George Chamberlain

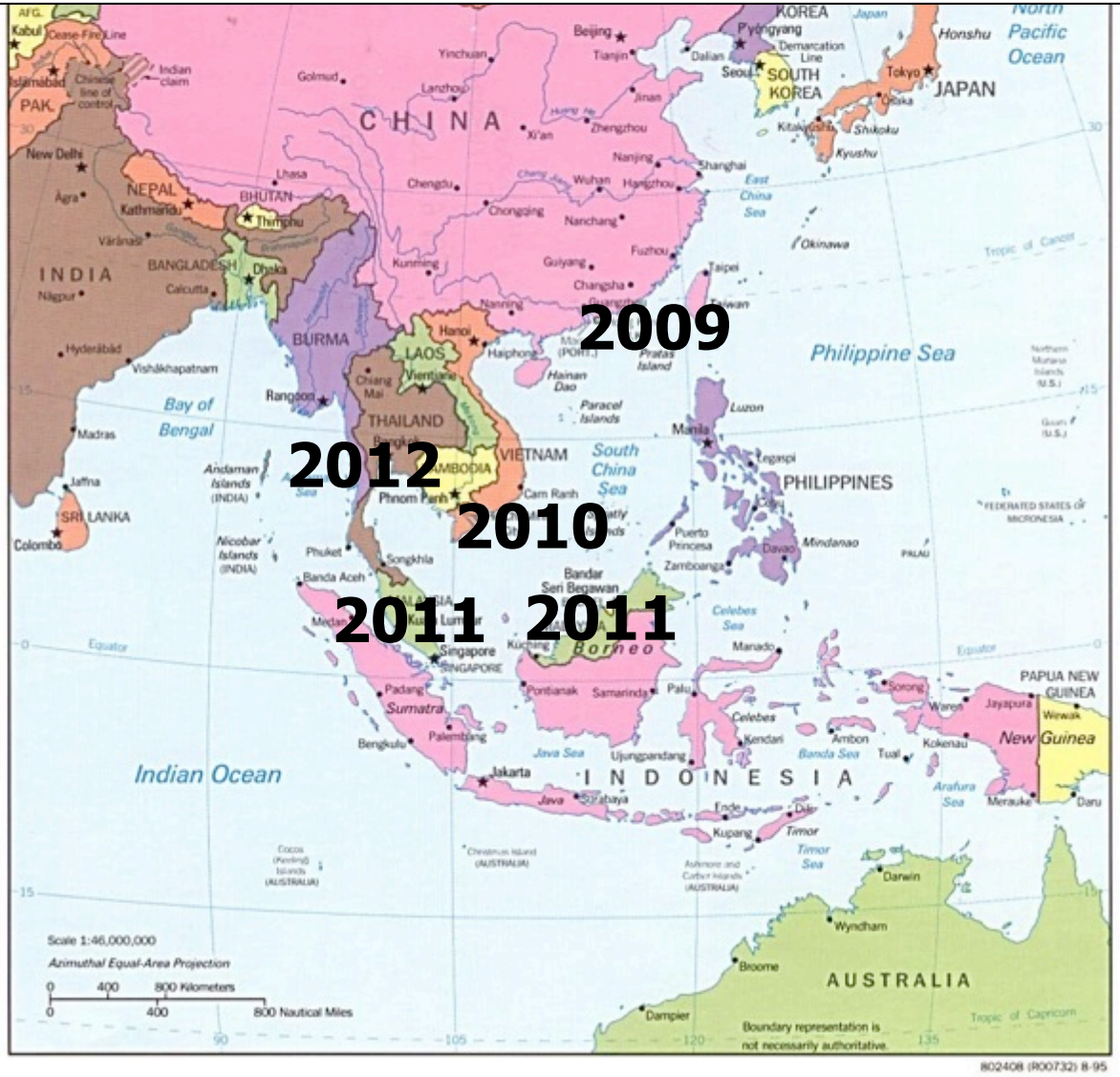
Global Aquaculture Alliance
United States

Dr. George Chamberlain, president of the Global Aquaculture Alliance since 1997, is also a former president of the World Aquaculture Society. With broad experience in farmed seafood, Chamberlain helped establish Integrated Aquaculture International in 2004. Now called iAqua, the company owns and manages breeding, nutrition and production facilities in Asia and the Americas. Chamberlain also developed shrimp feeds and production systems for Ralston Purina Co. and Monsanto.

EARLY MORTALITY SYNDROME

George Chamberlain

Spread of EMS/AHPNS in East and SE Asia



Gross Signs of EMS/AHPNS

- Atrophy of the hepatopancreas (HP).
- Often pale, yellowish or white within the HP connective tissue capsule.
- Black spots or streaks sometimes visible.
- HP does not squash easily between thumb & finger.

Juvenile *Penaeus vannamei* from Vietnam.
Left with EMS; right appears normal.

Itinerary for Mission 26 July to 6 August

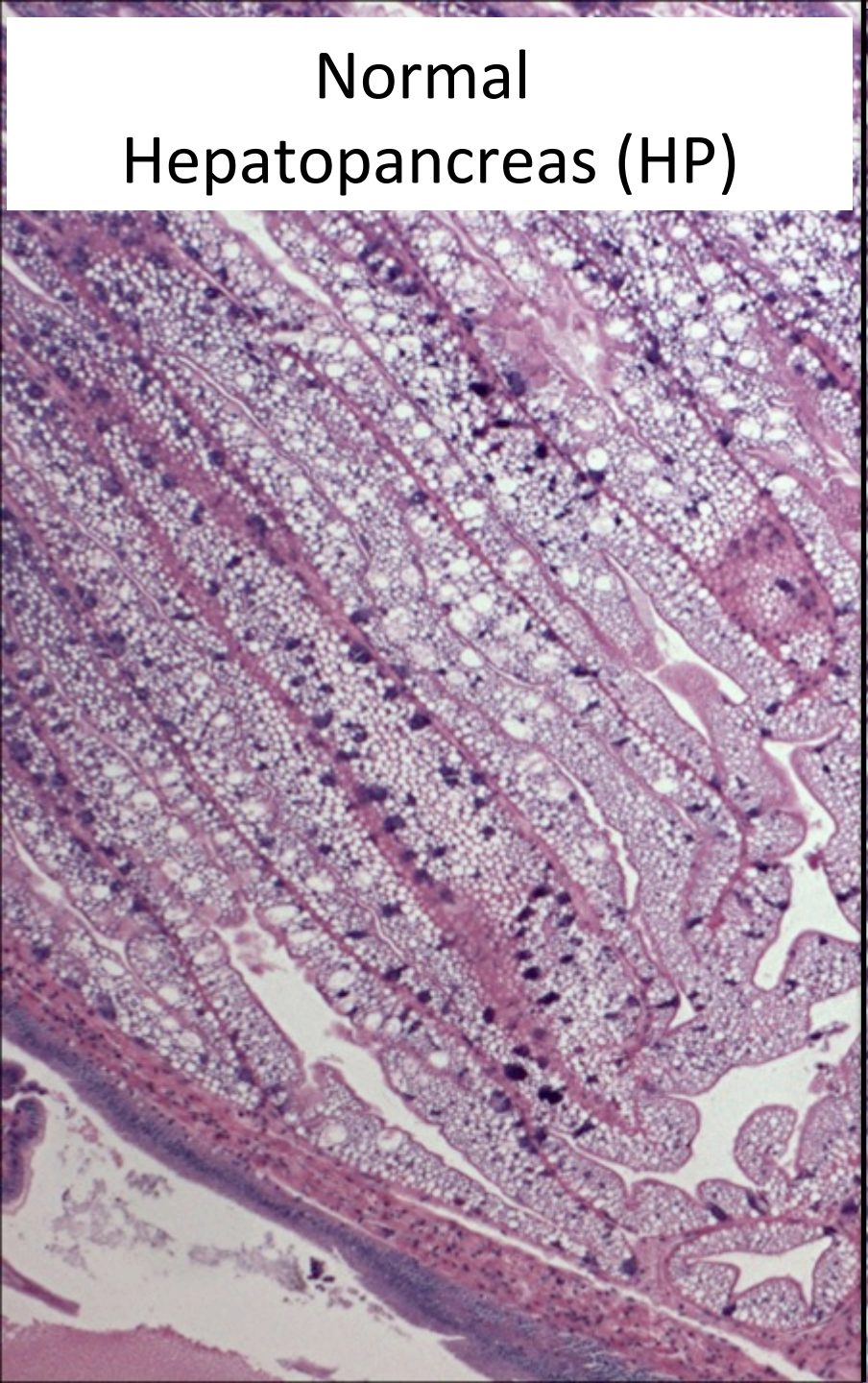
GOAL 2013

Don Lightner and Loc Tran (his graduate student)

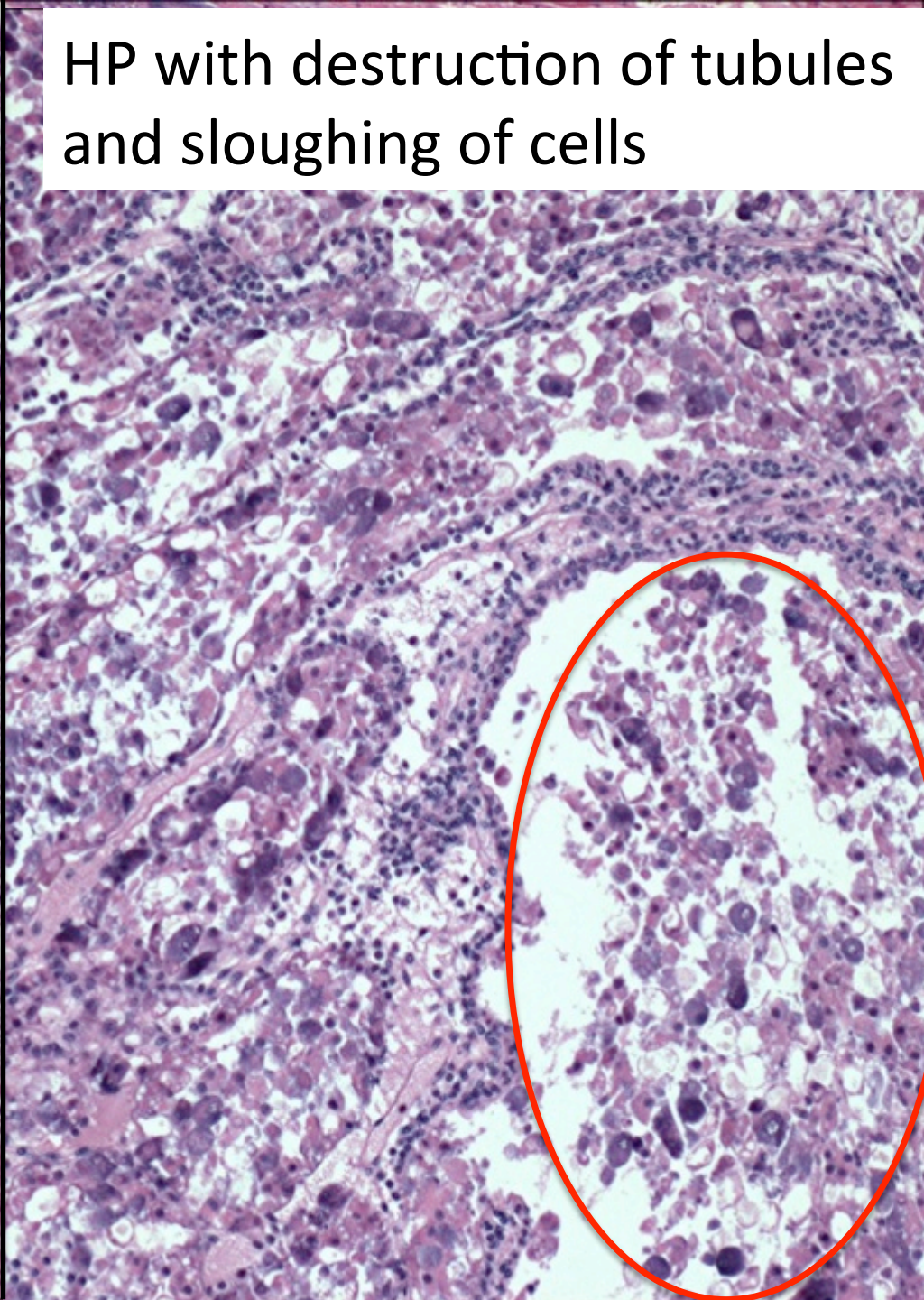
Research by Lightner's Team

- Two Phases of EMS/AHPNS
  - Acute phase
    - Dysfunction of HP tubule cells (R, B, F & later E-cells)
    - Sloughing of HP tubule epithelial cells
  - Terminal phase
    - Destruction of HP by opportunistic *Vibrio* spp.

Normal Hepatopancreas (HP)



HP with destruction of tubules and sloughing of cells



Lightner's Lab Considered Several Possible Causes

- **Infectious agent:**
  - per os & injection infectivity study using **frozen tissues.**
- **Toxic Algae:**
  - Algae populations were similar in affected and unaffected ponds.
- **Toxicant(s):**
  - Feed toxicity study.
  - Two commonly used crustacides tested.
  - Studies with sediments collected from affected areas.

  => **All studies failed to induce EMS pathology**

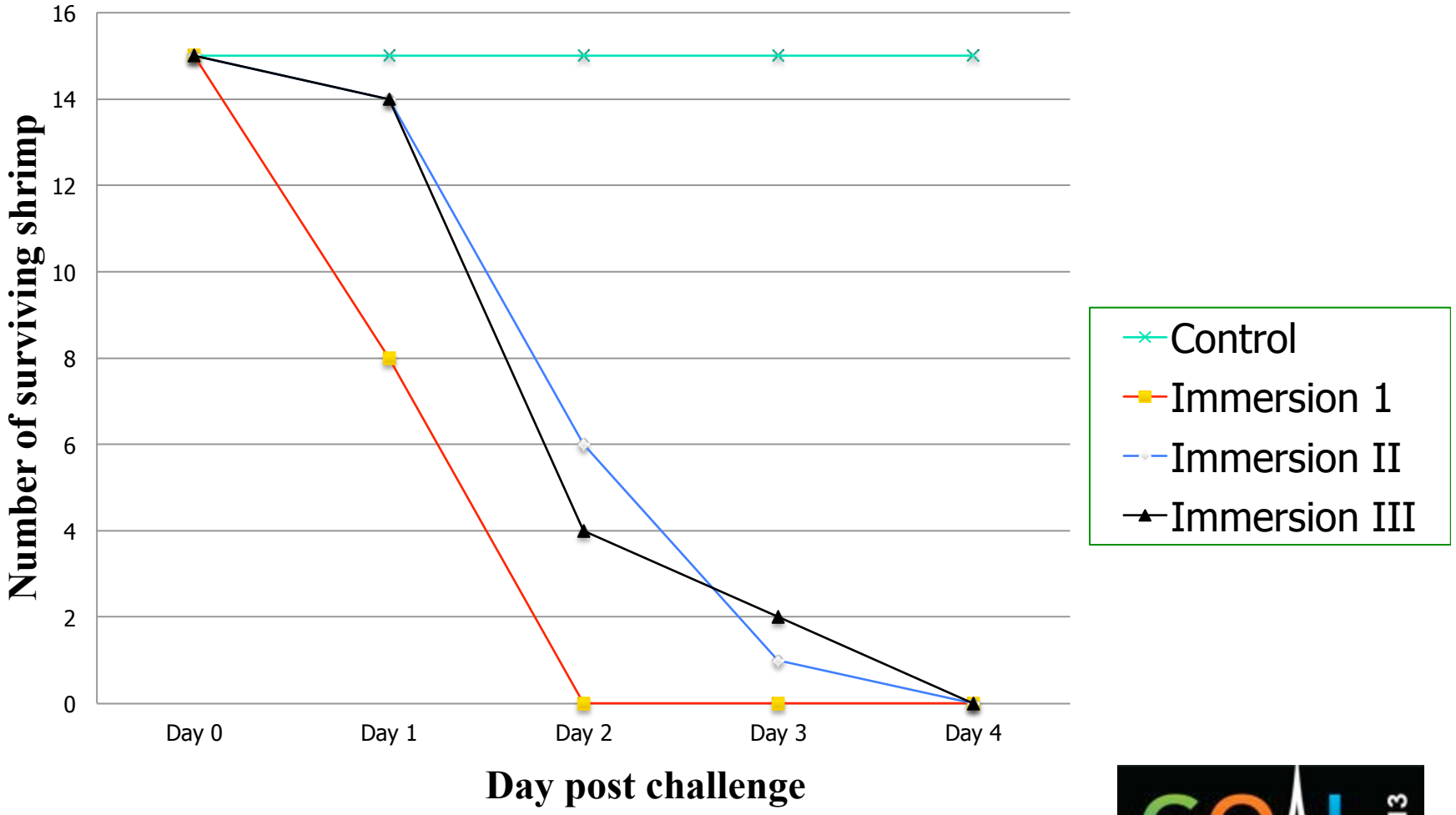
Break through came in July 2012 with infectivity studies in Vietnam

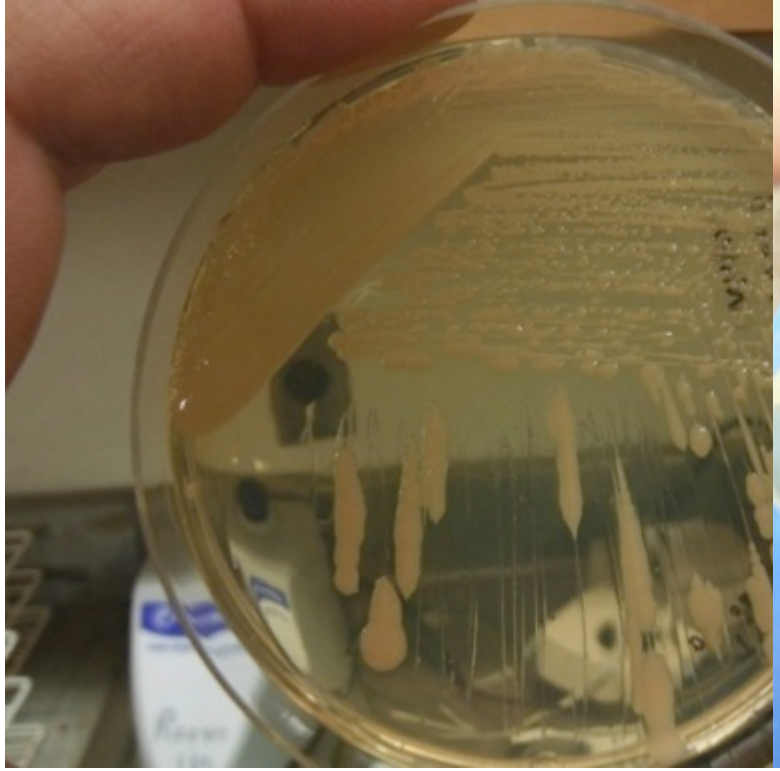
- **Per os study**
- SPF *P. vannamei* shrimp were fed infected tissues for 5 days.
- Tissue: Fresh *P. monodon* (the infected shrimp used for preparing the inoculums were kept alive/or chilled & never frozen).
- => This induced EMS typical pathology in SPF *P. vannamei.*
- *Only bacteria from EMS-infected shrimps' stomachs induced EMS.*
- **Conclusion:**
- ***EMS is infectious, caused by a bacterial agent(s) that can be found in the infected animals' stomachs.***

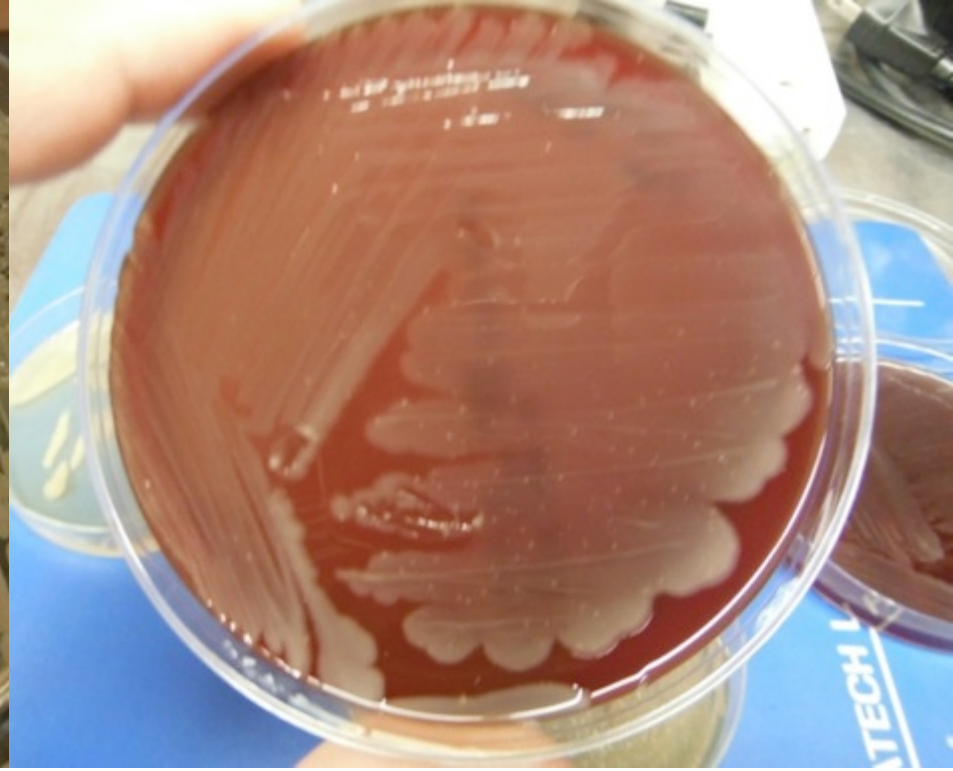
Immersion challenge test with bacteria grown in broth media

- Immerse SPF shrimp in 2.10^8 cells/ml for 15 min.
- 2.10^6 cells/ml added to the tanks/jars during the experiment.

Immersion resulted in rapid and complete mortality







- Identified agent as a strain of ***Vibrio parahaemolitycus.***
- This strain did not contain human toxins (TDH or TRH)

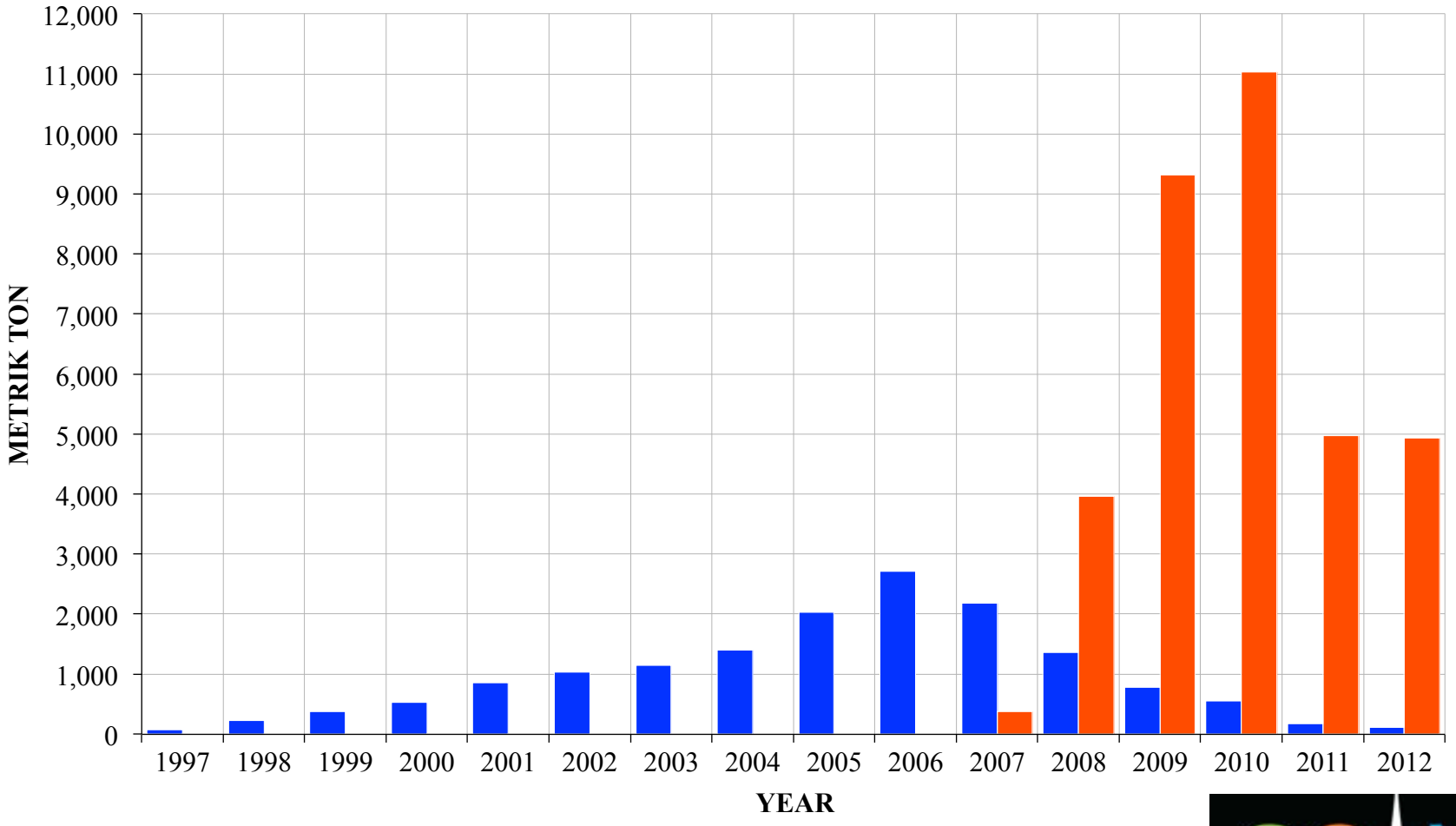
Summary of Lightner's Research

- ***EMS is caused by V. parahaemolyticus, transmitted via oral route; the agent colonizes shrimp stomach to produce toxin(s) causing HP dysfunction***
- Continuing Research:
  - Bacterial phage likely not involved.
  - Quorum sensing: Bacteria produce toxin only when the colony reaches a certain density (cell-to-cell signaling)
  - Characterization of toxin(s) and toxin producing gene(s) – studies are underway at UAZ-APL.
  - PCR and ELISA diagnostic methods underway at UAZ-APL.
  - Challenge lab is operational to evaluate which shrimp breeding lines and products control EMS/AHPNS

Experience at Agrobest in Malaysia

Noriaki Akazawa

TOTAL PRODUCTION (Monodon+Vannamei)

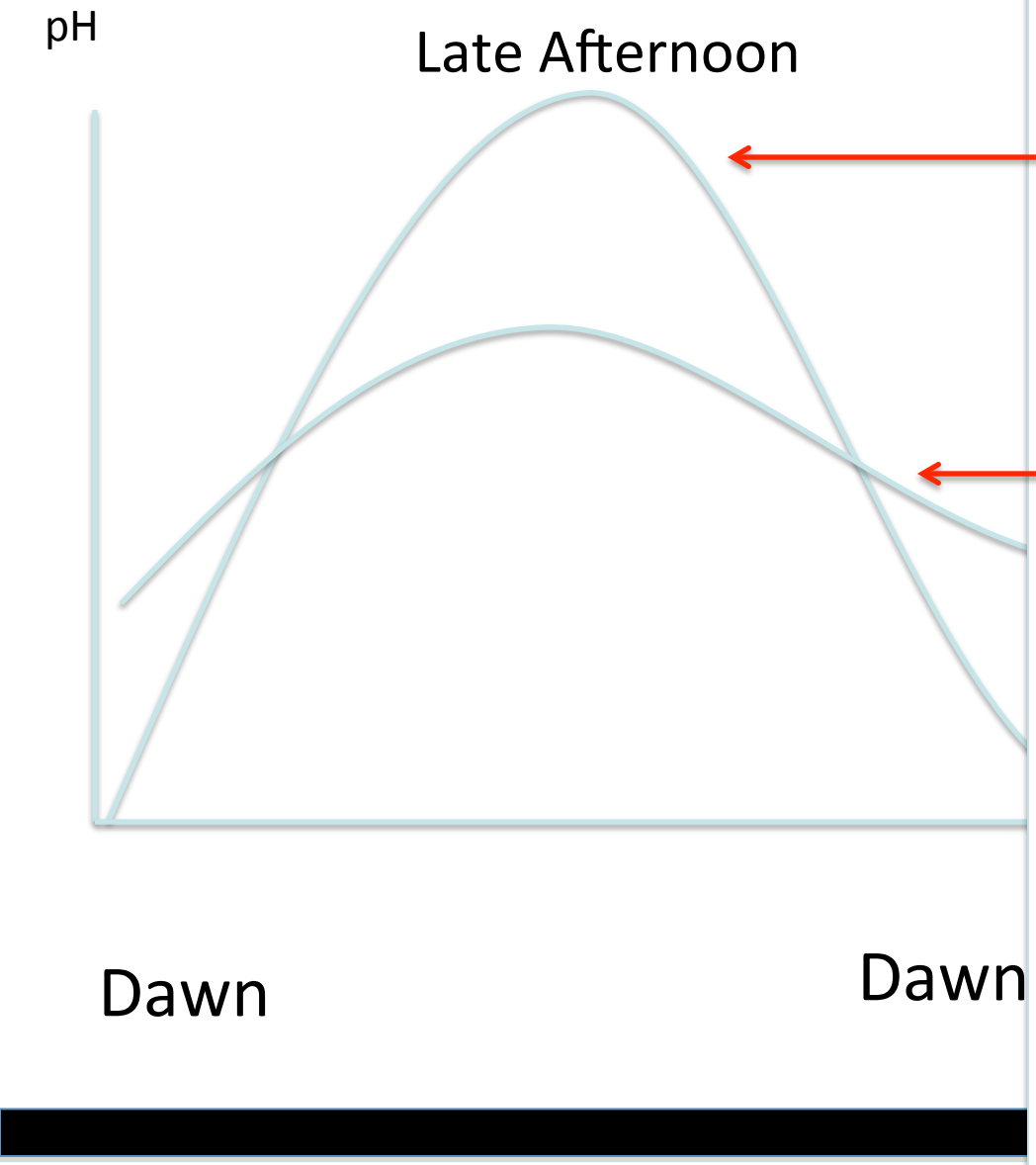


GOAL 2013

Chlorine gas injection system

Shot showing monofilament string about 2 m above pond

Learning to Overcome Early Mortality Syndrome



Key to management of pH is control of nutrients that stimulate algae.

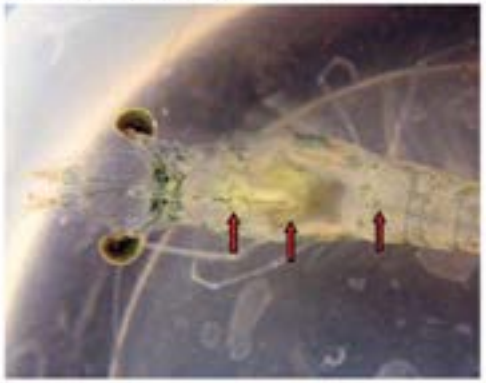
High phytoplankton density
Low alkalinity

Low phytoplankton density
High alkalinity

Lightner's and Akazawa's work is summarized in articles in the Advocate

challenge: ems/ahpns

EMS/AHPNS: Infectious Disease Caused By Bacteria



Gross signs of AHPNS in shrimp include an empty stomach (left), a pale atrophied hepatopancreas and an empty midgut.

Loc Tran
Aquaculture Pathology Laboratory
School of Animal and Comparative Biomedical Sciences
University of Arizona
Tucson, Arizona 85721 USA
thuloc@email.arizona.edu

Linda Nunan
Rita M. Redman
Donald V. Lightner Ph.D.
Aquaculture Pathology Laboratory
School of Animal and Comparative Biomedical Sciences
University of Arizona

Kevin Fitzsimmons Ph.D.
Department of Soil, Water and Environmental Science
University of Arizona
Tucson, Arizona, USA

Summary:
Asia's shrimp-farming industry has been heavily affected by early mortality syndrome or acute hepatopancreatic necrosis syndrome. Studies by the University of Arizona Aquaculture Pathology Laboratory identified the causative agent for AHPNS as a unique strain of *Vibrio parahaemolyticus* that can produce toxins responsible for the primary pathology in affected shrimp. Infected live shrimp and fresh shrimp tissues can transmit the disease to "clean" shrimp, but the agent is inactivated by freezing and thawing. Affected shrimp pose no human health concerns.

The shrimp-farming industry in Asia, the largest and most productive region in the world, was affected in 2009 by an emerging disease called early mortality syndrome or, more descriptively, acute hepatopancreatic necrosis syndrome. AHPNS began to cause significant production losses in southern China, and by 2012 had spread to farms in Vietnam, Malaysia and Thailand.

AHPNS has not only caused serious losses in terms of production and revenues in affected areas, but has also been responsible for secondary impacts on employment, social welfare and international market presence. The disease has caused significant shortages of shrimp products for the global market, which in turn impacted the global price of shrimp.

Pathology

AHPNS usually occurs within 45 days in shrimp ponds with newly stocked postlarvae of both black tiger shrimp, *Penaeus monodon*, and Pacific white shrimp, *Litopenaeus vannamei*. The gross signs of AHPNS are evident in pond-side examinations of affected shrimp accompanied by dissection and examination of the hepatopancreas organs of the shrimp.

Shrimp with early AHPNS show pale to white coloration of the hepatopancreas, as well as atrophy that can reduce the size of the organ by 50% or more. In the terminal phase of the disease, black streaks or spots due to melanin deposition from hemocyte activity appear in the hepatopancreas. Mortality in affected shrimp ponds can approach 100% within a few days of disease occurrence.

The histopathology of AHPNS presents as an acute progressive degeneration of the hepatopancreas from proximal to distal with dysfunction of tubular epithelial cells. Such cells round up and detach from the affected tubules, and become necrotic within the tubules or the gut lumen. In the terminal phase of AHPNS, the hepatopancreas shows marked hemocytic infiltration and development of massive secondary bacterial colonization that occurs in association with the necrotic and sloughed epithelial cells. This unique pathology suggested that the primary lesions in the hepatopancreas are mediated by a toxin.

Preliminary Studies

Two approaches were initially undertaken by the University of Arizona Aquaculture Pathology Laboratory (UAZ-APL) to determine the etiology of AHPNS. The studies sought to identify a possible environmental toxin in water,

challenge: ems/ahpns

Environmental Trigger For EMS/AHPNS Identified In Agrobest Shrimp Ponds

Shrimp held in aquaria with pH within the "safe zone" behaved normally, while those in aquaria outside the safe zone exhibited AHPNS symptoms and mortality.

Noriaki Akazawa
Agrobest Malaysia Sdn. Bhd.
Batu 20, Jalan Pekan-Nenasi
26600 Pekan, Pahang, Malaysia
agbest26@yahoo.co.jp

Mitsuru Eguchi
Kinki University
Nara, Japan

Summary:
In studies of early mortality syndrome/acute hepatopancreatic necrosis syndrome at a large integrated shrimp farm in peninsular Malaysia, results indicated that the disease originated with infected postlarvae and quickly spread throughout the farm. Subsequent data analysis and aquarium trials indicated the disease manifested only when a given environmental parameter, pH, was within a specific range. Survival rates have improved with management of the target parameter to avoid the zone of EMS/AHPNS susceptibility.

Agrobest Sdn. Bhd. is a large integrated shrimp farm located in the state of Pahang, Malaysia (see cover photo). It consists of 461 plastic-lined ponds with an average area of 0.75 ha. In 2010, the farm produced approximately 11,000 mt of Pacific white shrimp, *Litopenaeus vannamei*, and 500 mt of black tiger shrimp, *Penaeus monodon*.

Early mortality syndrome, also known as acute hepatopancreatic necrosis syndrome (AHPNS), appeared at Agrobest in early January 2011 in five ponds that were stocked with postlarvae from the same hatchery about one month earlier.

While the disease appeared to originate from this hatchery, not all ponds stocked with postlarvae from that hatchery were affected. The plankton blooms in the five affected ponds were an unusual color – dark green, almost black, which is more typically seen at the end of a production cycle. Environmental factors appeared to have a role in the manifestation of the disease.

Radiating Expansion

Within one week after the outbreak in the first five ponds, several neighboring ponds were affected with the disease. Within two months, it had spread throughout the farm. Typical mortality in the affected ponds was 70 to 80%, and all ages and sizes of shrimp were affected.

Based on the radial outward expansion of the disease from the initial epicenter, it appeared to be caused by a virulent pathogen, but repeated tests for all known viruses were negative. Shrimp production at Agrobest fell dramatically, as it did throughout Malaysia. Research on the AHPNS problem was quickly ramped up.

Environmental Interaction?

In an initial trial, stressed shrimp from an affected pond were transferred to aquaria with clean pond water. During the morning of their transfer, the shrimp were severely stressed, exhibited opaque muscle tissue and were inactive on the bottom. High mortality was expected.

However, by that afternoon, the shrimp in the aquaria had recovered normal coloration and activity. They continued to behave normally for an additional week, when the trial was discontinued.

In a second trial, non-infected shrimp were transferred to aquaria with water from a pond experiencing a die off from AHPNS. The shrimp remained unaffected, indicating that pond water in itself was not necessarily infective.

In a third trial, fresh dead shrimp from an infected pond were put in aquaria with non-infected shrimp. In some of the aquaria, the dead shrimp were placed in cages immersed in the aquaria that kept the dead animals from the live shrimp. There was no mortality in aquaria with caged dead shrimp, but mortality commenced in one or two days in aquaria that received dead shrimp outside cages. This indicated the pathogen could be transmitted through direct exposure to dead shrimp.

Despite the widespread outbreak at Agrobest, some ponds remained unaffected, which supported the hypothesis of environmental interaction with the disease. To better understand and manage the AHPNS, the Agrobest team intensified its collection of environmental data from each pond and teamed up with researchers at Kinki University in Japan to isolate, purify and sequence the DNA of the pathogen.