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ORIGINAL PAPER

Effects of ocean acidification and salinity variations on the physiology of osmoregulating and osmoconforming crustaceans

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Abstract

Survival, osmoregulatory pattern, oxygen consumption, energy spent on metabolism, ammonia excretion, type of oxidized energy substrate, and hepatosomatic index were evaluated in decapods (an osmoregulating crab, *Callinectes danae*, and an osmoconforming seabob shrimp, *Xiphopenaeus kroyeri*) exposed to carbon dioxide-induced water acidification (pH 7.3, control pH 8.0) and different salinities (20, 25, 30, 35, and 40‰) for 3 days. Compared to the animals kept at controlled pH, exposure to reduced pH resulted in the loss of osmoregulatory capacity in *C. danae* at all salinities, except for some hyporegulation at 40‰, and reduced oxygen consumption and ammonia excretion at 20 and 40‰. *Xiphopenaeus kroyeri* remained an osmoconformer in all evaluated conditions, except for some hyporegulation at 40‰, and when exposed to the reduced pH, it presented changes in oxygen consumption at all salinities and reductions in ammonia excretion at 20 and 35‰ compared to the control animals. Both species use protein as the main energy substrate and decrease the hepatosomatic index when exposed to reduced pH relative to the control. The observed changes may be associated with changes in the activity of enzymes related to osmoregulation, the use of amino acids as osmotic effectors of cell volume control and recovery, and the Bohr effect, and, because the gills are multifunctional organs related to osmoregulation, the changes may be related to acid–base control, nitrogen excretion, and respiration, with a change in one of these functions bringing about changes in the others.

Keywords Climate change · Acidification · *Callinectes* · *Xiphopenaeus* · Fishery · Osmoregulation

Introduction

The marine environment is changing faster due to increases in the levels of atmospheric CO_2 and consequent changes in the carbonate chemistry of surface waters, sea level rise, and temperature. The ocean pH may decrease from 8.1 to 7.7 by the end of the century and to pH 7.4 by 2300 (Caldeira and Wickett 2003; Orr et al. 2005). When carbonate chemistry of the seawater changes during ocean acidification, CO_2 excretion across the gills can be compromised, causing an increase in CO_2 in the hemolymph. Although these changes in pH can be buffered by physiological mechanisms, a rise in intracellular $[H^+]$ can disrupt key biological processes such as metabolism, protein synthesis, osmoregulation, cell volume control, and oxygen transport (Whiteley et al. 1999, 2001; Knapp et al. 2015; Lehtonen and Burnett 2016). An important factor that enhances the effects of ocean acidification on crustaceans is the fact that the gills are multifunctional organs responsible for the acid–base balance, osmoregulation, nitrogen excretion, and respiration (Evans 2005; Freire et al. 2008; Henry et al. 2012). Therefore, changes in one of its functions may bring about changes in the others. For example, some studies have shown that the acid–base balance of crabs to either hypercapnia or external changes in salinity is closely associated with ionoregulation because both homeostatic processes share the same mechanisms, such as the apical antiporters H^+/Na^+ and HCO_3^-/Cl^- (Cameron and Iwama 1987; Whiteley et al. 1999, 2001; Evans 2005; Henry et al. 2012). According to Whiteley (2011), marine species that are weak iono- and osmoregulators are more subject to the negative effects of

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ocean acidification because they have limited capabilities to compensate for acid–base changes.

Although some species may be more resilient than others and evolutionary processes could reduce some of the negative consequences expected with future climate change, the study of the physiology of marine animals contributes to the understanding of the effects that can cause biodiversity loss. However, a gap exists in the literature on the synergistic effects of the different factors related to climate change, despite evidence that such interactions may change physiological responses (Paganini et al. 2014; Mayor et al. 2015; Goncalves et al. 2017).

The species studied here, the blue crab *Callinectes danae* and the shrimp *Xiphopenaeus kroyeri*, are oceanic coastal species found in variable salinities, and therefore, are subject to the synergistic effects of ocean acidification and salinity. *C. danae* is a benthic crab, found in estuaries and in marine areas to depths of 75 m, and is an important resource exploited by fishermen (Severino-Rodrigues et al. 2001). The species is distributed throughout the Atlantic Ocean from Florida, USA, to the southern coast of Brazil (Melo 1996). The shrimp *X. kroyeri* is one of the main fishery resources of the coast of the state of São Paulo, Brazil (Ávila-da-Silva et al. 2007) and is distributed in the western Atlantic from Virginia (USA) to Rio Grande do Sul (Brazil) and in the eastern Pacific from Sinaloa (Mexico) to Paita (Peru) (Costa et al. 2003). Because it inhabits shallow water and has spatio-temporal distribution patterns and a reproductive season heavily dependent on temperature and salinity, *X. kroyeri* is considered a good model for studies on the effects of climate change (Fransozo and Nakagaki 1998; Costa et al. 2007; Almeida et al. 2012; Heckler et al. 2014). In addition, little is known about the osmoregulation and metabolism of either species when exposed to different salinities, although *C. danae* is frequently cited as an osmoregulating euryhaline species because of its wide distribution and similarity with known species of the genus *Callinectes* (Masui et al. 2002, 2005; Freire et al. 2011).

The effects of climate change need to be known because the distribution and abundance of marine organisms are directly or indirectly a function of biotic and abiotic features; therefore, we evaluated a set of physiological processes in *C. danae* and *X. kroyeri* exposed to carbon dioxide-induced water acidification and different salinities. The study of an osmoregulating and an osmoconforming species (*C. danae* and *X. kroyeri*, respectively) aims to compare crustaceans with different osmoregulatory patterns to understand whether the effects of climate change are stronger on species with one of these two patterns.

Materials and methods

Samples collection

Callinectes danae was collected in the estuarine of the Praia Grande, Brazil, (23º59′11.983″S, 46º24′11.311″W) using a traps. *Xiphopenaeus kroyeri* was collected in Santos Bay (23º59′54.139″S, 46º21′5.364″W) using a shrimp boat equipped with a net mesh of 20 mm. The crustaceans were collected in spring and summer of 2014. All animals used in the experiments were male in intermolt (Skinner 1962, 1985; Smith and Dall 1985). The carapace length of the *C. danae* ranged from 4.5 to 5.5 cm and the total length of the *X. kroyeri* ranged from 8 to 9 cm. The water variables at the collection site, salinity, pH and temperature, were verified, respectively, with refractometer, pH meter and thermometer. The animals were transported to the Laboratory of Aquatic Animals Physiology of UNESP, campus of the São Paulo State coast, in boxes containing water from the collection site with constant aeration. In the laboratory, they were acclimated to laboratory conditions during 4 days in tanks containing water with salinity (30‰), pH (8.0) and temperature (24 °C). The animals were daily fed with fish fillet. Experiments were performed with seven animals ($N = 7$).

Experimental procedures for the evaluation of oxygen consumption, ammonia excretion, atomic ratio *O/N*, osmoregulation and hepatosomatic index

4 days after acclimatization to the laboratory, animals were transferred to plastic containers with water of 20, 25, 30, 35 or 40‰ and pH of 7.3 or 8.0 (control) during 3 days. Animals were kept at the same temperature as the collection site (24 °C) with the help of electric heaters. The value of control pH was chosen after evaluating the place from where animals were collected and the reduction based on the simulation of future scenarios in which the pH of ocean waters might suffer a reduction of up to 0.7 units (Caldeira and Wickett 2003). Reduced pH was obtained by CO_2 bubbling in water using a cylinder equipped with manometer, diffuser, and solenoid valve (Dissanayake and Ishimatsu 2011). Animals were introduced into the plastic containers 2 days after the desired pH was reached. The pH of each container was also measured four times a day using a portable pH meter (Qualxtron, QX1500 Plus). Physical–chemical water parameters and associated variation, for each treatment, are shown in Table 1. Water samples were daily collected for total alkalinity (TA) determined by titration (Gran 1952). Mean total alkalinity, pH, temperature, salinity were introduced

Table 1 Seawater parameters, pH and salinity (‰) were daily measured and water samples (mean ± SE, $N = 7$) were collected for the analysis of total alkalinity (A_T; µmol kg^{-1})

Conditions	pH	‰	A_T (µmol kg^{-1})	pCO_2 (µatm)	Ω Cal	Ω Ar
pH 8.0 (control)	7.86 ± 0.10	20	2142.46 ± 197.8	743.86 ± 68.3	2.630 ± 0.3	1.64 ± 0.16
	7.99 ± 0.01	25	2459.18 ± 204.4	800.97 ± 66.7	3.307 ± 0.3	2.11 ± 0.18
	8.03 ± 0.03	30	2292.48 ± 13.0	745.43 ± 41.0	3.381 ± 0.2	2.19 ± 0.13
	7.96 ± 0.04	35	2897.83 ± 123.0	844.66 ± 35.9	4.622 ± 0.2	3.04 ± 0.13
	7.96 ± 0.04	40	2991.03 ± 150.3	839.14 ± 42.2	5.074 ± 0.2	3.35 ± 0.17
pH 7.3	7.33 ± 0.03	20	2642.49 ± 186.8	5001.48 ± 353.7	0.689 ± 0.1	0.427 ± 0.03
	7.37 ± 0.07	25	1824.65 ± 408.4	3251.48 ± 728.2	0.534 ± 0.1	0.340 ± 0.08
	7.36 ± 0.06	30	2727.66 ± 250.1	4631.35 ± 424.8	0.888 ± 0.1	0.576 ± 0.05
	7.36 ± 0.06	35	3350.99 ± 182.4	5484.74 ± 298.5	1.195 ± 0.1	0.785 ± 0.04
	7.38 ± 0.08	40	3346.42 ± 245.9	5342.32 ± 392.8	1.286 ± 0.1	0.849 ± 0.06

The CO_2-SYS software was used to calculate partial CO_2 pressures (pCO_2; µatm) and seawater saturation states for calcite (Ω Cal) and aragonite (Ω Ar)

into the CO_2SYS software to calculate CO_2 partial pressure (pCO_2) and saturation states of calcite (Ω Cal) and aragonite (Ω Ag) for each treatment, being shown as mean values for each tank. Ammonia concentration (Koroleff 1976) and water salinity were also daily monitored. They were daily fed with fish fillet, except on the last day when they were kept fasting to prevent the calorigenic effect of food on oxygen consumption (Rosas et al. 2001; Lemos et al. 2001; Augusto and Masui 2014). 3 days after, shrimp were withdrawn from the containers for the evaluation of oxygen consumption, ammonia excretion, atomic ratio *O*/*N*, osmoregulation and hepatosomatic index.

Evaluation of oxygen consumption, ammonia excretion and atomic ratio *O*/*N*

Oxygen consumption and ammonia excretion were measured on the last day of the experimental period (3th day) (Augusto and Masui 2014; Augusto and Valenti 2016). Animals were placed in individual respirometric chambers containing water for 30 min with aeration aiming at acclimatization and reduction of the stress caused by manipulation. After this period, aeration was removed and oxygen concentration within the chamber was measured with an oximeter (monitor and probe YSI, Models 53 and 5905, respectively). After 60 min, oxygen concentration was measured again. Control chambers without animals were kept under the same experimental conditions. Variations in oxygen concentration were calculated by the difference between the values obtained in samples and controls (no animals). After incubation, animals were killed by freezing, oven dried at 60 °C for 48 h and weighed (Marte, AS 2000C). Ammonia excretion was measured in samples of 10 ml of water obtained from respirometric chambers in the beginning and in the end of the experiment to assess oxygen consumption. Ammonia concentration in water samples was determined in triplicate by colorimetry (Koroleff 1976). Atomic ratio *O*/*N* was calculated by dividing the oxygen consumed (mol) by ammonia excreted (mol) (Mayzaud and Conover 1988). The ratio *O*/*N* indicates the type of energy substrate that the animal is predominantly consuming. Ratios between 3 and 16 are related to the use of protein; between 16 and 60 indicate the use of mixture of protein and lipid and ratios over 60 indicate the predominant oxidation of lipid (Mayzaud and Conover 1988).

Evaluation of the hemolymph osmolality and osmoregulatory capacity

Hemolymph samples were taken from the pericardial region located at the cephalothorax using an insulin syringe coupled to a #25-8 needle. Hemolymph osmolality was measured in 10 µl samples using a vapor pressure osmometer (Model 5500, Wescor). The osmoregulatory capacity, difference between the hemolymph osmolality and of the external medium in a given salinity, was calculated by ratio: Δ hemolymph osmolality/Δ medium osmolality (Lignot et al. 2000; Freire et al. 2003).

Evaluation of the hepatosomatic index (HSI)

The hepatopancreas was dissected and weighed to determine the HSI based on the ratio below:

HSI (%) = (hepatopancreas mass × 100)/body mass.

Statistical analysis

The effect of pH (8.0 or 7.3) and salinity (20, 25, 30, 35 or 40‰) on oxygen consumption, ammonia excretion, atomic ratio O/N, osmoregulation and hepatosomatic index was evaluated by the analysis of variance of two factors (ANOVA). The analyses were followed by Student–Newman–Keuls' multiple mean test (SNK) to find statistically different means. The analyses were done after examining

the conditions of normality of distribution and equality of variance using the Sigma Stat software and employing a minimal level of significance of $P = 0.05$.

Results

Mortality

Experimental exposure to different conditions of pH and salinity did not cause mortality in *C. danae* and *X. kroyeri* during the study period.

Osmoregulatory capacity

The osmolality of the hemolymph of the animals exposed to different pH and salinity levels is shown in Fig. 1. At the control pH, *C. danae* osmoregulated the hemolymph osmolality in all salinities, hyperregulating up to the isosmotic point of roughly 850 and then hyporegulating at all higher salinities. *Xiphopenaeus kroyeri* displayed an osmoconforming pattern at all salinities except 40S, where some hyporegulation was observed. Compared to the animals kept at the control pH, exposure to the reduced pH caused loss of the osmoregulatory capacity in *C. danae*, although both species showed some hyporegulation when exposed to 40‰ salinity.

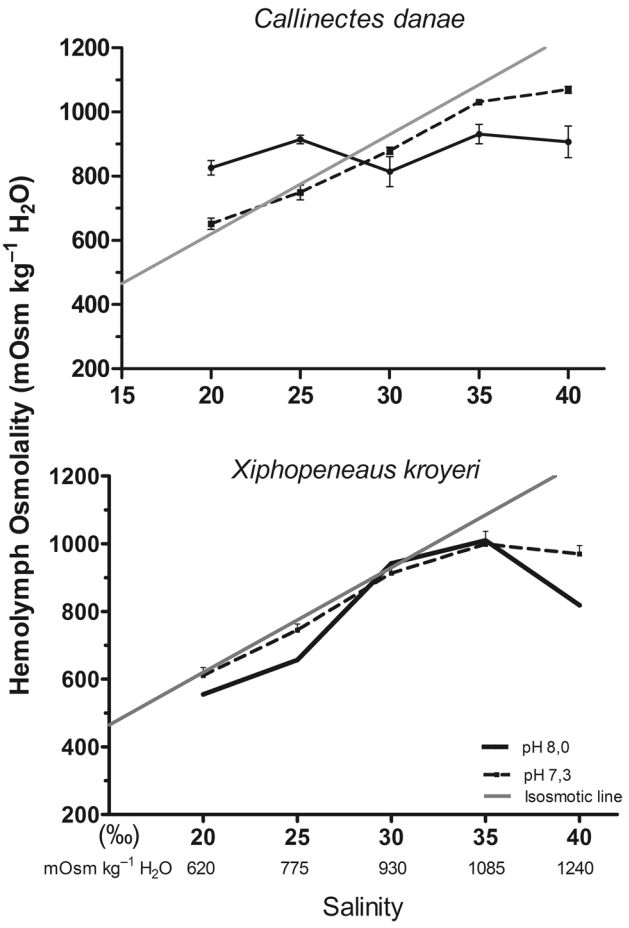

Fig. 1 Osmolality of the crab *C. danae* and of the shrimp *X. kroyeri* exposed to control (8.0) and reduced (7.3) pH conditions at different salinities (mean ± SEM, $N = 7$)

The osmoregulatory capacity of *C. danae* at the control pH was 1.05, a value similar to that of an osmoregulating species, but at the reduced pH, this value decreased to 0.88. In addition, the osmoregulatory capacity of *X. kroyeri* ranged from 0.73 (control pH) to 0.58, (reduced pH), characterizing the species as osmoconforming, regardless of the pH or salinity.

Oxygen consumption

Being kept at the control pH caused an increase in oxygen consumption of up to 220 and 150% in *C. danae* and *X. kroyeri*, respectively, after exposure to 40‰ salinity. Compared to the animals kept at the control pH, exposure to the reduced pH caused a decrease in oxygen consumption in *C. danae* at salinities of 20 and 40‰, but in *X. kroyeri*, reductions (at 20, 35, and 40‰) and increases (at 25 and 30‰) were observed (Fig. 2). In both species, the energy channeled into metabolism also underwent some changes. In *C. danae* kept at the control pH, the energy channeled into the metabolism at 40‰ (8.0 ± 0.8 kJ/ind/day) was higher than at 30 and 35‰ (4.8 ± 0.7 and 4.4 ± 0.8 kJ/ind/day), and in *X. kroyeri*, it was higher at 25 and 40‰ (approximately 3.8 kcal/ind/day). Comparing the animals kept at the control pH with those exposed to reduced pH, the energy channeled to metabolism decreased from 7.6 kJ/ind/day at the control pH to 3.8 kJ/ind/day at the reduced pH in *C. danae* kept at 20‰. In *X. kroyeri*, an increase from 1.2 to 2.8 kJ/ind/day was observed in the animals kept in 30‰.

Ammonia excretion

In *C. danae*, being kept at the control pH caused an increase in the ammonia excretion in the animals exposed to the salinities of 20 and 40‰ relative to the others, but in *X. kroyeri*, a reduction occurred only in the animals kept at 30‰ salinity. Compared to animals kept at the control pH, exposure to the reduced pH caused decreases in ammonia excretion in *C. danae* (20 and 40‰) and *X. kroyeri* (20 and 35‰) (Fig. 3).

O/*N* ratio and hepatosomatic index

The *O*/*N* ratio and the hepatosomatic index (HSI) of *C. danae* and *X. kroyeri* exposed to different pH and salinity conditions are shown in Table 2. The *O*/*N* ratio of both species kept under different pH and salinity conditions was lower than seven,

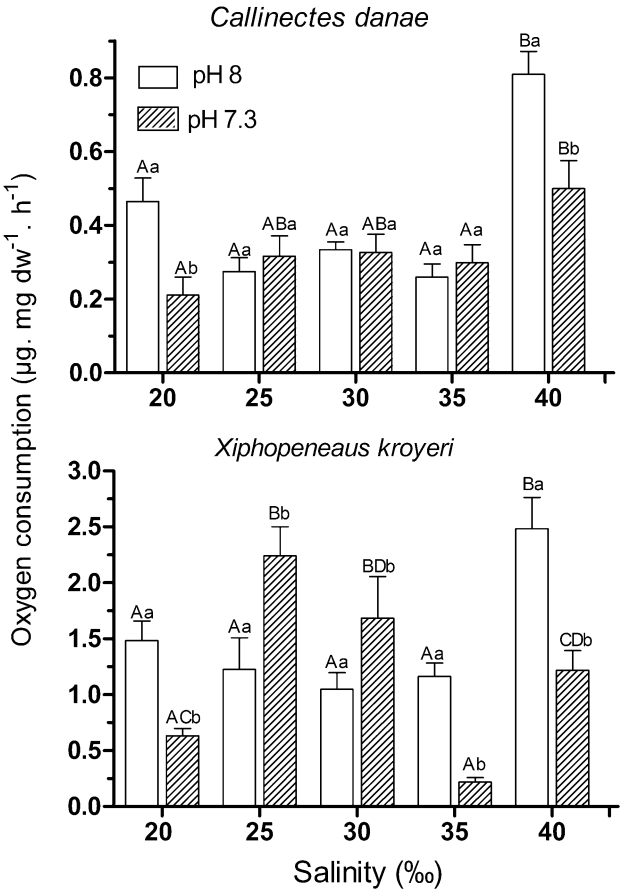

Fig. 2 Oxygen consumption of the crab *C. danae* and of the shrimp *X. kroyeri* exposed to control (8.0) and reduced (7.3) pH conditions at different salinities (Mean ± SEM, $N = 7$). Different uppercase letters indicate significant differences between the animals kept the same pH; different lowercase letters indicate significant differences between the animals kept at the same salinity ($P < 0.05$)

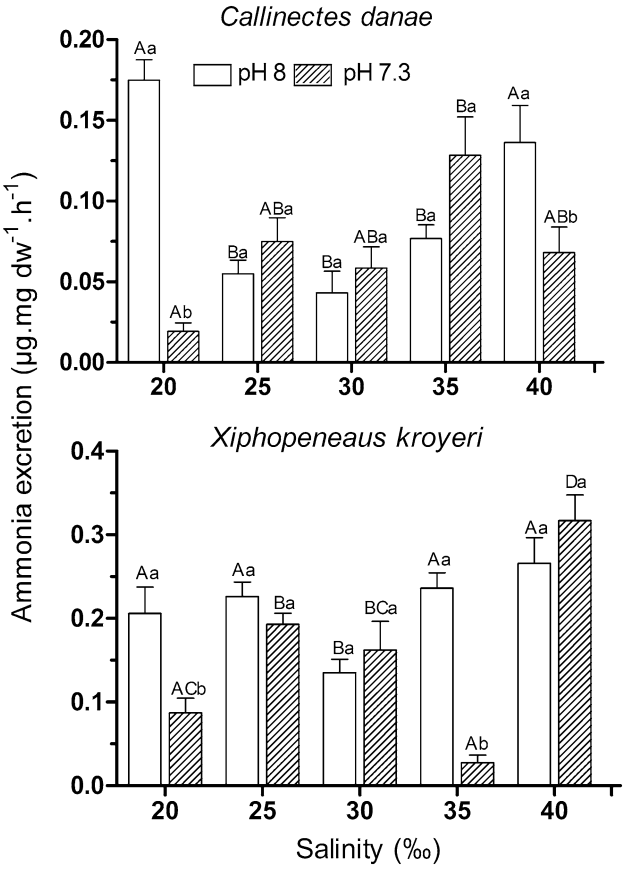

Fig. 3 Ammonia excretion of the crab *C. danae* and of the shrimp *X. kroyeri* exposed to control (8.0) and reduced (7.3) pH conditions at different salinities. (Mean ± SEM, $N = 7$). Different uppercase letters indicate significant differences between the animals kept the same pH; different lowercase letters indicate significant differences between the animals kept at the same salinity ($P < 0.05$)

which indicates the use of proteins as the main oxidized energy substrate (Mayzaud and Conover 1988).

The HSI of *C. danae* kept at the control pH increased at salinities of 20 and 35‰ relative to the intermediate salinities of 25 and 30‰. In *X. kroyeri*, the HSI of the animals kept at 25‰ was up to 450% higher than at the other salinities. Relative to the animals kept at the control pH, *C. danae* experienced a reduction in the HSI at salinities of 20 and 35‰ after exposure to reduced pH, but for *X. kroyeri*, the reductions occurred at all salinities, except for the shrimp exposed to 40‰ salinity.

Discussion

Exposure to reduced pH may have varying effects on the survival, physiology, morphology, and behavior of decapods. The effects can also vary depending on the association of pH with other variables such as salinity, temperature, oxygenation, and time of exposure. In *C. danae* and *X. kroyeri*, no mortalities were observed in the combinations of pH and salinity in which the animals were exposed to for a short time. This type of response is variable among crustaceans; reports exist of mortality after short-term exposure (Cameron and Iwama 1987) to no mortality after a long period of exposure (Dissanyake and Ishimatsu 2011). Similar responses to those observed in *C. danae* and *X. kroyeri* have been described in crustaceans such as the lobster *Homarus gammarus* (Arnold et al. 2009), the copepod *Acartia tsuensis* (Kurihara et al. 2008), and the intertidal barnacles *Semibalanus balanoides* and *Elminius modestus* (Findlay et al. 2010) after a long period of exposure to reduced pH.

Despite the absence of mortality, *C. danae* lost its osmoregulatory capacity at reduced pH and started to display an osmoconformer pattern. In contrast, in *Gammarus oceanicus*, osmoregulation is maintained during exposure to reduced pH, and no additional physiological costs

Table 2 Atomic *O*/*N* ratio and hepatosomatic index (HIS) of the crab *C. danae* and of the shrimp *X. kroyeri* exposed to control (8.0) and reduced (7.3) pH at different salinities (mean ± SEM, $N = 7$)

‰	*O*/*N*				HIS			
	C. danae		*X. kroyeri*		*C. danae*		*X. kroyeri*	
	pH 8.0	pH 7.3	pH 8.0	pH 7.3	pH 8.0	pH 7.3	pH 8.0	pH 7.3
20	1.9 ± 0.5^{Aa}	4.88 ± 0.6^{Ab}	3.94 ± 0.5^{Aa}	4.18 ± 0.5^{Aa}	6.85 ± 0.7^{Aa}	0.83 ± 0.5^{Ab}	2.03 ± 0.2^{ACa}	0.37 ± 0.1^{Ab}
25	2.68 ± 0.6^{ABa}	2.54 ± 0.5^{Ba}	4.06 ± 0.5^{Aa}	5.22 ± 0.5^{Aa}	3.0 ± 0.9^{BCa}	2.62 ± 0.7^{Aa}	7.25 ± 0.7^{Ba}	1.55 ± 0.4^{Ab}
30	4.61 ± 0.5^{Ba}	3.11 ± 0.5^{ABa}	4.42 ± 0.5^{Aa}	4.63 ± 0.6^{Aa}	2.364 ± 0.8^{Ca}	3.89 ± 0.7^{Ba}	3.90 ± 0.6^{Aa}	1.95 ± 0.6^{Ab}
35	1.86 ± 0.6^{Aa}	3.74 ± 0.6^{ABb}	3.48 ± 0.5^{Aa}	4.49 ± 0.5^{Aa}	6.95 ± 0.9^{Aa}	3.03 ± 0.6^{Bb}	3.29 ± 0.5^{ACa}	0.23 ± 0.1^{Ab}
40	2.47 ± 0.6^{ABa}	4.49 ± 0.5^{ABb}	4.99 ± 0.6^{Aa}	1.79 ± 0.6^{Bb}	5.24 ± 0.7^{ABa}	4.96 ± 0.7^{Ba}	1.3 ± 0.4^{Ca}	2.6 ± 0.4^{Aa}

Different uppercase letters in the same column indicate significant differences between the same physiological parameter analyzed; different lowercase letters in the same row indicate significant differences between the same physiological parameter analyzed ($P < 0.05$)

are generated because the active metabolic rate remains unchanged (Jakubowska and Normant-Saremba 2016). According to Gilles and Delpire (1997), euryhaline crustaceans, as with the species studied here, may use one of the two mechanisms in the presence of changes in the osmolality of the external environment: (a) extracellular anisosmotic regulation, which is used by osmoregulators, maintaining the osmolality of the hemolymph reasonably constantly through mechanisms of secretion/absorption of salts through the branchial epithelium, and (b) intracellular isosmotic regulation, which controls the cellular volume via changes in the concentration of osmotic effectors, mainly the free amino acids. This mechanism is mainly used in osmoconformation, when anisosmotic extracellular regulation mechanisms are not efficient in the prevention of cell volume changes. Therefore, the synergy between salinity and reduced pH seems to have altered the efficiency of the mechanisms related to extracellular anisosmotic regulation in *C. danae*. This loss may be because transporters such as Na^+/H^+ are shared by osmoregulation and acid–base control. Henry and Cameron (1982) found that *C. sapidus* osmoregulates up to 800 mOsm but that such regulation is accompanied by blood alkalosis, probably due to changes in the antiporter Na^+/H^+. The shrimps *Palaemon elegans* and *P. serratus* maintain ionic homeostasis after exposure to hypercapnia, but complete acid–base compensation was observed after 30 days of exposure, suggesting that the ionic homeostasis was maintained at the expense of the acid–base balance (Dissanayake et al. 2010). Additionally, in *C. danae* the synergy between the reduced pH and salinity may have decreased the activity of the enzymes related to salt transport as Na^+/K^+-ATPase and V-ATPase. The functioning of these enzymes is dependent of the energy, and especially at the salinity of 20‰, the oxygen consumption was reduced, and may have affected the ATP production. Finally, the change from the osmoregulating to the osmoconforming pattern can be seen as a strategy where a decrease in energetically costly processes occurs to reallocate energy resources.

In *X. kroyeri*, the maintenance of the osmoconforming pattern suggests that changes in pH and salinity do not affect the cell volume and composition to the point of causing mortality. However, some degree of hyporegulation is observed at 40‰. Although most of the marine crustaceans are osmoconformers, *X. kroyeri* is different from most of the peneid shrimps which hyper-osmoregulates at salinities below its iso-osmotic point and hypo-osmoregulate at higher salinities [P. *aztecus, P. duorarum, P. setiferus*, and *P. vannamei* (Castille and Lawrence 1981); *Crangon crangon* (Cieluch et al. 2005); *Litopenaeus stylirostris* (Diaz et al. 2004)]. Therefore, hyporegulatory capacity of the *X. kroyeri* in 40‰ may be a common feature of peneids related salt secretion and compensation of the passive NaCl influx. While the osmoconformers *Maja sp* and *Cancer pagurus* die within a few hours after transferred from seawater to a dilute medium (Pequeux 1995), the osmoconforming pattern of the *X. kroyeri* can be an adaptative strategy because it can save energy with osmoregulatory mechanisms.

The hemolymph pH regulation is important to maintain oxygen supply because reductions in the pH of extracellular fluid can decrease the affinity of respiratory pigments for oxygen, the so-called Bohr effect. On the other hand, exposure to stressful salinities usually increases oxygen consumption in crustaceans due to the increase in energy demand with the osmoregulatory processes (Rosas et al. 2001; Curtis et al. 2013; Li et al. 2017). The increase in oxygen consumption in *C. danae* and *X. kroyeri* kept at the control pH in 40S may be related to the increase in energy expenditure with salt secretion mechanisms, which increased in both species relative to intermediate salinities (approximately 72% in *C. danae* and 170% in *X. kroyeri*). Although not clear, some studies have suggested that Na^+/K^+-ATPase activity, one of the major enzymes involved in osmoregulation, ranges from 3 to 40% of total energy expenditure, indicating considerable cost to the individual (Pannevis and Houlihan 1992; Leong and Manahan 1997).

The loss of the osmoregulatory capacity of *C. danae* at the reduced pH was accompanied by a decrease in oxygen consumption at 20 and 40‰, probably due to the Bohr effect. However, metabolic processes produce a high concentration of acids, and the reduction in oxygen consumption may be an interesting strategy in animals exposed to reduced pH. In *X. kroyeri*, reductions in oxygen consumption were observed at salinities where animals are not commonly found in nature (20, 35, and 40‰), suggesting that the interaction among salinities different from those of the animal's low-pH habitat alter oxygen uptake. Reductions in oxygen uptake after exposure to elevated pCO_2 also occur in marine invertebrates such as the cuttlefish *Sepia officinalis* (Gutowska et al. 2010) and the peanut worm *Sipunculus nudus* (Langenbuch and Pörtner 2002). In *Metapenaeus joyneri* exposed to hypercapnia, the reduction in metabolism was accompanied by a 30% reduction in swimming ability, suggesting a consequent decrease in the activity pattern.

Ammonia excretion is another mechanism that may be affected by exposure to reduced pH. Our findings show that compared to the animals kept at the control pH, *C. danae* (20 and 40‰) and *X. kroyeri* (20 and 35‰) exhibited reductions in ammonia excretion after exposure to a pH of 7.3. Reductions in nitrogen excretion may occur due to an elevated concentration of extracellular H^+, leading to competition with the NH_4^+ in the apical antiporter NH_4^+/Na^+ and also because basic proteins can be used as buffers, reducing the catabolism of these proteins into amino acids (Dissanayake et al. 2010; Hans et al. 2014; Fehsenfeld and Weihrauch 2016). Some studies report that a reduced pH can inhibit the NH_4^+/Na^+ antiporter, which could affect ammonia excretion and salt absorption (Henry and Cameron 1982; Blanchard et al. 1998), which coincides with the loss of the osmoregulatory capacity of *C. danae* exposed to 20‰ salinity. Similar to *C. danae* and *X. kroyeri*, in *M. magister*, exposure to hypercapnia causes reduction in ammonia excretion and oxygen consumption, and these results are attributed to a reduction in amino acid catabolism or a change in the type of nitrogen excreta (Hans et al. 2014).

The observed changes in ammonia excretion in *X. kroyeri* and *C. danae* kept at the control pH demonstrate the effect of salinity on this parameter. The increase in ammonia excretion in *C. danae* at 20‰ may be due to the use of free amino acids as osmotic effectors, as demonstrated in other decapods exposed to a diluted medium [*C. sapidus* (Mangum et al. 1976), *Dilocarcinus pagei* (Augusto et al. 2007), *Palaemon northropi* (Augusto et al. 2009)]. However, the increase in ammonia excretion in *C. danae* at the 40‰ salinity may be related to protein oxidation and the consequent generation of energy for physiological processes, such as hyporegulation. Logically, in *C. danae* and *X. kroyeri*, oxidation of proteins prevails as a source of energy. Reductions in the *O*/*N* ratio were observed in animals exposed to reduced salinity (Shinji et al. 2012) or to elevated pCO_2 (Langenbuch and Pörtner 2002). Previous nonpublished data from our laboratory show that *X. kroyeri* oxidizes proteins in the different seasons of the year, unlike *Aegla platensis*, which displays seasonal fluctuations in the type of oxidized energy substrate (Oliveira et al. 2007). Therefore, although lipid oxidation provides more energy, which is interesting in situations that challenge homeostasis, *C. danae* and *X. kroyeri* mainly use proteins as an energy substrate. This is possibly a species-specific response or strategy, since lipid oxidation consumes twice as much oxygen as protein.

In both *C. danae* and *X. kroyeri*, reductions occur in the hepatosomatic index in various combinations of reduced pH and salinity. These responses suggest an increase in the use of stored energy without equivalent replacement due to several factors, ranging from reductions in the feeding rate to excessive use of stored energy incompatible with the species-specific replenishment capacity. In the estuarine crabs *Cyrtograpsus angulatus* and *Neohelice granulata* (Longo and Díaz 2015) and in the prawn *Penaeus latisulcatus* (Sang and Fotedar 2004), a reduction occurs in the glycogen stored in the hepatopancreas when the animals are exposed to a diluted medium, suggesting an increase in energy expenditure with osmoregulation. Moreover, crustacean species that are more tolerant to hypercapnia maintain a higher hemolymph HCO_3, which can impact several physiological mechanisms, including the use and accumulation of stored energy (Portner et al. 2004; Heuer and Grosell 2014).

Conclusions

Mortality is not the only problem resulting from climate change. Biodiversity loss can also occur through reductions in growth and reproduction. In terms of physiological changes, the species studied here exhibited reductions in metabolism under some experimental conditions, and the direct consequence could be a reduction in the energy available for active mechanisms, which influences the entire biology of the species. In addition, a reduction occurred in the HSI in both species, albeit more markedly in *X. kroyeri*. If all these changes persist for a long time, impairment may occur to the biodiversity, perhaps accompanied by economic losses, as *C. danae* and *X. kroyeri* are important fishing species. On the other hand, the loss of the osmoregulatory capacity by *C. danae* can influence the species' distribution, leading it to concentrate in certain areas with more favorable salinities and to compete for resources with the populations established there. In general, further studies are needed to demonstrate the exact mechanisms related to the effects demonstrated here, as well as for related species.

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