

# Tim Flegel



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For the past decade, Dr. Tim Flegel has led about 70 research scientists and post-graduate students at the Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), a joint operation of the Faculty of Science, Mahidol University and the National Science and Technology Development Agency.

He now serves as an NSTDA advisor and continues to research shrimp pathology and defense mechanisms.





# Research Progress on Causes of EMS

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# Work on bacteria done so far

- Many groups have isolated bacteria from EMS/AHPNS shrimp and tested them in bioassays
- Most of these have been *Vibrio* species
- So far, none of these have produced AHPNS pathology, even with some new types found
- Our group is using a shotgun sequencing approach to see if an un-culturable type may be involved



# Combined results 27 samples in 3 lots

- Highest difference between test and control ponds:
  - Burkholderiales:
    - *Burkholderiaceae* genus ***Ralstonia***
    - *Comamonadaceae* genus ***Delftia***
  - Actinomycetales:
    - *Microbacteriaceae* genus ***Leifsonia***
    - *Nocardiaceae* genus ***Rhododoccus***
    - *Actinosynnemataceae* genus ***Lentzea***
  - Aeromonadales:
    - *Aeromonadaceae* genus ***Aeromonas***
  - Vibrionales:
    - *Vibrionaceae* genus ***Photobacterium***
  - Pseudomonadales:
    - *Pseudomonadaceae* genus ***Pseudomonas***

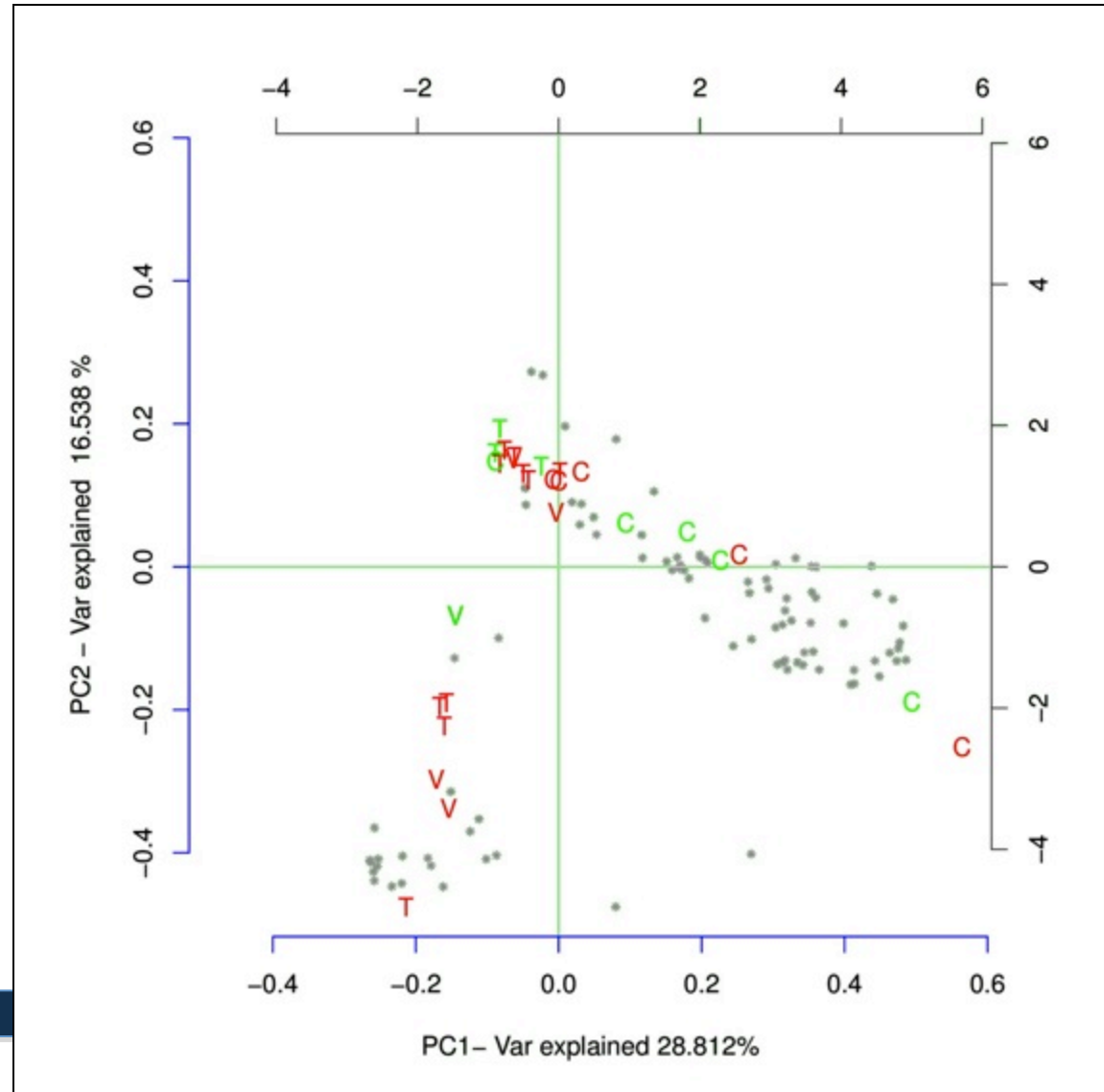


# Example principal component analysis

Red = outbreak  
Green = control

C = China  
T = Thailand  
V = Vietnam

The analysis showed that outbreak and control ponds could not be separated on the basis of bacterial profiles



# Summary for mass sequencing

- Analysis of 3 sequencing lots revealed no new or unique bacteria clearly linked to AHPNS
- Also no consistent species dominant in shrimp in affected ponds
- Lots 2 & 3 had more reads for *Vibrio* than lot 1 but little difference in test and control numbers
- However, the analysis is based on rRNA gene sequence and would not reveal phage interactions
- Isolates of the same species with and without a resident phage would behave differently
- Thus, a separate analysis is needed to reveal phage-bacterium partnerships



# Possible phage involvement?

- It is well-known that bacteriophages can transfer lethal toxin genes to bacteria:
  - *Corynebacterium diphtheriae* produces diphtheria toxin when lysogenized by beta phage.
  - *Vibrio cholerae* produces cholera toxin when lysogenized by CTX phage
  - *Vibrio harveyi* produces a lethal shrimp toxins when lysogenized by VHS1 phage and VHML phage
- Sometimes the lysogenized bacteria lose phages upon serial subculture (they become “cured”)
- We believe that this possibility should also be examined



# 2 phage bacterium pairs in hand

- One is our Rayong phage probably with *V. parahaemolyticus*
- Another phage in another lab (wishes to remain anonymous) probably with *V. fluvialis*
- Preliminary results for both pairs give no or low mortality with bacterial injection alone
- Some mortality with undiluted phage produced from bacterial cultures
- 100% mortality within 24 hours upon injection of bacterium plus phage
- These tests need to be repeated with diluted phage to confirm and to reproduce AHPNS





# Conclusions for EMS/AHPNS

- No conclusions regarding cause can yet be made
- Even the question of an infectious or non-infectious agent as the cause is still unanswered
- Disease outbreaks from different causes are still being lumped together under “EMS”
- The number of outbreaks that fit the AHPNS case definition in Thailand is still low (about 6%)
- All of the confirmed cases have been from the southeastern coast of the Gulf of Thailand
- Outbreaks from other unknown causes may be occurring without AHPNS pathology



An aerial photograph showing a large, dense mangrove forest in the foreground and middle ground. To the left, there are several rectangular aquaculture ponds, likely for shrimp farming, with some structures and roads nearby. The background shows a hilly landscape with more vegetation and some buildings. The sky is clear and blue.

The King's project at Kungkabaen

Thanks for your kind attention