Effect of Abrupt Environmental Deterioration on the Eruption of Vibriosis in Mari-Cultured Shrimp, *Penaeus indicus*, in Egypt

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ABSTRACT

Massive scale mortalities attacked, *Penaeus indicus*, cultured within two earthen pond shrimp farms at Muthallath El-Deeba Damietta on the North East coast of the Mediterranean Egypt, during the period extended from September to November 2014. Losses were rigorous in the farm use water directly from Lake Manzala approaching 100% compared to other draws water from El-Medan canal 40%. Mortalities started concomitantly with the onset of unstable climate in the Mediterranean sea. Epidemics hit premature shrimp after 4.5 month of culture. Affected shrimp showed signs of septicemia. Majority of investigated shrimp samples 77.7% were found to be infected. *Vibrio harveyi* 34.88, *Vibrio alginolyticus* 25.58, *Vibrio vulnificus* 22.09 and *Vibrio mimicus* 17.44% were solely retrieved from moribund shrimp specimens. Similar vibrios were also detected in water and sediments samples collected from source water and the studied farms. Results support considerable evidence links between deterioration of environmental water quality measures and the eruption of vibrio epidemics in the studied farms. Unfavorable values were recorded for un-ionized ammonia, nitrite, dissolved oxygen and some noxious heavy metals. Variable degenerative and proliferative changes were detected in histopathological studies.

Key words: *Penaeus indicus*, massive mortalities, vibrio outbreaks, environmental deterioration

INTRODUCTION

Shrimp farming has become one of the most profitable economic activities in many countries (De Silva, 2001; Vadher and Manoj, 2014). Regionally, the commercial farming of shrimp in Egypt goes back to 1985. Both extensive and semi-intensive culture systems are used. However, this industry faces major challenges. Disease outbreaks are always among the notorious problems (Sadek et al., 2002).

Epidemics relevant to microbial agents have augmented progressively along with the expansion and intensification of culture systems. Among the renowned category of bacterial pathogens responsible for colossal economic losses in shrimp culture, vibrios rank the most critical (Aguirre-Guzman et al., 2004; Longyant et al., 2008).

Vibrios comprise integral part of the autochthonous microbiota of marine ecosystem. They are widely distributed in culture facilities throughout the world causing array of detrimental infections to aquatic animals relevant to such habitats once unfavorable environmental conditions are established (Aguirre-Guzman et al., 2004; Moustafa et al., 2010; Elgendy et al., 2015).

Water-borne route is the principal transmission pathway for vibrio infections. Moreover, direct contact in crowded aquaculture conditions accelerates its spread (Kanno et al., 1989). Mucus
surfaces are considered as well important portals of entries. Vibrios also invade through the intestinal tract (Yan et al., 2007). Fins, gills skin either intact or abraded are important sites for attachment and invasion of vibrios (Spanggaard et al., 2000).

Episodes relevant to vibrios attack farmed aquatic animals year round particularly in late summer (Roberts, 2012). Relatively lower infections are noticed during cold periods since some vibrios virulence determents are lost during such conditions (Yan et al., 2007). Outbreaks are triggered by exposure to unfavorable culture conditions including temperature fluctuations, high stocking densities, decreased dissolved oxygen levels, higher values of water pH, increased organic material as well as silt loading (Diggles et al., 2000; Chenga et al., 2005; Chen et al., 2011).

Numerous species within genus vibrio, including V. harveyi, V. alginolyticus, V. anguillarum and V. vulnificus have long been considered among the most detrimental shrimp pathogens causing massive stock losses (Nash et al., 1992; Aguirre-Guzman et al., 2004; Gopal et al., 2005; Longyant et al., 2008).

The present study aimed to identify the potential bacterial agents incriminated in the serious mortalities recorded in two marine shrimp farms in Muthalath El-Deeba region at Damietta on the North East coast of Mediterranean, Egypt, during the period extended from September-November 2014. Further study describes the accompanied environmental measures triggered these infections as well as the histopathological alterations induced by these bacterial infections.

**MATERIAL AND METHODS**

**Disease history:** Two marine shrimp farms in Muthallath El-Deeba region at Damietta on the North East coast of the Mediterranean, Egypt, growing *Penaeus indicus* within earthen ponds (Fig. 1a) noticed unprecedented enormous mortalities during the period extended from September-November 2014. Water used in the first farm comes from Lake Manzala. The second investigated farm draws water from El-Medan canal. This canal extends between the Mediterranean and Lake Manzala. 80% of El-Medan canal water comes from the Mediterranean Sea while only 20% supplied from Lake Manzala. Mortalities were more rigorous approached 100% in the farm draws water directly from Lake Manzala (referred in the text by first farm). The other investigated farm use water from El-Medan canal (referred by second farm) noticed relatively lower mortalities 40% during the whole period of study, 3 months. The affected earthen-ponds in both studied farms were of semi-intensive type, stocking density was 15-20 m⁻¹. The culture conditions on the investigated farms included 25% daily water exchange and 40% pelleted feed (Zoo Control). Mortalities started in September when the water level increased in Lake Manzala a result of unstable climate in the Mediterranean Sea. Epidemics hit premature shrimp after 4.5 month of culture, size ranged between 20-25 g.

**Bacteriological examination:** Samples were taken aseptically from the hepatopancreatic tissues and haemolymph, collected from the heart. Ninety randomly collected *Penaeus indicus* samples, 45 specimens from each farm were investigated through the outbreaks affecting earthen ponds. Specimens were aseptically cultured into tryptic soy broth supplemented with 2% NaCl and transferred with the minimum time of delay in isothermal box with ice to the laboratory of Hydrobiology Department, National Research Centre Egypt, for further bacteriological investigations. Upon arrival to the lab, samples were incubated at 30°C for 24 h then routinely
spread onto Marine agar (Difco) and Thiosulphate Citrate Bile Salt sucrose agar, TCBS (Oxoid). The inoculated plates were incubated for 24-72 h at 30°C. Representative numbers of the different colonial types detected on the media were collected from plates and streaked on TSA supplemented with 2% NaCl for purity and identification.

Samples collected from invading *Tilapia zillii* present in the earthen ponds of cultured marine shrimp, fifteen specimens from each investigated farm were also processed for microbiology.

**Identification of bacterial isolates:** Identification of pure bacterial isolates was performed by studying their morphological and biochemical characteristics using traditional as well as API 20 E kit (Bio Merieux) following the criteria described in Buller (2004).

**Water quality examination:** Three water samples were obtained from different locations within every studied farm during mortalities. Samples were collected in sterile bottles for physical, chemical, heavy metals evaluations and microbiological analysis. Temperature, Dissolved Oxygen (DO), pH, un-ionized ammonia (NH₃), nitrites (NO₂), nitrates (NO₃) and some heavy metals (Iron, copper, zinc, cobalt, cadmium, nickel, chromium and lead) were measured according to methods adopted from APHA (1976).

**Enumeration of the total vibrio count in water samples and sediments:** Along with water samples, three random sediments specimens from each water source (Lake Manzala and El-Medan canal) as well as from every investigated farm were collected aseptically in sterile polythene bags. All the samples, water and sediments, were diluted serially and 0.1 mL aliquots were spread plated onto Thiosulfate Citrate Bile Salt Sucrose agar (TCBS) and incubated at 30°C for 24-72 h for enumeration of the total vibrios count according to Sung *et al.* (1999).

Furthermore, to analyze the composition of the vibrios population of each sample, 20 bacterial isolates were randomly selected from TCBS plates containing 20-200 colonies. These isolates were then purified by plating on Marine agar and identified using a battery of biochemical reactions included oxidase production, gram’s staining, sensitivity to O/129, motility and API20 E system according to Buller (2004).

**Histopathological examination:** Tissue specimens for histopathological techniques were taken on site from shrimp muscles, gills and hepatopancreas. Samples were transferred to laboratory fixed in Davidson’s fixative. After 48 h, specimens were transferred into 50% ethanol. The samples were routinely embedded in paraffin and sectioned at 5 μm thick. Tissue sections were routinely processed and stained with Hematoxylin and Eosin (H and E) and examined by light microscopy according to Bancroft and Gamble (1996).

**RESULTS**

**Clinical signs and postmortem lesions:** Shrimp losses were catastrophic (Fig. 1b). Moribund shrimps clearly manifested clinical septicemia. Body discoloration was distinctive since majority of succumbed shrimp displayed reddening of the shell, legs and tail area (Fig. 1c). Others exhibited blackening of body coloration. Some cases demonstrated sudden death with no disease signs. Internally, the gut was empty. Moreover, congestion and enlargement of hepatopancreatic tissue was frequently demonstrated (Fig. 1d).
Fig. 1(a-d): (a) Earthen ponds at Muthallath El-Deeba Damietta where outbreaks attack *Penaeus indicus*, (b) Mass mortalities in *Penaeus indicus* and some invading *Tilapia zillii*, (c) Moribund *Penaeus indicus* showing hemorrhages, redness of legs (swimmers) and tail area (uropods) and (d) *Penaeus indicus* showing congestion of hepatopancrease

Table 1: Vibrio infections in moribund shrimp and total viable vibrio count

| Bacterial isolates | *Site 1* | *Site 2* | No. of isolates | Total | *Prevalence | Sources | Average total viable vibrio count |
|--------------------|----------|----------|----------------|-------|-------------|---------|----------------------------------|
| V. harveyi         | 21       | 9        | 30             | 34.88 | Lake Manzala | 2.15×10^7 | 2.5×10^8 |
| V. alginolyticus   | 15       | 7        | 22             | 25.58 | El-medan canal | 1.35×10^4 | 0.2×10^6 |
| V. vulnificus      | 12       | 5        | 19             | 22.09 | Farm 1*      | 1.75×10^3 | 1.9×10^5 |
| V. mimicus         | 8        | 7        | 15             | 17.44 | Farm 2*      | 0.98×10^3 | 1.2×10^4 |

*Site 1: Farm use water from Lake Manzala, *Site 2: Farm use water from El-Medan canal, *Percentage was calculated according to the total number of isolates 86

**Bacteriological examination:** Vibrios were the sole bacteria isolated from moribund shrimp samples. Majority of investigated shrimp samples 77.7% were found to be infected. Vibrio infections were detected in 91.1% of specimens collected from the first farm that use water directly from Lake Manzala compared to 64.4% in the second farm which draws water from El-Medan canal. Bacteriological identification revealed a total number of 86 vibrio isolates. *V. harveyi* comprised 34.88% of isolates, *V. alginolyticus* 25.58%, *V. vulnificus* 22.09% while 17.44% was recorded for *V. mimicus* (Table 1). Phenotypic and biochemical characteristics of vibrio species retrieved from moribund shrimp samples are illustrated in Table 2.

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Table 2: Biochemical characteristics of retrieved vibrio species

| Gram-stainings          | Bacilli | Bacilli | Bacilli | Cocobacilli |
|-------------------------|---------|---------|---------|-------------|
| O/129 sensitivity (150 mg) | +       | +       | +       | +           |
| Motility                | +       | +       | +       |             |
| B-galactosidase production (OPNG) | -       | -       | -       | +           |
| Arginine dihydrolase production (ADH) | -       | -       | -       |             |
| Lysine decarboxylase production (LDC) | Variable | +       | +       | +           |
| Ornithine decarboxylase production (ODC) | Variable | +       | +       | +           |
| Citrate utilization (CTT) | Variable | Variable | +       | +           |
| H2S production (H2S)    | -       | -       | -       |             |
| Urease production (URE) | Variable | Variable | Variable | -           |
| Tryptophane deaminase production (TDA) | Variable | Variable | Variable | -           |
| Indole production (IND) | Variable | Variable | +       | +           |
| Acetoin production (VP) | Variable | Variable | +       | +           |
| Gelatinase production (CEL) | Variable | +       | Variable | +           |
| Acid from glucose (GLU) | Variable | -       | +       | +           |
| Acid from manitol (MAN) | Variable | +       | +       | +           |
| Acid from inositol (INO) | Variable | Variable | Variable | -           |
| Acid from sorbitol (SOR) | Variable | Variable | Variable | -           |
| Acid from rhamnose (RHA) | Variable | Variable | Variable | -           |
| Acid from sucrose (SAC) | Variable | Variable | Variable | -           |
| Acid from melibiose (MEL) | Variable | Variable | Variable | -           |
| Acid from amygdalin (AMY) | Variable | Variable | Variable | -           |
| Acid from arabinose (ARA) | Variable | Variable | Variable | -           |
| Cytochrome oxidase prod (OX) | Variable | Variable | Variable | -           |

Similar vibrio species were also obtained from invading *Tilapia zillii* as well as from all investigated water and sediments specimens collected from source water and the relevant farms indicating a potential source of infections. The total vibrio count in these specimens is illustrated in Table 1. The uppermost vibrio count was recorded in samples collected from Lake Manzala and the relevant farms.

**Water quality measures:** There is considerable evidence links eruption of vibrio epidemics in the studied farms and deterioration of environmental water quality measures in the studied farms. Unfavorable values were recorded for Dissolved Oxygen (DO), un-ionized ammonia (NH₃) and nitrite (NO₂). The measures recorded in the first farm were, 2.6, 1.53 and 0.95 mg L⁻¹, respectively compared to, 3, 0.87 and 0.76 mg L⁻¹, respectively in the second farm. Moreover, levels detected for some investigated heavy metals; Fe, Cu, Cd, Co, Ni, Pb, Cr and As were 0.70, 0.51, 0.14, 0.64, 0.86, 1.07, 0.05 and 30 ppm, respectively in sequential in the first farm compared to 0.61, 0.43, 0.13, 0.51, 0.66, 0.87, 0.012 and 19 ppm, respectively in the other farm (Table 3).

**Histopathological examination:** No evidence of inclusions bodies indicative for viral infections were detected in all investigated histopathological sections. Shrimp muscles showed haemocytic infiltration (Fig. 2a) edema, degeneration and Zenker’s necrosis in myocytes (Fig. 2b). Gills demonstrated melanization and degenerative changes in the lamellae (Fig. 2c). Variable extensive hepatopancreatic lesions were frequently detected including inflamed sinuses with bacterial plaques and cell debris (Fig. 2d). Furthermore, severe vacuolation, necrosis in hepatopancreatic epithelial cell and accumulation of sloughed cells in the lumen were also prominent (Fig. 2e). Furthermore, thickening of the basal lamina of invaded tubules with separation of cell lining from basal lamina as well as surrounding of hepatopancreatic tubules remnants by haemocytic infiltration were regularly noticed (Fig. 2f).
Table 3: Water quality measures recorded in the investigated shrimp earthen-ponds during mortalities

Results (Average)

| Items                        | Farm draw water from Lake Manzala | Farm draw from water El-Medan canal |
|------------------------------|-----------------------------------|-------------------------------------|
| Temperature                  | 16°C                              | 16°C                                |
| pH                           | 8.3                               | 8.6                                 |
| Dissolved oxygen (DO)        | 2.6 mg L\(^{-1}\)                | 3 mg L\(^{-1}\)                    |
| **Soluble cations (mEq L\(^{-1}\))** |                                     |                                     |
| Ca\(^{2+}\)                  | 100                               | 91                                  |
| Mg\(^{2+}\)                  | 150                               | 130                                 |
| Na\(^{+}\)                   | 688.7                             | 521                                 |
| K\(^{+}\)                    | 16.7                              | 13.6                                |
| **Soluble anions (mEq L\(^{-1}\))** |                                     |                                     |
| CO\(_3\)\(^{2-}\)           | -                                 | -                                   |
| HCO\(_3\)                    | 5.6                               | 5.1                                 |
| Chloride (Cl\(^{-}\))        | 750                               | 750                                 |
| Sulphate (SO\(_4\)\(^{2-}\)) | 199.8                             | 181.2                               |
| Salinity                     | 38\(^{\circ}\)\(_{a}\)           | 35\(^{\circ}\)\(_{a}\)             |
| Ammonia (NH\(_3\))           | 1.53 mg L\(^{-1}\)               | 0.87                                |
| Nitrite (NO\(_2\))           | 0.95 mg L\(^{-1}\)               | 0.76                                |
| Nitrate (NO\(_3\))           | 1.81 mg L\(^{-1}\)               | 0.91                                |
| Cobalt                       | 0.64 ppm                          | 0.51                                |
| Copper (Cu)                  | 0.51 ppm                          | 0.43                                |
| Iron (Fe)                    | 0.70 ppm                          | 0.61                                |
| Lead (Pb)                    | 1.07 ppm                          | 0.87                                |
| Nickel (Ni)                  | 0.86 ppm                          | 0.66                                |
| Arsenic (As)                 | 30 ppm                            | 19                                  |
| Cadmium (Cd)                 | 0.14 ppm                          | 0.13                                |
| Chromium (Cr)                | 0.05 ppm                          | 0.012                               |
| Phosphorus (P)               | 0.63 ppm                          | 0.73                                |

Fig. 2(a-f): Continue
Fig. 2(a-f): (a) Muscle tissue of *Penaeus indicus* showing infiltration of haemocytes (H and E, bar = 50 μm), (b) Muscle tissue of *Penaeus indicus* showing edema, Zenker’s necrosis in the myocytes and infiltration of haemocytes (H and E, bar = 50 μm), (c) Gills of *Penaeus indicus* showing melanization and degenerative changes in the lamellae (H and E, bar = 50 μm), (d) Hepatopancrease of *Penaeus indicus* showing inflamed sinuses with bacterial plaques and cell debris (H and E, bar = 50 μm), (e) Hepatopancrease of *Penaeus indicus* showing severe vacuolation, necrosis of hepatopancreatic epithelial cell and accumulation of sloughed cells in the lumen (H and E, bar = 50 μm) and (f) Hepatopancrease of *Penaeus indicus* showing thickening of basal lamina of invaded tubules and separation of cell lining from the basal lamina with remnants of hepatopancreatic tubules surrounded by hemocytic infiltration (H and E, bar = 50 μm)

**DISCUSSION**

Negative consequences of the expansion and intensification of aquaculture industry are innumerable. In essence, health problems are amongst the most argued issues (De Silva, 2001; Moustafa et al., 2014; Elgendy, 2013).

The most significant diseases of cultured penaeid shrimp, in terms of economic impacts, have infectious agents as their cause. Several mortalities stemming from microbial pathogenic agents are frequently noticed in many shrimp farming facilities worldwide (Flegel, 2006; Lightner et al., 2012). Among the bacterial pathogens distressing cultured shrimp, vibrios have long been considered the most devastating (Aguirre-Guzman et al., 2004). Vibrios have been incriminated in numerous disease problems in several marine shrimp farms (Vandenberghhe et al., 2003; Cano-Gomez et al., 2009). Epizootics relevant to this pathogen affect both wild and farmed marine fish species and has become a limiting factor for the development of intensive aquaculture industry worldwide (Elgendy et al., 2015; Moustafa et al., 2015).

Vibrios were the sole bacteria isolated from investigated shrimp samples. Majority of specimens 77.7% were found to be infected. Moreover, vibrio infections were detected in 91.1% of specimens collected from the first farm compared to 64.4% in the second farm. Bacteriological identification revealed a total number of 86 vibrio isolates. The upper most frequency of these infections was obtained from the first farm 65.11% compared to 32.55% from the second farm.

The higher total viable vibrio count was detected in water and sediments samples collected from the first farm, $1.75 \times 10^3$ CFU mL$^{-1}$ and $1.9 \times 10^5$ CFU g$^{-1}$, respectively compared to $0.98 \times 10^3$ CFU mL$^{-1}$ and $1.2 \times 10^4$ CFU g$^{-1}$, respectively in the second farm which possibly justify the
high frequency of infections in shrimp specimens collected from the first farm. Shrimp rearing water is a potential source of vibrios and a higher total vibrio count could enhance infection potentials. Vibrios infections can be transmitted through water and direct contact between intensively cultured aquatic animals accelerates its spread (Marco-Noales et al., 2001; Vaseeharan and Ramasamy, 2003).

The sea waves and strong wind noticed during the unstable climate affected Mediterranean Sea during the period of this study moved sediment in the water column. The high bacterial loads in these sediments together with the unfavorable culture environments were the main cause of shrimp deaths.

The vibrio count in water and sediments samples obtained from El-Medan canal $1.35 \times 10^4$ CFU mL$^{-1}$ and $2 \times 10^6$ CFU g$^{-1}$, respectively was lower than that recorded in Lake Manzala samples $2.15 \times 10^7$ CFU mL$^{-1}$ and $2.5 \times 10^8$ CFU g$^{-1}$, respectively. This possibly attributed to the large quantities of untreated domestic, industrial and agricultural drainage water, discharged annually into lake Manzala through several drain; Bahr El-Baqer Drains (domestic and industrial sewage), Hadous, Ramsis, El-Serw as well agricultural effluents via Faraskour Drains (Abdel-Baky et al., 1998; Mohamed, 2008).

The dilution of El-Medan canal water’s by large water quantities supplied from the Mediterranean Sea may partially clarify its relative low vibrio count since 80% of its water comes from the sea and only 20% supplied from Lake Manzala. Moreover, the magnitude of waves was stronger in Lake Manzala compared with El-Medan canal a result of large surface area of the lake. These circumstances may in part justify the relative lower mortalities noticed in the second farm compared to the first. Accumulation of sediment and other organic matter in shrimp rearing water enhances the growth and multiplication of vibrios (Vaseeharan and Ramasamy, 2003).

The uppermost frequency of isolation was recorded for *V. harveyi* 34.88% highlighting its critical impacts on shrimp culture. This vibrio species is viewed as the most virulent and prevalent pathogen for larval and grow-out shrimp culture (Liuxy et al., 1996; Alapide-Tendencia and Dureza, 1997; Soto-Rodriguez et al., 2010). Additionally, *V. harveyi* has long been reported on the critical list of the most serious pathogens for numerous marine aquatic organisms (Austin and Zhang, 2006; Soto-Rodriguez et al., 2012). The pathogenicity of this bacterium is relevant to the presence of many ruinous extracellular products (ECPs) including proteases, haemolysins, phospholipases, lipases, siderophores, chitinases and cytotoxins (Montero and Austin, 1999; Zhong et al., 2006; Won and Park, 2008; Defoirdt et al., 2010).

*V. alginolyticus*, *V. vulnificus* and *Vibrio mimicus* were also obtained from moribund shrimp samples, 25.58, 22.09 and 17.44%, respectively signifying their potential involvement in the recorded shrimp mortalities. Many publications reported the isolation of these pathogens from diseased penaeid shrimp as well as from their culture environments (Selvin and Lipton, 2004; Gopal et al., 2005; Raghunath and Karunasagar, 2007).

*V. alginolyticus* and *V. vulnificus* has long been considered as crucial pathogens for many marine aquatic animals particularly imuno-compromised individuals (Moustafa et al., 2015). Numerous epizootics relevant to both vibrios have been recorded in variety of cultured marine fishes and shellfish (Moustafa et al., 2014).

Considerable evidence that links emergence of vibrio epizootics to environmental deterioration in aquaculture is widely accepted (Moustafa et al., 2015). Diverse opportunistic pathogens are common in farm water. These microorganisms cause serious infections once unfavorable aquatic environmental conditions similar to that, noticed in the investigated farms are established.
The water quality measures were more adverse in the first farm. Dissolved Oxygen (DO), un-ionized ammonia (NH₃) and nitrite (NO₂) recorded 2.6, 1.53 m and 0.95 mg L⁻¹, respectively in the first farm compared to 3, 0.87 and 0.76 mg L⁻¹, respectively in the other farm. The concomitant coexistence of some noxious heavy metals exacerbates the case. Levels detected for some investigated heavy metals; Fe, Cu, Cd, Co, Ni, Pb, Cr and As were 0.70, 0.51, 0.14, 0.64, 0.86, 1.07, 0.05 and 30 ppm, respectively in the first farm compared to 0.61, 0.43, 0.13, 0.51, 0.66, 0.87, 0.012 and 19 ppm, respectively in the second farm. These values exceed safe limits. The marine high reliability trigger values (95% protection) recommended by ANZECC/ARMCANZ (2000) for Cu, Cd, Co, Ni, Pb and Cr are 1.3, 5.5, 1, 70, 4.4 and 4.4 μg L⁻¹, respectively. Furthermore, the toxicant guidelines for Fe, Cu, Cd, Ni, Pb and Cr recommended for protection of saltwater aquaculture production should be less than 10, 5, (0.5-5), 100, (1-7) and 20 μg L⁻¹, respectively ANZECC/ARMCANZ (2000).

The high levels of heavy metals in water and sediments samples of Lake Manzala could be attributed to industrial and agricultural discharges as well as spill of leaded petrol from fishing boats distributed in the Lake (Abdel-Moati and El-Sammak, 1997; Saeed and Shaker, 2008). Metal pollution contributed to weakening of the physiological condition of cultured shrimp. Several heavy metals are primary stressors as they enhance corticosteroid secretion accordingly trigger a broad suite of infectious diseases (Teles et al., 2005). There is reliable evidence linking synergistic interaction among some metals such as, copper and iron and emergence of several fish bacterial diseases (Esteve et al., 2012; Moustafa et al., 2014; Elgendy et al., 2015).

Copper has been reported as a trigger of some opportunistic diseases caused by numerous commensal fish bacteria (Rodsaether et al., 1977; Esteve et al., 2012). Many aquatic organisms became more vulnerable to infections on concomitant exposure to pathogens and copper (Carballo et al., 1995). In essence, the pathogenicity of vibrios increases with simultaneous exposure of fish to copper and iron (Esteve et al., 2012). Additionally, some other bacterial infections like edwardsiellosis also boost at high copper concentrations (Neal and Robson, 2000). It seems that copper-related repression of the immune response is the keystone in the pathway of such diseases (O’Neill, 1981; Dick and Dixon, 1985).

The histopathological alterations noticed in our study were distinctive and significantly suggestive for a systemic infection. The absence of inclusions or occlusion bodies excluded potential involvement of viral infections. Destruction of critical components of fish and shellfish immune defense mechanisms by vibrios extracellular products is thought to be the cornerstone behind detected pathological alterations (Aguirre-Guzman et al., 2004; Elgendy, 2007). It has been documented that vibrios produce variety of destructive extracellular products including haemolysins, proteases, collagenases, phospholipase and chitinases (Harris and Owens, 1999; Goarant et al., 2000; Ramaiah et al., 2000; Aguirre-Guzman et al., 2004).

Necrosis, rounding and sloughing of tubular hepatopancreatic epithelial cells were commonly detected. These changes possibly attributed to bacterial colonization and subsequent release of their toxic extracellular products including proteases that consequently degrade tissues forming balls of necrotic sloughed tissue blocking the gut of infected shrimp as well as disrupt the absorptive and secretory function of hepatopancreas (Lixuy et al., 1996; Robertson et al., 1998; Ambipillai et al., 2003).

The widespread haemocytic infiltrations in muscle, gills and hepatopancreas indicate their potential involvement in defense mechanisms against vibrios through recognition, phagocytosis, melanization and cytotoxic activities (Jiravanichpaisal et al., 2006).
CONCLUSION

Results extracted from the present study support considerable evidence links between deterioration of environmental water quality measures in the studied farms and the eruption of vibrio epidemics in the maricultured *Paneus indicus*. Stressful culture condition recorded in the investigated farms synergized with *Vibrio* species and caused the massive losses of *Penaeus indicus*.

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