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Environmental Factors and Acute Hepatopancreatic Necrosis Disease (AHPND) in Shrimp Ponds in Viet Nam: Practices for Reducing Risks

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Abstract

Water and sediment quality in shrimp ponds in Soc Trang, Bac Lieu and Ca Mau provinces in the Mekong River Delta, Viet Nam did not differ ($P > 0.05$) between ponds containing AHPND-infected shrimp and those with AHPND-free shrimp. However, there was evidence of wide-spread pollution in the sampling area, pond management procedures were often deficient, and general water quality conditions in some ponds were suboptimal. Farms in the study area take in water from the same canals into which they discharge effluent, favouring cross-contamination spread of shrimp diseases among farms. The risk of AHPND in shrimp ponds could be lessened by improvements in biosecurity and pond management that would result from adoption of good aquaculture practices (GAPs) described herein.

Keywords: AHPND, EMS, good aquaculture practices, pollution and shrimp farming, water quality in shrimp ponds.

Introduction

High mortality of shrimp during the first 30 days of culture for unknown reasons observed first in the People's Republic of China around 2009 was referred to as covert mortality disease and later as early mortality syndrome (EMS). The phenomenon subsequently spread to Viet Nam, Malaysia and Thailand, resulting in major losses of shrimp.

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Lightner et al. (2012) described the pathology of EMS and suggested that the malady be called acute hepatopancreatic necrosis syndrome (AHPNS). One possible cause proposed for AHPNS was a toxic agent of natural or anthropogenic origin (NACA 2012), and an environmental survey and monitoring effort was initiated in September 2012 as part of the Food and Agriculture Organization of the United Nations (FAO) programme TCP/VIE/3304 "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam."[1]

The cause of AHPNS was identified in early 2013 as a bacterial infection of the hepatopancreas of shrimp with a form of *Vibrio parahaemolyticus* that contains a phage (Tran et al. 2013a,b; Oanh et al. 2013). This cause of early mortality in shrimp is now known as acute hepatopancreatic necrosis disease (AHPND). Despite elucidation of the aetiology of AHPND, findings of the environmental study are important. The study provides critical information on conditions in culture ponds and their surroundings that can be useful in modifying farm operations and pond management to lessen the risk of AHPND.

Materials and Methods

The environmental study was conducted in Soc Trang, Bac Lieu, and Ca Mau provinces in the Mekong River Delta, Viet Nam. These provinces represent the major shrimp production area of Viet Nam (Table 1), and most of the intensive shrimp culture (farms using both feeding and aeration) in Viet Nam is done in these three provinces. Although intensive farms represent only a portion of shrimp aquaculture in Viet Nam, it was thought that owners of intensive farms could provide more reliable records of management inputs and current and previous production success than could owners of extensive and semi-intensive farms.

Table 1. Shrimp production for 2010 in the three major shrimp-farming provinces of Viet Nam.

Location	Pond Area (ha)	2010 Production (tonnes)	2010 Production ($kg.ha^{-1}$)
Viet Nam	722 000	449 652	622
Three major shrimp-producing provinces:			
Soc Trang	48 000	60 830	1 267
Bac Lieu	126 000	70 462	559
Ca Mau	265 000	108 847	411

Environmental Observations and Farm Survey

Six farms were selected in each province for observations and interviews with owners. Travel among farms and provinces by car allowed observations of canal networks that supply water to both agriculture and aquaculture, agricultural activities in the vicinity of shrimp farms, population distribution and industrial development.

[1] The report can be downloaded at http://www.fao.org/docrep/018/i3422e/i3422e00.htm.

Focus was mainly on specific activities and development that represented possible sources of pollution that might negatively impact shrimp farming. At each farm, observations of farm layout, water supply, condition of infrastructure and pond management were made, and farm owners were interviewed to obtain information on items listed in Table 2.

Table 2. List of information requested from shrimp farmers during interviews.

Location of farm	**Use of feed amendments**
Farm age	Aeration rate
Number of ponds	Water exchange practice
Area of ponds	Treatment of water used for exchange or replacement
Depth of ponds	
Source(s) of water	Specific chemicals used during crop, dose and application frequency:
Salinity range	
Pond bottom preparation:	• Fertilizers and minerals
• Dry-out time	• Liming materials
• Sediment removal practice	• Disinfectants
• Liming practice	• Zeolite
• Tilling practice	• Other
Water disinfection:	Probiotic use
• In reservoir – chemicals and concentrations	Usual appearance of water during culture
• In ponds before stocking – chemicals and concentrations	Survival and success in past
	Observations related to onset of EMS/AHPNS
Species stocked	
Postlarval health	Opinion about cause of EMS/AHPNS
Stocking density	Agricultural activities in vicinity
Expected production	Use of agricultural pesticides to disinfect ponds in past
EMS/AHPNS status	
Feed protein content	Other sources of pollution

Environmental Sampling

The environmental sampling was conducted between 11 September and 9 October 2012 in three rounds: 22 ponds and seven canals in the first round, 21 ponds in the second round and 11 ponds in the third round. The AHPND status (infected or not infected) of shrimp in ponds was ascertained by a shrimp disease specialist from Can Tho University who made the determination based on the gross clinical signs of AHPND as described by Lightner et al. (2012). Twenty-three of the study ponds were stocked with *Penaeus monodon* Fabricius 1798 and 31 were stocked with *Penaeus vannamei* Boone 1931. Of the *P. monodon* ponds, 16 (≈70 %) contained AHPND-infected shrimp, while 13 (≈58 %) of the *P. vannamei* ponds had infected shrimp.

Water samples were dipped from the surface, placed in appropriately sized bottles and preserved (Eaton et al. 2005) as follows: at 4 °C in dark [5-day biochemical oxygen demand (BOD_5), total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2^--N), and nitrate-nitrogen (NO_3-N)]; acidified to pH 2 with sulfuric acid and stored in the dark at 4 °C [total nitrogen (TN) and total phosphorus (TP)]; fixed in a BOD bottle [dissolved oxygen (DO)]; preserved with zinc acetate at pH 9 (total sulfide); particulate matter removed on Gelman GF/C glass fiber filters and frozen at -20 °C (chlorophyll *a*); treated with 1 mL.500 mL^{-1} concentrated nitric acid and stored at 4 °C (trace elements). Water samples also were collected from selected ponds, nine with AHPND-free shrimp and 13 with AHPND-infected shrimp, for pesticide and algal toxin analyses. These samples were dipped, placed in bottles and stored in the dark at 4 °C. Sediment samples also were collected from the upper 5-cm layer in these ponds for analyses for pesticides and trace elements. These samples were stored in plastic bags and held in the dark at 4 °C for later analysis. In May 2013, water samples for analysis for polychlorinated biphenyl compounds (PCBs) were collected from shrimp ponds and their water supply canal in each of the three provinces (n = 12). These samples were preserved in the same manner as pesticide samples.

Analyses of Samples

Water temperature, pH and salinity were measured *in situ* with a portable, multiprobe meter. Analyses for the common water quality variables, DO, BOD_5, TAN, NO_2^--N, NO_3^--N, TN, TP, total sulfide and chlorophyll *a*, as well as calculation of un-ionized ammonia nitrogen (NH_3-N) and un-ionized sulfide sulfur (H_2S-S), followed standard protocol (Eaton et al. 2005). The analyses were conducted at the Laboratory of Water Quality Management, College of Aquaculture and Fisheries, Can Tho University.

Trace metal, pesticide, and PCB analyses were made at the Laboratory Centre for Environmental Analysis and Technology Transfer, Institute for Agriculture and Environment, Hanoi, Viet Nam. The trace element analyses were by atomic absorption spectrophotometry using American Society for Testing and Materials methods (ASTM 2011). Pesticides and PCBs were determined by procedures recommended by the U.S. Environmental Protection Agency (http:/epa.gov/osw/hazard/testmethods/sw846/pdfs/8081b.pdf). The pesticide analyses included fenitrothion, deltamethrin and hexaconazole, which are common pesticides used in the Mekong Delta. Algal toxin analyses were made by the Laboratory of National Agro-Forestry-Fisheries Quality Assurance Department – Branch 6, Can Tho, Viet Nam. The mouse bioassay procedure was used (AOAC 2006).

The design of the survey was simple, and only basic statistical analyses – means, standard errors, Student t-test and analysis of variance with Duncan's multiple range test – were used in the data evaluation.

Results

Water Supply

Farms receive water from canals branching from estuarine reaches of rivers or the sea. In Soc Trang and Bac Lieu provinces, study farms were between 1 and 12 km from the sea, while in Ca Mau Province, distances to the sea ranged from 14 to 31 km. However, water in canals often traveled much farther than the straight-line distance from the sea to a farm. For example, a farm near Bac Lieu City was only 2 km from the sea, but it received water that flowed about 20 km in a canal before reaching the farm. Surface water in the Mekong River Delta seems almost totally interconnected – especially in the rainy season. Rice and other cereal grains are cultivated in the three provinces (http://www.gso.gov.vn/ default_en.aspx?tabid=469&idmid= 3&ItemID=13070). The areas devoted to this type of agriculture in 2010 were as follows: Soc Trang, 353 300 ha; Bac Lieu, 158 400 ha; Ca Mau, 125 800 ha. There is considerable use of insecticides and fungicides on these crops. Rice farming and other agriculture are done in freshwater areas, and water control structures greatly restrict the movement of brackishwater into freshwater canals. Nevertheless, freshwater areas drain into rivers, and pollution from freshwater areas can reach brackishwater canals that supply shrimp farms via river discharge into coastal areas. The population density of the Mekong River Delta is high; for the three provinces of this investigation, average population densities were as follows: Soc Trang, 394 people.km^{-2}; Bac Lieu, 354 people.km^{-2}; Ca Mau, 229 people.km^{-2} (http://www.gso.gov.vn/default_en.aspx?tabid=467&idmid= 3&ItemID=12941). High population density favours in a large amount of domestic and municipal wastewater, and in Viet Nam, wastewater usually is discharged untreated into canals and rivers. The minimum wet-season salinity sometimes falls to 0 ppt at a few farms; at most farms, the salinity remains above 5 ppt. Maximum, dry-season salinities of 10 to 42 ppt were reported. All farms have a wide fluctuation in salinity during a 12-month period.

Farms and their Management

Individual farms had different numbers of ponds; the smallest number was two, while the largest number was 28. Total production areas of individual farms ranged from 0.3 to 9.8 ha. Average depths of individual ponds ranged from 1.0 to 2.0 m, but most ponds averaged around 1.5 m in depth. Ponds at all farms were allowed to dry out between crops. Dry-out time was variable (7 to 60 days), but it did not exceed 15 days at the majority of farms. Sediment was removed from ponds between crops at all but two farms. At four farms practicing sediment removal, high-pressure hoses were used to wash sediment from ponds, but at others, a bulldozer was used to push sediment to pond edges. Pond bottom soil was limed between crops at all but one farm. Burnt lime, also known as quick or unslacked lime, was applied to pond bottoms at nine farms – application rates ranged from 250 to 2 000 kg.ha^{-1}. The recommended rate for disinfection of pond bottom soil with burnt lime is 4 000 to 5 000 kg.ha^{-1} (Hill et al. 2013).

At the other farms using liming material, agricultural limestone (pulverized limestone or marl) was applied at rates ranging from 500 to 2 500 $kg.ha^{-1}$. Agricultural limestone is not a disinfectant (Boyd and Tucker 1998). At five farms, pond bottoms were tilled with a disk harrow following liming. Water was pumped from water supply canals into farm reservoirs, into farm water supply canals, or in a few cases, directly into ponds. At farms with reservoirs, water was held in reservoirs for 4 to 8 hr for sedimentation. Chemical disinfectants were applied to water after it had been transferred to culture ponds. Calcium hypochlorite and iodine compounds were most commonly applied; other disinfectants reported to be used were: formalin, glutaraldehyde, peroxymonopersulfate, potassium permanganate and trichlorocyanuric acid. The farmers usually could not provide exact information on amounts of disinfectants applied, but the amounts of calcium hypochlorite used were much less than the recommended concentration of 10 $mg.L^{-1}$ of active chlorine (Hill et al. 2013). We could not find references to effective doses of the other compounds, but Hill et al. (2013) indicated that the dose of iodine needed for disinfection was considerably greater than for chlorine. The two farms that did not use chemical disinfectants passed the water from reservoirs through a fine filter (mesh not specified) when transferring it to production ponds.

All farms claimed to stock postlarvae (PL) tested free of specific pathogens (SPF PL). Of course, the PL were not tested for *V. parahaemolyticus*, because this bacterium had not been implicated as the cause of AHPND in 2012. Stocking densities varied from 35 to 120 $PL.m^{-2}$ for *P. vannamei* and from 25 to 40 $PL.m^{-2}$ for *P. monodon*. The crude protein content of feeds differed from 37 to 45 % – usually a lower protein-content feed was used for *P. vannamei* than for *P. monodon*. Fat content of feed was 4 to 8 %. Production expected by farmers based on previous crops depended upon species and stocking density, and varied from 6 to 16 $tonnes.ha^{-1}$ for *P. vannamei* and from 4 to 8 $tonnes.ha^{-1}$ for *P. monodon*. Three farms reported using water exchange during grow out. However, the amount of water exchange was small – only 10 to 20 % of pond volume two or three times during the production period. Make-up water was added to replace evaporation during the dry season at all farms. Owners of farms claimed to disinfect surface water before it was used for water exchange or replacement.

Two farms used groundwater to replace evaporation loss, and this water was not treated with a disinfectant before adding it to ponds. All farms applied mechanical aeration with "long-arm" paddlewheel aerators typically used in Asian shrimp farming. The amount of aeration was increased from early in the crop until harvest, and aeration rates at the end of the production period were reported to range from 10 to 32 $hp.ha^{-1}$. Based on maximum, expected production values given by farmers, aeration rates ranged from 312 to 1 000 kg $shrimp.hp^{-1}$ (average = 608 $kg.hp^{-1}$) for *P. vannamei* and from 250 to 466 kg $shrimp.hp^{-1}$ (average 357 $kg.hp^{-1}$) for *P. monodon*. An aeration rate of 1 hp for every 400 kg of production often is recommended for shrimp ponds in Asia (Boyd 2009). At the time of the survey, ponds were in the first 30 days of culture and had low biomass of shrimp. The amount of aeration per unit weight of shrimp per horsepower was well above the minimum recommended amount. All farms applied microbial products during the grow-out period with the belief that this would improve water and sediment quality.

These microbial products consisted of cultures of live microbial cells, and some products contained up to six of the following genera: *Aspergillus*, *Bacillus*, *Lactobacillus*, *Nitrobacter*, *Nitrosomonas*, *Paracoccus*, *Rhodobacter*, *Rhodococcus* and *Saccharomyces*. Other amendments used at all farms for the purpose of improving water quality were zeolite, iodine, potassium permanganate, calcium carbonate, dolomite, benzalkonium chloride, glutaraldehyde, *Yucca* extract, potassium monopersulfate, mineral mix, enzyme preparations and chelated copper. Various combinations of these amendments were used at different farms according to the judgment of the owners.

Water and Sediment Quality

Water in the canals that supplied farms in all three provinces was of relatively good quality, but it had high concentrations of nitrogen and phosphorus and contained a relatively great abundance of phytoplankton as indicated by elevated chlorophyll *a* concentrations (Table 3). Trace metal analyses were made on samples from only two canals, and concentrations of these substances were low (Table 4). Pesticide analyses were not made for canal water.

Table 3. Average concentrations of water quality variables in canals that supplied water to shrimp ponds in the three provinces.

Variable	**Soc Trang (n = 2)**	**Bac Lieu (n = 3)**	**Ca Mau (n = 2)**
Water temperature (^oC)	27.8	29.0	32.4
pH (standard units)	8.00	8.3	8.05
Salinity (ppt)	3.0	9.3	4.0
Dissolved oxygen ($mg.L^{-1}$)	3.70	5.91	4.34
5-Day biochemical oxygen demand ($mg.L^{-1}$)	3.0	4.0	9.0
Total ammonia nitrogen ($mg.L^{-1}$)	0.335	0.306	0.174
Un-ionized ammonia nitrogen ($mg.L^{-1}$)	0.028	0.043	0.015
Nitrite nitrogen ($mg.L^{-1}$)	0.131	0.110	0.052
Nitrate nitrogen ($mg.L^{-1}$)	0.095	0.087	0.192
Total nitrogen ($mg.L^{-1}$)	3.33	1.43	1.54
Total phosphorus ($mg.L^{-1}$)	0.351	0.10	0.280
Total sulfide ($mg.L^{-1}$)	0.052	0.059	0.126
Hydrogen sulfide ($mg.L^{-1}$)	0.004	0.003	0.011
Chlorophyll *a* ($\mu g.L^{-1}$)	78.4	26.6	14.5
Arsenic ($\mu g.L^{-1}$)	1.19		6.18

Table 3 Continued.

Variable	**Soc Trang (n = 2)**	**Bac Lieu (n = 3)**	**Ca Mau (n = 2)**
Cadmium (μg.L^{-1})	ND[1]		0.96
Copper (μg.L^{-1})	7.0		22.6
Lead (μg.L^{-1})	0.53		50.6
Mercury (μg.L^{-1})	ND		
Zinc (μg.L^{-1})	132		554

[1]ND = not detectable.

Table 4. Water analysis data for ponds in the Mekong Delta, Viet Nam that contained shrimp either positive or negative to AHPNS**.**

	1st Sampling[1]		**2nd Sampling[1]**		**3rd Sampling[1]**	
Water quality variable	**Negative (n = 9)**	**Positive (n = 13)**	**Negative (n= 11)**	**Positive (n = 10)**	**Negative (n = 5)**	**Positive (n = 6)**
Temperature (°C)	29.07±1.10	30.06±2.00	29.15±1.54	28.96±0.69	30.0 ±2.04	28.9±1.12
pH (standard units)	8.37±0.35	8.71±0.63	8.67±0.60	8.64±0.49	8.48±0.50	8.59±0.37
Salinity (ppt)	10.1±6.4	10.1 ± 5.4	12.8±5.3	12.4±6.9	4.6 ±3.6	9.2±5.3
Dissolved O_2 (mg.L^{-1})	7.1±1.6	7.73 ± 1.6	6.7 ± 1.8	6.4 ± 1.6	6.8±0.8	6.9±1.1
BOD_5 (mg.L^{-1})	10.6±8.0	9.4±8.2	4.1±4.7	2.4±3.4	4.6±0.5	1.9±0.2
Total ammonia N (mg.L^{-1})	0.55±0.61	0.18±0.29	0.08±0.15	0.20±0.38	0.73±0.10	0.30±0.18
NH_3 (mg.L^{-1})	0.07±0.07	0.04±0.05	0.09±0.05	0.08±0.10	0.38±0.42	0.16±0.17
Nitrite N (mg.L^{-1})	0.31±0.40	0.09±0.13	0.11±0.24	0.11±0.28	0.25±0.18	0.11±0.12
Nitrate N (mg.L^{-1})	0.29±0.63	0.04±0.04	0.49±0.58	0.50±0.45	0.99 ±0.57	0.88± 0.48
Total N (mg.L^{-1})	2.15±1.32	1.62±1.29	0.86±0.75	1.07±1.43	1.82±0.19	2.53±0.15
Total P (mg.L^{-1})	0.26±0.10	0.21±0.13	0.20±0.19	0.15±0.13	0.08 ± 0.04	0.04 ±0.04
Total sulfide (mg.L^{-1})	0.05±0.03	0.07±0.05	0.04±0.06	0.02±0.03	0.004±0.006	0.001±0.001
H_2S (mg.L^{-1})	0.003±0.002	0.006±0.010	0.002±0.004	0.002±0.004	ND	ND
Chlorophyll *a* (μg.L^{-1})	33.5±43.7	31.0±31.1	23.9±22.3	29.7±45.9		
Arsenic (μg.L^{-1})	0.10±0.05	0.01±0.06				
Cadmium (μg.L^{-1})	0.20±0.16	1.22±1.51				
Copper (μg.L^{-1})	49.6±38.7	44.2±52.3				
Lead (μg.L^{-1})	7.2±6.4	4.1±3.0				
Mercury (μg.L^{-1})	2.2±1.5	2.4±1.8				
Zinc (μg.L^{-1})	313±113	282±137				

[1]There were no differences at P = 0.05 between ponds with AHPNS-negative or AHPNS-positive shrimp for any variables as determine by *t*-tests.

[2]ND = not detectable.

Concentrations of water quality variables in pond waters were within similar ranges in all three provinces and for the three sampling rounds, but the important point is that there were no differences as determined by t-test ($P > 0.05$) between means for water quality variables with respect to AHPND status of shrimp for any of the three rounds of sampling. The upper limits (lower limit for DO) of the normal concentration ranges of critical water quality variables and for trace metals were established (Table 5) based on information from the literature by Boyd and Tucker (1998), Boyd (2000), Prapaiwong and Boyd (2012, 2014), Chin and Chen (1987), Lin and Chen (2001), and water quality fact sheets from the Southern Regional Aquaculture Center (http://srac.tamu.edu). One or more of these limits were exceeded in some ponds with shrimp of both AHPND categories. The number of un-ionized ammonia values above normal was considerably greater in ponds with AHPND-negative shrimp than in those with AHPND-positive shrimp, and the total number of instances of values of critical variables exceeding the limits of the normal ranges was somewhat greater for the ponds with AHPND-negative shrimp as a result.

Table 5. The number of ponds in the Mekong Delta, Viet Nam that contained shrimp either negative or positive to AHPNS that were outside acceptable concentration ranges of critical water quality variables.

Variable	**Negative (n = 25)**	**Positive (n = 29)**
Salinity, < 5 ppt	3	3
pH, > 9.0	5	7
Dissolved oxygen, < 4 mg.L^{-1}	1	0
Un-ionized ammonia nitrogen, > 0.05 mg.L^{-1}	12	4
Nitrite-nitrogen, > 0.5 mg.L^{-1}	6	3
Hydrogen sulfide, > 0.0025 mg.L^{-1}	4	3
Arsenic, > 5 µg.L^{-1}	0	0
Cadmium, > 0.25 µg.L^{-1}	2	2
Copper, > 50 µg.L^{-1}	4	3
Lead, > 5 µg.L^{-1}	5	5
Zinc, > 500 µg.L^{-1}	0	2
Total number instances with values outside normal range	42	33

The only pesticides detected in pond waters were the insecticides fenitrothion and deltamethrin. These two insecticides were at detectable concentrations in more ponds with AHPND-infected shrimp than in ponds without AHPND-infected shrimp, but there were no differences between the two categories of ponds with respect to average concentrations of the two insecticides (Table 6). The highest concentration of either insecticide was 0.5 µg.L^{-1}. Algal toxins and PCBs were not at detectable concentrations in any water samples.

Table 6. Mean pesticide concentrations and standard errors (SE) along with ranges ($\mu g.L^{-1}$) for water from ponds that contained either AHPNS-negative (n = 9) or positive (n = 13) shrimp.

Pesticide	**Number of ponds where detectable**		**Average concentration ± SE[1] (Ranges in parentheses)**	
	Negative	**Positive**	**Negative**	**Positive**
Water				
Fenitrothion	3	5	0.006 ± 0.0065 (ND[2] – 0.140)	0.106 ± 0.0411 (ND – 0.500
Deltamethrin	2	4	0.024 ± 0.0212 (ND–0.130)	0.035 ± 0.0174 (ND – 0.170)
Sediment				
Hexaconazole	4	5	20.4 ± 1.69 (ND – 24.5)	22.1 ± 1.34 (ND–27.0)
Deltamethrin	3	5	2.6 ± 0.90 (ND – 3.74)	3.1±1.43 (ND–4.54
Fenitrothion	1	6	1.0 ± 0.01 (ND – 1.0)	2.5±1.34 (ND–9.04)

[1]There were no differences at $P = 0.05$ between ponds with AHPNS-negative or AHPNS-positive shrimp for any variables as determine by *t*-tests.
[2]ND = not detectable.

There were no differences ($P > 0.05$) for trace metal concentrations in sediment between ponds with respect to AHPNS status of shrimp (Table 7). The concentrations of trace metals in sediment were within the range of concentrations of trace metals reported in shrimp pond sediment from ponds in several countries (Boyd et al. 1994).

Table 7. Trace metal concentrations and standard errors (SE) ($\mu g.kg^{-1}$) in sediment from ponds that contained either AHPNS-negative or positive shrimp.

Variable	**Negative (n = 9)**	**Positive (n = 13)**
Arsenic	5.95±0.781	5.85±0.560
Cadmium	0.23±0.033	0.22±0.017
Copper	26.2±1.34	24.8±2.08
Lead	37.6±1.31	35.4±1.67
Mercury	0.06±0.012	0.04±0.007
Zinc	168±4.0	160±5.6

Deltamethrin and fenitrothion, as well as the fungicide hexaconazole, were detected in sediment samples (Table 6). Although each of these pesticides was detected in more ponds with AHPND-positive shrimp than in ponds with shrimp unaffected by the disease, as with water, mean concentrations of the pesticides did not differ ($P > 0.05$) with respect to AHPND status of shrimp.

Discussion

The water supply situation for shrimp farms in the Mekong River Delta obviously is not ideal. Water often travels long distances in canals, providing ample opportunities for pollution from domestic, agricultural, aquacultural, industrial and natural sources. It is particularly problematic that discharges from shrimp farms in a particular area mix with water in canals from which all shrimp farms in the area take in water. Contamination of farm influent with farm effluent facilitates transfer of shrimp diseases among farms. Disinfection of water before using it for shrimp culture ponds should be considered a critical aspect of farm management in Viet Nam. The farmers visited in Viet Nam claimed to dry-out ponds and disinfect water before stocking shrimp, but there was wide variation from farm to farm in management practices. In particular, the methods of disinfection varied, and concentrations of disinfectants used also differed among farms. Concentrations of disinfectants used at all farms appeared to be too low to effect complete disinfection.

The farmers claimed to use SPF PL; however, the PL were not checked for *Vibrio parahaemolyticus* (with phage), because the causative agent had not yet been identified at the time of the farm survey. Most farmers claimed to use feeding trays or cast net samples for obtaining information on optimal feed input. However, the amount of aeration used in *P. vannamei* ponds was sometimes less than that considered necessary to maintain nighttime dissolved oxygen concentration above 3 $mg.L^{-1}$ (Boyd 2009) – especially during the last one or two months of culture. But, AHPND apparently occurred early in the shrimp crop when aeration was adequate. All farms used microbial products during culture, and the use of these products apparently had no influence on AHPND status of shrimp in ponds. There were no apparent differences in AHPND status of shrimp with respect to pond management. This does not necessarily mean that the disease cannot be influenced by management, because some PL may have been infected with AHPND in the hatchery, and even if they were AHPND-free when delivered to farms, the concentrations of disinfectants used at farms were too low to be effective.

Ponds had relatively high concentrations of nitrogen and phosphorus, and phytoplankton was abundant in most ponds during the first month of culture, as evident from elevated chlorophyll *a* concentrations. In some ponds, pH values were high, and concentrations of salinity, dissolved oxygen, un-ionized ammonia nitrogen, nitrite-nitrogen, hydrogen sulfide, arsenic, cadmium, copper, lead and zinc were outside optimal ranges for shrimp culture. Insecticides also were detected in some ponds, but PCBs and algal toxins were not found. There were, however, no differences in concentrations and ranges of potentially harmful substances between ponds with respect to AHPND status of shrimp. The onset of AHPND possibly could have been triggered by stress related to one or more water or sediment quality variables. However, infection of shrimp by AHPND requires the presence of the disease agent, and if *V. parahaemolyticus* was not present in ponds, stress would not have led to AHPND. This is confirmed by the observation that water quality was frequently outside optimal ranges for one or more variables in all ponds regardless of AHPND status of shrimp.

It is particularly important to note that the pH did not differ between ponds of the two AHPND categories. The claim that high pH is the environmental trigger for the disease because AHPND in shrimp repeatedly regressed at pH 7 but not at higher pH (8.5 to 8.8) (Akazawa and Eguchi 2013) is likely a mote point. Shrimp ponds typically have pH between 8.0 and 9.0, and there is no practical way – at least at present – for reducing pH below 8.0 in most ponds. Of course, it may be possible to manipulate pH in the shrimp gut to lessen the risk of AHPND, but there are no data to support this hypothesis. The report of the FAO project TCP/VIE/3304 "Emergency assistance to control the spread of an unknown disease affecting shrimps in Viet Nam" recommends the use of better biosecurity and application of good aquaculture practices as a means of lessening the risk of ANPNS (ANPHD). The objectives are to reduce the abundance of *Vibro* spp. (including *V. parahaemolyticus*) as much as possible before stocking PL, to stock PL that are free of the AHPND agent and other common disease-causing agents, to prevent entry of disease organisms during culture, and to maintain good water and sediment quality during grow out to avoid stressing shrimp. Some practices that should be adopted follow:

Biosecurity

- Banning broodstock and PL that have not tested free of specific pathogens (including *V. parahaemolyticus*).
- Stocking SPF shrimp – screening should include *V. parahaemolyticus*.
- Using closed culture systems where feasible.
- Disinfecting intake water before use (see Source Water below).
- Preventing entry of wild fish and other organisms by screening inflow (see Source Water below).
- Not transferring water or shrimp from AHPND-infected ponds to other ponds.
- Disinfecting nets and other equipment used in AHPND-infected ponds with calcium hypochlorite solution (50 mg.L^{-1} chlorine) or iodophor solution (200 mg.L^{-1} iodine) before reuse.
- Disinfecting water in ponds where the crop is lost to disease by treatment with calcium hypochlorite (50 mg.L^{-1} chlorine) before discharge.
- Using good aquaculture practices (GAPs) as described below.

Pond Bottoms

- Removing large accumulations of sediment – usually not necessary in semi-intensive culture.
- Drying pond bottoms for 2 weeks or more after harvest.
- Liming pond bottoms with burnt lime at 400 to 500 kg.1 000 m^{-2}.
- Treating bottoms with soil pH below 7.0 with agricultural limestone unless burnt lime has been applied as a soil disinfectant.
- Tilling to depths of 10 to 15 cm may be used to improve dry-out and incorporate liming materials into pond bottom soil.

Source Water

- Filtering water through 150–250 µm filter bags to remove wild fish and larger organisms that can be vectors of disease organisms. Note: this practice will not remove pathogenic bacteria.
- Holding source water in a reservoir for at least 2 weeks – for longer if possible – before filling ponds.
- Holding water in the culture ponds at farms without reservoirs for 2 weeks before stocking.
- Treating water for filling ponds with calcium hypochlorite (65 % chlorine) at 15 kg.1 000 m^{-3} before stocking PL. This can be done in the reservoir, or if there is no reservoir, treatment can be done directly in ponds.
- Applying fertilizer – preferably chemical fertilizer – to stimulate plankton growth before stocking the PL.

Postlarvae

- Using SPF PL from a reputable hatchery. *Vibrio parahaemolyticus* should be included in screening of PL.
- Acclimating the PL to pond water conditions before stocking.
- Using stocking rates reasonable for pond management strategy – especially the rate of water exchange and amount of aeration.

Feeding

- Using a feed of good quality that contains no more protein nitrogen and phosphorus than necessary.
- Storing feed in a dry place that does not have extreme temperatures.
- Using cast nets or feeding trays to assist in determining feeding rates.
- Appling feed in no greater quantities than animals will eat.
- Taking shrimp off feed if AHPND is observed. Gradually resume feeding if shrimp health improves.

Aeration

- Using about 10-hp of aeration for each 10 kg.ha^{-1} of daily feed input. The key is to avoid dissolved oxygen concentration below 3 mg.L^{-1} and preferably below 4 mg.L^{-1}.
- Positioning aerators to avoid erosion of pond bottoms and embankments.
- Using less aeration at the beginning of culture period and increasing the amount as feeding rate increases.
- Less aeration usually may be applied during the day than at night.

Fertilization during Grow out

- Using no more than 0.8 kg each of N and P_2O_5 per 1 000 m^2 per fertilizer application. Research shows that a fertilizer with an N:P_2O_5 ratio of about 1:2 or 1:3 is optimal – but there are many opinions about the best fertilization programme.

- Adjusting fertilizer application rate for plankton density. If Secchi disk visibility is less than 30 cm, delay application. In ponds with feeding, fertilization may not be necessary – especially after feeding rates reach 1 kg.1 000 m^{-2} (10 $kg.ha^{-1}$) per day.

Water Management during Grow out

- Disinfecting water in reservoirs before applying it to culture ponds to replace seepage and evaporation loss or for water exchange.
- Ceasing or reducing water exchange. At farms with large reservoirs, water may be exchanged between ponds and the reservoirs. Although water exchange often increases disease risk, its use may be necessary to lessen salinity in ponds supplied by seawater. But, no more than 5 % of pond volume per day is usually required to maintain salinity below 40–42 ppt by end of crop.

Water Quality Amendments

- Maintaining total alkalinity above 100 $mg.L^{-1}$. Liming materials may be applied as necessary, but do not make applications of burnt lime above 10 kg.1 000 m^{-2} at one time.
- Not using disinfectants (antimicrobial agents) during the culture period, because this practice is not effective, and these compounds may stress culture animals.
- Considering not applying probiotics. Although probiotics apparently are harmless, there is no evidence that they improve water quality.
- Considering not applying zeolite. Zeolite is ineffective in removing ammonia, but its use does not pose a risk to shrimp.
- Considering not applying molasses. The benefits of applying molasses for the purpose of preventing the growth of *Vibrio* spp. are unproven, but the treatment is harmless to shrimp in aerated ponds.

Water Quality Monitoring and Response

- Measuring dissolved oxygen (DO) daily in the early morning. If early morning DO concentration is below 3 $mg.L^{-1}$ regularly, more aeration is needed or the daily feed input should be lessened.
- Measuring salinity weekly. Little can be done to increase salinity, but if salinity becomes too high (over 40–42 ppt) water exchange can be used. Of course, water exchange increases disease risk.
- Measuring pH in morning and afternoon weekly. A pH above 9 in the afternoon often suggests poorly buffered water. Alkalinity should be measured, and if it is below 100 $mg.L^{-1}$, liming material should be applied. The pH in ponds varies daily – it is lowest in the morning and highest in the afternoon. It also is higher near the surface than near the bottom. Other than maintaining total alkalinity to assure buffering capacity, there is no proven way of lessening pH.

- Measuring total ammonia nitrogen concentration weekly. Values above 2 $mg.L^{-1}$ suggest excessive feed input, insufficient phytoplankton abundance to remove ammonia nitrogen effectively, or inadequate aeration.

Conclusion

Although AHPND is not caused by a toxic agent of environmental origin, environmental factors that stress shrimp will increase their susceptibility to this and other diseases. The main practices for lessening the risk of AHPND follow: use PL free of the causative agent and other specific pathogens; disinfect pond bottoms and pond water – including water added to replace evaporation and seepage; do not transfer water among ponds during culture; use management practices that assure good water quality during culture; and disinfect water in ponds in which the crop was lost to disease before releasing it into canals or other waterbodies.

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