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## Disease incidence in shrimp farms located in east coastal region of the Mekong Delta, Viet Nam

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### ABSTRACT

*A field study on the water quality parameters and prevalence of diseases was carried out in four locations in the Mekong Delta, Viet Nam. A total number of 960 samples (816 with disease signs and 144 without disease sign) of farmed shrimp were collected over 8 sampling months (2 production cycles). Samples were collected every two weeks and when abnormal behavior from cultured shrimp was observed. Five major groups of gross clinical signs were recorded among diseased shrimp samples. Diseases that appeared through the sampling months were: (1) atrophy and pale-colored hepatopancreas (HP) with the empty or little food in the mid gut; (2) empty/little/discontinued food in the midgut and (3) slow growth, HP atrophy and gut with discontinued food. White feces disease was not found in April but appeared for the rest of sampling months with the highest prevalence in July (39,2%) and the lowest prevalence in September (4,9%). White spot disease appeared from October to December (at the end of when the monsoon season and low temperature).*

## 1. INTRODUCTION

Brackish water shrimp are widely cultured in the Mekong Delta of Viet Nam which has been continuously developing with the expansion in cultural areas and production. However, along with intensification in shrimp farming, diseases have occurred more frequently and caused significant economic losses for shrimp farming in the Delta (Oanh & Phuong, 2012).

Climate change is causing an increase in temperature and salinity, especially in intensive cultural shrimp ponds. The increase in temperature and salinity due to climate change can affect the biological processes taking place in the water column and at the bottom of the pond. These processes can lead to changes in the water parameters and may cause environmental stress to

the cultured aquatic host species and suppress their immune system leading to changes in the susceptibility of the host to infectious diseases. At the same time, an increase in temperature and salinity may further increase the risk posed by diseases through alterations in the distribution, prevalence and virulence of pathogens (Halvell et al., 1999).

Diseases in farmed shrimp in the Mekong Delta have been reported to be associated with several pathogens (Phuong & Oanh 2012). White spot disease has been considered as a dangerous disease for shrimp culture since the nineties (Oanh et al., 2005). Acute hepatopancreatic necrosis disease (AHPND) or early syndrome mortality (EMS) is a bacterial disease which severely impacts shrimp farming all over the world including Viet Nam (Tran et al., 2013; Oanh et al., 2018). Besides,

investigating the presence of endoparasites in the digestive tract of cultured shrimp samples collected from farms in the Mekong Delta was recently reported (Oanh et al., 2021).

In this study, data on diseases and selected water quality parameters were collected in shrimp ponds for a period of 8 months to determine the prevalence of diseases and fluctuation of water quality parameters. The collected shrimp samples were also examined for parasites and bacterial isolation and identification were also performed.

## 2. MATERIALS AND METHODS

### 2.1. Collection and clinical examination of diseased shrimp samples

The study was conducted during 2 production cycles from April to July 2018 and from September to December 2018 at major shrimp cultural areas in Tra Vinh, Soc Trang, Bac Lieu, and Ca Mau provinces (Figure 1). Samples were collected in two-week intervals and when abnormal behavior from cultured shrimp was observed. Diseased shrimp sample collection was carried out following the methods described by Oanh et al. (2018). Lethargy or moribund but alive shrimp that showed obvious pathological signs of disease or without pathological signs in the same pond were collected (15-20 shrimp/pond). After being taken out from the pond using a cast-net, the collected samples were subjected to external clinical signs examination such as changes in body and hepatopancreatic (HP) color, HP atrophy/swollen, empty gut/little food, white spots in the shell and carapace according to the method described by Lightner (1996).

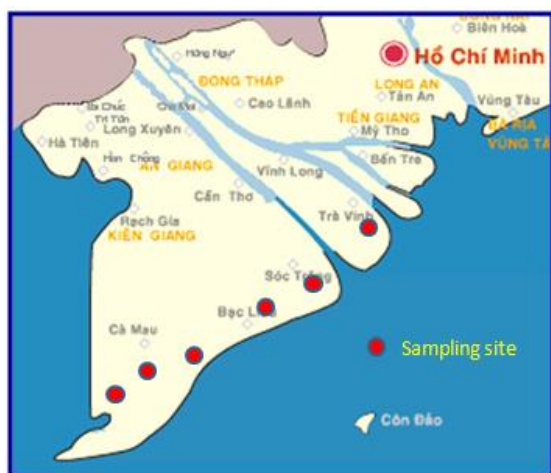


Figure 1. Map of sampling sites

### 2.2. Bacterial isolation and identification

After recording the clinical signs, the external surface of the shrimp was rinsed in 70 % ethanol and an incision was made over the head. Bacterial isolation from HP was taken by an inoculating loop and streaks on thiosulfate citrate bile salts sucrose (TCBS, Merck) agar plate and colony morphology was recorded after incubation for 24 hours at 28°C. Cell morphology was studied in Gram-stained preparations according to Hucker's modification method (Barrow & Feltham 1993). Selected isolates were subjected to DNA extraction (Bartie et al., 2006) and PCR detection of *V. parahaemolyticus* (using AP3 primers from Sirikharin et al., 2014).

### 2.3. Parasitological examination

The endoparasites present on the HP and intestine were detected by tissue scrapings with a drop of clean physiological saline water, covered with a clean cover slip (wet mount preparation), and examined by a light microscope.

### 2.4. Water sampling

Water samples were collected from each shrimp pond at the same time with diseased samples. Temperature, salinity and pH were measured while collecting water samples using a mercury thermometer, refractometer, and pH meter. Water from pond was collected in 500-ml sterile glass bottle following the method described by Boyd (1990) and transported to the laboratory in ice-cooled box to estimate dissolved oxygen (DO), nitrite (NO<sub>2</sub>), ammonium (NH<sub>4</sub><sup>+</sup>), ammonia (NH<sub>3</sub>) and COD using standard methods APHA *et al.* (2012).

### 2.5. Data analysis

The collected data were analyzed using Microsoft excel program.

## 3. RESULTS AND DISCUSSIONS

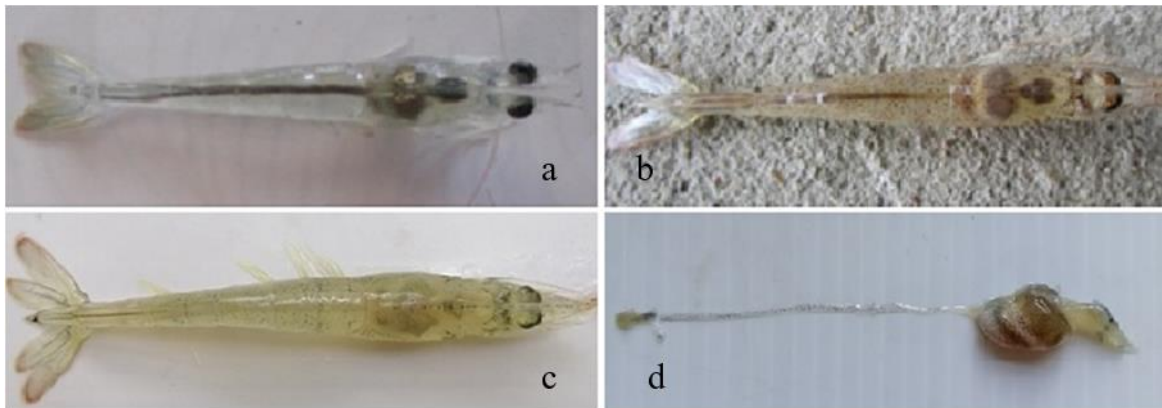
### 3.1. Pathological signs of sampled shrimp

A total of 816 shrimp samples, which show disease signs, were collected over the sampling period. There were 5 major groups of gross signs including (1) HP atrophy/pale and midgut with empty/discontinued food, (2) white feces, (3) midgut with empty/little/discontinued food, (4) slow growth, and (5) white spots in the shell.

Sampled shrimp in group 1 showed HP atrophy, pale to white and midgut with empty/discontinued food (Figure 2) which are typical pathological signs

of acute hepatopancreatic necrosis disease (AHPND). The AHPND gross sign appearance in the sampled shrimp was similar and as described by Tran *et al.* (2013). Pale to white hepatopancreas

appears due to pigmentation loss in the hepatopancreatic R-cells while approximately 50% of reduction of the expected size of the organ happens by the atrophy (Tran *et al.*, 2013).



**Figure 2. Healthy shrimp have a normal hepatopancreas with stomach and midgut filled with food in the (a). Diseased shrimp have atrophied, pale color hepatopancreas and little food in the midgut (b) or empty midgut (c). Stomach, hepatopancreas and midgut of shrimp in figure d**

Shrimp with white feces symptoms (group 2) showed yellowish white color in the midgut when the shrimp swim lethargically in the water and clearer when removing the shrimp from the pond (Figure 3A). Shrimp eat less or stop eating and the midgut has an incomplete, intermittent (broken) or empty with pale yellow or milky spots, especially at the gut connecting the shrimp's stomach and

hepatopancreas. The hepatopancreas of diseased shrimp is soft, pale yellow or milky white. Shrimp release opaque white (or yellowish white) feces floating on the water surface, especially at the corner of the pond where ending of the wind and were often seen in the feeding trays (Figure 3B).



**Figure 3. White feces disease. (A) The midgut of diseased shrimp has yellowish white color, (B) white feces segments in the feeding tray**

Sampled shrimp, which were noted as slow growth, did not show specific pathological signs but growth

retardation and variation in sizes about 2 months after stocking (Figure 5A).



Figure 4. Shrimp with empty (a)/little (b) /discontinued food (d) in the mid gut

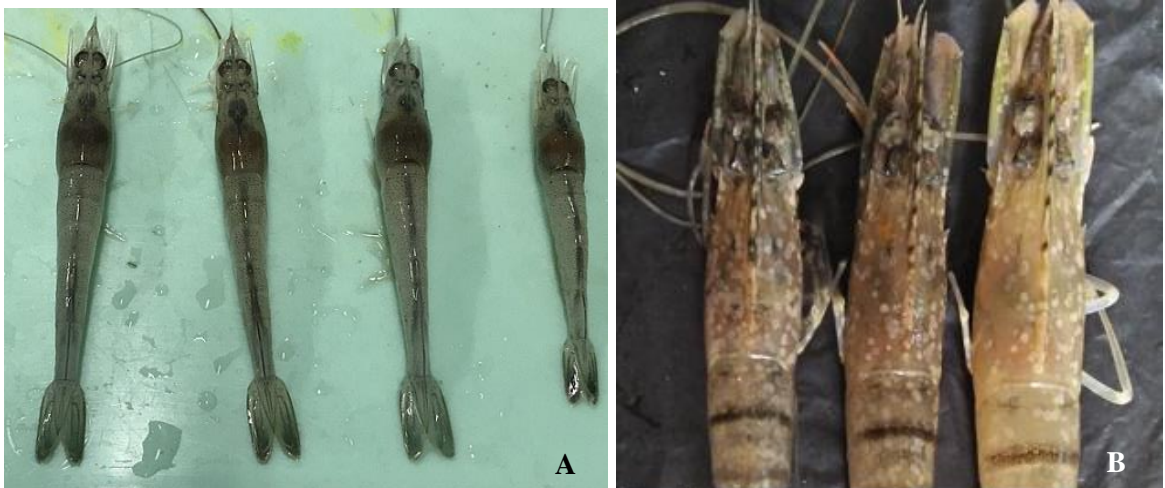


Figure 5. (a) Sampled shrimp with variation in sizes, (b) white spots in the shell

### 3.2. Bacterial isolation and identification

A total of 87 bacterial isolates were isolated from hepatopancreas and midgut of diseased shrimps.

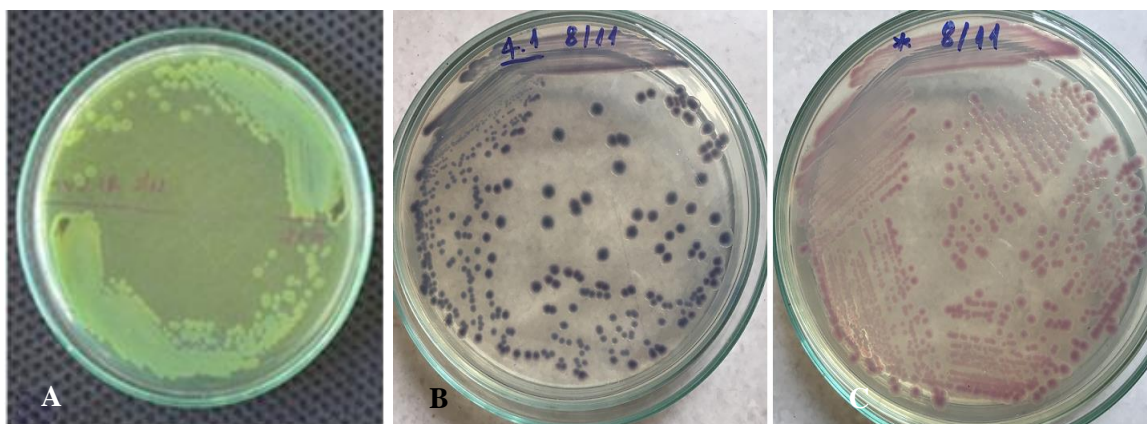
Results of isolation and colony morphology of bacterial isolates from each pathological group are presented in Table 1.

**Table 1. Characteristics of bacteria isolated from diseased samples**

Pathological signs	No. of isolates	Culture media	Colony morphology
atrophy and pale-colored hepatopancreas with empty or little food in the mid gut	25 (10 were selected for identification)	TSA <sup>+</sup>	yellow cream, swarming
		TCBS	green, round, convex, equal edges, d = 2-3mm
empty/little/discontinued food in the mid gut	14	TSA <sup>+</sup>	yellow cream, round, convex, equal edges, d= 2-3 mm
		TCBS	no colony
	9 (4 were selected for identification)	TSA <sup>+</sup>	yellow cream, not swarming
		TCBS	green, round, convex, equal edges, d = 2-3mm
	10	TSA <sup>+</sup>	yellow cream, not swarming
		TCBS	yellow, round, convex, equal edges, d = 1,5 mm
white feces	6	TSA <sup>+</sup>	yellow cream, round, convex, equal edges, d= 2-3 mm
		TCBS	no colony
	11	TSA <sup>+</sup>	opalescent, convex, equal edges, d = 1.5 mm
		TCBS	no colony
	8 (6 were selected for identification)	TSA <sup>+</sup>	yellow cream, not swarming
		TCBS	green, round, convex, equal edges, d = 2-3mm

The strains with green colonies on TCBS medium, after being selected, were inoculated with ChromAgar medium, showing that they were divided into two groups: the group with purple colonies and the group with the blue colony (Figure

6). Isolates are mobile, Gram-negative and short rod-shaped, positive reaction for catalase and oxidase and can ferment and oxidize glucose. PCR analysis confirmed bacterial isolates from diseased in group 1 were *V. parahaemolyticus*.

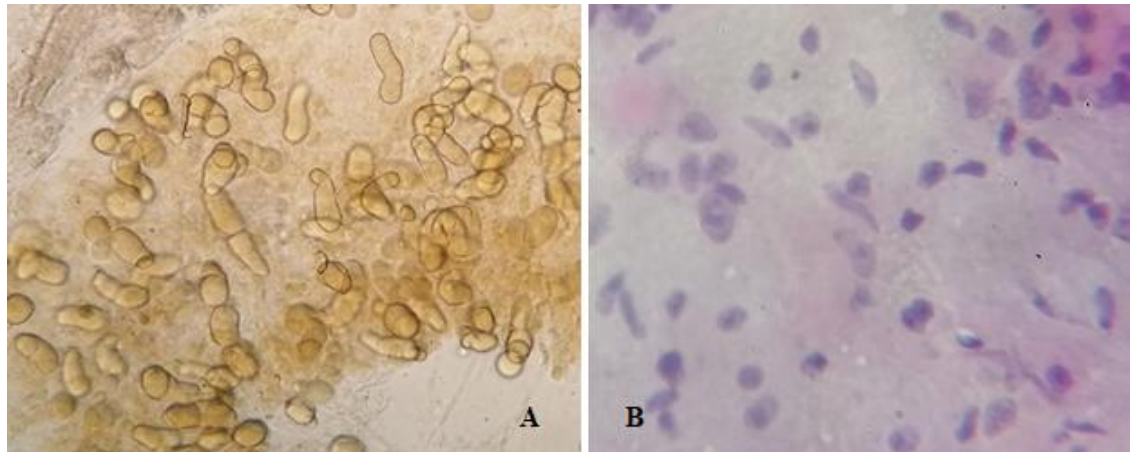


**Figure 6. Bacterial isolates from diseased shrimp samples. (A) Green colonies on TCBS medium; (B) blue and (C) purple colonies on ChromAgar medium**

**3.3. Parasitological examination**

The parasitological examination revealed that almost all the sampled shrimps were infected by

regarine parasite with different intensities (Figure 7).

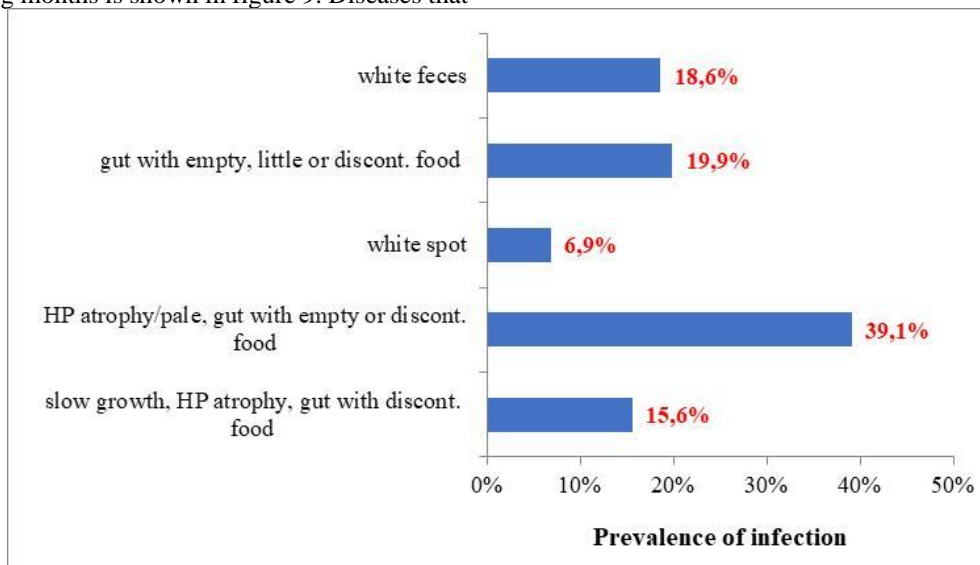


**Figure 7. Gregarine infection (20X). (A) Fresh smear of mid gut. (B) Giemsa stain of HP smear**

Gregarines are a diverse group of protozoan parasites that are common parasites in penaeid shrimp. They usually parasitize in the HP and the midgut of shrimp and their intermediate hosts are molluscs (mainly bivalve molluscs) and arthropods (Chakraborti & Bandyopadhyay, 2010). Gregarines are protozoan parasites that usually parasitize in the hepatopancreas and the midgut of shrimp. Infected shrimp do not show typical pathological sign but often grow slowly as the parasite takes nutrients from shrimp to grow.

appeared through the sampling months were: (1) atrophy and pale-colored hepatopancreas along with the empty or little food in the mid gut; (2) slow growth, HP atrophy and gut with discontinued food and (3) empty/little/discontinued food in the mid gut; (4) white feces disease was not found in April but appeared for the rest of sampling months with the highest prevalence in July (39,2%) and the lowest prevalence in September (4,9%); and (5) white spot disease appeared from October to December (at the end of the monsoon season and low temperature).

Prevalence of diseases by gross clinical signs by sampling months is shown in figure 9. Diseases that



**Figure 8. Prevalence of disease infection (%) from sampled shrimps**

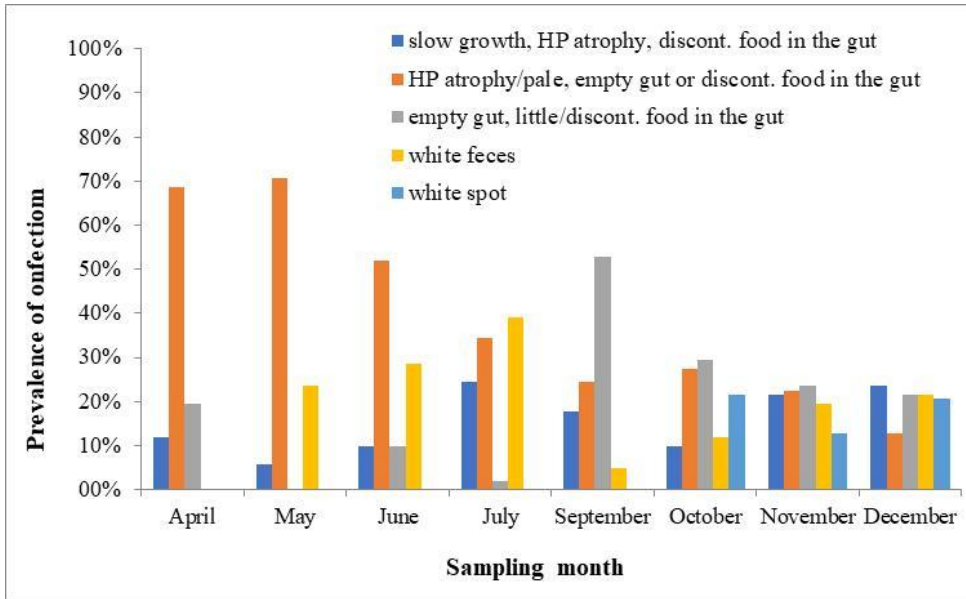


Figure 9. Prevalence infection (%) of collected samples by sampling months

3.4. Water quality parameters

Collected data on selected water parameters during the sampling periods is shown in Figure 10. During the sampling period, water parameters including pH, DO, nitrite, and NH<sub>4</sub><sup>+</sup> were not much fluctuated and were within a suitable range for shrimp growth.

Salinity in the sampling ponds ranges from 5-25 ‰, pH ranges from 7.5-9; DO ranges from 4.4-5; Nitrite ranges from 0.1-1; NH<sub>4</sub><sup>+</sup> is in the range of 0.2-1 and NH<sub>3</sub> is in the range of 0.2-2. Water temperature

fluctuated throughout sampling periods (the lowest was 29°C in December, increasing gradually and reaching the highest (32.5°C in May then gradually decreasing to October). The concentration of COD (mgO<sub>2</sub>/L) in water fluctuated highly throughout the sampling period, the lowest was 13.2 mgO<sub>2</sub>/L in December, increasing gradually and the highest was 84.4 mgO<sub>2</sub>/L in July. In the second culture cycle (from September to December), COD ranged from 13.2 to 21.6 mgO<sub>2</sub>/L.

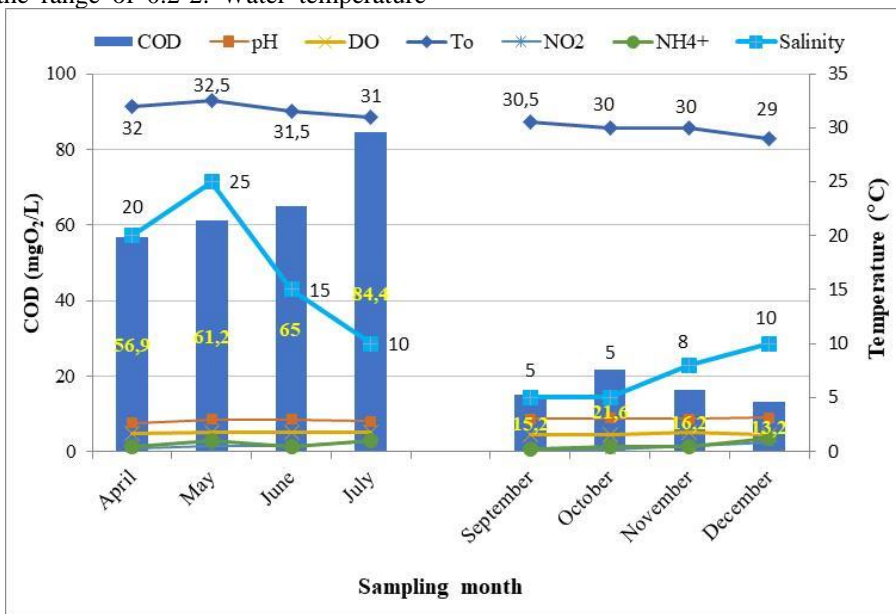


Figure 10. Fluctuation of water parameters in shrimp ponds during the sampling periods

Prevalence of diseases with pathological signs (1) atrophy and pale-colored hepatopancreas along with the empty or little food in the mid gut and (2) empty/little/discontinued food in the mid gut increased proportionally to water temperature (Figure 11).

Prevalence of remaining observed diseases (such as white feces, white spot and slow growth) were not found to be correlated with fluctuation of water salinity in the ponds. In addition, to water temperature, fluctuation of water temperature was not found to be correlated with observed pathological signs.

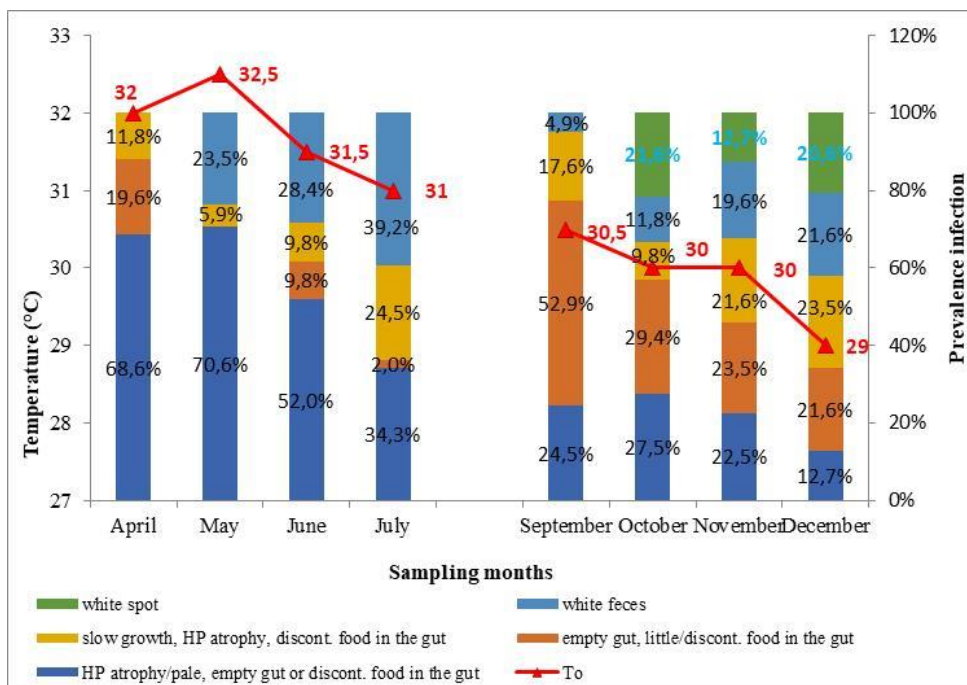


Figure 11. Fluctuation of temperature and disease prevalence by sampling months

#### 4. CONCLUSIONS

Diseases that appeared through the sampling months include (1) atrophy and pale-colored hepatopancreas with empty or little food in mid gut; (2) slow growth, HP atrophy and gut with discontinued food; and (3) empty/little/discontinued food in the mid gut. White feces disease was not found in April but appeared for the rest of sampling months with the highest prevalence in July (39,2%) and the lowest prevalence in September (4,9%). White spot disease appeared from October to December (at the end of when the monsoon season

and low temperature). Diseases with pathological signs (1) atrophy and pale-colored hepatopancreas along with the empty or little food in the mid gut and (2) empty/little/discontinued food in the mid gut increased proportionally to water temperature.

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