

# Development of a bacteriophage therapy with the potential for biocontrol of luminescent Vibriosis in marine hatcheries

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(Collaborative project)

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Funded by Proaqua Pty. Ltd.



**PROAQUA**

*Inputs for Aquaculture*



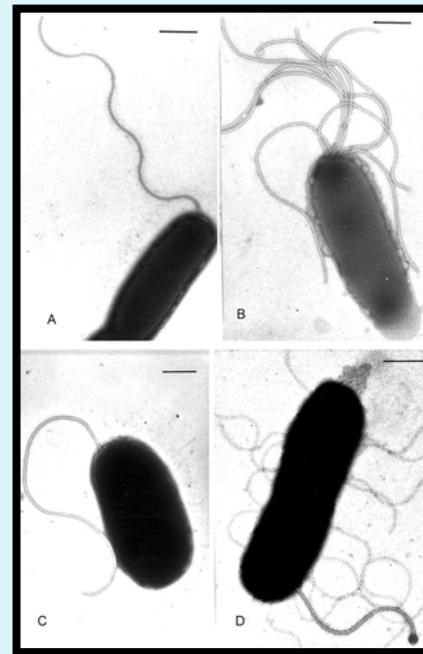
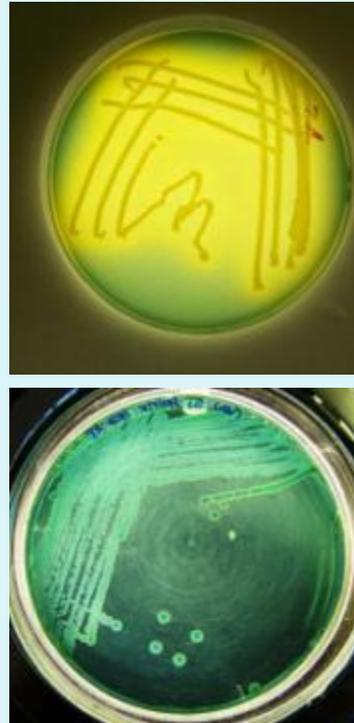
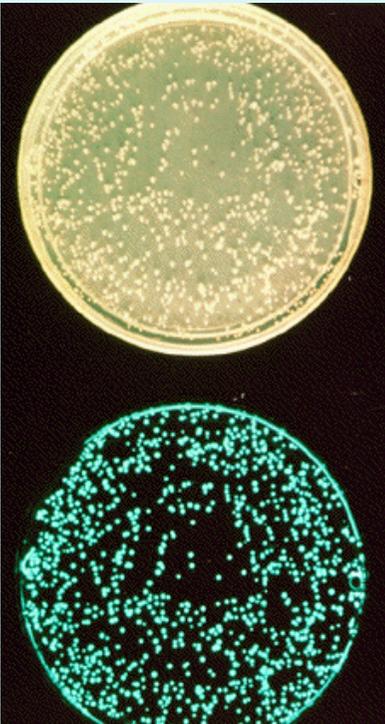
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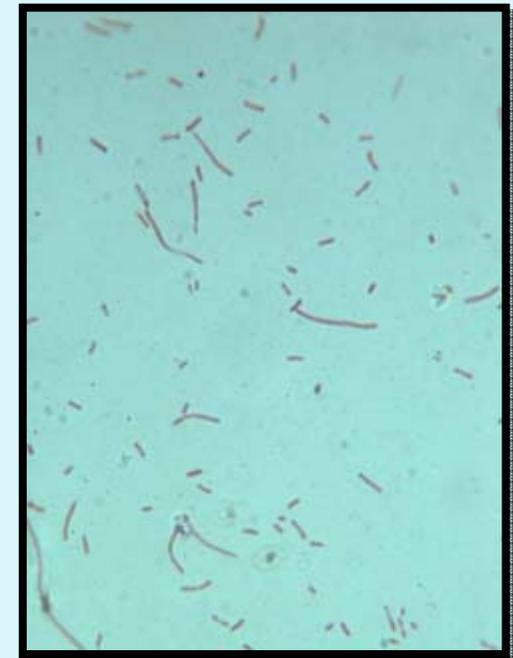
# Bacterial infections a major problem in marine hatcheries

- Mass mortalities 70-90% in hatcheries.
- Pathogenic *Vibrio* spp. mostly *V. harveyi* and closely related species (Moriarty, 1998).

<http://golgi.harvard.edu/hastings/images/bacteria.gif>



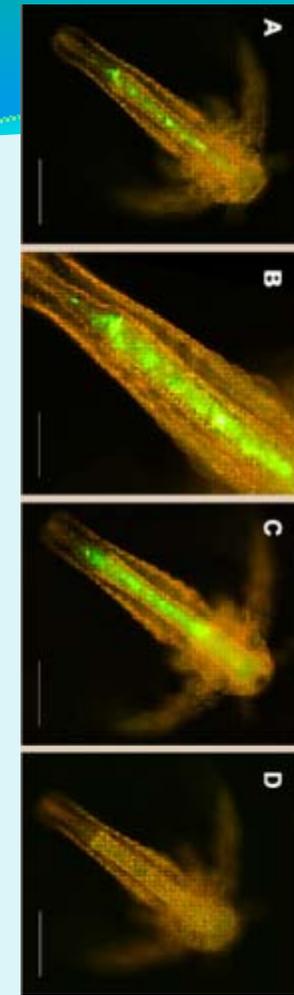
[lux.ibp.ru/info/history.htm](http://lux.ibp.ru/info/history.htm)



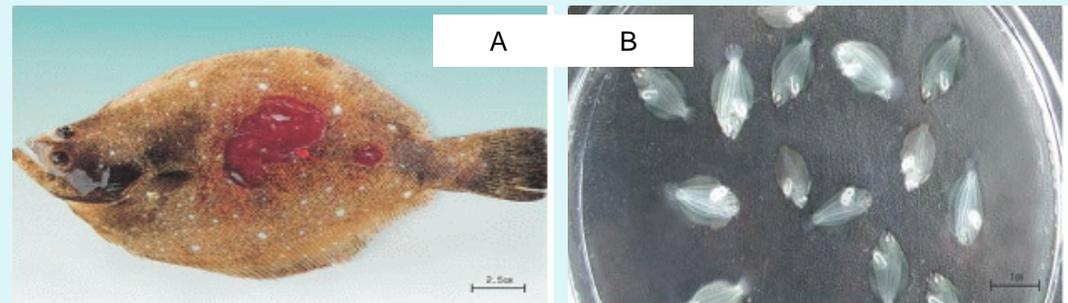
# Vibriosis

## Clinical signs

- Loss of appetite/empty gut.
- Erratic swimming behaviour/lethargy.
- Skin/cuticle lesions.
- Luminescence in culture water or animal gut.
- High mortalities.



[http://ucmexusrresults.ucr.edu/ucmexusrresults/UCMEXUS\\_GRANT\\_TRACKING.displayA?application\\_id=219#images](http://ucmexusrresults.ucr.edu/ucmexusrresults/UCMEXUS_GRANT_TRACKING.displayA?application_id=219#images)



Flounder infected with *Vibrio* sp. A: The external signs, B: An opaque intestine in flounder larvae infected with *V. ichthyenteri*.

<http://www.lib.noaa.gov/retiredsites/korea/diseases/bacterial.html>

# Methods of control

## Methods of bacterial control in hatcheries

- Complex water treatment systems including sand, carbon and cartridge filters, ultraviolet, ozone and chemical treatments.
- Separation of sections such as broodstock, spawning, hatching, live feeds and larval rearing.
- Probiotics, commercial or 'home grown'.



**DISEASE CONTROL REMAINS DIFFICULT**

# Solution? - Bacteriophage

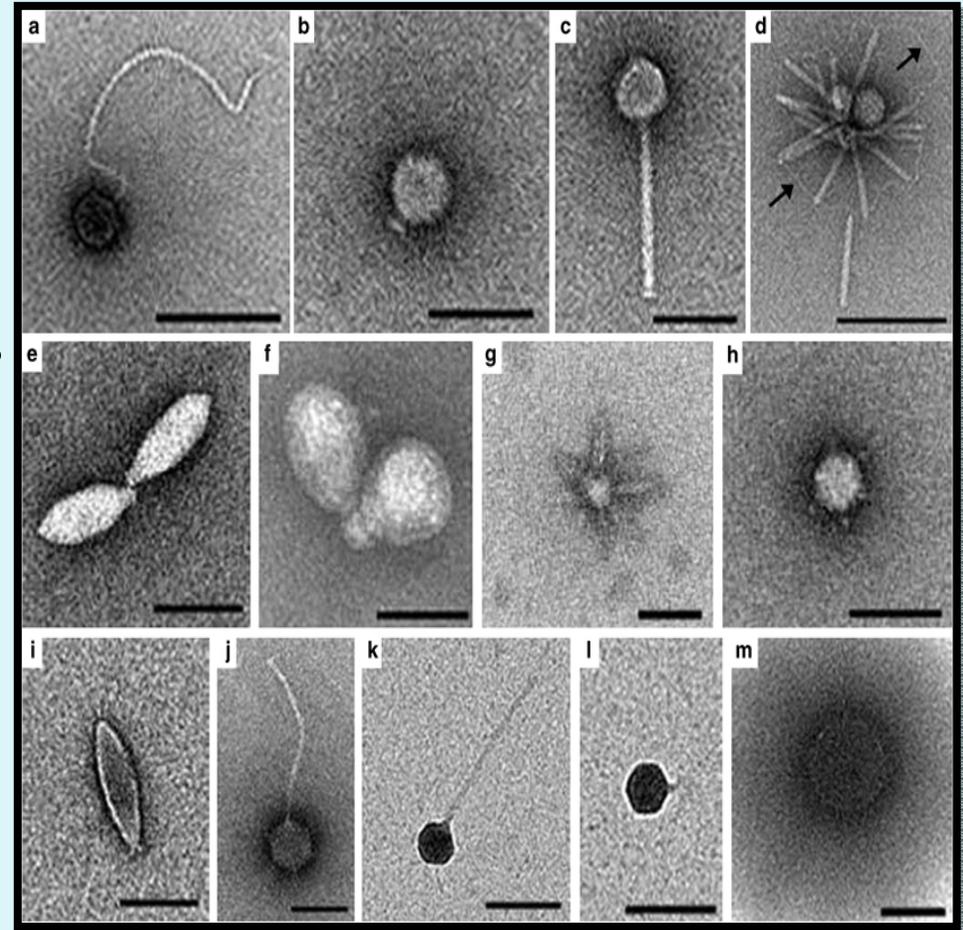
- Bacteriophage, commonly termed phage, is a group of viruses that infect bacteria.

[www.nature.com/.../v2/n5/full/ismej200818a.html](http://www.nature.com/.../v2/n5/full/ismej200818a.html)

- Phage are ubiquitous and are found where ever bacteria colonise including soil, water and intestinal tracks of animals

(Carlton, 1999).

- Many families with different morphologies and often specific hosts.



# Bacteriophage - History

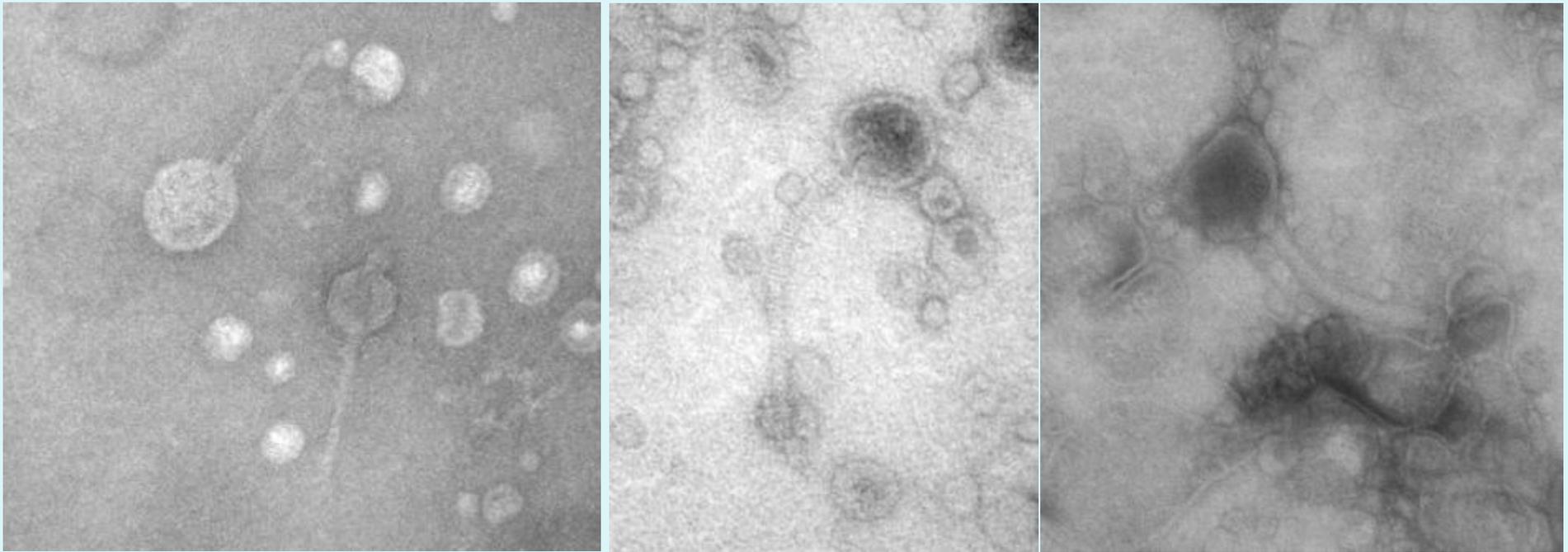
- Have been used for over 60 years in Eastern Europe as alternatives to antibiotics.
- In 2006 the United States Food and Drug Administration (FDA) approved using bacteriophages on cheese to kill *Listeria*.
- In 2007, the same bacteriophages were approved for use on all food products. GRAS status.
- The therapeutic potential for the use of phage in the control of vibriosis in aquaculture has been reported for fin fish (Nakai and Park, 2002) and prawns (Vinod, *et. al.*, 2006, Karunasagar, *et. al.*, 2007) with promising results.



Listex P100 is a suspension of *Listeria*-specific bacteriophages. It can be sprayed, added to the brine bath or be applied to the cheese surface. It can also be mixed into ripening cultures or in washing solutions.

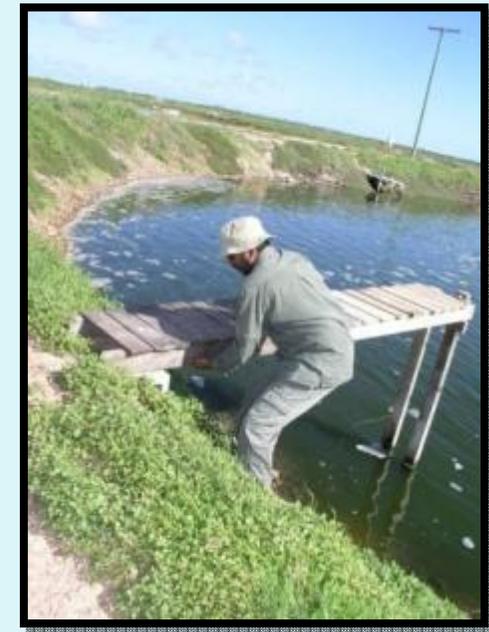
## Project Aim

**To develop a cocktail of bacteriophage that has the potential to control outbreaks of pathogenic *Vibrio* spp. in marine hatcheries.**



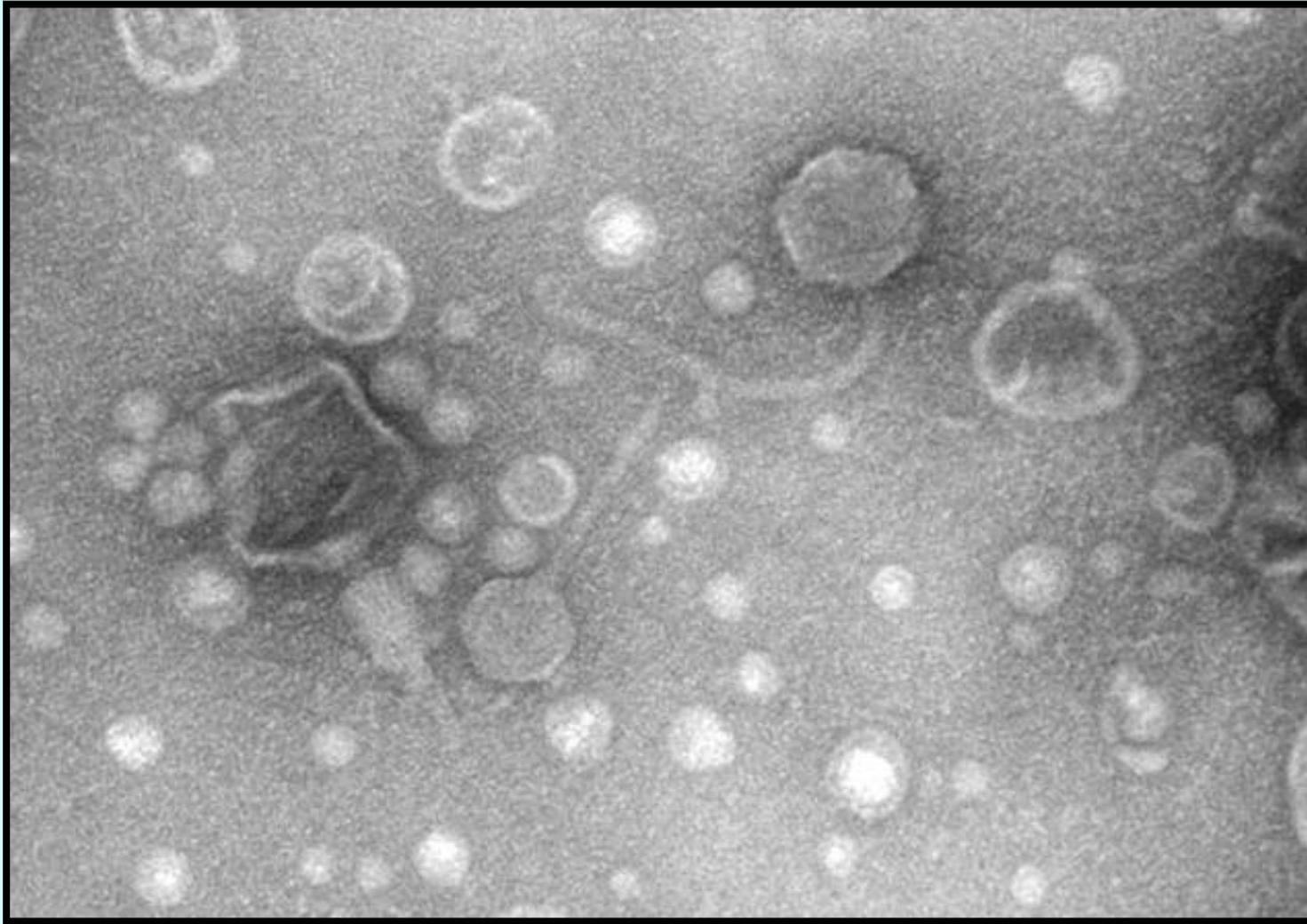
# Aims and Methods

1. Collection and isolation of bacteriophage.
  - Water samples were obtained from Australian prawn farms.
2. Determine the host ranges of bacteriophage isolates.
  - Over 90 *Vibrio* isolates tested against the phage isolates, including *V. harveyi*, *V. campbellii*, *V. alginolyticus*, *V. rotiferanus* and *V. parahaemolyticus*.



## Results - Determine the host ranges of bacteriophage isolates.

- Confirm the presence of bacteriophage in the samples using transmission electron microscopy (TEM).



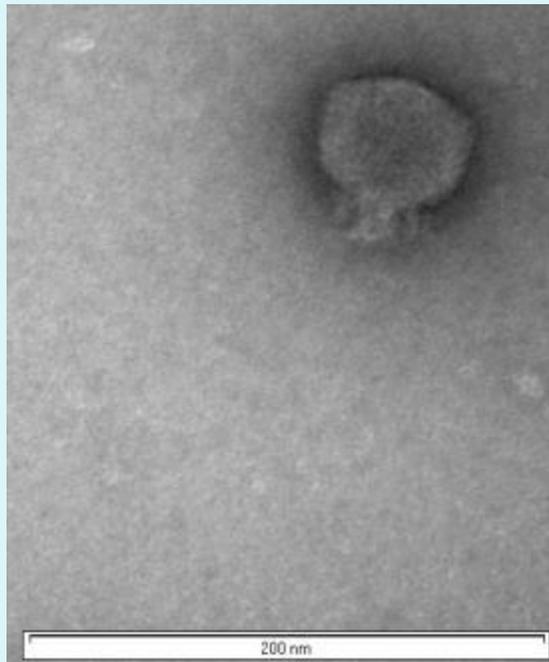
# Results - Purify enriched bacteriophage filtrates.

*Siphoviridae*

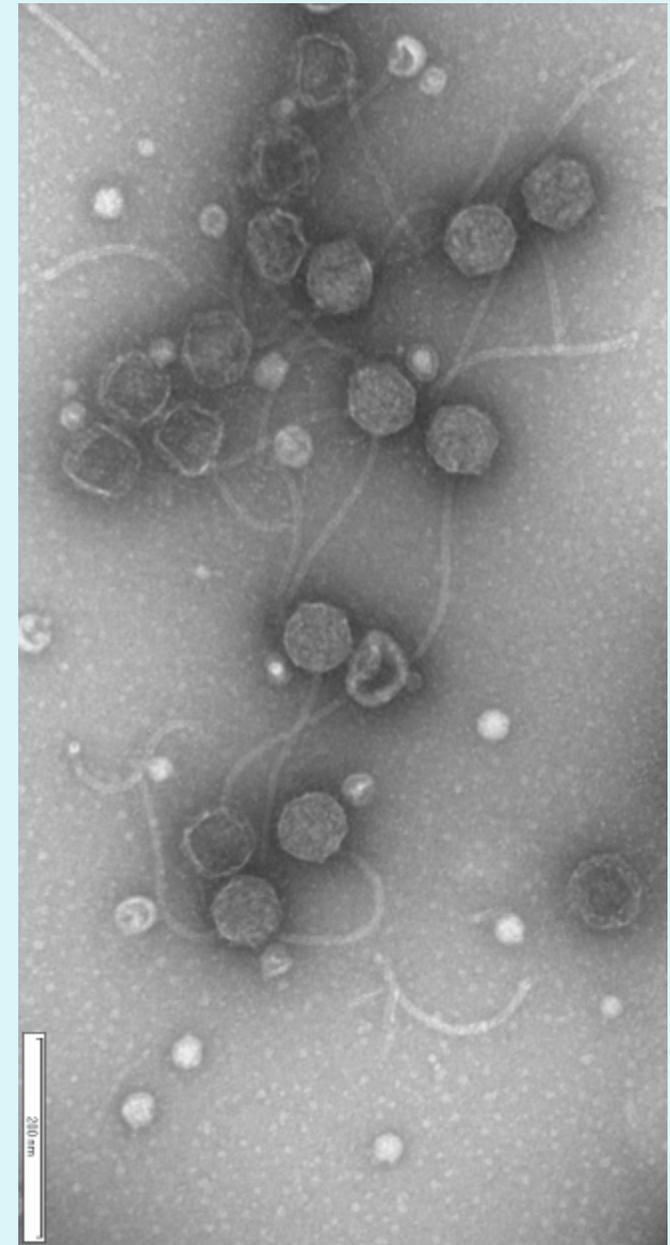
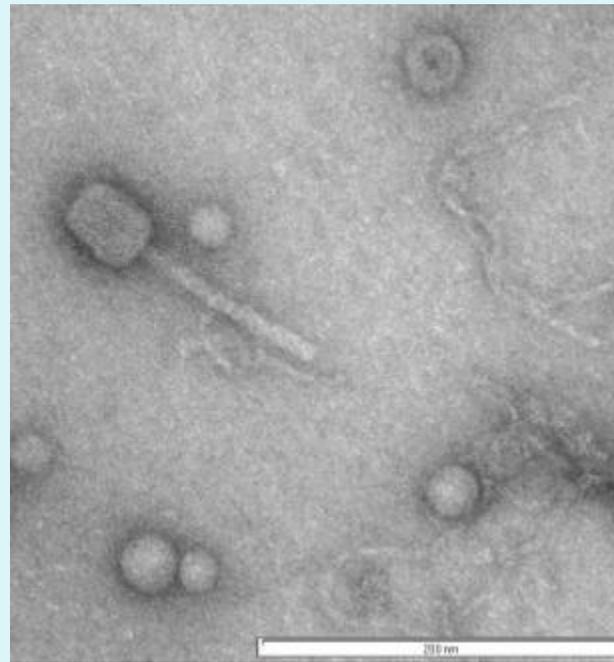
- Confirm the type of bacteriophage in 17 samples using TEM.

*(We acknowledge the technical, scientific and financial assistance from the Australian Microscopy and Microanalysis Research Facility (AMMRF) UQ).*

*Podoviridae*

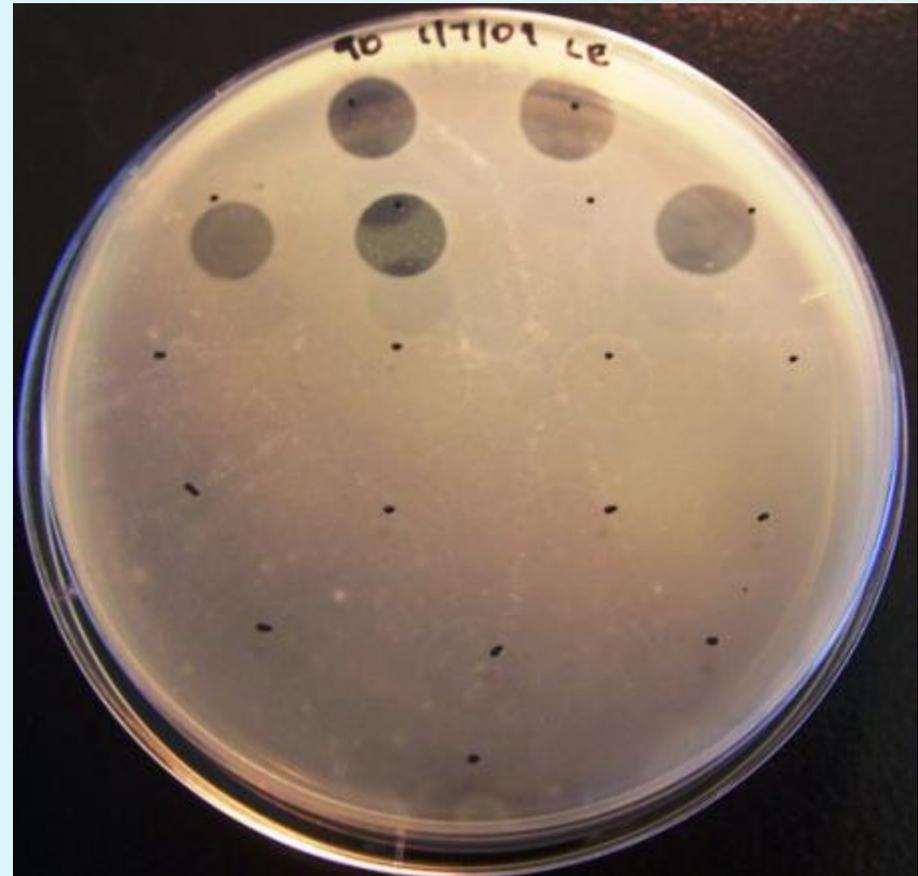


*Myoviridae*



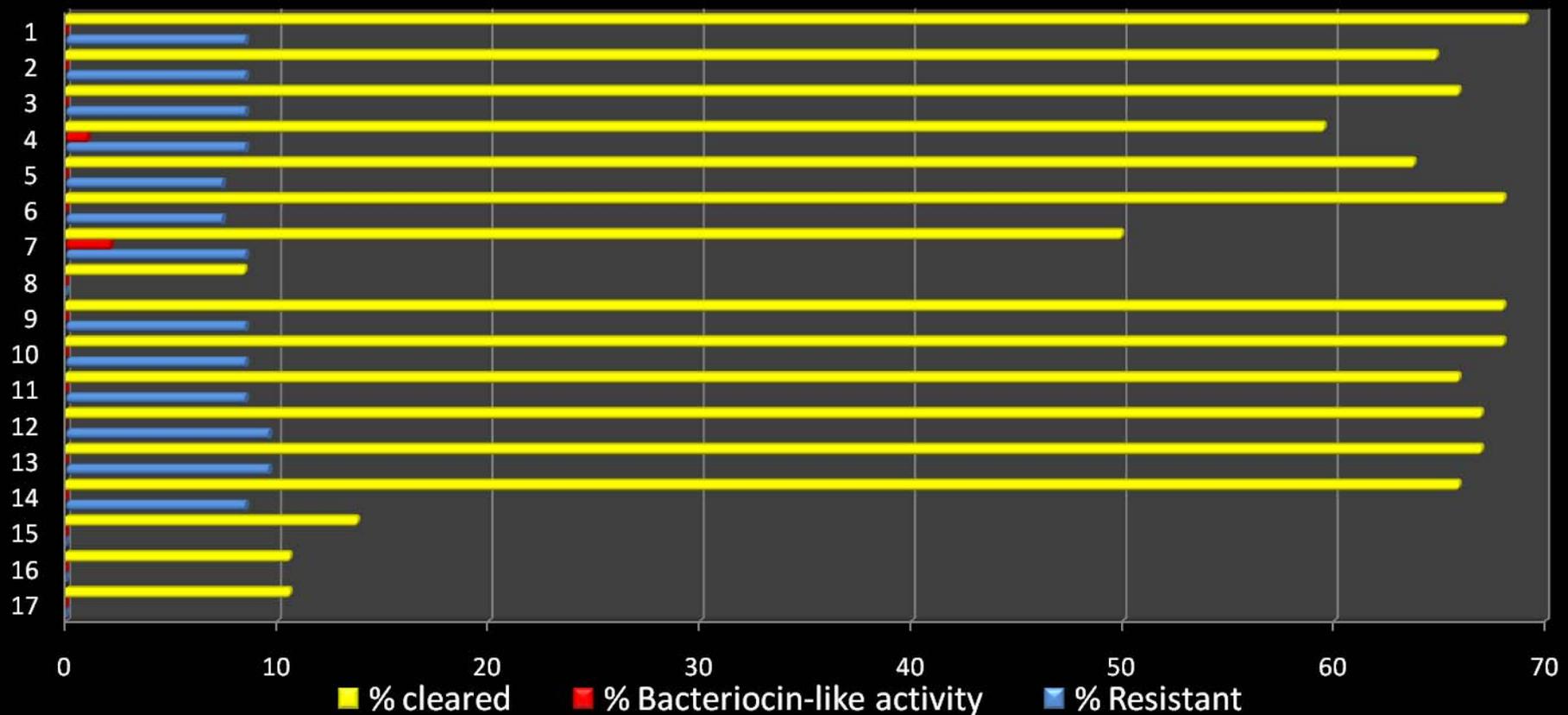
## Results - Determine the host ranges of bacteriophage isolates.

- Produce bacterial lawns to test phage host range.



# Results - Determine the host ranges of purified bacteriophage isolates.

- Over **90** *Vibrio* strains tested against **17** purified phage filtrates.
- 11 new filtrates plus 6 previously purified filtrates (Crothers-Stomps, 2007).
- Collectively, **77%** of these strains were susceptible to the phage filtrates.



# Toxicology trial

A total of 480 PL5 *P. monodon* were used in the trial.

- 15 x PL5 per replicate
- 4 replicates/treatment
- 3 phage cocktails
- 5 day trial

## OUTCOME:

No significant affect of phage preparations on post larval survival.



# Product development

In 2009 a small industry trial was performed with the assistance of a prawn hatchery.

- Two phage cocktails were trialled.
- Animals were treated before moult – Zoea to Mysis, Mysis to PL
- In the absence of a disease event - No detrimental affects observed.

With the assistance of marine hatcheries, further industry trials will be performed in the coming months.

- Establish the therapeutic potential of the bacteriophage isolates in the control of vibriosis in commercial marine hatcheries.



# Questions/Comments

**LAB INITIO**  
by Nick D Kim

