# Diseases of Crustaceans – Acute Hepatopancreatic Necrosis Disease (AHPND)

## **Signs of Disease**

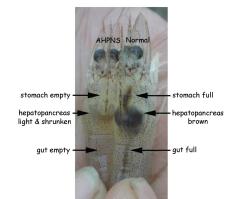
#### Disease signs at pond level

- Often pale to white hepatopancreas (HP) due to pigment loss in the connective tissue capsule.
- Significant atrophy (shrinkage) of HP.
- Often soft shells and guts with discontinuous contents or no content.
- Black spots or streaks sometimes visible within the HP.
- HP does not squash easily between thumb & finger.
- Onset of clinical signs and mortality starting as early as 10 days post stocking.
- Moribund shrimp sink to bottom.

#### Disease signs at animal level by histopathology

- Acute progressive degeneration of the HP accompanied initially by a decrease of R, B and F-cells followed last by a marked reduction of mitotic activity in E-cells.
- Progress of lesion development is proximalto-distal with dysfunction of R, B, F, and lastly E-cells, with affected HP tubule mucosal cells presenting prominent karyomegaly (enlarged nuclei), and rounding and sloughing into the HP tubule lumens.
- The sloughed HP cells provide a substrate for intense bacterial growth, resulting in massive secondary bacterial infection (putative *Vibrio* spp.) and complete destruction of HP at the terminal phase of the disease.

Note: The only definitive histopathology is the massive sloughing off of HP tubule epithelial cells in the absence of bacteria.



Gross signs of *Penaeus vannamei* affected by AHPND (left) compared to normal shrimp (right). Source: D Lightner/T. Flegel



Pale atrophied hepatopancreas of juvenile *Penaeus vannamei*, indicative of AHPND. Source: D Lightner



Pale atrophied hepatopancreas of juvenile *Penaeus monodon* from Vietnam with AHPND. Source: D Lightner

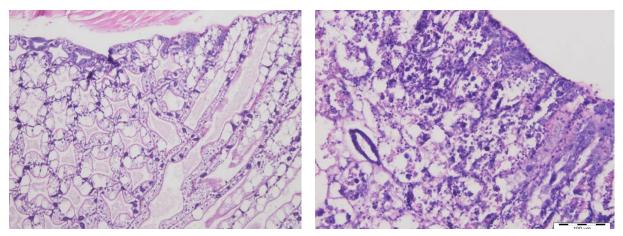
Page 1



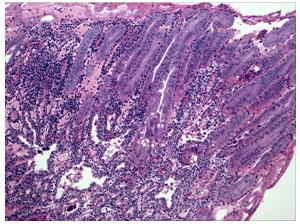
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## Acute Hepatopancreatic Necrosis Disease continued

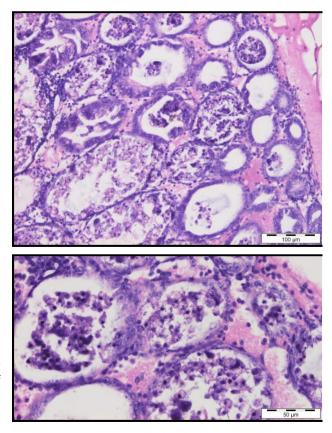


Key diagnostic feature of AHPND-affected hepatopancreas (HP) with medial sloughing of HP cells (right photo), compared with normal HP (left photo) with intact tubules and distinct F, B and R cells. Source: T. Flegel



Proximal-to-distal progression of lesion in the hepatopancreas with dysfunction of R, B, F and E cells. Source: D Lightner

The very distinctive stage of massive sloughing of HP cells; a feature to focus on for confirmative diagnosis of AHPND. Source: T Flegel





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Page 2

## Acute Hepatopancreatic Necrosis Disease continued

#### **Disease Agent**

The disease is caused by a pathogenic strain of *Vibrio parahaemolyticus* (Tran et al. 2013. Diseases of Aquatic Organisms, 105:45-55). Isolated pathogen should be subjected to bioassay studies following the challenge tests described in Tran et al. (2013).

## **Molecular Diagnostics**

A PCR method has been previously developed through cooperative research between Taiwan, Province of China and Thailand to detect isolates of AHPND bacteria. The primers for specific detection of the causative bacteria are publically available at no cost; detailed information on these primers can be obtained at <u>http://www.enaca.org/modules/news/article.php?article\_id=2015&title=primers-for-detection-of-bacterial-isolates-that-cause-ahpnd</u>. It should be noted that these primers (AP1 and AP2) have not been subjected to rigorous validation process and their performance for detection of the causative agent of AHPND has been evaluated over time.

In June 2014, a new and improved PCR method (called the AP3 primer method) for detection of AHPND bacteria has been developed by Thai scientists from Centex Shrimp and the Aquatic Animal Health Research Center (Sirikharin et. al.). It was entirely supported by research funding from Thailand. The sequence of the AP3 primer target, plus the primers and PCR protocol are given freely. Details can be obtained at <u>http://www.enaca.org/modules/news/article.php?article\_id=230&title=new-pcr-detection-method-for-ahpnd</u>. From the laboratory test results in screening AHPND bacterial isolates using this AP3 primer method, the team recommended that the previously announced AP1 and AP2 primer methods be replaced with this new method.

#### **Host Range**

AHPND affects both *Penaeus monodon* and *P. vannamei* (*P. chinensis* was also reported to be affected in China).

#### **Presence in Asia-Pacific**

AHPND has been officially reported in China and Vietnam (2010), Malaysia (2011), and Thailand (2012).





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## Acute Hepatopancreatic Necrosis Disease continued

#### **Prevention and Control**

The World Organisation for Animal Health (OIE) has developed an AHPND technical disease card that included information on susceptibility of *V. parahaemolyticus* to physical and chemical treatments. The disease card also includes information on the likelihood of AHPND transmission to wild or farmed shrimps from different shrimp commodities. The OIE disease card can be found at <a href="http://www.oie.int/fileadmin/Home/eng/Internationa\_Standard\_Setting/docs/pdf/Aquatic\_Commission/AHPND\_DEC\_2013.pdf">http://www.oie.int/fileadmin/Home/eng/Internationa\_Standard\_Setting/docs/pdf/Aquatic\_Commission/AHPND\_DEC\_2013.pdf</a>

#### **Further information**

Published reports from NACA and FAO on EMS, AHPNS and AHPND can be obtained at the following links:

http://www.enaca.org/modules/news/article.php?article\_id=1952 http://www.enaca.org/modules/wfdownloads/singlefile.php?cid=11&lid=1059 http://www.enaca.org/modules/podcast/programme.php http://www.fao.org/docrep/018/i3422e/i3422e.pdf

#### **Additional Notes:**

- AHPND was first reported in 2009 as a novel disease of unknown aetiology in shrimps and was initially named early mortality syndrome (EMS). A more descriptive term for the syndrome, acute hepatopancreatic necrosis syndrome (AHPNS), was later adopted. With the aetiological agent identified, the disease is now named AHPND.
- The common practice of many hatcheries are still routinely feeding live polychaete worms to broodstock to increase nauplii production, even though this may present a significant biosecurity risk and a possible source of AHPND. This practice should be discouraged as live polychaetes might harbor pathogens that can aggravate conditions in the presence of disease outbreaks including AHPND.

#### List of Experts:

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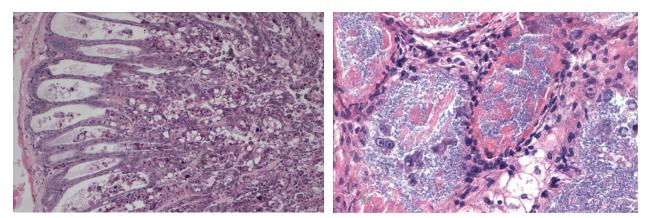
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# Other Photos of the Disease (courtesy of Dr. Loc Tran):



Gross signs of AHPND-affected shrimps showing empty stomach and midgut, with significant atrophied hepatopancreas.



Histopathology of shrimp hepatopancreas (HP) affected by AHPND. Left: acute cell sloughing of HP tubular epithelial cells. Right: terminal phase of AHPND showing heavy haemocytic infiltration and bacterial colonization.

Page 5



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