Environmental Studies: Assessment of Toxins in Feed, Sediment, and Water

Claude E. Boyd
Professor and Eminent Scholar in Environmental Issues,
School of Agriculture, Department of Fisheries and Allied Aquacultures
Auburn University, Alabama 36849 USA

Data were collected by Dr. Truong Quoc Phu and others of Can Tho University.

Objectives

The objective of the study was to compare water quality and sediment condition between EMS/AHPNS-infected and non-infected ponds. The reasoning was that possibly some normal water quality variable(s) or contamination with pesticides or other toxic chemicals might be the cause of the disease, and by conducting the survey of farms, such a factor could possibly be detected.

Selection of Farms and Sampling

- Farms representative of intensive farms in Soc Trang, Bac Lieu, and Ca Mau provinces were located by investigators from Can Tho University and samples were collected with standard water and sediment sampling equipment. Samples were preserved and sent to laboratories for analysis.

- Feed was obtained from farms.

Methods of Analyses

- Analyses of samples were done by Can Tho University and Ministry of Agriculture.
- General water analyses followed protocol by APHA.
- Pesticides were analyzed by USEPA methodology using gas chromotography.
- Trace metals were analyzed by atomic absorption spectrophotometry following ASTM procedures.
- Algal toxins were measured by mouse bioassay following the AOAC procedure.

Description of Samples

Type	Status	*P. monodon*	*L. vannamei*
Water:	Ponds	23	31
	Stocking rate (Pl/m^2)	28 $\pm$ 1	88 $\pm$ 4
	EMS/AHPNS-positive	16	13
Sediment:	Ponds	11	11
	EMS/AHPNS- positive	8	5
Feed:	Farms	7	6
	EMS/AHPNS-positive	6	2

Comparison among provinces and EMS/AHPNS infection status of shrimp with respect to average concentrations and standard errors (SE) of critical water quality variables.

Province	Inspection status	Mean ± SE	Duncan's test ranking (P = 0.05)
		pH	
Ca Mau	Negative	8.32 ± 0.173	A
Soc Trang	Positive	8.51 ± 0.183	A
Bac Lieu	Negative	8.58 ± 0.196	A
Bac Lieu	Positive	8.65 ± 0.144	A
Soc Trang	Negative	8.68 ± 0.172	A
Ca Mau	Positive	8.81 ± 0.183	A
		Salinity (ppt)	
Ca Mau	Positive	6.0 ± 1.30	A
Ca Mau	Negative	6.7 ± 1.38	A
Soc Trang	Negative	7.7 ± 1.30	A
Soc Trang	Positive	7.8 ± 1.38	A
Bac Lieu	Positive	15.4 ± 1.08	B
Bac Lieu	Negative	18.0 ± 1.47	B

Comparison among provinces and EMS/AHPNS infection status of shrimp with respect to average concentrations and standard errors (SE) of critical water quality variables.

Province	Inspection status	Mean ± SE	Duncan's test ranking (P = 0.05)
	Dissolved oxygen (mg/L)		
Ca Mau	Negative	6.4 ± 0.54	A
Soc Trang	Positive	6.9 ± 0.57	A
Ca Mau	Positive	6.9 ± 0.57	A
Soc Trang	Negative	7.0 ± 0.54	A
Bac Lieu	Negative	7.1 ± 0.61	A
Bac Lieu	Positive	7.3 ± 0.44	A
	Un-ionized ammonia nitrogen (mg/L)		
Bac Lieu	Positive	0.016 ± 0.0186	A
Bac Lieu	Negative	0.040 ± 0.0250	A
Ca Mau	Positive	0.057 ± 0.0230	A
Soc Trang	Negative	0.067 ± 0.0220	A
Ca Mau	Negative	0.068 ± 0.0220	A
Soc Trang	Positive	0.078 ± 0.0220	A

Comparison among provinces and EMS/AHPNS infection status of shrimp with respect to average concentrations and standard errors (SE) of critical water quality variables.

Province	Inspection status	Mean ± SE	Duncan's test ranking (P = 0.05)
		Nitrite nitrogen (mg/L)	
Bac Lieu	Positive	0.027 ± 0.0745	A
Ca Mau	Positive	0.095 ± 0.0949	AB
Bac Lieu	Negative	0.162 ± 0.1015	AB
Ca Mau	Negative	0.184 ± 0.0895	AB
Soc Trang	Positive	0.281 ± 0.0949	AB
Soc Trang	Negative	0.331 ± 0.090	B
		Hydrogen sulfide (mg/L)	
Soc Trang	Negative	0.001 ± 0.0024	A
Bac Lieu	Negative	0.001 ± 0.0027	A
Bac Lieu	Positive	0.001 ± 0.0020	A
Ca Mau	Negative	0.004 ± 0.0024	A
Ca Mau	Positive	0.004 ± 0.0025	A
Soc Trang	Positive	0.007 ± 0.0025	A

Grand means and standard errors for water quality variables in ponds with (positive) or without (negative) EMS/AHPNS-infected shrimp.

Variable	Negative	Positive	(T ≤ t) P
Water temperature (°C)	29.3 ± 0.30	29.4 ± 0.29	0.702
pH (standard units)	8.52 ± 0.100	8.66 ± 0.098	0.181
Salinity (ppt)	10.2 ± 1.22	10.7 ± 1.09	0.811
Dissolved oxygen (mg/L)	6.84 ± 0.309	7.09 ± 0.294	0.327
5-Day biochemical oxygen demand (mg/L)	9.27 ± 1.733	8.59 ± 1.774	0.723
Total phosphorus (mg/L)	0.246 ± 0.023	0.215 ± 0.0250	0.234
Chlorophyll a (μg/L)	35.2 ± 7.70	33.1 ± 7.21	0.893

Grand means and standard errors for water quality variables in ponds with (positive) or without (negative) EMS/AHPNS-infected shrimp.			
Variable	Negative	Positive	$(T \leq t)$ P
Total ammonia nitrogen (mg/L)	0.35 ± 0.093	0.20 ± 0.055	0.119
Un-ionized ammonia nitrogen (mg/L)	0.064 ± 0.0130	0.045 ± 0.0130	0.590
Nitrite nitrogen (mg/L)	0.23 ± 0.068	0.12 ± 0.038	0.188
Nitrate nitrogen (mg/L)	0.17 ± 0.078	0.06 ± 0.012	0.128
Total nitrogen (mg/L)	1.65 ± 0.202	1.49 ± 0.205	0.279
Total sulfide (mg/L)	0.059 ± 0.0110	0.061 ± 0.0090	0.898
Hydrogen sulfide (mg/L)	0.002 ± 0.0010	0.003 ± 0.0020	0.324

Grand means and standard errors for water quality variables in ponds with (positive) or without (negative) EMS/AHPNS-infected shrimp.

Variable	Negative	Positive	$(T \leq t)$ P
Arsenic (μg/L)	2.79 ± 0.714	2.81 ± 0.350	0.415
Cadmium (μg/L)	0.21 ± 0.075	0.28 ± 0.044	0.298
Copper (μg/L)	41.0 ± 17.32	50.1 ± 13.62	0.240
Lead (μg/L)	6.8 ± 2.96	5.0 ± 1.04	0.586
Mercury (μg/L)	ND	ND	---
Zinc (μg/L)	269 ± 36	324 ± 39	0.351

ND = not detectable.

The number of ponds in each of the two categories (shrimp negative and shrimp positive to EMS/AHPNS) that were outside normal, acceptable 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	0
Un-ionized ammonia nitrogen, > 0.05 mg/L	12	4
Nitrite-nitrogen, > 0.5 mg/L	6	3
Hydrogen sulfide, > 0.0025 mg/L	4	3
Arsenic, > 5 µg/L	0	0
Cadmium, > 0.25 µg/L	2	2
Copper, > 50 µg/L	4	3
Lead, > 5 µg/L	5	5
Zinc, > 500 µg/L	0	2
Values above normal range	42	33

Trace metal concentrations and standard errors (SE) (μg/kg) in sediment from ponds that contained either EMS/AHPNS-negative or positive shrimp.

Variable	Negative (n = 9)	Positive (n = 13)	$(T \leq t)$ P
Arsenic	5.95 ± 0.781	5.85 ± 0.560	0.919
Cadmium	0.23 ± 0.033	0.22 ± 0.017	0.889
Copper	26.2 ± 1.34	24.8 ± 2.08	0.640
Lead	37.6 ± 1.31	35.4 ± 1.67	0.346
Mercury	0.06 ± 0.012	0.04 ± 0.007	0.311
Zinc	168 ± 4.0	160 ± 5.6	0.286

Comparison of sampling dates.			
EMS/AHPNS status of shrimp	1st	2nd	3rd
pH			
Negative	8.37	8.67	8.52
Positive	8.71	8.64	8.66
Salinity (ppt)			
Negative	10.1	12.8	10.2
Positive	10.1	12.4	10.7

Comparison of sampling dates.			
EMS/AHPNS status of shrimp	1st	2nd	3rd
Dissolved oxygen (mg/L)			
Negative	7.1	6.7	6.8
Positive	7.7	6.4	7.1
Un-ionized ammonia nitrogen (mg/L)			
Negative	0.07	0.09	0.064
Positive	0.04	0.08	0.045
Nitrite nitrogen (mg/L)			
Negative	0.31	0.11	0.23
Positive	0.09	0.11	0.12
Hydrogen sulfide (mg/L)			
Negative	0.003	0.002	0.002
Positive	0.006	0.002	0.003
Copper (mg/L)			
Negative	49.6	---	41.0
Positive	44.2	---	50.1

Mean pesticide concentrations and standard errors (SE) along with ranges (μg/L) for water from ponds that contained either EMS/AHPNS-negative (n = 9) or positive (n = 13) shrimp.

Pesticide	Number of ponds where detectable		Average concentration ± SE (Ranges in parentheses)		(T ≤ t)
	Negative	Positive	Negative	Positive	P
Fenitrothion	3	5	0.006 ± 0.0065	0.106 ± 0.0411	0.105
			(ND – 0.140)	(ND – 0.500	
Deltamethrin	2	4	0.024 ± 0.0212	0.035 ± 0.0174	0.695
			(ND – 0.130)	(ND – 0.170)	

ND = not detectable.

Mean pesticide concentrations and standard errors (SE) (µg/kg) in sediment from ponds that contained either EMS/AHPNS-negative (n = 9) or positive (n= 13) shrimp.

	Number ponds where detectable		Average concentration ± SE (Ranges in parenthesis)		(T ≤ t) P
Pesticide	Negative	Positive	Negative	Positive	
Hexaconazole	4	5	20.4 ± 1.69	22.1 ± 1.34	0.912
			(ND – 24.5)	ND – 27.0)	
Deltamethrin	3	5	2.6 ± 0.90	3.1 ± 1.43	0.727
			(ND – 3.74)	(ND – 4.54)	
Fenitrothion	1	6	1.0 ± 0.01	2.5 ± 1.34	0.228
			(ND – 1.0)	(ND – 9.04)	

ND = not detectable.

New pesticides used in Vietnam since 2010.

Class	2011	2012	2013
Insecticides	Bensultap	Fluazinam**	Annonin*
	Diflubenzuron	Metallocarb	Cyantraniliprole
	Karajin*	Spirotetramat	Transfluthrin
	Pyriproxyfen		
	Tebrufenpyrad		
Fungicides	Myclobutanil	Amisulbrom	Bronopol
	Trifloxystrobin	Dazomet	Fludioxonil*
	Zinam	Fluopicolide	Pyrimethanil
		Penconazole	Triflumizole
		Picoxystrobin	
Herbicides	Fenclorium	Benazolin	Metamifop
		Oxaziclomefone	Mestrione
		Clopyralid	Nicosulfuron
			Pentoxazone

*Not new chemical compound.

**Actually is a fungicide, but listed as insecticide.

Mean concentrations and standard errors (SE) for contaminants in feed from farms where ponds contained either EMS/AHPNS-negative or positive shrimp.

Variable	Negative (n = 5)	Positive (n = 8)	(T ≤ t) P
Arsenic (µg/kg)	0.40 ± 0.056	0.72 ± 0.125	.081
Mercury (µg/kg)	ND	ND	---
Ethoxyquin (µg/kg)	5.56 ± 3.275	30.10 ± 7.891	0.039
Butylated hydroxylanisole (µg/kg)	1.26 ± 0.758	0.81 ± 0.213	0.726
Butylated hydroxyltoluene (µg/kg)	2.16 ± 0.845	6.00 ± 1.053	0.027

Conclusions

- None of the water quality or sediment quality variables that were monitored differed with EMS/AHPNS status of ponds.
- Feed differed with EMS/AHPNS status only for BHT.
- Water quality was often impaired and pesticides detectable in ponds regardless of EMS/AHPNS status.

Conclusions (continued)

- **Better water quality management in ponds is needed, and certainly, shrimp in ponds with impaired water quality would be more susceptible to diseases in general.**

- **Apparently, there is some evidence that high pH is possibly the trigger for onset of EMS/AHPNS, but this hypothesis is not supported by pH data collected in the environmental monitoring effort.**

Conclusions (continued)

- **Although the environmental sampling results did not support the pH-trigger hypothesis, the pH data for ponds were not collected at the same time of day. This possibly affected averages and ranges obtained for EMS/AHPNS-negative and positive ponds. Thus, the pH-trigger hypothesis should be subjected to further investigation, both in the laboratory and ponds.**

pH Management in Ponds

- Because of the pH-trigger hypothesis, some information on pH management in ponds will be presented.

Effects of pH on fish, shrimp, and other aquatic life.

pH	Effects
4	Acid death point
4 – 5	No reproduction[1]
4 – 6.5	Slow growth of many species[1]
6.5 – 9	Optimum range
9 – 11	Slow growth and adverse reproductive effects
11	Alkaline death point

[1]Some fish in rivers flowing from jungles do very well at low pH.

Important Points about pH

- The pH in ponds varies over a 24-hr period, being lowest near dawn and highest in the afternoon.
- The main reason for daily pH variation in ponds usually is phytoplankton photosynthesis and respiration.
- In some ponds where waters are poorly-buffered, i.e. low total alkalinity, pH may be above 9 throughout a 24-hr period. This results from near depletion of inorganic carbon for photosynthesis.

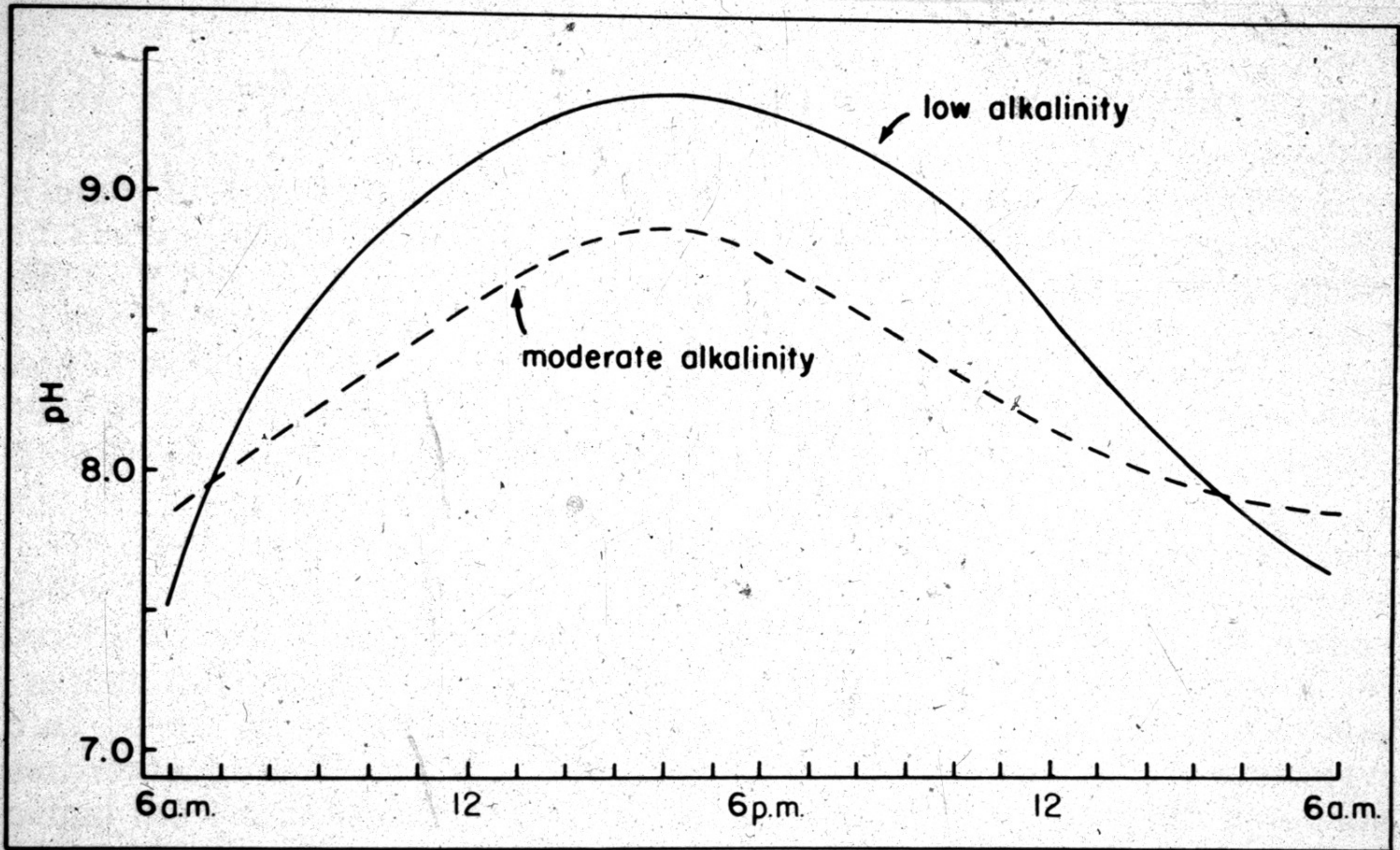

FIG. 2.2. Diel fluctuation in pH of pond waters.

Photosynthesis by Green Plants

$$6CO_2 + 6H_2O \xrightarrow[\text{Nutrients}]{\text{Light}} C_6H_{12}O_6 + 6O_2$$

Respiration

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

Important Points (continued)

- Highest pH usually is in surface water. Shrimp live on the pond bottom, and pH in water column is not always indicative of the pH of water around the shrimp.
- Low pH can usually be increased by use of liming material at an affordable cost.
- Seawater is naturally of higher pH than typical freshwaters, and aquaculture inputs favor an increase in normal pH.
- There is no simple procedure for lowering pH.

pH Management

- Acidic bottom soils ($pH < 7.0$) should be limed between crops.
- If water has a total alkalinity below 90 mg/L, it should be limed with agricultural limestone.
- Practice moderate stocking densities and good feed management to avoid excess nutrients leading to dense phytoplankton blooms that favor high pH.
- Avoid using burnt lime (CaO) and hydrated lime [$Ca(OH)_2$] in large amounts; they cause high pH.

pH Management (continued)

- In aerated ponds, application of molasses or other readily-decomposable source of organic matter might reduce pH by increasing carbon dioxide concentration.

- Commercial, mineral acids and alum reduce pH initially, but they consume alkalinity and might possibly increase the daily pH swing.

- Dyes or other sources of turbidity to limit light penetration can lessen pH. But, they are expensive.

pH Management (continued)

- Accurate pH measurement in water and soil requires a pH meter with glass electrode.

- Soil pH testers are notoriously inaccurate – despite their appeal.

CORNING
pH meter model 7
temperature °C
calibrate


PH
KELWAY SOIL TESTER