Viruses of Freshwater Mussels

expanding the invertebrate "virosphere" in the pursuit of mussel health and conservation

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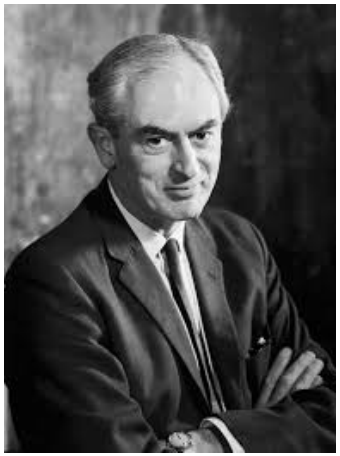
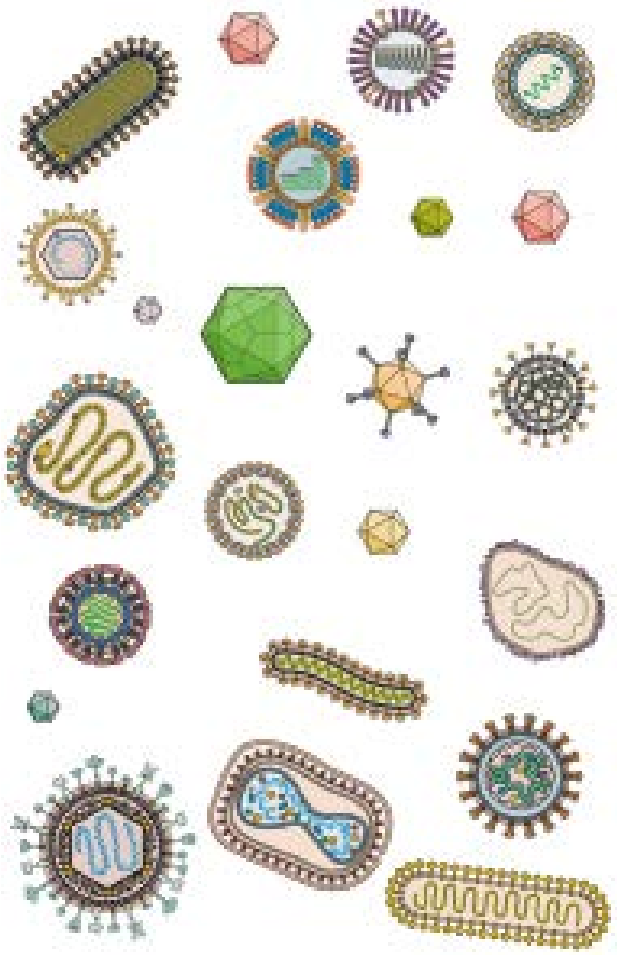
Viruses

- “A piece of bad news wrapped up in a protein"



- Peter Medawar
  (1960 Nobel Prize for work on immunity and organ transplantation)



Viruses

- Annoyingly small
  - Hard to see/diagnose/confirm
- Obnoxiously diverse
  - No unifying genetic features
- Irritatingly clever
  - Evolve around vaccines and antivirals
- Maddeningly emergent
  - Ebola, SARS, West Nile, Zika, HIV, etc.

Viruses of bivalves

Risk in Brief

Hepatitis A Virus in Shellfish

Centre for Food Safety, Hong Kong

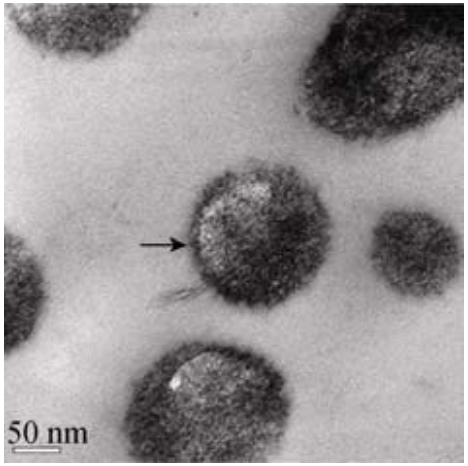
Oyster herpes

- OsHV-1 mVar
  - *Malacoherpesviridae*; *Ostreavirus*
- Massive mortality in *Crassostrea gigas* since 1998
- France → Portugal → Spain → Italy → Ireland →Norway → Australia → New Zealand →Asia
- No vaccines or treatments
- Epidemiologically informed management is the current best strategy.

Pernet, F., C. Lupo, C. Bacher and R. J. Whittington (2016). Infectious diseases in oyster aquaculture require a new integrated approach. *Philos Trans R Soc Lond B Biol Sci* 371(1689).

Viruses of Unionids: $n = 1$

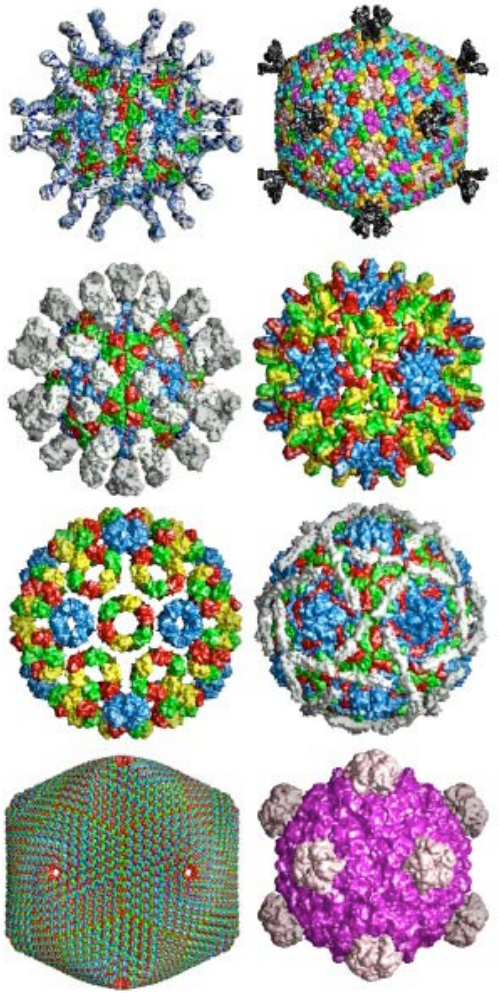
- Lea plague Virus (HcPV) in *Hyriopsis cumingii* (triangleshell)
- *Arenaviridae* (very distant relative of Lassa virus)
- "Explosive" epidemics in China, in freshwater pearl aquaculture facilities



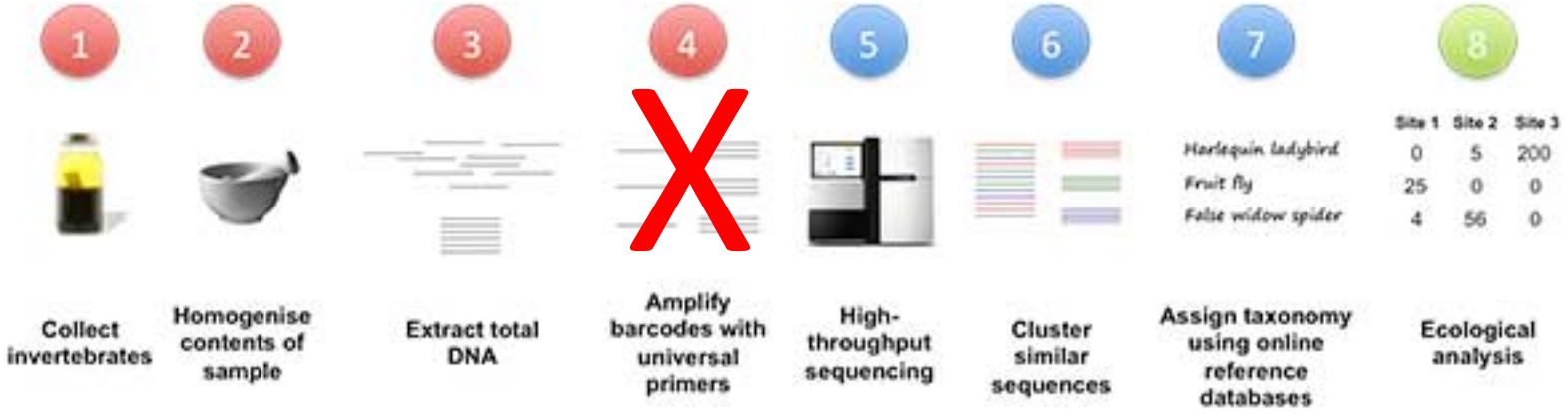
Zhong et al. (2011). *Acta Hydrobiologica Sinica* 35(4): 666-671.

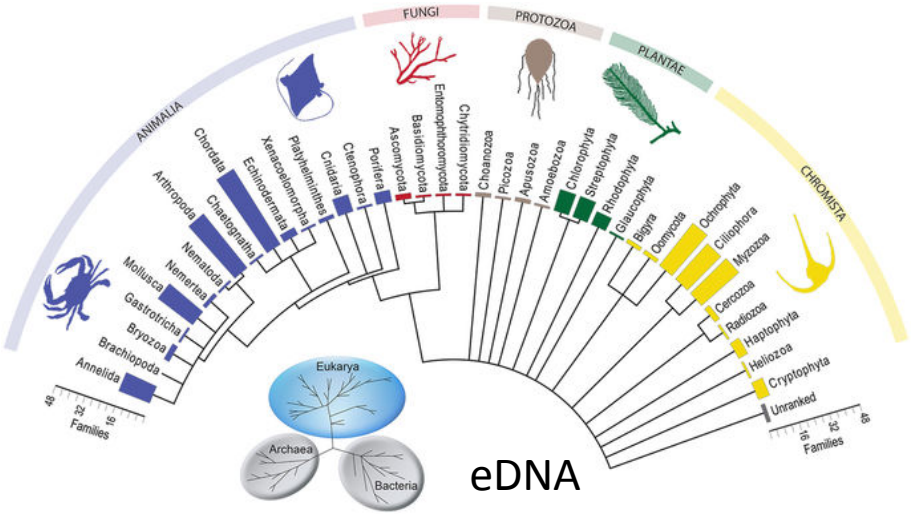
Next-Generation Sequencing (NGS)

Magelhaens Schiff „Vittoria". Nach einem alten Stich.



Metabarcoding

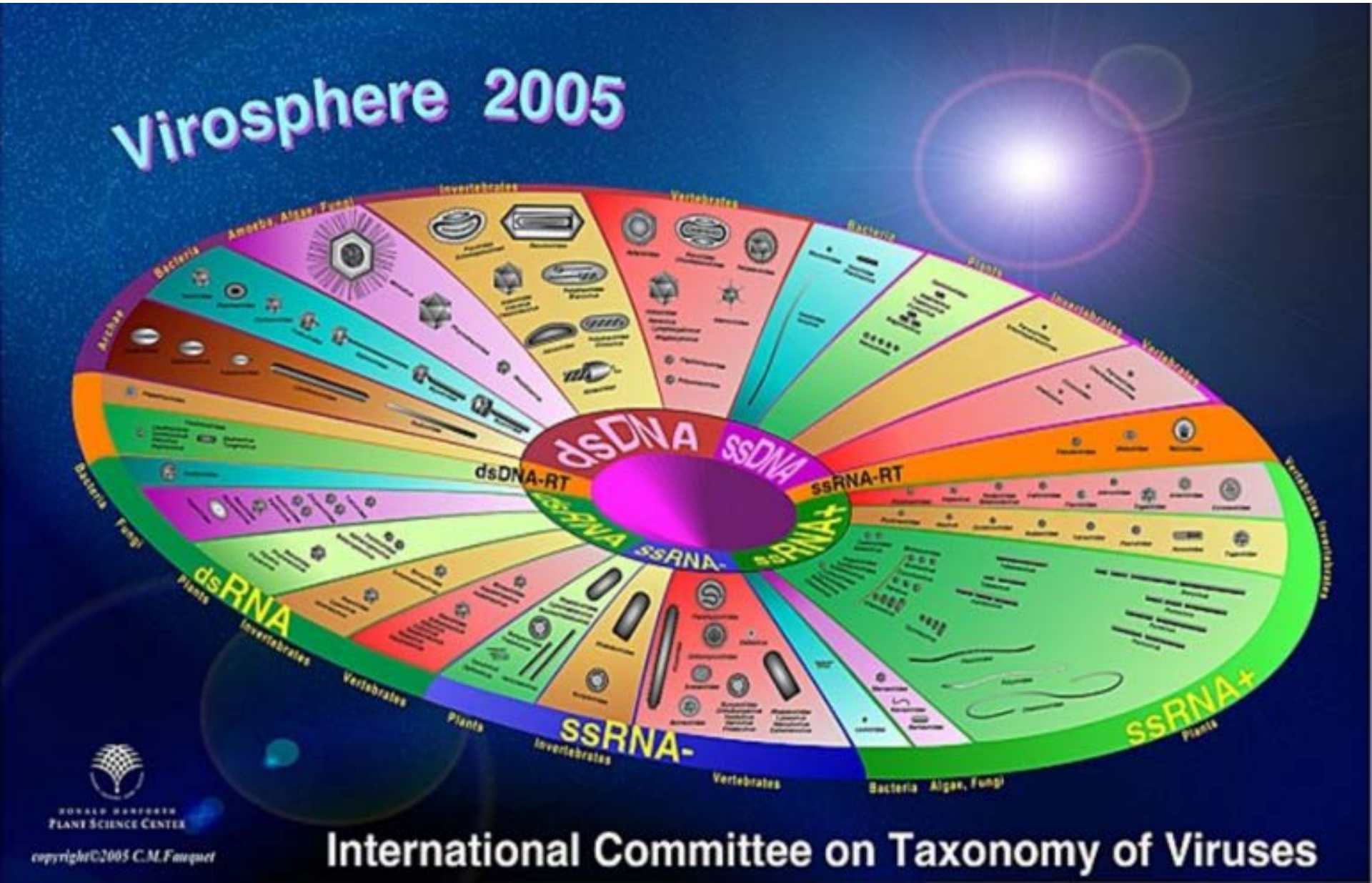




eDNA

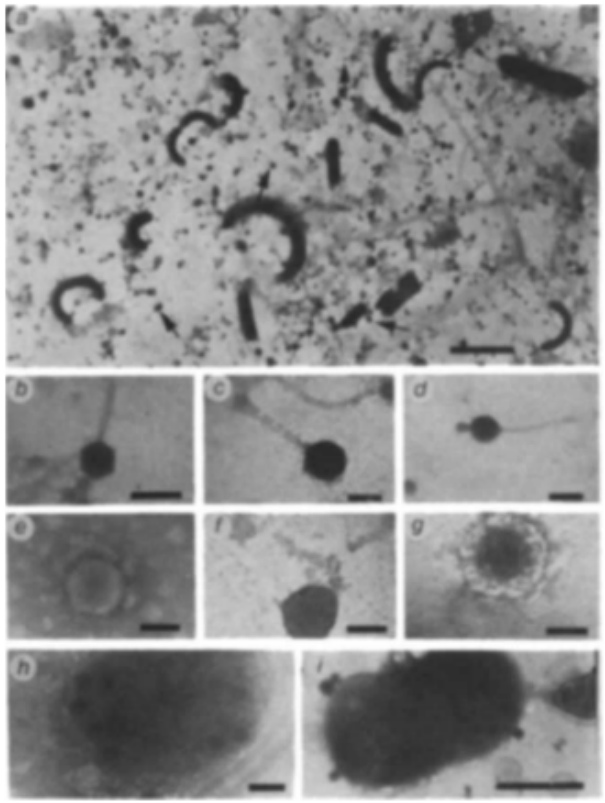
earth microbiome project

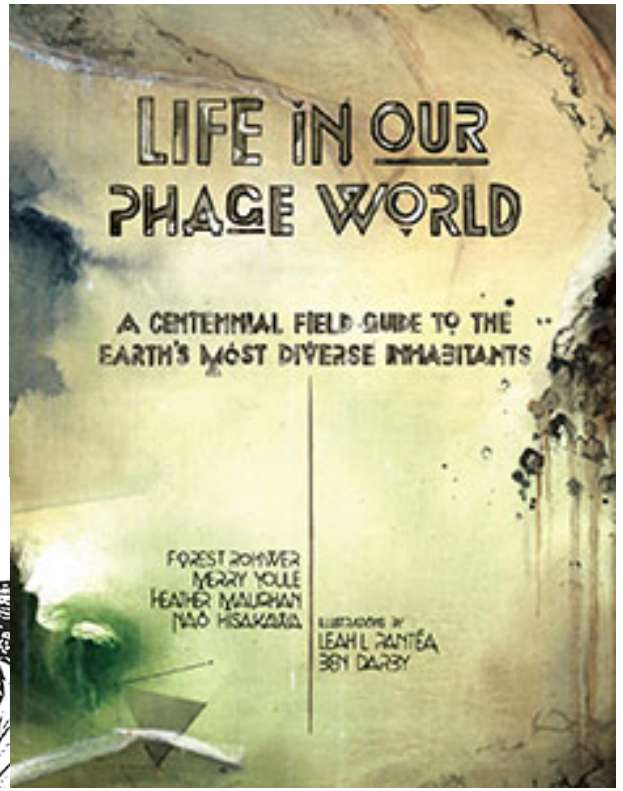
The "Virosphere"



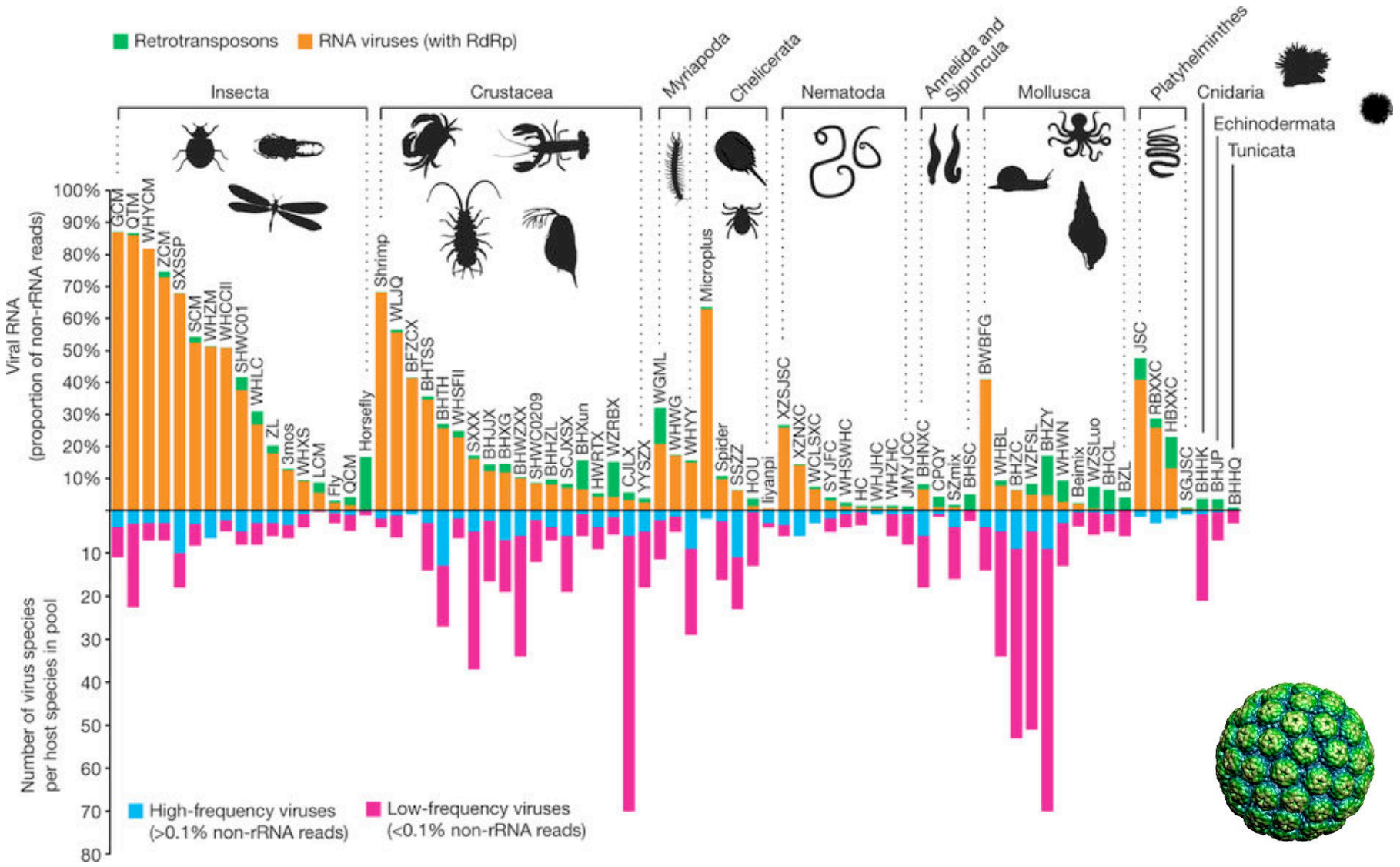
Phages: there are a lot

- "Using a new method for quantitative enumeration, we have found up to 2.5 x 10^8 virus particles per millilitre in natural waters."
  - Bergh, O., K. Y. Borsheim, G. Bratbak and M. Heldal (1989). High abundance of viruses found in aquatic environments. *Nature* 340(6233): 467-468.





Shi, M., X. D. Lin, J. H. Tian, L. J. Chen, X. Chen, C. X. Li, X. C. Qin, J. Li, J. P. Cao, J. S. Eden, J. Buchmann, W. Wang, J. Xu, E. C. Holmes and Y. Z. Zhang (2016). **Redefining the invertebrate RNA virosphere**. *Nature* doi: 10.1038/nature20167.



- Examples
  - The smoking gun
  - The red herring (?)
  - "Musselbola"

The Clinch River, TN

- Massive die-off in Fall, 2017
- Multiple species, but not all equally affected
- Some sites severely affected, others less so
- Some animals at affected sites moribund while others at the same sites healthy (apparently).

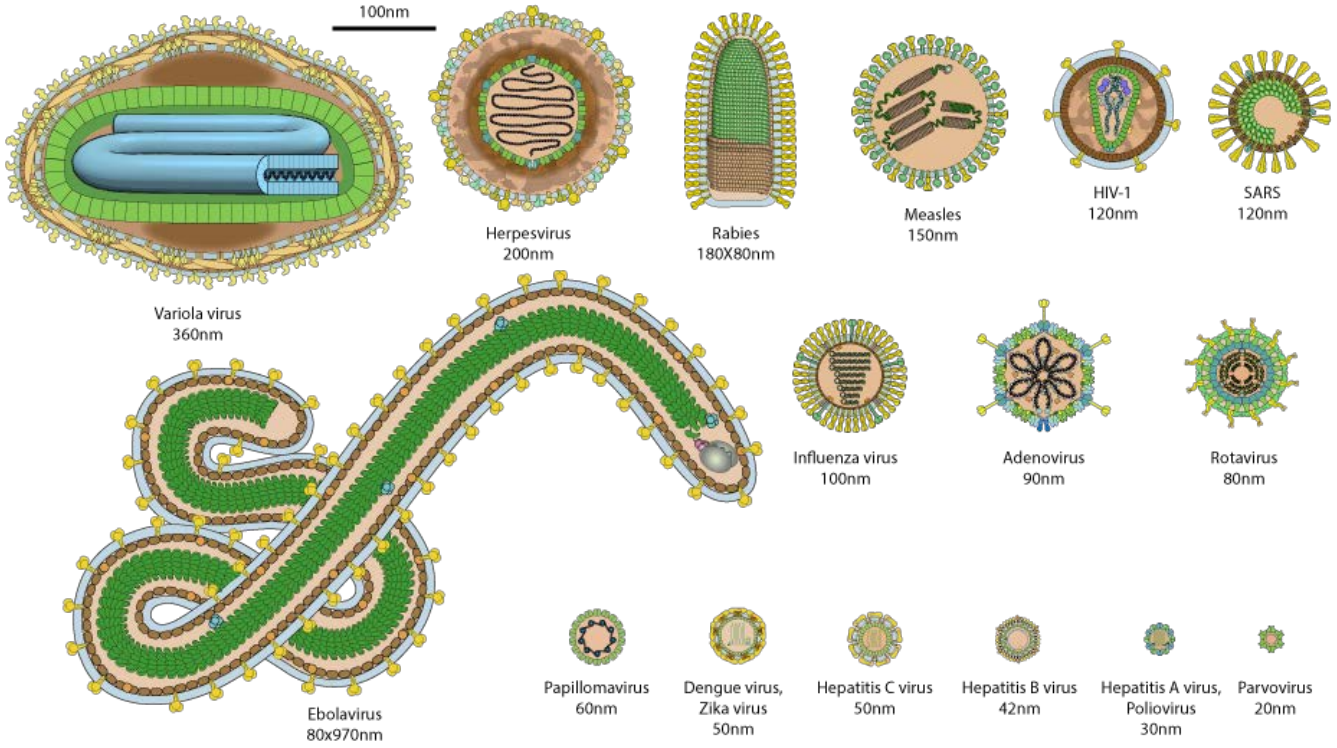
[Talk to Jordan Richard]

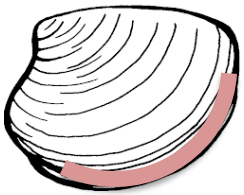


Samples and results

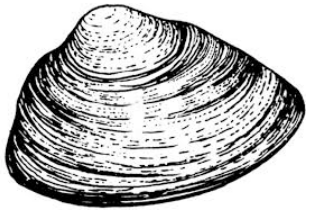
- Hemolymph from 84 mussels
  - 61 pheasantshells (*Actinonaias pectorosa*)
  - 18 muckets (*Actinonaias ligamentina*)
  - 5 purple wartybacks (*Cyclonaias tuberculata*)
- 2 dates
  - 10/20/2017 and 11/3/2017
- 4 sites
  - Kyle's Ford
  - Sycamore Island
  - Frost Ford
  - Wallen's Bend.
- New viruses discovered.

New viruses: cool, but so what?



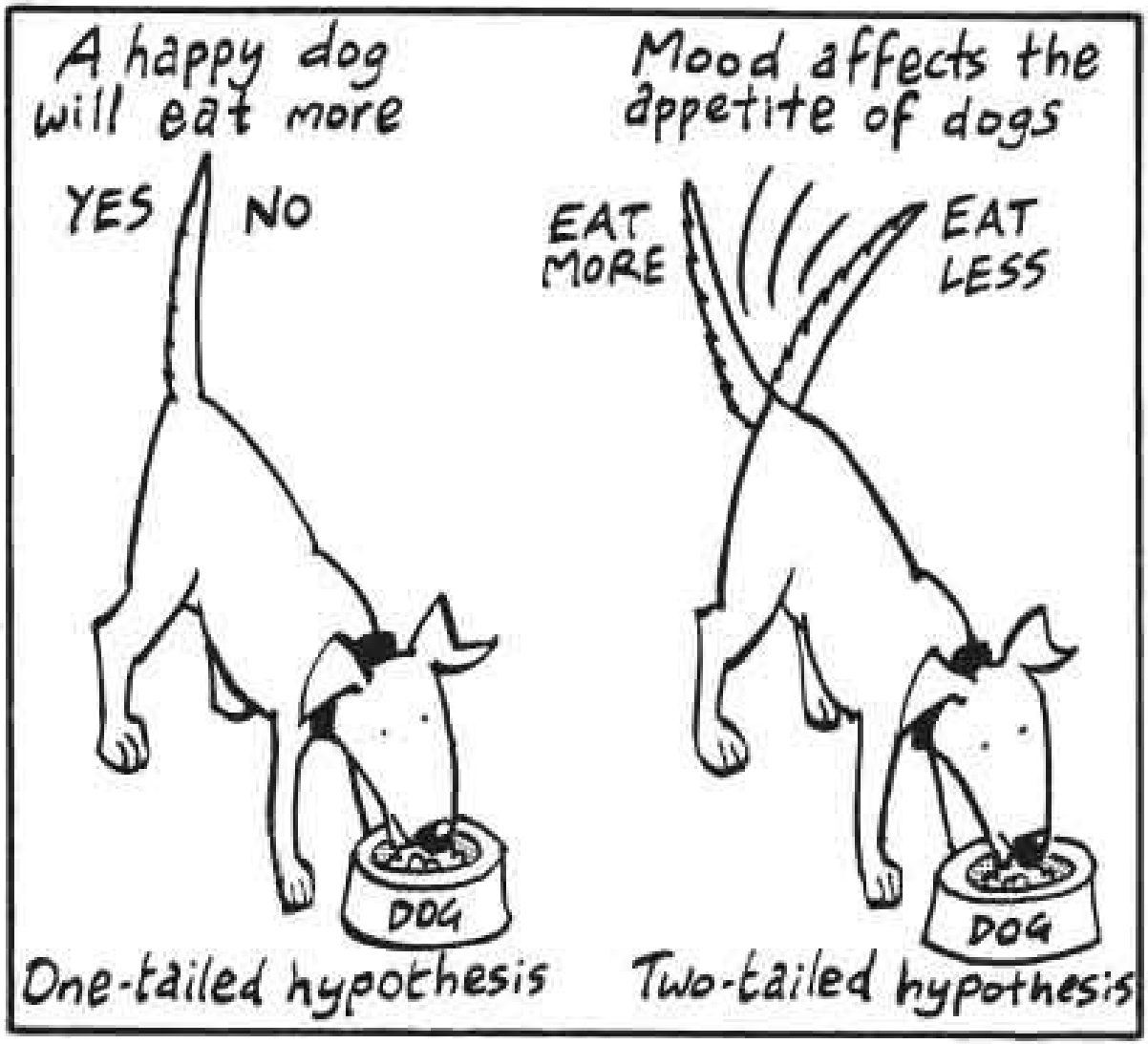


Case-Control Study

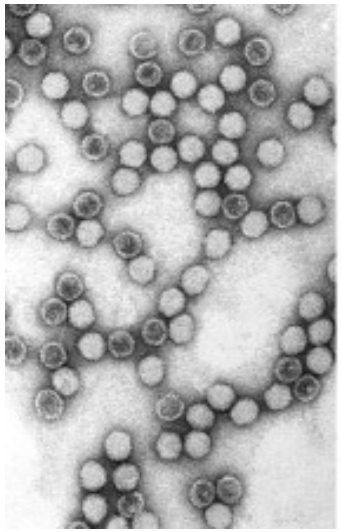


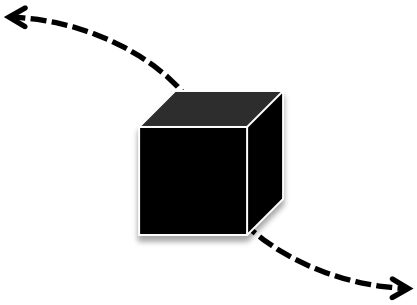
Plan: analyze data in terms of a case-control study design, with controls being apparently healthy mussels and cases being moribund mussels.

What we have from all this



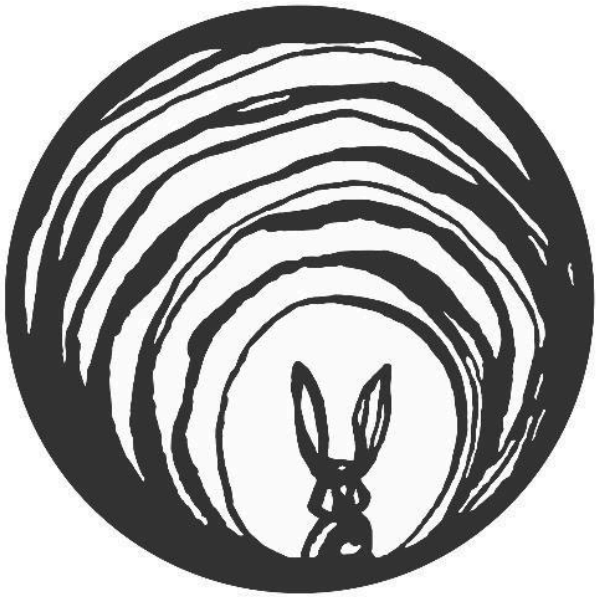
Hypothesis





Caveats

- Small sample size
- Type 1 error
- Direction of causality
  - Virus causes disease.
  - Disease causes virus.
  - Something else causes both.
- All sorts of bias (probably)



The key: incisive comparisons

Epidemiology and Risk Assessment

- **Count** cases or health events, and describes them in terms of time, place, and person (or **mollusk**)
- **Divide** the number of cases by an appropriate denominator to calculate rates; and
- **Compare** these rates over time or for different groups of people (or **mollusks**).

Principles of Epidemiology in Public Health Practice, Third Edition
An Introduction to Applied Epidemiology and Biostatistics

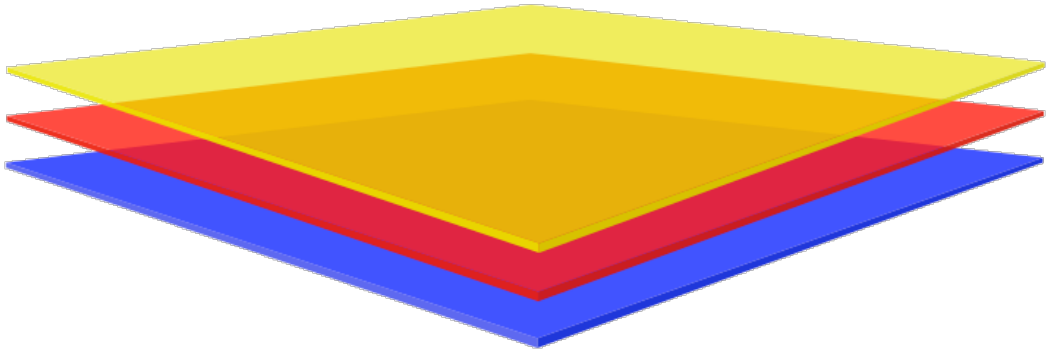
Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Some relevant comparisons

- Sick *vs.* healthy
- Affected species *vs.* Unaffected species
- Affected locations *vs.* Unaffected locations
- Before *vs.* After

Some next steps

- Bacteriology (including microbiomes)
- Parasitology
- Toxicology
- Metabolomics
- Other -omics
- Virus isolation
- Specific diagnostics (e.g. qPCR)
- Additional sampling (species and locations)
- Experimental studies (infection/translocation)
- Risk assessment (*informed* risk factor analysis and ecological/epidemiological modeling).



The Plan

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Contact info for questions

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