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Litopenaeus vannamei (Boone) post-larval survival related to age, temperature, pH and ammonium concentration

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Abstract

Transport of post-larvae shrimp used in aquaculture is an important element of successful cultivation because of the potential for stress during stocking procedures. To find optimum transport conditions, several bioassays were performed in the laboratory to evaluate survival of whiteleg shrimp *Litopenaeus vannamei* 5–30-day-old postlarvae under conditions similar to those encountered during transport from the hatchery to nursery and shrimp ponds. Postlarvae were exposed for 4 h to different temperatures and pH levels ammonia concentrations. Survival was significantly reduced after a 4 h exposure to pH 9 and was inversely related to temperature with or without 7 mg L^{-1} of ammonia. The 15- and 20-day-old postlarvae had higher survival rates than other ages. The lowest survival occurred in alkali conditions (pH 9), with 7 mg L^{-1}ammonia at 30 and 32 °C. To assure optimal survival of postlarvae during transfer from the hatchery to the nursery and shrimp ponds, we recommend temperatures below 28 °C, pH no higher than 8, no ammonia and post-larval age at least 15 days.

Keywords: *Litopenaeus vannamei*, shrimp aquaculture, ammonium toxicity, environmental effects, post-larval transport

Introduction

Marine shrimp farming is an important aquaculture industry in the world with a production greater than 1 million metric tonnes in the year 2000 (Tacon 2003). The shrimp culture has expanded in the last two decades, providing economic benefits at national, regional, community and household levels (Neiland, Solely, Varley & Whitmarsh 2001). *Litopenaeus vannamei* (Boone) has become the primary cultured species in the Americas from USA to Brazil, including México over the past 20–25 years. Beginning in 1996, this specie was introduced into Asia on a commercial scale in Mainland China and Taiwan Province of China and subsequently spread to the Philippines, Indonesia, Vietnam, Thailand, Malaysia and India. Total production for 2002 in both regions has been estimated to be more than 500 000 metric tonnes, nearly 40% came from the American region (Briggs, Funge-Smith, Subasinghe and Phillips 2004).

One of the most important problems in shrimp farming is the toxicity of nitrogen waste products such as ammonia and nitrites (Losordo 1991). Ammonia is toxic in high concentrations, and is generated by excretion and ammonification of unconsumed feed, organic wastes and sediments (Chen, Ting, Lin & Lin 1990; Wright 1995; Zhao, Lam & Guo 1997). The situation is exacerbated in closed and intensive shrimp farming operations (Chen, Chin & Lee 1986; Chen, Liu & Lin 1988; Chen & Kou 1993), leading to reduced growth and survival that causes major economic losses.

Aquatic organisms mostly excrete ammonia (Campbell 1973), a compound highly soluble in water. Ammonia results from catabolism of proteins. Cellular and ingested proteins are hydrolysed to form a pool of amino acids that can be used to form new proteins for growth and basic protein turnover. Amino acids are de-aminated to form ammonia if they are not used to form new proteins (Wright 1995). In seawater, the total ammonia (ammonia-N) can be found in an ionized form such as ammonium ion (NH_4^+), and in a non-ionized form (NH_3-N). The latter is toxic and its concentration depend on ammonia-N, pH, salinity and temperature (Warren 1962; Trussell 1972; Skarheim 1973; Whitfield 1974; Emerson, Russo & Thurston 1975; Armstrong, Chipendale, Knight & Colt 1978; Bower & Bidwell 1978; Chen & Sheu 1990).

There are many published results concerning ammonia toxicity in fish, mollusks and crustaceans (Johnson 1985; Lewis Jr & Morris 1986; Chen Ting *et al*. 1990; Chen, Liu & Lei 1990; Chen & Lin 1991, 1992a–c; Noor-Hamid, Fortes-Romeo & Parado-Estepa 1994). At high concentrations, NH_3-N is toxic because it permeates cell membranes quite easily, affecting intracellular pH and transamination reactions (Colt & Armstrong 1981; Chen & Lei 1990; Hargreaves 1998). Some sublethal effects on marine organisms are modification of urea/ammonium excretion (Chen & Lin 1995) reduced ingestion (Frías-Espericueta, Harfush-Melendez & Páez-Osuna 2000), and reduced growth rate (Colt & Armstrong 1981; Allan, Maguire & Hopkins 1990; Chen & Lin 1992a, b; Noor-Hamid *et al*. 1994). High ammonia content affects the immune system of *Marsupenaeus japonicus* (Bate) (Jiang, Yu & Zhou 2004) and *L. vannamei* (Liu & Chen 2004). Reduced survival and growth because of sublethal and lethal effects of ammonia toxicity are relevant for aquaculture operations.

Resistance to ammonia toxicity varies with ontogenetic development. Larvae and post-larvae shrimp are more susceptible than adults (Chin & Chen 1987; Chen & Chin 1988; Lin, Thuet, Trilles, Mounet-Guillaume & Charmantier 1993; Zhao *et al*. 1997). This was also shown in studies of *L. vannamei* (Frías-Espericueta, Harfush-Melendez, Osuna-López & Páez-Osuna 1999; Chin & Chen 2001), but there have been few studies concerned with this effect in relation to immature shrimp stages in different farming environments.

From spring to summer in the Gulf of California, large quantities of post-larvae shrimp are moved from more than 30 hatcheries to about 300 shrimp farms having different environmental conditions. Although shrimp farmers know that environmental conditions during larval transport should be controlled, sometimes these conditions change in a time-dependent manner during transport, particularly ammonia-N concentration, temperature and pH. Excretion increases ammonia concentration, and respiration can lower seawater pH in the transport containers. At farm ponds, water temperature and pH can change every time the ponds are stocked, depending on season, latitude, photosynthesis rate and applications of hydrated lime. Increased alkalinity causes NH_3-N accumulation in the haemolymph (Chen & Kou 1991, 1993) and increased ammonia toxicity (Warren 1962; Skarheim 1973; Armstrong *et al*. 1978; Chen & Chin 1989; Lin *et al*. 1993; Noor-Hamid *et al*. 1994; Zhao *et al*. 1997). In this study, we attempt to establish the combination of environmental conditions that improves survival of *L. vannamei* postlarvae under short-term exposure to ammonia-N. To this end, we tested age, temperature, and pH factors to find an optimal set of conditions to reduce risks affecting survival during the stocking of shrimp ponds.

Materials and methods

Approximately 15 000 *L. vannamei* postlarvae 1-day-old (pl_1) obtained from a hatchery in La Paz, B.C.S., México (APSA, Acuacultores de la Península), were shipped to the laboratory at CIBNOR and acclimated for 5 days at 28 °C, pH 8.2 and salinity 38 g L^{-1} in a 1000 L tank containing filtered seawater. During acclimation, the specimens were fed a commercial diet (PIASA, 40% protein) and live *Artemia salina nauplii*. Every 5 days, post-larvae aged 5, 10, 15, 20 and 30 days (pl_5, pl_{10}, pl_{15}, pl_{20} and pl_{30}), in four temperature treatment groups of 640 specimens were placed under different sets of conditions. Each temperature group was acclimated at their respective temperature (26, 28, 30 and 32 °C) and further subdivided into 32 groups of 20 postlarvae for other bioassays. Two sets of four 60 L tanks A and B of filtered seawater were prepared (Fig. 1): The four tanks of set A were used for acclimation of postlarvae, each at its respective temperature. Those in set B were for exposure to stresses, each at the same temperature as the corresponding tank in Set A. Each tank in Set A contained an acrylic rack of 32 plastic 100 mL mesh-bottom containers, each with 20 postlarvae. Set B tanks had a similar acrylic rack with 32 plastic bottles of 100 mL of stress solution. These bottles were chosen to have a diameter somewhat larger than that of the containers in Set A. Alignment of the containers in the two sets of racks was identical, so that the containers in Set A could be easily and simultaneously inserted into the bottles of Set B. Eight stress solutions were

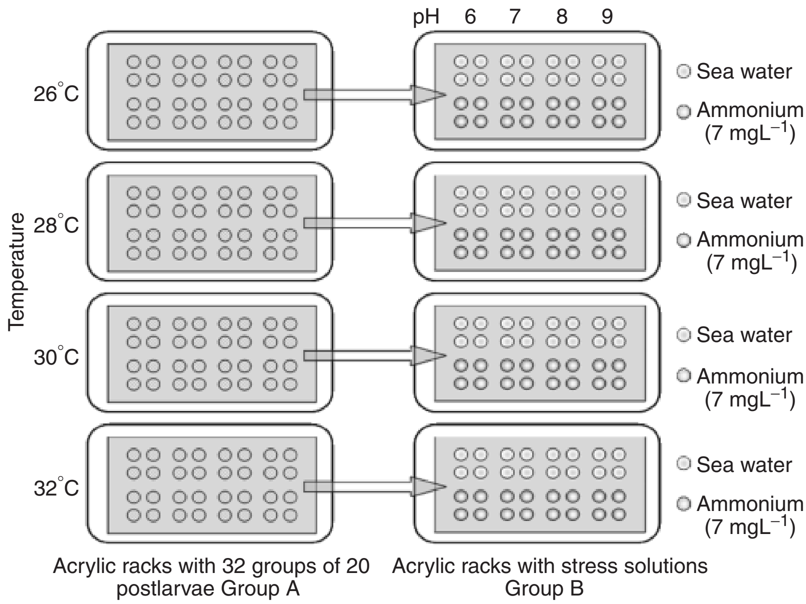

Figure 1 Bioassay module, comprising eight acclimation tanks at four temperatures (26, 28, 30 and 32 °C). Set A consists of four acrylic racks with 32 plastic mesh-bottom containers (100 mL), each with 20 postlarvae. Set B consists of four acrylic racks of 32 plastic bottles with 100 mL of solutions (four replicates with ammonia-N seawater solutions (0 and 7 mgL^{-1}) at four pH levels (6, 7, 8 or 9).

prepared with all combinations of four different pH values (6, 7, 8 or 9) with two concentrations of ammonia (0 or 7.0 mgL^{-1} ammonia-N). Each of the four racks of Set B was divided into eight sets of four replicates, each set for a different combination of pH and concentration of ammonia-N. The pH was stabilized with NaOH or HCl solution and a pH meter (Model 44, Beckman-Coulter, Fullerton, CA, USA).

When all stress conditions were properly adjusted, the four racks of Set A with the test specimens were put into the respective racks of Set B with the stress solutions, taking care to immerse all the postlarvae simultaneously. After exposure for 4 h, the four racks of Set A were returned to the Set A tanks and mortality in each bottle was recorded (Vega & De La Cruz 1988). Survival was calculated as proportions (prop) and then transformed to arcsen$\sqrt{\text{prop}}$ according to Daniel (1997). Transformed data were analysed by a two-way ANOVA and significance was set at $P \leq 0.001$. Homogeneous groups were identified according to the Tukey test at $\alpha = 0.05$.

Results

The results of the bioassay showed that survival of *L. vannamei* postlarvae after 4 h was affected by exposure to pH ($F_{(3, 576)} = 2836.74$, $P < 0.001$), ammonia level ($F_{(1, 576)} = 850.77$, $P < 0.001$), and the interaction between both factors ($F_{(3, 576)} = 788.97$, $P < 0.001$). According to the Tukey test, survival of *L. vannamei* postlarvae was significantly reduced in the interaction between ammonia-N (7 mg L^{-1} seawater) at pH 9, with a similar but minor effect on stresses solutions without ammonia at pH 9, and no effect at pH 6, 7 and 8, independent of ammonia level, temperature and age (Table 1).

In the stresses solutions without ammonia-N at pH 9, the increase in temperature from 26 to 32 °C decreased survival of postlarvae ($F_{(3, 72)} = 162.46$, $P < 0.001$), independent of age (Table 2). In the stresses solutions with 7 mg L^{-1} ammonia-N at pH 9, the increase in temperature in the same levels mentioned above also decreased survival of postlarvae ($F_{(3, 72)} = 103.36$, $P < 0.001$), independent of age (Table 3). The Tukey test showed differences among all the temperature levels in both cases.

The age of postlarvae at the time of exposure to stresses had a significant effect on their survival ($F_{(5, 72)} = 11.34$, $P < 0.001$) independent of temperature. The highest survival occurred in 15- and 20-day-old postlarvae according to the Tukey test (Table 3). It is noteworthy that 25–30-day-old postlarvae had reduced survival when the temperature was

Table 1 Survival (%) of *Litopenaeus vannamei* postlarvae exposed for 4 h to eight combinations of seawater solutions (with ammonia-N (7 mg L^{-1}) and no ammonia), at pH (6, 7, 8 or 9), each combination included survival data from four temperatures (26, 28, 30 and 32 °C) and different ages (pl_5, pl_{10}, pl_{15}, pl_{20} and pl_{30})

pH	No ammonia	Ammonia-N (7 mg L^{-1})	Average
6	100.0^a	100.0^a	100.0
7	100.0^a	100.0^a	100.0
8	100.0^a	99.6^a	99.8
9	89.7^b	48.4^c	69.1
Average	97.4	87.0	92.2

Letters denote homogeneous groups according to the Tukey test at $\alpha = 0.05$.

Table 2 Survival (%) of *Litopenaeus vannamei* postlarvae exposed for 4 h to seawater solutions at pH 9 (no ammonia added), at four temperatures (26, 28, 30 and 32 °C) and different ages (pl_5, pl_{10}, pl_{15}, pl_{20} and pl_{30}), each combination included survival data from four replicates

	Temperature (°C)				
Age (days)	26	28	30	32	Average
5	100.0	100.0	83.8	83.8	91.9^{aa}
10	100.0	100.0	90.0	83.8	93.4^{aa}
15	100.0	100.0	90.0	83.8	93.4^{aa}
20	100.0	90.0	82.5	88.8	90.3^{aa}
25	96.3	93.8	87.5	50.0	81.9^{bb}
30	100.0	98.8	87.5	63.8	87.5^{aa}
Average	99.4^a	97.1^b	86.9^c	75.6^d	89.7

Letters denote homogeneous groups according to the Tukey test at $\alpha = 0.05$.

Table 3 Survival (%) of *Litopenaeus vannamei* postlarvae exposed for 4 hr to sea water solutions at pH 9 (with ammonia-N (7 mg L^{-1}), at four temperatures (26, 28, 30 and 32 °C) and different ages (pl_5, pl_{10}, pl_{15}, pl_{20}, and pl_{30}), each combination included survival data from four replicates

	Temperature (°C)				
Age (days)	26	28	30	32	Average
5	83.8	28.8	25.0	10.0	36.9^{aa}
10	91.3	31.3	27.5	6.3	39.1^{aa}
15	93.8	62.5	56.3	23.8	59.1^{bb}
20	95.0	65.0	45.0	32.5	59.4^{bb}
25	62.5	61.3	25.0	8.8	39.4^{aa}
30	83.8	67.5	28.8	47.5	56.9^{bb}
Average	85.0^a	52.7^b	34.6^c	21.5^d	48.4

Letters denote homogeneous groups according to the Tukey test at $\alpha = 0.05$.

$\geq$ 30 °C, independent of the presence of ammonia (Tables 2 and 3).

Discussion

pH effect

Other species of shrimp show similar responses as found in our study. Armstrong *et al.* (1978) found an interaction between pH and ammonia toxicity in *Macrobrachium rosenbergii* (De Man). Straus, Robinette and Heinen (1991) showed that LC_{50} (NH_3-N) is reduced at high pH (alkali conditions) for postlarval and juvenile *M. rosenbergii*. Chen and Chin (1989) found that an increase in pH from 6.4 to 9.1 reduced LT_{50} and LT_{100} of *Penaeus monodon* (Fabricius) postlarvae (pl_{12}–pl_{15}) at two ammonia concentrations at 30 °C. At pH 9.1, 30 °C and 25 gL^{-1} salinity, 50% of the *P. monodon* postlarvae died within 13 h, in 0.07 mg L^{-1} ammonia-N seawater. Noor-Hamid *et al.* (1994) showed that an increase in pH from 7.0 to 8.5 (28 °C, 30 g L^{-1} salinity) significantly reduced the LT_{50} of *P. monodon* zoea, mysis and postlarvae. These authors stated that pH rangeing from 7.0 to 8.0 is safe for rearing larvae.

Increased concentrations of ammonia in seawater with alkaline conditions allow the accumulation of ammonia in shrimp hemolymph to above-normal levels (6–7 mg L^{-1} ammonia-N) in a 4 h period (Chen & Kou 1993). In their study, Chen and Kou stated that diffusion of NH_3-N is the most important form of ammonia excretion because the NH_3-N levels inside the body are generally greater than the levels found in the environment. However, when the pH of seawater increased NH_3–N concentration, diffusion of NH_3-N can be reversed, causing accumulation of ammonia in hemolymph, increasing ammonia toxicity. Our results support this conclusion, that is, with pH 9 and 7 mg L^{-1} ammonia seawater, survival rates declined.

Ammonia is a necessary and beneficial compound for life because it is the base for amino acids synthesis and fundamental for protein anabolism (Lehninger 1995), but exposure to > 1 mg L^{-1} NH_3-N concentrations in the environment for more than 12 h can be lethal for *L. vannamei* (Frías-Espericueta *et al.* 1999, 2000; Lin & Chen 2001). Even lower concentrations can be toxic for short-term exposure ($<$12 h) if seawater reaches alkaline pH, as shown in this study.

Effect of temperature and ammonia

Although there are few studies about the effect of temperature associated with ammonia toxicity, some studies showed that ammonia toxicity in penaeid postlarvae increases with temperature. Kir, Kumlu and Eroldoğan (2004) found that low temperature increased *Penaeus semisulcatus* (De Haan) juvenile tolerance to total ammonia-nitrogen and non-ionized ammonia when the temperature was reduced from 26 to 14 °C. Young-Lai, Charmantier-Daures and Charmantier (1991) found that LC_{50} (NH_3–N) increased from 3.25 to 5.12 mg L^{-1} over 96 h in adult American lobster *Homarus americanus* (H. Milne-Edwards) when the temperature was reduced from 20 to 5 °C. Chen, Tu and Yang (1989) showed that LC_{50} (NH_3-N) for kuruma shrimp *M. japonicus* postlarvae (pl_2), tolerance for 24 h at 30 °C was 1.94 mg L^{-1} and for 48 h it was 1.49 mg L^{-1}. Lin *et al*. (1993) found that LC_{50} (NH_3-N) of postlarvae (pl_1) of this species at 25 °C was 2.30 and 1.70 mg L^{-1}, for 24 and 48 h respectively. These results showed that LC_{50} was lower when temperature increased in the presence of ammonia. Several explanations have been suggested: (a) higher temperatures increase metabolic rate. In *L. vannamei*, the respiratory rate increased as temperature was increased from 25 to 30 °C (Martínez-Palacios, Ross & Jimenez-Valenzuela 1996); (b) increased ammonia concentration increases respiratory rate in *Fenneropenaeus chinensis* (Osbeck) (Chen & Nan 1993; Chen & Lin 1995) and *L. vannamei* (Racotta & Hernández-Herrera 2000); (c) increased temperature increases ammonia excretion in crustaceans (Regnault 1987) and (d) increased temperature increases the toxic form NH_3–N in water according to the equations of Bower and Bidwell (1978). According to our results, survival rates of *L. vannamei* postlarvae are affected during a 4 h exposure to combined temperature and pH above 28 °C and pH 9, and this effect is modulated by ammonia concentration.

Effects of post-larval age

Consistent with our results, others reported that LC_{50} (NH_3–N) increases with ontogenetic development and decreases with length of exposure to ammonia (Zhao *et al*. 1997). Chen *et al*. (1989) found an increase in the LC_{50} (NH_3–N) of *M. japonicus* postlarvae (pl_2–pl_{12}) with exposure for 12 and 24 h. Chen, Liu and Nan (1991) mention that the LC_{50} (NH_3–N) for 24 h exposure under the same experimental conditions (30 °C, pH 8.1, 36 g L^{-1} salinity) is reduced in *Metapenaeus ensis* (De Haan) postlarvae (pl_{10}) in contrast to postlarvae (pl_1), suggesting that, although an increase in age improves resistance to the toxic effect of ammonia, the effect also depends on the species.

Our results showed that there is a higher tolerance to ammonia under alkaline conditions at 28 °C when postlarvae are older than 15 days. However, when the temperature is higher (30–32 °C), no improvement in tolerance to ammonia occurs in postlarvae (pl_{20}). Again, this suggests that postlarvae at age 15–20 days are more resistant to changes in environmental conditions, such as temperature, pH and ammonia (Table 3). Hence, we conclude that postlarvae ($pl_{15–20}$) appear to be the best age group for surviving the transfer from hatcheries to farm ponds.

This finding agrees with other studies on the resistance of postlarvae to the effects of temperature and salinity. In the Sao Paulo shrimp *Farfantepenaeus paulensis* (Pérez-Farfante), postlarvae showed increased resistance to changes in salinity and temperature until they reached 18 days (Andreatta 1999). McGraw, Davis, Teichert-Coddington and Rouse (2002) reported that *L. vannamei* postlarvae less than 15 days old have higher mortality with a change in salinity than older postlarvae. Lin and Chen (2001) found the LC_{50} (NH_3-N) for a 96 h exposure progressively declines in *L. vannamei* juvenile when salinity is reduced from 35 to 15 g L^{-1}. The same effect was also reported in *Fenneropenaeus penicillatus* (Alcock) (Chen & Lin 1991) and *F. chinensis* (Chen & Lin 1992c).

Combined effect of pH, temperature, ammonia and age

The combination of alkaline conditions (pH > 9), high temperature (> 28 °C) and ammonia (7 mg L^{-1}) during a 4 h stress exposure affects the survival of *L. vannamei* postlarvae younger than 15 days old. This has implications for hatcheries, nurseries, stock transport and stocking of farm ponds for reducing mortality from ammonia toxicity.

There is a risk that rearing conditions of *L. vannamei* in hatcheries and nurseries with autotrophic cultures and heating equipment, even with control systems, will reach alkaline conditions and temperatures warmer than 28 °C. Improvement in temperature and pH control can help to avoid mortality from ammonia toxicity. Although there is a tendency to increase the age of postlarvae shipped to farm ponds, sometimes hatchery personnel move postlarvae younger than 15 days. Maintaining strict control of temperature, pH and ammonia during transfer can reduce the risk of mortality of postlarvae more than

15 days old. Ammonia concentration inside the containers during transport frequently reaches 7 mg L^{-1} or higher. In this case, survival in transport containers might not be affected if the production of CO_2 from postlarval respiration causes acidic pH. The situation changes when postlarvae are transferred to nursery tanks or ponds. In farm ponds, temperature and pH can be higher than in transport containers, depending on the season, time of day, tank productivity and use of hydrated lime. In this case the ammonia accumulated in the transport container must be removed before stocking, low temperatures maintained and pH controlled (<8) to reduce the risk of mortality during the transfer to grow-out ponds.

Transfers at night prevent alkalinity generated by photosynthesis during the day (Boyd 1990). Dissolved oxygen should be kept close to saturation because lower oxygen concentration increases the toxicity of ammonia, as reported for other peneid species (Allan *et al*. 1990; Wajsbrot, Gasith, Krom & Samocha 1990). In ponds treated with hydrated lime ($Ca(OH)_2$) before stocking, the deleterious effect of alkalinity is a considerable risk.

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References

Allan G.L., Maguire G.B. & Hopkins S.J. (1990) Acute toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved-oxygen levels. *Aquaculture* **91**, 265–280.

Andreatta E.R. (1999) *Repovoamento de Lagoas Costeiras em Santa Catarina: Producao de Pos-Larvas e Estimativa de recaptura do camarao rosa Farfantepenaeus paulensis (Decapoda, penaeidae)*. Doctoral thesis, Universidade Federal de Sao Carlos, Brazil, 148 pp.

Armstrong D.A., Chipendale D., Knight D.W. & Colt J.E. (1978) Interaction of ionized and un-ionized ammonia on short term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. *Biological Bulletin* **154**, 15–31.

Bower C.E. & Bidwell J.P. (1978) Ionization of ammonia in seawater: effects of temperature, pH and salinity. *Journal of the Fisheries Research Board of Canada* **35**, 1012–1016.

Boyd C.E. (1990) *Water quality in ponds for aquaculture*. Alabama Agricultural Experiment Station, Auburn University. Birmingham, AL, USA, 482 pp.

Briggs M., Funge-Smith S., Subasinghe R. & Phillips M. (2004) Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. FAO-RAP Publication 2004/10, 92pp.

Campbell J.W. (1973) Nitrogen excretion. *Comparative Animal Physiology.* W. B. Saunders, Philadelphia, pp. 279–316.

Chen J.C. & Chin T.S. (1988) Joint action of ammonia and nitrite on tiger prawn *Penaeus monodon* postlarvae. *Journal of the World Aquaculture Society* **19**, 143–148.

Chen J.C. & Chin T.S. (1989) Effect of Ammonia at different pH levels on *Penaeus monodon* postlarvae. *Asia Fisheries Science* **2**, 233–238.

Chen J.C. & Kou Y.Z. (1991) Accumulation of ammonia in the hemolymph of *Penaeus japonicus* exposed to ambiente ammonia. *Diseases of Aquatic Organisms* **11**, 187–191.

Chen J.C. & Kou Y.Z. (1993) Accumulation of ammonia in the haemolymph of *Penaeus monodon* exposed to ambient ammonia. *Aquaculture* **109**, 177–185.

Chen J.C. & Lei S.C. (1990) Toxicity of ammonia and nitrite to *Penaeus monodon* juveniles. *Journal of the World Aquaculture Society* **21**, 300–306.

Chen J.C. & Lin C.Y. (1991) Lethal effects of ammonia and nitrite on *Penaeus penicillatus* juveniles at two salinity levels. *Comparative Biochemistry and Physiology* **100**, 477–482.

Chen J.C. & Lin C.Y. (1992a) Effect of ammonia on growth of *Penaeus penicillatus* juveniles. *Comparative Biochemistry and Physiology C Pharmacology Toxicology and Endocrinology* **101**, 443–447.

Chen J.C. & Lin C.Y. (1992b) Effects of ammonia on growth and molting of *Penaeus monodon* juveniles. *Comparative Biochemistry and Physiology C Pharmacology Toxicology and Endocrinology* **101**, 449–452.

Chen J.C. & Lin C.Y. (1992c) Lethal effects of ammonia on *Penaeus chinensis* Osbeck juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* **156**, 139–148.

Chen J.C. & Lin C.Y. (1995) Responses of oxygen consumption, ammonia-N excretion and urea-N excretion of *Penaeus chinensis* exposed to ambient ammonia at different salinity and pH levels. *Aquaculture* **136**, 243–255.

Chen J.C. & Nan F.H. (1993) Effects of ammonia on oxygen consumption and ammonia-N excretion of *Penaeus chinensis* after prolonged exposure to ammonia. *Bulletin of Environmental Contamination and Toxicology* **51**, 122–129.

Chen J.C. & Sheu T.S. (1990) Effect of ammonia at different pH levels on larval *Penaeus monodon*. *Journal of the Fisheries Society of Taiwan* **17**, 59–64.

Chen J.C., Chin T.C. & Lee C.K. (1986) Effects of ammonia and nitrite on larval development of the shrimp (*Penaeus monodon*). In: *The First Asian Forum* (ed. by J.L. Maclean, L.B. Dizon & L.V. Hosillos). Asian Fisheries Society, Phillipines, pp. 657–662.

Chen J.C., Liu P.C. & Lin Y.T. (1988) Superintensive culture of red-tailed shrimp *Penaeus penicillatus*. *Journal of the World Aquaculture Society* **19**, 127–131.

Chen J.C., Tu C.C. & Yang W.S. (1989) Acute toxicity of ammonia to larval *Penaeus japonicus*. *Journal of the Fisheries Society of Taiwan* **16**, 261–270.

Chen J.C., Liu P.C. & Lei S.C. (1990) Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. *Aquaculture* **89**, 127–137.

Chen J.C., Ting Y.Y., Lin J.N. & Lin M.N. (1990) Lethal effects of ammonia and nitrite on *Penaeus chinensis* juveniles. *Marine Biology* **107**, 427–431.

Chen J.C., Liu P.C. & Nan F.H. (1991) Acute toxicity of ammonia to larval *Metapenaeus ensis*. *Asian Fisheries Science (Asian Fisheries Society, Manila Philippines)* **4**, 41–51.

Chin T.S. & Chen J.C. (1987) Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. *Aquaculture* **66**, 247–253.

Chin T.S. & Chen J.C. (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* **259**, 109–119.

Colt J.E. & Armstrong D.A. (1981) Nitrogen toxicity to crustaceans, fish and molluscs. In: *Proceedings of the Bio-Engineering Symposium for Fish Culture* (ed. by L.J. Allen & E.C. Kinney). American Fisheries Society, pp. 34–47.

Daniel W.W. (1997) *Bioestadística*. Base para el análisis de ciencias de la salud, Editorial Limusa. 399 pp.

Emerson K., Russo R.L. & Thurston R.V. (1975) Aqueous ammonia equilibrium calculations: effects of pH and temperature. *Journal of the Fisheries Research Board of Canada* **32**, 2379–2388.

Frías-Espericueta M.G., Harfush-Melendez M., Osuna-López J.I. & Páez-Osuna F. (1999) Acute toxicity of ammonia to juvenile *Penaeus vannamei* Boone. *Bulletin of Environmental Contamination and Toxicology* **62**, 646–652.

Frías-Espericueta M.G., Harfush-Melendez M. & Páez-Osuna F. (2000) Effects of ammonia on mortality and feeding of postlarvae shrimp *Litopenaeus vannamei*. *Bulletin of Environmental Contamination and Toxicology* **65**, 98–103.

Hargreaves J.A. (1998) Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture* **166**, 181–212.

Jiang G., Yu R. & Zhou M. (2004) Modulatory effects of ammonia-N on the immune system of *Penaeus japonicus* to virulence of white spot syndrome virus. *Aquaculture* **241**, 61–75.

Johnson E.L. (1985) Ambient water quality for ammonia – 1984., *Environmental Protection Agency*, EPA 440/5-85-001, USA.

Kir M., Kumlu M. & Eroldoğan O.T. (2004) Effects of temperature on acute toxicity of ammonia to *Penaeus semisulcatus* juveniles. *Aquaculture* **241**, 479–489.

Lehninger A.L. (1995) Bioquímica. Ediciones Omega, Barcelona, España. pp 735.

Lewis Jr. W.M. & Morris D.P. (1986) Toxicity of nitrite to fish: a review. *Transactions of the American Fisheries Society* **115**, 183–195.

Lin Y.C. & Chen J.C. (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. *Journal of Experimental Marine Biology and Ecology* **259**, 109–119.

Lin H.P., Thuet P., Trilles J.P., Mounet-Guillaume R. & Charmantier G. (1993) Effects of ammonia on survival and osmoregulation of various development stages of the shrimp *Penaeus japonicus*. *Marine Biology* **117**, 591–598.

Liu C.H. & Chen J.C. (2004) Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to Vibrio alginolyticus. *Fish & Shellfish Immunology* **16**, 321–334.

Losordo T.M. (1991) Engineering considerations in closed recirculating systems. In. Aquaculture Systems Enginnering. *Proceedings of the World aquaculture Society and the American Society of Agricultural Engineers*, WAS 22nd Annual Meeting, 16–20 June 1991, San Juan, Puerto Rico. pp. 58–69.

Martínez-Palacios C., Ross L.G. & Jimenez-Valenzuela L. (1996) The effects of temperature and body weight on the oxygen consumption of *Penaeus vannamei*, Boone, 1931. *Journal of Aquaculture in the Tropics* **11**, 59–65.

McGraw W.J., Davis D.A., Teichert-Coddington D. & Rouse D.B. (2002) Acclimation of *Litopenaeus vannamei* Postlarvae to low salinity: influence of age, salinity endpoint, and rate of salinity reduction. *Journal of the World Aquaculture Society* **33**, 78–84.

Neiland A.E., Soley N., Varley J.B. & Whitmarsh D.J. (2001) Shrimp aquaculture: economic perspectives for policy development. *Marine Policy* **25**, 265–279.

Noor-Hamid S., Fortes Romeo D. & Parado-Estepa F. (1994) Effect of pH and ammonia on survival and growth of the early larval stages of *Penaeus monodon Fabricius*. *Aquaculture* **125**, 67–72.

Racotta I.S. & Hernández-Herrera R. (2000) Metabolic responses of the white shrimp. *Penaeus vannamei*, to ambient ammonia. *Comparative Biochemistry and Physiology Part A* **125**, 437–443.

Regnault M. (1987) Nitrogen excretion in marine and fresh water crustacea. *Biological reviews of the Cambridge Philosophical Society* **62**, 1–24.

Skarheim H.P. (1973) Tables of the fraction of ammonia in the undissociated form for pH 6 to 9, temperature 0 to 30 °C. TDS 0 to 3.000 mg/l and salinity 5 to 35 g/kg. Sanitary Engineering Research Laboratory, University of California, Berkeley, Report 75-5, 33pp.

Straus D.L., Robinette H.R. & Heinen J.M. (1991) Toxicity of un-ionized ammonia and high pH to post-larval and juvenile freshwater shrimp *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society* **22**, 128–133.

Tacon A.J. (2003) Revisión del estado mundial de la acuicultura. Análisis de las Tendencias de Producción de la Acuicultura. *FAO Circular de Pesca (No. 886, Rev.)* **2**, 5–41.

Trussell R.P. (1972) The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. *Journal of the Fisheries Research Board of Canada* **29**, 1505–1507.

Vega A.J. & De La Cruz S.A. (1988) Efecto de la temperatura, la salinidad y el pH sobre las larvas de camarón blanco *Penaeus schmitti*. *Revista de Investigaciones Marinas (Cuba)* **9**, 125–132.

Wajsbrot N., Gasith A., Krom M.D. & Samocha T.M. (1990) Effect of dissolved oxygen and the molt stage on the acute toxicity of ammonia to juvenile green tiger prawn *Penaeus semisulcatus*. *Environmental Toxicology and Chemistry* **1**, 497–504.

Warren K.S. (1962) Ammonia toxicity and pH. *Nature* **195**, 47–49.

Whitfield M. (1974) The hydrolysis of ammonium ions in sea water – a theoretical study. *Journal of the Marine Biological Association of the United Kingdom* **54**, 565–580.

Wright P.A. (1995) Nitrogen excretion: three end products, many physiological roles. Review. *The Journal of Experimental Biology* **198**, 273–281.

Young-Lai W.W., Charmantier-Daures M. & Charmantier G. (1991) Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. *Marine Biology* **110**, 293–300.

Zhao J.H., Lam T.J. & Guo J.Y. (1997) Acute toxicity of ammonia to the early stage-larvae and juveniles of *Eriocheir sinensis* H. Milne-Edwards, 1853 (Decapoda:Grapsidae) reared in the laboratory. *Aquaculture Research* **28**, 517–525.