VIRUSES RULE THE SEA

CMORE SUMMER COURSE

2012

GRIEG STEWARD CENTER FOR MICROBIAL OCEANOGRAPHY: RESEARCH AND EDUCATION DEPARTMENT OF OCEANOGRAPHY UNIVERSITY OF HAWAII AT MANOA

OUTLINE OF THE TALK

- Introduction to viruses
- Why viruses rule the sea
- Why viral diversity matters
- Vignettes of some of our work:
 - 1. RNA viruses
 - 2. Isolation of new virus-host systems
 - 3. Fractionation of cells and Viruses

Let's ask some experts



André Lwoff (1902-1994) Nobel Prize 1965

Sir Peter Medawar (1915-1987) Nobel Prize 1960

Let's ask some experts



André Lwoff (1902-1994) Nobel Prize 1965

Sir Peter Medawar (1915-1987) Nobel Prize 1960

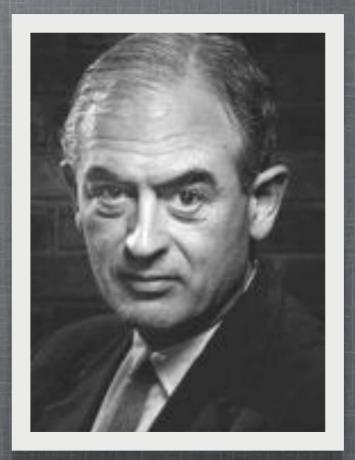
A VIRUS IS A VIRUS!

Let's ask some experts



André Lwoff (1902-1994) Nobel Prize 1965

A VIRUS IS A VIRUS!

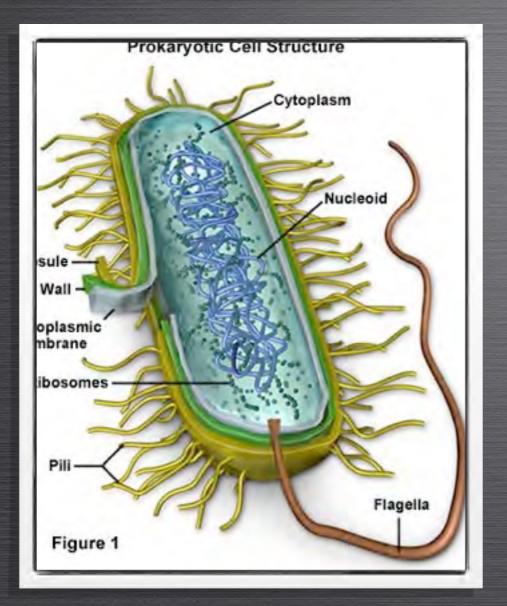


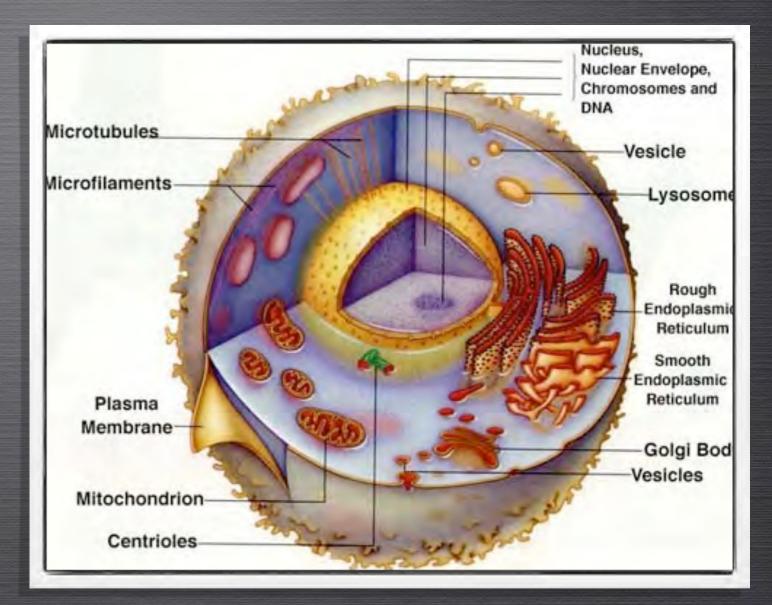
Sir Peter Medawar (1915-1987) Nobel Prize 1960

A VIRUS IS A PIECE OF BAD NEWS WRAPPED IN PROTEIN

PROKARYOTE

EUKARYOTE

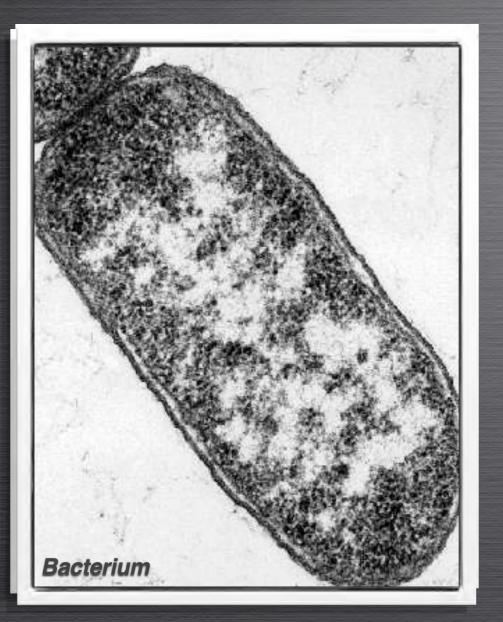


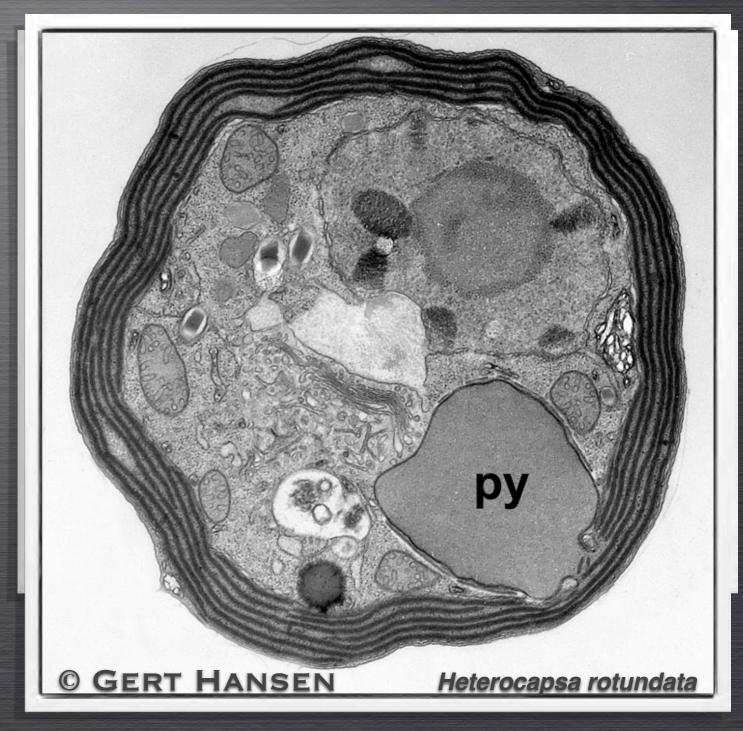


CELLULAR LIFE

PROKARYOTE

EUKARYOTE



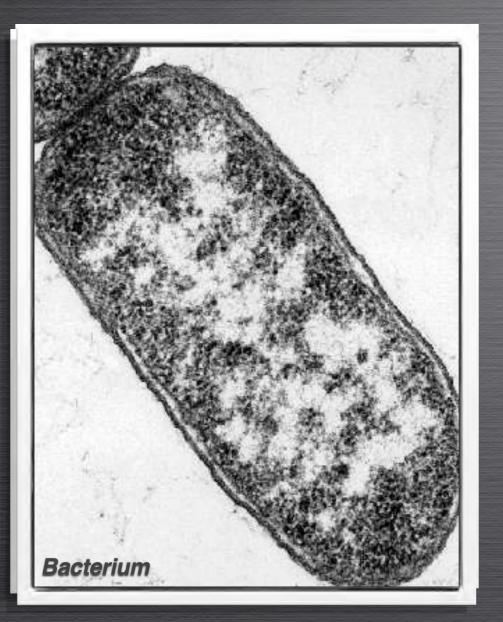


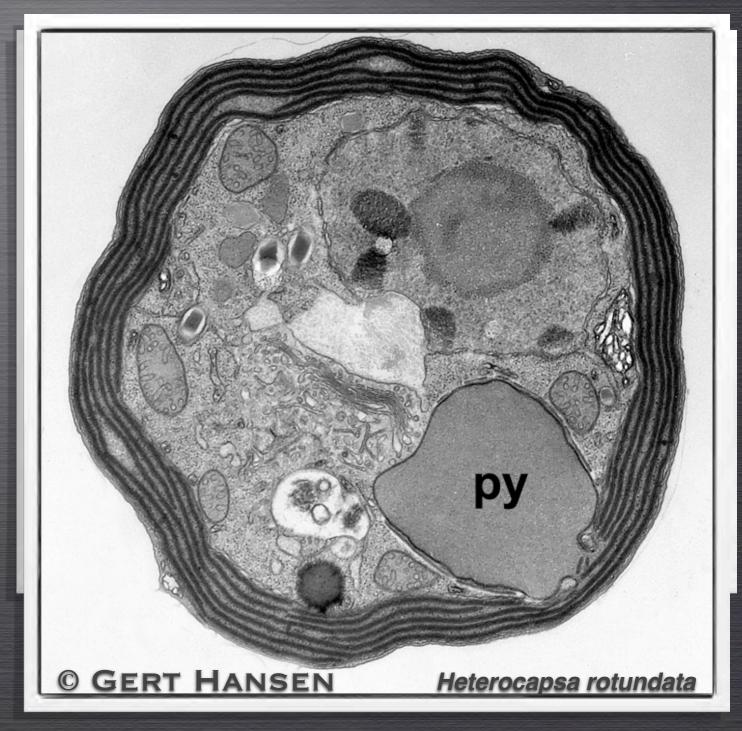
CELLULAR LIFE

Friday, June 29, 2012

PROKARYOTE

EUKARYOTE

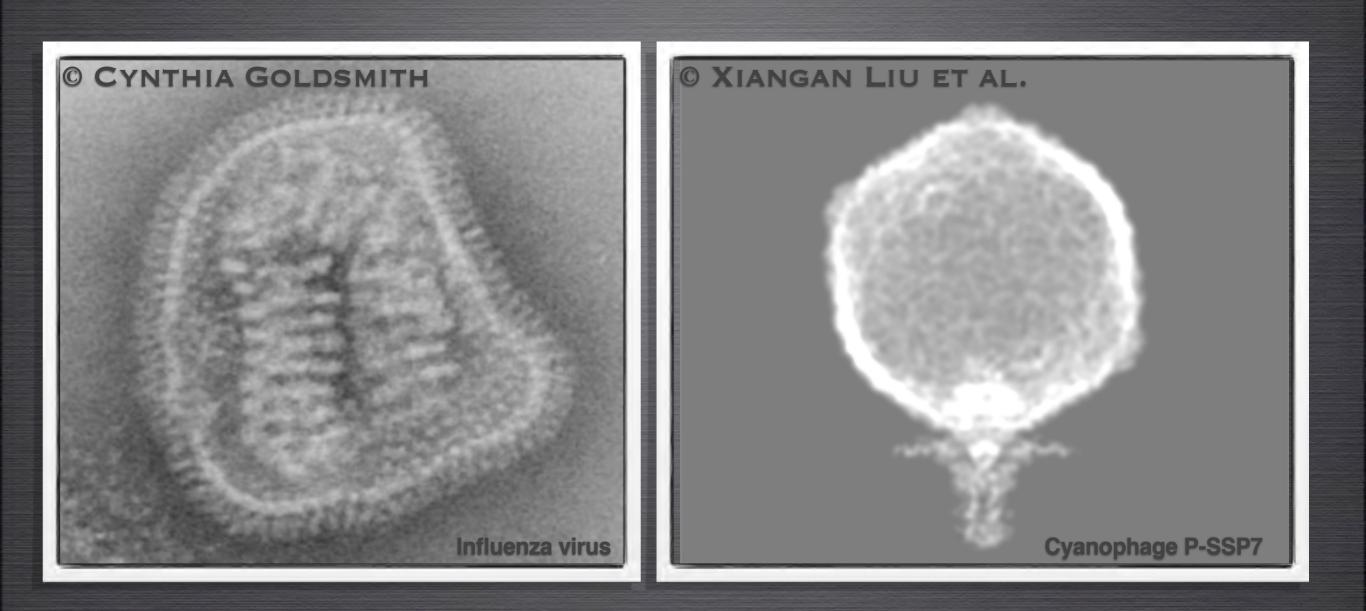




CELLULAR LIFE

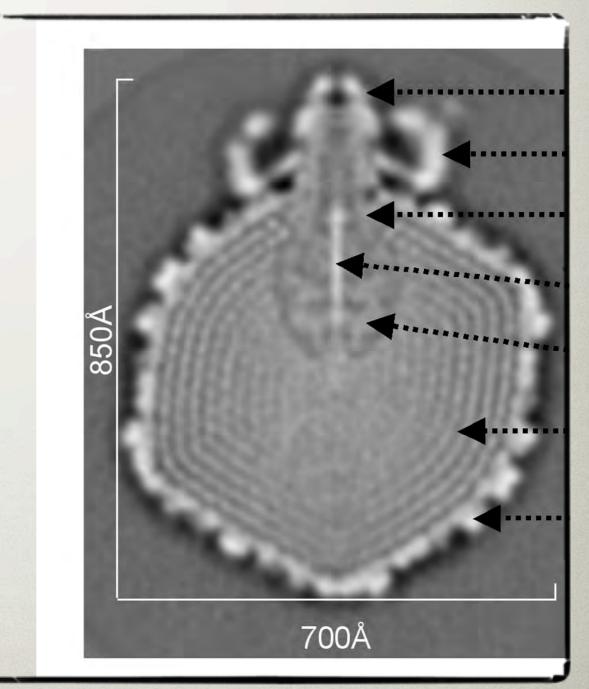
CAN WE CALL VIRUSES "ACELLULAR"?

Friday, June 29, 2012



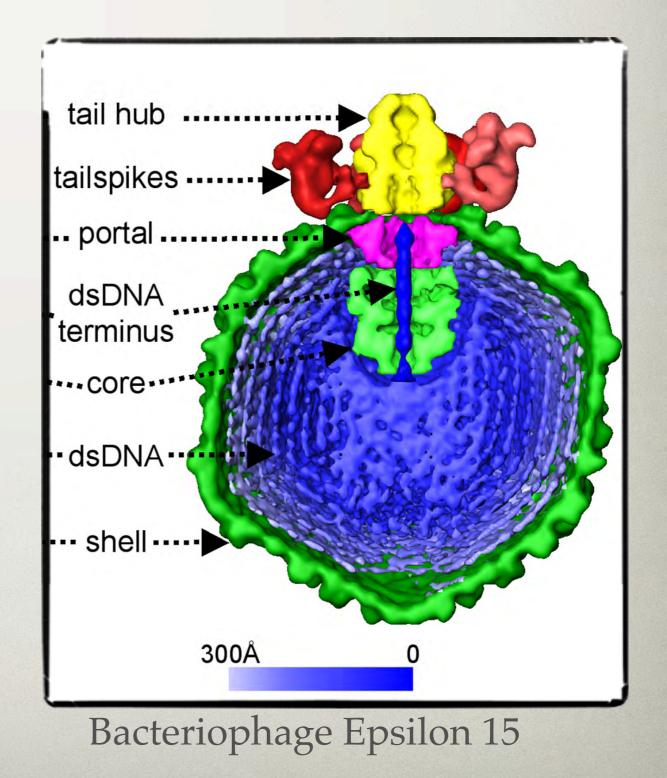
VIRUSES ARE ALSO "CELLULAR" BUT THESE "CELLS" HAVE NO METABOLISM

- Infectious agents that can replicate only inside a host cell
- Relatively simple:
 - A set of instructions (DNA or RNA)
 - A protective coat
 - No motility
 - No metabolism



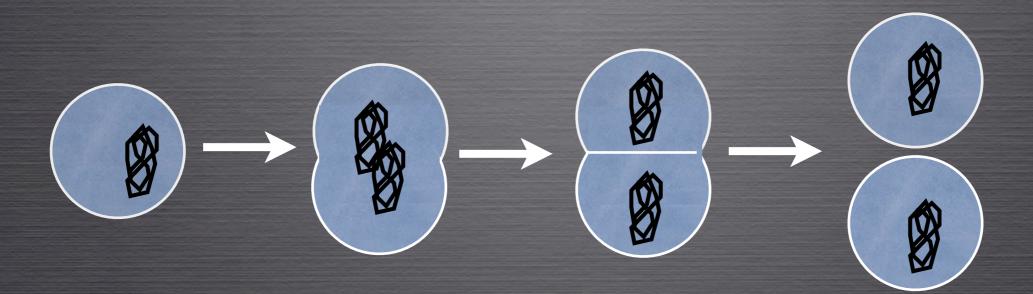
Bacteriophage Epsilon 15

- Infectious agents that can replicate only inside a host cell
- Relatively simple:
 - A set of instructions (DNA or RNA)
 - A protective coat
 - No motility
 - No metabolism

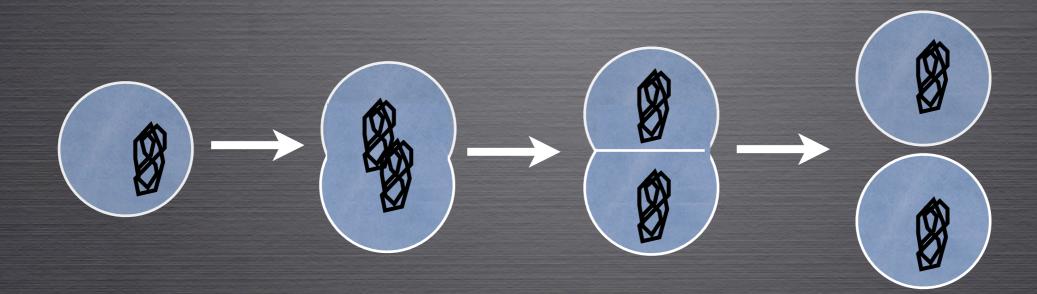


PROKARYOTES AND EUKARYOTES: REPLICATION BY FISSION

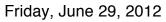
PROKARYOTES AND EUKARYOTES: REPLICATION BY FISSION



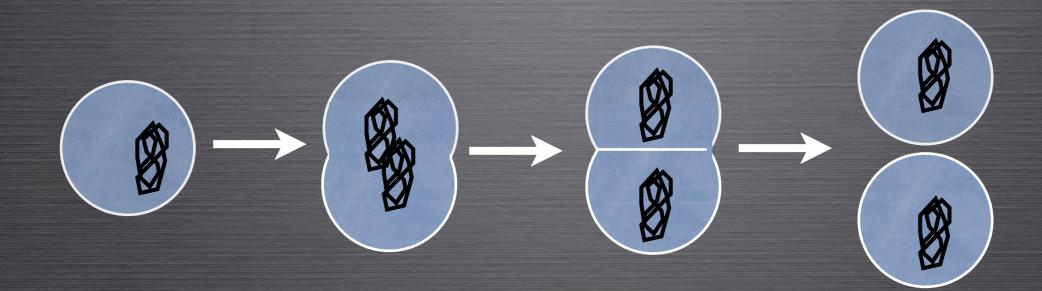
PROKARYOTES AND EUKARYOTES: REPLICATION BY FISSION



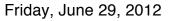
VIRUSES: REPLICATION WITHIN CELLS BY ASSEMBLY



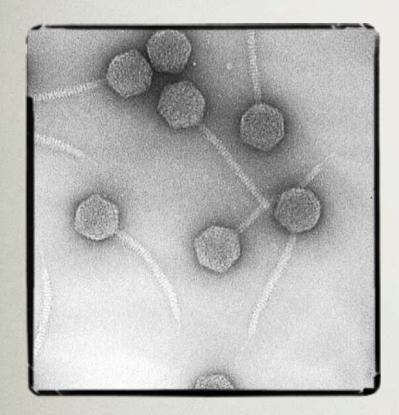
PROKARYOTES AND EUKARYOTES: REPLICATION BY FISSION



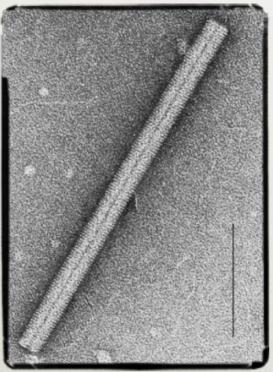
VIRUSES: REPLICATION WITHIN CELLS BY ASSEMBLY



MANY SHAPES AND SIZES



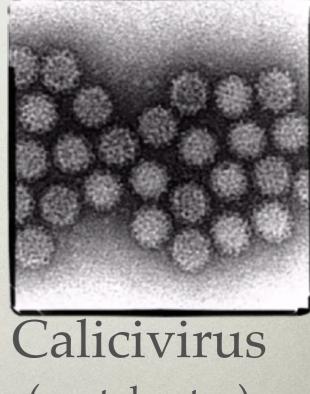
Siphovirus (bacteria)



Tobamovirus (Tobacco plant)

Influenza (Humans, birds, pig, seals)

50 nm



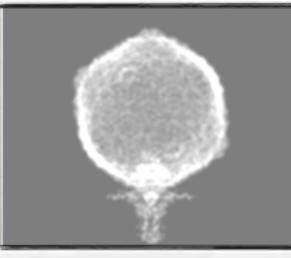
(vertebrates)

THE CONTINUUM OF EXTRACHROMOSOMAL GENETIC REBELS

- Tranposons and Inteins no direct mechanism of cell entry or exit (must hitch a ride)
- **Plasmids** direct transfer between hosts, no explicit extracellular stage
- Viruses cell entry and exit mechanisms with extracellular stage and distinct phenotypes

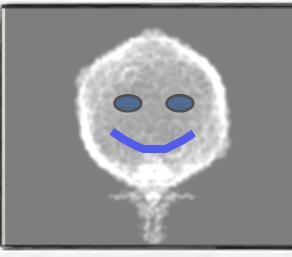
THE CONTINUUM OF EXTRACHROMOSOMAL GENETIC REBELS

- **Tranposons and Inteins** no direct mechanism of cell entry or exit (must hitch a ride)
- Plasmids direct transfer between hosts, no explicit extracellular stage
- Viruses cell entry and exit mechanisms with extracellular stage and distinct phenotypes



THE CONTINUUM OF EXTRACHROMOSOMAL GENETIC REBELS

- **Tranposons and Inteins** no direct mechanism of cell entry or exit (must hitch a ride)
- Plasmids direct transfer between hosts, no explicit extracellular stage
- Viruses cell entry and exit mechanisms with extracellular stage and distinct phenotypes



SO, ECOLOGICALLY, WHAT ARE VIRUSES?

• Predators?

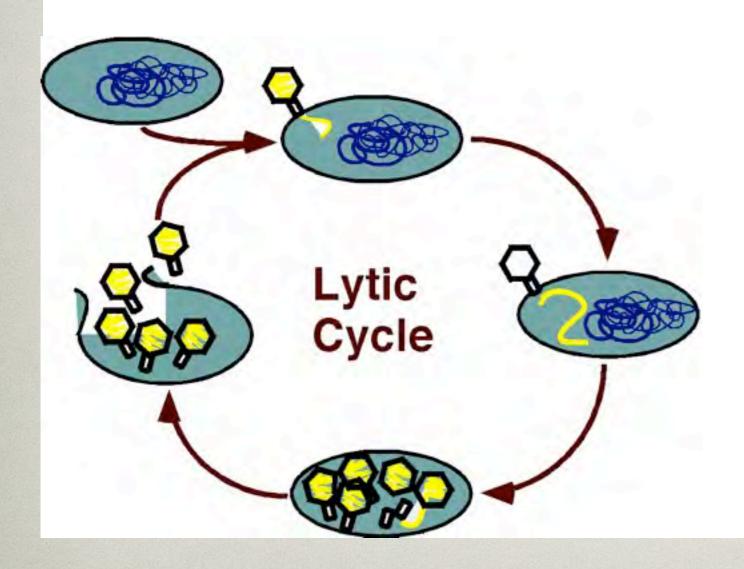
Pathogens?

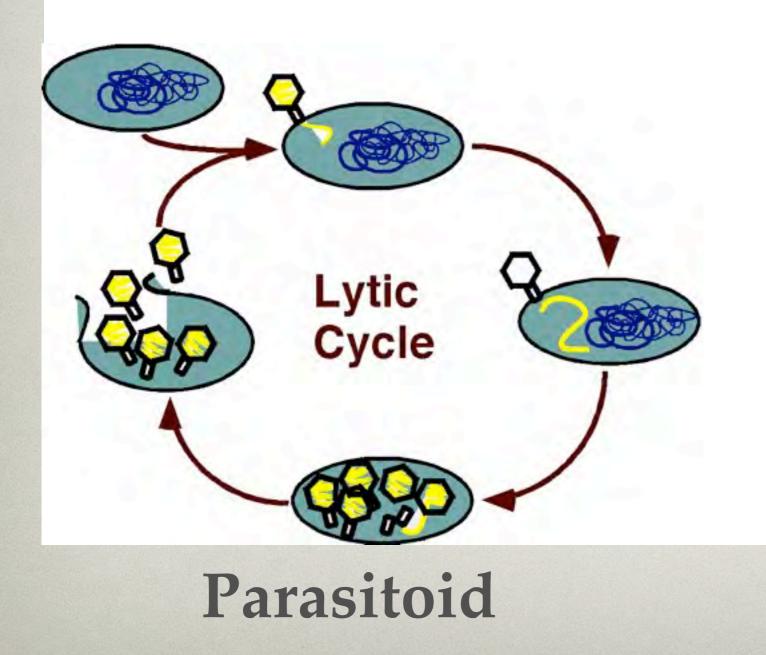


SO, ECOLOGICALLY, WHAT ARE VIRUSES?

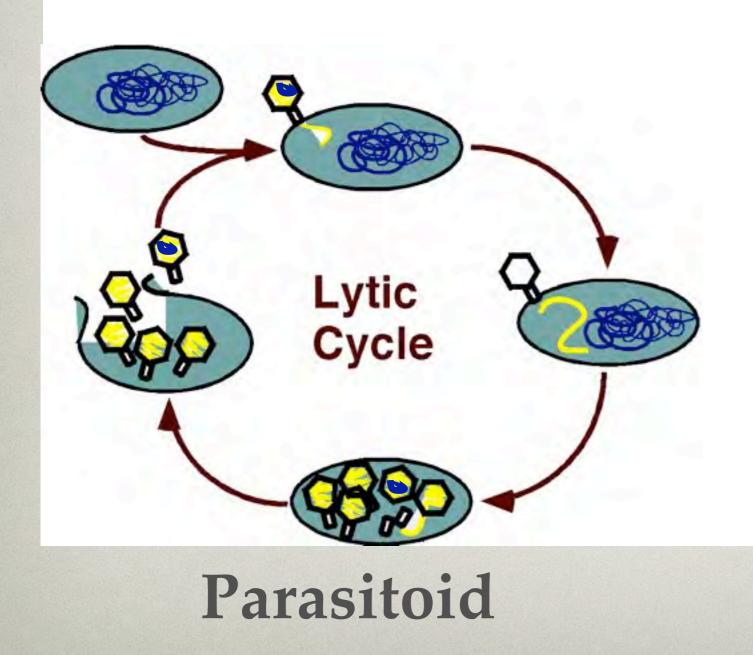
Predators?

Pathogens?
Symbionts?

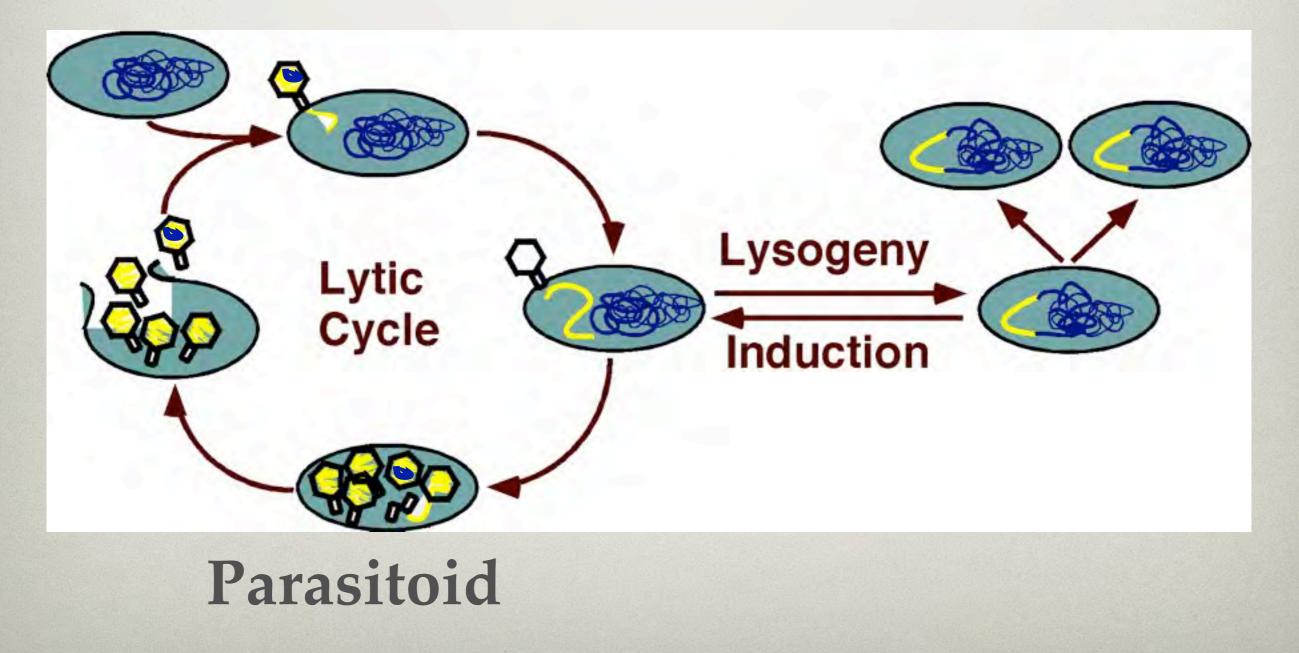




Transduction

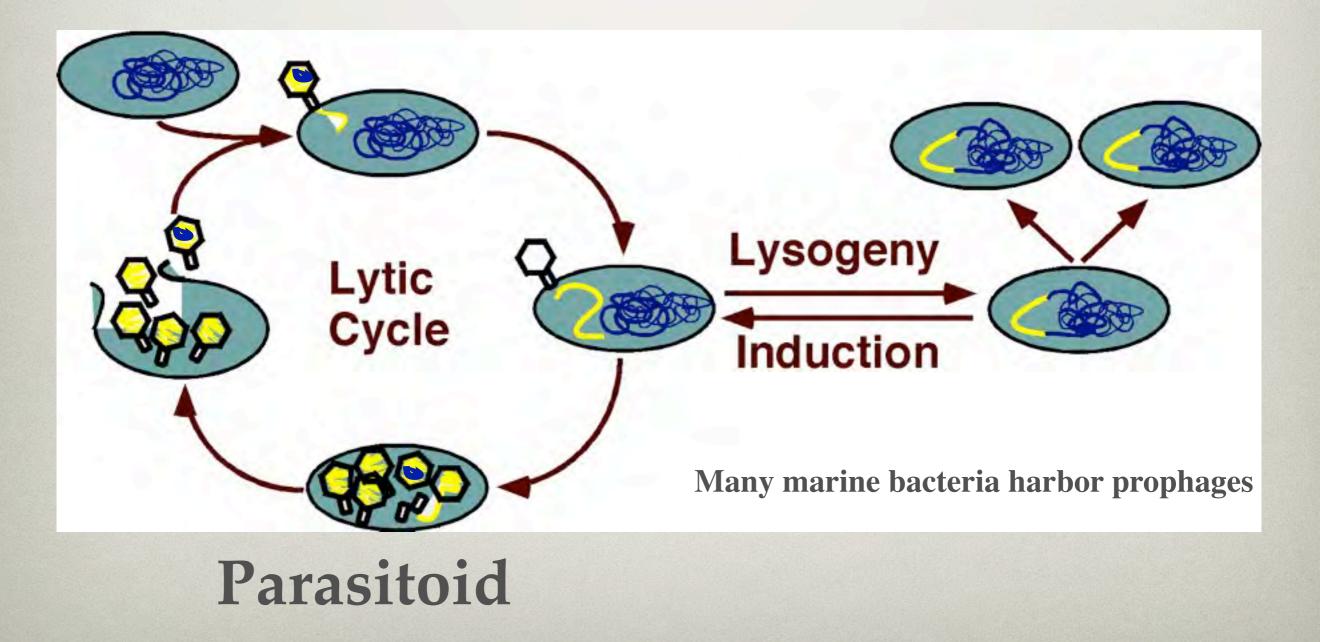


Transduction



Friday, June 29, 2012

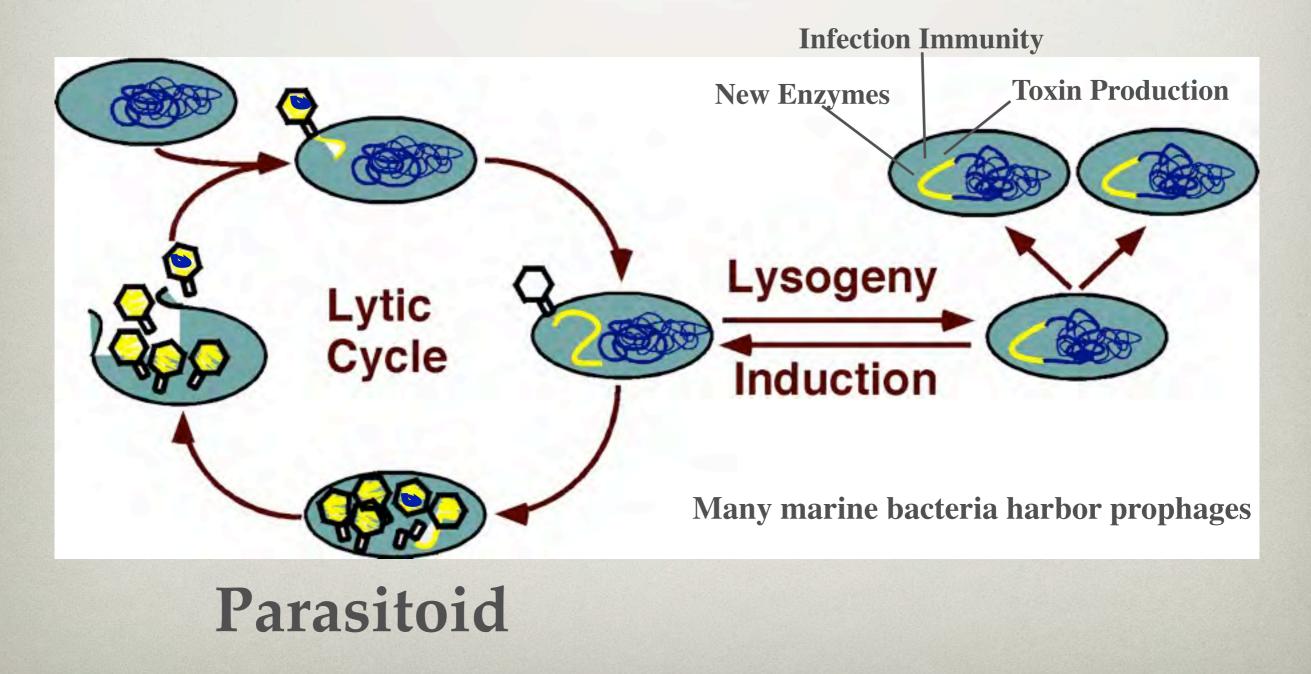
Transduction



Friday, June 29, 2012

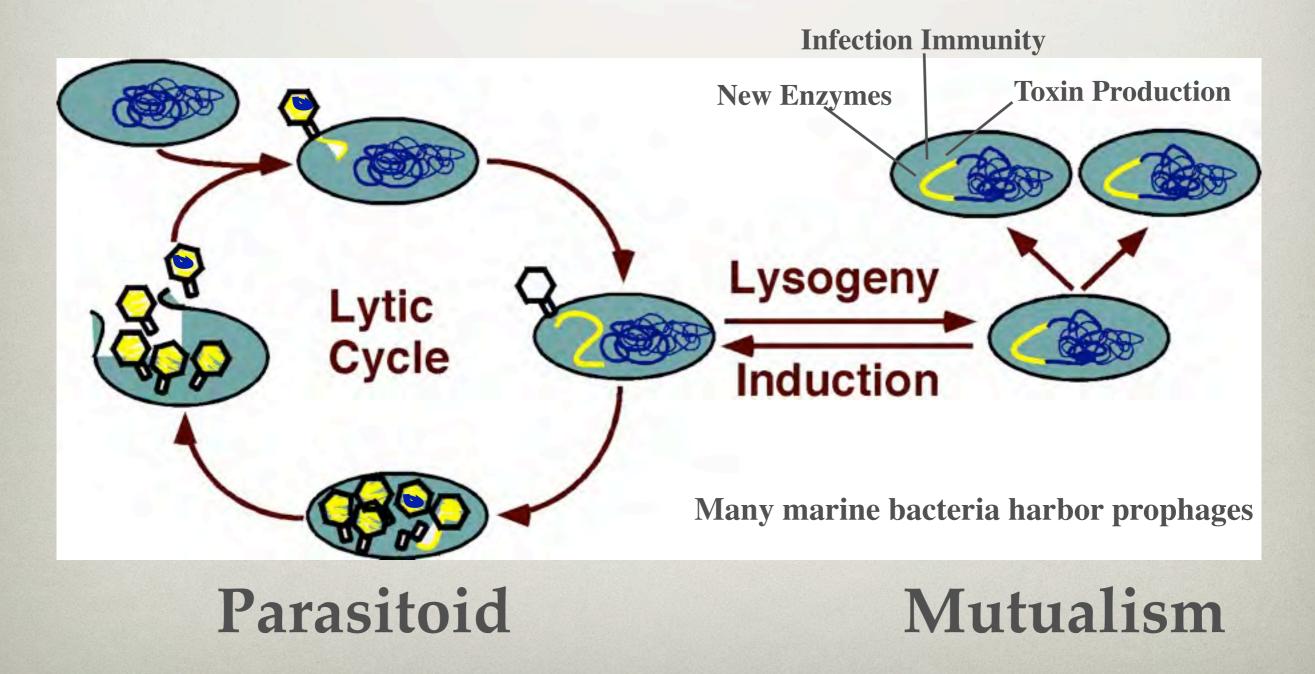
Transduction

Lysogenic Conversion



Transduction

Lysogenic Conversion



INFECTION CAN BE LETHAL AND UGLY



Friday, June 29, 2012

INFECTION CAN BE BEAUTIFUL



CAN VIRUSES MAKE US HUMAN?

Apparently, YES!

1 Image by Jane Ades, NHGRI

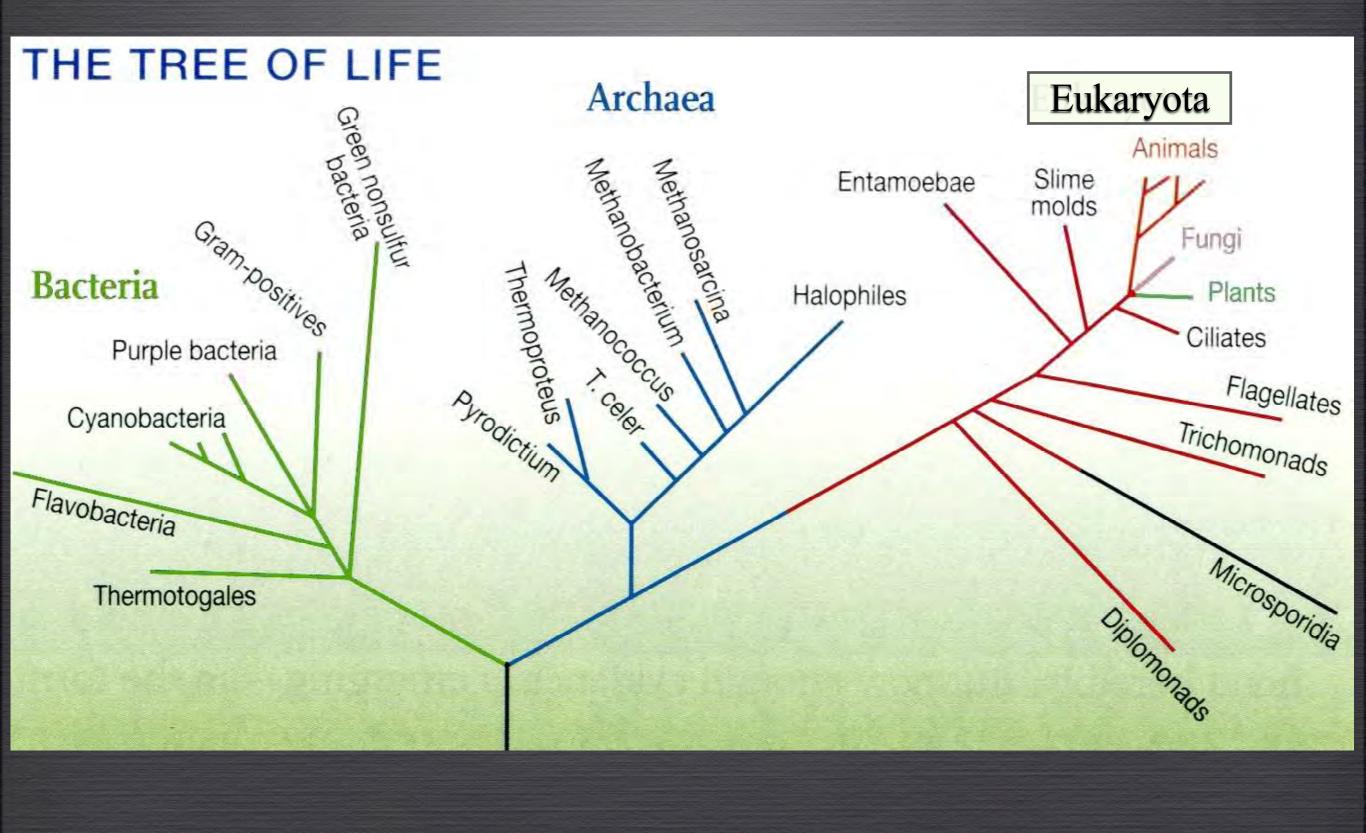
CAN VIRUSES MAKE US HUMAN?

essay by L.P. Villareal (2004) Proc. Am. Phil. Soc.

Apparently, YES!

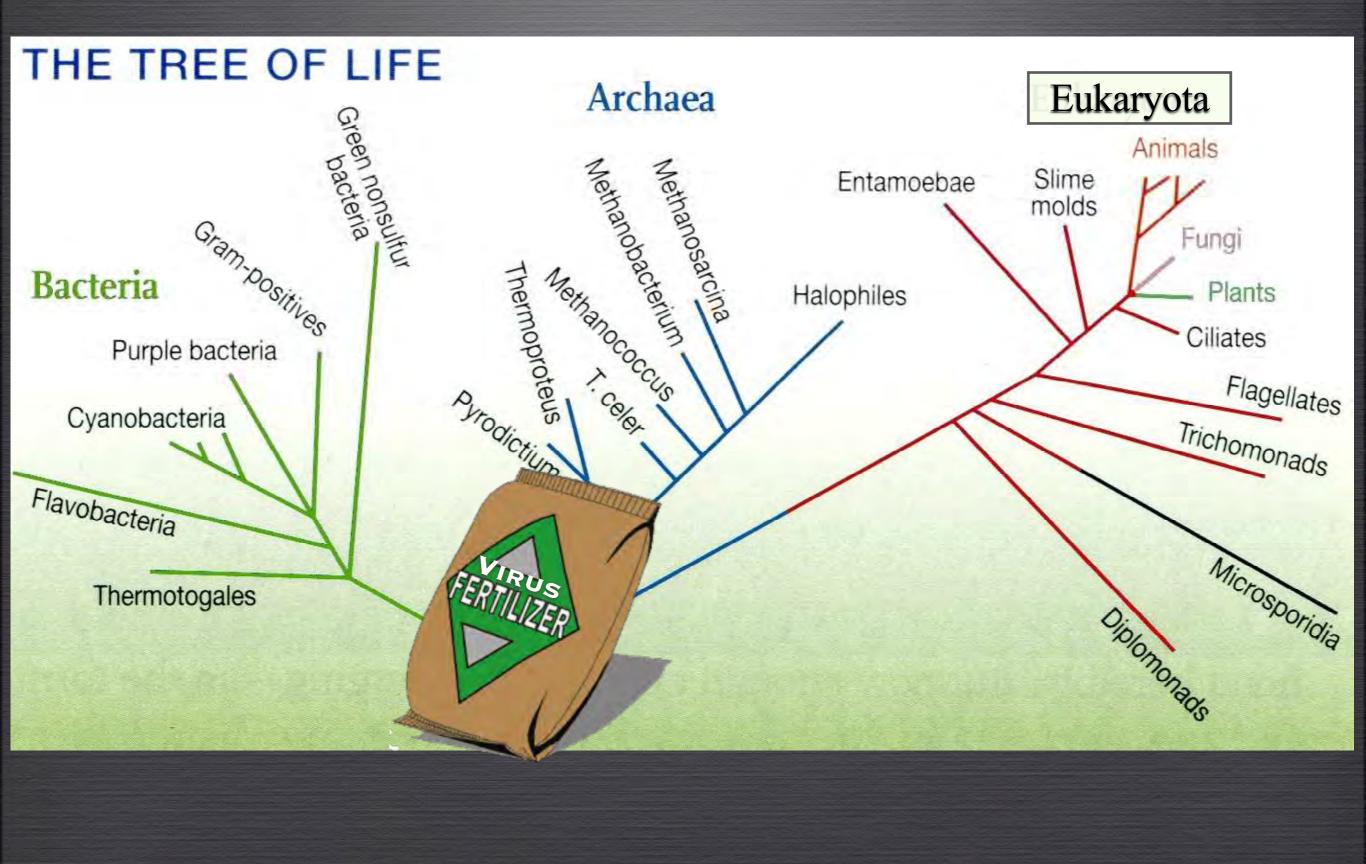
Image by Jane Ades, NHGRI

WHERE ARE THE VIRUSES?



Friday, June 29, 2012

WHERE ARE THE VIRUSES?



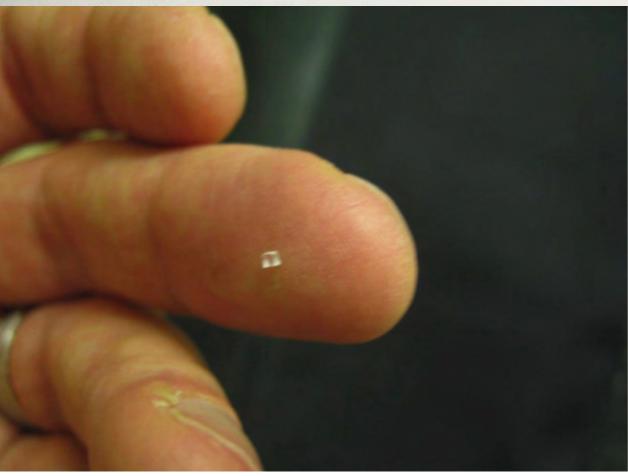
Friday, June 29, 2012

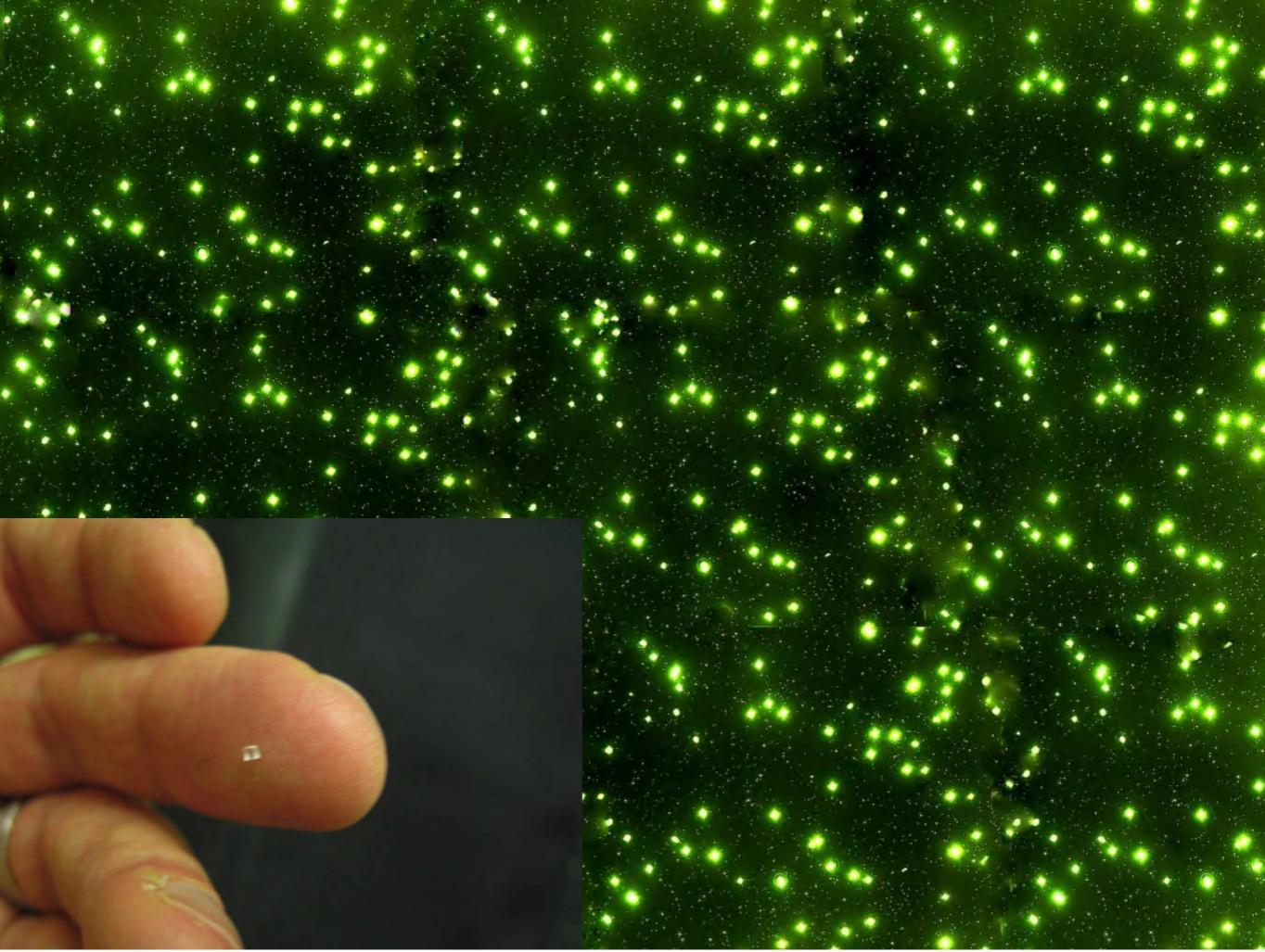
VIRUSES RULE THE SEA!!

- They are abundant
- Every living thing is subject to viral infections (from bacteria to whales)
- They affect biogeochemical cycles
- They drive evolution

VIRUSES DOMINATE THE PLANKTON

VIRUSES DOMINATE THE PLANKTON

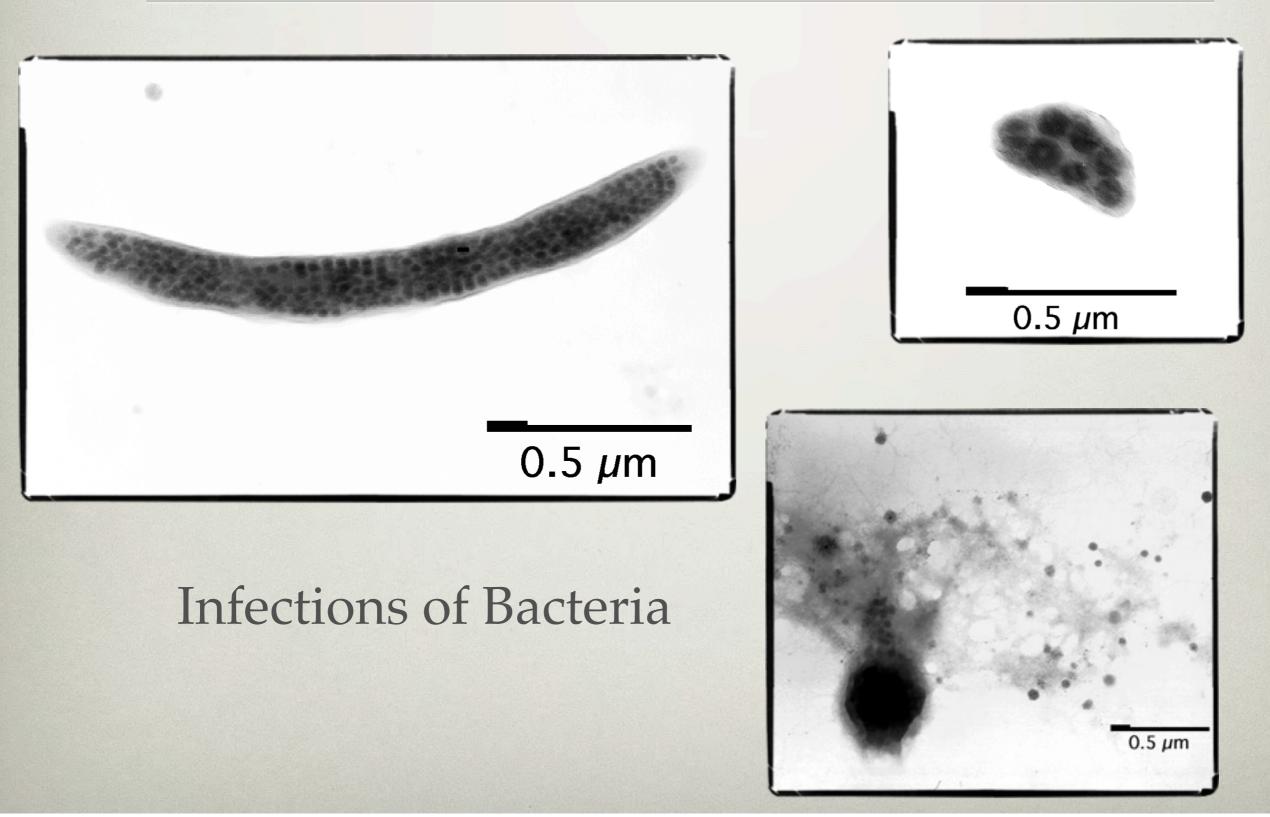




Viruses are relatively host specific

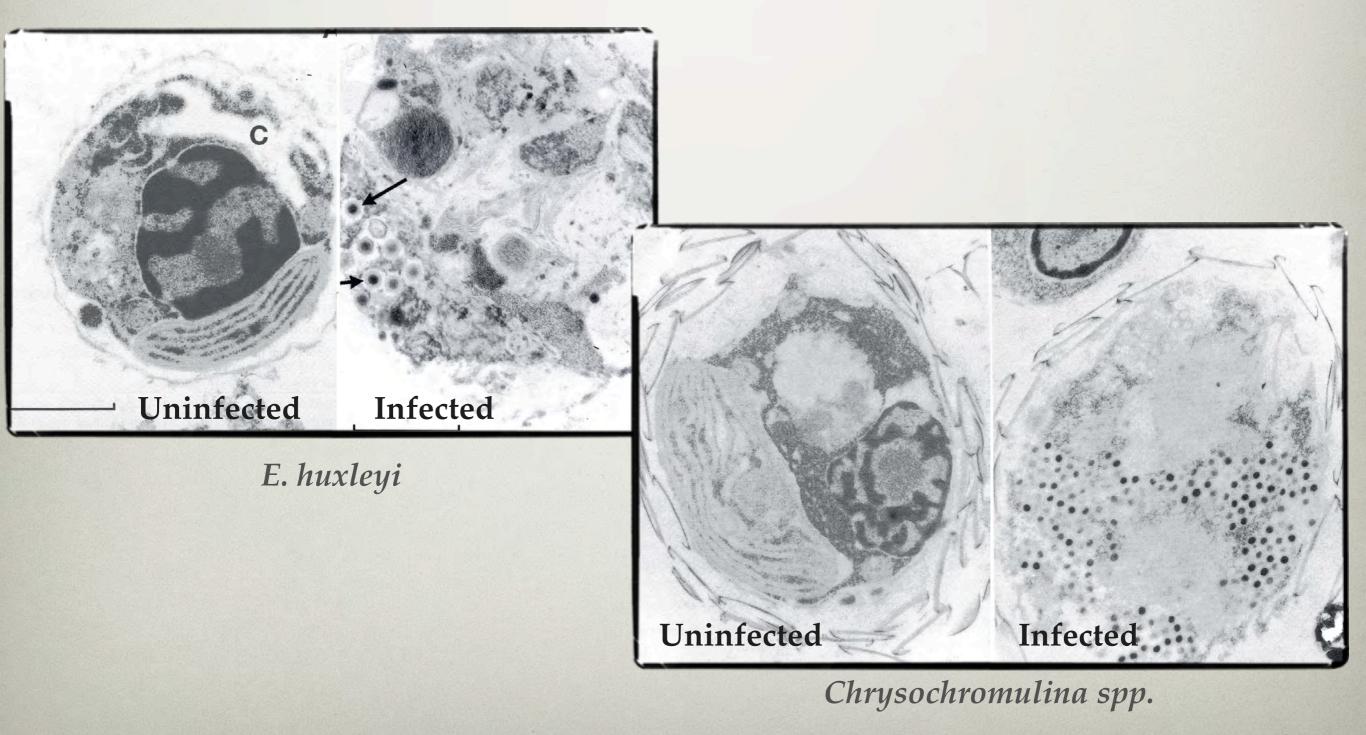
- Many viruses infect only one species or even only one strain within a species
- Some viruses have broad host range
- Occasionally, viruses can mutate and "jump" hosts

VIRUSES KILL PLANKTON



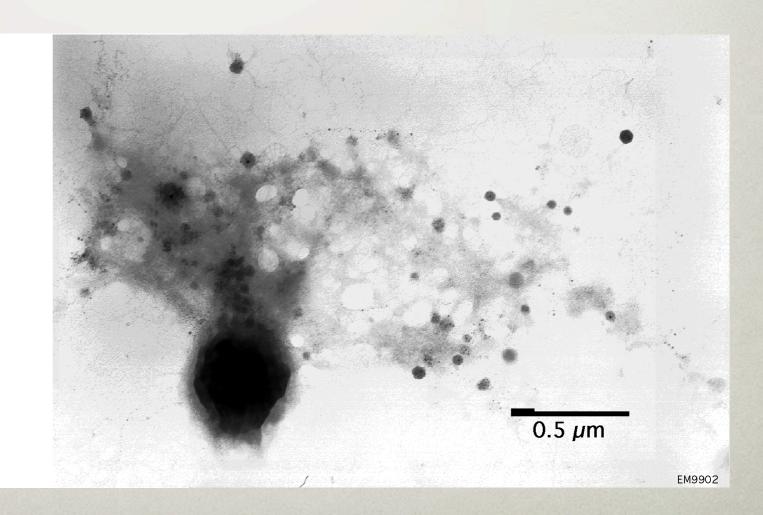
VIRUSES KILL PLANKTON

Infections of Protists



- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion

- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion

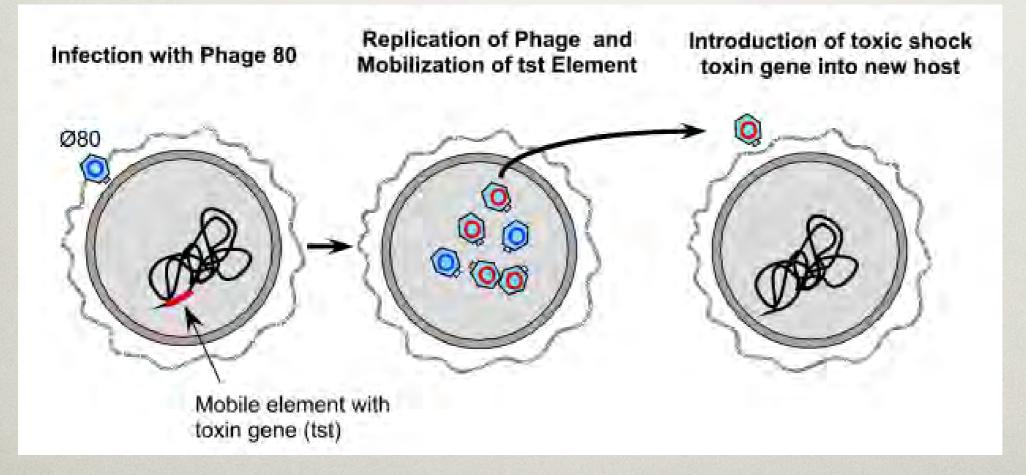




0.5 *µ*m

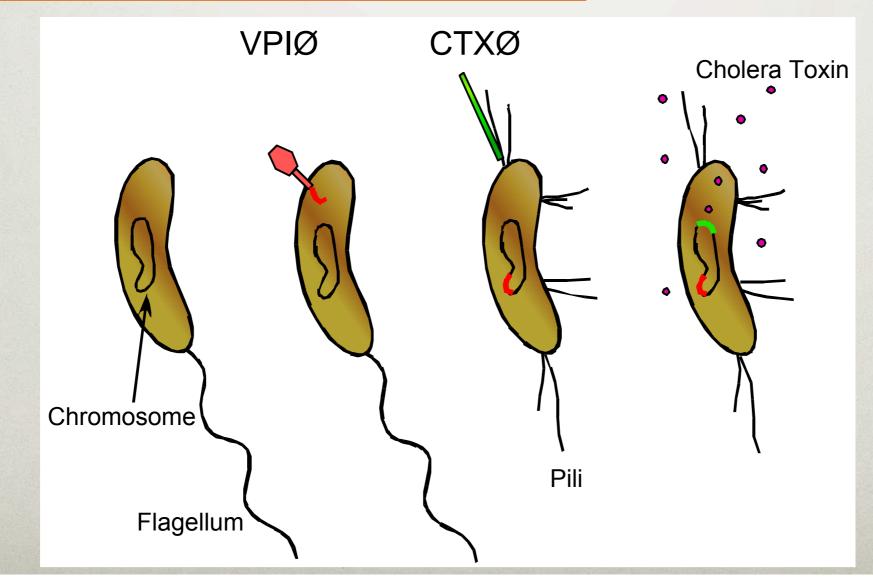
- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion

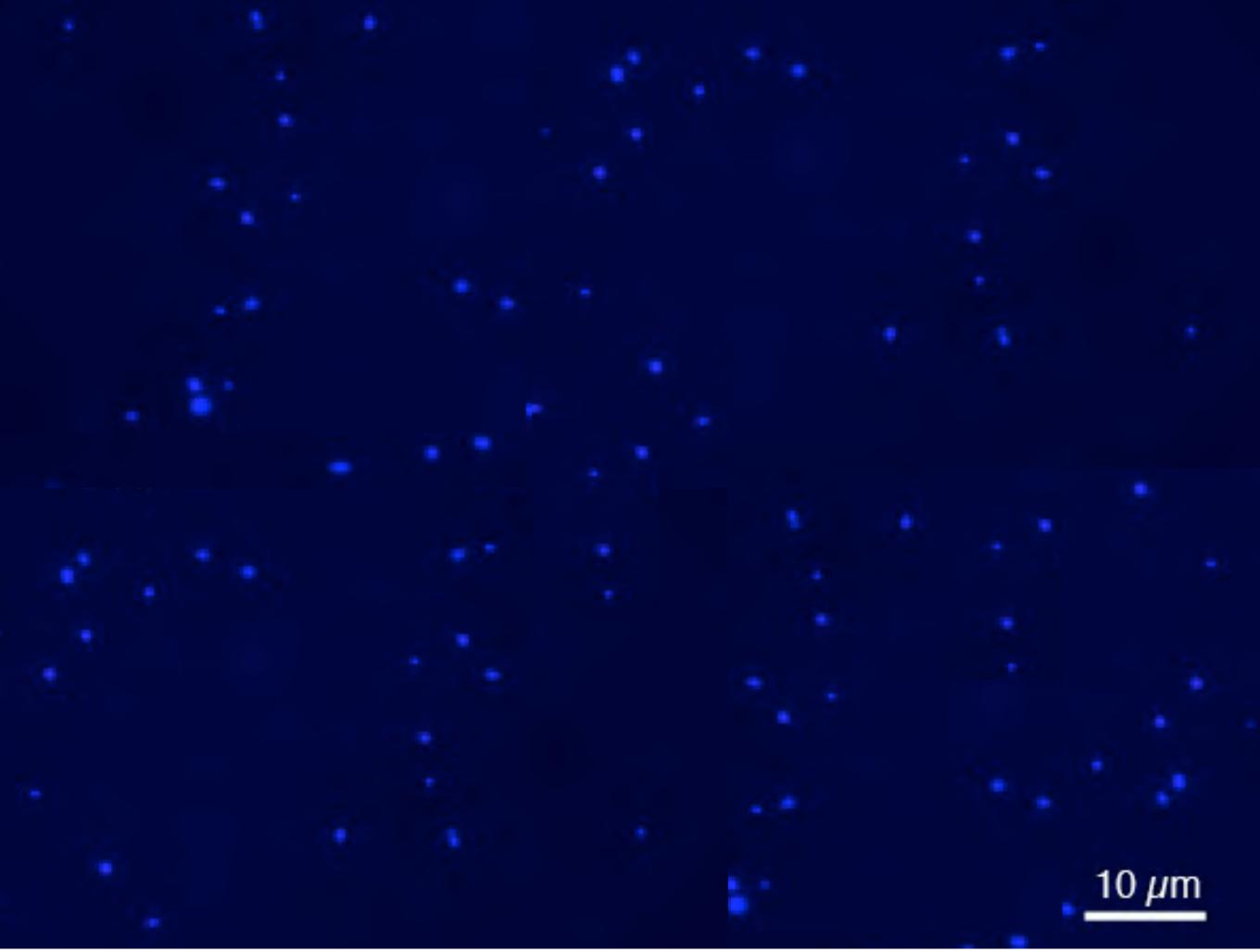
- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion

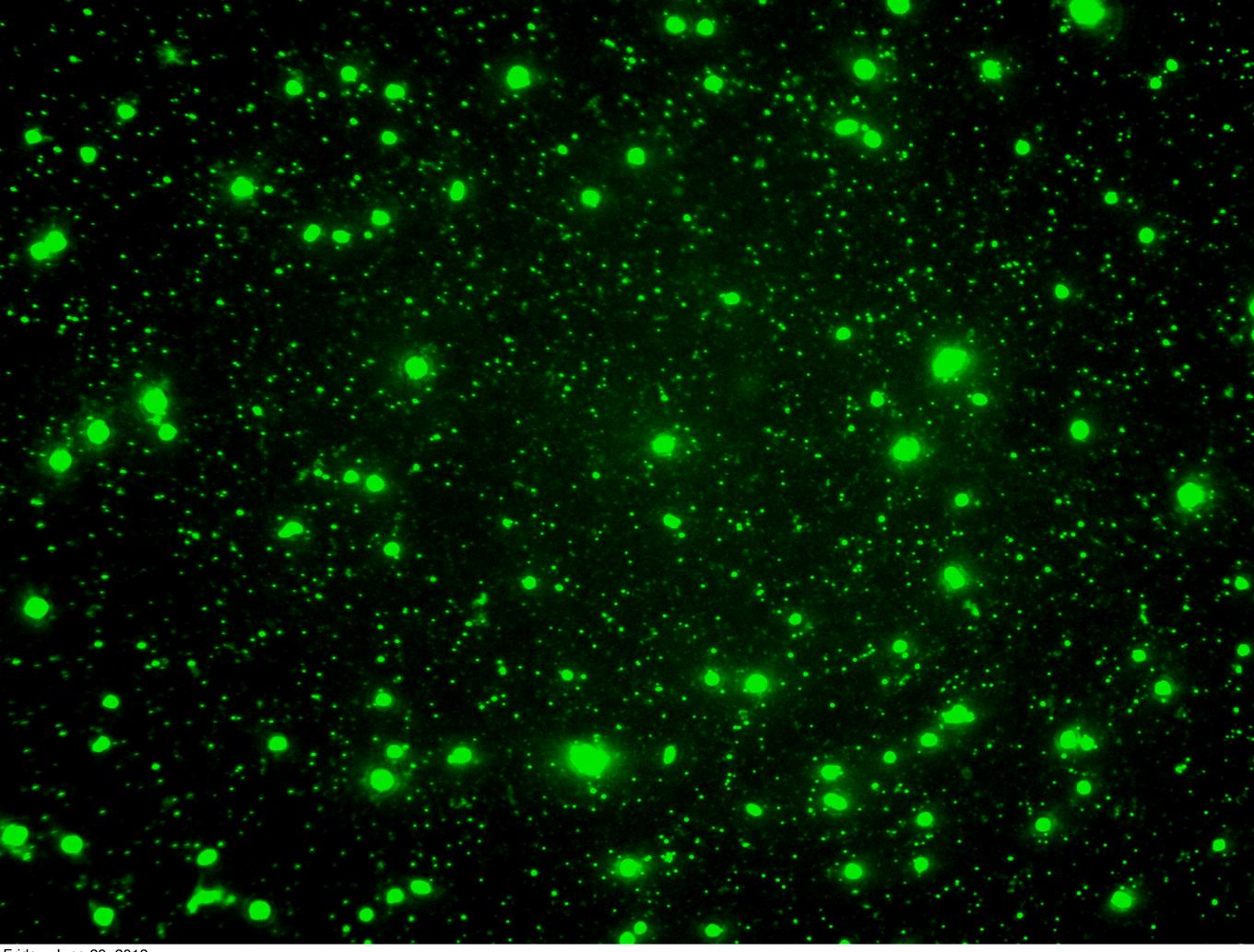


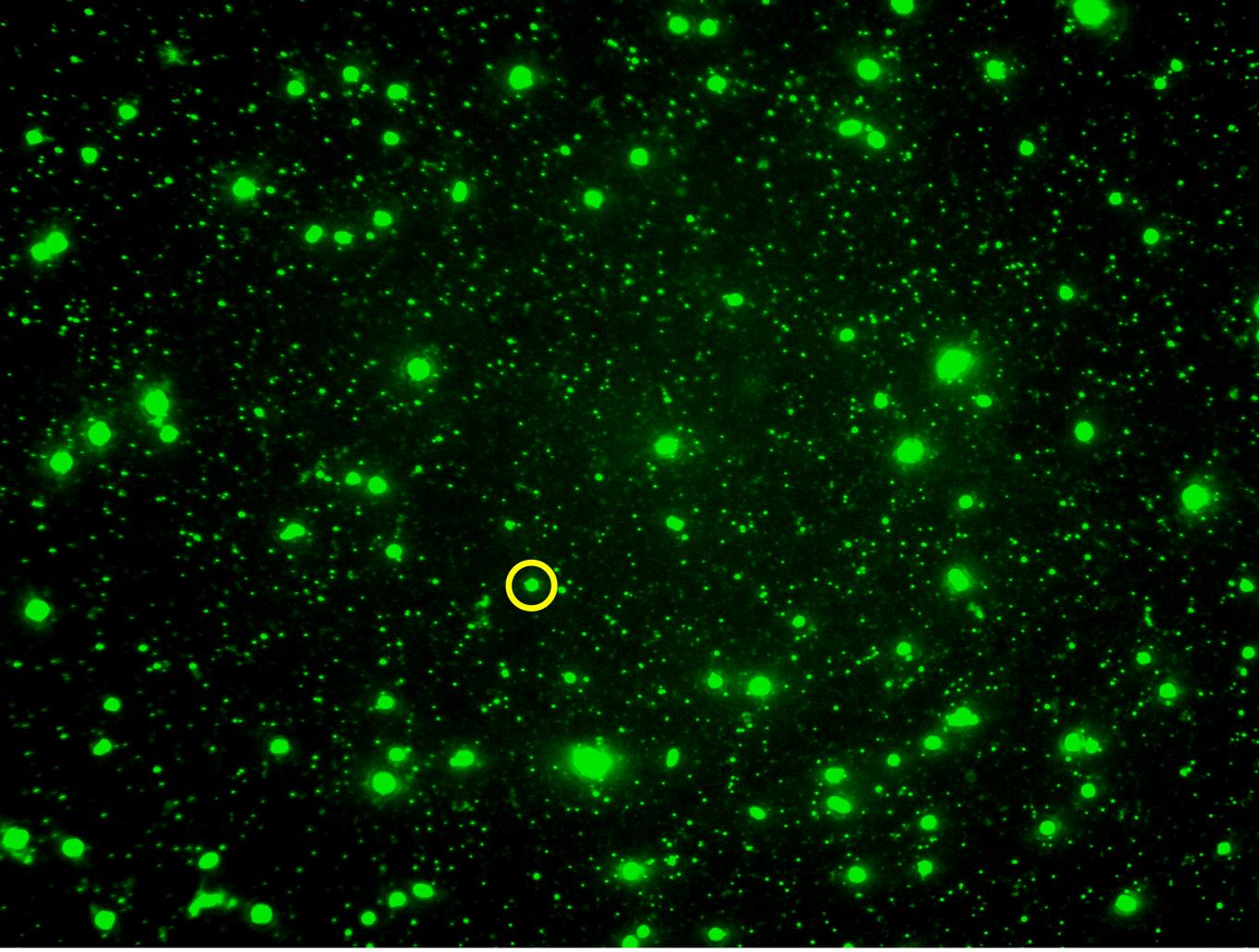
- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion

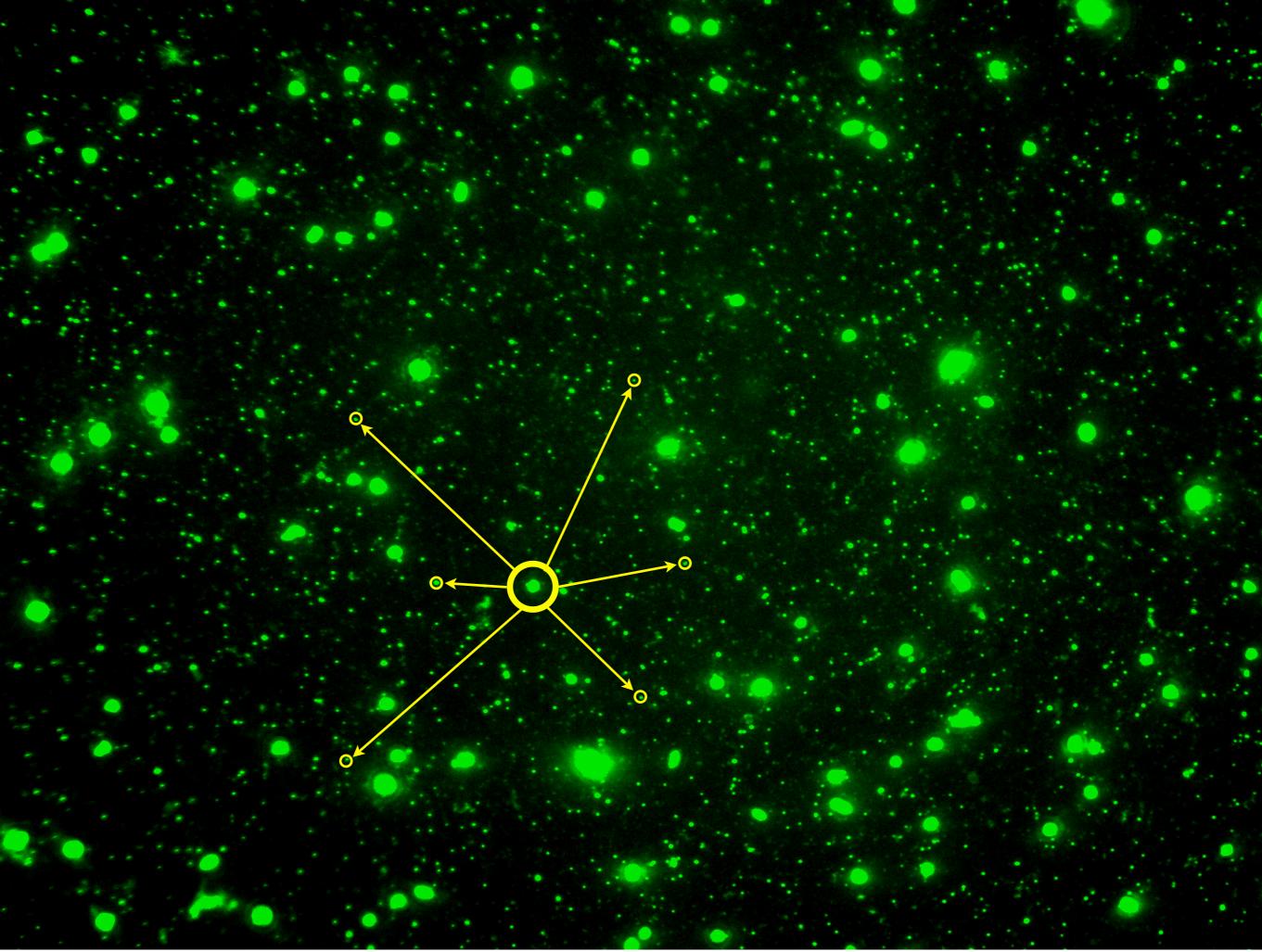
- Viruses make things HAPPEN!:
 - Mortality
 - Horizontal Gene Transfer
 - Lysogenic Conversion











>1 BILLION INFECTIONS PER DAY IN EACH CUBIC METER To Understand the ECOLOGY OF THE PLANKTON NEED TO KNOW THE "EXTENDED GENOTYPE"

> >1 BILLION INFECTIONS PER DAY IN EACH CUBIC METER

MARINE VIRAL ECOLOGY LAB (MARVEL)

Marine Virus Team



Jackie Mueller Chris Schvarcz Gordon Walker Teresa Porter **Alex Culley**

Vibrio Ecology Team



Olivia Nigro





La' Toya James Shaun Giancaterino

MARVEL PROJECTS

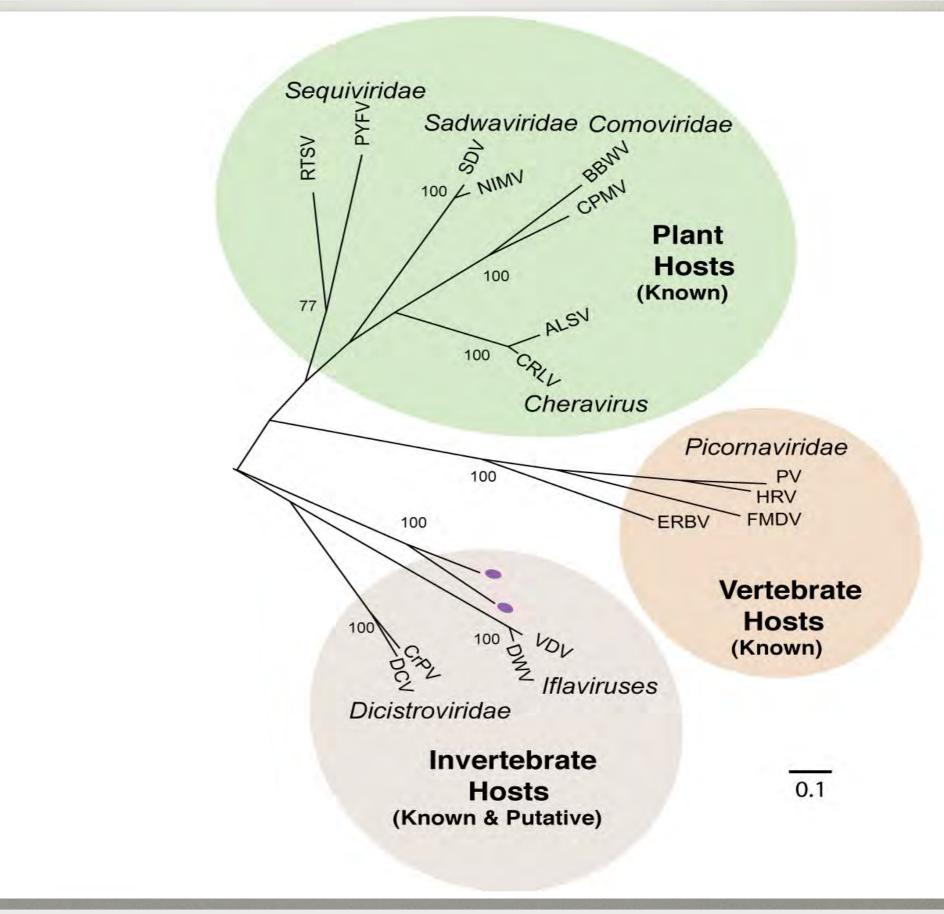
(selected)

- RNA viruses rising stars
- New host-virus systems a growing appreciation
- Physical Fractionation a new dimension

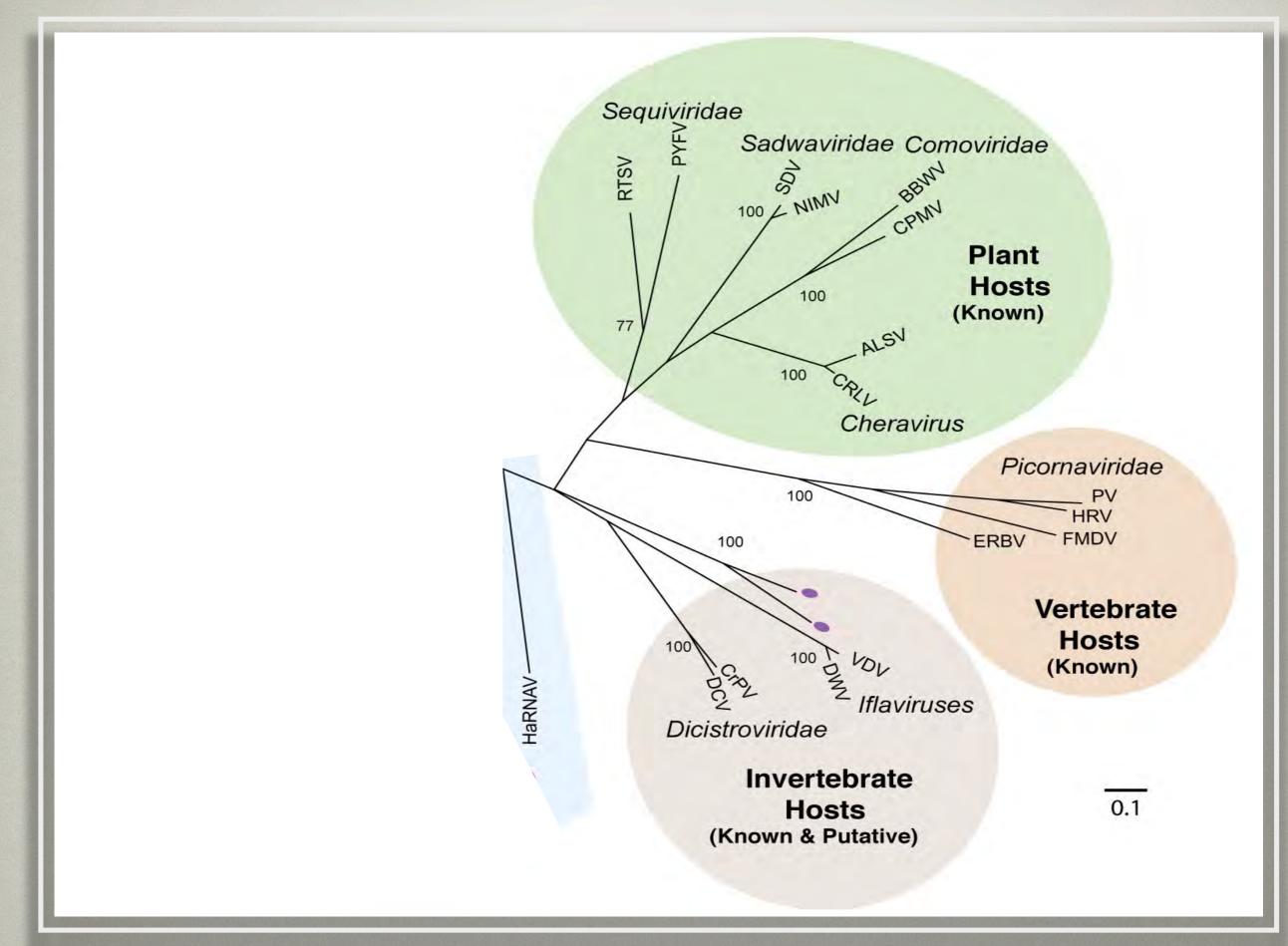
WHAT ABOUT RNA VIRUSES?

MOST WORK HAS BEEN DONE ON DNA-CONTAINING VIRUSES

- DNA easier to work with
- RNA viruses were assumed to be rare
 - assume most are bacteriophages
 - most phages contain DNA
- Metagenomic surveys seem to confirm this (95% of recognizable sequences are phage-like)

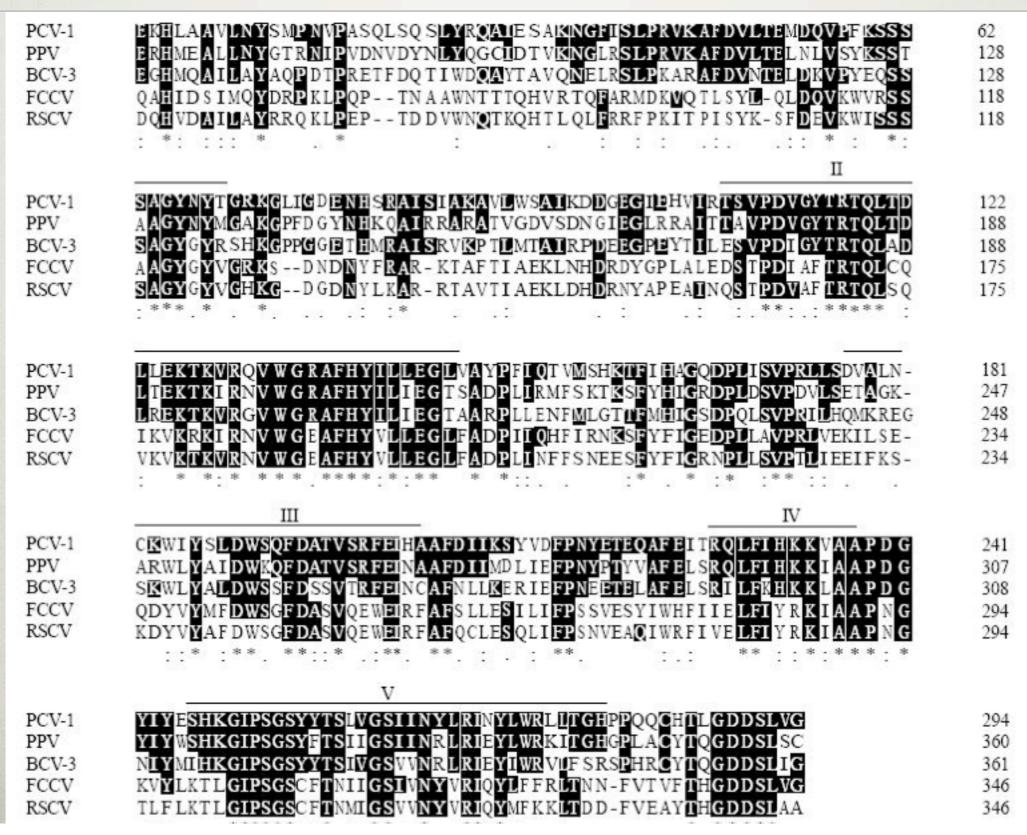


Tree of the known Picornavirales based on RdRP

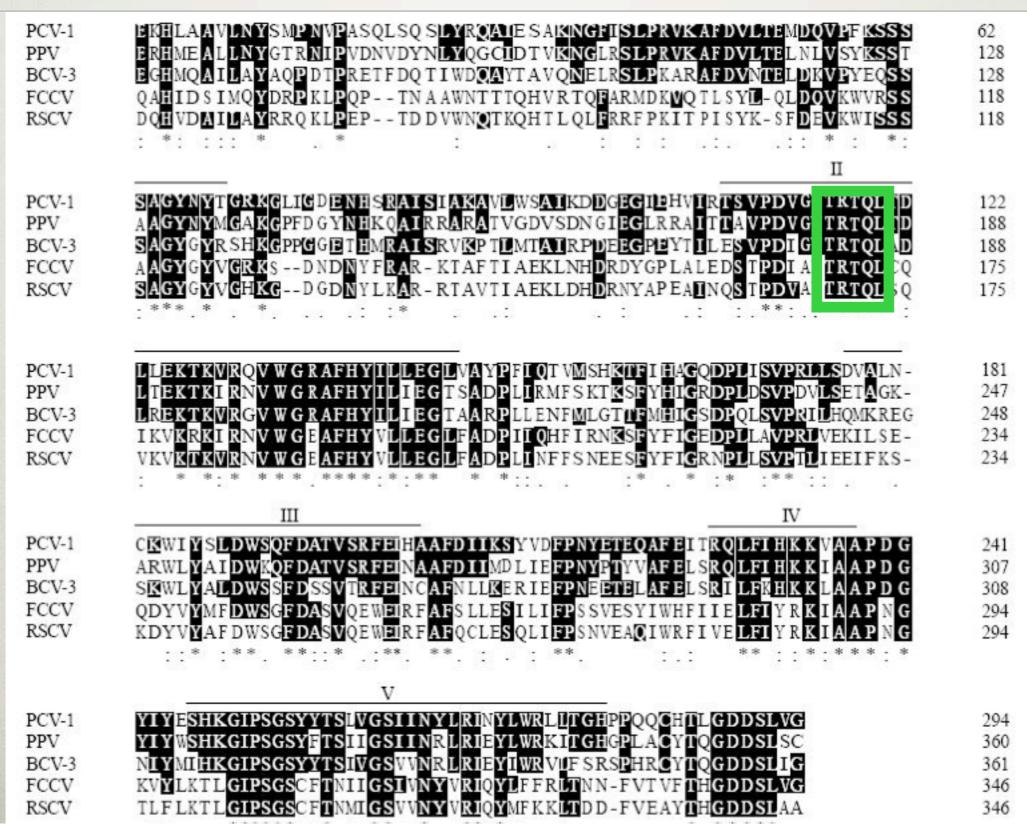


Tree of the known Picornavirales based on RdRP

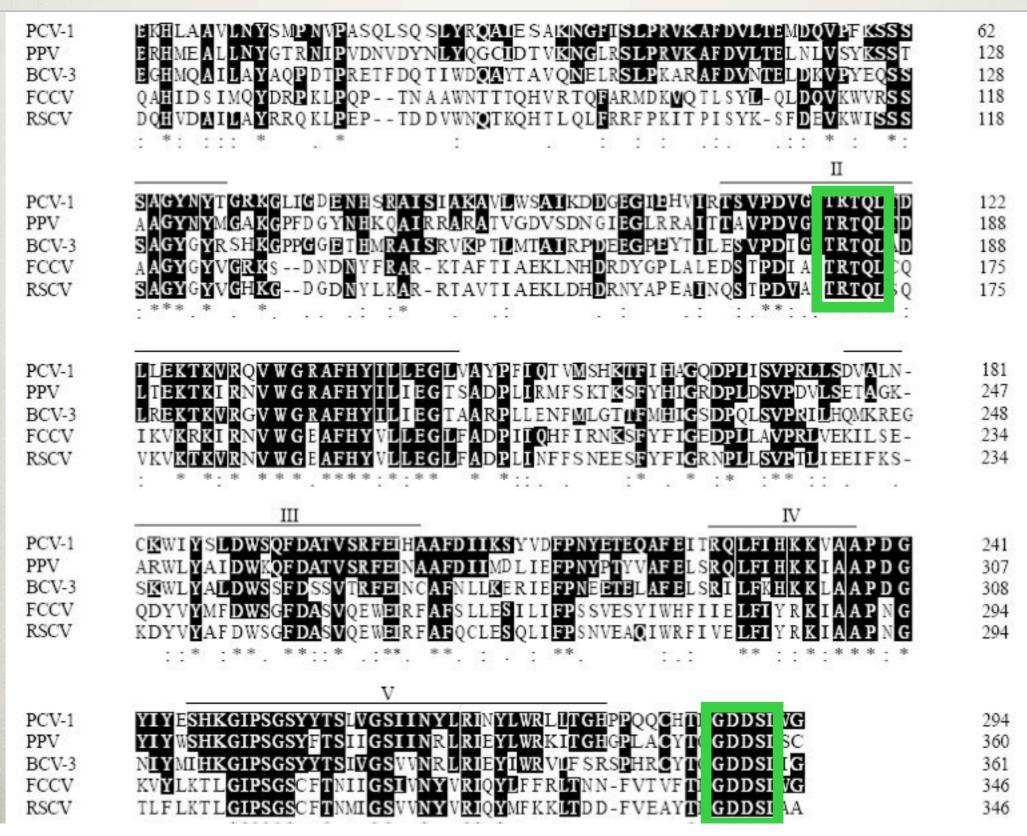
FIND CONSERVED REGIONS TO DESIGN PRIMERS

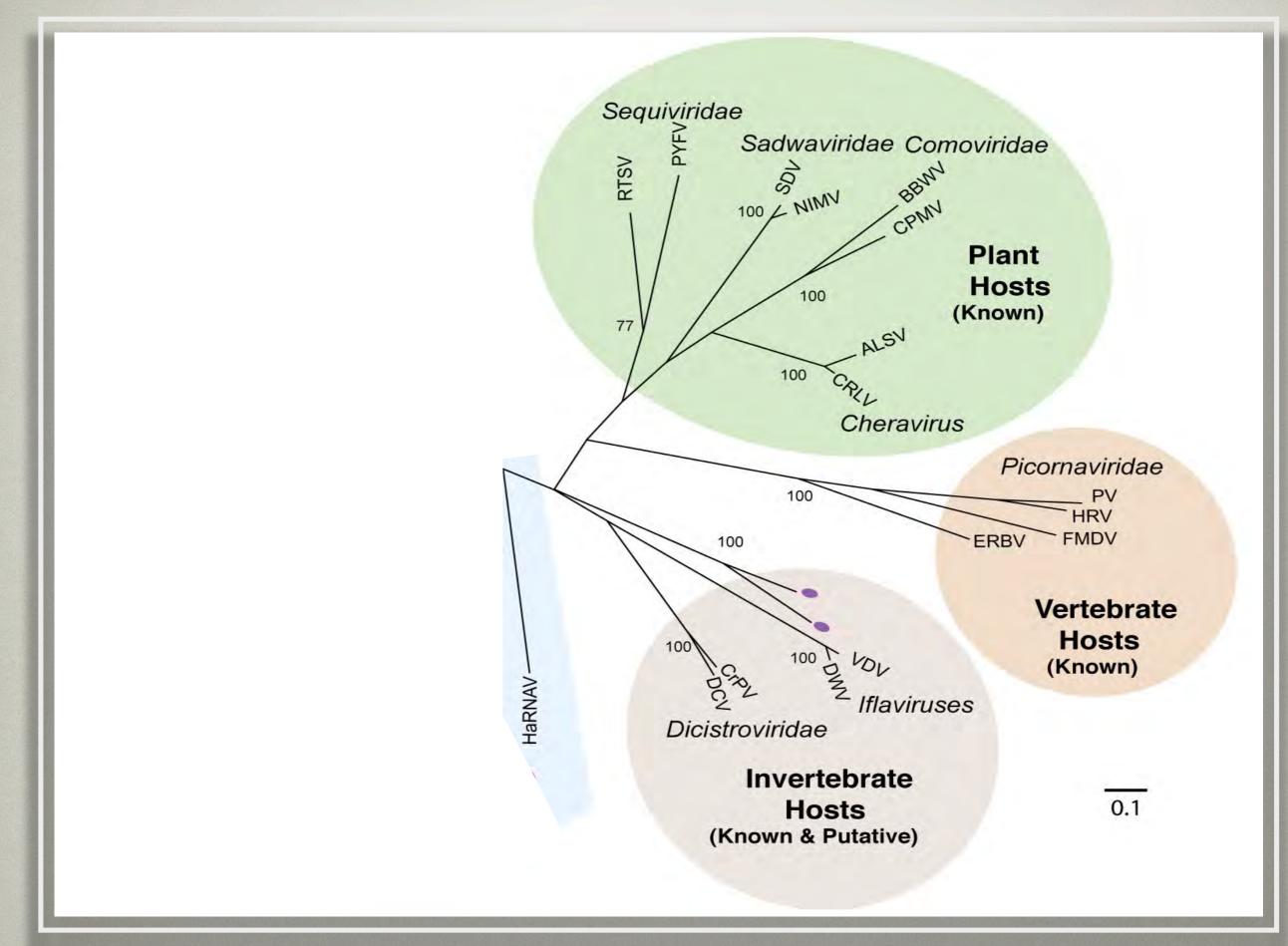


FIND CONSERVED REGIONS TO DESIGN PRIMERS

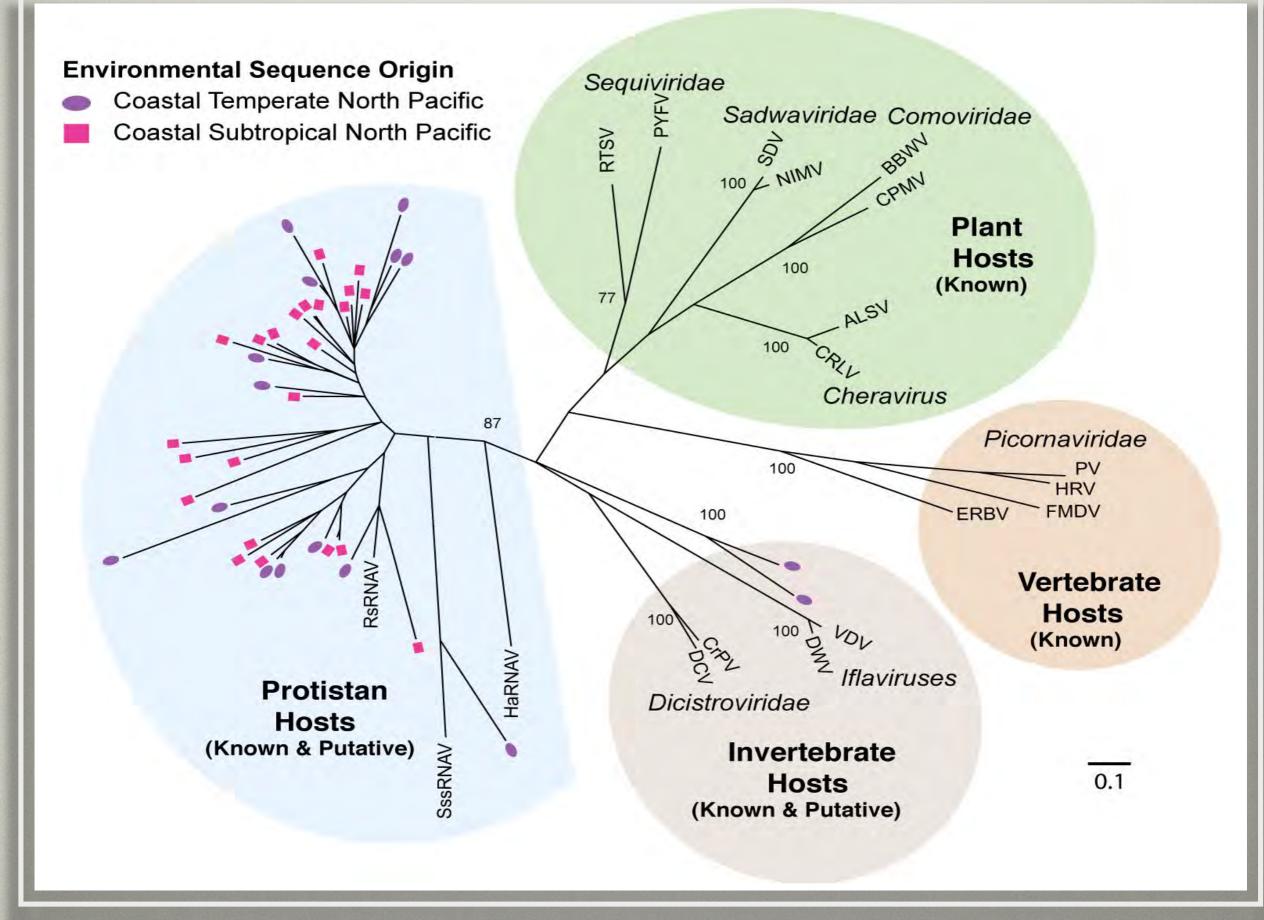


FIND CONSERVED REGIONS TO DESIGN PRIMERS





Tree of the known Picornavirales based on RdRP



Tree of the known Picornavirales based on RdRP

DO RNA VIRUSES MATTER?

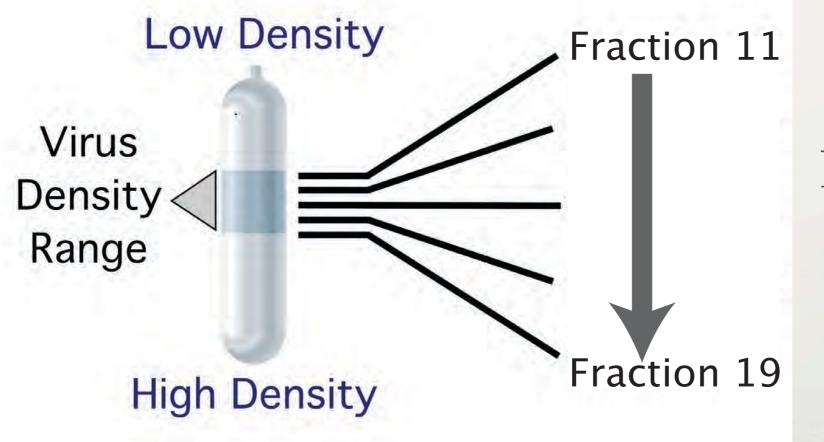
- Picorna-like viruses very common
- RNA viral metagenomes dominated by picornavirads and other eukaryote-infecting types
- No RNA-containing phages!

Are RNA viruses bit players or major stars in the pageant of the sea?

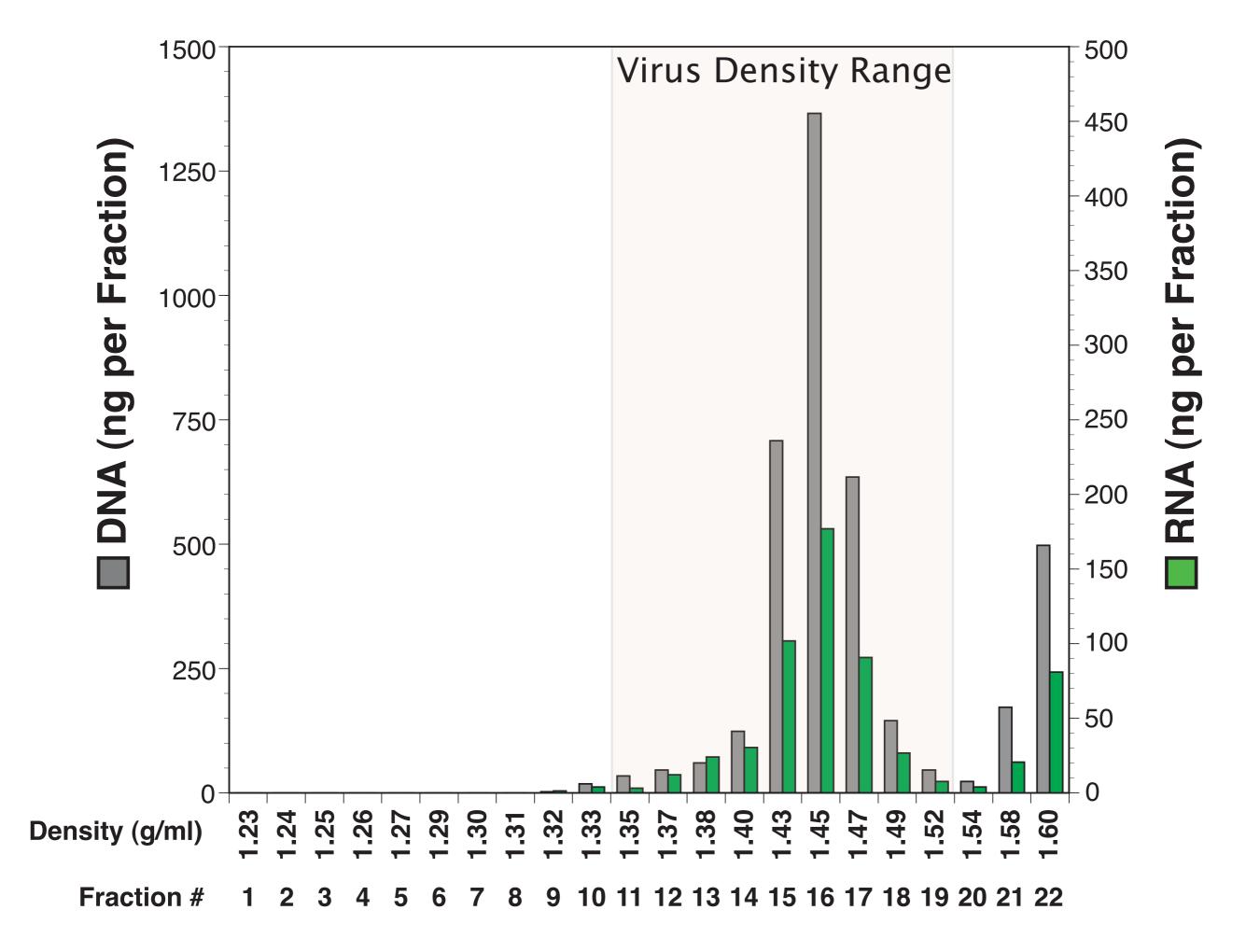
DO RNA VIRUSES MATTER?

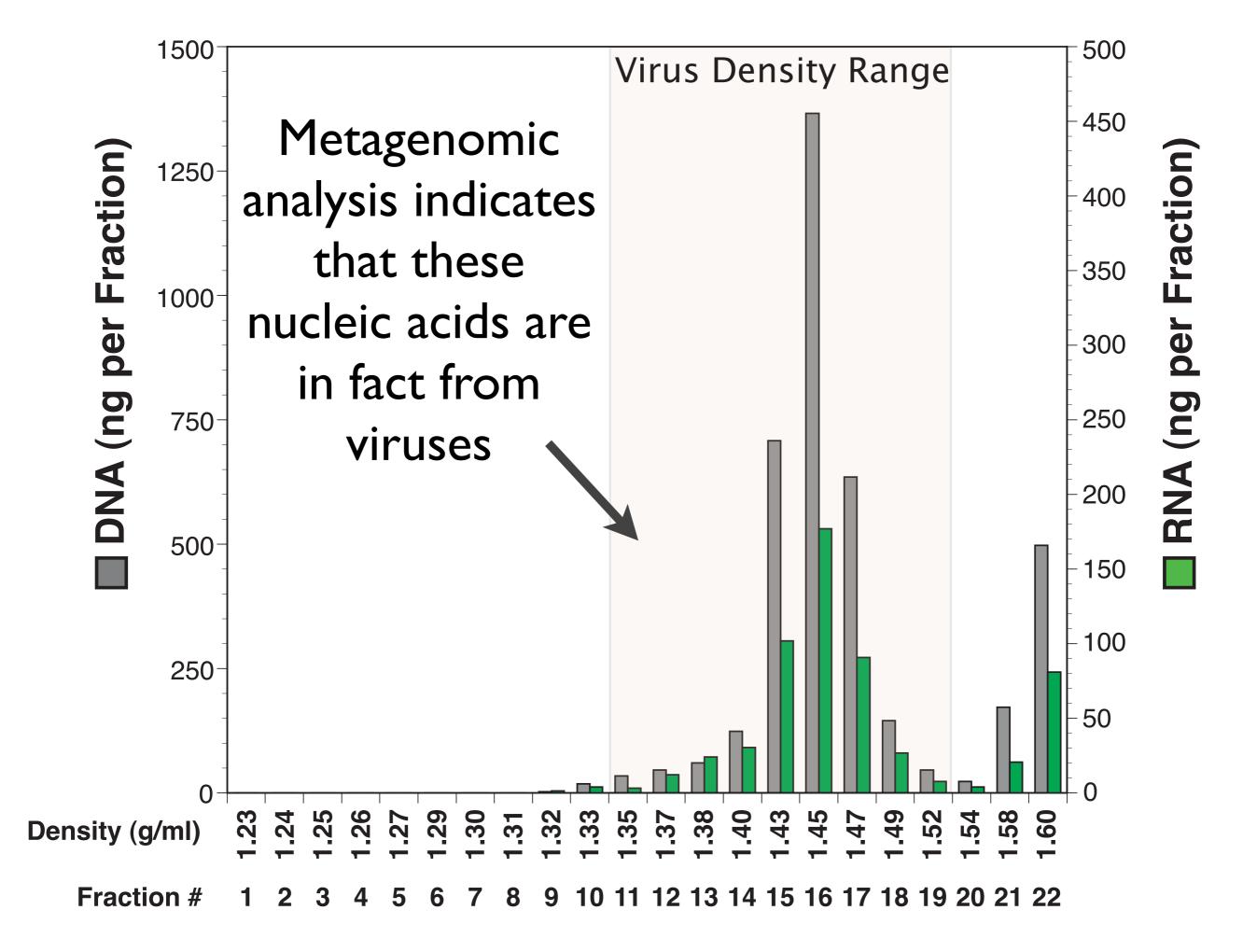
- Picorna-like viruses very common
- RNA viral metagenomes dominated by picornavirads and other eukaryote-infecting types
- No RNA-containing phages!

FRACTIONATION OF VIRUSES IN CsCl GRADIENT



Extract and Quantify DNA and RNA from each fraction





FROM NUCLEIC ACID MASS TO VIRAL ABUNDANCE

Assume:

RNA viruses are single-stranded and average 8 kb DNA viruses are double-stranded and average 50 kb

FROM NUCLEIC ACID MASS TO VIRAL ABUNDANCE

Assume:

RNA viruses are single-stranded and average 8 kb

DNA viruses are double-stranded and average 50 kb

	Genome Copies (billions per liter)					
	Date 01	Aug 2009	07 July 2010	08 July 2010		
RNA	Virus	2.98	0.33	0.28		
DNA	Virus	1.62	0.62	0.30	Average	
% RNA Vir	ruses	65%	35%	48%	49%	

FROM NUCLEIC ACID MASS TO VIRAL ABUNDANCE

Assume:

RNA viruses are single-stranded and average 8 kb

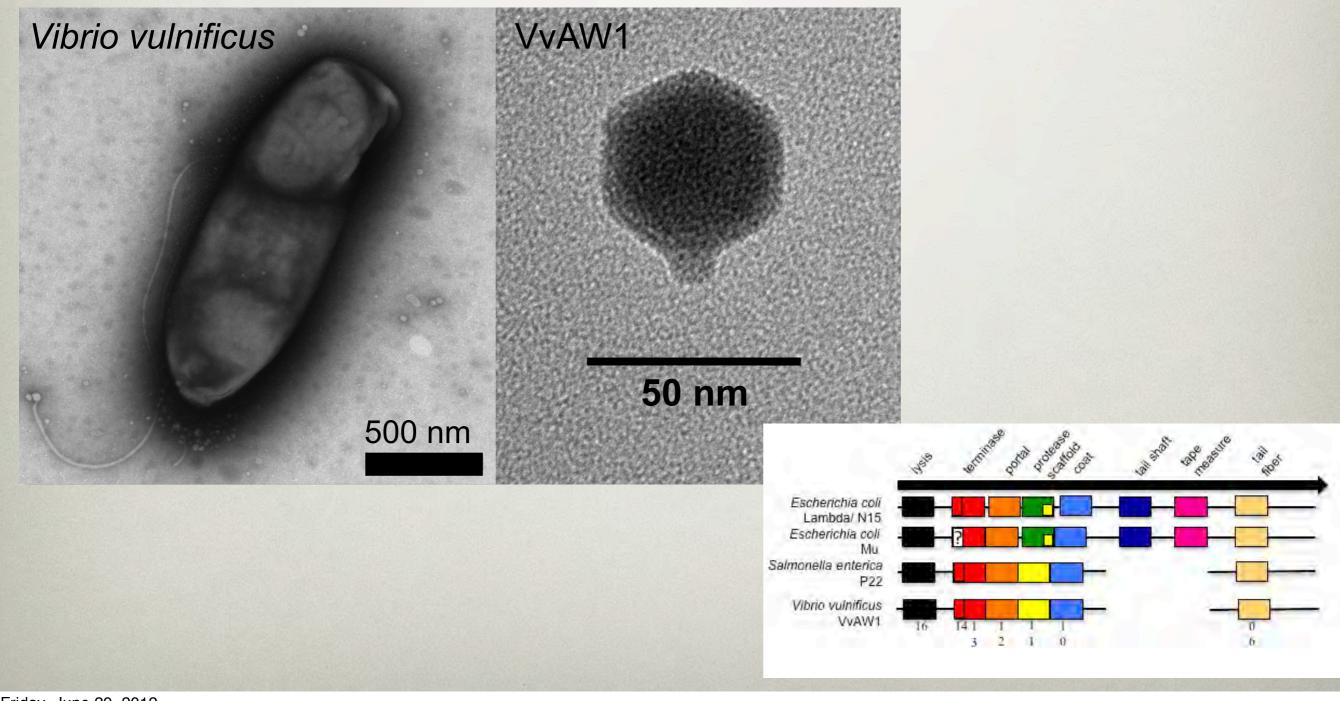
DNA viruses are double-stranded and average 50 kb

	Genome Copies (billions per liter)					
Date	01 Aug 2009	07 July 2010	08 July 2010			
RNA Virus	2.98	0.33	0.28			
DNA Virus	1.62	0.62	0.30	Average		
% RNA Viruses	65%	35%	48%	49%		

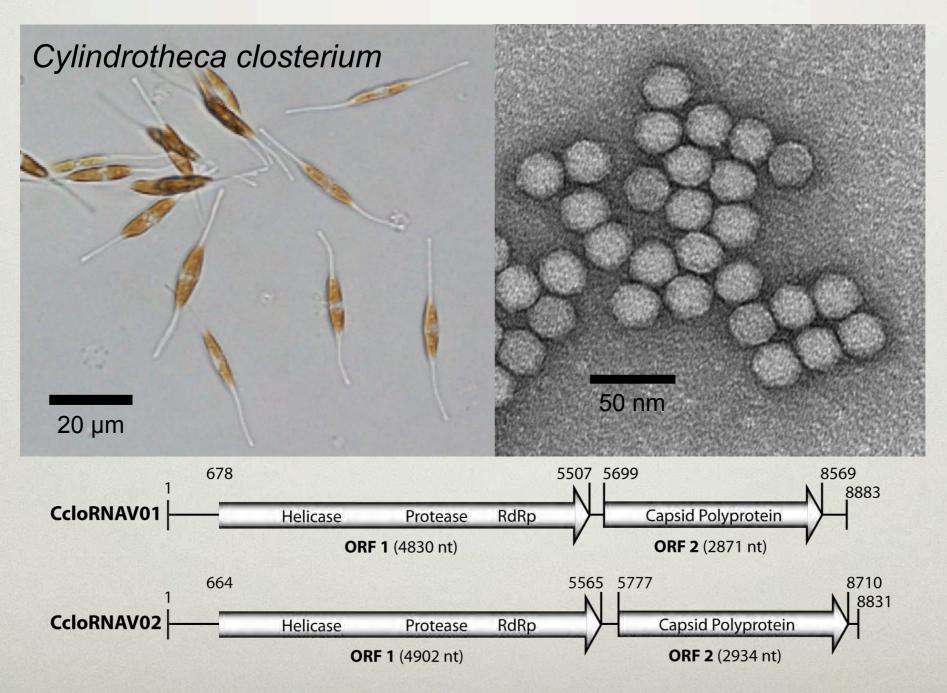
We have been missing HALF of the viruses in the sea

CULTIVATION OF VIRUS-HOST SYSTEMS

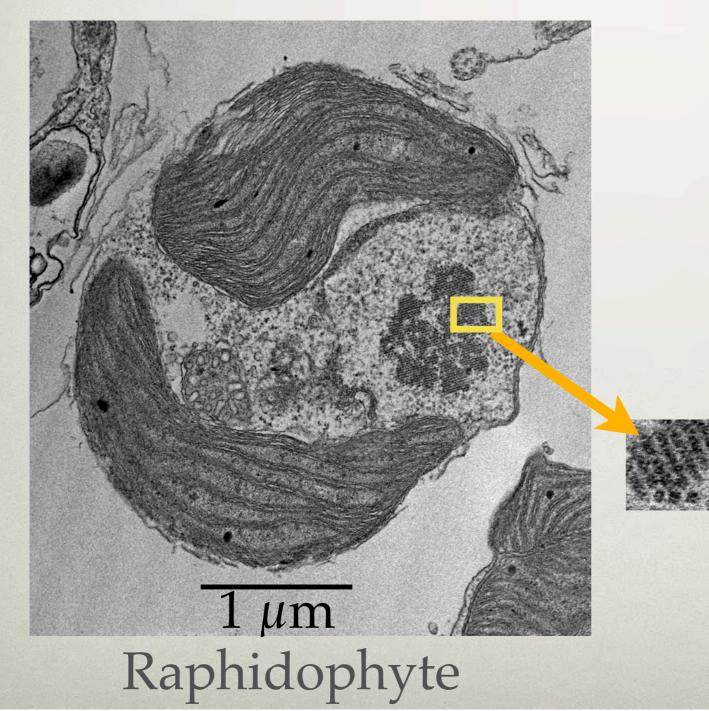
First genome of a pathogenic Vibrio vulnificus



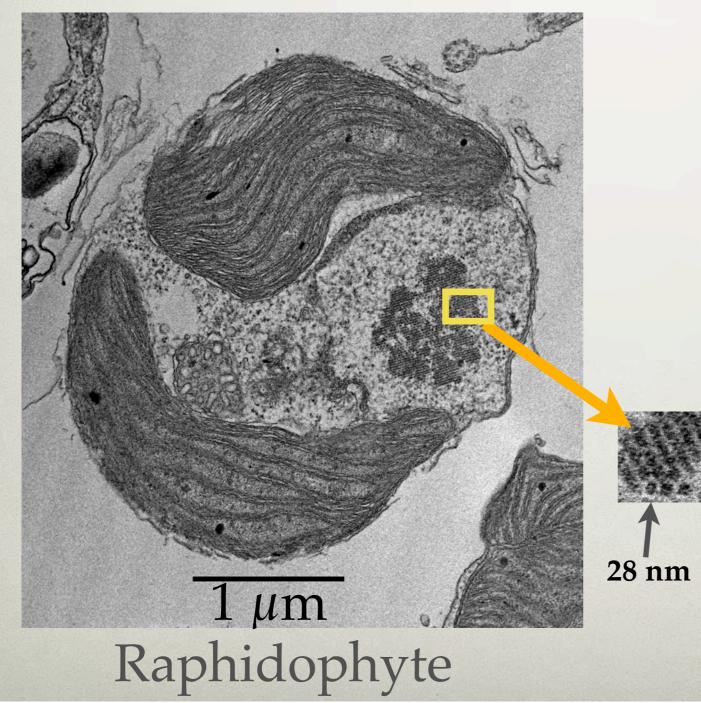
First genomes of viruses infecting pennate diatoms



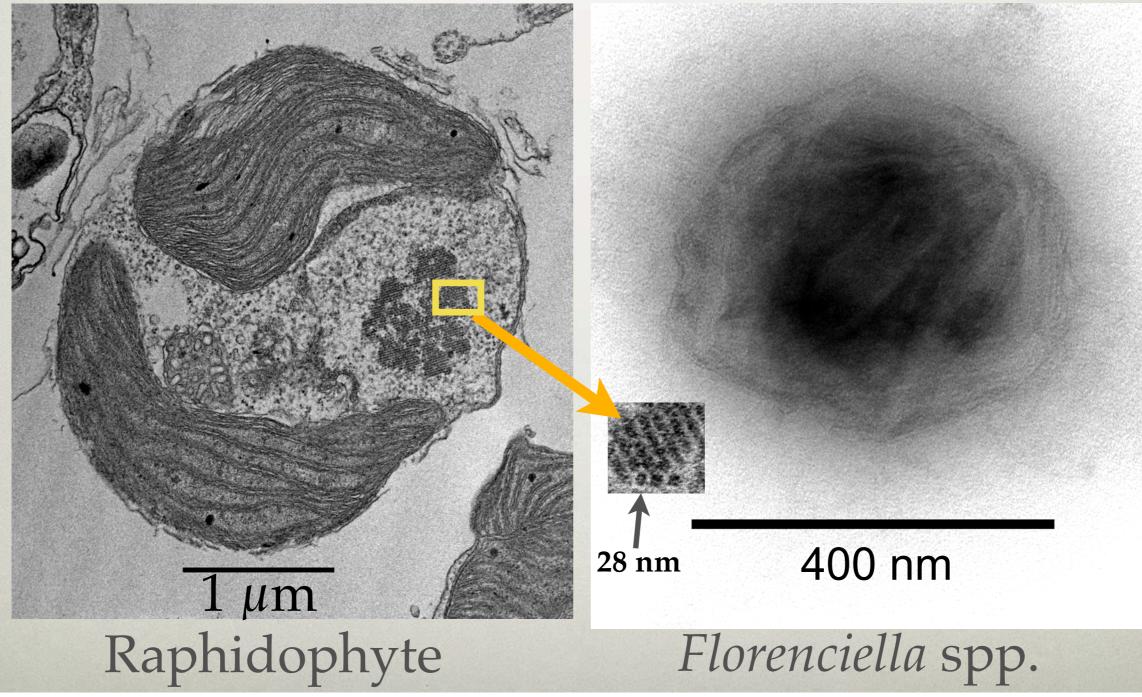
Smallest and largest viruses yet reported for marine phytoplankton



Smallest and largest viruses yet reported for marine phytoplankton



Smallest and largest viruses yet reported for marine phytoplankton



ACKNOWLEDGMENTS

- CMORE
- National Science Foundation
- Sea Grant
- The MarVEL Personnel

Pac

Pacific Islands Ocean Observing System

OS

center for microbial oceanography: research and education

