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Ammonia and nitrite toxicity to pacific white-leg shrimp *Litopenaeus vannamei*

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Abstract
Litopenaeus vannamei, also known as the Pacific white leg shrimp/ White leg shrimp/ Pacific white shrimp or King prawn, attaining a maximum length of 230 mm; with a carapace length of 90 mm. Adults live in the ocean, at depths of up to 72 m, while juveniles live in estuaries. The rostrum is moderately long, with 7-10 teeth on the dorsal side and 2-4 teeth on the ventral side. Pacific white leg shrimp are widely distributed throughout tropical Pacific waters, from Mexico to as far south as northern Peru. It is restricted to areas where the water temperature remains above 20°C throughout the year. This is the most important cultivated shrimp species in the world. In any culture system, ammonia and nitrite, form the two main inorganic forms of nitrogen, especially in an intensive shrimp culture system, ammonia and nitrite increase exponentially over time in the grow-out ponds, in spite of frequent water replacement. Higher ammonia and nitrite levels may deteriorate water quality resulting in high mortality and low growth rate in penaeid shrimps. In this article an attempt has been made to review several works related to ammonia and nitrite toxicity to Pacific White Shrimp, which would help researchers and farmers to understand the several paths of ammonia and nitrite toxicity and plan measures to reduce its impact.

Keywords: Ammonia, nitrite toxicity to pacific white-leg shrimp, *Litopenaeus vannamei*

Introduction
In many countries the common vannamei culture practices being followed are semi-intensive and intensive culture system. Generally ammonia originates from excretion of cultured animals and from ammonification of unconsumed food or organic detritus is the most common toxicant. Nitrite, formed from ammonia by *Nitrosomonas* spp., is rather more toxic than ammonia to crustaceans (Armstrong, 1979) [34]. In intensive shrimp farming, built-up of nitrogenous waste in the form of ammonia, nitrite and nitrate from uneaten food and the waste products from the shrimp continuously degrade the culture environment. Since ammonia and nitrite are extremely toxic to shrimp compared to nitrate, control of ammonia and nitrite is the second most important factor impacting survival and growth of cultured organisms, followed by dissolved oxygen (Ebeling *et al*. 2006) [4]. Therefore, the accumulation of ammonia and nitrite may have detrimental effects on prawn rearing.

Exposure of *Penaeus vannamei* to ammonia and nitrite
Ammonia toxicity
The cause of toxicity of ammonia is mainly based on the irritative properties of the compound. Ammonia is the main end product of protein catabolism in crustaceans and can account for 60–70% of nitrogen excretion with only small amounts of amino acids, urea and uric acid (Chen and Kou 1996a, b) [17, 18]. While mammals convert nitrogenous wastes into other forms of nitrogen such as urea, fish and crustaceans excrete ammonia in an unaltered form. This is possible since in natural conditions ammonia is instantly diluted to safe levels by the surrounding water. Fish and crustaceans lack the ability to convert ammonia to the less toxic, carbamoyl phosphate compound and therefore, aquatic species are especially prone to toxic effects of ammonia at highly concentrated levels.
In water, ammonia is present in both ionized (NH_4^+) and un-ionized (NH_3) state, with NH_3 as the toxic form due to its ability to diffuse across cell membranes (Fromm and Gillete 1968; Emerson *et al*., 1975) [42]. The unionized form of ammonia is the more toxic species to aquatic organisms due to its ability to gain entry through the gills.

The lipid soluble, un-ionized form can readily pass through cell membranes (Boardman *et al*. 2004) [11], whereas the ionized form does not readily cross hydrophobic microphores in the gill membrane (Svobodova *et al*., 1993). The unionised ammonia can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and molluscs (Smart, 1978) [92].

Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia, muscle depolarization, hyper excitability, convulsions and finally death (Ip *et al*., 2001). NH_4^+ is also toxic, especially at low pH levels (Allan *et al*. 1990) [4]. Ammonia is oxidized to nitrite and nitrate by *Nitrosomonas* and *Nitrobacter* bacteria (Sharma and Ahlert, 1977). Ammonia and its intermediate product of oxidation, nitrite, are the most common toxicants in culture systems and are toxic to fish, molluscs and crustaceans (Colt and Armstrong, 1981) [34].

The physiological changes in aquatic organisms due to ammonia exposure vary. The effect of ammonia relates to site specific irritation. Caglan, *et al* (2005) [13] analyzed the gills of tilapia that had been exposed to chronic ammonia tests and concluded that ammonia was responsible for gill hyperplasia as well as lamella fusion. The hyperplasia and lamella fusion resulted in restricted water flow over the gills, leading to respiratory stress on the organism. Similar results, as well as epithelial pitting of the gills, were observed when rainbow trout were tested and examined using scanning electron microscopy (Kirk and Lewis 1993) [61]. Exposure of Pacu fish to different concentrations of ammonia-N caused an elevation in total hemoglobin and blood glucose (Barbieri and Bondioli 2015) [7]. The sub-lethal effects induced decrease in growth rate and resistance to diseases and poor food conversion (Kuttchantran, 2013) [63]. In Nile tilapia (*O. niloticus*), El-Sayed (2015) studied the effects of ammonium nitrate on the hematological parameters and the serum attributes and found a parallel disturbance in all parameters with increase of ammonia concentration.

In penaeid shrimp, high concentrations of ammonia may affect growth rates and survival, and can in extreme cases cause mortality (Wickins 1976; Zin & Chu 1991; Chen & Lin 1992) [19, 110, 105]. Ammonia damages the gills and reduces the ability of haemolymph to transport oxygen while increasing oxygen consumption by tissues (Chien 1992; Racotta and Herna´ndez-Herrera 2000) [31, 84]. Osmoregulatory capacity decreases with increasing ammonia concentration and exposure time (Lin *et al*. 1993). Ammonia may also increase the moulting frequency of shrimps (Chen and Kou 1992) [20]. Ammonia is also thought to cause damage to the central nervous system (Wright 1995) [107].

High ammonia content affects the immune system of *Marsupenaeus japonicus* (Bate) (Jiang *et al*. 2004) [57] and *L. Vannamei* (Liu and Chen 2004) [21]. Reduced survival and growth because of sublethal and lethal effects of ammonia toxicity become relevant in aquaculture operations.

Effect of ammonia on survival and growth

A number of studies have been conducted on the lethal effects of ammonia at various life stages of penaeid shrimps, such as *Penaeus chinensis* (Chen and Lin 1992) [22], *P. monodon* (Chen and Lei 1990) [23], *P. paulensis* (Ostrensky and Wasielesky 1995) [80], *P. penicillatus* (Chen and Lin 1991) [24], *P. semisulcatus* (Wajsbrot *et al*. 1990) [103], and *Metapenaeus ensis* (Nan and Chen 1991) [24]. Lethal toxicity tests can be acute or chronic depending on the time of exposure. In most cases, acute tests are performed over a period of 2 - 7 days, while chronic tests are longer than 7 days. Concentrations leading to 50% mortality vary depending on the organism being tested.

Previous studies have shown that 48 h median lethal concentrations (LC_{50}) for ammonia-N to varying species of shrimp, to range from 30 and 110 mg/l TAN at full strength seawater depending on size and age (Chen *et al.* 1990a, Chen *et al.* 1990b, Ostrensky and Wasielesky 1995, Fri´as-Espericueta *et al*. 1999, Kir and Kumlu 2006) [25, 80, 26]. For *Penaeus monodon* and *Metapenaeus macleayi* juveniles, LC_{50}'s were determined using 96 hr acute tests. The results showed the respective LC_{50}'s to be 1.69 and 1.39 mg/l NH_3-N (Allan *et al*. 1990) [4]. Other authors, through studies with various genera and species, have concluded that the toxicity of ammonia to specific species is dependent on time and concentration. A study using *Penaeus semisulcatus* post larvae (PLs) found that the tolerance to ammonia-N decreased with decreasing salinity. Specifically, the shrimp tested at 40 ppt salinity were tolerant to ammonia-N levels 2.9 times higher than those at 15 ppt over 48 h (LC_{50}'s of 32.5 and 11.2 mg/l TAN, respectively) (Kir and Kumlu 2006) [60].

Elevated ammonia levels can also lead to reduced growth of species raised in intensive aquaculture systems. Wickins (1976) [105] showed that a concentration of 0.45 mg/l NH_3-N led to a 50% decrease in growth of five species of penaid shrimp. The author also concluded that a concentration of above 0.10 mg/l NH_3-N breached maximum acceptable levels for reduced growth over a three week chronic test (Wickins 1976) [105]. The median lethal concentration of ammonia to *Penaeus japonicus* has been reported by Chen *et al*. (1989) [26] for larvae, and by Kou and Chen (1991) [24] for juveniles.

Nitrite Toxicity

Nitrite is an intermediate product of ammonia either in the bacterial nitrification of ammonia or in the bacterial denitrification of nitrate. It has been reported that concentration of nitrite increased directly with culture period and might reach as high as 4.6 mg/l nitrite-N (nitrite as nitrogen) in pond water (Chen *et al*., 1989) [28]. Accumulation of nitrite in pond water may deteriorate water quality, reduce growth, increase oxygen consumption and ammonia excretion, and even cause high mortality of shrimp (Chen and Chen, 1992; Cheng and Chen, 1998) [29, 30]. Elevated nitrite in water has also been reported to increase the susceptibility of giant freshwater prawn *Macrobrachium rosenbergii* to pathogen *Lactococcus garvieae* (Cheng *et al*., 2002) [30]. Nitrite toxicity is not related to site specific irritation. Instead, the toxicity of nitrite is a function of the effects on the circulatory and immune systems of aquatic organism. Nitrite enters the blood stream and inhibits the binding of oxygen to the iron molecule of hemoglobin (Hargreaves, 1998) [51]. The nitrite toxicity mechanism acts on the process of oxygen transport. In other words, nitrite binds to hemocyanin, converting it into meta-hemocyanin, which is unable to transfer oxygen to the tissues.

Previous studies have demonstrated that the increase in nitrite in the environment leads to nitrite accumulation in the hemolymph, which immunosuppresses the *L. Vannamei* and

increases their susceptibility to *Vibro alginolyticus* infections (Tseng and Chen 2004) [30]. Barbieri *et al*. (2014) [18] observed an increase in oxygen consumption and ammonia excretion in *Litopenaeus schmitti* juveniles exposed to increasing concentrations of nitrite. Several researchers evaluated the acute toxicity of the nitrogenous compounds in penaeid shrimps (Lin and Chen 2004; Gross *et al*. 2004, Campos *et al*. 2012; Barbieri *et al*. 2014) [18, 50].
Nitrite also competes with chloride for transfer across erythrocyte membranes leading to the oxidation of haemoglobin to met-hemoglobin. Consequently, excessive nitrite levels in culture systems can cause depressed growth, increased susceptibility to disease, and eventual mortality. However, this competition with chloride decreases the detrimental effects of nitrite in marine waters and makes nitrite more dangerous in freshwater aquaculture. In crustaceans, ambient nitrite reduces their thermal tolerance and induces methaemocyanin formation, causes hypoxia in tissues and diminishes the respiration efficiency (Alcaraz and Carnegas 1997) [3].

Effect of nitrite on growth and survival

The acute lethal effects of nitrite on aquatic organisms is not as pronounced as ammonia at low concentrations, yet its toxicity is still of concern. The effects of nitrite stress on immune responses to *Vibrio alginolyticus*, a common bacterial disease in marine aquaculture systems was examined by Tseng and Chen (2004) [18]. They found that shrimp exposed to nitrite between 5 and 22 mg/l showed significantly reduced resistance to bacterial infection. The study was conducted through analysis of haemocyte (invertebrate red blood cells) counts (Tseng and Chen 2004). In another study that explored the acute effects of nitrite on *L. vannamei* shrimp over 48 h revealed LC50s of 142.2, 244.0, and 423.9 mg/l nitrite-N for 15, 25, and 35 ppt salinity respectively (Lin and Chen 2004) [18]. *Macrobrachium malcolmsonii* juveniles were subjected to nitrite stresses in the presence of the bacteria *A. hydrophila*. The authors concluded that increased nitrite stress led to a reduction in immune response to *A. hydrophila* (Chand and Sahoo 2006) [15]. In aquacultural systems, an increase in ammonia concentration is followed by a decrease in ammonia that is indirectly proportional to a rise in nitrite, as NH_3 is oxidized to NO_2. Gross *et al*. (2004) [50] also explored the acute effects of nitrite to *L. vannamei* in low salinity waters. When reared in water with 2 ppt salinity, the 48 h LC value was determined to be approximately 15 mg/l NO_2-N (Gross *et al.* 2004) [50], significantly lower than seen in the Lin and Chen (2003) experiments. The median lethal concentration (LC_{50}) of ammonia and nitrite has been estimated for penaeid shrimp postlarvae, such as *Penueus monodon, P. chinensis, P. puulensis.* and *P. juponicus* (Chin and Chen 1987; Chen and Chin 1988; Chen and Lin 1991; Lin *et al*. 1993; Ostrensky and Wasielesky 1995) [29, 30, 80].
The mean 48-h LC_{50} of un-ionized ammonia and nitrite for postlarvae of several penaeids has been estimated at 1.29 mg/l NH_3-N (24-h mg/l ammonia-N) and 170 mg/l nitrite-N (Wickins, 1976) [105]. The effect of nitrite has been widely studied in freshwater animals (Lewis and Morris 1986) [66]. In these organisms, nitrite induces reversible methaemoglobin formation, which is unable to transport oxygen to tissues (Russo, 1985) [89]. In crustaceans, incorporation of nitrite in haemolymph may reduce haemocyanin levels. Nitrite has also been found to oxidize the respiratory pigment (Needham, 1961) [75]. There are few studies available on the toxic action of nitrite in marine organisms. There are direct evidences that *P. setiferus* postlarvae are highly sensitive to ammonia and nitrite on short-time and chronic exposures (Alcaraz *et al*. 1997) [3]. For *P. setiferus* postlarvae, nitrite was much less toxic than ammonia. The acute toxicity of nitrite increased with time of exposure. The 24-h, 48-h and 72-h LC50 values for nitrite were 268.1, 248.8 and 167.3 mg/l nitrite-N. Thus, tolerance of *P. Setiferus* postlarvae to nitrite decreased 7 and 38% at 48-h and 72-h exposure with respect to the 24-h LC_{50} values. The lethality of nitrite on the juveniles of penaeid shrimp has been provided for fleshy shrimp *Fenneropenaeus chinensis* (Chen *et al*., 1990) [30], *P. monodon* (Chen and Lei, 1990), red-tailed shrimp *Fenneropenaeus penicillatus* (Chen and Lin, 1991), and sand shrimp *Metapenaeus ensis* (Chen *et al*., 1990). The reported 96-h LC_{50} varied from 37.71 to 54.76 mg/l for nitrite-N. However, little information is available on the lethality of nitrite at different salinity levels for penaeid shrimp (Chen and Lin, 1991) [31]. According to Lin and Chen (2003) [29], there is an inverse relationship between salinity and nitrite toxicity such that the toxicity increases with the reduction in salinity, making juvenile *L. vannamei* more susceptible to nitrite in hypo-osmotic conditions.
The environmental chloride can inhibit the uptake of nitrite and mortality due to nitrite, suggesting a method of managing nitrite toxicity in aquaculture production systems (Tomasso, 2012). The gills provide a selective interface between the external and internal environment, constituting a multifunctional organ responsible for gas exchange, ion transport, nitrogenous excretion, volume adjustment, and acid–base regulation (Lucu & Towle 2003). High levels of nitrite in water are potential factors triggering stress in aquatic organisms (Lewis and Morris, 1986) [66].
The toxicity of nitrite to crustaceans has been studied by several authors (Cheng and Chen, 1999; Chen and Lee, 1997) [29 30]. Elevated environmental nitrite has been reported to induce methaemocyanin formation, cause hypoxia in tissue, and impair the respiratory metabolism of penaeid shrimps (Nan and Chen, 1991; Chen and Chen, 1998) [31, 32]. However, very little is known about the effect of nitrite on the crustacean immune system. Ambient nitrite-N at 1.59 mg/l has been reported to decrease phagocytic activity of freshwater prawn *Macrobrachium rosenbergii* against *Lactococcus garvieae*, but increase the respiratory burst of prawn. However, nothing is known regarding the effect of nitrite stress on the immune response and pathogen resistance of penaeid shrimps.

Haematology

Shrimp farming witnessed impressive growth in many developing countries where this activity attained great economic and social importance. However, the shrimp industry has always been affected by infectious diseases, mainly of bacterial and viral etiology (Mohney *et al*., 1994; Hasson *et al*., 1995; Flegel, 1997) [73, 52, 44] causing heavy loss of production. Therefore, sustainable shrimp farming largely depends on health management and control of diseases in the shrimp and immune system is a tool to assess the shrimp health (Rodríguez *et al*., 1995) [87]. Many authors had already studied the physiological stress responses in crustaceans (Lorenzon *et al*., 2008, Fotedar and Evans, 2011) [69, 45]. Hemolymph chemistry has been the primary

means for assessing the effects of various stress inducing factors such as air exposure, changes in temperature and salinity, low dissolved oxygen and other stressors associated with fishing operations, live holding and transport.

Stress responses may either be primary, secondary or tertiary responses (Iwama *et al*., 1999) [56]. Primary responses represent the initial neuroendocrine/ endocrine response to the body's altered condition. In crustaceans, this involves the rapid release of crustacean hyperglycemic hormone (CHH) from the sinus gland, which acts to meet an increasing demand for energy (Fanjul-Moles, 2006) [3]. This leads to secondary stress responses, typically observed as elevated hemolymph glucose, formed through the mobilization of intracellular glycogen (Patterson *et al*., 2007) [82], increased lactate, and a host of other physiological and hematological changes that cascade from metabolic acidosis and the accumulation of metabolic end products (Taylor and Whiteley, 1989; Whiteley and Taylor, 1992; Paterson *et al*., 2005) [104, 86, 1]. Tertiary responses are whole-animal changes that occur because of energetic repartitioning resulting from stress, such as reductions in feeding, growth, predator avoidance, disease resistance, and reproduction. Elevated CHH and glucose are adaptive physiological responses that help restore homeostasis in the body, while other physiological changes are maladaptive.

In crustacean immune defense system haemocytes play a central role. First, they remove any foreign particles in the hemocoel by phagocytosis, encapsulation and nodular aggregation (Söderhäll and Cerenius, 1992) [3]. Second, haemocytes take part in wound healing by cellular clumping and initiation of coagulation processes through the release of factors required for plasma gelation (Johansson and Söderhäll, 1989; Omori *et al*., 1989; Vargas-Albores *et al*., 1998.) [58, 102] and carriage and release of the prophenol oxidase (proPO) system (Johansson and Soderhall, 1989; Hernández *et al*., 1996) [58]. They are also involved in the synthesis and discharge in the haemolymph of important molecules, such as α_2-macroglobulin (α_2M) (Rodríguez *et al*., 1995; Armstrong *et al*., 1990) [34, 87], agglutinins (Rodríguez *et al*., 1995) [87] and antibacterial peptides (Destoumieux *et al*., 1997; Schnapp *et al*., 1996; Lester *et al*., 1997) [36, 90, 65]. A hemogram consists of the total haemocyte count (THC) and the differential haemocyte count (DHC). For the DHC, most researchers agree with the identification of three cell types in penaeid shrimp namely large granule haemocytes (LGH), small granule haemocytes (SGH) and agranular haemocytes or hyaline cells (HC) (Rodríguez *et al*., 1995; Van de Braak *et al*., 1996) [87, 101].

Total haemocyte count has been used as a measure of stress in crustaceans, because it may reflect immune suppression. For crustaceans, some information exists on the importance of THC in pathogen resistance. In *Pacifastacus leniusculus*, Persson *et al*. (1987) [83] reported a relationship between haemocyte number and its resistance to the parasitic fungus *Aphanomyces astaci*. They demonstrated that a decrease in the haemocyte number of crayfishes harbouring *A. astaci* as a latent infection resulted in an acute infection with incomplete melanization of fungus hyphae, leading to the death of the crayfish. Le Moullac *et al*. (1997) [64] observed that *Penaeus stylirostris* with low THC due to hypoxia situation, became more sensitive to infections with highly virulent *Vibrio alginolyticus*. In *P. japonicus* (Tsing *et al*., 1989.) [100] and *P. stylirostris* (Le Moullac *et al*., 1998) [64], the highest haemocyte number was found during the postmoult stage, while the lowest was associated with the intermoult stage. Similar variations were seen in *Sicyonia ingentis* (Hose *et al*., 1992) [54] in which the most important release of haemocytes from hematopoietic tissue occurs during postmoult stage. As far as DHC is concerned, the highest number of LGH in *P. stylirostris* and *S. ingentis*, occurs in intermoult (Le Moullac *et al*., 1997; Hose *et al*., 1992) [64, 54].

Crustaceans have an open circulatory system in which the haemolymph carries out several physiological functions. One of these function is the transport of molecules such as the respiratory protein (hemocyanin) which is the most abundant molecule of the haemolymph (60% to 95 % of total protein) (Djangmah, 1970) followed by the clotting protein and other humoral components. Chisholm and Smith (1994) [33] found a relation between the protein concentration and water temperature, showing low plasma protein concentrations when temperatures are at their lowest and highest in the year. The concentration of total proteins is also related to the moult cycle of the shrimp. In *P. japonicus*, Chen and Cheng (1993) have reported lower levels of protein concentration during postmoult stage (41.37 mg ml^{-1}) as opposed to higher levels (74.90 mg ml^{-1}) found in early premoult.

Hemolymph glucose is one of the traditional indicators of stress in lobsters and crabs, and increases in glucose have been reported for a wide range of stressors, including emersion, handling and disease in clawed lobsters (Lorenzon *et al*., 2007; Basti *et al*., 2010) [69, 10], rock lobsters (Paterson *et al*., 2005) [81], and crabs (Barrento *et al*., 2009; Woll *et al*., 2010). Glycogen is the principal reserve of carbohydrates for crustaceans and constitutes the primary source of energy during intense or protracted exercise; therefore, high levels of glucose in the hemolymph reveal increased energetic investment (Briffa and Elwood, 2001). Giomi *et al*. (2008) showed that the additive effect of high temperature on emersion is strongly reflected in glucose concentration. However, recent studies with both rock lobsters and clawed lobsters show that glucose concentrations can increase or decrease rapidly depending upon duration of exposure to air and elevated temperature (Ridgway *et al*., 2006; Basti *et al*., 2010) [86, 10]. Many of the physiological parameters mentioned above are useful in understanding the mechanisms involved in stress responses.

Stress induced changes on enzymes

Marine crustaceans are under the influence of numerous environmental factors such as natural environmental changes according to daily or seasonal rhythms, environmental stress from contaminants or physico-chemical changes. Sub-optimal temperature or unsuitable salinity level in water may interact in an antagonistic, additive or synergistic manner with toxicants like ammonia, nitrite and many others thereby causing changes in the tolerance capacity of aquatic animals.

When an organism is subjected to stresses such as chemical, physical, biological (i.e. pathogen infection) upon sudden shortage of oxygen, abnormal oxidative reactions in the aerobic metabolic pathway result in the formation of excess amounts of singlet oxygen and the subsequently generated radicals (sometimes called ''free radicals''). These radicals can impair lipids, proteins, carbohydrates and nucleotides (Yu, 1994), which are important parts of cellular constituents, including membranes, enzymes and DNA.

Radical damage can be significant because it can proceed as a chain reaction. Consequently, mortality can occur due to severe destruction by massive radicals generated from acute stresses or long-term chronic stresses.

Fish respond to toxicants by altering their enzyme activities and the inhibition or induction of these enzyme activities has been used to indicate tissue damage (Nemcsok and Boross, 1982) [76]. Many enzymes like carboxyl esterase (CE), lactate dehydrogenase (LDH), alkaline and acid phosphates (ALP, ACP), glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (GOT and GPT) are measured as useful biomarkers to determine cellular impairment and cell rupture. Transaminases such as GOT and GPT play a vital role in protein and carbohydrate metabolism and act as an indicator for tissue damage (Nemcsok *et al*., 1981; Nemcsok and Boross, 1982) [76, 77].

Aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (GOT) and alanine aminotransferase (ALT) or glutamate pyruvate transaminase (GPT) are enzymes involved in the transfer of amino groups from one specific amino acid to another. Therefore, higher values indicate a greater transfer of amino groups, or the greater metabolic waste of amino acids in the tissue. AST and ALT activities are usually used as general indicators of the functioning of vertebrate liver. High AST and ALT generally, but not definitively, indicate a weakening or damage of normal liver function. AST and ALT may be indirectly related to oxidant metabolites so they serve as indicators of oxidative status. For finfish, AST and/or ALT have been used extensively in studies that evaluate finfish response to toxins (heavy metal pollutants and pesticides), stress caused by temperature changes, low oxygen, starvation, pH, ammonia, nitrite, disease, health, therapeutics monitoring and nutrition. The crustacean hepatopancreas is assumed homologous to the mammalian liver and pancreas (Gibson and Barker, 1979) and is responsible for major metabolic events, including enzyme secretion, absorption and storage of nutrients, molting and vitellogenesis (Chanson and Spray, 1992). Several aminotransferases in different tissues and organs including the hepatopancreas of crustaceans have been studied, including AST and ALT in lobster Homarus americanus (Devereaux, 1986) [37], kynurenine aminotransferase in tiger prawn (Meunpol *et al*., 1998) [72], and D-alanine oxidase and D-aspartate oxidase in several crustacean species (D'Aniello and Giuditta, 1980) [35].

Lactate dehydrogenase (LDH) is also used as indicative criteria of exposure due to chemical stress and anaerobic capacity of tissue (Diamantino *et al*., 2001; Rendon-von Osten *et al*., 2005) [38, 85]. LDH is present in all tissues and normally associated with cellular metabolic action; it is used as potential marker for assessing the toxicity of a chemical (Agrahari *et al*., 2007). Any changes in protein and carbohydrate metabolism may cause change in LDH activity (Abston and Yarbrough, 1976). Chemical stress alters the normal LDH activity patterns (Diamantino *et al*., 2001) [38]. Elevated LDH activity in gills suggests that the aerobic catabolism of glycogen and glucose has shifted towards the formation of lactate, which may have adverse long-term effects on the organisms (Szegletes *et al*., 1995) [95]. Increased release of LDH into the medium may indicate damage in the integrity of cell membranes or heart muscle (Nemcsok *et al*., 1984) [79]. Changes in food availability strongly affect LDH activity in white muscle. However, LDH activity (and that of other metabolic enzymes) tends to remain constant in brain, independent of changes in environmental food quality or quantity (Yang and Somero, 1993; Kawall *et al*., 2002) [59, 108]. LDH is central to burst swimming performance because its activity allows for the continuance of energy production critical for muscle contraction during functional hypoxia. A decrease in LDH activity because of low food availability directly impacts swimming performance, causing a decline in the ability of an individual to escape from predators or capture prey. Conversely, brain LDH activity, while low, is conserved during starvation, presumably to allow the individual to survive until conditions are more ideal for active movement and growth. Thus, the measurement of alteration in the LDH activity in gill, liver and kidney can be used as a biomarker indicating stress.

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