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Survival, growth, and physiological responses of advanced juvenile freshwater crayfish (*Cherax quadricarinatus*), reared at low temperature and high salinities

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ABSTRACT

The effects of stress caused by low temperature, high salinities, and a combination of both low temperatures and high salinities were evaluated in advanced (~5 g) juvenile freshwater crayfish (*Cherax quadricarinatus*). Ten animals were weighed and assigned to each of the following combination of two temperature and three salinity treatments: 27 °C (optimum for this species) or 20 °C and 0, 5, and 10 g/L salt concentrations. After 30 days in each treatment, oxygen consumption and weight were recorded, together with hemolymph levels of glucose, sodium, potassium, and free amino acids (FAA). Glycogen level was determined in hepatopancreas and abdominal muscle, while FAA levels were measured in abdominal muscle and hemolymph. A significant decrease of weight gain was seen at the combination of 20 °C and 10 g/L salinity. A marked hyperglycemia was seen at the lower temperature, at any salinity tested. At the same temperature, there was a concomitant decrease of glycogen, in both hepatopancreas and muscle. Both sodium and potassium hemolymphatic levels significantly increased with increasing salinity, but only at 20 °C. No changes were seen in hemolymphatic FAA levels, but they increased in abdominal muscle at higher salinities, in correspondence with the sodium hemolymphatic increase, in order to regulate cellular osmolarity. Although advanced juveniles of *C. quadricarinatus* did not exhibit reduced survival or growth at 20 °C or 10 g/L salinity, the combination of relatively low temperature and high salinity significantly reduces growth, suggesting that the combination of these two factors is a stressful condition for these crayfish juveniles.

1. Introduction

Redclaw crayfish *Cherax quadricarinatus* (Von Martens) is a native species to the tropical region of Queensland, northern Australia. In this natural habitat, temperature ranges from 26 to 29 °C during the summer, and 21–22 °C during the winter (Karpus et al., 2003). This species has a high commercial potential, and has been cultured for several decades in Australia and other countries (Medley et al., 1994). *C. quadricarinatus* was introduced several years ago to Argentina, although the subtropical to temperate climate of this country is marginal for its culture. This species can survive at a winter temperature as low as 10 °C, but growth at this temperature is practically arrested (Karplus et al., 1998). This is a serious problem in temperate countries, mainly during grow-out of juveniles in external ponds. In farms, juveniles achieving a body weight around 1 g are suitable for being grown-out, but at this size a reduced growth occurs at 20 °C (Jones, 1997). Meade et al. (2002) reported both reduced survival and reduced growth of newly-hatched juveniles of *C. quadricarinatus* cultured at a temperature of 22 °C or lower. Although the size of juveniles at the beginning of the grow-out period has been considered (Barki and Karplus, 2004), the effect of temperature on growing of advanced juveniles of *C. quadricarinatus* has not been previously reported.

Although *C. quadricarinatus* mostly live in freshwater, they exhibit a wide tolerance to increased salinity, especially during the dry season or after flooding, when they can passively move toward the sea, exposed to salinities near that of sea water (Jones, 1997). In farms, this species may be exposed to brackish water (up to 25 g/L) for a short period (24 to 48 h), at the end of the grow-out period, in order to improve their flavor as seafood (Jones, 1997; Konosu and Yamaguchi, 1982). At higher salinities *C. quadricarinatus* release free amino acids to achieve an osmotic balance; this higher pool of free amino acids is responsible for taste of the flesh. After an experimental exposure to salinities as high as 25 g/L for 3 weeks, adult *C. quadricarinatus* showed a 90% survival, together with a significant increase of free amino acid in muscle (Prymaczok et al., 2008). However, few data are available for juveniles of the same species concerning either tolerance or physiological responses to salinity changes. For instance, a reduced survival rate (75 to 40%) was observed in *C. quadricarinatus* juveniles maintained for 70 days at salinities higher than 5 g/L (Meade et al., 2002).

As part of a program aimed at improving the culture of *C. quadricarinatus* in cooler latitudes and brackish environments, the current study was designed to evaluate the survival, growth, and metabolic

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rates, as well as several hemolymphatic and muscle metabolites in advanced (~5 g) *C. quadricarinatus* juveniles, after a gradual acclimation to the following experimental conditions: a) 20 °C (a relatively low temperature), b) 5 and 10 g/L salinity (relatively high salinities) and c) a combination of both low temperature and high salinities.

2. Materials and methods

2.1. Experimental design

Male, intermolt *C. quadricarinatus* juveniles were purchased from a local dealer (*Ecopeces*, Santa Fe, Argentina). Once in the laboratory, sixty advanced juveniles (~5 g) were maintained for 2 weeks at a temperature of 27 ± 1 °C, photoperiod 14:10 (L:D) in large aquaria containing dechlorinated tap water (pH = 7.5 hardness = 80 mg/L as $CaCO_3$ equivalents). Water was filtered through charcoal and resin filters. The juveniles were fed daily *ad libitum* commercial fish pellets (Tetra Color®, 50% crude protein) and fresh leaves of *Elodea sp*.

After this 2 week acclimation period, each juvenile was weighed on an analytical balance (precision ± 0.01 g, see Table 1), after gently removing the excess of water with a paper towel. Then, each animal was individually placed in a 1.5 L glass container filled with 1 L of the appropriate solution. Ten animals were randomly assigned to each of the following experimental groups:

1) Temperature = 27 °C, freshwater (tap water, as indicated above)
2) Temperature = 27 °C, salinity = 5 g/L
3) Temperature = 27 °C, salinity = 10 g/L
4) Temperature = 20 °C, freshwater
5) Temperature = 20 °C, salinity = 5 g/L
6) Temperature = 20 °C, salinity = 10 g/L

Increased salinity was achieved by adding artificial sea salts (Marine Mix, Germany) to freshwater at a gradual rate of 1 g/L per day. Temperature was changed from 27 to 20 °C (groups 4 to 6) at a rate of 0.5 °C per day, by placing the individual containers in a thermostat controlled incubator. Once the desired values of both temperature and salinity were attained in all groups (*i.e.*, after 2 weeks), the animals were maintained at constant conditions for 30 days. Water temperature, salinity and pH were checked daily and maintained at a precision of ± 0.1 °C, ± 0.1 g/L and ± 0.01 pH units. If necessary to maintain a constant salinity, distilled water was added to compensate for water evaporation, while pH was maintained at 7.5 by adding a strong acid or base. During the entire experiment, the juveniles were fed three times a week and the water was replaced once a week. Mortality was recorded to estimate the percentage of survival. At the end of the experiment, the following determinations were made:

Growth: weight gain (WG) was calculated as follows: WG = ((Wf − Wi)/Wi) × 100, where Wf and Wi are the body weight measured at the end and the beginning of the experiment, respectively.

Metabolic rate: the oxygen consumption rate of each animal was determined in closed glass chambers, fitted with a polarographic oxygen electrode (LUTRON DO-5510, sensitivity: 0.1 mg/L) connected to a computer by means of an analog–digital converter. Oxygen consumption of all animals was determined at the same conditions of temperature and salinity maintained throughout the experiment. Water inside the chamber was continuously stirred with a magnetic stirrer placed at the bottom of each chamber and enclosed in a plastic mesh to prevent it from injuring the animals. Animals were placed in the chambers 5 min before starting each oxygen consumption determination. Animals exhibited minimal activity during the 10 min recording period. Oxygen concentration in chambers always ranged between 7 and 5 mg/L. After each recording, animals were weighed (± 0.01 g) to allow calculation of weight specific metabolic rates.

A sample of hemolymph (100 to 200 μL) was taken within 10 min of the end of the metabolic measurements from the base of the 4th or 5th pereiopod of each animal, by using a hypodermic syringe fitted with a 27 G needle. Each hemolymphatic sample was then centrifuged at 3000 rpm for 15 min, three aliquots were taken from the supernatant and frozen at −20 °C for no more than one month, until levels of ions, glucose, and free amino acids (FAA) were determined. Immediately after hemolymph withdrawal, crayfish were anesthetized in an ice-cold bath, and the hepatopancreas and abdominal muscle were quickly dissected, frozen, and stored at −70 °C until analysis of glycogen and/or FAA.

Glycogen and glucose levels: Glycogen was extracted from hepatopancreas and muscle by using the method described by Van Handel (1965), to be then hydrolyzed with HCl followed by neutralization with Na_2CO_3, according to the method of Geary et al. (1981). Glucose equivalents from those tissues, as well as hemolymph glucose, were assessed with a colorimetric enzymatic kit (Wiener lab, Argentina).

Ions and FAA levels: sodium and potassium hemolymphatic levels were measured with a flame photometer (Crudo Caamaño SRL, Argentina), after appropriate dilution of the samples. FAA levels in both hemolymph and muscle were measured using a fluorometric method based on the reaction of amino acids with O-phthaldehyde (OPA), in the presence of β-mercaptoethanol (MET) as a reducing agent (Fisher et al., 2001). This technique was previously optimized for adults of the studied species (Prymaczok et al., 2008). Briefly, a sample of hemolymph weighing 2 mg was homogenized with 0.1 M perchloric acid in 1:20 (w/v) ratio and centrifuged at 15,000 *g* for 10 min. The supernatant was then neutralized with 2 M KOH (1:20 ratio), and centrifuged as above after the samples were cooled in ice water for about 10 min. The supernatant (40 μL) was mixed with 3 ml of OPA-MET reagent and after 2 min, the fluorescence was read in a Bowman fluorometer, using an excitation wavelength of 340 nm and an emission wavelength of 440 nm.

2.2. Data analysis

The Fisher exact test (Sokal and Rohlf, 1981) was used to compare the survival rate among groups. The remaining variables measured were analyzed by a two-way ANOVA (temperature and salinity as factors), followed by the *post-hoc* Tukey test for multiple comparisons (Sokal and Rohlf, 1981). A 95% confidence level was always considered.

3. Results

Only one crayfish died during the entire experiment, in the combination of 20 °C and freshwater. Animals maintained at 20 °C showed a significant lower weight gain (WG) than those held at 27 °C, but only at the salinity of 10 g/L (Fig.1). The metabolic rate of juvenile crayfish exposed to both 5 and 10 g/L salinities was not significantly different to that of crayfish exposed to freshwater at both temperatures ($p > 0.05$, Table 1). At the same time, the experimental group exposed to 20 °C did not show significant differences ($p > 0.05$) in metabolic rate with respect to the group maintained at 27 °C, when comparing for the same salinity (Table 1).

Table 1
Body weight and metabolic rate of *C. quadricarinatus* juveniles used in the 30-d experiment. Values are means ± standard errors. No significant ($p > 0.05$) differences were detected among groups in either initial body weight or ending metabolic rate.

Temperature (°C)	Salinity (g/L)	Initial body weight (N = 10 in all cases)	Final body weight	Metabolic rate (μg O_2/min/g)	Final N
27	0	4.39 ± 0.22	6.79 ± 0.31	1.10 ± 0.16	10
27	5	4.74 ± 0.24	7.29 ± 0.35	1.96 ± 0.40	10
27	10	4.35 ± 0.21	6.81 ± 0.36	1.30 ± 0.34	10
20	0	4.44 ± 0.26	6.25 ± 0.35	1.59 ± 0.15	9
20	5	4.38 ± 0.27	6.28 ± 0.43	2.29 ± 0.23	10
20	10	4.71 ± 0.31	6.63 ± 0.42	2.17 ± 0.20	10

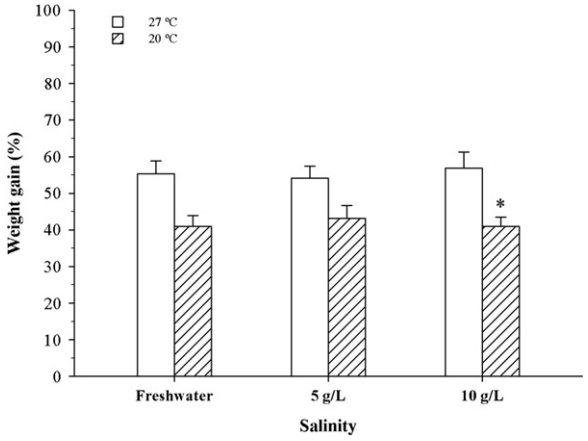

Fig. 1. Effect of both temperature and salinity on weight gain of *C. quadricarinatus* juveniles, at the end of the 30-d experiment. Mean values ± standard error are indicated. Number of animals is indicated in Table 1. Asterisks indicate significant differences ($p<0.05$) between temperatures, for each salinity.

Hemolymphatic glucose levels were significantly ($p<0.05$) higher at 20 °C, compared to 27 °C, at all salinities tested. On the other hand, the effect of salinity on glucose level was more evident at 20 °C, since both 5 and 10 g/L had significantly ($p<0.05$) increased glucose when compared to freshwater, while at 27 °C only 10 g/L exhibited a significant ($p<0.05$) increase (Fig. 2). Concerning glycogen reserves in the hepatopancreas, only at 5 g/L of salinity a significant ($p<0.05$) difference was found between 27 °C and 20 °C (Fig. 3A). In the same tissue, crayfish held at 27 °C had significantly higher glycogen levels ($p<0.05$) at both salinities assayed, while significantly ($p<0.05$) increased glycogen levels were only detected at 10 g/L, for 20 °C (Fig. 3A). In abdominal muscle, significant ($p<0.05$) differences in glycogen levels were detected between both temperatures at any salinity tested, while a significant ($p<0.05$) increase was seen between either 5 or 10 g/L and freshwater, but only at 20 °C (Fig. 3B).

Concerning hemolymphatic ions, a significant ($p<0.05$) increase in both sodium and potassium was detected at 20 °C, compared to 27 °C, for both 5 and 10 g/L. At 20 °C, 10 g/L caused a significant ($p<0.05$) higher sodium levels than freshwater (Fig. 4A), while the effect of salinity on potassium was only significant ($p<0.05$) while comparing 5 g/L and freshwater, at 20 °C (Fig. 4B). As for FAA, no changes were detected in hemolymph (overall mean = 2.54 μmol/g), but in abdominal muscle a significant ($p<0.05$) increase was

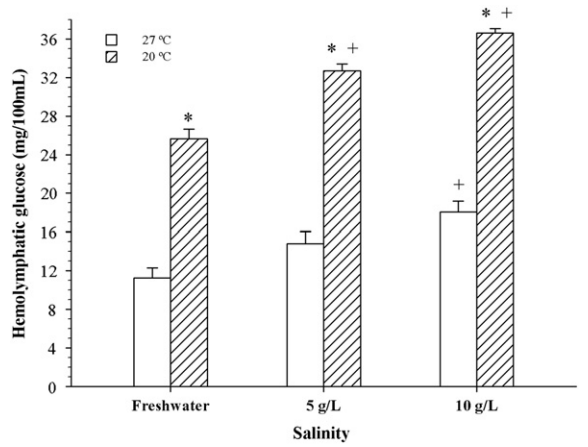

Fig. 2. Hemolymphatic glucose levels of *C. quadricarinatus* juveniles, at the end of the 30-d experiment. Mean values ± standard error are indicated. Number of animals is indicated in Table 1. Asterisks indicate significant differences ($p<0.05$) between temperatures, for each salinity. Crosses indicate significant differences ($p<0.05$) of any salinity with respect to freshwater, for each temperature.

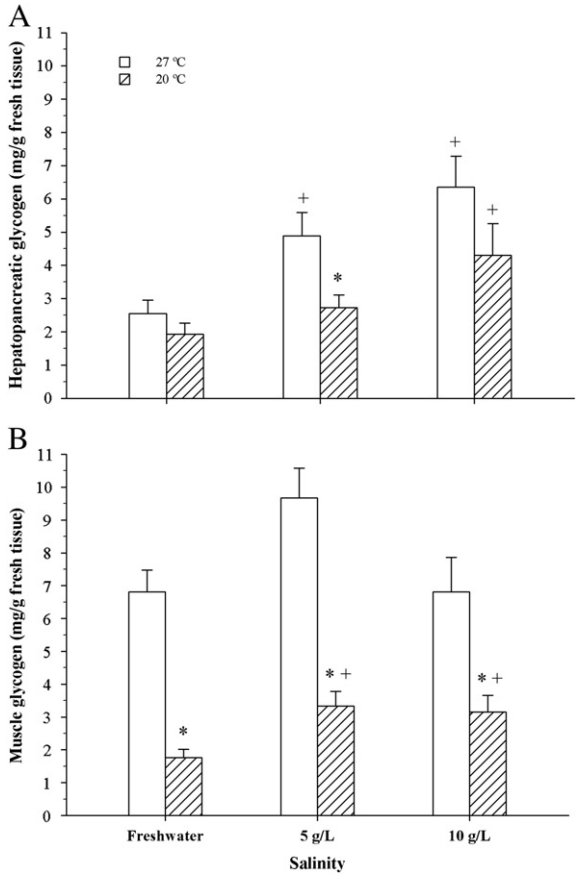

Fig. 3. Glycogen levels in A) hepatopancreas and B) abdominal muscle of *C. quadricarinatus* juveniles, at the end of the 30-d experiment. Mean values ± standard error are indicated. Number of animals is indicated in Table 1. Asterisks indicate significant differences ($p<0.05$) between temperatures, for each salinity. Crosses indicate significant differences ($p<0.05$) of any salinity with respect to freshwater, for each temperature.

detected at 20 °C, at the salinity of 5 g/L; comparing between salinities, both 5 and 10 g/L had significantly ($p<0.05$) increased levels of FAA in abdominal muscle than freshwater, but only at 20 °C (Fig. 5).

4. Discussion

Both temperature and salinity are among the most relevant factors influencing survival and growth of cultured crayfish species, together with food and water quality (Jones, 1997). Meade et al. (2002), in a previous study of newly-hatched (~10 mg) juveniles reported that temperatures below 28 °C significantly reduced weight gain of juveniles cultured in freshwater. For the same species, similar results were also reported by Austin (1995) for early juveniles (~20 mg) and by Jones (1997) for intermediate juveniles (~1 g). Concerning the effect of salinity on early juveniles of *C. quadricarinatus*, a decreased weight gain at a salinity of 10 g/L or higher has been reported by Meade et al. (2002), while no differences were found by Austin (1995) among salinities ranging from freshwater to 14 g/L.

Advanced juveniles of *C. quadricarinatus* used in the current study (~5 g of wet weight, at the beginning of the experiment) did not show a significant decrease in either weight gain or survival at 20 °C, compared to 27 °C, when cultured in freshwater. Salinity *per se* did not seem to affect weight gain, at least up to 10 g/L. By comparing these results with those of early juveniles mentioned above, we can remark that the higher tolerance showed by advanced juveniles would allow culturing them at lower temperatures or higher salinities than early juveniles. However, at the combination of 20 °C and 10 g/L of salinity a significantly diminished weight gain was observed

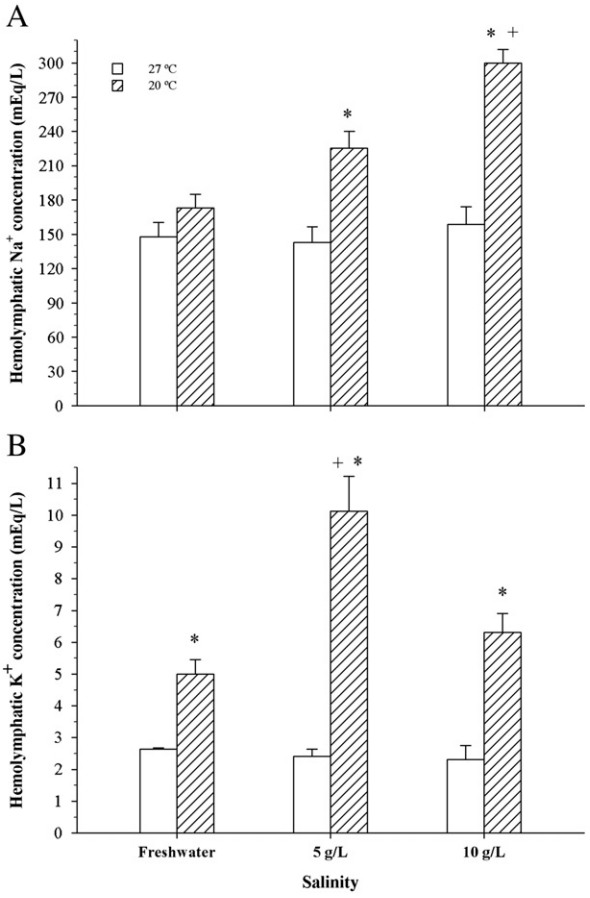

Fig. 4. Hemolymphatic ion concentrations in juveniles of *C. quadricarinatus*, at the end of the 30-d experiment; A) sodium, B) potassium. Mean values ± standard error are indicated. Number of animals is indicated in Table 1. Asterisks indicate significant differences ($p<0.05$) between temperatures, for each salinity. Crosses indicate significant differences ($p<0.05$) of any salinity with respect to freshwater, for each temperature.

in advanced juveniles. For management purposes on farms, these results should be taken into account at the time of transferring juveniles to external ponds for grow-out; in freshwater, a temperature as low as 20 °C would not significantly affect growth of juveniles of a body weight ~5 g, but a decreased growth of such advanced juveniles

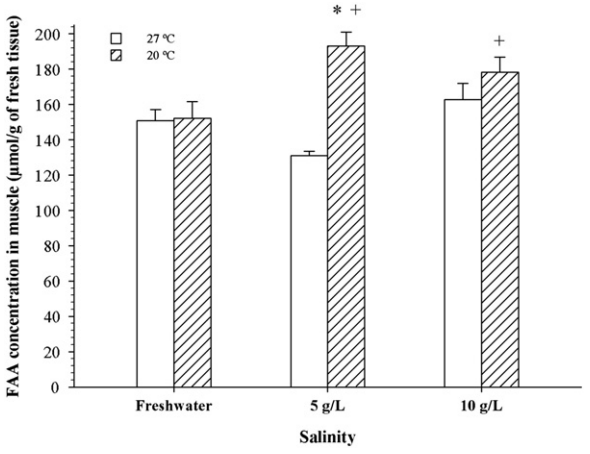

Fig. 5. Free amino acid (FAA) levels in abdominal muscle of *C. quadricarinatus* juveniles, at the end of the 30-d experiment. Mean values ± standard error are indicated. Number of animals is indicated in Table 1. Asterisks indicate significant differences ($p<0.05$) between temperatures, for each salinity. Crosses indicate significant differences ($p<0.05$) of any salinity with respect to freshwater, for each temperature.

would be expected if simultaneously a relatively high salinity (*i.e.* 10 g/L) was maintained.

Metabolic rate of advanced juveniles acclimated to 20 °C (and measured at the same temperature), was not significantly different from that of animals acclimated to (and measured at) 27 °C, therefore indicating a metabolic compensation after acclimation to low temperature, as described for several ectotherm species (Randall et al., 2002). Similarly, salinity did not affect the metabolic rate of advanced juveniles. According to Meade et al. (2002), metabolic rate of early juveniles was also not affected by salinity. Salinity fluctuations may be found in northern Australia, due to water evaporation and tidal influence (Bayly and Williams, 1973). Therefore, the stability of metabolic rate at higher salinity may have an adaptive value for *C. quadricarinatus*, even at the early stages of the life cycle. *Litopenaeus vannamei*, a euryhaline shrimp, also did not exhibit changes in metabolic rate when exposed to different salinities; however, a reduction of growth rate was eventually detected (Walker et al., 2009).

Hepatopancreatic glycogen level decreased in the group maintained at 20 °C, but only at the lower salinity, while muscle glycogen decreased at the same temperature at any salinity assayed. At 20 °C glucose in hemolymph increased more than 100%, in comparison with the group held at 27 °C, at any salinity tested. These results suggest that acclimation of advanced *C. quadricarinatus* to a temperature as low as 20 °C demands a marked mobilization of energetic reserves, as was also reported for other stressful conditions, such as hypoxia during emersion (Chang et al., 1998; Webster, 1996) and exposure to pollutants (Lorenzon et al., 2004). In this context, the crustacean hyperglycemic hormone (CHH) has been suggested as a relevant endocrine factor involved in the hyperglycemic response to several kinds of stressors, including temperature and salinity (Lago-Lestón et al., 2007, Lorenzon et al., 2007, Sook Chung et al., 2010). This hormone typically produces an increase of glycemia, by using the glycogen stored in hepatopancreas and other tissues (reviewed by Fanjul-Moles, 2006, Sook Chung et al., 2010). It has been also reported that CHH may have some role in osmoregulation, likely controlling ion fluxes and/or Na^+/K^+ ATPase activity (Serrano et al., 2003; Sook Chung et al., 2010, Spanings-Pierrot et al., 2000). The higher salinity assayed in the current study also caused, at both temperatures, an increase in glycemia, but to a lesser extent than the increment produced by the low temperature. Nevertheless, a potentiation of the effect of low temperature (20 °C) by high salinity (10 g/L) was clearly seen, either in terms of weight gain, glycogen utilization from muscle, or glycemia increase. Similar results were found by Lago-Lestón et al. (2007) in the euryhaline shrimp *L. vannamei*, *i.e.*, temperature had a greater effect on CHH gene expression than salinity, while salinity showed significant effects only when temperature was far from the optimum.

Hemolymphatic levels of both sodium and potassium measured in freshwater, at 27 °C, averaged 147.78 and 2.65 meq/L respectively. These levels are similar to those reported for other freshwater crustaceans (Wheatly and Gannon, 1995; Wheatly et al., 1996). Our results also show that these values can significantly change at a combination of low temperature (20 °C) and relatively high salinity. Concentration of sodium, the main cation determining blood osmolarity, did not change with salinity at the temperature of 27 °C, but when *C. quadricarinatus* juveniles were maintained at 20 °C, a significant increase of hemolymphatic sodium was seen at the higher salinity assayed. Increase in hemolymph sodium levels was also reported in *Procambarus zonangulus*, at temperatures non-optimal for the species, while increased hemolymphatic sodium was seen in *Procambarus clarkii* and *P. zonangulus* at relatively high salinities (Newsom and Davis, 1994).

Tolerance to salinity represents an interesting advantage in crayfish aquaculture for two main reasons: the possibility to settle new farms in areas of brackish water, and the better palatability of crayfish meat after a controlled exposure to relatively high salinities caused by an increased level of FAA in muscle induced by a higher hemolymphatic osmolarity

(Konosu and Yamaguchi, 1982; McCoid et al., 1984; Papadopoulos and Finne,1986).Higherlevelofhemolymphaticionsat20 °Cwasaccompanied byasignificantincreaseofFAAinabdominalmuscle,atbothsalinitiestested. Asreportedforseveralcrustaceanspecies,thisresponserepresentsacompensatory mechanism, aimed at maintaining the isosmotic regulation of the intracellular compartment, therefore avoiding an osmotic gradient with the extracellular medium and consequently a net flux of water (Gilles,1997,ParmegianiJahnetal.,2006;Fariaetal.,2011).

As previously reviewed (Freire et al., 2008, Gilles 1997, Vogt, 2002), crustaceans regulate hemolymphatic concentration of the main osmolites such as proteins, glucose, Na^+, Ca^{2+}, Cl^-, as well as other compounds which contribute less to hemolymph osmolarity such as nitrogenous non-protein compounds and FAA. Regulation of the main osmolite concentrations is the mechanism that most crustaceans utilize to produce an osmotic hemolymphatic pressure of 350 mOsm or higher (Mantel and Farmer, 1983). A combination of both low temperature and high salinity produced an increase in sodium hemolymphatic concentration, together with an increased in the FAA levels of muscle. FAA make a minor contribution to the osmotic pressure in hemolymph. Among crayfish species, a concentration of 2.67 mol/g of FAA was reported in *Cherax destructor* (Dooley et al., 2000), this level representing less than 1% of the osmotically active hemolymphatic solutes. The corresponding average value estimated in the current study was very close to that of *C. destructor*. Moreover, it did not change with either low temperature or high salinity.

In a previous study made on adults of *C. quadricarinatus* (Prymaczok et al., 2008), a high survival rate was observed at 27 °C, at salinities ranging from 0 to 25 g/L, the hemolymphatic isosmotic point corresponding to an external salinity of 15 g/L. In these animals, no changes in muscle FAA levels were seen at salinities ranging from 0 to 15 g/L, but at higher salinities FAA increased above freshwater control up to 3.5 fold, similarly to the results found in the freshwater anomuran *Aegla franca* (Coelho de Faria et al., 2011). As mentioned by Freire et al. (2008), freshwater crayfish can increase urine osmolality when exposed to saline media, although they never produce hyperosmotic urine. This is in accordance with both the constancy in FAA muscle levels seen in *C. quadricarinatus* at salinities below the isosmotic point, and the subsequent FAA increase at higher salinities. Similarly, advanced *C. quadricarinatus* juveniles (current study) have not shown, at 27 °C, any significant variation in muscle FAA at salinities up to 10 g/L, in correlation with constant sodium hemolymphatic levels. However, an increase of both sodium and FAA levels was seen in the same juveniles exposed to those salinities at a temperature of 20 °C. This may indicate the possibility that low temperatures reduces the capacity to produce urine more concentrated than in freshwater, either acting as a stress factor, or by another mechanism.

We can conclude that, although advanced juveniles of *C. quadricarinatus* can survive and grow relatively well at low temperature (20 °C) or high salinities (up to 10 g/L), a reduction in both growth rate and energetic reserves, together with an increase in both hemolymphatic sodium concentration and FAA levels of muscle, occurs when they are exposed to the combination of these environmental factors.

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References

Austin, C.M., 1995. Effect of temperature and salinity on the survival and growth of juvenile redclaw (Cherax quadricarinatus). Freshwater Crayfish 10, 419–426.

Barki, A., Karplus, I., 2004. Size rank and growth potential in redclaw crayfish (*Cherax quadricarinatus*): are stunted juveniles suitable for grow-out? Aquaculture Research 35, 559–567.

Bayly, I.A.E., Williams, W.D., 1973. Inland Waters and Their Ecology. Logman, Australia.

Chang, E.S., Keller, R., Chang, S.A., 1998. Quantification of crustacean hyperglycemic hormone by ELISA in hemolymph of the lobster, *Homarus americanus*, following various stresses. General and Comparative Endocrinology 111, 359–366.

Dooley, P.C., Long, B.M., West, J.M., 2000. Amino acids in hemolymph, single fibres and whole muscle from the claw of freshwater crayfish acclimated to different osmotic environments. Comparative Biochemistry and Physiology 127A, 155–165.

Fanjul-Moles, M.L., 2006. Biochemical and functional aspects of crustaceans hyperglycemic hormone in decapod crustaceans: review and update. Comparative Biochemistry and Physiology 142C, 390–400.

Faria, S.C., Augusto, A.S., Mc Namara, J.C., 2011. Intra- and extracellular osmotic regulation in the hololimnetic Caridea and Anomura: a phylogenetic perspective on the conquest of fresh water by the decapod Crustacea. Journal of Comparative Physiology 181 B, 175–186.

Fisher, G.H., Arias, L., Quesada, L., D'Aniello, S., Errico, F., Di Fiore, M.M., D'Aniello, A., 2001. A fast and sensitive method for measuring picomole levels of total free amino acids in very small amounts of biological tissues. Amino Acids 20, 163–173.

Freire, C.A., Onken, H., McNamara, J.C., 2008. A structure–function analysis of ion transport in crustacean gills and excretory organs. Comparative Biochemistry and Physiology 151A, 272–304.

Geary, N., Langhans, W., Scharrer, E., 1981. Metabolic concomitants of glucagons induced suppression of feeding in the rat. American Journal of Physiology 241, R330–R335.

Gilles, R., 1997. "Compensatory" organic osmolytes in high osmolarity and dehydration stresses: history and perspectives. Comparative Biochemistry and Physiology 117 A, 279–290.

Jones, C.M., 1997. The Biology and Aquaculture Potential of the Tropical Freshwater Crayfish *Cherax quadricarinatus*. Department of Primary Industries, Brisbane, Australia.

Karplus, I., Zoran, M., Milstein, A., Harpaz, S., Eran, Y., Joseph, D., Sagi, A., 1998. Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel III. Survival in earthen ponds under ambient winter temperatures. Aquaculture 166, 259–267.

Karplus, I., Gideon, H., Barki, A., 2003. Shifting the natural spring-summer breeding season of the Australian freshwater crayfish Cherax quadricarinatus into the winter by environmental manipulations. Aquaculture 220, 277–286.

Konosu, S., Yamaguchi, K., 1982. The flavour components in fish and shellfish. In: Martin, R.E. (Ed.), Chemistry and Biochemistry of Marine Food Products. The Avi Publishing Company Westport, Connecticut, pp. 367–404.

Lago-Lestón, A., Ponce, E., Muñoz, M.A., 2007. Cloning and expression of hyperglycemic (CHH) and molt-inhibiting (MIH) hormones mRNAs from the eyestalk of shrimps of *Litopenaeus vannamei* grown in different temperature and salinity conditions. Aquaculture 270, 343–357.

Lorenzon, S., Edomi, P., Giulianini, P.G., Mettulio, R., Ferrero, E.A., 2004. Variation of crustacean hyperglycemic hormone (CHH) level in the eyestalk and haemolymph of the shrimp *Palaemon elegans* following stress. Journal of Experimental Biology 207, 4205–4213.

Lorenzon, S., Giulianini, P.G., Martinis, M., Ferrero, E.A., 2007. Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. Comparative Biochemistry and Physiology 147A, 94–102.

Mantel, L., Farmer, L.L., 1983. Osmotic and ionic regulation. In: Bliss, D.E. (Ed.), The Biology of Crustacea, Vol. 5. Academic Press, New York, pp. 53–161.

McCoid, V., Miget, R., Finne, G., 1984. Effect of environmental salinity on the free amino acid composition and concentration in Penaeid shrimp. Journal of Food Science 49, 327–330.

Meade, M.E., Doeller, J.E., Kraus, D.W., Watts, S.A., 2002. Effects of temperature and salinity on weight gain, oxygen consumption rate, and growth efficiency in juvenile red-claw crayfish *Cherax quadricarinatus*. Journal of World Aquaculture Society 33, 188–198.

Medley, P.B., Nelson, R.G., Hatch, L.U., Rouse, D.B., Pinto, G.F., 1994. Economic feasibility and risk analysis of Australian red claw crayfish *Cherax quadricarinatus* aquaculture in the southeastern United States. J. World Aquaculture Soc. 25, 135–146.

Newsom, J.E., Davis, K.B., 1994. Osmotic responses of hemolymph in red swamp crayfish (*Procambarus clarkii*) and white river crayfish (*P. zonangulus*) to changes in temperature and salinity. Aquaculture 126, 373–381.

Papadopoulos, L.S., Finne, G., 1986. Effect of environmental salinity on sensory characteristics of penaeid shrimp. Journal of Food Science 51, 812–814.

Parmegiani Jahn, M., Cavagni, G.M., Kaiser, D., Kucharski, L.C., 2006. Osmotic effect of choline and glycine betaine on the gills and hepatopancreas of the *Chasmagnathus granulata* crab submitted to hyperosmotic stress. Journal of Experimental Marine Biology and Ecology 334, 1–9.

Prymaczok, N.C., Medesani, D.A., Rodríguez, E.M., 2008. Levels of ions and organic metabolites in the adult freshwater crayfish, *Cherax quadricarinatus*, exposed to different salinities. Mar. Freshwat. Behav. Physiol. 48, 121–130.

Randall, D.J., Burggren, W., French, K., 2002. Eckert Animal Physiology: Mechanisms and Adaptations. W.H. Freeman and Company, New York.

Serrano, L., Blanvillain, G., Soyez, D., Charmantier, G., Grousset, E., Aujoulat, F., Spanings-Pierrot, C., 2003. Putative involvement of crustacean hyperglycemic hormone isoforms in the neuroendocrine mediation of osmoregulation in the crayfish *Astacus leptodactylus*. Journal of Experimental Biology 206, 979–988.

Sokal, R.R., Rohlf, F.J., 1981. Biometry, second ed. Freeman, New York.

Sook Chung, J., Zmora, N., Katyama, H., Tsutsui, N., 2010. Crustacean hyperglycemic hormone (CHH) neuropeptides family: functions, titer, and binding to target tissues. General and Comparative Endocrinology 166, 447–454.

Spanings-Pierrot, C., Soyez, D., Van Herp, F., Gompel, M., Skaret, G., Grousset, E., Charmantier, G., 2000. Involvement of crustacean hyperglucemic hormone in the control of gill ion transport in the crab *Pachygrapsus marmoratus*. General and Comparative Endocrinology 119, 340–350.

Van Handel, E., 1965. Estimation of glycogen in small amount of tissue. Analytical Biochemistry 11, 256–265.

Vogt, G., 2002. Functional anatomy. In: Holdrich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, New York, pp. 64–87.

Walker, S.J., Neill, W.H., Lawrence, A.L., Gatlin III, D.M., 2009. Effect of salinity and body weight on ecophysiological performance of the Pacific white shrimp (*Litopenaeus vannamei*). Journal of Experimental Marine Biology and Ecology 380, 119–124.

Webster, S.G., 1996. Measurement of crustacean hyperglycaemic hormone levels in the edible crab *Cancer pagurus* during emersion stress. Journal of Experimental Biology 199, 1579–1585.

Wheatly, M.G., Gannon, A., 1995. Ion regulation in crayfish: freshwater adaptation and the problem of molting. American Zoologist 35, 49–59.

Wheatly, M.G., de Souza, S.C.R., Hart, M.K., 1996. Related changes in hemolymph acid–base status, electrolytes, and ecdysone in intermoult crayfish (*Procambarus clarkii*) at 23 °C during extra-cellular acidosis induced by exposure to air, hyperoxia, or acid. J. Crust. Biol. 16, 216–277.