ISSN: 2347-5129
IJFAS 2015; 2(4): 271-276

www.fisheriesjournal.com
Received: 05-01-2015
Accepted: 25-02-2015

Shimaa A. H. El Far
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Riad H. Khalil
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Talaat T. Saad
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Mahmoud El-Tanekhy
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Hany M. R. Abdel-Latif
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Edfina, Behera, P.O. Box: 22758, Egypt.

Correspondence
Hany M. R. Abdel-Latif
Department of Poultry and Fish diseases, Faculty of Veterinary Medicine, Alexandria University, Edfina, Behera, P.O. Box: 22758, Egypt.

Occurrence, characterization and antibiotic resistance patterns of bacterial communities encountered in mass kills of pond cultured Indian prawn (*Fenneropenaeus indicus*) at Damietta governorate, Egypt

Shimaa A.H. El Far, Riad H. Khalil, Talaat T. Saad, Mahmoud El- Tanekhy, Hany M.R. Abdel-Latif

Abstract
Infectious diseases especially those caused by bacterial and viral pathogens are considered to be serious economic loss factors in shrimp farming. In our investigation, Mass kills of cultured Indian Prawns (*Fenneropenaeus indicus*) (*F. indicus*) were observed during winter season last December 2014, whereas there was sharp decrease in water temperature (17–18 ^{0}C) at Damietta governorate, Egypt. Samples were subjected to full clinical, bacteriological and histopathological examinations. Water samples were taken to determine any physicochemical abnormalities as well as heavy metal contents. Results showed that the infected *F. indicus* have darkening of the hepatopancreas, gill fouling, dark discoloration of the carapace, loss of the abdominal appendages and tail necrosis. Bacterial communities retrieved were belonged to Vibrio, Enterococcus and Pseudomonas genera. Their prevalence % was found to be as following; *V. harveyi* (56%), *V. parahaemolyticus* (12%), *V. vulnificus* (17%), *E. faecalis* (7%) and *Ps. fluorescence* (8%). Antibiogram testing indicates that all bacterial isolates were markedly sensitive to Florfenicol, Cephradine and Norfloxacin, while resistant to Gentamycin, Oxytetracycline and Amoxicillin. Water examinations revealed elevated nitrite (NO2), unionized ammonia (NH3), organic matter and hydrogen sulphate as well as Copper (Cu), Iron (Fe) and Cadmium (Cd). We can conclude that the abrupt change in the physicochemical properties of water can be considered as stress factors which suppress the immune status of the Indian prawns, so that bacterial pathogens were encountered.

Keywords: Fenneropenaeus indicus – Bacterial infections – gill fouling – Antibiogram.

1. Introduction
The world production of both captured and farmed shrimp is around 6 million tones, about 60 percent of which enters the world market. Shrimp is now the most important internationally traded fishery commodity in terms of value. Annual exports of shrimp are currently worth more than US$10 billion, or 16 percent of all fishery exports [1].
Shrimp accounts for about 20% of the value of exported fishery products over the past 20 years [2]. Imports into developed countries accounted for about 40% of intra-developed countries trade, while about 60% comes from developing countries; out of the exports from developing countries 80% goes to developed countries and only 20% stays in the group [3]. Also, shrimps are one of the major aquaculture products of export importance from the tropics.
Bacteria are among the groups of microorganisms causing serious losses in shrimp culture throughout the world. Members of the genus Vibrio, including *V. parahaemolyticus* and *V. harveyi*, have been described as the main pathogenic species in shrimp and are responsible for most of the larval deaths. These pathogens cause serious infections, decreased production both in the hatchery and grow-out ponds, reduced feed conversion and growth rates in surviving individuals, thus having a negative impact on the overall financial efficiency of the business.
Vibriosis is a bacterial disease caused by gram-negative, motile, facultative anaerobe bacteria of the family Vibrionaceae. It is ubiquitous throughout the world and all marine crustaceans, including shrimp, are susceptible. Vibrio species are the eminent microorganisms in the marine environment and usually constitute the majority in the normal microflora of farmed and wild penaeid shrimp. They become opportunistic pathogens when the natural defense

mechanisms are suppressed [4]. In intensive systems, shellfish species are often exposed to stressful conditions due to the high stocking density, leading to secondary vibriosis.

Therefore, this study mainly focused on isolation and identification of bacterial pathogens encountered in mass kills of cultured *F. indicus* during winter season at Damietta governorate, demonstration of their relation to water quality parameters.

2. Material and Methods

2.1. Sample collection

In our investigation, a total number of one hundred and fifty (150) earthen pond cultured Indian prawns (*Fenneropenaeus indicus*) of body weight averaged (12 ± 5 g) were collected from private farms at Damietta governorate, Egypt during outbreak in winter season where there is a sudden sharp decrease in water temperature ranged (17-18 °C). Prawns were transferred to the laboratory of the Department of Poultry and fish diseases, Faculty of veterinary medicine, Alexandria University as soon as possible. The collected specimens were subjected to full clinical, postmortem (PM) lesions, and bacteriological examinations.

2.2. Gross clinical and Postmortem (PM) examination

The external examination was carried out by naked eyes for any clinical abnormalities on the external body surface and carapace. For internal examination, the carapace was removed and the subcuticular tissues were examined by naked eyes for any abnormalities. Hepatopancreas, stomach and musculature were examined for any deviation in the size or consistency [5].

2.3. Bacterial isolation and identification

The prawn surfaces were swapped with 70% ethyl alcohol for surface disinfection and then inocula were taken from Hepatopancreas under complete aseptic condition to be cultured on tryptic soya broth (Difco, Detroit, MI, USA) and incubated at 20 ^{0}C for 24 - 48 hrs then sub-cultured on TCBS media (Thiosulphate Citrate Bile salts). After incubation period, a single colony from each suspected isolate was picked up and re-streaked on a new plate of its perused selective culture media and re-incubated at the same conditions. When pure colonies have been grown, a loopful of each pure culture was streaked onto a nutrient agar slope or semisolid agar medium to be used as stock culture for further biochemical and serological identification. Bacterial isolates were stored in 15% glycerol and kept at – 80 ^{0}C.

The criteria used for identification of the isolates are based on colonial characteristics (colony morphology and arrangement) and gram staining of the microorganisms [6]. Bacterial isolates were presumptively identified using conventional biochemical tests including catalase with 3% hydrogen peroxide solution, cytochrome oxidase with oxidase strips (Remel), motility in a motility test medium (BD Biosciences, MA), citrate utilization using simmons's citrate (Remel), sugar utilization using triple sugar iron (TSI, Remel), oxidation/fermentation of glucose using of basal media with glucose as the sole carbohydrate source (BD Biosciences), and esculin hydrolysis using bile esculin agar (Remel). Further biochemical testing was performed using API20E and 20NE tests (Bio-Me´rieux, NC), with isolates incubated at 20 °C. Results were interpreted at 24-48 hours according to the manufacturer's instructions. Enrichment and identification were done [7].

2.4. Antibiogram test

The antibiotic discs were obtained from (**Oxoid, England**). The graduated rule to 0.5 mm was used for reading the diameter of the zones of inhibition twice at right angles. Antibiotic discs used were Cefotaxime (CTX) (30 mg), Gentamycin (CN) (10 mg), Florfenicol (FFC) (30 mg), Norfloxacin (NOR) (10 mg), Doxycycline (DO) (30 mg), Cephradine (CE) (30 mg), Oxytetracycline (OT) (30 mg), Amoxicillin (AML) (10 mg) and Erythromycin (E) (15 mg). Susceptibility to several antibiotics was determined using the disc diffusion technique [8].

The inoculum was prepared in 0.85% saline, and turbidity was adjusted to 0.5 McFarland's standard (approximately 2 x 10^8 CFU / ml). Petri dishes of Mueller-Hinton ager supplemented with 1% NaCl were streaked with 1 ml of the prepared inoculum, then put the different antimicrobials discs and incubated at 25 ^{o}C for 24-48 hours. The diameter of the zones of inhibition (point at which no growth is visible) was read twice at right angles with a ruler graduated to 0.5 mm.

2.5. Histopathological studies

Following complete necropsy of the freshly dead Prawns, specimens were collected from the hepatopancreas and mid gut for histopathological examination. Thereafter, these specimens were rapidly fixed in 10% natural formalin buffered phosphate for at least 24 hours, after that the specimens washed by running tap water then dehydrated through ascending grads of ethanol, cleaning in by chloroform and embedded in paraffin wax at 60 C. Paraffin block were prepared and from which 5 microns thick sections were obtained by microtome. These sections were stained by Hematoxylin and eosin stain (H & E) [9].

2.6. Collection of water samples

2.6.1. Assessment of physico-chemical water properties

The tools used for determination of Physico-chemical properties of water quality were namely; Dissolved Oxygen meter for measuring the level of Dissolved oxygen in the water, Salinometer for measuring of % of water salinity, PH meter for measuring the pH values and Kits for measuring the levels of unionized ammonia and Sulphate in the water (USA, Virginia Company, lot. No .201134).

2.6.2. Spectrophotometric method for detection the levels of heavy metals in water and fish tissues

The method for analysis of the heavy metals in water [10] was carried out using Atomic Absorption Spectrophotometry (Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK,) instrument was used to detect the heavy metals. The concentrations of heavy metals were expressed as mg / l for water. Atomic absorption spectrophotometer instrument was used to determine As, Zn, Cu, Pb, Hg, and Cd concentrations which were expressed as µg / g dry weight in the Toxicology Unit of Central Laboratory, Faculty of Veterinary medicine, Alexandria University, Egypt.

3. Results

3.1. Results of clinical examination

Specimens of *F. indicus* showed gill fouling (Photo 1a), dark and red discoloration of the carapace (Photo 1b) with sloughing of the abdominal appendages (Photo 2), and tail. Generalized cloudiness of the musculature and rigidity of

appendages, gills were yellow or pale red, Hepatopancreas region was dark with slight redness.

Photo 1: Cultured *F. indicus* showing gill fouling (arrow) (1 a) with dark discoloration of the carapace (arrow) with erosions of the tail (arrow) (1 b).

Photo 2: Cultured *F. indicus* showing erosions of the abdominal appendages (arrows).

3.2. Results of bacteriological examinations

According to the morphological and biochemical reactions (Table 1), identification revealed the isolation of Vibrio harveyi, *V. vulnificus*, *V. parahaemolyticus*, Enterococcus faecalis and Ps. fluorescence. The prevalence (%) of bacterial isolates (Table 2 and Figure 1) was found to be as following; *V. harveyi* (56%), *V. parahemolyticus* (12%), *V. vulnificus* (17%), *E. faecalis* (7%) and Ps. fluorescence (8%).

Table 1: Morpho-biochemical characteristics of bacterial isolates retrieved from mass kills of *F. indicus* from Damietta governorate.

Items	*V. vulnificus*	*V. parahemolyticus*	*V. harveyi*	*Ps. fluorescence*
Gram staining reaction	Gram negative	Gram negative	Gram negative	Gram negative
Cell morphology	Curved Rods	Straight Rods	Rods	Rod bacilli
Motility	+	+	+	-
Catalase test	+	+	+	+
Cytochrome oxidase test	+	+	+	+
Vogus Proskauer test	+	-	-	-
Growth on 8% NaCl	-	+	-	-
Growth on TCBS	+	+	+	-
Hemolysis of RBCs	hemolytic	Hemolytic	hemolytic	Non hemolytic
Sensitivity to 0/129 disc	+	+	+	-
Production of:-				
H2S	-	-	-	-
Indole (peptone H2O)	-	+	-	-
Urease	-	-	-	-
Utilization of:-				
Citrate	+	+	+	+
D-Mannitol	+	+	+	-
Glucose	-	+	-	-
Lactose	-	-	-	-
L-Arabinose	-	+	-	-
Maltose	-	+	-	-
Sorbitol	-	-	-	-
Sucrose	-	-	-	-

Table 2: Prevalence percentage (%) of bacterial isolates retrieved from mass kills of *F. indicus*.

Bacterial isolates	No. of Isolates	Prevalence %
E. faecalis	4	6.67
Ps. fluorescence	5	8.33
V. harveyi	34	56.67
V. parahemolyticus	7	11.67
V. vulnificus	10	16.67
Total number of isolates	60	

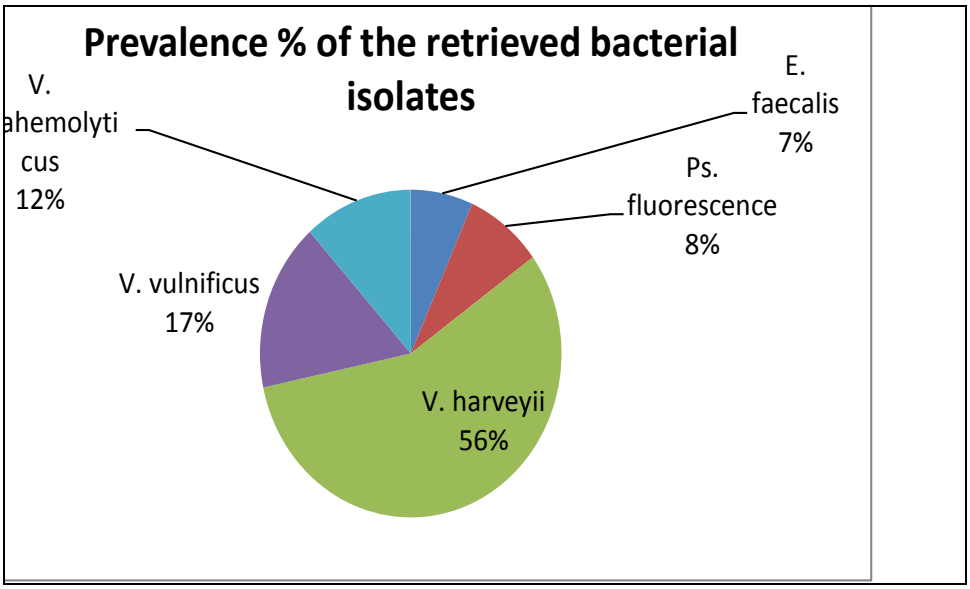

Fig 1: Histogram showing the prevalence % of the retrieved bacterial isolates from diseased cultured *F. indicus*.

3.3. Results of Antibiogram test

Table 3: Results of Antibiogram test for the bacterial isolates retrieved.

	V. harveyii	*V. parahemolyticus*	*V. vulnificus*	*Ps. fluorescence*	*E. faecalis*
Amoxicillin	-	-	-	+	++
Cefotaxime	++	++	+	++	++
Cephradine	++	+++	++	++	+++
Doxycycline	+	+	+	++	++
Erythromycin	++	++	+	++	+++
Florfenicol	+++	+++	++	+++	+++
Gentamycin	-	-	-	-	-
Norfloxacin	++	++	++	+++	+++
Oxytetracycline	-	-	-	-	-

(-) resistant, (++) sensitive and (+++) markedly sensitive

3.4. Results of histopathological findings

The results of histopathological findings of the diseased cultured *F. indicus* were illustrated in Fig. 2, 3 and 4.

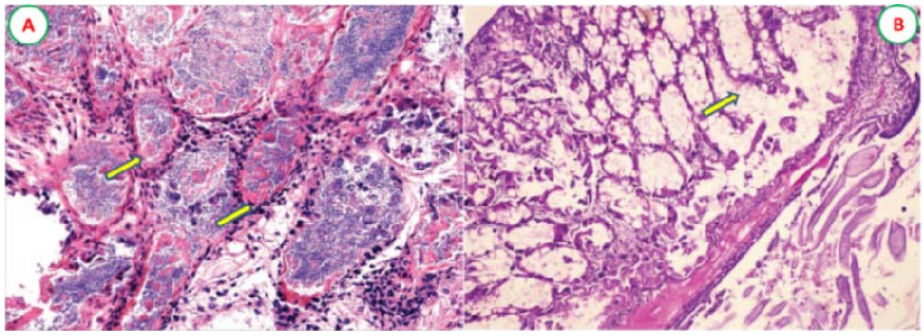

Fig 2: Hepatopancreas of *F. indicus* showed epithelial cells, intratubular bacterial colonization, and haemocytic infiltration (arrows) (A) and sloughed tubules (B) (arrow) (40 x magnifications), H & E stain.

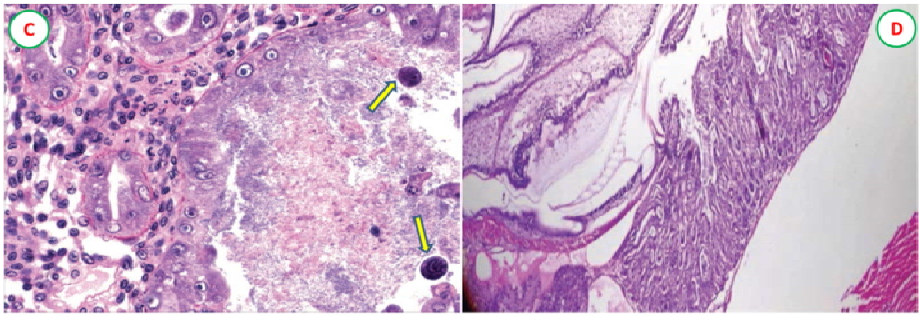

Fig 3: Hepatopancreas of *F. indicus* showed haemocyte infiltration with bacterial colonization (arrows) (C) while, hepatopancreas, stomach and mid gut showed mixed infection with bacterial disease and fungus (D), (40 x magnifications), H & E stain

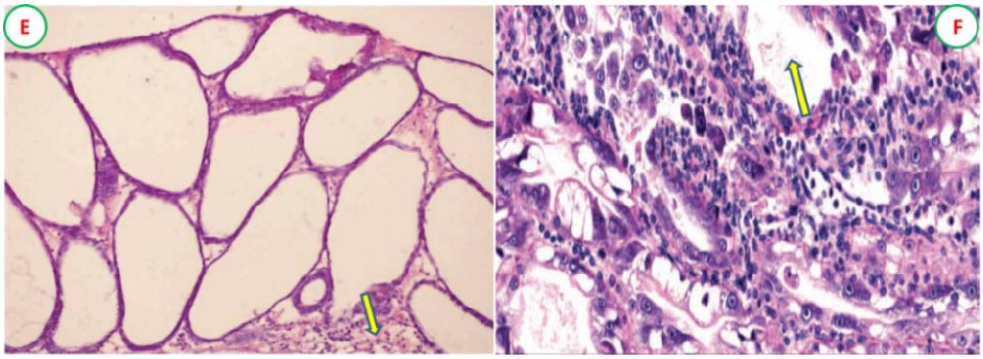

Fig 4: Hepatopancreas of *F. indicus* showed mixed infection with bacteria and fungus (arrows) (E) (40 x magnifications) and acute sloughing of tubular epithelial cells, haemocyte infiltration (arrows) (F) (20 x magnification), H & E stain.

3. 5. Results of physicochemical properties of water

Table 4: Results of physicochemical properties of water in comparison with the permissible limits.

Water parameters	Reading	Permissible limits (PL) [11]
Dissolved oxygen (mg / L)	5.6	5-6
Salinity (PPT)	30 - 34	-----
Temperature (0C)	23-24	-----
Nitrite (NO2) (mg / L)	0.04	0.01
Unionized ammonia (NH3) (mg / L)	0.05	0.01
Organic matter (mg / L)	3.79	2-3
Hydrogen Sulphate (mg / L)	153.1	70-120
PH	8.4	8.0 - 8.5

3.6. Results of heavy metal content in water (mg / L).

Table 5: Results of heavy metal content in water (mg / L) in comparison with the permissible limits.

Heavy metals (mg / L)	Reading	Permissible level (PL) [12]
Copper (Cu)	0.68	0.2
Zinc (Zn)	0.66	2.0
Cadmium (Cd)	ND	0.004
Lead (Pb)	0.22	0.050
Mercury (Hg)	ND	0.001
Nickel (Ni)	0.004	0.001
Iron (Fe)	1.420	1.00

4. Discussion

In the present study, the clinical picture of Indian prawn, F. indicus was characterized by Specimens of *F. indicus* showed gill fouling, dark and red discoloration of the carapace with sloughing of the abdominal appendages, and tail. Generalized cloudiness of the musculature and rigidity of appendages, gills were yellow or pale red, Hepatopancreas region was dark with slight redness were also noticed. Such obtained results are nearly agree with those recorded by Peddie and Wardle [13] who mentioned that multitude of infections caused by bacteria belonging to the genus vibrio and infections commonly known as black shell disease, tail rot, necrosis, brown gill disease. Also, such finding nearly agree with those mentioned by Khuntia *et al*. [14] and Eissa *et al*. [15] who recorded that disease signs like red discoloration, loss of appendages, black coloration of the body and gills.

Regarding the bacteriological investigations and according to the morphological and biochemical results of the bacterial isolates revealed from the examined Indian prawn, F. indicus were identified as *V. harveyi* (56%), *V. parahemolyticus* (12%), *V. vulnificus* (17%), E. faecalis (7%) and Ps. fluorescence (8%). These results agreed with that met by Alcaide *et al*. [16], Slevin and Lipton [17], Buller [18] and Noga [19].

In the present study, the total prevalence of vibriosis was (64.4%) among the examined Indian prawn, F. indicus. These results are nearly agreed with Hisbi *et al*. [20]. Also, Musa and Wei [21] mentioned that (54.2%) of Vibrio isolates from lesion and hepatopancreas from total isolates. These differences may be attributed to type of shrimp spp., site of sampling, water quality and size of shrimp collected.

Regarding the histopathological studies, our results nearly in concordance with that reported by Lavialla-pitogo *et al*. [22], Ambipillai *et al*. [23] and Khuntia *et al*. [14].

Water examinations revealed elevated nitrite (NO2), unionized ammonia (NH3), organic matter and hydrogen Sulphate as well as Copper (Cu), Iron (Fe) and Cadmium (Cd). Pollutants have been implicated in outbreaks of disease in fish populations either by reactivation of carrier states or by predisposition to infection by common waterborne pathogens [24]. Generally, Heavy metals are chemical stressors and the development of disease will reflect interactions between the host, the disease causing situation and stressors [25] and this may be attributed to suppression of immune system and immune response which provide opportunities for entering of many pathogens [26].

5. Conclusions

We can conclude that the abrupt change in the physicochemical properties of water, as well as sudden sharp decrease in water temperature can be considered as stress factors which suppress the immune status of the Indian prawns, so that bacterial pathogens were encountered.

6. References

1. FAO. Global study of shrimp fisheries, 2008, 1-359.
2. CAC. Discussion Paper on Risk Management Strategies for Vibrio spp. in Seafood. Food and Agriculture Organization / World Health Organization, Rome, Italy,

2002.
3. Josupeit H. Trade flows between developed and developing countries. Rome, Italy: FAO, 2005. http://www. globefish.org/dynamisk.php4?id=2504. [17.02.06].
4. Lightner DV. Handbook of shrimp pathology and diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA, 1993.
5. Austin B, Austin DA. Methods for the microbiological examination of fish and shellfish"; Ellis Harward limited halsted press, Newyork, Brisban, Toranto, 1989, 59-68.
6. Austin B, Austin D A. Bacterial Fish Pathogens: Disease of Famed and wild Fish, Edn 2, Chichester: Ellis Horwood, 1993.
7. ISO / TS 21872-2. Microbiology of food and animal feeding stuff- Horizontal method for the detection of presumptive enteropathogenic Vibrio spp.-part 2: Detection of species other than Vibrio parahemolyticus and Vibrio cholera, Edn 1, ISO Copyright office published in Switzerland, 2007, -22.
8. Barry A, Thornsberry C. Susceptibility test and diffusion test procedures. In: Manual of Clinical Microbiology, Washington DC 1991, 1526-1542.
9. Roberts RJ. Fish Pathology, Edn 3, W. B. Saunders, Philadelphia, PA, 2001.
10. APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater. 1995; Edn 19, Washington DC.
11. WHO, World Health Organization. Guidelines for drinking water quality. Geneva, 1984, 111.
12. FAO/WHO. List of maximum levels recommended for contaminants by the Joint FAO/ WHO Codex Alimentarius Commission. Second Series. CAC/FAL, Rome, 1984; 3:1–8.
13. Peddie S, Wardle R. The impact and control of vibriosis in shrimp culture worldwide. Aquaculture Health International issue 2005; (2):4-5.
14. Khuntia CP, Das BK, Samantaray BR, Samal SK, Mishra BK. Characterization and pathogenicity studies of Vibrio parahemolyticus isolated from diseased freshwater prawn, Macrobrachium rosenbergii (de Man), 2008.
15. Eissa IAM, Derwa HIM, Abd-Elrehim AAM. Epizootiological studies on vibriosis in marine shrimp, Penaeus japonicus in Suez-Bay. SCVMJ, 2011; XVI (2):85–92.
16. Alcaide E, Carmen A, Rafael T, Rafael O. Isolation and characterization of Vibrio parahemolyticus causing infection in Iberian toothcarp Aphanius iberus. Diseases of aquatic organisms 1999; (35):77-80.
17. Selvin J, Lipton AP. Vibrio alginolyticus associated with white spot disease of Penaeus monodon. Diseases of aquatic organisms 2003; (57):147–150.
18. Buller NB. Bacteria from fish and other aquatic animals: A practical identification manual; CABI publishing, London; ISBN 0 85199 738, 2004; (4):1-361.
19. Noga EJ. Fish disease: Diagnosis and treatment"; 2nd Edition, published by Wiley-Blackwell, 2010, 1-536.
20. Hisbi D, vandenberghe J, Robles R, Verdonck L, Swing J, Sorgeloos P. Characterization of Vibrio and related bacteria associated with shrimp penaeus monodon larvae in Indonesia. Asian Fisheries science 2000; (13):57-64.
21. Musa N, Wei LS. Outbreak of Vibriosis in Mantis shrimp (*Squilla* ap.). World Journal of agricultural sciences 2008; 4(2):137-139; ISSN 1817-3047.
22. Lavilla-Pitogo CR, Lean˜o EM, Paner MG. Mortalities of pond-cultured juvenile shrimp, Penaeus monodon, associated with dominance of luminescent vibrios in the rearing environment. Aquaculture 1998; (164):337–349.
23. Ambipillai L, Sobhana KS, George KC, Sanil NK. Histopathological survey of cultured shrimps in Cochin, Kerala. J mar biol Ass India 2003; 45(2):178–185.
24. Wedemeyer GA, Meyer FP, Smith L. Book 5: Environmental stress and fish diseases. TFH Publications Inc, Neptune City, NJ. 192, 1976.
25. Austin B, Austin DA. Bacterial fish pathogens: disease of farmed and wild fish, Edn 3 (revised). Springer-Praxis series in aquaculture and fisheries. Springer-Verlag, London, 1999.
26. Storelli M, Giacominelli-Stuffler R, Marcotrigiano G. Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. Bull Environ Contam Toxicol 2000; (68):201–210.