Occurrence of diseases and selected water quality parameters in cultured striped catfish (*Pangasianodon hypophthalmus*) ponds in the Mekong Delta of Vietnam

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Abstract. A field study on prevalence of diseases and on selected water quality parameters was carried out in major catfish cultural areas in the Mekong Delta, Vietnam. A total number of 1,602 samples (1,339 with disease signs and 263 without disease sign) of farmed stripped catfish, *Pangasianodon hypophthalmus*, were collected over 12 sampling months from July 2018 to June 2019. Four major groups of gross clinical signs were recorded among the diseased fish samples. Ectoparasite infections (*Dactylogyrus* spp. on the gill: 46.8%, *Trichodina* spp. on the skin: 25.5%) accounted for the highest proportion (41.3%) of diseases, followed by hemorrhagic disease by *Aeromonas hydrophila* infection (20.5%), disease with white nodules in internal organs by *Edwardsiella ictaluri* infection (15.8%) disease, pale gills and liver (5.4%) and jaundice (0.5%). The proportion of the hemorrhagic disease increased in the higher temperature season, whereas the white nodule disease was more common at low temperatures (about 27-29°C), when the water resource comes from the upper stream, creating high COD concentration. Besides, ectoparasitic infections occurred all year round in the catfish ponds, especially in the rainy season combined with adverse environmental parameters of the pond. The results suggest that precautionary measures should be taken at the major hotspots of disease, during the onset of the disease.

Key Words: bacterial disease, environmental parameters, parasitic disease.

Introduction. Striped catfish, *Pangasianodon hypophthalmus*, is the most important cultured freshwater fish species in the Mekong Delta, Vietnam which has been continuously developing with the expansion in cultural areas and production. Its exportation significantly contributes to the national aquaculture export in Vietnam (De-Silva & Phuong 2011). However, diseases have been one of the major challenges in the sustainable development of *P. hypophthalmus* farming sector. The intensification process of the culture practices resulted in more frequent disease outbreaks and were more difficult to control in the recent decade (Dung et al 2015).

Recently, climate change is causing changes in the water parameters that may cause environmental stress to the cultured aquatic species, suppressing their immune system and leading to changes in the susceptibility of the host to infectious diseases. As the same time, increases in temperature and salinity may further increase the risk posed by diseases, through alterations in the distribution, prevalence and virulence of pathogens (Harvell et al 1999). According to Phuong & Oanh (2010), the outbreak of diseases in *P. hypophthalmus* farms has always been primarily caused by stress induced factors that include localized environmental degradation, wastes from agricultural activities, improper caring, high stocking density, low quality of stocked seed, and is of a seasonal nature, all making the *P. hypophthalmus* stock susceptible to infectious pathogens.

Diseases in *P. hypophthalmus* culture in the Mekong Mekong Delta have been reported to be associated several pathogens, including fungi, ento- and ecto-parasites as well as bacteria (Phuong & Oanh 2010). Bacteria and parasites were the most common pathogens causing diseases that affect the survival rate of farmed *P. hypophthalmus*

(Dung et al 2008; Phan et al 2009). The annual cycle of bacterial pathogen infections have been responsible for the major epizootics affecting *P. hypophthalmus* production. Losses resulting from bacterial diseases have been estimated to be as high as 50% of total production compared with others (Phuong et al 2007). The most common bacterial disease in *P. hypophthalmus* is haemorrhagic septicaemia caused by *Aeromonas hydrophila*. It causes up to 80% mortality with clinical signs of hemorrhages on the head, mouth and at the base of fins, a red, swollen vent and the presence of pink to yellow ascitic fluid (Dung et al 2008). Besides, the disease with white nodules in internal organs as clinical signs (also named bacillary necrosis of Pangasius), which caused by *Edwardsiella ictaluri* is responsible for severe economic losses, especially in fingerlings and juvenile *P. hypophthalmus* (Dung et al 2008; Crumlish et al 2010).

In this study, a survey on the diseases and selected water quality parameters was carried out in *P. hypophthalmus* cultural areas, in An Giang, Dong Thap and Can Tho provinces, for a period of 12 months, to determine the prevalence of diseases with the fluctuation of water quality parameters. The collected *P. hypophthalmus* samples were examined for external parasites and bacterial isolation and identification were performed.

Material and Method

Collection and clinical examination of diseased fish samples. The study was conducted from July 2018 to June 2019, on major *P. hypophthalmus* cultural areas in An Giang, Dong Thap and Can Tho provinces (Figure 1). Collected samples were lethargic fish with obvious clinical signs of disease and live fish without clinical sign, in the same pond (15-20 fish collected from each pond). After being taken out from the pond, the collected fish were subjected to clinical examination for the changes in color and any external gross lesions like wounds, hemorrhages, ulcers or eroded skin on the external body surface (skin, gills, eye and mouth), according to the method described by Austin & Austin (1993).

Figure 1. The location of sampling sites.

Parasitological examination. The ectoparasites present on the skin, gills and fins were detected by tissue scrapings with a drop of clean physiological saline water, covered with a clean cover slip (wet mount preparation). The prepared smear was examined by light microscope and the observed parasites were identified to the genus level, as described by Lom et al (1992) and by Ha & Te (2007).

Bacterial isolations and PCR analysis. The external abdominal surface fish samples were disinfected by swabbing with 70% ethanol and an incision was made over the peritoneal cavity. The internal clinical signs were recorded and inoculating loops were used to take a little bit of kidney sample and streak it on trypton soya agar (TSA, Merck) plates. The colony morphology was recorded after an incubation for 24-48 hours, at 28°C. The cell morphology was studied in Gram-stained preparations from the same agar plates, according to Hucker's modification method (Barrow & Feltham 1993). Isolated bacteria were subjected to DNA extraction, using the method of Bartie et al (2006), and to PCR detection of *E. ictaluri* and *A. hydrophila*, according to the protocol of Panangala et al (2007).

Water quality parameters. Water samples were collected from each *P. hypophthalmus* pond at the same time with the diseased fish samples. Temperature, salinity and pH were measured while collecting water samples using a mercury thermometer, a refractometer and a pH meter. Water from the *P. hypophthalmus* pond was collected in 500 mL sterile glass bottle following the method described by Boyd (1990) and transported to the laboratory in ice-cooled boxes to estimate the dissolved oxygen (DO), nitrite (NO_2), ammonium (NH_4^+), ammonia (NH_3) and COD, using the standard methods (APHA et al 2012). The collected data were analyzed using the Excel software.

Results

Clinical signs. A total number of 1,602 samples (1,339 with disease signs and 263 without any disease sign) were collected over the sampling period. Collected samples displayed 4 major groups of gross signs including: hemorrhages, white nodules in internal organs (liver, spleen and kidney), pale red color of the gill and liver and yellow color of the body and internal cavity. In addition to obvious gross clinical signs, collected diseased samples were noted with ectoparasitic infection.

Hemorrhages. Diseased fish had signs of hemorrhage in the head, eyes, on the body, anus and fin roots. A number of cases have been reported with extruded eyes. Internal signs of diseases includes: (i) hemorrhage and red fluid in the internal cavity; (ii) hemorrhage on the liver, intestines and swimming bladder; (iii) congestioned and swollen kidney and spleen, with blood cells (Figure 2).

Figure 2. Pathological signs of hemorrhagic disease: (A) hemorrhage in the head, eyes and fins (arrow); (B) hemorrhage on the internal organs, red fluid in the internal cavity, swollen liver and head kidney (arrow) (original photos).

White nodules in internal organs. Pale color of the body and slow growth. Typical internal clinical sign showing white patches in liver, kidney and spleen. Hemorrhages in liver, stomach and swimming bladder were observed in some samples (Figure 3).

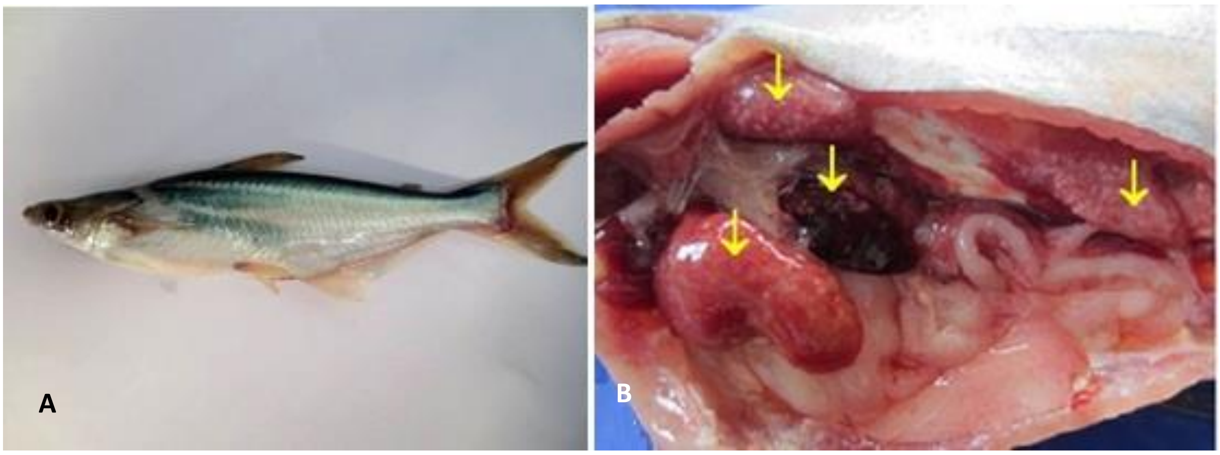

Figure 3. Pathological signs of white patches in internal organs: (A) un-apparent external clinical sign; (B) white patches in internal organs (liver, kidney and spleen) (arrow) (original photos).

Pale color in the gills and liver. Moribund fish samples swim slowly near the surface of the water. Pale skin and gill color with a lot of released mucus, hemorrhages at the base of the fins, anemia in the gills and internal organs (obviously in liver and spleen) were observed in those samples (Figure 4).

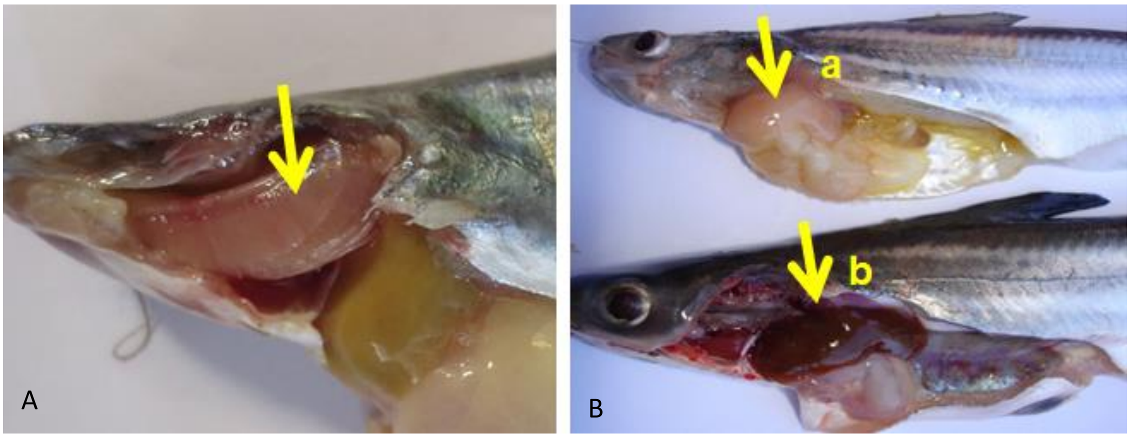

Figure 4. (A) Pale gills; (B) Pale red color in the liver (a) in comparison to normal liver (b) (original photos).

Yellow color of the body and internal cavity (jaundice). Yellow coloration on skin, fins, muscle and abdominal cavity (Figure 5) were seen.

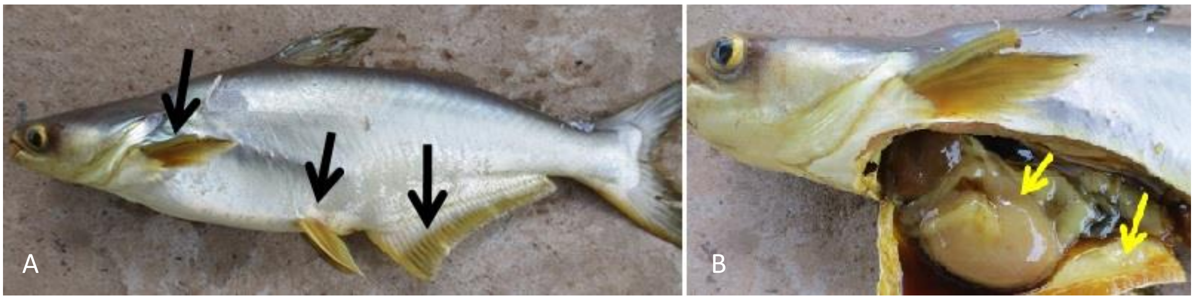

Figure 5. Yellow coloration on skin (A) and in abdominal cavity (B) (original photos).

Bacterial isolation and identification. A total of 512 bacterial isolates were obtained from fish showing white nodules in the internal organs and fish with hemorrhagic sign, but not from fish with jaundice. Based on the pathological signs and morphological characteristics, bacterial isolates are divided in two main groups as shown in Table 1.

Table 1

Characteristics of bacteria isolated from diseased samples

Morphological characteristics	Group of bacterial isolates	
	Hemorrhagic diseased fish	*White nodules in internal organs*
Colony morphology	Yellow cream, round and convex; 2-3mm in diameter	Clear white, round and convex; 1 mm in diameter
Bacterial shape and gram stain	Short rod-shape, Gram negative	Rod-shape, Gram negative
Percentage (%)	61	39

Bacterial isolation from hemorrhagic diseased P. hypophthalmus. There are 312/512 (61%) isolates from samples showing signs of hemorrhage. All bacterial isolates are characterized by gram negative, short rod-shaped, developed after 18-24 h on TSA medium, creating creamy yellow colonies, round, convex and measuring 2–3 mm in diameter (Figure 6). The PCR result showed that all isolates displayed a 209 bp band specific for *A. hydrophila* (Figure 8A).

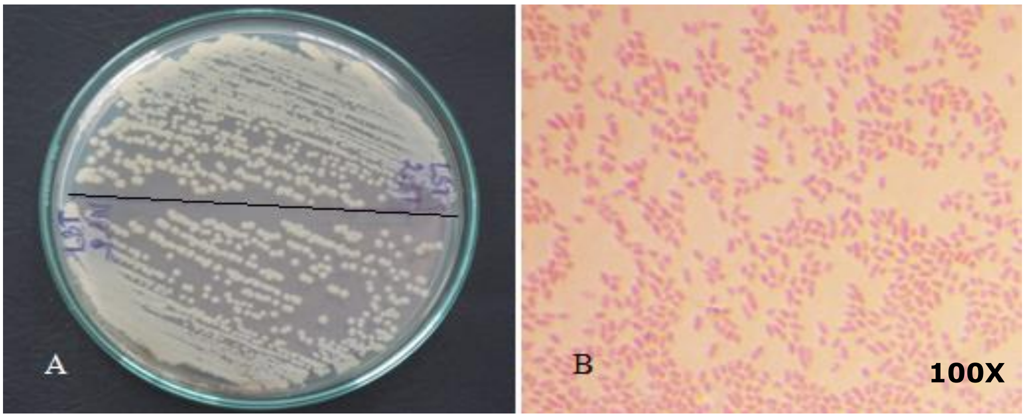

Figure 6. Bacterial isolation from hemorrhagic diseased catfish samples. (A) colony morphology on TSA plate; (B) Gram-negative bacteria and short rod-shaped.

Bacterial isolation from diseased P. hypophthalmus samples with white nodules in the internal organs. There are 200/512 (39%) isolates from samples showing signs of white nodules in internal organs. The group of bacteria has the characteristic of Gram negative, rod, developed after 48 h on TSA medium, small and round, clear white colonies about 1 mm in diameter (Figure 7). The PCR result showed that all isolates displayed a 407 bp band specific for *E. ictaluri* (Figure 8B).

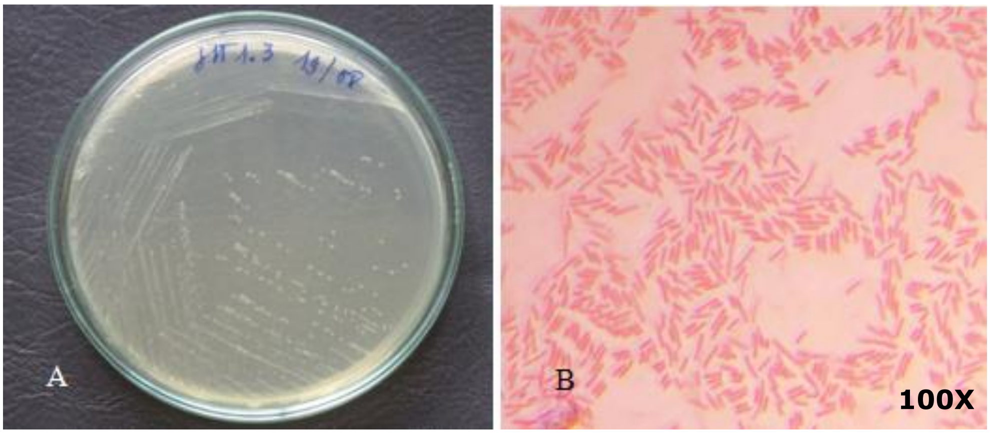

Figure 7. Bacterial isolation from catfish samples with white nodules in the internal organs. (A) colony morphology on TSA plate; (B) Gram-negative bacteria and rod-shaped.

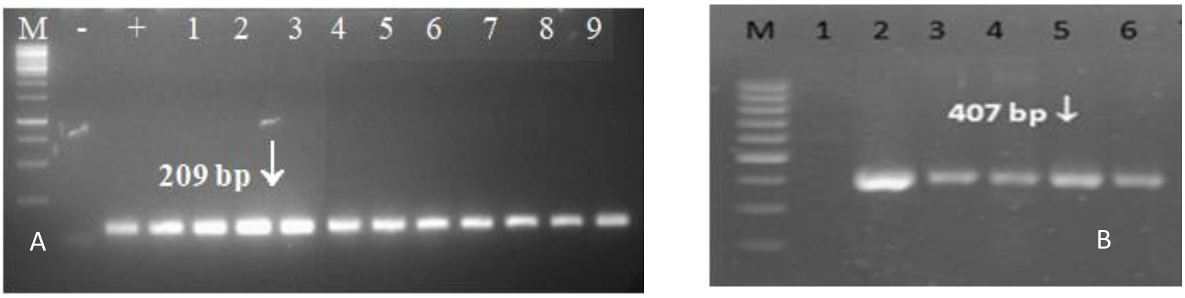

Figure 8. (A) PCR product using specific primers for *Aeromonas hydrophila*. M: 1kb DNA ladder; (-): negative control (water); (+): positive control; (1-9): bacterial DNA from hemorrhagic diseased fish; (B) PCR product using specific primers for *Edwardsiella ictaluri*. M: 1kb DNA ladder; (1): negative control (water); (2): positive control; (3-6): bacterial DNA from samples with white nodules in the internal organs.

Parasitological examination. Two groups of ectoparasites were observed: *Dactylogyrus* spp., on the gills, and *Trichodia* spp., on the gills and skin mucus. These parasites caused small wounds on the body of infected fish and more mucus release (Figure 9).

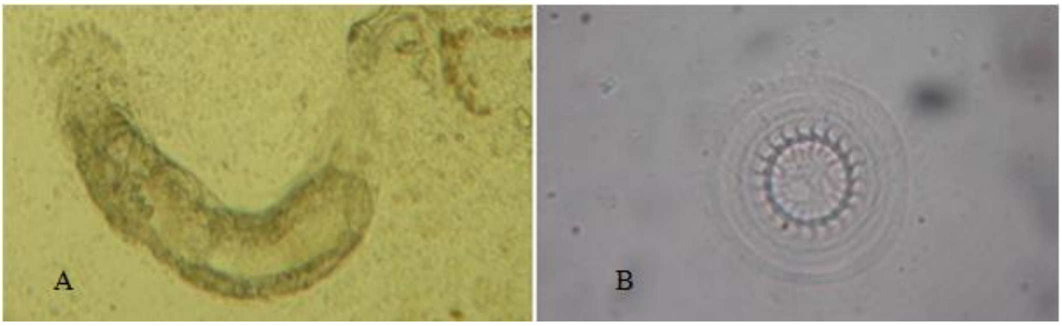

Figure 9. (A) *Dactylogyrus* sp. on the gill; (B) *Trichodia* sp. on skin mucus.

Prevalence of diseases. A prevalence of diseases by gross clinical signs is shown in Figure 10. The highest proportion is caused by ectoparasites (41.3%), of which the prevalence was of 46.8% for the *Dactylogyrus* spp., on the gill, and 25.5% for the *Trichodia* spp., on skin and mucus; the prevalence of other disease causes was: 20.5% for the haemorrhagic disease, 15.8% for the white nodules in internal organs, 5.4% for the pale gills and liver and 0.5% for the yellow color of the body and internal cavity.

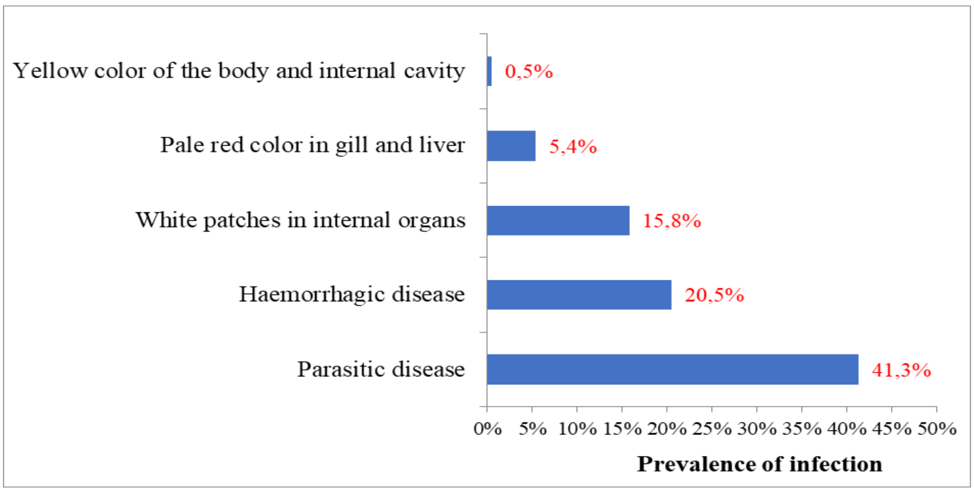

Figure 10. Prevalence of infection (%) in collected samples during the sampling periods.

Prevalence of diseases according to the observation of the gross clinical signs, detailed by sampling month, is shown in Figure 11. Diseases that appeared year-round were hemorrhagic disease caused by *A. hydrophila*, white nodules in internal organs caused by *E. ictaluri* and parasitic disease. *P. hypophthalmus* is cultured in ponds often infected with ectoparasites, at a high infection rate. The month with the lowest prevalence of ectoparasitic infections was June (22.6%) and with the highest it was February (60%). The rate of hemorrhagic diseased fish appeared with a higher frequency in May, June and July (36.7%, 41.9% and 31.6%, respectively). White nodules in internal organs occurred from July to December. There were samples co-infected with hemorrhagic and white nodules in internal organs but with a low rate (1.2%). Pale gills and liver was recorded in the period from April to October and jaundice (yellow color of the body and internal cavity) was recorded only on one sampling time, in May.

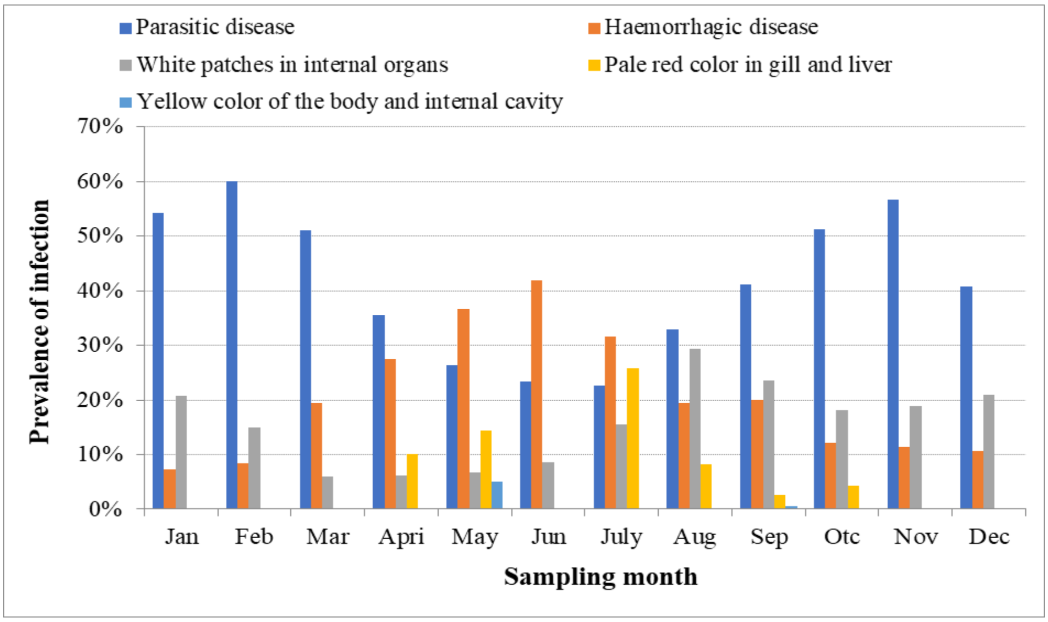

Figure 11. Prevalence of infection (%) of collected samples by sampling months.

Water quality parameters. Collected data on the selected water parameters during the sampling periods is shown in Figure 12. During the sampling period, water parameters including salinity, pH, DO, nitrite, $NH4^+$, NH_3 did not significantly fluctuated and remained within a range suitable for the *P. hypophthalmus* growth. Salinity in the sampling ponds was 0‰, pH ranged from 6.5 to 8; DO ranged from 4 to 5 mg L^{-1}; nitrite ranged from 0.1 to 1 mg L^{-1}; $NH4^+$ was in the range of 0.1-0.5 mg L^{-1} and NH_3 was in the range of 0.009-0.03 mg L^{-1}. Water temperature fluctuated throughout the sampling periods (the lowest was 27°C in January, increasing gradually and reaching the highest level, 32°C, in May, then gradually decreasing until the end of the year). The concentration of COD in water highly fluctuated through the sampling period; the lowest was 28.8 O_2 mg L^{-1} in April, increasing gradually, and the highest was 142.4 O_2 mg L^{-1} in September; it was maintained at high levels in October and November (118, 4 and 116.8 O_2 mg L^{-1}, respectively); the next months, COD contents ranged from 57.6 to 81.6 O_2 mg L^{-1}.

Fluctuations of water temperature and of COD, and disease prevalence by sampling months are shown in Figure 13 and Figure 14, respectively. Hemorrhagic disease increased in proportion of the temperature; when the temperatures were between 30 and 32°C, the rate of hemorrhagic disease in *P. hypophthalmus* ranged from 20 to 42% (Figure 13). White patches in the internal organs were more common at low temperatures (about 27-29°C) (Figure 13) and when the water resource originated from upper streams, creating a high COD concentration (Figure 14). Ectoparasite infections occurred all year round in catfish ponds, especially in the rainy season and in high COD concentration conditions (Figure 14).

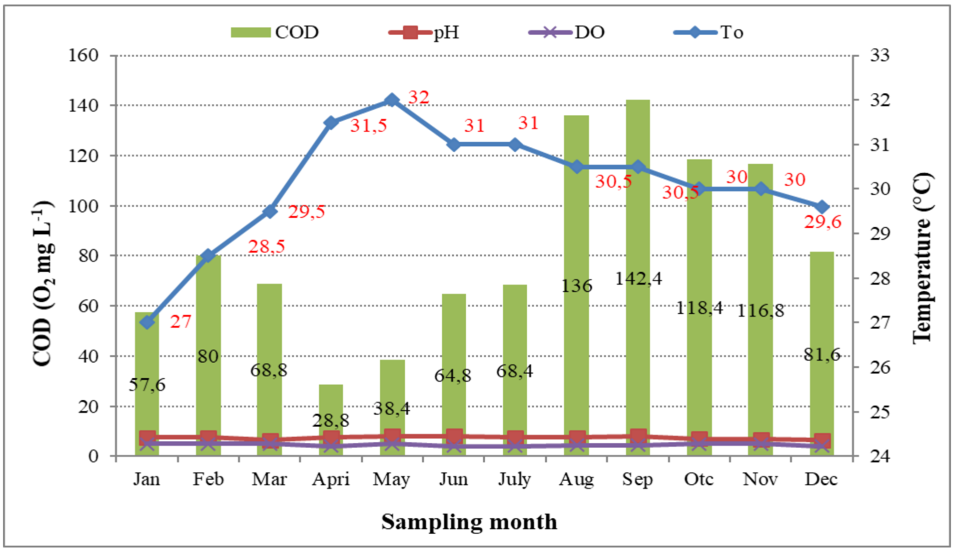

Figure 12. Fluctuation of water parameters in catfish ponds, during the sampling periods.

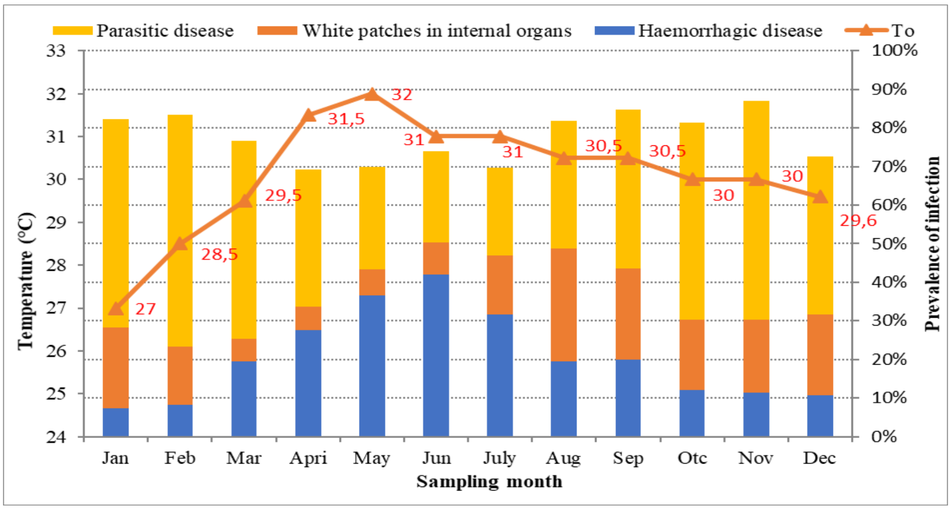

Figure 13. Fluctuation of temperature and disease prevalence by sampling months.

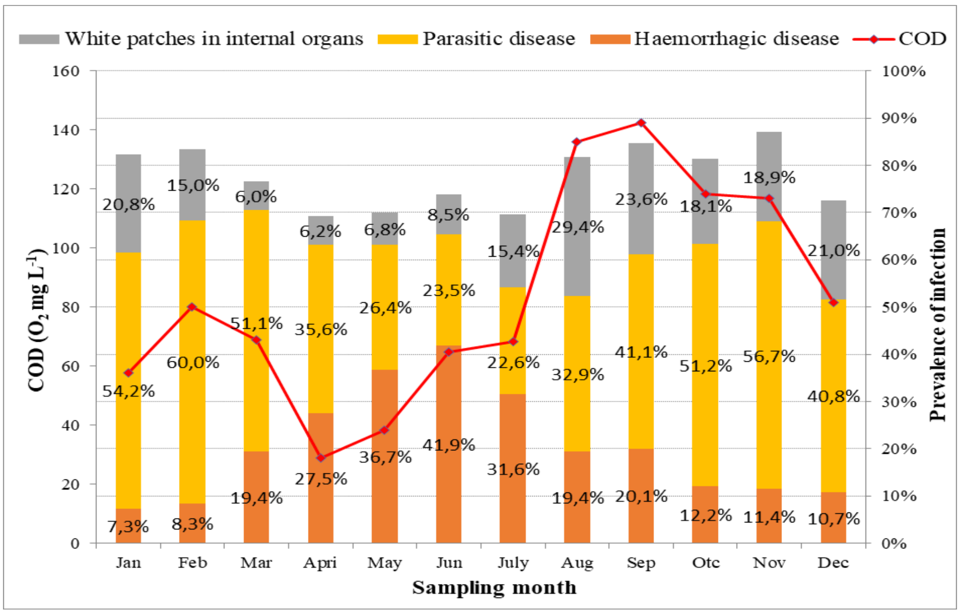

Figure 14. Fluctuation of COD and disease prevalence by sampling months.

Discussion. *P. hypophthalmus* culture has been developed very rapidly in Vietnam and has a great contribution to the nation's aquaculture production. However, emergence and spread of diseases has greatly increased over the past few years along with rapid expansion and intensification of *P. hypophthalmus* farms, especially under the fluctuation of environmental water parameters due to climate change. To address these concerns, we conducted a field study for 12 months (2 culture cycles) to record diseases and selected water quality parameters in major *P. hypophthalmus* cultural areas in the Mekong Delta. An overall prevalence of 41.3% ectoparasites was recorded, including *Dactylogyrus* spp., the major parasites of class Monogenea, observed on the gills of collected catfish samples. Besides, *Trichodina* spp. parasites were also found on the skin mucus and gills. *Trichodina* spp. are very common in *P. hypophthalmus* ponds, being present on the skin, fin, and gills of the fish (Hoffman, 1998).

Prevalence of the hemorrhagic disease was observed in proportion of 20.5% among the collected samples and *A. hydrophila* was isolated and identified from the hemorrhagic diseased fish. Previous studies had similar records: hemorrhagic disease is the most popular disease of *P. hypophthalmus* farms, caused by *A. hydrophila* (Ly et al 2009; Crumlish et al 2010). The clinical signs showed that petechial hemorrhage appear around the mouth and on the abdominal area, fins, anus; hemorrhage appear also in the internal organs (liver, kidney, spleen, intestine and swim bladder). The hemorrhagic disease usually appears almost all year round, especially when fish are shocked and in low water quality conditions (Dung et al 2015), with a high mortality causing serious losses to farmers. The disease with white nodules in internal organs as clinical signs (also named bacillary necrosis of *Pangasius*), caused by *E. ictaluri*, was recorded with a prevalence of 15.8%, with clinical signs similar to previous studies (Ferguson et al 2001; Crumlish et al 2002). The clinical signs of pale gills and liver and yellow color of the body and internal cavity were recorded with the prevalence of 5.4% and 0.5%, respectively. These diseases have been reported in *P. hypophthalmus* farms a few years ago, but the causative agents which are responsible for the clinical signs of those diseases have not yet been identified. Further research is necessary for their identification and for determining the possible prevention and treatments.

In this study, ranges of water parameters suitable for the *P. hypophthalmus* growth including salinity, pH, DO, nitrite, $NH4^+$, NH_3 were determined. Measured data of water parameters throughout the sampling period showed that there was a correlation between occurrence of disease, temperature and concentration of COD in the pond water. Ectoparasitic infestation occurred all year round in catfish ponds, especially in the rainy season, combined with adverse environmental parameters in ponds. The parasites observed in the catfish samples can cause lesions on the body of the fish, destroying the gill filaments, obstructing the respiratory activity and reducing the resistance of the fish to the opportunistic pathogens infection, leading to mass mortality in the cultured ponds.

Conclusions. Hemorrhagic diseases caused by *A. hydrophila*, bacillary necrosis of *Pangasius* disease caused by *E. ictaluri* and ectoparasite infestation appear year-round in cultured *P. hypophthalmus*, according to this survey. Hemorrhagic disease increased in proportion of the temperature, whereas white nodules in the internal organs were more common at low temperatures (about 27-29°C) and when the water resource comes from the upper stream, creating high COD concentrations. Ectoparasite infestation occurred with a high prevalence in the rainy season, combined with adverse environmental parameters in ponds. Diseases with clinical signs of pale gills and liver, or jaundice were recorded with a low prevalence and their causative agents remain to be investigated. The results of the survey suggest that precautionary measures should be taken at the major hotspot of disease, during the first months of the disease onset.

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Conflict of interest. The authors declare no conflict of interest.

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