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Acute Toxicity of Sodium Metabisulphite in Larvae and Post-Larvae of the Land Crab, *Cardisoma guanhumi*

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Abstract Sodium metabisulphite (SMB) is used in marine shrimp aquaculture to prevent the occurrence of black spot. The release SMB into the estuarine environment from shrimp farm pond effluents has been reported. This study evaluated the susceptibility of larvae and post-larvae of land crab, *Cardisoma guanhumi* to this salt. A decrease in dissolved oxygen and pH occurred with increasing concentration of SMB and exposure time. LC_{50} values after 48 h of exposure were 34 ± 1.1 mg/L, 31.1 ± 1.9 mg/L, and 30.6 ± 0.5 mg/L for I zoea larvae, megalopa larvae and stage I juveniles, respectively.

Keywords Sodium metabisulfite · Blue land crab · Brachyura · LC_{50} · Neotropical species

Marine shrimp farming has grown into one of the largest and most import aquaculture activities in Brazil (Rodrigues 2005; FAO 2006). However, a negative environmental impact, affecting important mangrove areas, estuary and bays, is associated with this activity (Macintosh and Phillips 1992; Phillips et al. 1993; Primavera 1994; Clay 1997). One of the main problems associated with shrimp farms is the discharge of sodium metabisulphite (SMB) from farm ponds (Barbiere Jr and Ostrensky 2002). This salt is largely used to avoid melanosis, also called black spot, in marine shrimp. It also inhibits the proliferation of microorganisms, an attribute that, together with its antioxidant property, validates the wide use of sodium metabisulphite by the food industry (Jamieson et al. 1985; Carvalho et al. 2011). Although the American Agency of Food and Drugs (FDA) has recommended 6.25 kg of SMB to 500 L water (12.5 g/L), shrimp farms in Brazil have largely ignored this guideline under the excuse that the recommended concentration is not enough to inhibit melanosis. The actual concentrations used by farmers ranges from 25 to 50 kg metabisulphite for 500 L water (50 g a 100 g/L) (Valença and Mendes 2004). The SMB reacts with the oxygen dissolved in water, releasing the gas sulphur dioxide (SO_2). This combines readily with water, resulting in acidic substances such as sulfuric acid (H_2SO_4), sulfurous acid (H_2SO_3) (Nickelson and Cox 1977; Fazio and Warner 1990; Atkinson et al. 1993) and sodium acid sulfate ($NaHSO_4$) (Ogawa et al. 1983, 1985). The release of SMB in water bodies without previous treatment may result in the deaths of estuarine animals, mainly fish and crabs, causing trophic alterations in the ecosystem (Silva 1988; Macintosh and Phillips 1992; Figueiredo et al. 2006).

The land crab, *C. guanhumi* Latreille, 1828, is an important resource in the Brazilian coastal area. It is exploited by populations living in the region due its great economic value (Taissoun 1974; Wolcott 1988). This crab inhabits areas adjacent to mangroves, preferably above the upper inundation thresholds (Taissoun 1974). They depend on the coastal water environment for larval release (Hartnoll 1981). Ovigerous females migrate to the margin of the estuarine channels to release their larvae, which are dispersed to the open sea during the ebb tide (Shanks et al. 2002). In the open sea, the zoeal larvae pass through five

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stages of development before they undergo metamorphosis into megalopa. In the latter phase, they are able to settle and metamorphose into the post-larval juvenile I stage, which represents the early life of a benthic crab in estuaries. Subsequently, crabs migrate to adjacent areas of the estuary, above the high tide level (Costlow Jr and Bookhout 1968; Hadfield 1986; Caley et al. 1996; Anger 2001). Due to the dependency of the larval and post-larval life-stages of *C. guanhumi* on the estuarine environment, it is important to evaluate the sensitivity of these early life-stages to potential environmental stressors. Data are lacking on the effects of SMB on the land crab. The present study was conducted to determine the sensitivity of, larvae (zoea I and megalopa) and post-larvae (juvenile I) of the land crab *C. guanhumi* to SMB.

Materials and Methods

Three egg-bearing *C. guanhumi* females were collected from an estuarine creek close to the city of Caravelas, Bahia, northeastern Brazil. They were placed in tanks (with aeration) covered with black plastic material filled with marine water (30 ppt and pH 7.7 ± 1). The water employed in this study was collected from the abrolhos channel (17°42′495″ S, 039°00′002″ W), Caravelas, Bahia, Brazil. After collection, the water was directed to the Laboratory of CEPENE-IBAMA (Brazilian governmental bureau), mechanically filtered (10 μm) and then placed in a 200 L constantly aerated plastic container equipped with a biological filter.

Approximately 1,000 zoea were collected and transferred to plastics beakers (300 mL) filled with marine water. They were kept for 24 h without food and subsequently used in toxicity tests. For the megalopa experiment, about 500 zoea I larvae were transferred to plastics beakers (300 mL) filled with marine water, and reared until metamorphosis into the megalopa stage. They were fed newly hatched *Artemia nauplii*. When individuals reached the megalopa stage, they were individualized to prevent cannibalism, acclimated for 24 h without food, and subsequently used in toxicity tests. In order to obtain juveniles I, some other megalopae (about 500 larvae) were reared**.** This group of individuals was fed *A. nauplii*. Once they reached the juvenile stage, individuals were acclimated for 24 h without food and subjected to the toxicity treatments. In order to evaluate the toxicological effects of SMB on larvae and post larvae, we determined the acute toxicity test measure of the 48 h $CL_{50\ldots 48h}$ (the lethal concentration to 50 % of the organisms exposed for a period of 48 h) from the IBAMA's manual (1987). Concurrently, following the same methodology described above, we carried out the standard test using KCl (potassium chloride) as the reference substance to assess the quality of the set of organisms used in the experimental tests and validate the results.

After the acclimatization period, organisms were subjected to preliminary screening test using ample variations in SMB concentrations. The period of exposure for this experiment was 48 h. With this test we aimed to determine (1) the highest concentration of SMB that would resulting in no mortality; (2) the lowest concentration incurring in total mortality of organisms in the zoea I, megalopa and juvenile I stage. The results of the test were used to design the definitive test. For zoea I, the acclimatization period lasted 24 h. After that, 10 larvae were transferred into plastic containers (250 mL) filled with sea water (30 ppt). SMB at 6 different concentrations and a control treatment ($Na_2S_2O_5$, 0.0 mg/L) were set up, using five replicates per treatment. The containers were numbered in accordance with their concentrations. Salinity, dissolved oxygen, temperature and pH were recorded. For megalopa and juveniles I, the acclimatization lasted 48 h. Then, five individuals of each stage (megalopa and juvenile I) were subjected to six different concentrations of SMB and a control treatment ($Na_2S_2O_5$, 0.0 mg/L). Salinity, dissolved oxygen (DO), temperature and pH were recorded. The concentrations of SMB used in the preliminary tests were as follows: 100, 50, 25, 12.5, 6.25, 3.12 and 0.0 mg/L. Simultaneously, a preliminary test using potassium chloride (KCl) as the reference substance was conducted at concentrations of 400, 200, 100, 50, 25, 12.5 and 0.0 mg/L. Such experiment followed the same procedure as the SMB tests.

In the final test, after 24 h of acclimatization, zoea larvae I were subjected to nine different concentrations of SMB (12.5, 17.1, 21.8, 26.5, 31.2, 35.9, 40.6, 45.3 and 50.0 mg/L) and a control concentration ($Na_2S_2O_5$, 0.0 mg/L). Ten larvae per container were used at each test concentration, in five replicates. When testing the megalopa and juvenile I stages, the organisms were subjected to nine different concentrations of SMB (concentration range obtained from screening), plus the control solution ($Na_2S_2O_5$, 0.0 mg/L), after they had been acclimated for 48 h. Only one individual was placed in each container for the megalopa and juvenile I tests, to avoid cannibalism. Five larvae (5 replicates) and a control ($Na_2S_2O_5$, 0.0 mg/L) were used in each treatment. Individual mortality was recorded every 12 h until the end of experiment (48 h). The definitive test with potassium chloride (KCl) was carried out following the same methodology described for the definitive test with SMB, with concentrations of: 0.0, 100, 112.5, 125, 137.5, 150, 162.5, 175, 187.5, 200 mg/L.

For zoea I, megalopa and juvenile I exposed to SMB for 48 h, the analysis of the LC_{50} values were calculated by the trimmed Spearman–Karbe method (Hamilton et al. 1977).

The straight lines equation in Excel 2003 was applied to analyze the chemical parameters of DO and pH at the different SMB test concentrations.

Results and Discussion

The analysis of the chemical data for the SMB tests on larvae (zoea and megalopa) and post-larvae (juvenile I) showed a significant variation in dissolved oxygen (DO) and pH (Fig. 1). The ranges for DO and pH in the control tanks were 6.5–6.7 and 7.73–7.77, respectively. This relationship between DO and pH, and concentration, is due to the reductive action of the SMB. Upon reacting with the OD in the solution it promotes a reduction, releasing SO_2 (sulfur dioxide) (Ogawa et al. 1983, 1985; Silva 1988; Meng and Zhang 1990). SO_2 combines easily with water, causing another chemical reaction that results in the production acidic by-products such as sodium acid sulfate (Ogawa et al. 1995; Paiva 2004; Aragão et al. 2008; Carvalho et al. 2011), sulfuric acid (H_2SO_4) and sulfuric acid (H_2SO_3) (Nickelson and Cox 1977; Fazio and Warner 1990; Atkinson et al. 1993). The decreases in the DO and pH corresponded to increases in the concentrations of SMB. The lowest values were observed in the group that had the highest concentration of SMB ($Na_2S_2O_5$, 50 mg/L) (Fig. 2). The salinity and temperature varied only slightly in the tests, averaging at 30 ± 1°C and 27° ± 1°C, respectively.

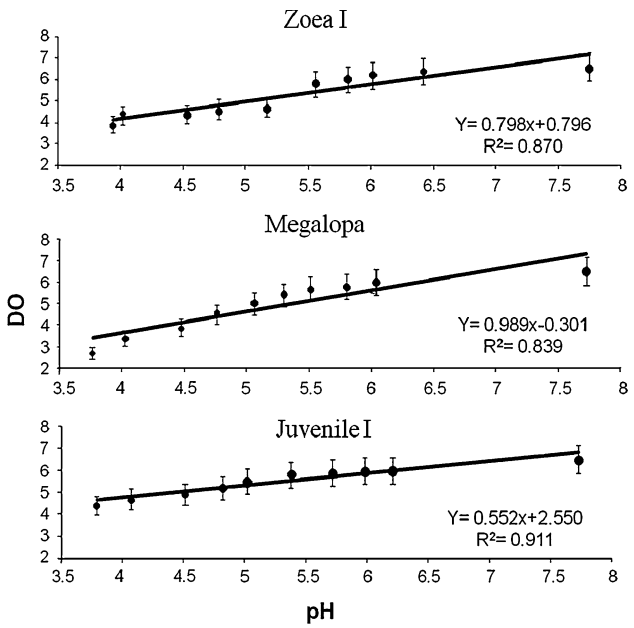

Fig. 1 Relationship between the dissolved oxygen (DO) and pH for zoea I, megalopa and juvenile I at the end of experiment (48 h)

The dissolved oxygen (DO) after 48 h ranged from 6.6 ($Na_2S_2O_5$, 0.0 mg/L) to 3.9 ($Na_2S_2O_5$, 50 mg/L) for zoea I ($y = 0.798x + 0.976$; $R^2 = 0.870$), 6.5 ($Na_2S_2O_5$, 0.0 mg/L) to 2.7 ($Na_2S_2O_5$, 50 mg/L) for megalopa ($y = 0.989x - 0.301$; $R^2 = 0.839$) and 6.5 ($Na_2S_2O_5$, 0.0 mg/L) to 4.4 ($Na_2S_2O_5$, 50 mg/L) for juvenile I ($y = 0552x + 2.550$; $R^2 = 0.911$) (Fig. 2). In the control groups ($Na_2S_2O_5$, 0.0 mg/L), few changes were observed in the DO at the end of 48 h, which ranged from 6.7 to 6.6 mg/L, 6.6 to 6.5 mg/L and from 6.7 to 6.5 mg/L for zoea I, megalopa and juvenile I organisms, respectively (Fig. 2).

At the end of 48 h a considerable decrease in the pH was observed. It ranged from 7.75 ($Na_2S_2O_5$, 0.0 mg/L) to 3.94 mg/L ($Na_2S_2O_5$, 50 mg/L) for zoea I ($y = -0.074x + 7.494$; $R^2 = 0.985$), 7.73 ($Na_2S_2O_5$, 0.0 mg/L) to 3.76 ($Na_2S_2O_5$, 50 mg/L) for megalopa ($y = -0.071x + 7.260$; $R^2 = 0.961$) and 7.73 ($Na_2S_2O_5$, 0.0 mg/L) to 3.79 ($Na_2S_2O_5$, 50 mg/L) for juvenile I ($y = -0.073x + 7.385$; $R^2 = 0.979$) (Fig. 2). Few variations in pH were observed in the control groups ($Na_2S_2O_5$, 0.0 mg/L) where ranges were 7.77–7.75 (zoea I), 7.74–7.73 (megalopa), and 7.74–7.73 (juvenile I) (Fig. 2).

The definitive test with KCl resulted in 48 h LC_{50} values of 138.9 mg/L (CI = 131.0–147.2 mg/L) for zoea I larvae, 137.5 mg/L (CI = 130.3–147.2 mg/L) for megalopa larvae, and 135.3 mg/L (CI = 123.1–147.1 mg/L) for juveniles I animals. Because the data presented here are new with respect to employing these organisms in acute toxicity tests, our results for the KCL as a reference substance should be tested in future studies in order to validate the set of test organisms.

The larval and juvenile life-stages were quite similar in their sensitivities to SMB. LC_{50} values after 48 h of exposure were 34.1 ± 1.1 mg/L (CI = 30.4–38.3 mg/L), 31.1 ± 1.9 mg/L (CI = 26.4–36.7 mg/L), and 30.6 ± 0.5 mg/L (CI = 26.4–35.5 mg/L) for zoea I larvae, megalopa larvae, and juvenile I animals, respectively (Fig. 3).

At the end of the experiment (48 h), total mortality (100 %) was observed for both larval stages (zoea I and megalopa) and for the post-larval juvenile I stage at the highest concentration (50 mg/L of SMB). In tests with megalopa and juvenile I animals, 100 % mortality was observed after 24 h of exposure to a solution of 50 mg/L of SMB. Exposure of juvenile I animals to a concentration of 45.3 mg/L SMB resulted in total mortality after 36 h of exposure. This did not happen when other stages were tested, and indicated that the juvenile I life-stage had greater sensitivity to the toxic effects of the contaminant. In the two lowest concentrations of SMB (12.5 and 17.1 mg/L) no mortality was observed, even after 48 h, demonstrating that it had no immediate effect on the larvae and post-larvae tested (Fig. 4). These data, despite differences between developmental stages, are compatible with the

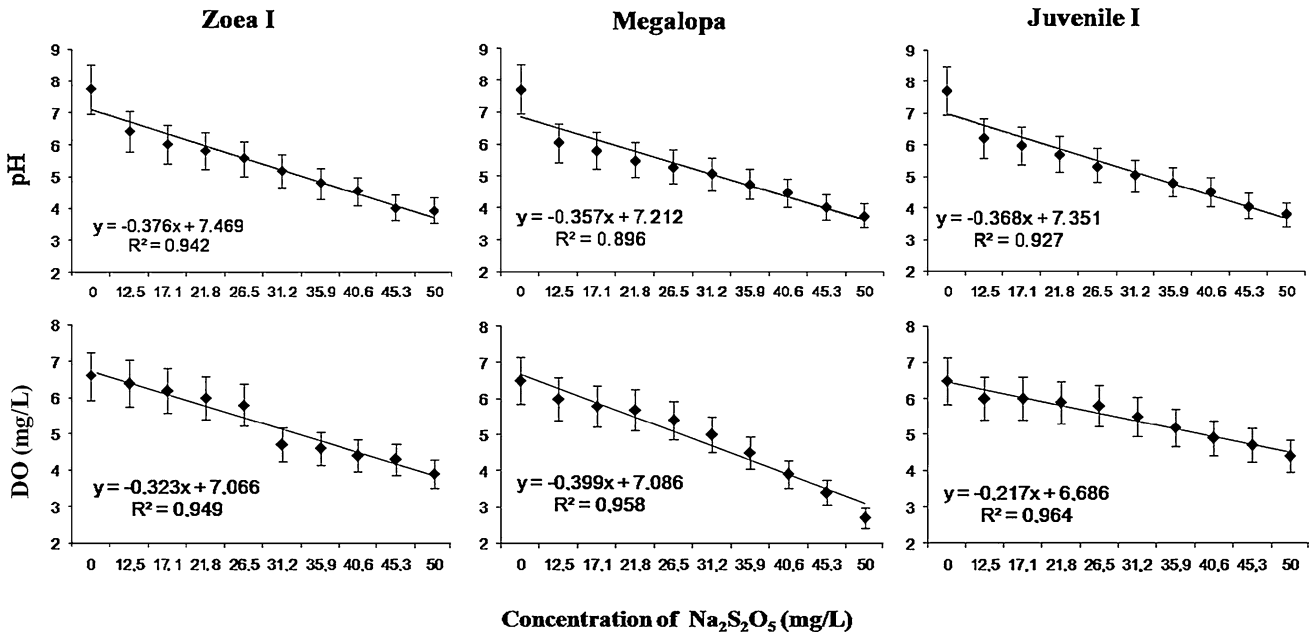

Fig. 2 Relation between dissolved oxygen (DO) and pH with sodium metabisulphite (SMB) concentrations in the end of the experiment (48 h)

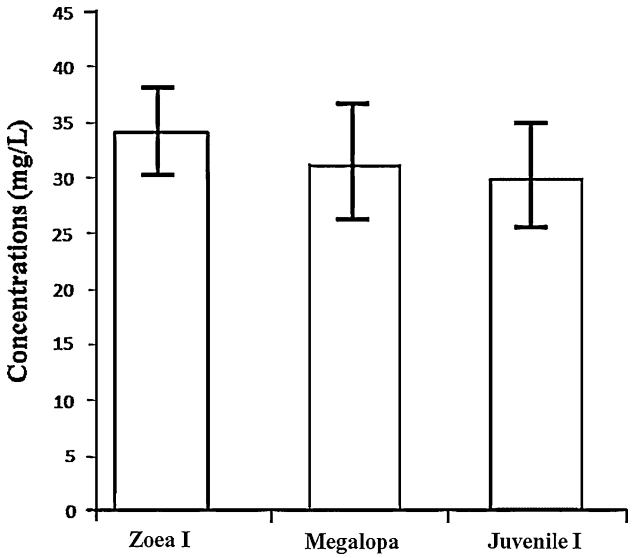

Fig. 3 $LC_{50...48\ h}$ for the different stages of development of *C. guanhumi* when exposed to the SMB, with their confiance interval (CI) of 95 % upper (Zoea I, 38.3; Megalopa, 36.7 and Juvenile I, 35.5) lower (Zoea I, 30.4; Megalopa, 26.4 and Juvenile I, 26.4)

idea that the mortality rate of organisms is dependent on the duration of exposure, as well as toxicant concentration.

One result of the experimental tests is that zoea I have low mortality after 12 h of exposure, even when the concentrations of SMB (50 mg/L) were high. This result, coupled with the characteristics of the estuary and the export strategy of zoae, may indicate that SMB contamination may not be very relevant to this stage, because the individuals will be transported to coastal areas without it or with much diluted concentrations of SMB. However, despite the low mortality, chronic effects that may cause future morpho-physiological problems may result from even a short exposure. Further studies on the subject should be conducted, while taking into account the relative toxicity to SMB.

Zoea I larvae have also been shown to the slightly more tolerant of SMB than megalopa larvae and juvenile I stage animals. To the zoea I larvae, this may be due to the high levels of reserves arising from the egg vitellus, which confer some natural resistance to the larvae (Burggren and Mc Mahon 2008; Anger 1995, Lindquist and Carroll 2004; Lindquist et al. 2009) by providing a natural protection from the toxic substances found in the environment (Anger and Dawirs 1981; Anger et al. 1985; Anger 1996; Souza et al. 2006). When we compare the concentrations of SMB set by the FDA (12.5 g/L SMB) (Valença and Mendes 2004) with those used in our shrimp rearing study (<50 mg/L), a big difference becomes obvious. If we then compare the FDA approved concentrations with those usually employed in shrimp farms (between 50 and 100 g/L of SMB (Valença and Mendes 2004), the environmental threat becomes even more obvious. This fact is of particular concern when discharges occur in small bodies of water that do not have large volume to dilute the contaminant.

The DO and pH were correlated with the mortality of the larvae and post-larvae of *C. guanhumi* (Fig. 5). An increased vulnerability to this salt follows the development stages: zoea I larvae are slightly more tolerant, compared with the megalopae and juveniles. The greater tolerance to

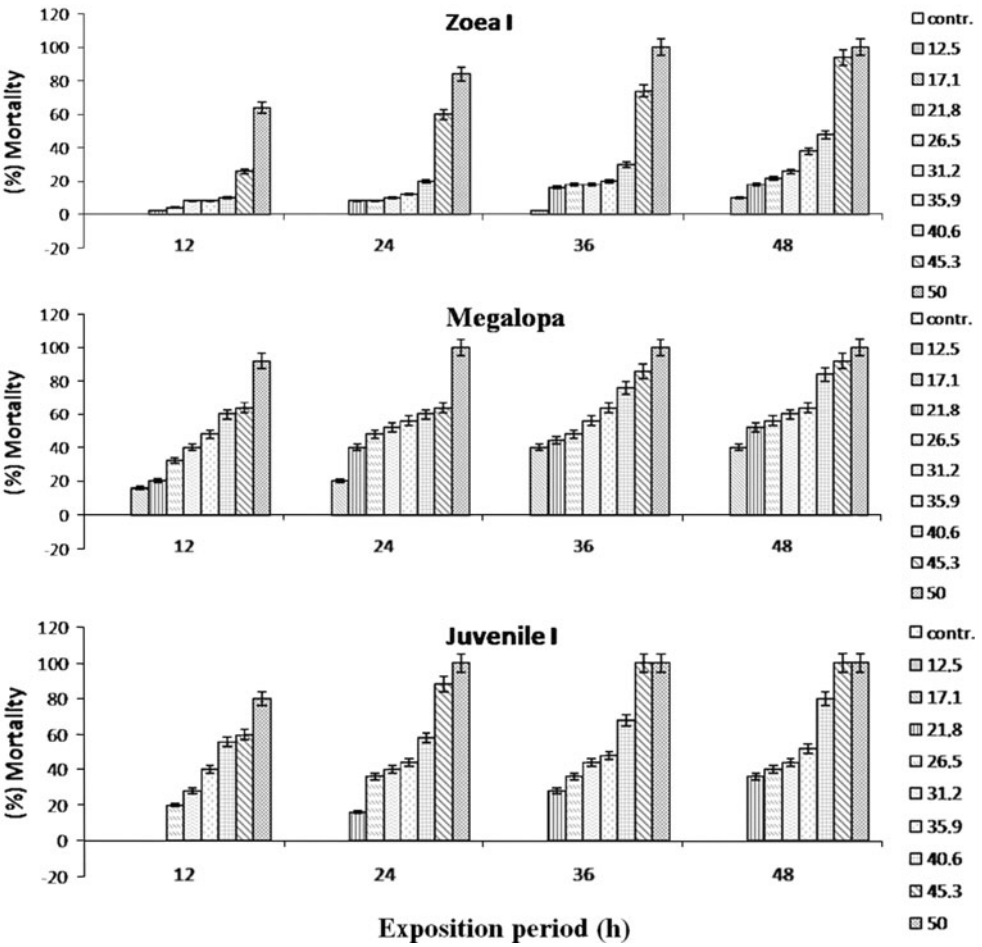

Fig. 4 Relationship between the mortality of the zoea I, megalopa and juvenile I of the *C. guanhumi* with SMB concentrations (mg/L) and time of exposure (h)

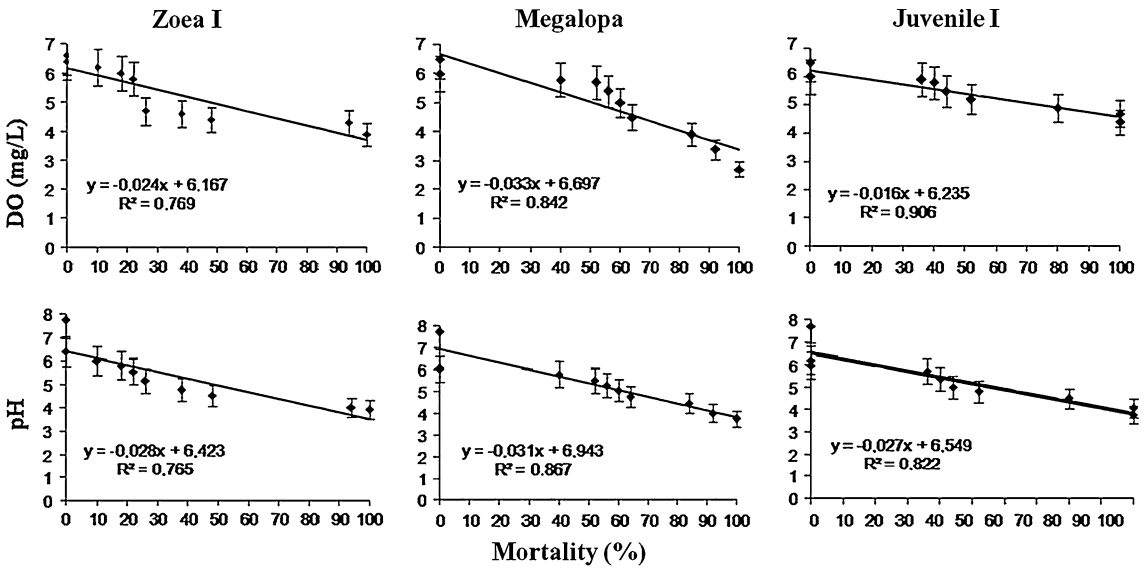

Fig. 5 Relationship between the DO and pH with the mortality of the zoea I, megalopa and juvenile I at the end of the experiment (48 h)

unfavorable environmental conditions displayed by individuals in the zoea I stage in this study appears to be common in estuarine species. According to Anger (2001) the larval stages of estuarine species have morpho-physiological adaptations which, during development and transition to the benthic environment, make them less likely to

survive in adverse abiotic conditions. Among those conditions, the pH and DO are factors that have the greatest influences on the development of larvae of brachyuran crustaceans (Burggren and Mc Mahon 2008; Lindquist et al. 2009).

The decrease in DO in the water and consequent acidification may be considered as the main cause of mortality in larvae (zoea I and megalopa) and post-larvae. Badaró-Pedroso et al. (2002) reported that acute toxicity in conditions of dissolved oxygen and pH may contribute to the mortality of shrimp. This lethal effect in the environment results mainly from low DO, which is known to cause death in other animals and plants as well (Aragão et al. 2008).

In our experiment with zoea I, megalopa and juvenile I, the DO decreased with concentrations of SMB increasing. The reaction of the SMB with the oxygen in the the water results in sodium acid sulphate and bisulphate ions, and consequently in a reduction in dissolved oxygen (de Araújo and de Araújo 2004; Cruz 2004; Aragão et al. 2008), and also in the pH. The water then becomes acidic, which may influence the homeostasis of ecosystems (Silva 1988). The reaction of the SMB with the water indicates that indiscriminate discharge of residues from shrimp tanks into adjacent ecosystems pose an environmental risk. Mangroves, estuaries and coastal areas, mainly in the northeastern region of Brazil, exhibit abiotic characteristics, with average values of pH >6 and >4 dissolved oxygen (Paiva et al. 2008). Due to those conditions they are regarded as a marine life nursery and as an important natural resource for the majority of the people who live near the coast.

Thus, an effective monitoring of shrimp farming and a strategy to mitigate its effects are necessary to prevent damage caused by the improper use of SMB by inland shrimp farming. Inappropriate application rates of SMB have been reported in shrimp farms in Brazil, and no recommended criterion for the use of this substance has been set (Figueiredo et al. 2005). Despite the extensively reported dangerous toxic effects of metabisulphite on human beings and the environment (de and de 2004; Figueiredo et al. 2005; Aragão et al. 2008), little information on how it affects aquatic organisms is available. For this reason, in order to design appropriate environmental laws for the manipulation of the SMB in estuarine effluents, further studies are needed. The results of this study indicate that SMB has an elevated toxicological effect on the early life stages of the land crab *C. guanhumi*. Small concentrations of SMB are sufficient to cause damage in larvae and post-larvae, and the primary causative agent of mortality is the change in dissolved oxygen and pH resulting from chemical reactions induced by the contaminant.

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