Diseases & Disease Management in Shrimp Aquaculture

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Aquaculture Pathology Laboratory
The World Organization for Animal Health (OIE, Paris, France)
Reference Laboratory
USDA-APHIS Approved & ISO 17025 Accredited Laboratory
School of Animal & Comparative Biomedical Science
The University of Arizona, Tucson, Arizona, USA
Agenda

- Introduction of Aquaculture Pathology Laboratory

- Components of Sustainable Shrimp Aquaculture
  - Importance of disease in shrimp aquaculture

- Major Diseases in Shrimp Aquaculture
  - White Spot Disease - a continuing threat to shrimp aquaculture
  - EMS/ AHPND - a game changer in shrimp aquaculture
  - EHP - an emerging threat to shrimp aquaculture

- Antibiotics in Aquaculture: Use & Detection

- Management of Diseases in Shrimp Aquaculture
Aquaculture Pathology Laboratory

- Pre-eminent place for infectious disease research in shrimp
- Our Missions: Disease Diagnostic & Other Services to Shrimp Industry Worldwide, Education & Training and Basic Research.

Main Campus

West Campus

Founding Director: Prof. Donald V. Lightner (Retired)
Aquaculture Pathology Laboratory- Core Teams


Histopathology

PCR Diagnostics

Microbiology & Molecular Biology

Program Coordinator

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Domains of Shrimp Disease Diagnostics in Aquaculture Pathology Laboratory

Pathology

Microbiology

Molecular Diagnosis

**North America**
2 Countries

**Latin America**
9 Countries

**Europe**
6 Countries

**Africa/Middle East**
6 Countries

**Asia**
5 Countries

**Oceania**
2 Countries

Live and frozen shrimp, feed and feed ingredients are screened for shrimp pathogens.

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Key Components in Sustainable Shrimp Aquaculture

GENETICS: Genetically superior SPF/SPR stock

NUTRITION: High Quality Balanced Diet

BIOSECURITY: Disease Management

Healthy Harvest
Aquaculture is the only way to increase the seafood supply.
Global Trends in Shrimp Aquaculture

FAO Data

Shrimp production per region

Funete: (GOAL 2016-2017)

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Shrimp Production in SE Asia

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Species included are *L. vannamei*, *R. monodon* and *other*. *M. rosenbergii* is excluded.
Shrimp Production in the Americas

Source: GOAL 2019, FAO 2017

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Major Shrimp Producing Countries in Asia & the Americas

Shrimp Aquaculture in Asia: 2010 – 2014
Major Producers

China is expected to recover by 2017. Thailand will only see a partial recovery, being displaced from second to fifth place in the region. Production increases in all major farming nations are expected by 2017. The largest increases are expected in Indonesia.


Shrimp Aquaculture in Latin America: 2010 – 2017
Major Producers

Ecuador is ramping up production as output from other countries declines. Production in 2017 is expected to be 22% higher than in 2013. Brazil expects to reach 85,000 tons by 2017. Mexico heavily impacted by EMS in 2013 and 2014: production down by 50%, with near full recovery by 2017.

Americans ate 16.1 pounds of seafood per capita... of which shrimp is 4.6 pounds in 2018...

In 2018, the United States imported $22.4 billion worth of edible seafood products and exported $5.6 billion..

Sea food trade deficit in the US reaches ~16.8 billion in 2018 of which ~7.0 billion is shrimp.
Per capita seafood consumption in the U.S.

Top 10 Consumed Seafoods in US
Source: AboutSeafood.com

Per capita seafood consumption in the US in lb.

2017 vs. 2016 Top 10 Rankings

<table>
<thead>
<tr>
<th>Species</th>
<th>2017</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp</td>
<td>4.40</td>
<td>4.10</td>
</tr>
<tr>
<td>Salmon</td>
<td>2.41</td>
<td>2.18</td>
</tr>
<tr>
<td>Canned Tuna</td>
<td>2.10</td>
<td>2.10</td>
</tr>
<tr>
<td>Tilapia</td>
<td>1.08</td>
<td>1.18</td>
</tr>
<tr>
<td>Alaska Pollock</td>
<td>0.78</td>
<td>0.96</td>
</tr>
<tr>
<td>Pangasius</td>
<td>0.71</td>
<td>0.89</td>
</tr>
<tr>
<td>Cod</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Crab</td>
<td>0.52</td>
<td>0.54</td>
</tr>
<tr>
<td>Catfish</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>Clams</td>
<td>0.31</td>
<td>0.34</td>
</tr>
</tbody>
</table>


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Major Species of Farmed Shrimp

Pacific white shrimp
*Penaeus vannamei*

Blue shrimp *Penaeus stylirostris* (top & bottom)
*P. vannamei* (middle)

Black tiger shrimp
*Penaeus monodon*
GOAL 2016 Survey: Top Issues in Shrimp Aquaculture

- Diseases
- International market prices
- Production costs - Feed/Fishmeal
- Seed stock quality & availability
- Product quality control
- Access to disease-free broodstock
- Production costs - Others
- Environmental management
- Feed quality and availability
- Production costs - Fuel

Not Important, Moderately Important, Extremely Important

Asia, Americas

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Evolution of Pathogen Discovery in Shrimp Aquaculture

1966/1972 - CP (Unestam)
1971 – BMNV (Momoyama)
1974 – BP (Couch)
1977 – MBV (Lightner & Redman)
1982 – HPV (Lightner & Redman)
1981 – IHHNV (Lightner)
1984 – F. solani (Fusariosis) (Colorni)
1985 – NHP (Mendoza et al)
1989 – YHV (Limsuwang, Chantanachookin)
1991 – RPS (Lu et al)
1993 – TSV (Hasson et al.)
1994 – WSSV (Inouye et al & Takashi et al)
1995 – MrNV (Arcier)
1996 – MoV (Walker)
1996 – PRDV (Inouye)
2001 – IMNV (Lightner et al)
2006 – MSGS (Sritunyalucksana et al)
2006 – LSNV (Srisala)
2007 – CMNV (Zhang et al.,)
2010 – AHPND/EMS (Tran et al)
2009 – EHP
2009 – S. khirikhana (Prachumwat)
2020 – PmMDV (Penzes et al.)
2002 – WSSV (Inouye et al & Takashi et al)
2014 – SHIV (Qiu et al.)
2015 – Wenzhou viruses (Li et al)
2017 – MrGV (Hooper et al.)
2018 – MrGV (Hooper et al.)
2019 – S. khirikhana (Prachumwat)

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OIE-Listed Crustacean Diseases-2019

Viral:
- Taura Syndrome – TSV
- White Spot Disease – WSSV
- Yellow Head Disease – YHV/GAV
- Infectious Hypodermal & Hematopoietic Necrosis Virus – (IHHNV)
- Infectious Myonecrosis Virus – IMNV (listed May 2007)
- White Tail Disease – MrNV (listed May 2007)

Bacterial:
- Necrotizing Hepatopancreatititis bacterium-NHP-B
- Acute Hepatopancreatic Necrosis Disease-AHPND (listed in 2016)

Fungal:
- Crayfish plague- Aphanomyces astaci
Estimated losses caused by OIE-listed shrimp virus diseases since their emergence

<table>
<thead>
<tr>
<th>Virus – region</th>
<th>Year of emergence</th>
<th>Product loss to industry</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHNV – Americas</td>
<td>1981</td>
<td>$0.5–1 billion</td>
</tr>
<tr>
<td>YHV – Asia</td>
<td>1991</td>
<td>$0.5 billion</td>
</tr>
<tr>
<td>TSV – Americas</td>
<td>1991/92</td>
<td>$1–2 billion</td>
</tr>
<tr>
<td>TSV – Asia</td>
<td>1999</td>
<td>$0.5–1 billion</td>
</tr>
<tr>
<td>WSSV – Asia</td>
<td>1992/93</td>
<td>$6 billion</td>
</tr>
<tr>
<td>WSSV – Americas</td>
<td>1999</td>
<td>$1–2 billion</td>
</tr>
<tr>
<td>IMNV – Americas</td>
<td>2004</td>
<td>$100–200 million</td>
</tr>
<tr>
<td>IMNV – Asia</td>
<td>2006</td>
<td>$1 billion (estimated)</td>
</tr>
</tbody>
</table>

*Acute Hepatopancreatic Necrosis Disease (AHPND)/ Early mortality syndrome (EMS), resulted ~$7.5 billion losses in Southeast Asia and a loss of 100,000 jobs since 2011.

*Davies & Shinn, 2016

Lightner et al., 2012. J. Inv. Pathol. 110: 174-183
White Spot Disease- A Threat to Sustainability of Shrimp Aquaculture Worldwide
THE WHITE SPOT VIRUS PANDEMIC
(Year of First Occurrence by Location)


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Some Recent WSD Outbreaks

• 2017: USA- Crayfish in Louisiana
• 2016: Australia- *P. monodon*
• 2013: Kingdom of Saudi Arabia- *P. indicus*
• 2010-2012: Mexico, in *Penaeus vannamei*:
• 2012: Brunei – in *P. stylirostris*
• 2012: Madagascar – in *P. monodon*
• 2011: Saudi Arabia – in *P. indicus*
• 2011: Mozambique – in *P. monodon*
White Spot Disease Clinical Signs

- Sudden reduction in feeding, lethargic.
- Red discoloration of body, soft, loose shells
- White spots 0.5 to 2 mm under cuticle
- Up to 100% mortality within 3 days of onset of disease signs.

*Natural & experimental hosts is >100 species (decapods, non-decapod crustaceans, polychete etc.)*
White Spot Disease Histopathology

Intranuclear inclusion bodies
Early Mortality Syndrome (EMS)/ Acute Hepatopancreatic Necrosis Disease (AHPND)- Most Lethal Bacterial Disease in Shrimp Aquaculture
AHPND Geographic Distribution

**Eastern Hemisphere**
- 2009 = China
- 2010 = Vietnam
- 2011 = Malaysia
- 2012 = Thailand
- 2015 = Philippines, Myanmar, Bangladesh

**Western Hemisphere**
- 2013 = Mexico
- 2017 = Texas, USA
Diseased shrimp become lethargic and anorexic.
Pale & atrophied HP
Sloughing of epithelium in HP tubule
Soft darker shell and mottling of the carapace.
A Schematic Representation of Shrimp Digestive System

Esophagus (E)
Gastric mill (GM)
Hepatopancreas (HP)
Mid gut (MG)
Hind gut (HG)
Anus (A)

Histopathology of AHPND Affected Shrimp

Healthy Shrimp

AHPND Affected Shrimp

Detection of AHPND in Texas, USA

**PCR Screening of TX-Samples**

- **pirB 392 bp**
- **pirA 284 bp**
- **230 bp**

**Identification of Vibrio sp. in Texas samples**

- **pirA** 284 bp
- **345 bp**
- **482 bp**

**Genotyping Texas isolates of V. parahaemolyticus**

- **pirA** 284 bp

**Dhar et al., 2019. Dis Aquatic Org. 132: 241-247.**
Enterocytozoon hepatopenaei (EHP)-
A Fungal Pathogen Threatening Shrimp Aquaculture
Enterocytozoon hepatopenaei (EHP)

- First described in *Penaeus monodon* from Thailand.
- Severely retarded growth of cultured shrimp & “Size Variability”.
- It infects only the tubules of the hepatopancreas, which damages the ability of this critical organ to absorb nutrition from digested feed.
- Causes chronic mortality in severe cases.
- EHP does not require intermedia host.
- EHP Infection increases susceptibility to AHPND and secondary vibriosis (Aranguren et al., 2016).
Enterocytozoon hepatopenaei (EHP) Clinical signs
**EHP Geographic Distribution**

- **Asia:** Thailand, Vietnam, India, China, and Indonesia
- **Americas:** Venezuela

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Histopathology of EHP Infected Shrimp

- **EHP Plasmodium**
- **EHP Spores**
- Sloughed off epithelial cells in the lumen of Hp tubule, Loss of lipid droplets
- Giemsa Staining

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Developing a Non-lethal Sampling Method for EHP Detection

Antibiotics in Shrimp Aquaculture

• Commonly used during the production cycle of shrimp (larval and growth phases)

• Associated problems include
  • Human health problems
  • **Bacterial resistance**
    • Altered biochemical composition of the sediment
  • Accumulation of antibiotic residues in shrimp also alters the intestinal flora in humans and causes food poisoning and allergies (Ma et al., 2006)

The article describes how the uncontrolled use of antibiotics is affecting the American population
Antibiotic Resistant Bacteria Isolated From Shrimp

• The biggest concern **Antimicrobial Resistance**

• *Klebsiella* spp. resistant to reported tetracycline and nalidixic acid have been isolated from shrimp imported and sold in the USA (Nawaz et al., 2012)

• The multi-drug resistant *Klebsiella* spp. are an important reservoir and could represent a potential risk for humans
How Are Antibiotic Residues Detected in Shrimp

Screening Methods:
- Thin layer chromatography (TLC)
- Enzyme linked immunosorbent assay (ELISA)
- Nouws antibiotic test (NAT)
- Commercial ampoule test (Premi Test)

Confirmation Methods:
- High performance liquid chromatography (HPLC)
- Liquid chromatography/mass spectrometry (LC-MS)
FDA Refusals of Shrimp Imports

Most refusals are associated to:

- Chloramphenicol
- Nitrofurans

In January 2019, 26 shrimp consignments were rejected due to banned antibiotics (FDA, 2019).

[Graph showing FDA refusals of shrimp entry lines for veterinary drug residues]

http://www.shrimpalliance.com/fdas-refusals-antibiotic-contaminated-shrimp
<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Treatment of</th>
</tr>
</thead>
</table>
| Oxytetracyclin (for medicated feed) (FAO and FDA) | **Furunculousis in salmonids** *(Aeromonas salmonicida)*  
**Gafkemia in lobsters** *(Aerococcus viridans)*.  
**Hemorrhagic septicaemia** *(Aeromonas hydrophila, A. sobria and Pseudomonas)*.  
**Cold water disease in salmonids** *(Cytophaga psychrophilia)*.  
**Columnaris disease** in salmonids *(Chondrococcus (Flexibacter) columnaris)*.  
**Enteric redmouth disease** *(Yersinia ruckeri)*.  
**Pseudomonas disease** in catfish and salmonids.  
**Ulcer disease in salmonids** *(Haemophilus piscium)*. |
| Florfenicol Premix (FAO and FDA)  | **Furunculousis in salmonids** *(Aeromonas salmonicida)*.                     |
| Sarafloxacin (FAO)               | **Furunculousis in salmonids** *(Aeromonas salmonicida)*.                     |
| Erythromycin (FAO)               | **Bacterial kidney disease** in salmonids *(Renibacterium salmoninarum)*.  
**Streptococciosis** in yellowtail in Japan. |
| Sulphonamides potentiated with trimethoprim or ormethoprim (FAO &FDA) | **Furunculousis, enteric redmouth disease and vibriosis.** |
## Antibiotics Banned in Aquaculture

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Country</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectinomycin</td>
<td>USA</td>
<td>Use is limited by the ready development of bacterial resistance</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>USA</td>
<td>Use is limited by the ready development of bacterial resistance</td>
</tr>
<tr>
<td>Cloramphenicol</td>
<td>Argentina, Canada, EU,</td>
<td>Induced human aplastic anemia</td>
</tr>
<tr>
<td></td>
<td>Japan, US</td>
<td></td>
</tr>
<tr>
<td>Rifampin</td>
<td>Not labelled in the USA or Canada for use in animals, including food-producing animals</td>
<td>Tumorigenicity and tetragenic effects on experimental animals</td>
</tr>
</tbody>
</table>
Follow “The 5 ONLY Rules” to Handle Antimicrobial

#1 Only use antimicrobials when prescribed by a veterinarian
#2: Only when needed: antimicrobials do not cure every infection
#3: Only obtain antimicrobials from authorized source & retailers
#4: Only use the dosage and follow length of treatment and withdrawal period as prescribed
#5: Only use antimicrobials when associated, with good animal health care.

https://oie-antimicrobial.com/
Challenges: What to include?

Perspectives in Disease Management in Shrimp Aquaculture

- Preventing pathogen entry in the culture system will continue to remain as a cornerstone in disease management.

- Use of SPF broodstocks & PLs
  - Since EHP can cause low levels of chronic infection, screening of broodstocks and PLs could be even more important for managing this disease.

- Development of genetically resistant line of shrimp
  - Highest success with Taura Syndrome disease
  - Some lines of shrimp are resistant/tolerant Infectious Hypodermal & Haematopoietic Necrosis Disease
  - Low levels of resistance against WSD reported
  - AHPND resistant lines are being developed
Perspectives in Disease Management in Shrimp Aquaculture

- Pond management:
  - Since both AHPND and EHP can be detected in pond sediment, avoid high concentration of organic matter/sediment in the pond.
  - Water exchange to reduce organic matter, lined pond.
  - Since biofloc that can competitively eliminate microbial pathogens, maintaining biofloc and low stocking density could be another avenue to managing these diseases.

- Chemotherapeutics, Probiotics, prebiotics, organic acids, immunostimulants & many other organic products can help to avoid large-scale mortalities.

- Functional feed – containing disease therapeutics (e.g. Functional Feed against AHPND).
The Disease Triangle: Pathogen, Host & Environment

- **Pathogen in wild population** (plankton etc)
- **HOST** *Penaeus vannamei*
- **OPTIMUM ENVIRONMENT** Temperature / salinity

**Trigger:**
- Poor management
- Overpassing Carrying capacity
- Biosecurity breach

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Thank You!
On behalf of
Aquaculture Pathology Laboratory