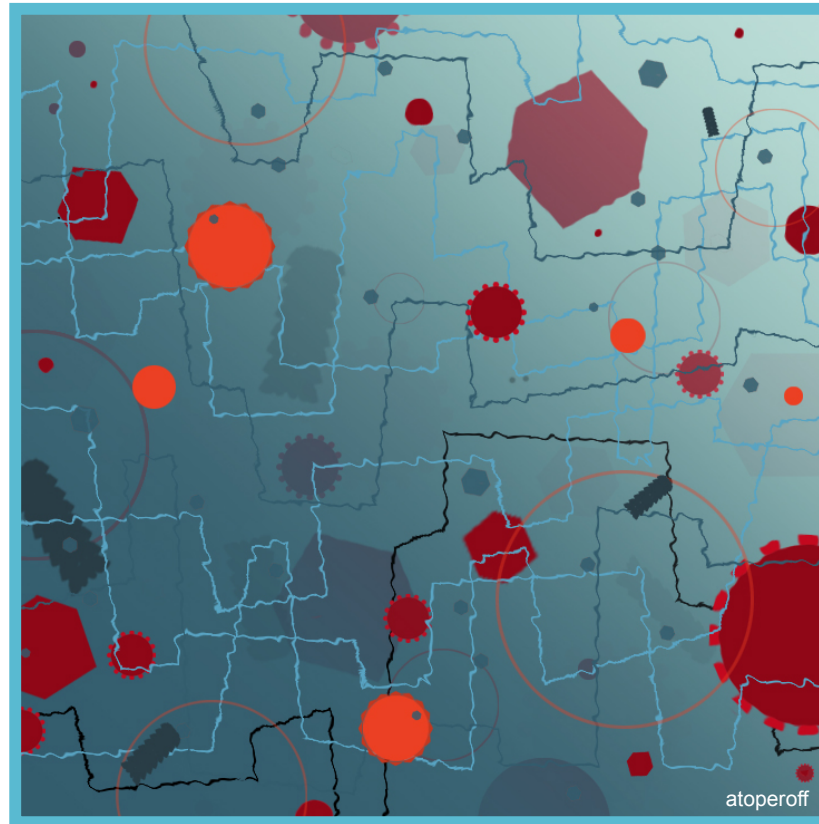
Based on:

- the phylogenetic analysis of the RdRP
- a unique genome organization within the *Picornavirales*
- infects a protist

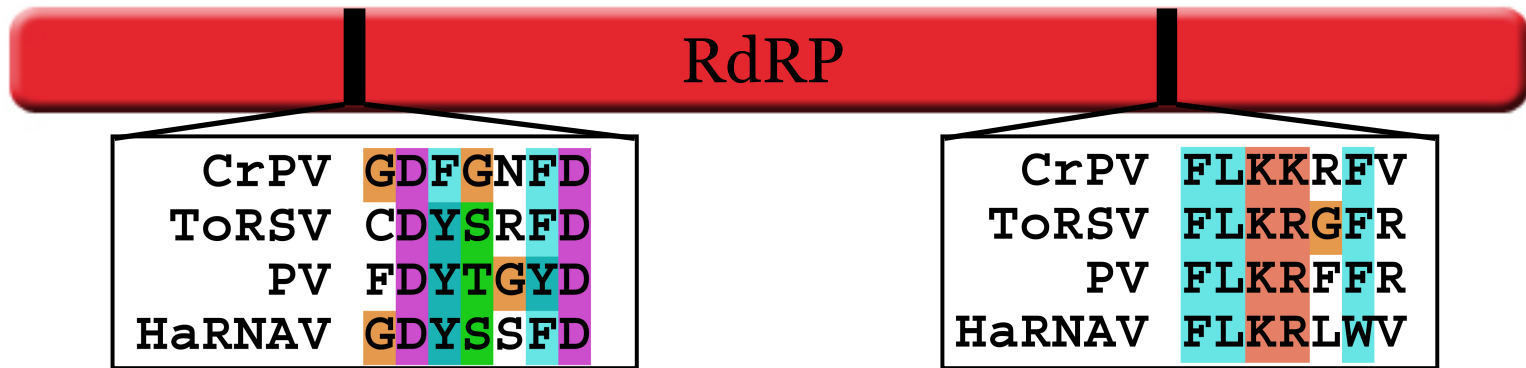
HaRNAV has been classified as the type species for the new family

***Marnaviridae***

## Characterization of the marine RNA virus community



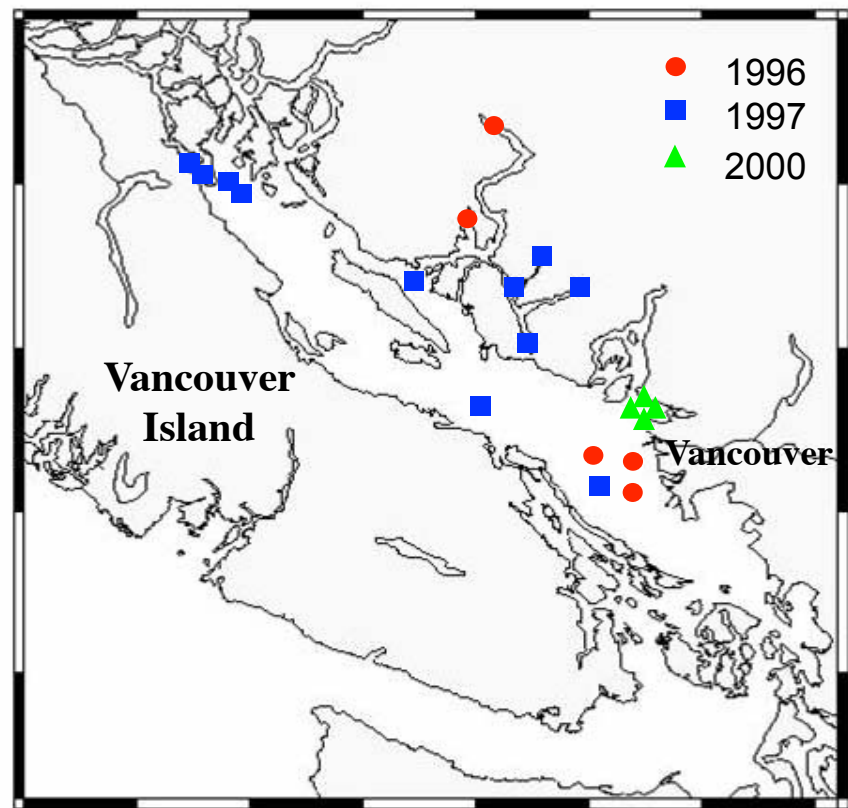




- Primers were designed from regions of the RNA-dependent RNA polymerase (RdRP) conserved among picornavirads

- Degenerate RT-PCR assay used to survey viral communities from coastal B.C.

- Amplification occurred from different stations, in different years & seasons



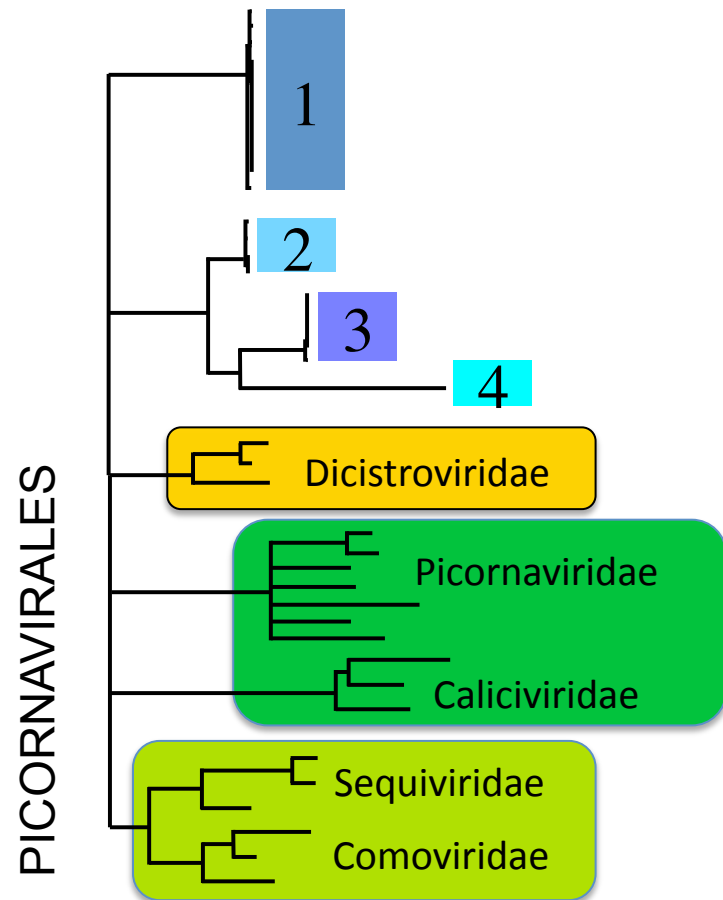
- Canonical RdRP motif “GDD” present in all novel sequences

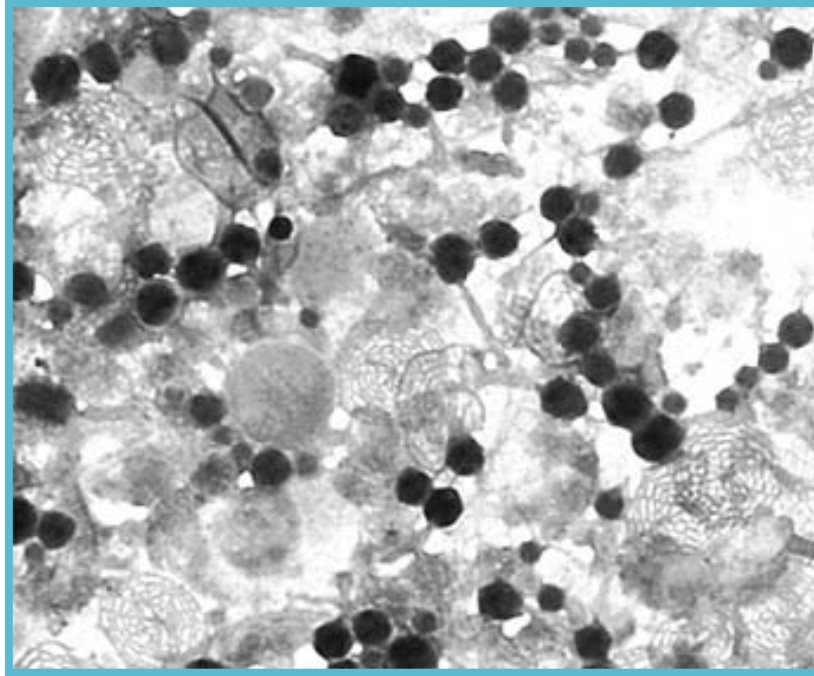
- Sequence database searches resulted in significant similarity to picornavirads

- Suggests sequences are viral in origin

FRP896-1	KENVSLLT	GDD	VMG
JP800-6	KENVSLLT	GDD	VMG
JP700-1	KENVSLLT	GDD	VMG
JP800-7	KENESLLT	GDD	VMG
JP800-2	KHHVALFT	GDD	ICG
JP800-3	KHHVALFT	GDD	ICG
JP800-9	KHHVALFT	GDD	ICG
JP800-5	KHHVALFT	GDD	ICG
JP800-10	KHRVALFT	GDD	ICG
JP700-2	ADVCNLAC	GDD	IACN
FRP896-2	RSHVALIV	GDD	NAA
FRP896-3	RSHVALIV	GDD	NAA
FRP896-4	RSHVALIV	GDD	NAA
FRP896-5	RSHVALIV	GDD	NAA
FRP896-6	RSHVALIV	GDD	NAA
JP800-4	RSHVALIV	GDD	NAA
JP800-8	RSHVALIV	GDD	NAA
JP500-1	RSHVALIV	GDD	NAA
FRP896-7	RSHVALIV	GDD	SAA
JP800-1	RSHVALIV	GDD	NAA
JP800-11	RSHVALIV	GDD	HAA

- All environmental sequences clustered outside of established taxa
- Using tree as a guide, 4 novel families of RNA viruses?



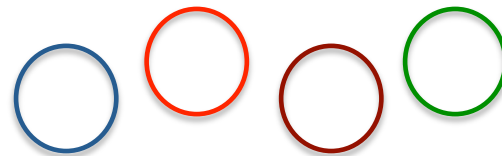


[fuhrmanlab.usc.edu](http://fuhrmanlab.usc.edu)

**Marine RNA virus metagenomics:  
characterization of the total RNA virus community**

- Metagenomics- genomic analysis of nucleic acids extracted directly from communities in environmental samples
- The metagenomic approach is culture independent and requires no assumptions about the composition of the community

All genomes extracted from a microbial community

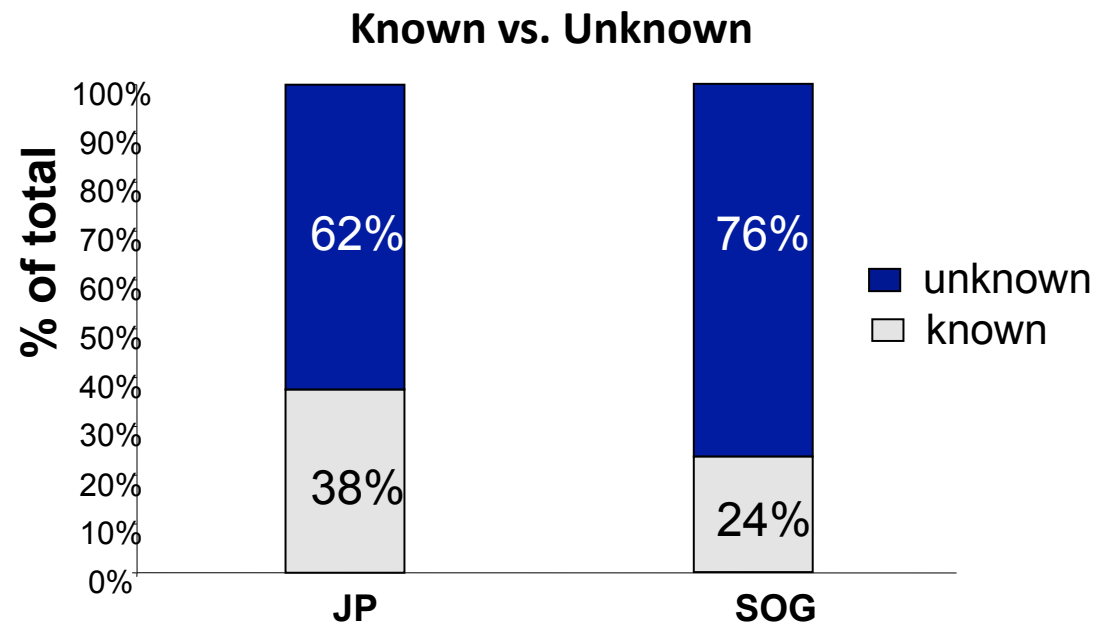


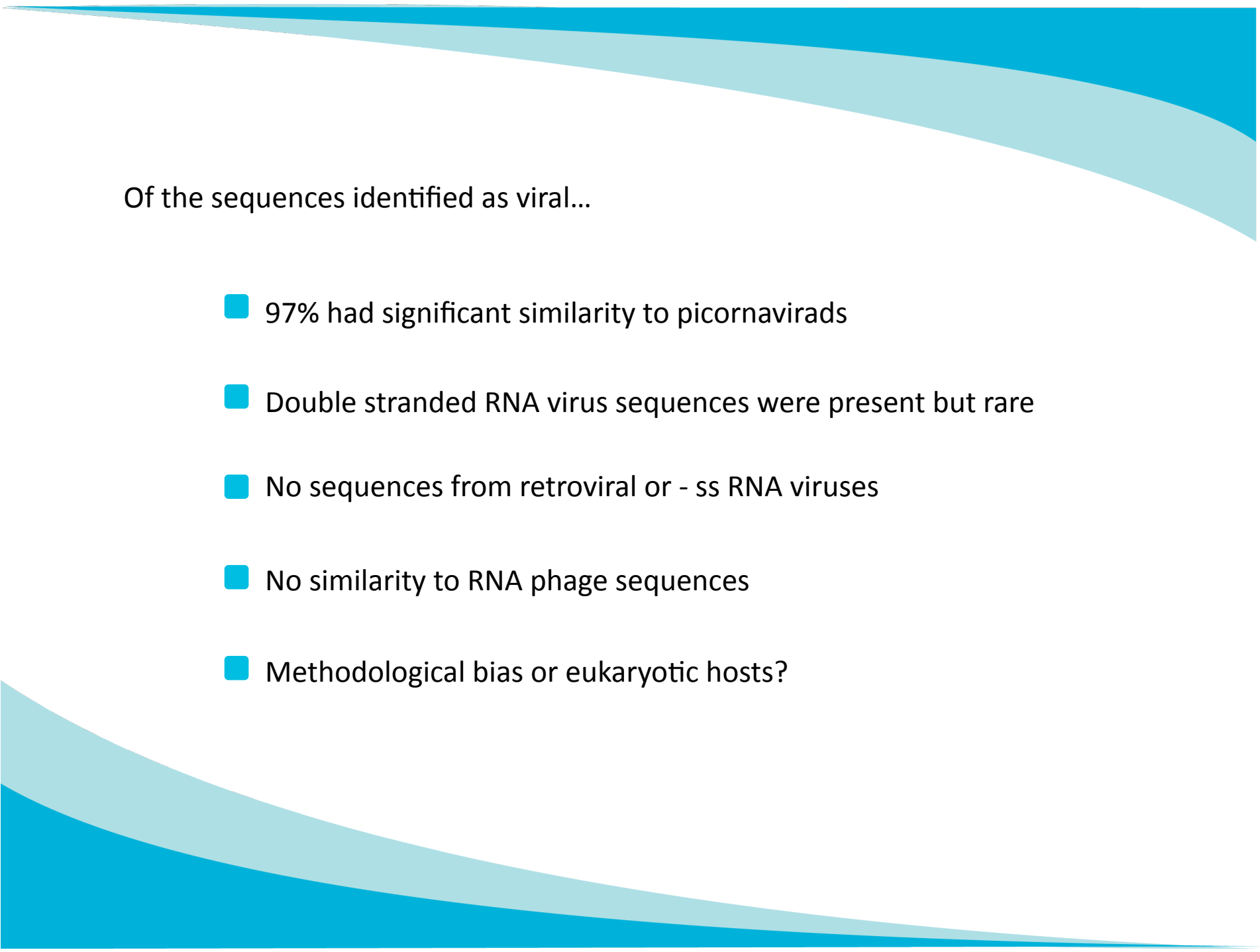
Genetic material is sheared into fragments and sequenced



Sequence information is used to characterize the community

- Metagenomic libraries were constructed from two stations
- A majority of environmental sequences had no significant similarity to known sequences
- Suggests that most marine RNA viruses are unrecognizable



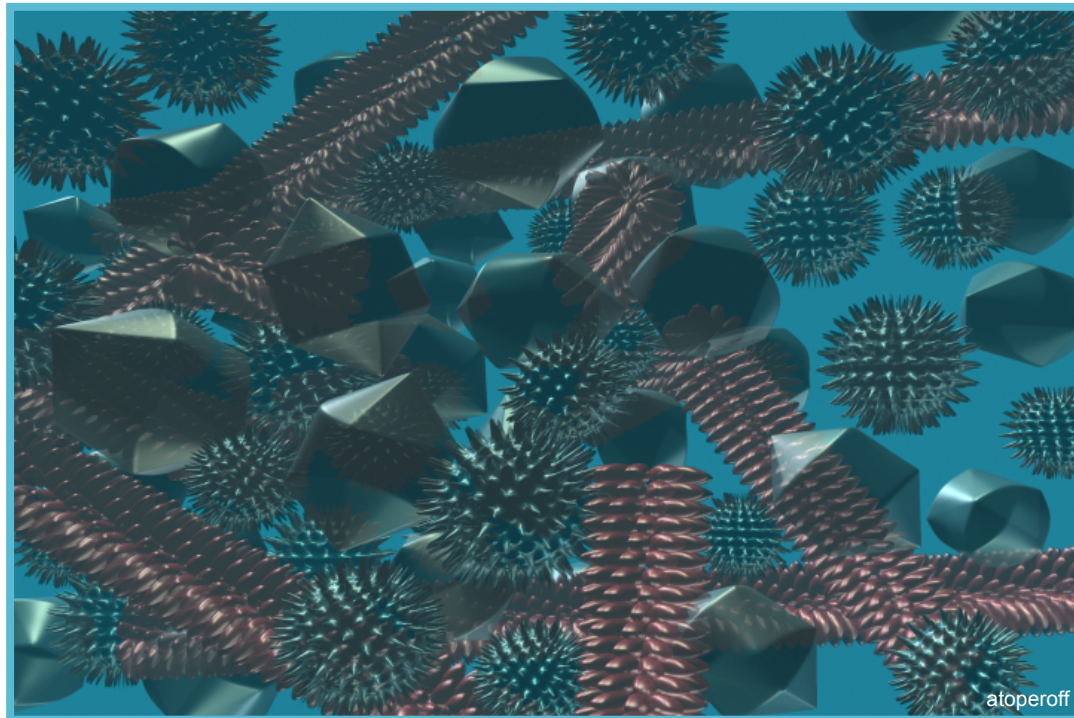


Of the sequences identified as viral...

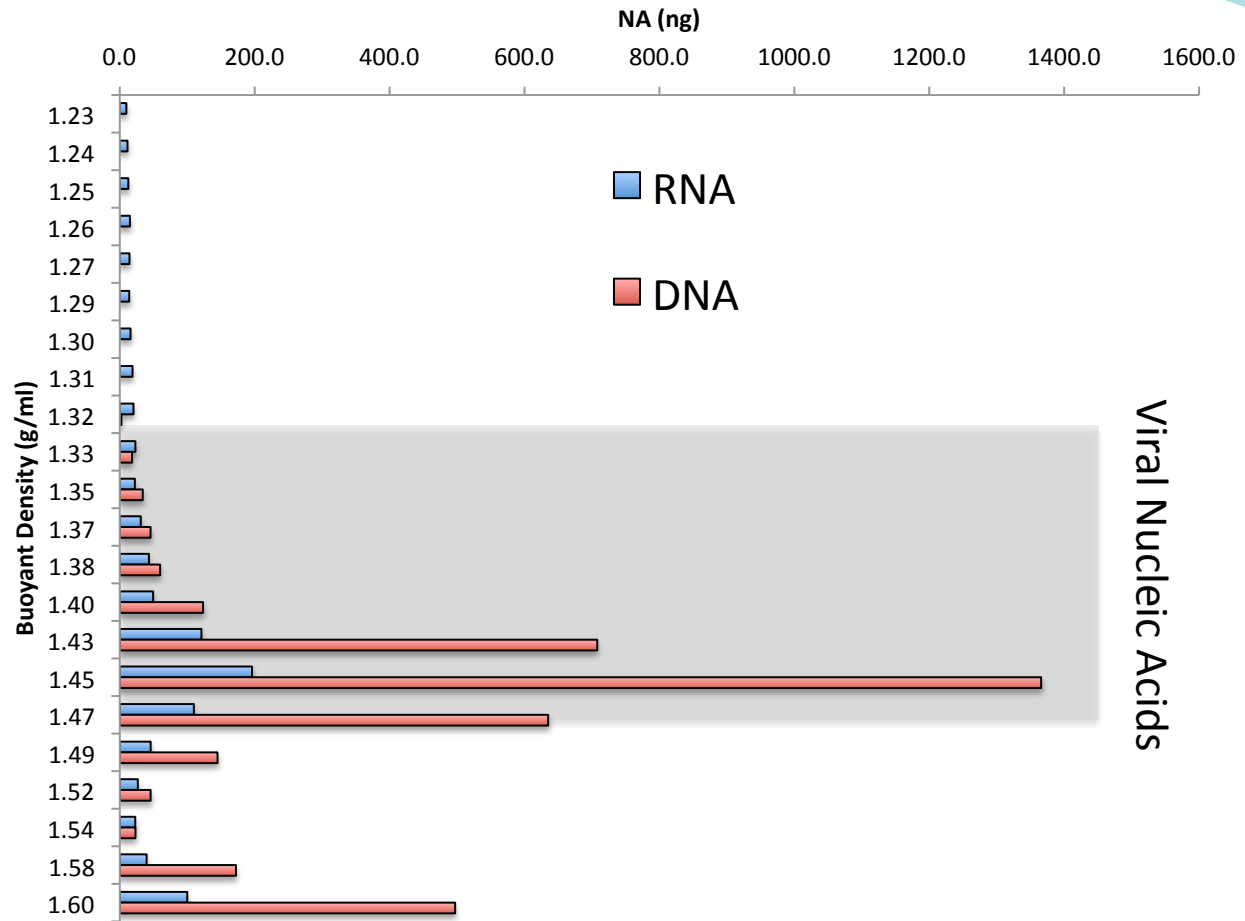
- 97% had significant similarity to picornavirads
- Double stranded RNA virus sequences were present but rare
- No sequences from retroviral or - ss RNA viruses
- No similarity to RNA phage sequences
- Methodological bias or eukaryotic hosts?



## Estimating the abundance of RNA viruses



- Viruses harvested and purified on a density gradient to measure total viral NA





**Assumptions:**

- All RNA viruses in this sample are single stranded with an average genome size of 8 kb
- All DNA viruses in this sample are double stranded with an average genome size of 50 kb

**Abundance estimate in Kaneohe Bay:**

$3.5 \times 10^9$  RNA virus genomes  $L^{-1}$

$2.0 \times 10^9$  DNA virus genomes  $L^{-1}$

**Abundance estimate from station ALOHA:**

$4.2 \times 10^8$  RNA virus genomes  $L^{-1}$

$8.5 \times 10^8$  DNA virus genomes  $L^{-1}$





## Conclusions

- RNA viruses are a dynamic, widespread and persistent component of the marine virus community
- A vast majority of marine RNA viruses have not been isolated
- Most recognizable marine RNA viruses have + ss genomes that are distantly related to picornavirads
- Most recognizable marine RNA viruses infect protists
- If most of the distinct environmental phylotypes have different hosts, our data imply that a diverse assemblage of marine protists may be infected with viruses at any given time
- Marine RNA viruses can be more numerous, but do not appear to be as diverse, as DNA viruses

## Why study marine RNA viruses?

- **Biological Oceanography:** Greater understanding of a significant source of mortality for marine plankton
- **Microbiology:** New insight into a driving force in microbial evolution
- **Evolutionary Biology:** New taxa of RNA viruses may lead to a better understanding of RNA viral evolution
- **Virology:** New model viruses from the marine environment may result in greater insight into RNA virus life history
- **Industry:** RNA viruses are pathogens of economically important species of fish, invertebrates and protists
- **Bio Tech:** Sources of novel genetic diversity and viral vectors

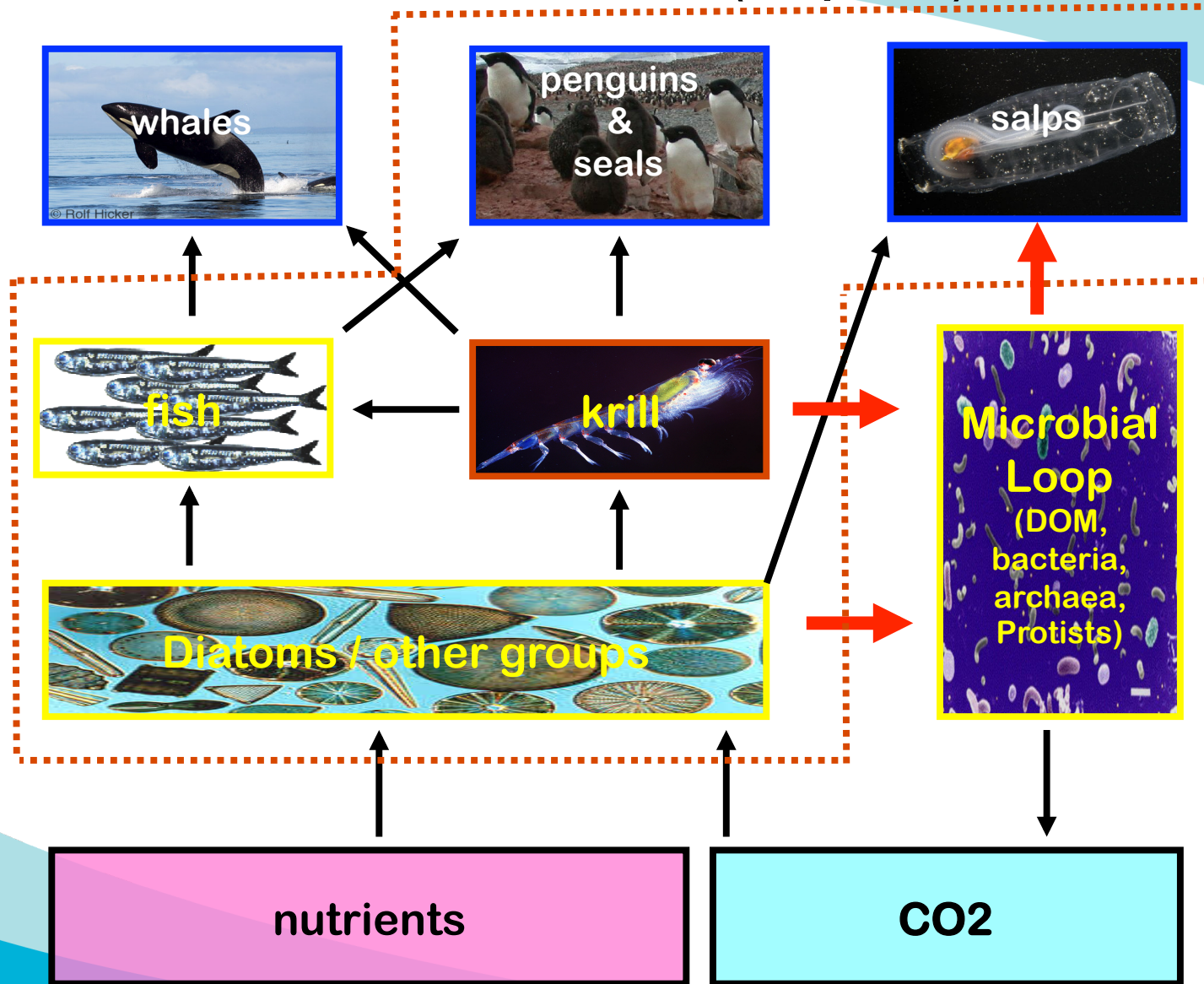
# Future research



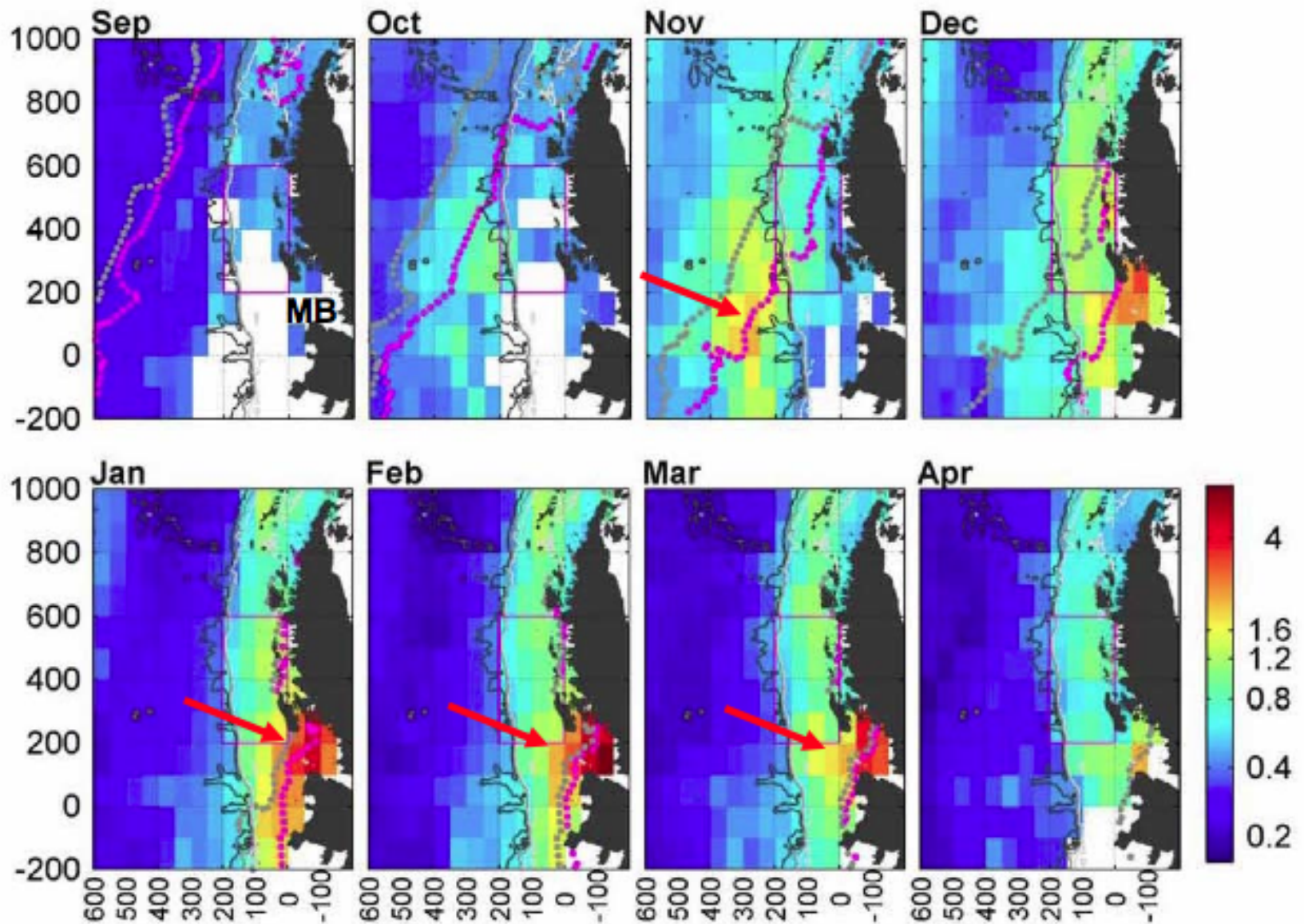
## Viruses in the Western Antarctic Peninsula

- WAP has experienced a rise in mean winter temperature of 6 °C over the last 60 yrs
- Palmer LTER program has been active for the past 20 yrs

# Palmer LTER Marine Food web (simplified)



Courtesy of H. Ducklow



Courtesy of H. Ducklow

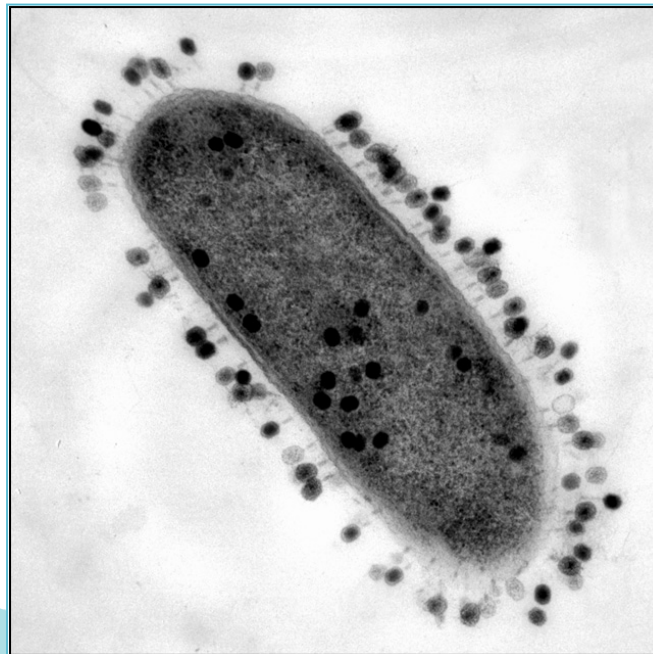
[mg Chl-a m<sup>-3</sup>]



## Viral contributions to summer bloom dynamics in the Western Antarctic Peninsula

### Primary research objective

- Better understand the contributions of viruses to the mortality of prokaryotic and eukaryotic plankton during the summer bloom



J. Wertz



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