Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 274 (2008) 398–407
Aquaculture
www.elsevier.com/locate/aqua-online

Combined effect of exposure to ammonia and hypoxia on the blue shrimp *Litopenaeus stylirostris* survival and physiological response in relation to molt stage

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Received 14 October 2006; received in revised form 20 November 2007; accepted 20 November 2007

Abstract

The effect of ambient ammonia, hypoxia and combination of both on survival and the physiological and immunological response of the blue shrimp *Litopenaeus stylirostris* in relation to molt stage was studied. Shrimp were submitted to 44.0-71.5 mg l^{-1} total ammonia-N corresponding to 2.0 mg l^{-1} unionized ammonia NH_3-N and/or to 1.5 mg O_2 l^{-1} (4.3 kPa) for 24 hours. Survival was recorded and the molt stages of both dead and surviving shrimp determined. Only shrimp in intermolt and premolt stages were sampled for analysis of haemolymph. Haemolymph was assayed for osmoregulatory capacity (OC), magnesium ion (Mg^{2+}), calcium ion (Ca^{2+}), total proteins, oxyhaemocyanin, lactate, glucose and total haemocyte count (THC).

Low mortalities were recorded for shrimp submitted independently to ammonia or hypoxia. Seventy five percent of dead shrimp were in early post molt (stage A) in ammonia treatment, while hypoxia affected mainly late premolt animals (stage D_2). A synergic effect of ammonia and hypoxia combination ($A+O_2$ treatment) on mortality was observed, affecting nearly exclusively shrimp in late premolt stage D_2.

Analysis of molt stage repartition at the end of the experiment suggests that ammonia treatment may have accelerated molting.

The common physiological response of shrimp to the different treatments was characterized by a reduced OC and an increase in Ca^{2+}. Increase in Mg^{2+} could not be validated by the statistical analysis, as well as glycaemia variations. Plasmatic lactate level increased and THC decreased in shrimp submitted to hypoxia and the combination of hypoxia and ammonia. Total proteins concentration was reduced in ammonia and $A+O_2$ treatments. The effect was more pronounced in late premolt shrimp than in intermolt shrimp. Combination of ammonia and hypoxia led to a physiological response stronger than this observed for ammonia-alone and/or hypoxia-alone treatments, except for oxyhaemocyanin.

The effects of each external factor (ammonia, hypoxia) and the combination of both, and internal one (molt stage) are discussed.

Keywords: Ammonia; Haemolymph constituents; Hypoxia; *Litopenaeus stylirostris*; Molt stage; Penaeid shrimp; Stress; Survival

1. Introduction

Farming of shrimp in semi-intensive culture system may be subjected to environmental variations such as fluctuations of dissolved oxygen, temperature, or accumulation of potentially toxic compounds like ammonia or nitrite (Chien, 1992). Variations of environmental factors are not necessarily independent of each other, and combination of several factors which may be stressful for shrimp could be encountered at the same time in a pond. A consequence of shrimp being stressed may be a decrease in immune defence and an increased susceptibility to pathogens (Le Moullac and Haffner, 2000; Horowitz and Horowitz, 2001).

L. stylirostris is the only species cultivated in New Caledonia. It is the second exported product of the country, but its culture is still a developing industry. Those shrimp benefit an almost virus-free status but are commonly exposed to seasonal mortalities due to vibriosis either during the cold or the warm seasons (Costa et al., 1998; Goarant et al., 2006). Variations in environmental factors seem to have an impact on the mortalities observed (Mermoud et al., 1998).

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doi:10.1016/j.aquaculture.2007.11.033

In New Caledonia, several farms do not use aerators in their ponds. In these conditions, dissolved oxygen may reach critical values, in particular in the last part of grow-out, and shrimp can be exposed to short-term hypoxia (oxygen level from 3 mg l^{-1} to less than 1 mg l^{-1}). It has been shown that short-term decreases of DO concentration can have a negative effect on shrimp osmoregulation (Charmantier et al., 1994; Mugnier and Soyez, 2005) and immune system (Direkbusarakom and Danayadol, 1998; Le Moullac et al., 1998; Le Moullac and Haffner, 2000; Mikulski et al., 2000; Cheng et al., 2002; Burgents et al., 2005; Jiang et al., 2005), and may decrease resistance to diseases (Mikulski et al., 2000; Cheng et al., 2002). Moreover, ammonia-N, which is the principal end-product of nitrogenous compounds, accumulates in ponds and has a deleterious effect on fish and crustaceans (Colt and Armstrong, 1981). Of the ammonia species, the unionised form NH_3-N, which is pH-, temperature- and salinity- dependent, is the most toxic to aquatic life. The effects of ammonia-N on shrimp or other decapods physiological response or immune resistance are relatively well documented (Wajsbrot et al., 1990; Young-Lai et al., 1991; Chen and Cheng, 1993a; Lin et al., 1993; Schmitt and Uglow, 1997; Racotta and Hernandez-Herrera, 2000; Harris and al., 2001; Cheng and Chen, 2002; Cheng et al., 2003a; Jiang et al., 2004; Liu and Chen, 2004), including for *L. stylirostris* (Mugnier and Justou, 2004).

The present work aimed at studying the survival and physiological/immunological response of juvenile *L. stylirostris* to sublethal levels of ammonia NH_3-N and hypoxia, either separately or combined, in relation to molt stage. Few works have been carried out so far on the short-term effects of the combination of two or more stressors on shrimp, mainly on survival (Allan et al., 1990; Wajsbrot et al., 1990; Chen and Lin, 1992b; Chen et al., 1996; Martinez et al., 1998; Parado-Estepa, 1998; Lin and Chen, 2001; Kir et al., 2004; Zhang et al., 2006), and very few on physiological effects (Mugnier and Soyez, 2005; Li et al., 2006). In the present study, survival was considered in relation to the molt stage, and physiological response was studied in intermolt and late premolt shrimp. Premolt animals are more sensitive to stress than intermolt (Wajsbrot et al., 1990) and less resistant to experimental infection with pathogenic *Vibrio* than intermolt animals (Le Moullac et al., 1997; Cheng et al., 2003b; Liu et al., 2004).

Some physiological indicators measured in the haemolymph and known to be indicative of a stress response were selected for this study. In seawater, penaeid shrimp hypo-osmoregulate, and variation of osmoregulatory capacity (OC), which is the difference in osmotic concentration between haemolymph and surrounding water, was studied. It is a non-specific indicator commonly used for detecting physiological stress, including in *L. stylirostris* (Lignot et al., 2000). Previous work have shown that total proteins concentration, which can serve as a significant source of metabolic energy for crustaceans (Claybrook 1983), decreased under ammonia stress (Chen et al., 1993, Chen and Cheng 1993a; Mugnier and Justou, 2004). A possible change in oxyhaemocyanin concentration under hypoxia can be expected, as it is the main protein in the haemolymph and is implied in several functions like oxygen transport, enzymatic activities, osmoregulation or buffering (Paul and Pirow, 1997/98).

If anaerobic metabolism occurs, such as under hypoxic conditions, lactate formation and its increase in the haemolymph could be expected (McMahon, 2001; Racotta et al., 2002). At the opposite, a decrease was observed in *L. stylirostris* submitted to ammonia (Mugnier and Justou, 2004). Mg^{2+}, which plays an important role as a co-factor in enzyme systems and as a modulator of the hemocyanin of crustacean arthropods (Morritt and Spicer, 1993), increases in shrimp haemolymph under different stress conditions (Boglio, 1995; Mugnier and Justou, 2004). Ca^{2+} is implicated in haemolymph buffering, and hypoxia may induce an increase of Ca^{2+} in haemolymph (Hagerman and Uglow, 1982, McMahon, 2001). Variations of glycaemia have been observed under several different environmental and physiological conditions, including hypoxia (Hagerman et al., 1990; Hall and van Ham, 1998; Schmitt and Uglow, 1998; Racotta et al., 2002) and ammonia (Racotta and Hernandez-Herrera, 2000; Mugnier and Justou, 2004). In addition, the number of total haemocyte in the haemolymph, considered as an immunological indicator (Rodriguez and Le Moullac, 2000) was measured, haemocytes being involved in most of the immune mechanisms in crustacean (Johansson et al., 2000).

2. Material and methods

2.1. Experimental animals (Table 1)

The experiment was conducted on three occasions -referred as experiments E1 to E3- in the aquaculture facilities of IFREMER in New Caledonia, on the same population of juvenile (average weight 6.3±0.1 g, N=744) *L. stylirostris* reared in an earthen pond. For each experiment, shrimp were transported to eight 200 l indoor tanks (29-32 shrimp per tank) with aerated sea water. Mean temperatures and salinities are reported in Table 1. They were acclimated at least for 5 days before the experiment started (Soyez, 1997) and were fed commercial pellets. Shrimp were not fed 12 h before and during the experiment and water renewal was stopped during the experiment.

2.2. Moult stage determination

Six moult stages were defined according to the retraction of the epithelium within setae of the antennal scale (Drach, 1939; Chan et al., 1988). Shrimp were classified as A and B respectively for the early and late post molt stages, C for intermolt and D_0, D_1, D_2 for premolt stages. D_2 was the late premolt stage prior to ecdysis, when epidermis is at maximal retraction and it is possible to distinguish the developing seta.

2.3. Experimental procedure

Experimental conditions of temperature, salinity, osmotic pressure and pH are reported in Table 1.

Table 1
Experimental conditions, density, acclimation and weight of shrimp in the experiments E1 to E3

	E1	E2	E3
Temperature (°C)	24.8±0.5	25.9±0.2	26.1±0.5
Salinity (‰)	30.1	31.9	32.4
Osmotic Pressure (mOsm kg^{-1})	971	1025	1055
pH	7.74±0.21	7.91±0.06	7.82±0.31
Shrimp/tank	29	32	32
Acclimation period (days)	5	7	6
Mean weight (g)	5.6±0.2	6.6±0.2	6.6±0.1