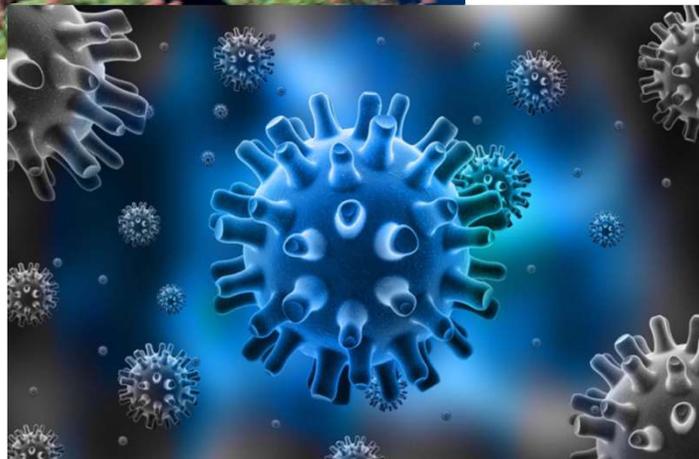




Vírus: aspectos ecológicos em ambientes aquáticos e aplicação tecnológica



Profa. Dra. Dolores U. Mehnert
Laboratório de Vírus Entéricos Humanos e Animais

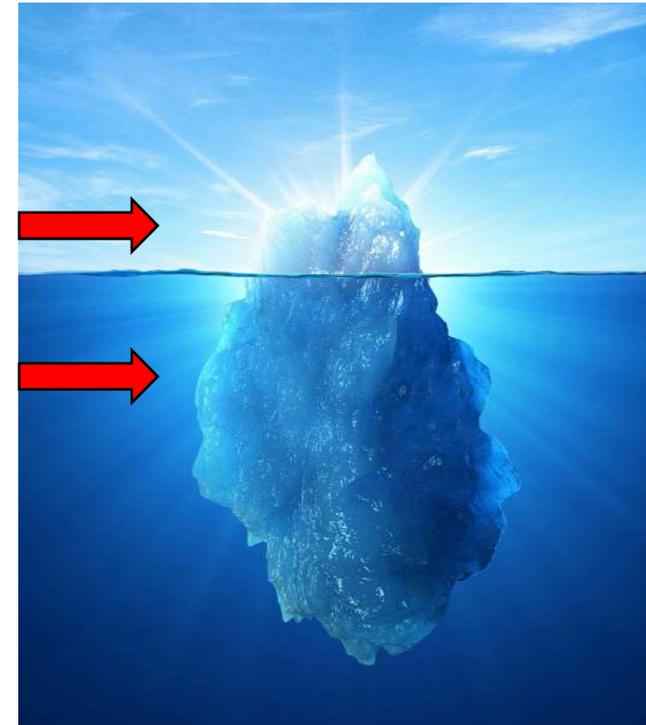
VÍRUS

Doenças

Aspectos ecológicos

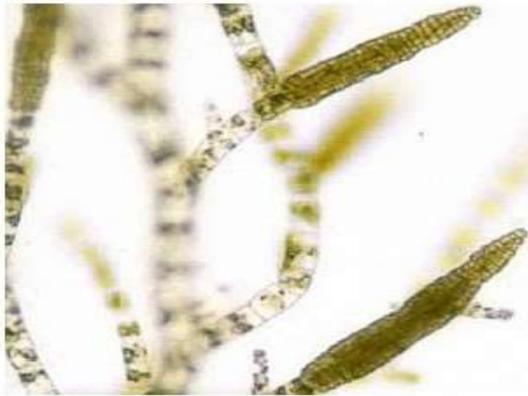
Ambiente aquático

- Partículas de 0,2 a 200 μm
- Elevadas Concentrações:
 - 10^6 partículas/mL (oceano profundo)
 - 10^8 partículas/mL (região costeira)
- Estima-se nos oceanos: 4×10^{30} vírus



Virioplâncton

Hospedeiros

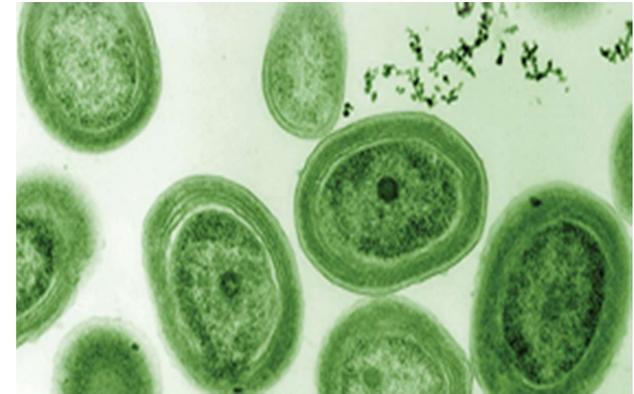


Detalle de los esporangios. Galicia, © Ignacio Bárbara

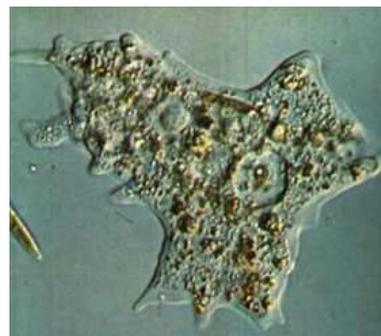
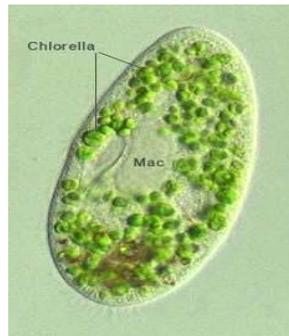
- Algas macroscópicas



- Algas unicelulares



- Cianobactérias



- Protozoários

- Bactérias

- Archaea

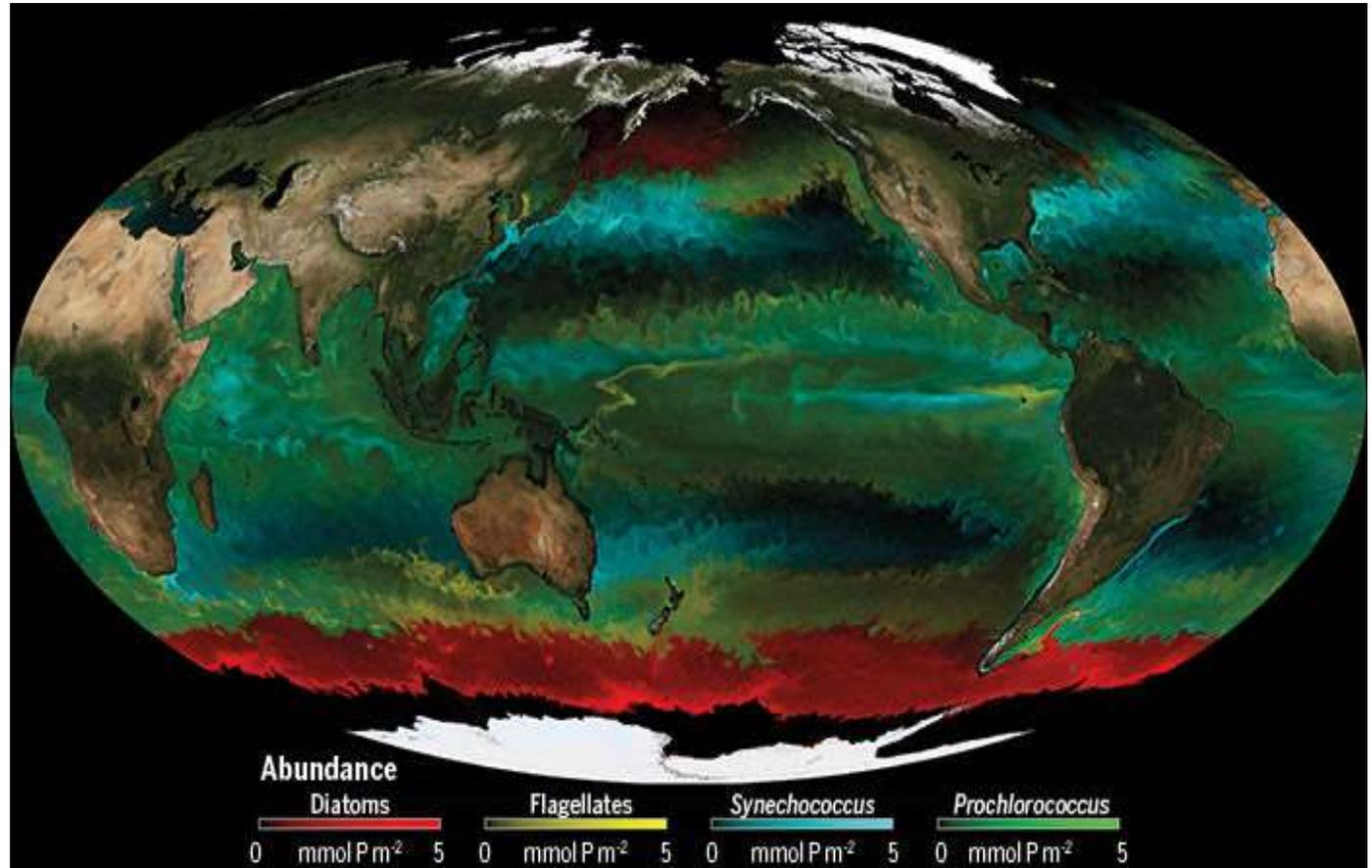
Cianobactérias

Organismos unicelulares,
fotossintéticos.
 10^5 cels/ mL



Synechococcus (0,8 a 1,5 μm)

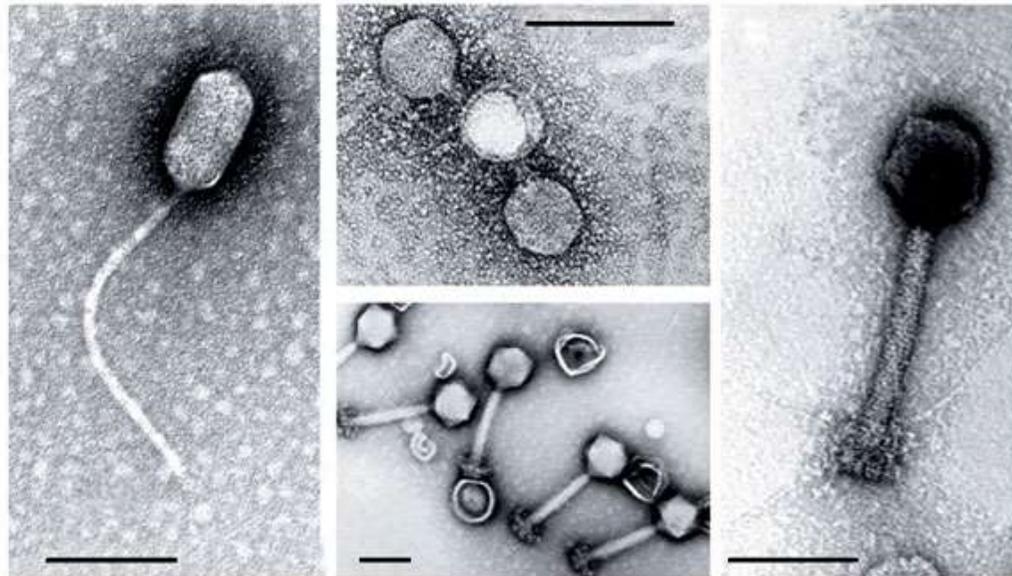
Prochlorococcus (0,5 a 0,8 μm)



MAGE: MIT DARWIN PROJECT, ECCO2, MITGCM/OLIVER JAHN (MIT), CHRIS HILL (MIT), MICK FOLLOWS (MIT), STEPHANIE DUTKIEWICZ (MIT), DIMITRIS MENEMENLIS (JPL)

http://www.sciencemag.org/news/2017/03/meet-obscure-microbe-influences-climate-ocean-ecosystems-and-perhaps-even-evolution?utm_source=sciencemagazine&utm_medium=facebook-text&utm_campaign=pennychisholm-11726

Vírus de cianobactérias



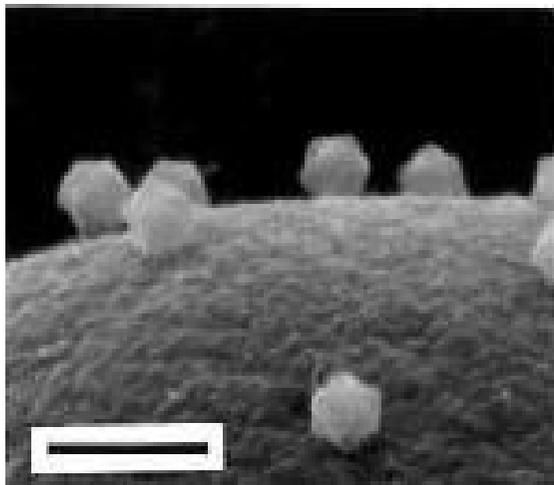
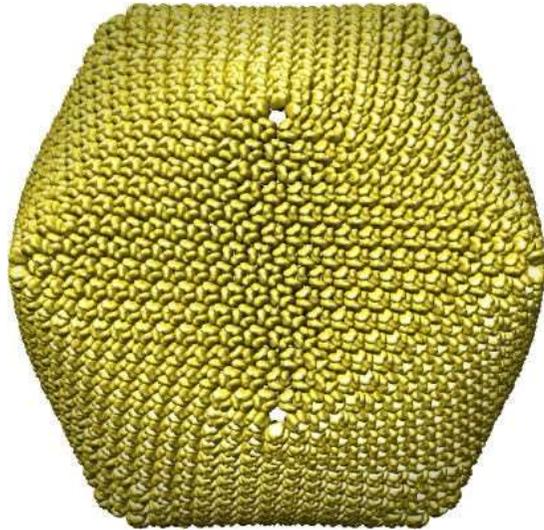
Fagos DNA fita dupla

Podovirus P-SSP7

Myovirus P-SSM2
P-SSM4

Courtesy M. Sullivan/MIT

Família *Phycodnaviridae*



- Vírus líticos
- Infectam algas eucarióticas em todos os ambientes aquáticos
- Tamanho 100 a 220 nm de diâmetro
- Genoma DNA dupla fita de 160 Kb a 560 Kb
- 6 Gêneros

Gênero *Chlorovirus*

PBCV-1

(vírus *Chlorella* de *Paramecium bursaria*)

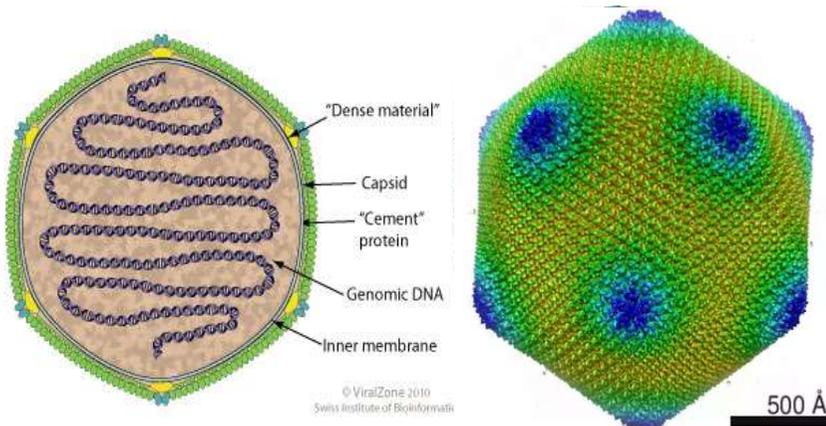
Tamanho em torno de 200 nm

Simetria icosaédrica

Sem envoltório

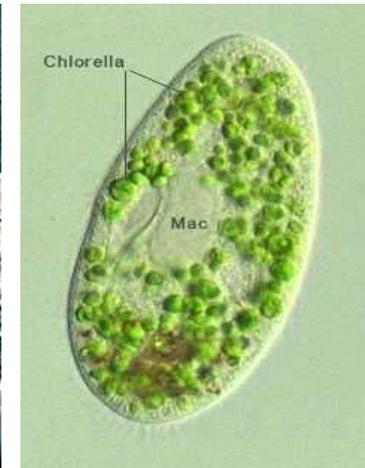
Hospedeiro: Algas verdes unicelulares do gênero *Chlorella*

- vida livre
- zoochlorella ou *Chlorella-like* (simbiontes de paramécios)



Chlorella NC64A - 16 tipos virais

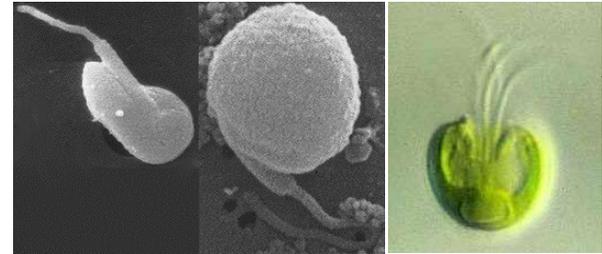
Chlorella Pbi - 5 tipos virais



Vírus de algas

Gênero Prasinovirus

Vírus: MpV-1 *Micronomas pusilla*
(Prasinophyceae, Gênero *Micromonas*, alga unicelular)



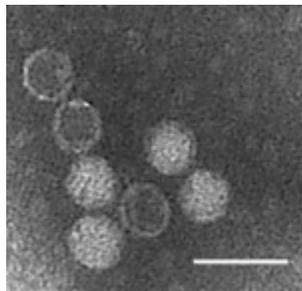
Gênero Raphidovirus



Heterosigma akashiwo RNA virus (HaRNAV)

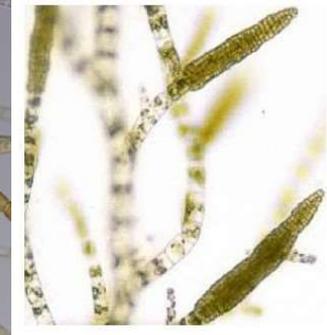
Heterosigma akashiwo nuclear inclusion virus (HaNIV)

Gênero Phaeovirus – membros presentes em ambiente marinho



Vírus: EsV-1 -*Ectocarpus siliculosus*

Vírus: FsV-1 e FsV-2 - *Feldmannia* sp

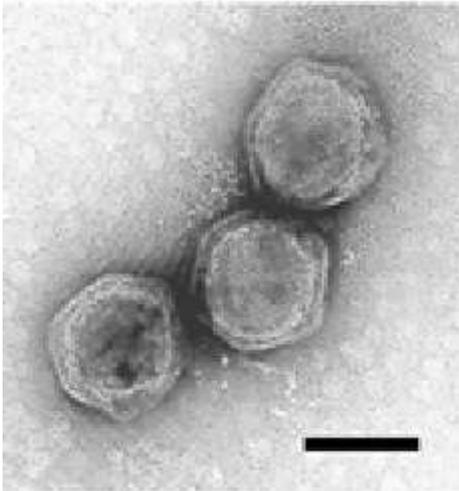


Detalle de los esporangios. Galicia, © Ignacio Bárbara

Vírus de algas

Gênero *Prymnesiovirus*

Phaeocystis globosa virus 1

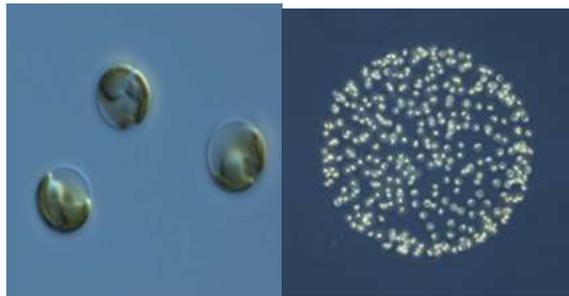


Hospedeiros:

Phaeocystis globosa - águas de clima temperado e tropicais

Phaeocystis. antarctica - águas da Antártica

P. pouchetii – águas árticas



Floração com produção de espuma
(Matriz polissacarídica)

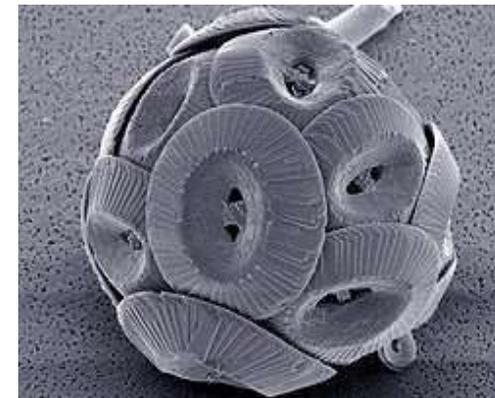
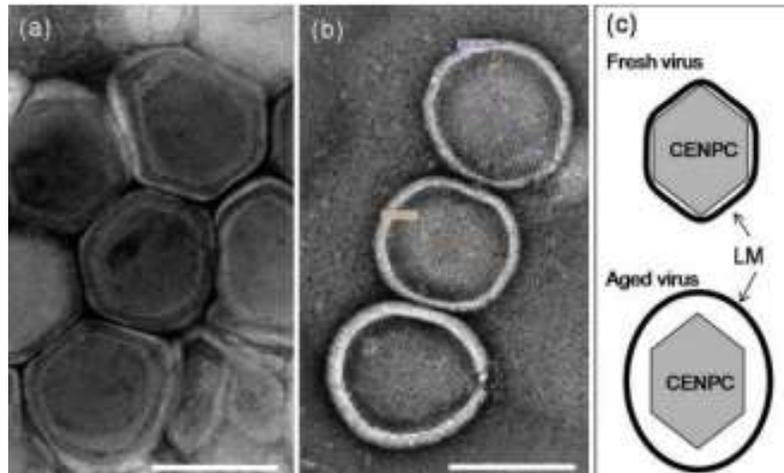
<http://www.gtresearchnews.gatech.edu/single-celled-transformers/>

Gênero *Coccolithovirus*

Vírus de algas

Coccolithovirus 86 (EhV-86)

Hospedeiro: *Emiliana huxleyi*



Maiores vírus marinhos em termos de genoma conhecidos – “vírus gigantes”

Genoma DNA fita dupla codificando 472 proteínas

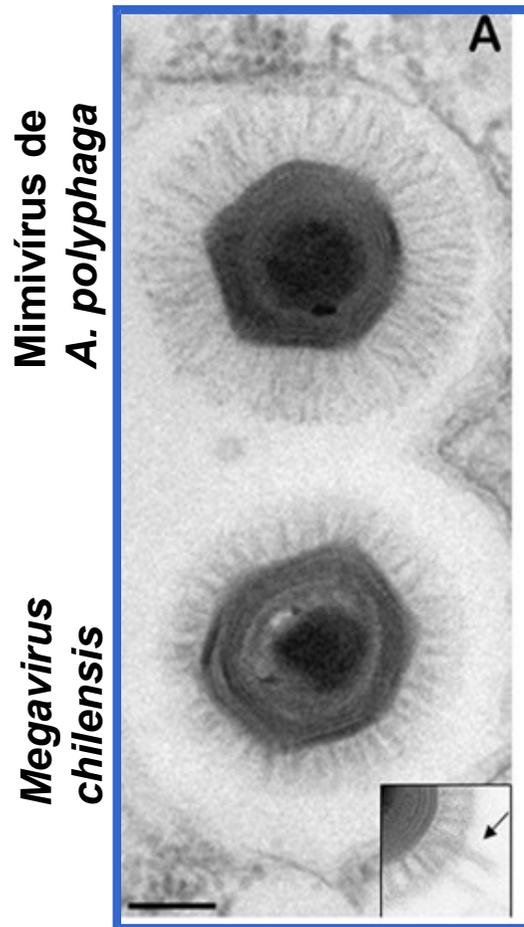
10 espécies e só 2 sequenciadas - EhV-86 e EhV-203



Floração - 2004

Megavírus (Vírus Gigantes)

Hospedeiros: amebas de vida livre (*Acanthamoeba*)



Genoma DNA fita dupla com cerca de 1.000 genes e 1.259.197 pares de bases

Capsídeo icosaédrico - cerca de 390 a 440 nm

Recobertos por camada de fibras

~75 nm (Mega)

~120 nm (Mimi)

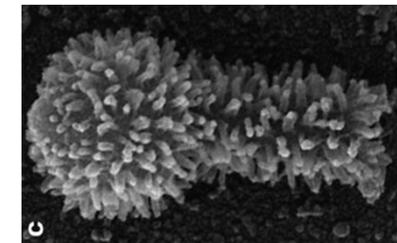
Pithovirus sibericum



Pandoravirus dulcis
P. salinus



Tupanvirus



Genomas de 1,5 a 2,5 megabases

Bacteriófagos

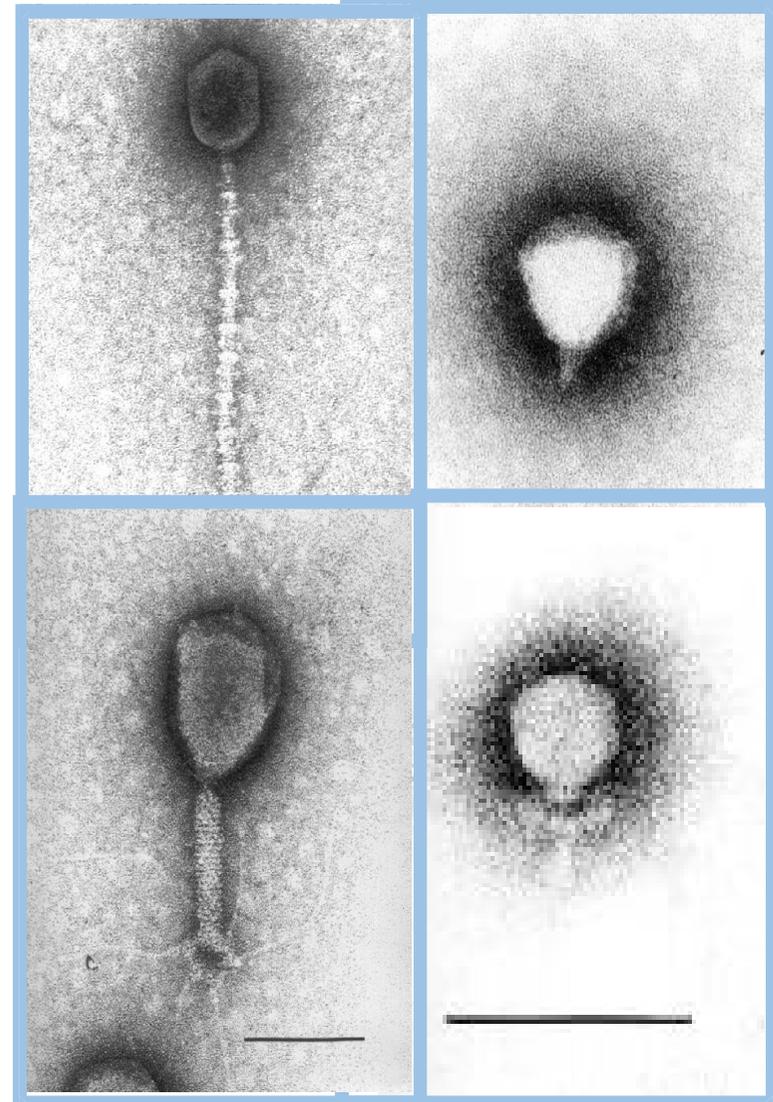
- Mais numerosos
- Tamanho de 30 – 70 nm
- Causam 40 a 75% das mortes

Famílias

Siphoviridae caudas longas e flexíveis;

Podoviridae cauda curta;

Myoviridae cauda contrátil
(maioria dos cianofagos)



Vírus de Archaea

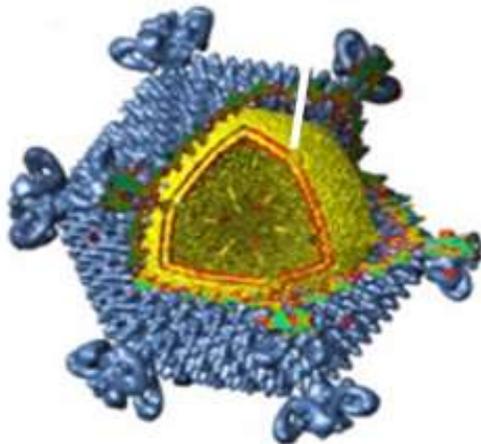
Estrutura

Genoma

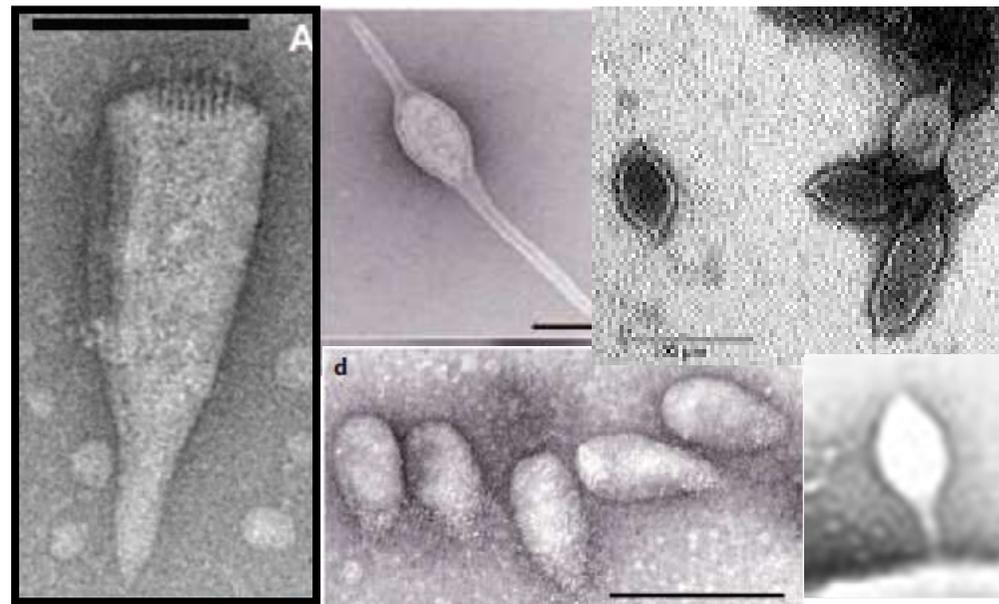
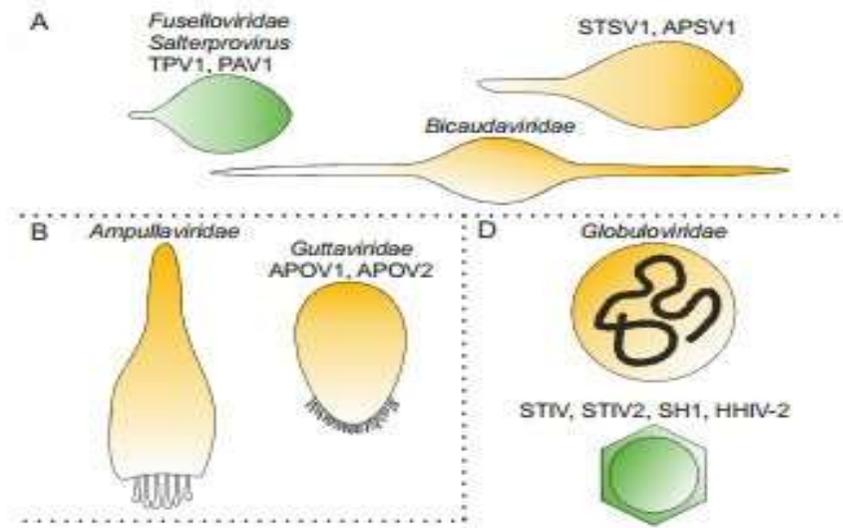
DNA ds ou DNA ss
Tamanho de 14 a 50kb
Linear ou circular

Envoltório

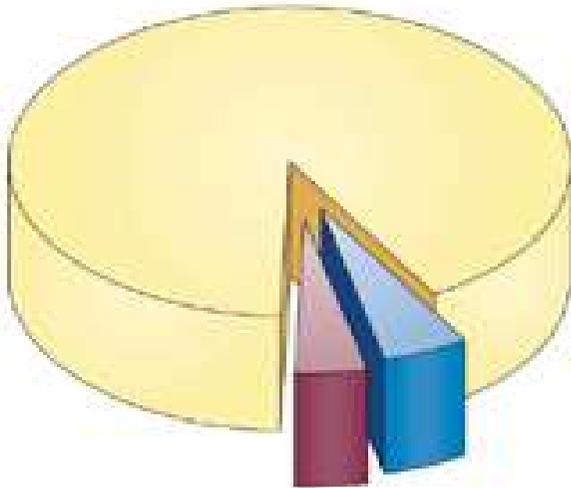
Presente em algumas espécies



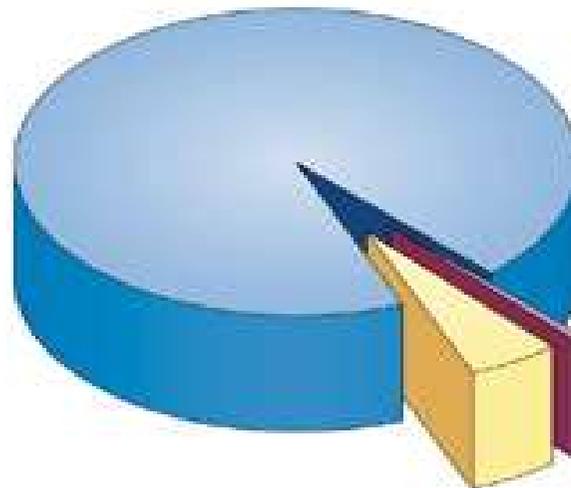
Formas variadas



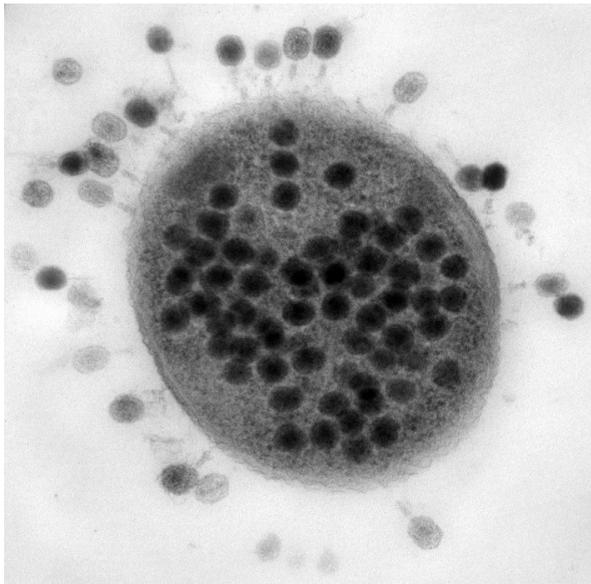
Biomass



Abundance



Nature Reviews | **Microbiology**



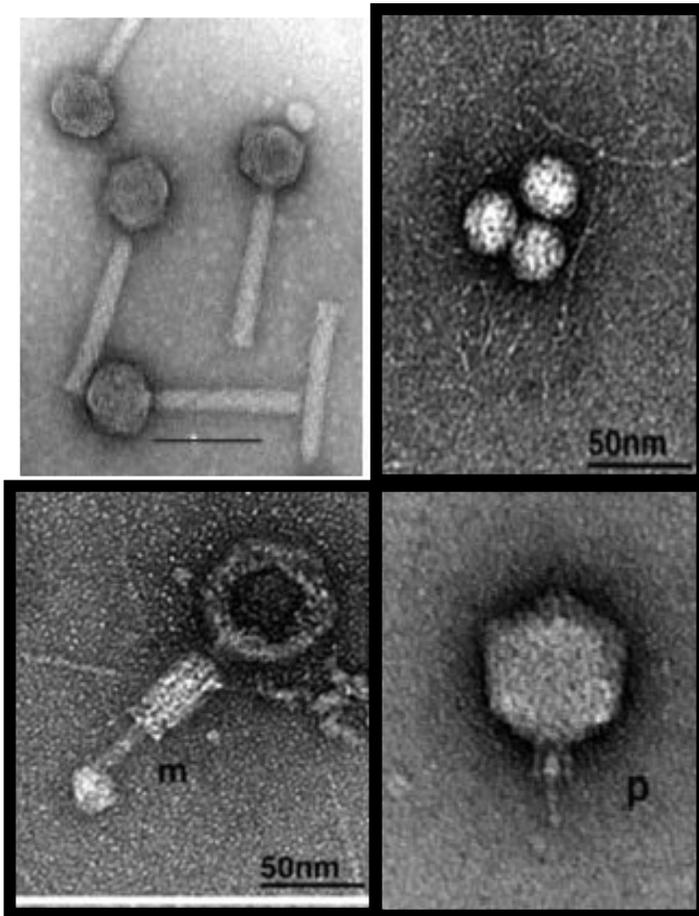
VÍRUS

Concentração 15x maior do que as bactérias e arqueias

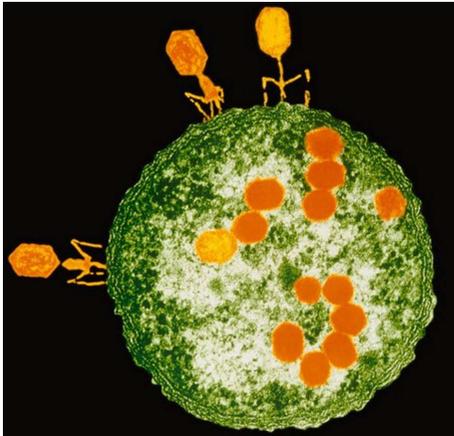
Por que tão numerosos?



Funções do virioplâncton nos ecossistemas aquáticos



- Causam 40 a 75% das mortes
- Ciclagem de nutrientes
- Retenção de nutrientes na zona eufótica
- Influência direta sobre o clima global
- Manutenção do equilíbrio populacional dentre os produtores primários (Hipótese *Kill the Winner*)

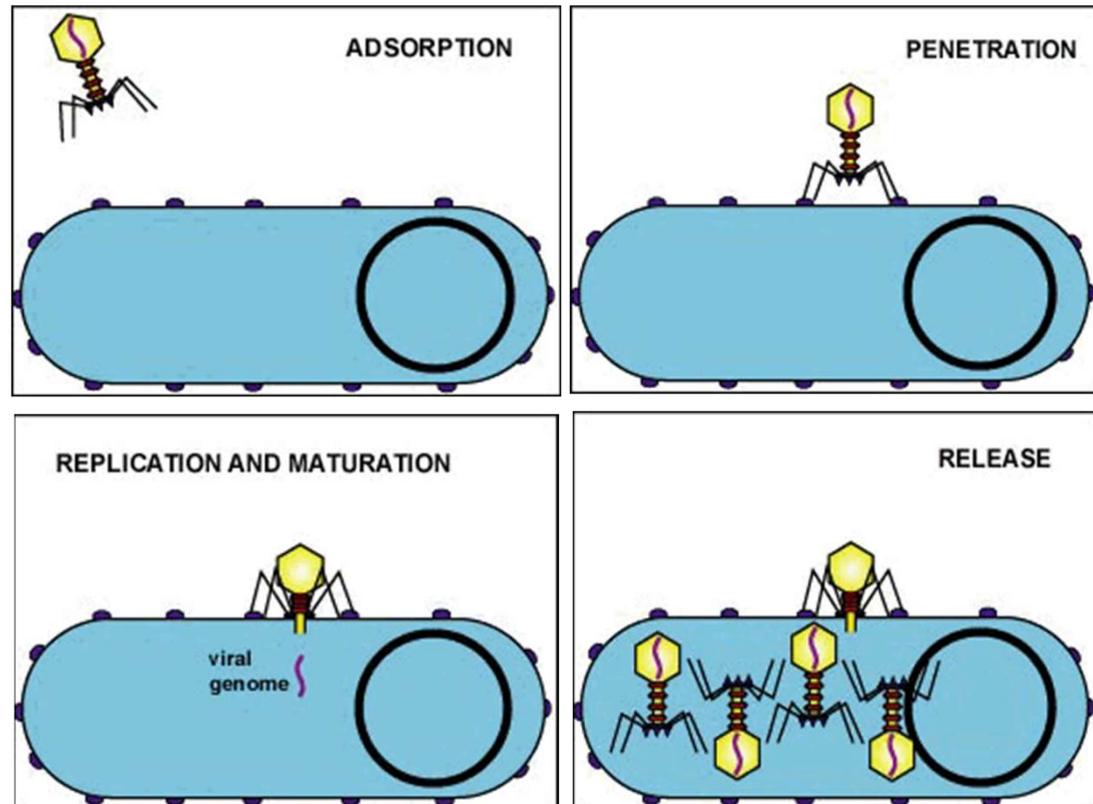


Fagos virulentos (T4)

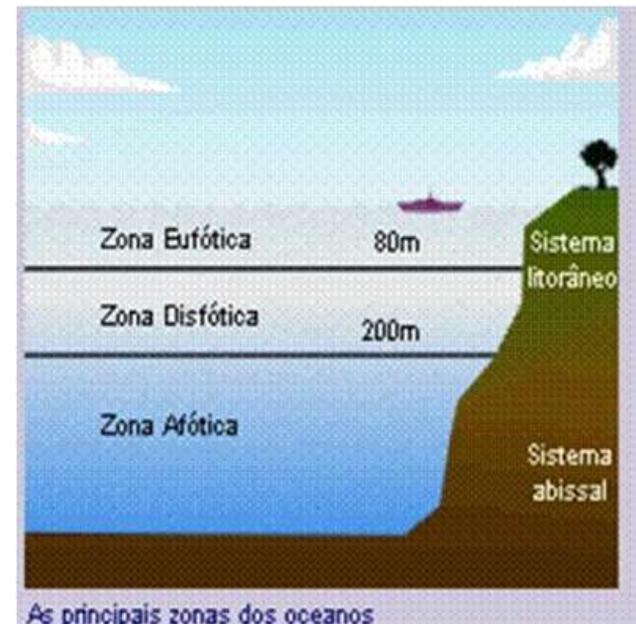
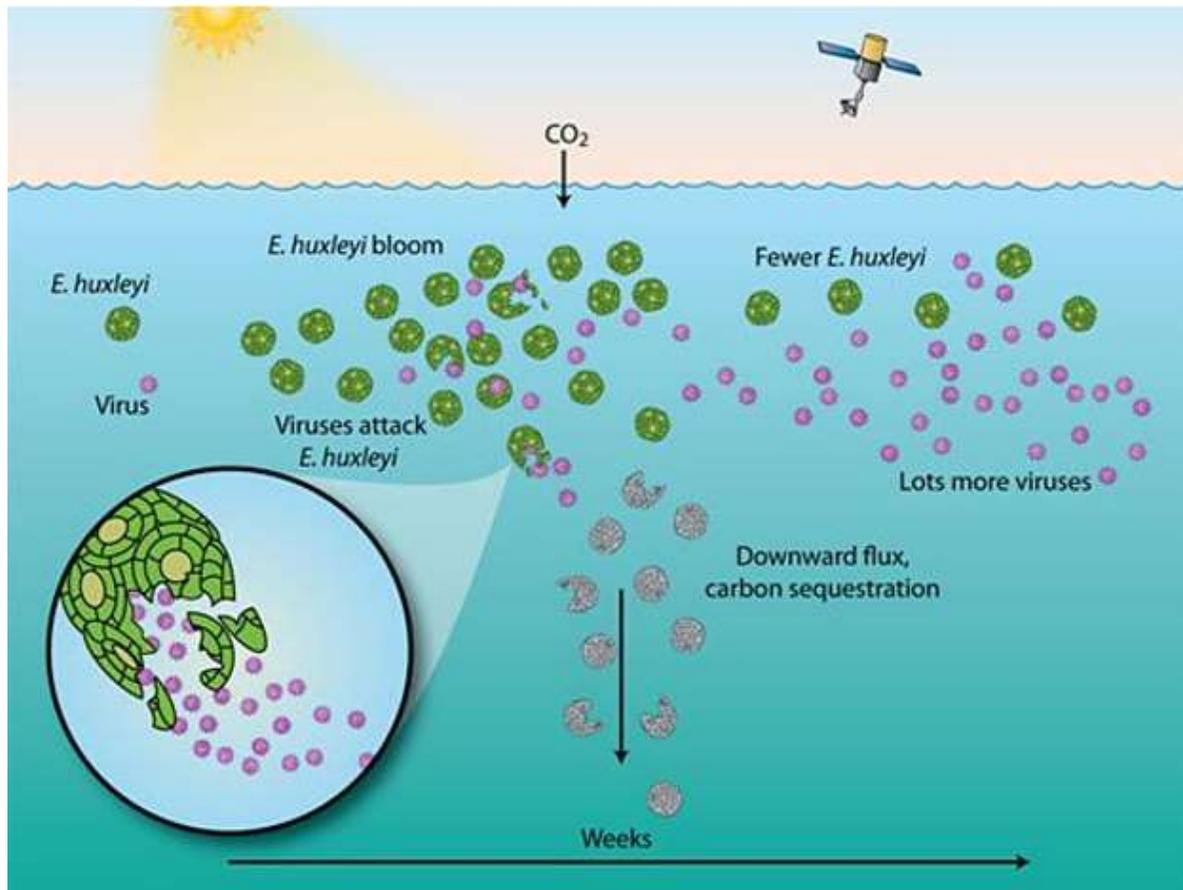
- ✓ Estabelecem só ciclo lítico
- ✓ Infectam as bactérias hospedeiras, injetam seu ácido nucléico e progênie formada é liberada por lise

1. Ciclo lítico

<https://www.youtube.com/watch?v=Ms2xhuKgmDI>



Funções do virioplâncton nos ecossistemas aquáticos



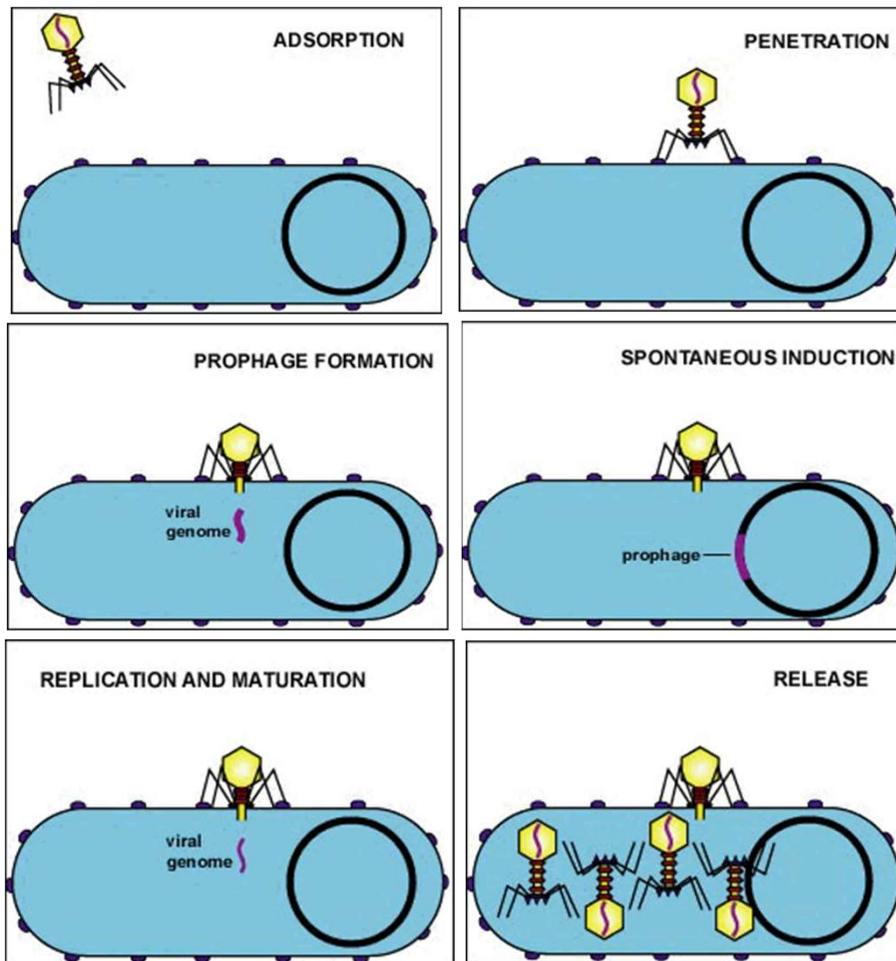
Hipótese "Kill the Winner"

" a população mais bem sucedida deve ser eliminada"

Fagos temperados (λ)

✓ Estabelecem tanto ciclo lítico como lisogênico.

2. Ciclo lisogênico



✓ Quando a bactéria hospedeira está em um ambiente de condições nutricionais pobres optam pelo ciclo lisogênico (só serão capazes de produzir poucos vírus).

✓ Podem então incorporar no genoma da bactéria ou formar um plasmídio

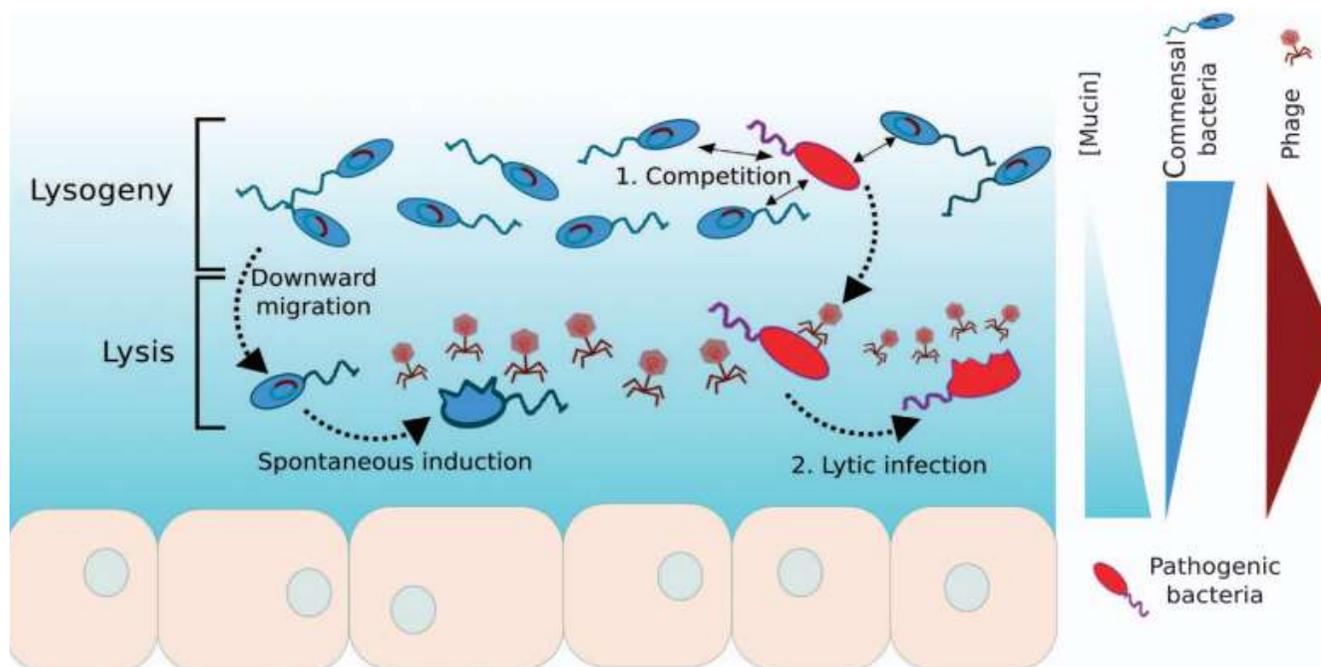
✓ Quando saem deste estado, passam a se multiplicar e progênie é liberada por lise.

<https://www.youtube.com/watch?v=JhyBNdukhPE>

Relações parasita-hospedeiro: controle de populações no meio ambiente aquático

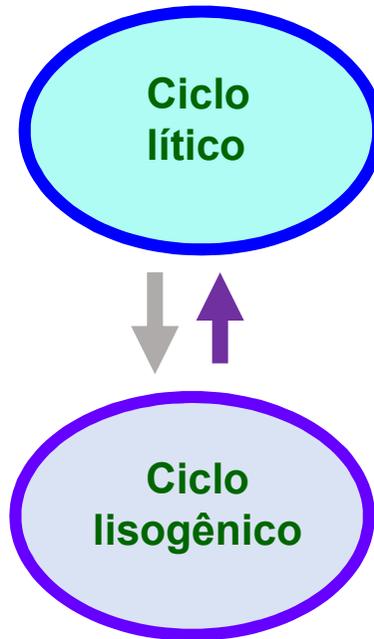
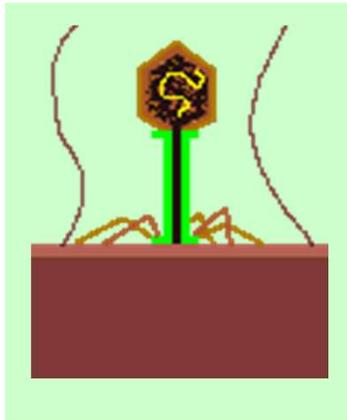
2. Piggyback-the-Winner (PtW)

A lisogenia predomina em altas taxas de abundância e crescimento microbiano



Funções do virioplâncton nos ecossistemas aquáticos

✦ Transferência horizontal de genes



Variabilidade genética

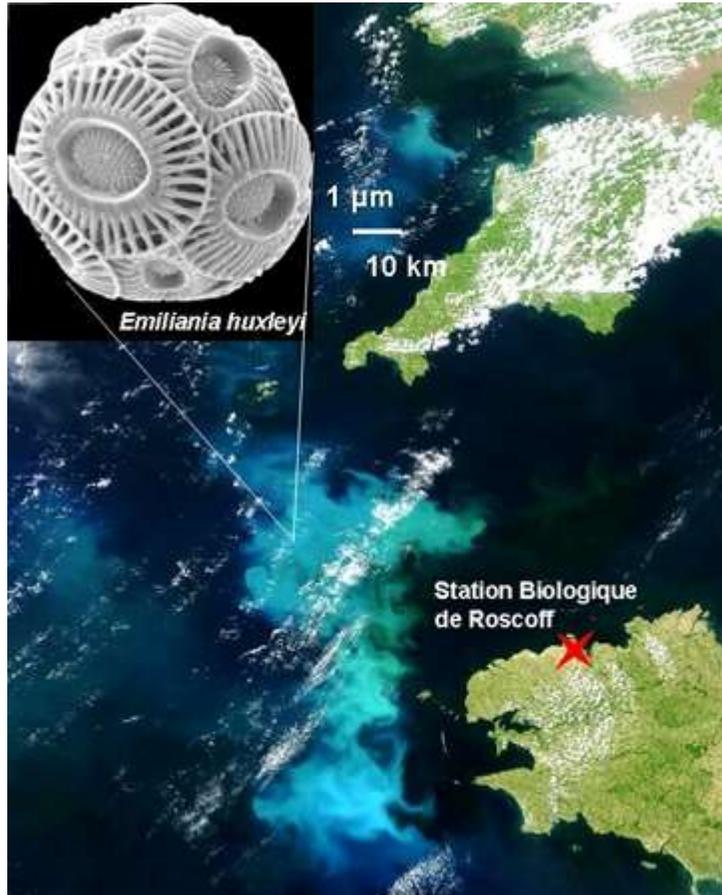


Red Queen Hypothesis
(adaptar e proliferar para sobreviver, coevolução)



Evolução

Gênero *Coccolithovirus*



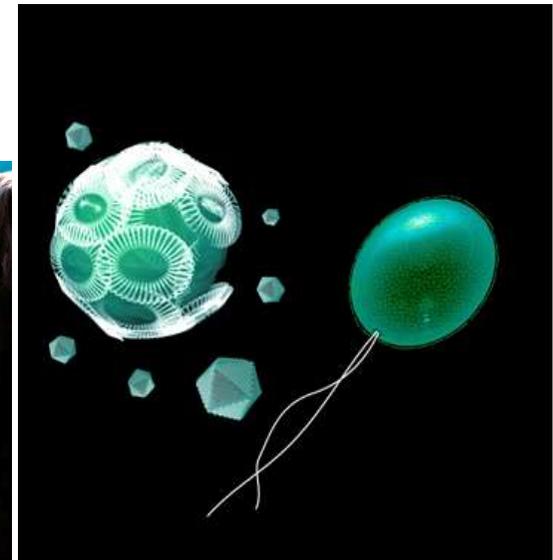
Floração na costa da Cornualha em 2004 vista do espaço

<http://www.nhm.ac.uk/nature-online/species-of-the-day/biodiversity/climate-change/emiliana-huxleyi/biology/index.html>

Manipulação do ecossistema por vírus

The 'Cheshire Cat' escape strategy in response to marine viruses

<http://www2.cnrs.fr/en/1294.htm>



Cocolito
Diploide → haploide (resistente)

Manipulação do ecossistema por vírus

Ex. genes virais para aquisição de fosfato são ativados no hospedeiro qdo este está em ambiente com baixa concentração de fosfato



Coccolithophorid phytoplankton



Diploid, non-motile



Haploid, motile and virus resistant

'Cheshire Cat' effects on phytoplankton



Horizontal gene transfer



GTAs

Gene Transfer Agents



Eukaryotic viruses



Phage



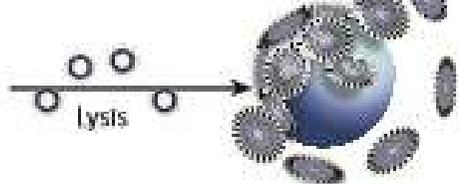
Niche expansion, new metabolisms



Bacterial mortality



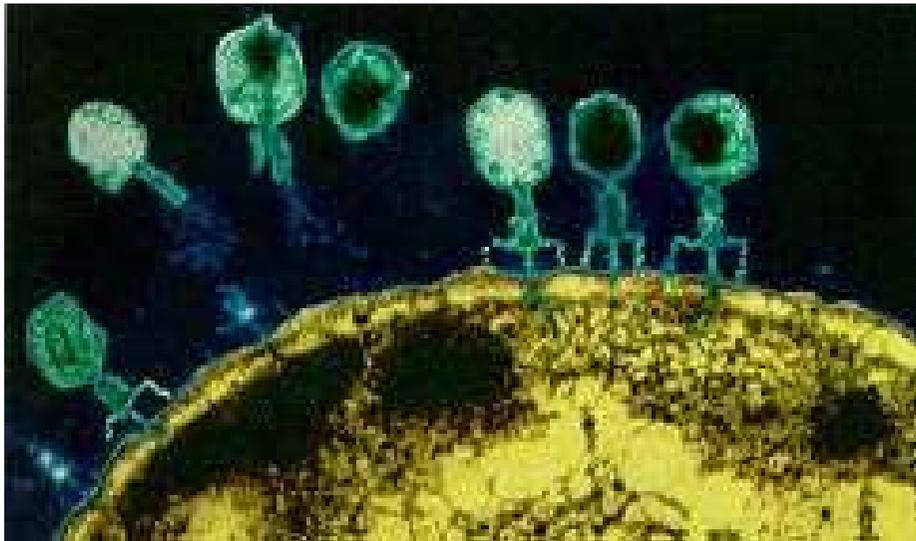
'Red Queen' effects on phytoplankton through predator-prey interactions



Lysis

Fatores que influenciam a produção e a distribuição viral em ambientes aquáticos

- ✦ Produtividade e densidade das populações de hospedeiros, especialmente de bacterioplâncton
- ✦ Fatores físicos: temperatura da água, luminosidade e profundidade

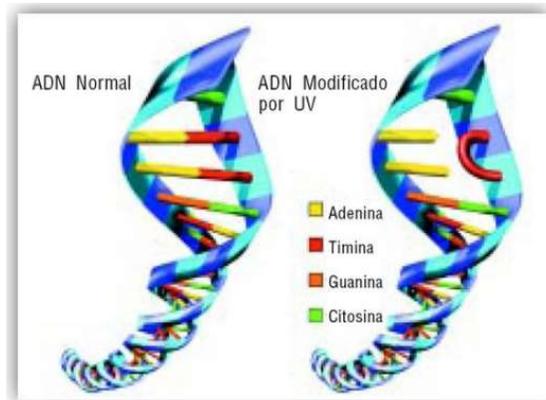


- ✦ Gradientes de salinidade
- ✦ Variação sazonal

Fatores que influenciam a produção e a distribuição viral em ambientes aquáticos

Infectividade, inativação e decaimento viral

✦ Perda da infectividade: Luz UV



infectividade restaurada por fotoreativação (vírus usa mecanismo do hospedeiro)

✦ Remoção dos vírus da coluna d'água

Sedimentação por adsorção em material particulado

Predação por protistas flagelados

Desintegração das partículas virais por moléculas bioativas como exoenzimas, proteases e nucleases.

*Funções do virioplâncton nos ecossistemas
aquáticos*

EQUILÍBRIO

no ambiente aquático e na Natureza



Como posso encontrar em vírus na Natureza?

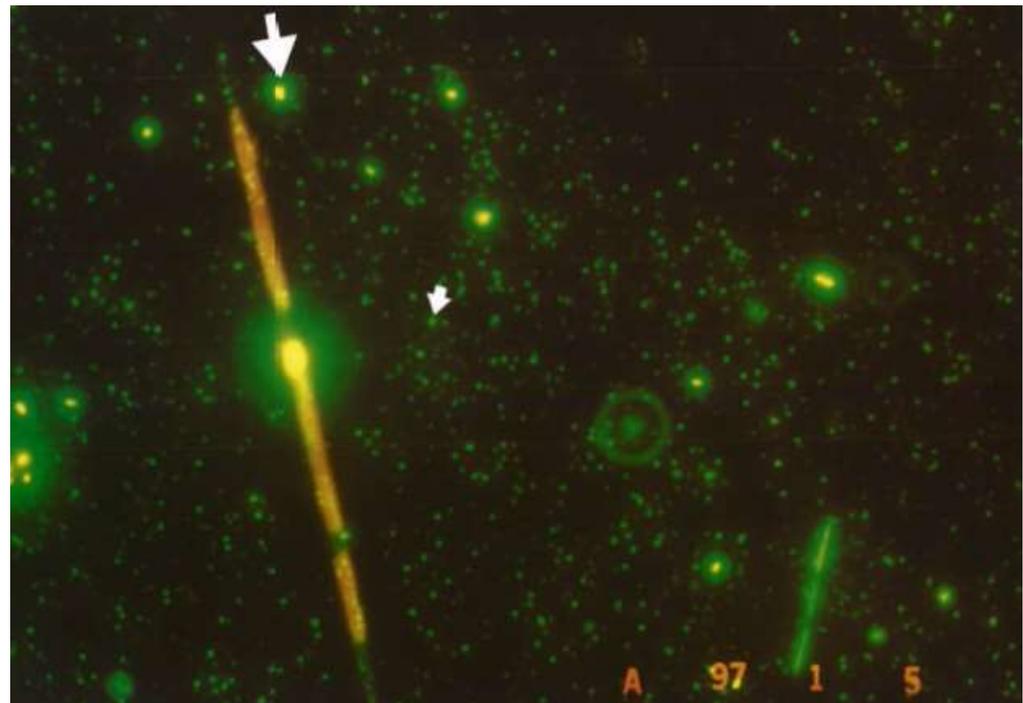
Métodos para detecção de vírus

Cultivo de algas
Chorella e ensaios de
plaqueamento viral



Métodos moleculares

Ensaio quantitativo
Microscopia de epifluorescência
com SyBR green

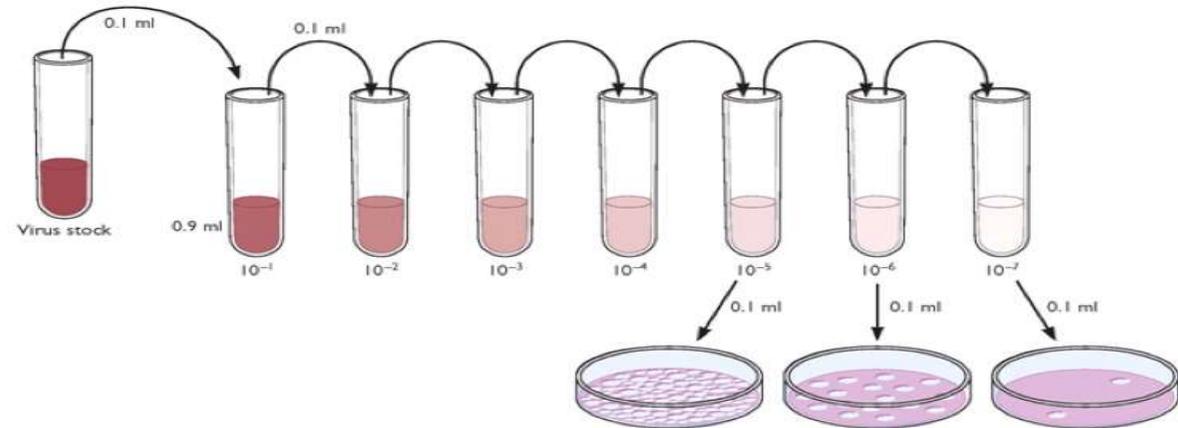


Métodos para detecção de vírus

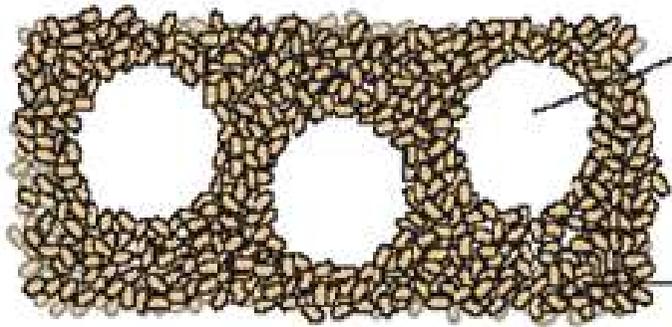
Método de plaqueamento

Bacteriófagos

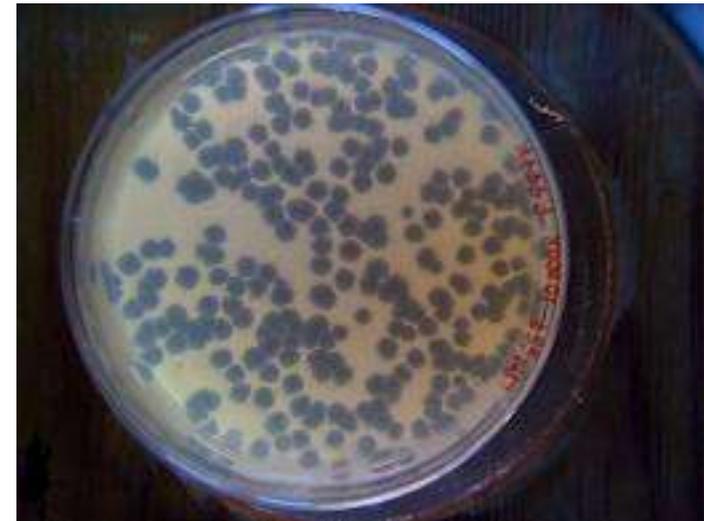
- ✓ Detecção
- ✓ Isolamento
- ✓ Quantificação



(b) Lytic plaques

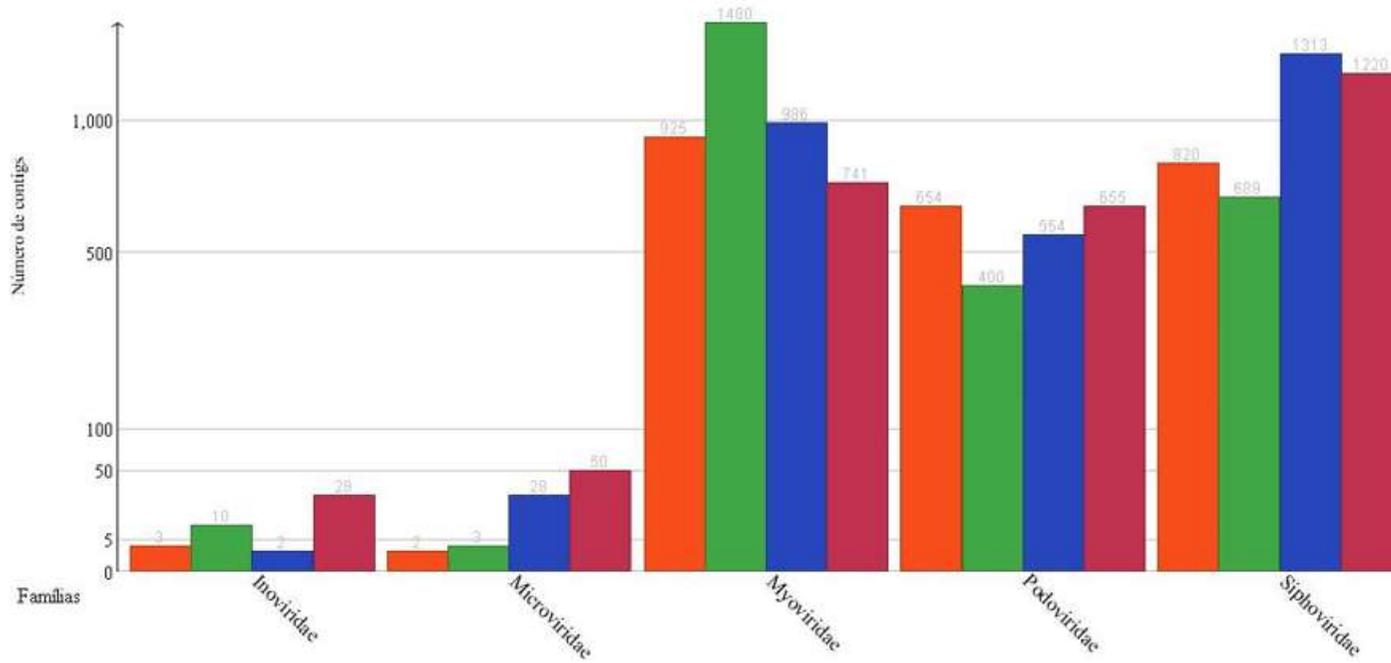
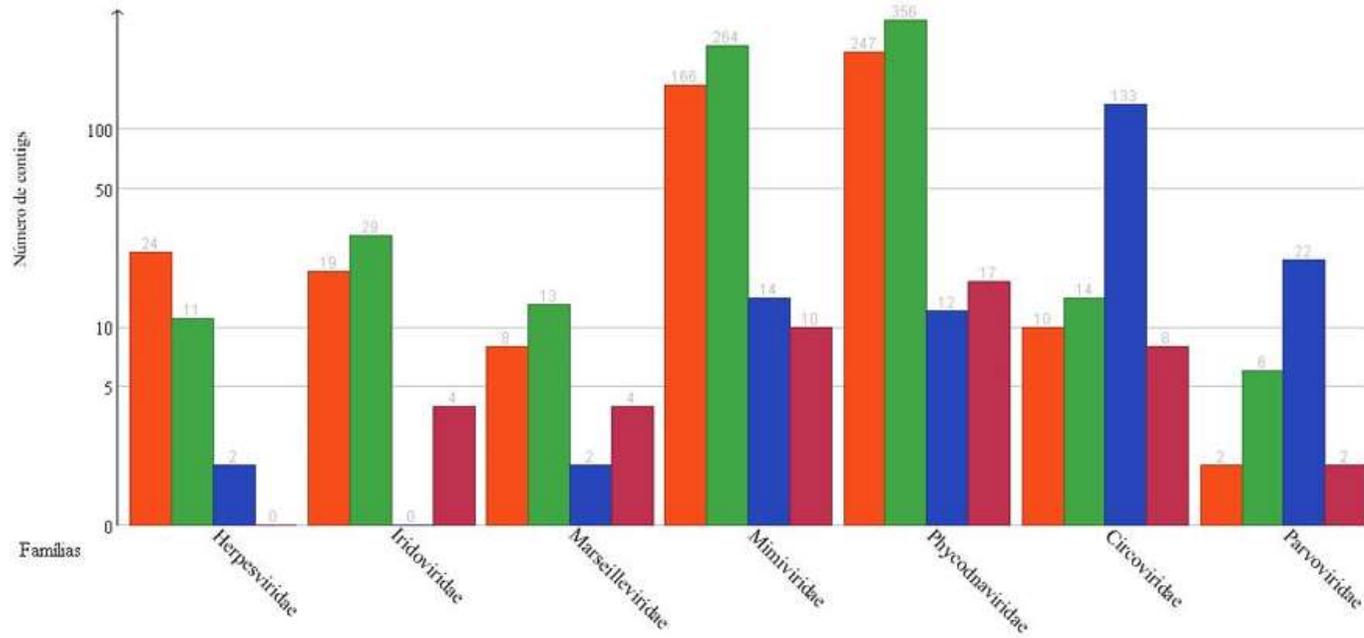


No. Unidades formadoras de placa
(UFP/mL)



Legend (Ponto de Coleta):

Gua_Manancial SL_Manancial SL_Esgoto TS_Esgoto

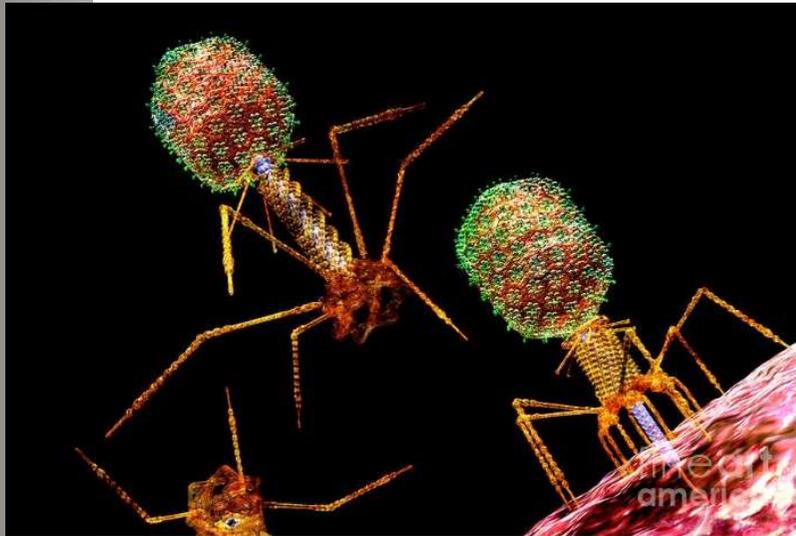


Sempre considerar que as interferências no ambiente macroscópico podem ter repercussões no microscópico...



...que só serão observadas com o passar do tempo e qdo estas se manifestarem já podem ser, muitas vezes, irreversíveis.





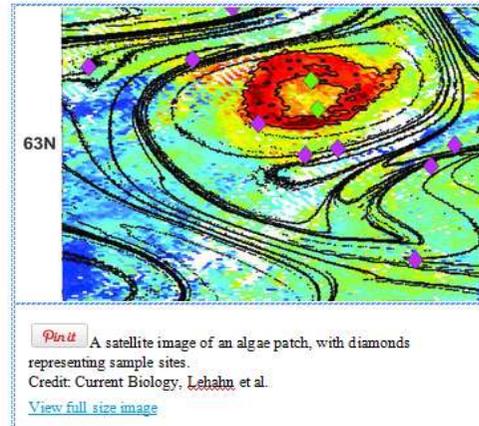
Fagos
aspectos ecológicos com
aplicação tecnológica

Controle biológico de florações de algas

<http://www.livescience.com/47489-viruses-kill-algae-blooms.html>

Viruses Deflate Huge Algal Blooms at Sea

Laura Geggel, Staff Writer | August 21, 2014 01:57pm ET



Gobs of microscopic organisms called algae may have met their match in viruses that can invade their cells, ultimately leading to death, new research suggests.

The findings may help researchers refine models that forecast [algal blooms](#) and the influence these microscopic plants have on the climate, experts say.



Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Virology 341 (2005) 80 – 90

VIROLOGY

www.elsevier.com/locate/yviro

Characterization of different viruses infecting the marine harmful algal bloom species *Phaeocystis globosa*

A.-C. Baudoux, C.P.D. Brussaard*

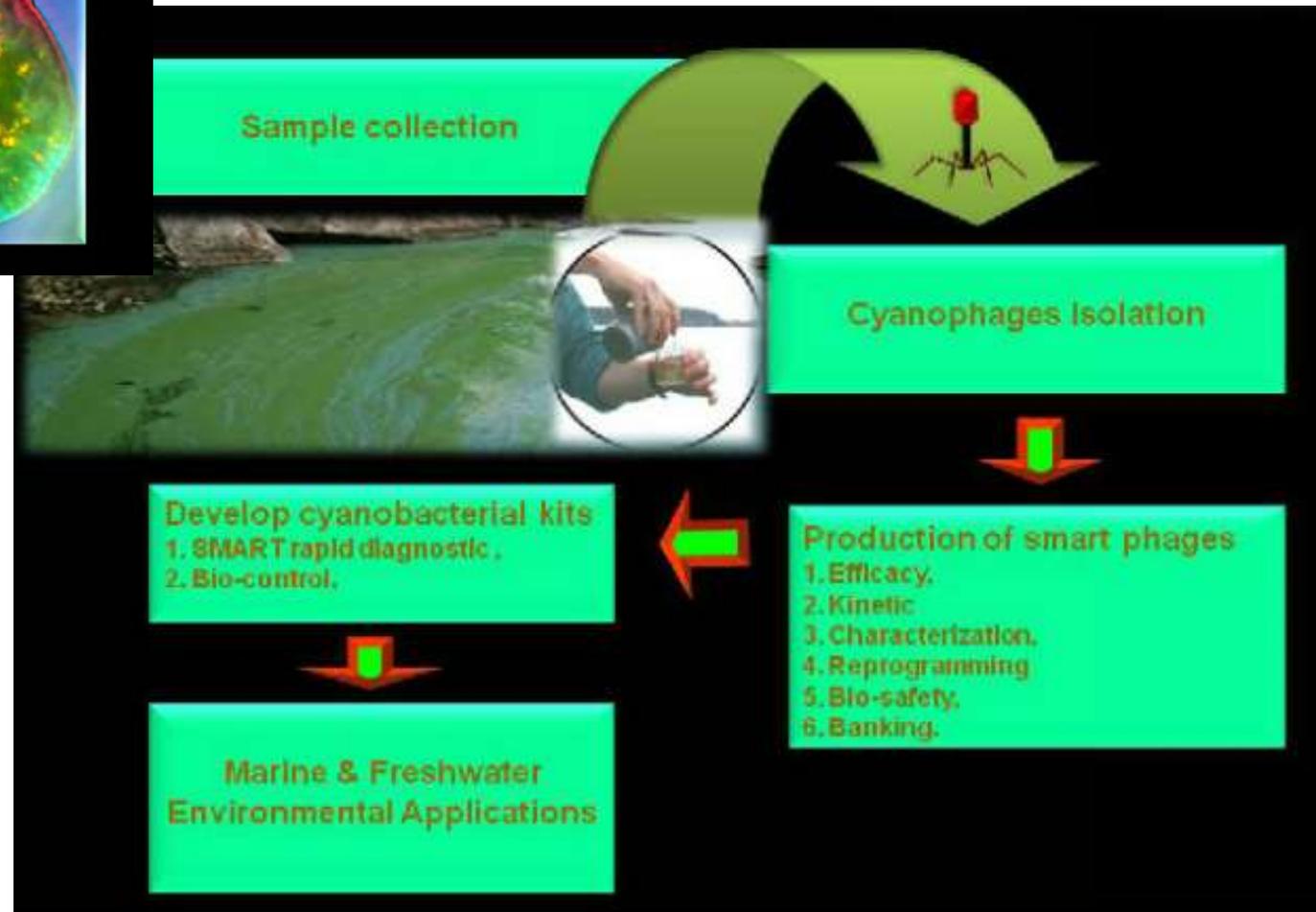
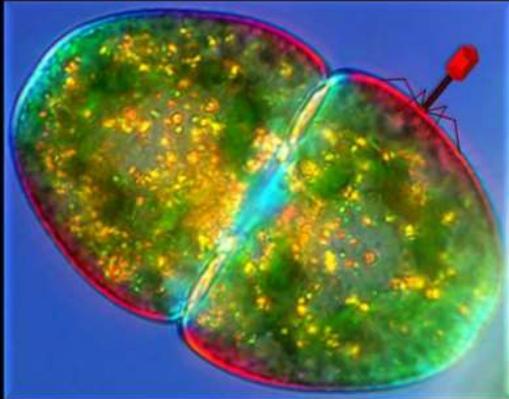
Department of Biological Oceanography, Royal Netherlands Institute for Sea Research, PO Box 59, NL-1790 AB Den Burg, The Netherlands

Received 6 April 2005; returned to author for revision 3 May 2005; accepted 5 July 2005

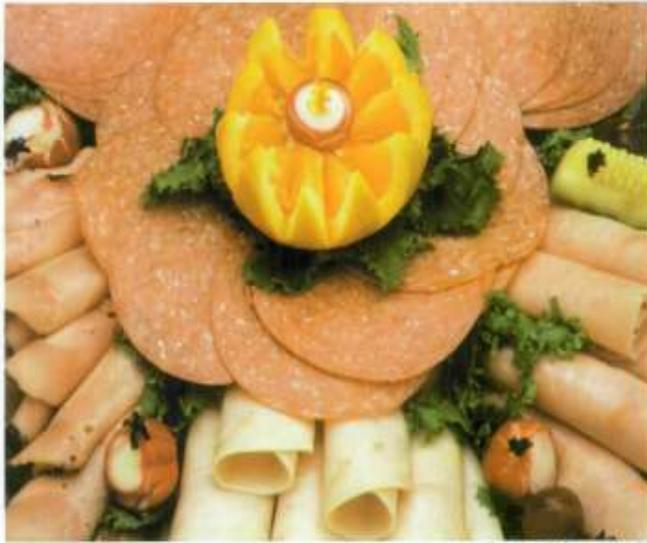
Available online 2 August 2005

Controle de florações de algas

Cyanophage-Biotechnology Innovation



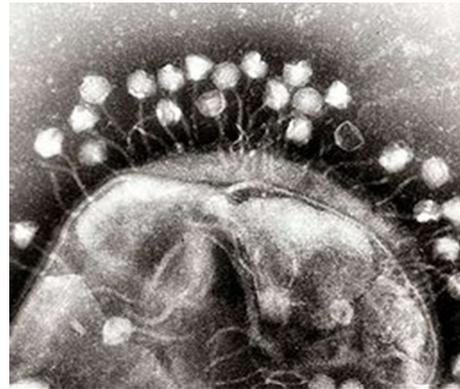
Aditivos alimentares



Bacteria-Eating Virus Approved as Food Additive

By Linda Iltis

Not all viruses harm people. The Food and Drug Administration has approved a mixture of viruses as a food additive to protect people. The additive can be used in processing plants for spraying onto ready-to-eat



2006 – FDA regulamenta uso para controle bacteriano em alimentos: [Listeria monocytogenes](#). LMP-102 (ListShield by Intralytix) para carnes vermelhas e de ave industrializadas.

Pathogen

Escherichia coli
O157:H7

Salmonella

Campylobacter

Listeria monocytogenes

Enterobacter sakazakii

Staphylococcus aureus

Aditivos alimentares

Letters in Applied Microbiology ISSN 0266-8254

UNDER THE MICROSCOPE

Bacteriophages and their application in food safety

P. García, B. Martínez, J.M. Obeso and A. Rodríguez

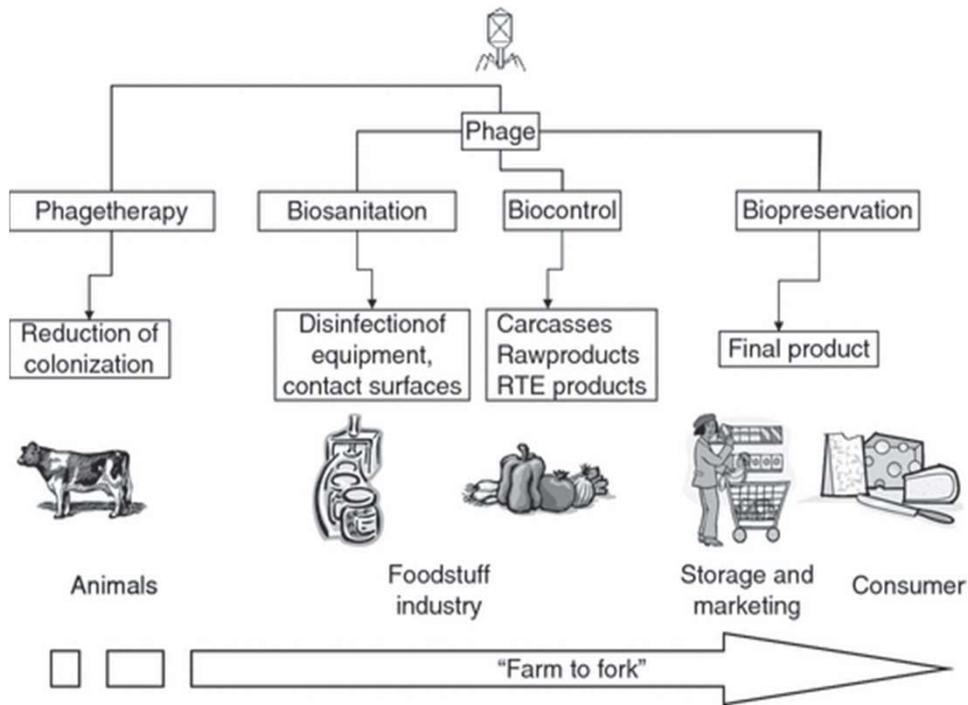
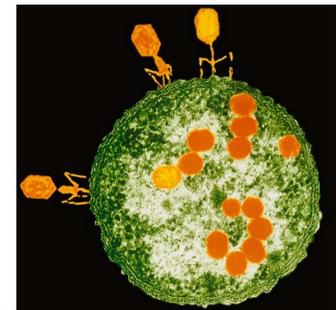


Table 2. Main advantages of bacteriophages as biocontrol tools in food safety

History of safe use
Ubiquitous in nature including food ecosystems
Natural commensals of humans and animals
Extensive clinical use in Eastern Europe
Highly active and specific
No adverse effects on the intestinal microbiota
Innocuous to mammalian cells
Autoreplicative
Can be active against biofilms ←
Genetically amenable
Versatile use along the food chain
Phage therapy, biosanitation, biopreservation
Tools for detecting pathogens
Source of potent antimicrobials
Endolysins and other peptidoglycan hydrolases



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<http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2008.02458.x/full#f1>

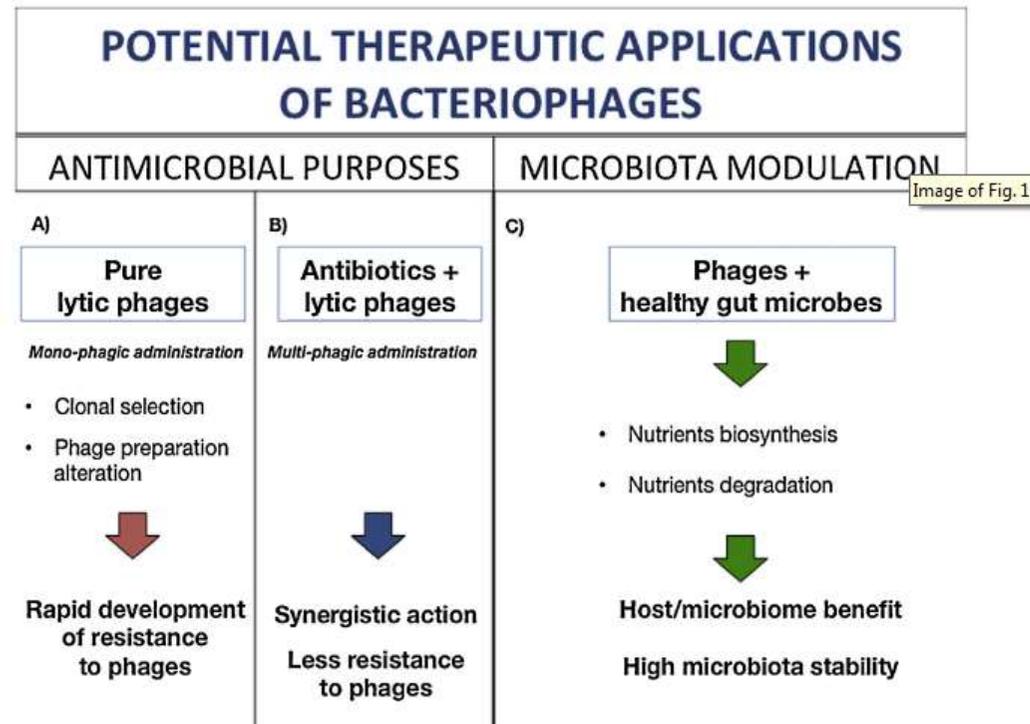
Fins terapêuticos

1941 – Georgia

Tratamento de infecções por bactérias multi-resistentes

Controle da microbiota intestinal

E. Scarpellini et al. / Digestive and Liver Disease 47 (2015) 1007–1012



Fagos “delivery”



The EpiBiome Process



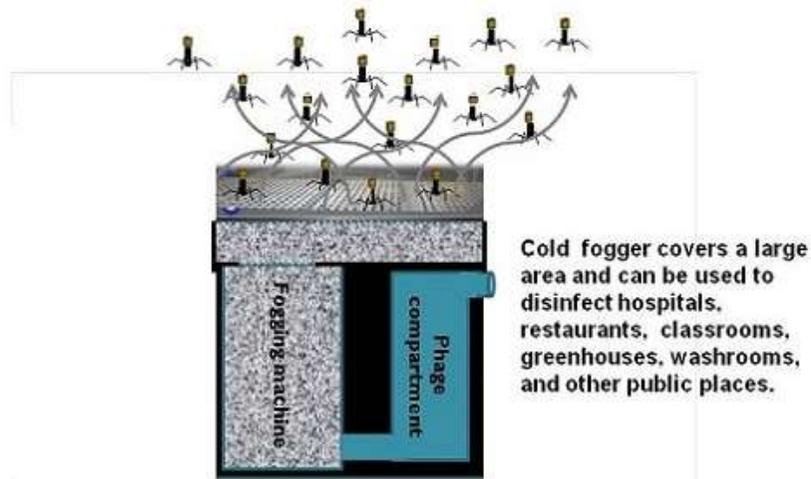
[/www.epibiome.com/products-services/phage-based-technologies/](http://www.epibiome.com/products-services/phage-based-technologies/)

Desinfecção de sistemas de nebulização: hospitalar, industrial, agrícola



Phage Technology
Overcoming barriers to optimize phage technology

Mobile phage fogging system



Cold fogger covers a large area and can be used to disinfect hospitals, restaurants, classrooms, greenhouses, washrooms, and other public places.

Phage cold fogging system: Is a high speed aerosol droplets (less than 15 micron in diameter) systems & is wireless connected with the target bacterial-alarm system.

Clean water from commercial-industrial grey water

Our PhytoPhage solutions can be applied to clean polluted water and for cleaning and recycling commercial and industrial wastewater streams

http://appliedbioresearch.co/Phage_Technology.html



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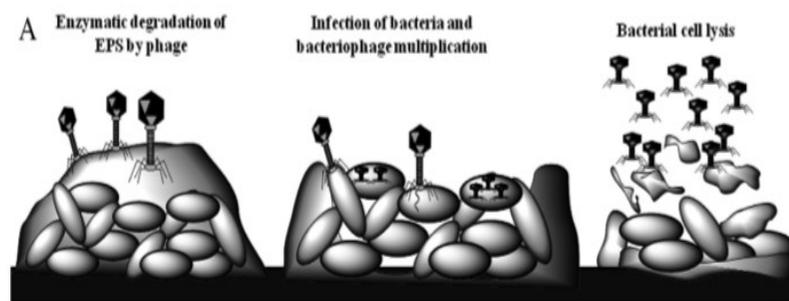
journal homepage: www.elsevier.com/locate/ibiod

Microorganisms: Induction and inhibition of corrosion in metals

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K. Alasvand Zarasvand, V.R. Rai / International Biodeterioration & Biodegradation 87 (2014) 66–74



Controle de *souring* por BRS em poços de produção de petróleo

Paper No.
11222



Phage of sulfate reducing bacteria isolated from high saline environment

ABSTRACT

Sulfate reducing bacteria (SRB) cause significant problems in the petroleum industry through fouling, corrosion of metal pipelines and infrastructure, and by degradation of petroleum in reservoirs (reservoir souring) and storage facilities. Current control efforts include regular cleaning and the use of chemical biocides that are both toxic to the environment and are not always effective. We are actively investigating the potential use of phage for controlling SRB. Phage are natural, bacteriolytic viruses that are highly specific for bacterial hosts and harmless to all other life forms, including humans. The use of phage requires knowledge of the specific bacterial target. Here, we identify the bacteria present in an oil brine and mud sample and report phage efficacy experiments combating the growth of these H₂S



Alguma dúvida?