Effects of Heavy Metal and Pollutants on the Non-special Immunity of the Shrimp and Crab

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Abstract: This paper attempted to review and evaluate existing information about the effects of heavy metal, ammonia-N, nitrite and the organic pollutants on the non-special immune response of the shrimp and crab, which providing theory for improving the self-immunity of shrimp and crab by meliorating cultural environment. In addition, it could provide information for further study on this field.

Keywords: Shrimp and crab, non-special immune, heavy metal, pollutants

Introduction

Recently, shrimp and crab culture is currently suffering serious losses due to infectious diseases all over the world. Disease outbreak in aquatic animals can be caused by many reasons, like wastewater from processes of industrial and agricultural production, feed and feces from aquaculture, remains of dead shrimp and crab or offing beach pollutions. All that made aquaculture environment more and more deteriorated yearly. Ecologically integrated-control on diseases of the shrimp and crab, analyzing disease resistibility of shrimp and crab, infection of pathogens and environmental physiology mechanism of immune regulation affected by environmental factors are concerned as strategies to prevent and cure diseases. Environmental stress from heavy metal and pollutants seems to be important factor for determining reduction of immunocompetence and is signalled by the appearance or the increased prevalence of diseases in shrimp and crab. Environment quality of waters and influences of environmental factors on non-special immune of the shrimp and crab have become new research hotspots. This paper summarized researches of effects of environmental factors (heavy metal, ammonia-N, nitrite, pollutants) on the non-special immune of shrimp and crab at home and abroad, in order to accumulate research information of non-special immune of the shrimp and crab for further research.

1  Influence of several environmental factors on the non-special immune of the shrimp and crab

1.1  Effects of heavy metal on the non-special immune of the shrimp and crab

Heavy metal is not only poisonous to the shrimp and crab, affects the development or even results in
mortality, but also is harmful to human health through biologic enrichment and food chain transfer. The effect mechanisms of heavy metal for shrimp and crab growth, development and survival are complicated, and few studies on effects of heavy metal on shrimp and crab immunity are known. If Carcinus maenas exposed to 500µg·L⁻¹ Cd²⁺ for 30d, heamocyte phagocytic ability to bacteria will decrease [2].

Macrobrachium rosenbergii exposed to 1µg·L⁻¹ mercuric chloride over a 30-day period, and exhibited hyperplastic gill lamellae engorged with haemocytes. And then, haemocytes were released into the interlamellar spaces through necrotic regions and they covered the entire gill lamellae, and total haemocyte counts (THC) were reduced during exposing to Hg²⁺[3]. Palaemon elegans was exposed to 0.1 mg·L⁻¹Cu²⁺, 0.05 mg·L⁻¹Hg²⁺, 0.1 mg·L⁻¹Cd²⁺, 0.5 mg·L⁻¹Cr²⁺, 1.0 mg·L⁻¹Zn²⁺ or 10.0 mg·L⁻¹Pb²⁺, respectively, and after the initial 8 h, THC of Palaemon elegans in each group decreased, while after 16 h, it recovered to the original level[4]. This study showed that host itself had certain adaptability to heavy metal, and low heavy metal ion concentration was less poisonous to the host.

As a common disinfectant and remedial medicine of shrimp and crab culture, although CuSO₄ is effective to diseases of shrimp and crab, at the same time, long-term usage of CuSO₄ could affect their immunity. Low concentration (0.1 mg·L⁻¹) of CuSO₄ could decrease the sensitivity to Lactococcus garvieae, and the sensitivity increased with CuSO₄ concentration elevating, but when CuSO₄ concentration was higher than 0.2 mg·L⁻¹, superoxide anion increased in Macrobrachium rosenbergii [5]. Heavy metals of different concentrations have special effects on shrimp and crab. 9.6 µg·l⁻¹ Hg²⁺ and 60.5 µg·l⁻¹Cu²⁺ could enhance the heamocyte phogocytic ability of Macrobrachium malcolmsonii, however, the THC was reduced in 90.8 µg·l⁻¹ Cu²⁺, and the heamocyte phogocytic ability was lower in 21.4 µg·L⁻¹ Hg²⁺ [6]. Concentrations of Cu²⁺, Co²⁺, Mn²⁺ in seawater were 40, 40, 20 µg·L⁻¹, respectively, AKP could be obviously activated during Mysis period, but decreased in higher concentrations; low levels of Cu²⁺ made the activities of AKP and ATPase increase. AKP activity peaked at 40 µg·L⁻¹. ATPase activity was obviously higher in the condition of Cu²⁺ (ranging from 40 to 80 µg·L⁻¹) than that in the control, but higher concentration could restrain AKP and ATPase activity. 20 µg·L⁻¹ Co²⁺ could enhance the activities of AKP and ATPase, but Ni²⁺ could reduce the activity of them. Because of direct relationship between the activities of AKP, ATP and growth, effects of heavy mental on shrimp larvae development are nearly accordant with the activities of AKP and ATPase, so the activities of AKP and ATPase could be regarded as an index whether the environmental factors were suitable to survival of shrimp larvae or not [7-9]. Therefore, although high level of heavy metal is harmful to shrimp and crab, minimum or infinitesimal heavy metal ions are good for increasing hosts immune, and further study on the mechanisms are necessary.

Litopenacus vannamei were exposed to 0, 1, 5, 10 or 20 mg·L⁻¹ Cu²⁺ for 24, 48 and 96 h, respectively, to determine THC, PO activity, respiratory burst, phagocytic activity and clearance efficiency to Vibrio alginolyticus. The results showed that Litopenacus vannamei exposed to 1 mg·L⁻¹ Cu²⁺ or higher for 24 h, and exhibited a decrease in all the above four indices, while the groups exposed to 20 mg·L⁻¹ Cu²⁺, had an obviously increase in respiratory burst, and the immunity of Litopenacus vannamei could be restrained by 1 mg·L⁻¹ or higher concentration of Cu²⁺, furthermore, sensitivity of Litopenacus vannamei to
V. alginolyticus has been enhanced [10].

Cu²⁺ and Zn²⁺ levels in seawater significantly affected the humoral immune factors of Scylla serrata. At low concentration of no-Waterborne Cu²⁺ (≤60µg·L⁻¹) and Zn²⁺ (≤100µg·L⁻¹), ceruloplasmin (CP), PO activity both in heanolymph, the activities of superoxide dismutase (SOD), Antibacterial (Ua), Lysozyme(Ul) in hepatopancreas, heanolymph and muscle of Scylla serrata were enhanced with Cu²⁺ and Zn²⁺ levels’ increasing. Whereas when Cu²⁺ Zn²⁺ levels increased to 75 µg·L⁻¹ and 125 µg·L⁻¹, respectively, the activities of PO, SOD, Ua and Ul all declined. Activities of ACP and AKP were not affected obviously except those in hepatopancreas were restrained significantly; while in different Zn²⁺ levels, activities of ACP and AKP decreased significantly. Thus it could be seen that, Cu²⁺ and Zn²⁺ in water has significant effects on humoral immune factors of Scylla serrata, and good immunity could be maintained by effective controlling of Cu²⁺ and Zn²⁺ concentration (ranging from 15 - 60 µg·L⁻¹ and 25 - 100 µg·L⁻¹), respectively [11].

Wu et al. (2005) reported that study on the effects of 3 kinds of heavy metal (Cu²⁺, Zn²⁺, Cd²⁺) on SOD activities in hepatopancreas, gill, and haemolymph of Litopenacus vannamei, showed that SOD activities changed significantly with prolonged exposure of these ion (P<0105). The SOD activities of all test objectives changed with a single peak under the exposure of Cu²⁺ (0.1 - 1 mg·L⁻¹), and the activities of hepatopancreas, gills and blood of SOD changed with the extension of time, the activities of the hepatopancreas of SOD was inhibited obviously by the Zn²⁺ under 10 mg·L⁻¹, and the activities of the hepatopancreas and gill of SOD was also inhibited obviously by the Cd²⁺ under 0.5 mg·L⁻¹, respectively, while 0.125 mg·L⁻¹ had no-significant effect on that of gill. The SOD activities of hepatopancreas, gill and blood all increased first and then decreased under the prolonged exposure of Zn²⁺ (<10 mg·L⁻¹) and Cd²⁺ (<0.25 mg·L⁻¹). There was an obvious dose-time response relationship between test metal ions and SOD activity. The SOD activities decreased in order of hepatopancreas>gill> haemolymph, while the toxicity of test metal ions was in order of Cd²⁺>Cu²⁺>Zn²⁺ [12].

1.2 Effects of ammonia-N on non-special immunity of shrimp and crab

Ammonia-N is the most common pollutants in aquaculture ponds, which refers to summation of ammonia and ionic ammonia, and the ratio of ammonia and ionic ammonia is related to pH, temperature and salinity [13]. Ammonia-N dominatingly comes from extraction by cultured shrimp and crab, amination of nitric organism (remains of feedstuff and feces), 40% - 90% of nitric waste of shrimp and crab is excreted out of the epithelial cells in the terms of ammonia-N, which in water affects the development, moulting, oxygen-consumption, excretion of ammonia-N, hemocyanin level and total protein in haemolymph, osmosis regulation, ion concentration and Na⁺,K⁺-ATPase activities of shrimp and crab [14-15]. When NH₄-N level was 1.01 mg·L⁻¹ or higher, Macrobrachium rosenbergii hypoxia caused the susceptibility to V. alginolyticus increasing [16], the susceptibility to V. alginolyticus of Litopenaeus vannamei had a direct correlation with NH₄-N level, and increased under NH₄-N concentration ranges from 0 to 20.00 mg·L⁻¹ [17]. When environmental NH₄-N was 2.5 mg·L⁻¹, after 20d, Fenneropenaeus chinensis THC decreased, while activities of PO, SOD, peroxidase, Lysozyme and antibacterial were decreased [14]. Macrobrachium
rosenbergii exposure to the concentration of NH$_4$-N 0.55, 1.68 and 3.18 mg·L$^{-1}$, respectively. After 7d, PO activity was significantly reduced and generation of reactive oxygen decreased too, while changes of THC were no-significant, and phagocytic activity and clearance efficiency decreased, and susceptibility to Lactococcus garvieae increased [18,19]. Under Litopenacus vannamei exposure to the concentration of NH$_4$-N 0, 1.10, 5.24, 11.10 and 21.60 mg·L$^{-1}$, respectively, THC, hyalinocytes and granulocytes had no significant changes; while that in concentration of NH$_4$-N was 5.24 mg·L$^{-1}$, PO activity dropped significantly, but after 7d it increased again. The PO activity decreasing was not because of THC or granulocytes decreasing; in Litopenacus vannamei, concentration of superoxide anion was affected by that of NH$_4$-N in water. Exposing to the concentration of NH$_4$-N 21.6 mg·L$^{-1}$, 11.2 mg·L$^{-1}$ and 21.22 mg·L$^{-1}$, respectively, for 7 d, SOD activity lowered obviously, and phagocytic activity and clearance efficiency of phagocyte decreased too [17].

Under L. stylirostris exposure to 1.5 mg·L$^{-1}$ and 3.0 mg·L$^{-1}$ NH$_4$-N, respectively, both PO activity and THC (mainly the hyaline cell) decreased. It was found that the amount of the transcript encoding ProPo and peroxinectin decreased by 60.5%, which explained why haemocytes PO activity was reduced under ammonia-N stress in molecular level [20]. Ammonia-N affects the THC, activities of PO, AKP and ACP in Fenneropenaeus chinensis [21]. Macrobrachium rosenbergi exposure to 1 mg·L$^{-1}$ ammonia-N for 1 d, 4d, 7d and 10d, respectively, PO activities both in haemolymph and muscle decreased, while that in hepatopancreas increased slightly; SOD activities in different tissues and organs increased first, and then decreased; phosphatase activity in different tissues and organs increased [22]. L. stylirostris exposure to the concentration of NH$_4$-N 21.6mg·L$^{-1}$, 11.2 mg·L$^{-1}$ and 21.22 mg·L$^{-1}$, respectively, THC, PO activity in haemocytes, Lysozyme and antibacterial activity changed significantly (p<0.05). With ammonia-N concentration increasing, THC, Lysozyme activity and antibacterial activity decreased significantly, while PO activity and susceptibility to pathogenic bacteria increased, so it was suggested that changed range of NH$_4$-N should be better not exceed 0.5 mg·L$^{-1}$ or NH$_4$-N should not be maintained at a high level (>0.5 mg·L$^{-1}$) for a long time [23]. NH$_4$-N stress could result in declining haemocyte counts and phagocytic percentage, decreasing the activities of phagocytic, lysozyme, phenoloxidase, Superoxide dismutase, damaging no-specific immune system of Eriocheir sinensis. The reaction degree is positively related to the stress of NH$_4$-N concentration and time length treated [24]. Total haemocyte count decreased while ambient ammonia increased, Superoxide dismutase (SOD) activity in haemolymph was stimulated by lower ambient ammonia concentration after short time exposure and depressed by higher ammonia concentration. Therefore, a depression in immunity [25].

Shrimp and crab immunity could be reduced under ammonia-N stress, and concurrently, susceptibility to pathogenic bacteria was increased accordingly, and it was much easier to cause diseases, so effective methods should be taken during water management in shrimp and crab culture to control the NH$_4$-N concentration in ponds, which could avoid shrimp and crab diseases outbreak.

1.3 Effects of nitrite on non-special immunity of shrimp and crab

After NO$_2^-$ in water gets into the haemolymph of shrimp and crab, oxyhemocyanin can be turned into
deoxyhemocyanin, and haemocytes oxygen affinity could be reduced, then the ability of carrying oxygen decreased, which is toxic to the aquatic organism [23]. In addition, NO$_2^-$ also affects the excretion of nitric waste, protein level, osmosis regulation and ion concentration in Haemolymph [26,27]. Higher NO$_2^-$ concentration in water could result in decreasing the activities of PO, SOD, and lysozyme in Fenneropenaeus chinensis [28]. When NO$_2^-$ concentration was 1.68 mg·L$^{-1}$, Macrobrachium rosenbergii disease resistibility will be reduced significantly, and phagocytotic activity and clearance efficiency to bacteria decreased, whereas more reactive oxygen produced. Higher level of reactive oxygen in body is obviously toxic to the organism, while the body disease resistibility decreased, and susceptibility to Lactococcus garvieae increased, and total mortality caused by infection had a positive relation to NO$_2^-$ level [16].

Reactive oxygen generated by Macrobrachium nipponense increased with levels of Nacl and Sodium polyphosphate increasing, while SOD activity increased slightly at first, and then decreased. This results indicated that immune system of shrimp has certain resistibility to low concentration of poison, but will be restrained with the concentration of the poison increasing, and shrimp immunity decreased, even death was caused by diseases [30].

The studies of effects of nitrite-N (NO$_2^-$N) on Litopenacus vannamei immune indices showed that after exposure to 0, 0.98, 4.94, 9.87 and 19.99 mg·L$^{-1}$ NO$_2^-$N for 96h, respectively, THC and PO activities in different groups decreased, while respiratory burst (release of superoxide anion), exposure to 9.87 and 19.99 mg·L$^{-1}$ NO$_2^-$N significantly increased (P<0.05). The results showed that higher level of NO$_2^-$N in water inhibited the immunity of Litopenacus vannamei and enhanced susceptibility to pathogeny, reactive oxygen level and maybe cytotoxic levels [31].

Exposure to each NO$_2^-$N level, reactive oxygen intermediates (ROIs) of Macrobrachium nipponense was obviously increased, which has a positive correlation with NO$_2^-$N level. Sublethal exposure to NO$_2^-$N for 24 h, ROIs increased by 97 % than the control, while activities of SOD, CAT and glutathione peroxidase (GPX) were 67 %, 80.6 % and 82.7 % lower than those of the control, respectively. The results showed that unbalance of peroxidative ability and antioxidative ability is one toxic mechanism of NO$_2^-$N to shrimp. After exposure to 1 mg L$^{-1}$ NO$_2^-$N for 1 d, 4 d, 7 d and 10 d, respectively, PO activity in both haemolymph and muscle of Macrobrachium rosenbergii decreased, while that in hepatopancreas increased slightly; SOD activities in different tissues and organs increased first and then decreased, however, phosphatase activity increased [22].

NO$_2^-$N stress could be toxic to shrimp and crab, also could decrease immunity of shrimp and crab, and increase susceptibility to pathogens, so it was much easier to cause shrimp and crab diseases, and hence, effective methods should be taken to control the NO$_2^-$N concentration in water management of shrimp and crab culture.

1.4 Effects of pollutants in water on shrimp and crab non-special immunity

Pollutants is a primary pollutant which affects the growth, development and survival of shrimp and
crab, resulting in reducing shrimp and crab immunity, both in natural and culture water. Pollutants not only cause the immunity of shrimp and crab reduced, but also have seriously toxic effects in cell and molecule level, causing pathological changes and numerous death. Up to the present, studies on effects of pollutants on shrimp and crab immune system are very few, and toxic mechanism is not clear yet. At present, dominant effects of pollutants on enzymes related to non-special immunity, small molecule active material and changes of qualities, kinds, ratio and function of haemocytes have been studied, in order to find the alternative regulation of these indices, and to regard those much more related to pollutant levels as indicators of shrimp and crab immunity during culture.

Formulated feed was used for pollutant source to study effects of organic pollution on *Fenneropenaeus chinensis* body’s intraenvironment and extraenvironment, and susceptibility to pathogens, respectively. The results showed that DO level apparently decreased, while COD, NH\textsubscript{3}-N and NO\textsubscript{2}-N rapidly increased in experimental pools; susceptibility to pathogens was elevated by 1 - 3 times; activities of SOD, PO and Lysozyme deceased by 22 %, 50 % and 28 % - 86 %, respectively. THC increased about 50 %, which was related to some toxicant produced by decomposing formulated feed in the aquatic environment, made water worse, reduced activities of enzyme which was related to disease resistibility and promoted outbreaks of diseases, and especially decrease of generation of ammonia-N and DO activity was significant. Propiconazole affecting the non-activated haemocytes will stimulate *Litopenacus vannamei* haemocytes to generate superoxide anion; if it affected activated haemocytes by yeast polysaccharide, levels of superoxide anion could be reduced. *Crangon crangon* living in harbour dredge spoils and polluted by PCBs, PAHs and heavy metal, both THC and PO activity in haemocytes was lower than those living in clean sand. *Crangon* exposed to sublethal concentration (0.05-500µg·L\textsuperscript{-1}) of PCB\textsubscript{15} and PCB\textsubscript{17}, respectively, and THC and PO activities in haemocytes decreased, while PCB\textsubscript{17} has no great effects on disease resistibility.

Studies indicated that Monocrotophos stress could reduce activities of PO and SOD in *Fenneropenaeus chinensis*, and this change could be enhanced with Monocrotophos concentration increasing and time prolonging, and the main reason was that the structures of tissue and cell were damaged by Monocrotophos, resulting in capability of synthesizing enzyme decreased. Further study will be conducted to determine whether there was direct effects on the activities of SOD and PO under Monocrotophos stress. Monocrotophos stress may inhibit the recognition abilities and eliminate pathogens. In addition, Lysozyme activity can be reduced under Monocrotophos stress.

Shrimp and crab culture has been puzzled by pollutants, and to eliminate pollutants is very important way to avoid shrimp and crab diseases outbreaks, therefore, studies on rapidly decomposing and transforming of pollutants in shrimp and crab culture is essential and practical meanings for promoting safe and healthy culture of shrimp and crab.
2 Several suggestions about studies on effects of environmental factors on the non-special immunity of shrimp and crab

Up to the present, knowledge about immunology of shrimp and crab is not very systemic, composition and character of shrimp and crab immune system, generating regulation and character of immune factors, and the mechanisms of cell immunity and humoral immunity all have not been studied clearly yet; evaluating system of effects of environmental factors on non-special immunity of shrimp and crab has not been set up, which badly affected the implementation of preventing methods which were used to increase the resistibility. Accordingly, to focus studies on effects of environmental factors on non-special immunity of cultured shrimp and crab, has crucial academic meanings and practical values.

Further studies on effects of environmental factors on non-special immunity include the followings:

1) To study composition and character of immune system of shrimp and crab, generating regulation and character of immune factors, and the mechanisms of cell immunity and humoral immunity further and systemically, to set up immune parameter data-base of healthy shrimp and crab, for example, THC, phagocytic activity, respiratory burst, PO activity, SOD activity, antibacterial peptide, agglutinin, bacteriolysis activity and antibacterial activity and so on. In order to evaluate exactly health of cultured shrimp and crab, and improve the culture environment, prevent and control the disease outbreaks in time.

2) To study effects of interaction of environmental factors on immunity of shrimp and crab, and to set up general-technic system which is to be used for water regulation in safe and healthy culture of shrimp and crab.

3) To study mechanism of effects of environmental factors on immunity of shrimp and crab, and study further effects of changes of environmental factors on expression of immune genes, especially, by modern molecular biology technique.

4) To really do well in environmental factors monitoring and diseases detecting in safe and healthy culture, to discuss relation between environmental factors and immune parameters; to set up methods and indices system to evaluate health of shrimp and crab rapidly and exactly.

In addition, shrimp and crab disease outbreak is nearly related to environmental quality, and after hosts getting in touch with pollutants, non-special immunity in shrimp and crab will incisively change. Therefore, if changes of non-special immune indices in shrimp and crab can be used to monitor degree of environment pollution, there will be certain developing and practical foreground in environmental monitoring practice.

Acknowledgements

This work was funded in part by a grant from the National High Technology Research and
Development Program of China (863 Program) (2007AA091406), a grant from the Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection (JLCBE05007), and a grant from NingBo Natural Science Foundation (2006A610086).

References


几种环境因子对虾蟹类非特异性免疫的影响
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摘要：综述了近 10 a 国内外有关重金属、氨态氮、亚硝酸盐、有机污染物等几种环境因子对虾蟹类非特异性免疫影响的研究成果，以期为通过改善养殖环境条件提高虾蟹类自身免疫抗病力提供理论依据，同时，为进一步深入开展该领域的研究工作积累资料。

关键词：虾蟹类；非特异性免疫；重金属；污染物