Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(6): 1258-1262

Received: 20-09-2018
Accepted: 24-10-2018

Patel MP
Assistant Professor, Fisheries Research Station, Junagadh Agricultural University, Okha, Gujarat, India

Dr. Vadher KH
Associate Professor, Department of Aquaculture, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Dr. Tank PR
Assistant Professor, Department of Aquaculture, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Correspondence
Dr. Tank PR
Assistant Professor, Department of Aquaculture, College of Fisheries Science, Junagadh Agricultural University, Veraval, Gujarat, India

Virulence of *Vibrio* spp. to pacific white shrimp *Litopenaeus vannamei* juveniles

Patel MP, Dr. Vadher KH and Dr. Tank PR

Abstract
This study was conducted to evaluate the virulence of *Vibrio harveyi* MTCC 7954, *V. parahaemolyticus* MTCC 451 and *V. vulnificus* MTCC 1145 to Pacific white shrimp *Litopenaeus vannamei* juveniles. *Vibrio* spp. were injected to juvenile shrimps (2.28 ± 0.19 g) with a lethal dose of 1 x 10^7 CFU/shrimp while control without *Vibrio* spp. and reared for 10 days. Significantly higher and earlier mortality was recorded with *V. parahaemolyticus* than other *Vibrio* strains and control. Total mortality of juvenile shrimps was recorded in 6 days with *V. parahaemolyticus* injection followed by *V. vulnificus* in 7 days while *V. harveyi* produced 63.33% ± 10.00% mortality in 10 days of the test period. Higher virulence exhibited by *V. parahaemolyticus* to juveniles shrimp with median lethal time value 4.13 days than *V. vulnificus* and *V. harveyi* with values of 4.96 and 8.57 days respectively.

Keywords: Virulence, *Vibrio harveyi, Vibrio parahaemolyticus, Vibrio vulnificus,* shrimp, *Litopenaeus vannamei,* Probit

Introduction
The aquaculture of shrimp has rapidly grown to a major industry worldwide. The aquaculture production practices have been intensified to a greater extent both in technological and practical measures to meet the global demand [1]. Pacific white shrimp *Litopenaeus vannamei* has potential to grow fast. Problem related to diseases and deterioration of environmental parameters often occurs resulting in serious economic losses [2]. Hence, disease is considered as a one of the critical limiting factor [3] in the shrimp aquaculture. However, the growth of the aquaculture industry is hampered by unpredictable mortalities mostly caused by pathogenic microorganisms. *Vibrio* is the most important bacterial pathogens of cultured shrimps responsible for number of diseases and mortality among all [4]. *Vibrio* are in Vibrionaceae family, Gram-negative facultative anaerobes, short to medium, coma-shaped rods found in fresh, estuarine and marine ecosystems [5]. Pathogenic strain of *Vibrio harveyi, V. parahaemolyticus* and *V. vulnificus* are able to cause massive epidemics in *Penaeus monodon* [6, 7]. *V. harveyi* and *V. parahaemolyticus* @ 10^5 to 10^7 CFU/ml developed red disease syndrome in *Penaeus monodon* and *L. vannamei* [8-12]. *V. harveyi* shown higher virulence than *V. alginolyticus, V. vulnificus, V. fischeri* and *V. parahaemolyticus* in *P. monodon* which were isolated from the host infected with white-spot syndrome virus and shell disease outbreaks from the shrimp farms located in peninsular India [13] while such study in *L. vannamei* was lacking. *V. harveyi* associated with white gut disease (WGD), loose shell syndrome (LSS) and red disease (RD); *V. parahaemolyticus* with RD and tail nacrosis (TN) and *V. vulnificus* with LSS in shrimp farms and hatcheries [14-17]. Researchers studied the pathogenicity of *V. harveyi* [18-21], *V. parahaemolyticus* [22] and *V. vulnificus* [23] on *L. vannamei* [15]. The present study was formulate to find out comparative virulence of *Vibrio harveyi, V. parahaemolyticus, V. vulnificus* associated with *L. vannamei* disease and mass mortality.

2. Materials and methods
All experimental works were carried out at Fisheries Research Station, Junagadh Agricultural University, Okha, Gujarat, India (lat 22˚28'42.3"N; long 69˚04'40.8"E).

2.1 Procurement and acclimatization of shrimp *L. vannamei* juveniles
Shrimp juveniles were collected from pond of the research station and acclimatized in 1000 l FRP tanks for 10 days prior to the experiment. During the acclimatization period, shrimps

were fed with commercial feed *ad libitum*, provided continuous aeration and water exchanged every 48 hours. Shrimp juveniles were observed for health. Physico-chemical parameters of seawater were maintained within ambient limits with salinity 35 ppt, pH 7.9 to 8.2, temperature 28 to 30 ˚C and dissolved oxygen more than 6 ppm.

2.2 Procurement and culture of *Vibrio* spp. strains

Three pathogenic bacterial strains *Vibrio harveyi* MTCC 7954, *V. parahaemolyticus* MTCC 451 and *V. vulnificus* MTCC 1145 were procured from the Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh, India. They were grown in Tryptone Soya (TS) broth (M011 HiMedia, India) at streaked on TCBS agar (M189, HiMedia, India). They were incubated at 35 ˚C for 24 to 48 hours. Working strains were stored in a refrigerator (Haier, India) at 4 ˚C. Bacterial strains of *Vibrio* spp. were also preserved in TS broth containing 15% (v/v) glycerol solution at -80 ˚C in ultra-freezer (EIE-414, India) [24, 19]. TS broth cultured *Vibrio* spp. strain pellets were harvested by centrifuging in refrigerated centrifuge (Remi R-8C, India) at 3000 g for 10 minutes at 4 ˚C. Supernatant fluid was decanted and the pelleted cells washed with phosphate buffer saline thrice. Concentration of *Vibrio* spp. strains were adjusted to 10^8 CFU/ml by adding sterile normal saline solution (0.85% NaCl) equal to its standardized absorbance. Bacterial concentration absorbance was standardized by spectrophotometrically (Spectronic 21D, USA) at 600 nm wavelength using serial dilution method [20].

2.3 Injection of *Vibrio* spp. to *L. vannamei*

Healthy shrimp juveniles (2.28 ± 0.19 g, n=10) in the intermolt stage were collected from acclimatisation tank and gently injected with 0.1 ml 10^8 CFU/ml *Vibrio harveyi*, *V. parahaemolyticus* and *V. vulnificus* suspension into the abdominal segment using sterile 1 ml capacity syringe with 31 gauge needle resulting in dose of 10^7 CFU/shrimp. The concentration of injection were based on previous research [8, 23] and preliminary challenge test. The experiment was conducted on 10 shrimp juveniles in each treatment of *Vibrio* sp. in triplicates sets. For control, same volume of sterile normal saline solution (0.85% NaCl) without *Vibrio* spp. was injected to shrimp juveniles [19, 20, 23].

2.4 Rearing of *L. vannamei* and observation

Injected shrimps were released in glass jar filled with 10 l cartridge filtered and UV sterilized seawater. Continuous aeration was provided by aerator and fed with commercial feed (Manamei brand, Avanti). They were observed periodically for health, moribund and mortality up to 10 days. The physico-chemical parameters salinity, temperature, pH and dissolved oxygen during the experiment were 35 ppt, 28 ± 2 ˚C, 8.1 ± 0.2 and > 6 ppm respectively. Dead shrimps were removed from glass jars and recorded. Samples from hepatopancreas of moribund shrimps were aseptically streaked on TCBS agar and observed growth of *Vibrio* spp. after 24 hours of incubation at 35 ˚C.

2.5 Statistical analysis

The experiment was arranged in triplicate. All results were presented as means ± their standard deviations. A two way ANOVA of complete randomized design analysis of the data to test for significant differences between treatments at a *P* value <0.05% and mean comparison were performed [25]. Median lethal time (LT_{50}, time period in which 50% of shrimp juveniles were died at a dose of *Vibrio* sp. @ 1 x 10^7 CFU/shrimp) value was estimated using Probit method [13, 26-29].

3. Results and discussion

The results of the tests are presented in Table 1 and graphically illustrated in Figure 1. Infected shrimps showed lethargic movement led to death. All the strains of *Vibrio* spp. produced significantly higher mortalities than the controls (P<0.05). *V. parahaemolyticus* produced significantly higher and earlier mortality than other *Vibrio* strains. *V. parahaemolyticus* produced total mortality of *L. vannamei* juveniles in 6 days followed by *V. vulnificus* in 7 days while *V. harveyi* produced 63.33% ± 10.00% mortality in 10 days of the test period. Infection of *Vibrio* spp. were confirmed on TCBS agar plates by hemolymph streaking from the moribund and dead juveniles. Mortality of *L. vannamei* was increased with the increase in the time period (days) of infection but not significant. Median lethal time values of *Vibrio* spp. at intramuscular dose of 1 x 10^7 CFU/shrimp to *L. vannamei* are presented in Table 2. Highest virulence showed by *V. parahaemolyticus* with lower median lethal time value to *L. vannamei* which was recorded 4.13 days. *V. vulnificus* and *V. harveyi* showed 4.96 and 8.57 days LT_{50} value respectively (Table 2).

The results of this study is near similar with lethal dose of *V. harveyi* @ 2.7 x 10^6 to 1.4 x 10^7 CFU/shrimp on *L. vannamei* by Huang *et al.* [30]; and *V. harveyi* @10^5 to 10^7 CFU/ml and *V. parahaemolyticus* @10^5 to 10^7 CFU/ml on *Penaeus monodon* by Alapide and Dureza [8]. Zheng and Wang found virulence of *V. vulnificus* @ 10^7 CFU/shrimp on *L. vannamei* [23]. Aguirre-Guzman *et al.* used lethal dose of 10^6 CFU/ml of *V. parahaemolyticus* in *L. vannamei* [10] and similar infection dose of *V. harveyi* in *P. monodon* by Karunasagar *et al.* [11].

Results of the present study differ with higher virulence recorded by Manilal *et al.* on *Vibrio* spp. isolated from *P. monodon* of South-East coast of India with lower LD_{50} value i.e. 2.8 x 10^3 CFU/shrimp of *V. harveyi*, 7.4 x 10^4 CFU/shrimp of *V. parahaemolyticus* and 8.6 x 10^5 CFU/shrimp of *V. vulnificus*. They found higher virulence of *V. harveyi* than other *Vibrio* spp. [13]. This study also differs with result of higher virulence with lower lethal dose values 1 x 10^3 CFU/g to 2.5 x 10^4 CFU/g of *V. harveyi* and 1.5 x 10^4 CFU/g to 3 x 10^4 CFU/g of *V. parahaemolyticus* by Jayshree *et al*. They isolated *Vibrio* spp. from diseased shrimp *Penaeus monodon* from culture ponds of coastal Andhra Pradesh [14]. This difference might be due to different shrimp species, its juvenile size and isolation of *Vibrio* spp. from host which is more virulent in nature.

Table 1: Mortality (%) of *L. vannamei* juveniles after injection with pathogenic *Vibrio* strains (mean ± SD, n=3)

Duration-D (Days)	Treatment- Pathogens (T)				Mean (D)
	T1 (*V. harveyi*)	T2 (*V. para- haemolyticus*)	T3 (*V. vulnificus*)	T4 (Control)	
D0 (0)	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00
D1 (1)	00.00 ± 0.00	06.67 ± 5.77	03.33 ± 0.00	00.00 ± 0.00	02.50
D2 (2)	00.00 ± 0.00	16.67 ± 5.77	06.67 ± 0.00	00.00 ± 0.00	05.83
D3 (3)	03.33 ± 5.77	23.33 ± 5.77	20.00 ± 10.00	00.00 ± 0.00	11.67
D4 (4)	06.67 ± 5.77	43.33 ± 5.77	33.33 ± 10.00	00.00 ± 0.00	20.83
D5 (5)	16.67 ± 10.00	70.00 ± 10.00	53.33 ± 5.77	00.00 ± 0.00	35.00
D6 (6)	23.33 ± 10.00	96.67 ± 5.77	63.33 ± 11.55	00.00 ± 0.00	45.83
D7 (7)	33.33 ± 5.77	100.00 ± 0.00	90.00 ± 5.77	00.00 ± 0.00	55.83
D8 (8)	43.33 ± 5.77	100.00 ± 0.00	100.00 ± 0.00	00.00 ± 0.00	60.83
D9 (9)	56.67 ± 5.77	100.00 ± 0.00	100.00 ± 0.00	00.00 ± 0.00	64.17
D10 (10)	63.33 ± 10.00	100.00 ± 0.00	100.00 ± 0.00	00.00 ± 0.00	65.83
Mean (T)	21.82	60.00	50.30	00.00	

Treatment	S.Em. ±	C.D. at 5%	CV%
T (Pathogen)	0.88	2.49	15.37
D (Duration)	1.47	4.12	
D X T	2.93	NS	

ANOVA

SV	DF	SS	MS	FCal	Signi.
T	3	74000.00	24666.67	957.56	*
D	10	86737.88	8673.79	336.72	*
T X D	30	41983.33	1399.44	54.33	*
Error	88	2266.67	25.76		
Total	131	204987.88			

Note: '*' indicates significance at 0.05%

Table 2: Median lethal time LT_{50} value of *L. vannamei* juveniles after injection with *Vibrio* strains @ $1x10^7$ CFU/shrimp

	Treatment- Pathogens (T)		
***Vibrio* strains**	**T1 (*V. harveyi*)**	**T2 (*V. parahaemolyticus*)**	**T3 (*V. vulnificus*)**
LT_{50} value (days)	8.57	4.13	4.96
Equation	Y=5.27x + 0.09	Y=5.64x + 1.53	Y=4.42x + 1.93
R^2	0.982	0.981	0.982

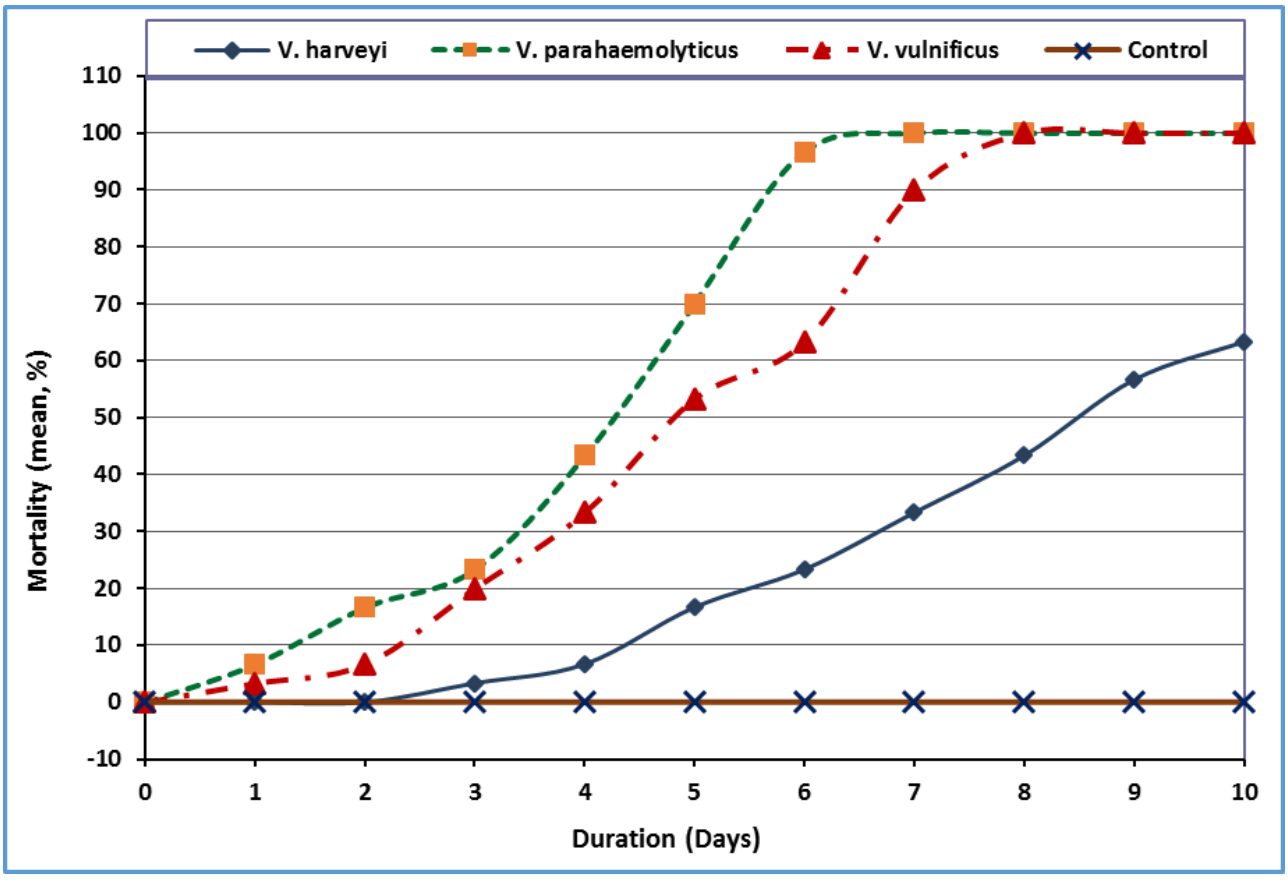

Fig 1: Mortality of *L. vannamei* juveniles challenged with pathogenic *Vibrio* strains

4. Conclusion

Vibrio harveyi, V. parahaemolyticus and *V. vulnificus* @ 1x 10^7 CFU/shrimp are virulent to shrimp *L. vannamei. V. parahaemolyticus* found more virulent than *V. vulnificus* and *V. harveyi* to *L. vannamei* in laboratory conditions.

5. Acknowledgements

The experiment was conducted as part of in-service Ph.D. study. Authors are grateful to authority of Junagadh Agricultural University, Junagadh for granting necessary permissions. The authors are also grateful to Research Officer, Fisheries Research Station, JAU, Okha for providing required laboratory facilities and manpower and Principal, College of Fisheries, JAU, Veraval for providing bacterial strains, bacterial media, chemicals and requirements.

Reference

1. Tuan TN, Duc PM, Hatai K. Overview of the use of probiotics in aquaculture. International Journal of Research in Fisheries and Aquaculture. 2013; 3(3):89-97.
2. Balcázar JL, De Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D, Muzquiz JL. The role of probiotics in aquaculture. Veterinary Microbiology. 2006; 114(3-4):173-86.
3. Purivirojkul W, Khidprasert S. Diseases and parasites on cultured fairy shrimps, *Branchinella thailandensis* and *Streptocephalus sirindhornae*. Proceeding of Asian Pacific Aquaculture. 2009, 3-6.
4. Sivakumar N, Sundararaman M, Selvakumar G. Probiotic effect of *Lactobacillus acidophilus* against vibriosis in juvenile shrimp (*Penaeus monodon*). African Journal of Biotechnology. 2012; 11(91):15811-15818.
5. Thompson FL, Iida T, Swings J. Biodiversity of vibrios. Microbiology and Molecular Biology Reviews. 2004; 68(3):403-431.
6. Nash G. Vibriosis and its control in pond-reared *Penaeus monodon* in Thailand. Diseases in Asian Aquaculture. 1992, 143-155.
7. Lavilla-Pitogo CR, Baticados MC, Cruz-Lacierda ER, Leobert D. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture. 1990; 91(1, 2):1-3.
8. Alapide-Tendencia EV, Dureza LA. Isolation of *Vibrio* spp. from *Penaeus monodon* (Fabricius) with red disease syndrome. Aquaculture. 1997; 154(2):107-114.
9. Labrie L, Roque A, Gomez-Gil B, Turnbull JF. Effect of methyl parathion on the susceptibility of shrimp *Litopenaeus vannamei* to experimental vibriosis. Diseases of Aquatic Organisms. 2003; 57(3):265-270.
10. Aguirre-Guzmán G, Sánchez-Martínez JG, Pérez-Castañeda R, Palacios-Monzón A, Trujillo-Rodríguez T, De La Cruz-Hernández NI. Pathogenicity and infection route of *Vibrio parahaemolyticus* in American white shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society. 2010; 41(3):464-470.
11. Karunasagar I, Pai R, Malathi GR, Karunasagar I. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture. 1994; 128(3, 4):203-209.
12. Soto-Rodriguez SA, Gomez-Gil B, Lozano R. 'Bright-red'syndrome in Pacific white shrimp *Litopenaeus vannamei* is caused by *Vibrio harveyi*. Diseases of Aquatic Organisms. 2010; 92(1):11-19.
13. Manilal A, Sujith S, Selvin J, Shakir C, Gandhimathi R, Kiran GS. Virulence of vibrios isolated from diseased black tiger shrimp, *Penaeus monodon*, Fabricius. Journal of the World Aquaculture Society. 2010; 41(3):332-343.
14. Jayasree L, Janakiram P, Madhavi R. Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). Journal of the World Aquaculture Society. 2006; 37(4):523-532.
15. Tank PR. Effect of probiotics on growth, survival and disease resistance of *Litopenaeus vannamei* (Boone, 1931) larvae. Ph.D. Thesis submitted to Junagadh Agricultural University, Junagadh, Gujarat, India, 2018.
16. Baticados MC, Lavilla-Pitogo CR, Cruz-Lacierda ER, De La Pena LD, Sunaz NA. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 1990; 9(2):133-139.
17. Mohney LL, Lightner DV, Bell TA. An epizootic of vibriosis in Ecuadorian pond-reared *Penaeus vannamei* Boone (Crustacea: Decapoda). Journal of the World Aquaculture Society. 1994; 25(1):116-125.
18. Vieira FN, Buglione CC, Mourino JP, Jatobá A, Martins ML, Schleder DD, *et al*. Effect of probiotic supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi*. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2010; 62(3):631-638.
19. Kongnum K, Hongpattarakere T. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish & Shellfish Immunology. 2012; 32(1):170-177.
20. Zokaeifar H, Balcázar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A, *et al*. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology. 2012; 33(4):683-689.
21. Karthik R, Hussain AJ, Muthezhilan R. Effectiveness of *Lactobacillus s*p. (AMET1506) as probiotic against vibriosis in *Penaeus monodon* and *Litopenaeus vannamei* shrimp aquaculture. Biosci. Biotechnol. Res. Asia. 2014; 11:297-305.
22. Balcázar JL, Rojas-Luna T, Cunningham DP. Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp (*Litopenaeus vannamei*) following immersion challenge with *Vibrio parahaemolyticus*. Journal of Invertebrate Pathology. 2007; 96(2):147-150.
23. Zheng CN, Wang W. Effects of *Lactobacillus pentosus* on the growth performance, digestive enzyme and disease resistance of white shrimp, *Litopenaeus vannamei* (Boone, 1931). Aquaculture Research. 2017; 48(6):2767-2777.
24. Khuntia BK. Basic microbiology- An illustrated laboratory manual. Daya Publishing House, Delhi, India. 2011, 299.
25. Snedecor GW, Cochran WG. Statistical Methods, Lowa State Universiy Press, Iowa, U.S.A. 1967, 435.
26. Finney DJ, Tattersfield F. Probit analysis. Cambridge University Press, Cambridge. 1952, 318
27. Escobedo-Bonilla CM, Audoorn L, Wille M, Alday-Sanz V, Sorgeloos P, Pensaert MB, *et al*. Standardized white spot syndrome virus (WSSV) inoculation procedures for intramuscular or oral routes. Diseases of Aquatic

Organisms. 2006; 68(3):181-8.
28. Howe NR, Quast WD, Cooper LM. Lethal and sublethal effects of a simulated salt brine effluent on adults and subadults of the shrimps *Penaeus setiferus* and *P. aztecus*. Marine Biology. 1982; 68(1):37-47.
29. Dean TL, Richardson J. Responses of seven species of native freshwater fish and a shrimp to low levels of dissolved oxygen. New Zealand Journal of Marine and Freshwater Research. 1999; 33(1):99-106.
30. Huang HH, Liu XL, Xiang JH, Wang P. Selection of *Vibrio harveyi*-resistant *Litopenaeus vannamei* via a three-round challenge selection with a pathogenic strain of *V. harveyi*. Fish & Shellfish Immunology. 2013; 35(2):328-33.