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Effect of sudden salinity change on *Penaeus latisulcatus* Kishinouye osmoregulation, ionoregulation and condition in inland saline water and potassium-fortified inland saline water

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Abstract

Two trials were conducted to determine the effect of sudden decrease in salinity of raw and potassium-fortified inland saline water on western king prawn *Penaeus latisulcatus* osmoregulation, ionoregulation and condition. Prawns were subjected to salinity decrease over 1 h from 32 to 25 ppt in the first trial and from 27 to 20 ppt in the second trial in three water types: inland saline water with potassium fortified to 100% and 80% of the marine water concentration (IS100, IS80), and raw inland saline water (ISW). In the first trial condition and ingestion rate were monitored over 19 days following salinity change. In the second trial condition, haemolymph osmo- and iono-regulation were recorded over 48 h following salinity change. In the first trial, 100% mortality was observed in ISW by day 13, with final survival 94% in IS80 and 100% in IS100. Tail muscle moisture content increased significantly ($P < 0.05$) over time in both trials and in all water types, suggesting loss of energy reserves. In the second trial, serum osmolality, sodium concentration and osmoregulatory capacity decreased following salinity change, stabilising by 24 h in IS100 and IS80 but continuing to decrease till 48 h in ISW, suggesting partial breakdown of osmoregulatory function in the potassium-deficient medium. Prawns were stronger regulators of divalent than monovalent cations. These trials demonstrate that potassium-deficient inland saline water requires fortification with potassium to allow prawn survival and efficient osmoregulation.

Keywords: Inland saline water; Ionoregulation; Osmoregulation; *Penaeus latisulcatus*; Potassium; Salinity

1. Introduction

Inland saline water is often deficient in potassium in comparison with marine water and may experience rapid salinity changes due to heavy rainfall. Salinity is an important environmental factor affecting euryhaline penaeid growth and survival (Chen and Lin, 1994b; Kumlu and Jones, 1995). Prawns are exposed to sudden decreases in salinity in nursery areas such as brackish water lagoons and estuaries as a result of tides, rainfall and wind (Dalla Via, 1986; Kumlu and Jones, 1995). In these environments, the capacity of a prawn to adapt to varying salinity is a major factor determining survival (Ferraris et al., 1987). Previous research has demonstrated that several prawn species can tolerate sudden change in salinity, with haemolymph osmolality and ion concentrations stabilising within 12–48 h (Parado-Estepa et al., 1987; Dall et al., 1990; Chen and Lin, 1994a,b; Kumlu and Jones, 1995; Fang et al., 1999; Diaz et al., 2001). Intermoult penaeids are typically excellent iono- and osmoregulators when compared with other crustaceans (Mantel and Farmer, 1983; Ferraris et al., 1986; Parado-Estepa et al., 1989).

Osmoregulatory capacity, and organosomatic indices and moisture content can be used as tools to monitor the physiological condition and the effect of stressors in crustaceans (Huner et al., 1990; Lignot et al., 2000; Fotedar, 2004; Sang and Fotedar, 2004a; Prangnell and Fotedar, submitted for publication). Prawn tail muscle moisture content, haemolymph osmotic and ionic concentrations change with salinity, with haemolymph divalent cations tending to be more strongly regulated than

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monovalent cations (Dall and Smith, 1981; Sang and Fotedar, 2004a). Exposure to stress often disrupts ionic and osmotic regulation in osmoregulating crustaceans (Lignot et al., 2000).

A candidate species for culture in inland saline water is the western king prawn (*Penaeus latisulcatus*), which is distributed throughout the Indo-West Pacific region (Dore and Frimodt, 1987; Potter et al., 1991). Salinity influences *P. latisulcatus* distribution within a temperate estuary, with larger prawns more susceptible to declining salinity (Potter et al., 1991). This species has an isosmotic range of 29–32 ppt and optimum culture range of 22–34 ppt in marine water (Sang and Fotedar, 2004a) and are also found in hypersaline waters (Dall et al., 1990).

Previous research has shown that potassium fortification of inland saline water is essential for prawn growth and survival (Saoud et al., 2003; Prangnell and Fotedar, 2005; Rahman et al., 2005). Haemolymph potassium is important for osmolality maintenance in prawns and, while it is regulated to some extent, changes with the potassium concentration in the external medium (Dall and Smith, 1981; Vargas-Albores and Ochoa, 1992). A potassium deficiency in the external medium may reduce the osmoregulatory capacity of prawns (Prangnell and Fotedar, submitted for publication), which could restrict their ability to tolerate sudden changes in salinity. There is no information available on how *P. latisulcatus* will tolerate a sudden decrease in salinity in inland saline water.

The present study was conducted to determine the effect of sudden salinity decrease in inland saline water and potassium-fortified inland saline water on western king prawn osmoregulation, ionoregulation, survival and condition indices.

2. Materials and methods

Experimental animals for both trials were caught from the Peel Inlet (32°55″S 115°43″E) in Western Australia, using drag nets in water up to 1.5 m deep. They were then held in 5000 L tanks containing marine water, procured from near Hillarys, W.A. (31°48″S, 115°44″E), for 2 days at the Curtin Aquatic Research Laboratory until the commencement of the trials. The trials were conducted in 125-L black plastic tanks, each with a diameter of 59 cm and covered with a sheet of black plastic (with ventilation holes) to ensure prawns remained in the tanks. Each tank in trial 1 had an external bio-filter and protein skimmer running continuously. Each tank in trial 2 was aerated through a single air stone. Inland saline water was procured from a lake at Wannamal, Western Australia (31°15″S, 116°05″E) on two separate occasions. The salinity of the water obtained for trial 1 was 60 ppt, which was reduced to the desired salinity of 32 ppt by adding deionised water. The salinity of the inland saline water obtained for trial 2 was 27 ppt.

Anhydrous potassium chloride was used to fortify inland saline water to approximately 100% and 80% of the concentration of potassium in marine water (IS100 and IS80). Prangnell and Fotedar (2005) estimated the minimum medium potassium requirement for rearing *P. latisulcatus* in inland saline water from Wannamal, WA at 76% of the marine water concentration. Therefore, IS80 was chosen as one of the test water types in the present trials. Survival, condition and feed ingestion rate (trial 1), and short-term condition, osmoregulation and ionoregulation (trial 2) were tested in IS100 (control) and IS80 and compared with un-fortified inland saline water (ISW). There were three replicates of each water type in trial 1 (9 tanks) and five in trial 2 (15 tanks). The ionic composition of the inland saline water used in these trials was analysed using ICP spectroscopy by "SGS", Queens Park, W.A. (Table 1).

In both trials, prawns were acclimated to each water type by being held in 125-L tanks (trial 1) or 250-L tanks (trial 2) containing marine water and the respective water types were siphoned in over 3 h. Deionised water was used to decrease salinity in each trial. Whenever prawns were sampled, they had their total weight (g) measured on electronic scales after having excess moisture removed with paper towel, total length (mm) and carapace length (mm) measured with calipers, and gender recorded. They were then frozen for later dissection. Sampled prawns in trial 2 also had haemolymph withdrawn prior to measuring. Water samples were taken at the commencement of each trial to measure the ionic concentrations in each water type.

In trial 1, 36 inter-moult prawns, with an initial mean weight of 3.71 ± 0.13 g were acclimated to each water type, 108 prawns in total. Twelve prawns were then placed in each of the nine tanks and were held for 3 days before salinity change. Mortalities were replaced during this time. Prawns were starved for 24 h prior to salinity change. At the conclusion of the 3 days, six prawns were sampled from each water type (two per tank). This sampling time was called "pre-salinity change."

The remaining 10 prawns in each tank were then subjected to a salinity change of 32 ppt to 25 ppt over 1 h. Ten minutes following salinity change, four prawns from each tank were sampled. This sampling time was called "post-salinity change." Following this, the remainder of the prawns in the tanks were measured and then returned to their respective tanks. Survival and ingestion rate were then monitored over 18 days. All surviving prawns were sampled at the conclusion of the experiment.

Table 1
The ionic composition of each water type used, in comparison with marine water (MW)

Component	Trial 1			Trial 2			MW
	ISW	IS80	IS100	ISW	IS80	IS100	
Salinity (ppt)	32	32	32	27	27	27	32
Osmolality (mOsm/kg)	854	869	873	769	779	782	893
Na^+ (mg/L)	8026	8026	8026	7337	7337	7337	9433
K^+ (mg/L)	80	322	390	68	230	290	353
Ca^{2+} (mg/L)	592	592	592	323	323	323	347
Mg^{2+} (mg/L)	1537	1537	1537	1293	1293	1293	1132
S^{2-} (mg/L)	–	–	–	442	442	442	846
Cl^- (mg/L)	15,800	16,070	16,150	15,200	15,420	15,500	16,161
SO_4^{2-} (mg/L)	1614	1614	1614	1323	1323	1323	2528
Hardness ($CaCO_3$) (mg/L)	7802	7802	7802	6133	6133	6133	5582
Na/K ratio	100.7:1	25.0:1	20.6:1	107.9:1	31.9:1	25.3:1	26.7:1
Mg/Ca ratio	2.6:1	2.6:1	2.6:1	4.0:1	4.0:1	4.0:1	3.3:1