Biochemical and Biophysical Research Communications 461 (2015) 475–480

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Acid–base balance and changes in haemolymph properties of the South African rock lobsters, *Jasus lalandii*, a palinurid decapod, during chronic hypercapnia

Jarred L. Knapp [a], Christopher R. Bridges [b], Janina Krohn [b], Louwrens C. Hoffman [a], Lutz Auerswald [a, c, *]

[a] Department of Animal Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[b] Institute of Metabolic Physiology, Heinrich-Heine-Universität, D-40225 Düsseldorf, Germany
[c] Branch: Fisheries Management, Department of Agriculture, Forestry and Fisheries, Roggebaai 8012, South Africa

ARTICLE INFO

Article history:
Received 23 March 2015
Available online 12 April 2015

Keywords:
Chronic hypercapnia
Molecular structure change
Haemoprotein haemocyanin
Acid-base balance
Ocean acidification

ABSTRACT

Few studies exist reporting on long-term exposure of crustaceans to hypercapnia. We exposed juvenile South African rock lobsters, *Jasus lalandii*, to hypercapnic conditions of pH 7.3 for 28 weeks and subsequently analysed changes in the extracellular fluid (haemolymph). Results revealed, for the first time, adjustments in the haemolymph of a palinurid crustacean during chronic hypercapnic exposure: 1) acid-base balance was adjusted and sustained by increased bicarbonate and 2) quantity and oxygen binding properties of haemocyanin changed. Compared with lobsters kept under normocapnic conditions (pH 8.0), during prolonged hypercapnia, juvenile lobsters increased bicarbonate buffering of haemolymph. This is necessary to provide optimum pH conditions for oxygen binding of haemocyanin and functioning of respiration in the presence of a strong Bohr Effect. Furthermore, modification of the intrinsic structure of the haemocyanin molecule, and not the presence of molecular modulators, seems to improve oxygen affinity under conditions of elevated pCO_2.

1. Introduction

Exposure to acute and chronic hypercapnia leads to various responses in the few different crustacean species investigated so far [1], making generalisation difficult and more research necessary. In marine crustaceans, some responses can be observed in the extracellular fluid. Haemolymph separates the animals' environment from the intracellular space and mediates external impacts before they affect cellular metabolism and oxygen supply of the cells. For example, tight control of the extracellular pH via regulation of acid-base balance is essential for oxygen supply and gas exchange [2]. Marine invertebrates are exposed to natural hypercapnia during events such as upwelling episodes, low oxygen events and – in the medium to long-term – ocean acidification (OA).

The West Coast rock lobster, *Jasus lalandii*, is a cold-water palinurid decapod supporting a valuable commercial fishery in South Africa and Namibia [3]. The species lives mainly along the West Coast of Southern Africa in the Benguela Current System in shallow coastal waters [4]. This environment is characterised by 1) frequent upwelling events, 2) periods of low-oxygen in some areas due to algal decay and bacterial respiration and is potentially threatened by 3) declining pH due to OA. During 1) and 2), the pH can drop to levels as low as 6.6 for several days [5]. These events have been forecast to become more frequent and severe due to OA [6,7]. Currently, global pH levels are decreasing at rates between 0.0014 and 0.0024 units yr^{-1} [7] and by the year 2300, a global pH of about 7.3 is expected [8]. Due to the already low pH levels in the Benguela Current system, extended periods of high acidity could occur earlier.

Chronic responses to hypercapnia are currently unknown for this species and, in fact, other palinurid decapods. In general, there is a lack of chronic studies on crustacean hypercapnia that exceed a few weeks [1]. The aim of the present study was therefore to elucidate the responses at the extracellular level in *J. lalandii* during true long-term exposure (28 weeks) to hypercapnia.

* Corresponding author. Branch: Fisheries Management, Department of Agriculture, Forestry and Fisheries, Roggebaai 8012, South Africa.
E-mail addresses: jarredmail@yahoo.com (J.L. Knapp), bridges@hhu.de (C.R. Bridges), janina.krohn@aol.com (J. Krohn), LCH@sun.ac.za (L.C. Hoffman), Lutz.Auerswald@gmail.com (L. Auerswald).

http://dx.doi.org/10.1016/j.bbrc.2015.04.025
0006-291X/

2. Materials and methods

2.1. Lobsters

J. lalandii juveniles were collected from oyster settlement plates of an offshore oyster farm in the Langebaan lagoon, Western Cape, South Africa. They were transported within ±2 h to holding tanks at the research aquarium in Cape Town in plastic bags filled to 50% with 4.5 l seawater, bubbled with oxygen before sealing. Bags were placed into a polystyrene container with ice bricks to ensure T_A below 20 °C. In Cape Town, they were maintained in flow through tanks for four months prior to experimentation (pH ranged from 7.9 to 8.1; T_A from 8.4 to 16.8 °C) and fed a mixed diet of mussel (*Chromomytilus meridionalis*; *Mytilus galloprovincialis*), sardines (*Sardinops sagax*) and Maasbunker (*Trachurus trachurus)* twice a week *ad libitum*. Feeding was discontinued three days before handling.

2.2. Hypercapnic exposure

Lobsters were weighed (*w*), carapace length (*CL*) measured and placed into individual perforated 750 ml plastic containers. Twelve individuals (mean ± S.E.; *w*: 2.3 ± 0.4 g; *CL*: 16.0 ± 0.8 mm) were placed into a 1000 l tank with normocapnic seawater and 12 individuals (*w*: 2.1 ± 0.3 g; *CL*: 15.5 ± 0.7 mm) into a 1000 l tank with hypercapnic seawater. Student's t-test revealed no difference between *w* and *CL* between the two groups. Tanks were well mixed and aerated. Lobsters were acclimatized for a week to around 18 °C after which the pH in one tank was lowered in two steps in five days from approximately 8.1 to 7.3 using a pH controller (7074/2, TUNZE, Germany) containing a solenoid valve (7074.111) and a pH electrode (7070.110) attached to a 9 kg CO_2 bottle (technical). Lobsters remained under these experimental conditions for 195 days (~28 weeks). Seawater (~80%) was exchanged with pre-conditioned seawater twice a week. Seawater parameters pH and T_A were measured five times a week, A_T and salinity twice weekly. These conditions are summarised in Table 1. Seawater pCO_2, HCO_3^- and CO_3^{2-} were calculated using measured pH, T_A, salinity and A_T [9] as constants in CO2SYS software [10], using dissociation constants refitted by Ref. [11] from Ref. [12] and KSO_4 from Ref. [13]. Lobsters were fed *ad libitum* five days a week, 3 days with pelleted feed (Nutrafin max, A6792U), the other two, fresh mussel (*Choromytilus meridionalis* and *Mytilus galloprovincialis*). Food and moults were removed and water quality monitored by measuring NH_4^+ concentration (Ammonia test kit, Sera, Germany). The latter never exceeded 0.4 mg l^{-1}.

2.3. Haemolymph sampling

At the time of haemolymph sampling, lobsters had the following size: Normocapnia: *w* 10.7 ± 0.8 g, *CL* 26.6 ± 0.7 mm; hypercapnia: *w* 9.6 ± 0.5 g, *CL* 25.6 ± 0.4 mm. Student's t-test revealed no differences in *w* and *CL* between the groups. Pre-branchial haemolymph (max. 0.3 ml) was extracted from the arthrodial membrane at the base of the fifth pair of pereiopods by syringe with hypodermic needle (Neomedic 1 ml, 29 G); avoiding tail flips by securing the abdomen with a firm grip. The sample was placed in a 0.5 ml Eppendorf tube for acid-base analysis or frozen at −80 °C for haemocyanin studies. Subsequently, the moult stage of each lobster was determined microscopically from setagenic stages of pleopods, based on a moulting stage range of AB, C, through to D_4 [14].

2.4. Haemolymph acid-base balance

pH was measured within 20 s after sampling using a Orion 3 star pH meter equipped with an Orion 8220 BNWP micro pH electrode (Thermo Scientific, USA). Calibration was performed with NBS precision buffers (Applichem, Germany) at the same temperature as that of ambient seawater of the lobster tanks. A haemolymph subsample (50 µl) was immediately injected into a de-gassing (magnetic stirrer) chamber containing 200 µl of 100 mM H_2SO_4 and liberated total CO_2 (cCO_2) determined by CO_2 analyser (SBA4, PP Systems, USA) using CO_2-free N_2 (technical) as carrier gas (50 ml min^{-1}) calibrated against freshly made $NaHCO_3$ standards (1–10 mM). From measured pH and cCO_2 values, pCO_2, and [HCO_3^-] were calculated using derivatives of the Henderson Hasselbalch equation (I and II). The required solubility coefficient αCO_2 and dissociation constant pK'_1 of carbonic acid were adopted as described previously for *Carcinus maenas* [15].

$$\text{I.}\quad pCO_2 = \frac{cCO_2}{10^{pH-pK'_1} \times \alpha CO_2 + \alpha CO_2}$$

$$\text{II.}\quad HCO_3^- = cCO_2 - \alpha CO_2 \times pCO_2$$

Ca^{2+}, Mg^{2+} (Diaglobal, Germany) and L-lactate concentrations (Roche, Germany) were determined by commercial kits on small subsamples from each individual. Haemocyanin concentration was determined spectrophotometrically (335 nm) in 1:50 haemolymph *vs. J. lalandii* Ringer solution (0.52 M NaCl, 0.015 M $MgSO_4$, 0.013 M $CaSO_4$, 0.005 M KCl, 0.005 M $NaHCO_3$, pH 7.8), calculated using an extinction coefficient $\varepsilon_{335} = 0.233$ ΔE units mg^{-1} ml^{-1} [16].

2.5. Oxygen affinity of haemocyanin

After transporting frozen samples to Düsseldorf, aliquots from each treatment were pooled due to their small individual volumes; subsequently the clotted haemolymph was re-suspended by means of a pestle, and centrifuged for 15 min at 17 100 g and 4 °C (Eppendorf, Germany). Aliquots (1.5 ml) from each pool were dialysed (1:1000 ratio haemolymph *vs*. Ringer) at 4 °C for 48 h against two changes of standard dialysis Ringer (0.017 M NaCl, 0.006 M $CaSO_4$, 0.003 M $MgSO_4$, 0.013 M KCl, and 0.012 M $NaHCO_3$, pH 7.8). Aliquots of full and dialysed haemolymph were transferred into 2 ml reaction tubes and centrifuged for 15 min at 13 000 rpm (4 °C). Subsequently, 200 µl supernatant was centrifuged (Airfuge ultracentrifuge, Beckman, USA) for 30 min at 160 000 g. The top 100 µl were removed and the remaining 100 µl re-suspended with the haemocyanin pellet, doubling the concentration of haemocyanin. From these preparations, oxygen affinity curves were established and analysed for whole and dialysed haemolymph using a spectrophotometric method on 5 µl haemocyanin samples in a diffusion chamber [17] modified as described previously [18,19]. The non-

Table 1
Seawater conditions recorded during exposure to normocapnic and hypercapnic conditions during a period of 28 weeks.

	Salinity (‰)	T (°C)	pH	A_T (µmol kg^{-1})	pCO_2 (Torr)	HCO_3^- (mmol l^{-1})	CO_3^{2-} (mmol l^{-1})
Normocapnia	34.4 ± 0.1	18.8 ± 0.1	8.02 ± 0.02	1903 ± 8	0.27 ± 0.01	1.58 ± 0.02	0.14 ± 0.01
Hypercapnia	34.4 ± 0.0	18.8 ± 0.1	7.32 ± 0.01	1925 ± 7	1.57 ± 0.03	1.88 ± 0.01	0.04 ± 0.00

Values are given as means ± S.E.

Table 2
Haemolymph parameters of juvenile lobsters after exposure to normocpanic and hypercapnic conditions for 28 weeks.

Haemolymph parameter	pH	cCO_2 (mmol l^{-1})	pCO_2 (Torr)	HCO_3^- (mmol l^{-1})	Ca^{2+} (mmol l^{-1})	Mg^{2+} (mmol l^{-1})	L-lactate (mmol l^{-1})	Haemocyanin (mg ml^{-1})
Normocapnia	7.760 ± 0.025	4.2 ± 0.4	1.7 ± 0.2	4.1 ± 0.4	12.8 ± 0.8	17.5 ± 0.6	0.4 ± 0.1	22.3 ± 2.0
Hypercapnia	7.655 ± 0.061	8.5 ± 1.2*	3.9 ± 0.2*	8.4 ± 1.2*	9.2 ± 0.6*	19.4 ± 1.5	0.3 ± 0.1	13.9 ± 2.1*

Values are mean ± S.E. (n = 12). *denotes significant difference to normocapnic value ($p < 0.05$, Student's t-test).

bicarbonate buffer capacity was calculated from carbon dioxide titration of 50 µl aliquots in a Radiometer BMS II system (Radiometer, Denmark), used to measure haemolymph pH at carbon dioxide tensions provided by gas mixing pumps (Wöstoff, Germany).

3. Results

3.1. Haemolymph acid-base balance

Measured pH in the haemolymph from hypercapnic incubated lobsters was lower than in the normocapnic group, whereas cCO_2 had doubled to 8.5 mM under hypercapnia compared with normocapnia (Table 2). Accordingly, calculated pCO_2 and $[HCO_3^-]$ were 3.9 Torr compared to 1.7 Torr and bicarbonate 8.4 mM compared to 4.1 mM under normocapnia, respectively. $[Ca^{2+}]$ was significantly lower (28%) under hypercapnic conditions, whereas $[Mg^{2+}]$ changes were slight and not significant (Table 2). Lactate concentrations were relatively low at 0.4 to 0.3 mM in both groups. pH and $[HCO_3^-]$ values were similar within the normocapnic group and most values below the 2 Torr isopleth (Fig. 1A). They varied considerably, however, in both pH and $[HCO_3^-]$, in the hypercapnic group and are assembled along the 4 Torr pCO_2 isopleth with no value below the 2 Torr isopleth (Fig. 1B). The non-bicarbonate buffer line of haemolymph from the hypercapnic group is elevated and shifted parallel compared with that of the normocapnic lobsters (Fig. 1C). At the same time, haemocyanin concentration in the haemolymph decreased by 38% under hypercapnic exposure indicating a decrease in oxygen carrying capacity.

3.2. Oxygen affinity of haemocyanin

In Fig. 2 oxygen affinity is depicted in whole blood and dialysed (Fig. 2A) and dialysed blood (Fig. 2B) together with cooperativity measured as n50. The slope of the regression lines indicate the Bohr coefficient, -0.42 in normocapnic whole haemolymph and higher at −0.66 in hypercapnic whole haemolymph. Dialysis had no effect on the Bohr coefficient of hypercapnic or normocapnic haemolymph (Fig. 2A).

Oxygen binds cooperatively to *J. lalandii* haemocyanin. Maximal cooperativity (Hill coefficient $n_{50} = 4.15$) was found in dialysed haemolymph from normocapnic lobsters but was marginally smaller ($n_{50} = 3.92$) in dialysed haemolymph from hypercapnic lobsters. Cooperativity was lower in both whole haemolymph samples, again with that of hypercapnic haemolymph slightly lower ($n_{50} = 3.22$) than in normocapnic haemolymph.

A full oxygen saturation curve was constructed using the haemocyanin from the juvenile lobsters that were exposed to normocapnia, using a 0.1% CO_2 to set pH. The curve is described by the equation: $y = -2E{-}08x^5 + 7E{-}06x^4 - 0.0006x^3 + 0.0141x^2 - 0.0846x + 0.152$ ($R^2 = 0.9957$, pH = 7.216, 15 °C) (not shown here).

3.3. Moult stage

Each experimental group consisted of a cohort of lobsters that were in a similar range of moult stages. In the normocapnic group, two lobsters were at stage AB, three at C, two at D_0, four at D_1 and one at D_4. In the hypercapnic group, three lobsters were at stage AB, five at C, three at D_1 and one at D_2.

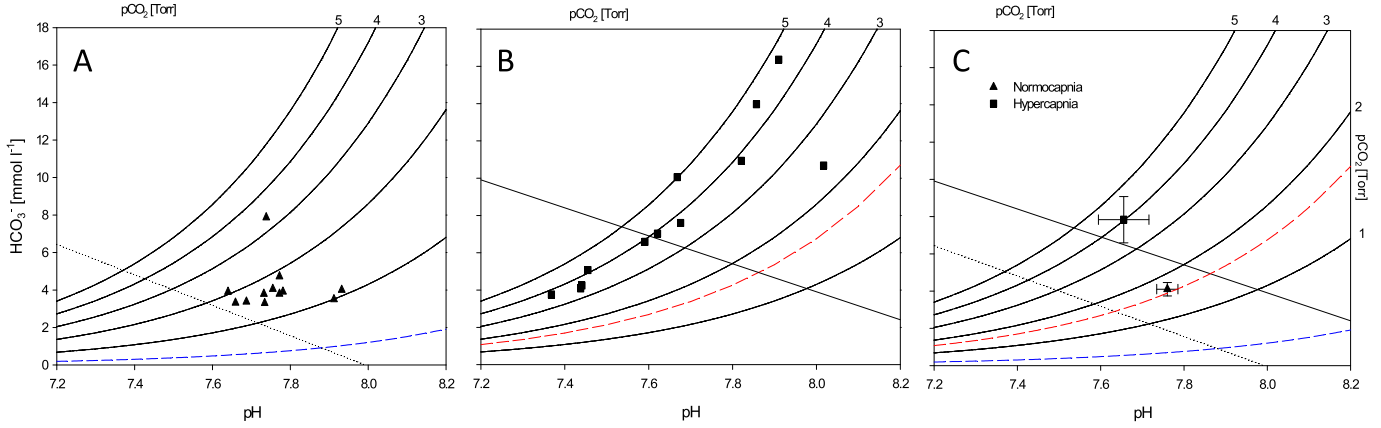

Fig. 1. Henderson-Hasselbalch (pH-bicarbonate) diagrams for haemolymph of juvenile lobsters incubated for 28 weeks. (A) Individual values from 12 lobsters exposed to normocapnic conditions, (B) individual values from 12 lobsters exposed to hypercapnic conditions and (C) mean ± S.E. from values depicted in (A) and (B). pCO_2 isopleths were derived from the Henderson-Hasselbalch equation. Values for the first dissociation constant (pK'_1) and Solubility coefficient (αCO_2) were derived from Ref. [23] and were: $pK'_1 = 6.01$, $\alpha CO_2 = 0.044$ (18 °C). Blue dashed line = normocapnic seawater isopleth, red dashed line = hypercapnic seawater isopleth. Dotted black line = normocapnic non-bicarbonate buffer line, solid black line = hypercapnic non-bicarbonate buffer line (see also materials and methods). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

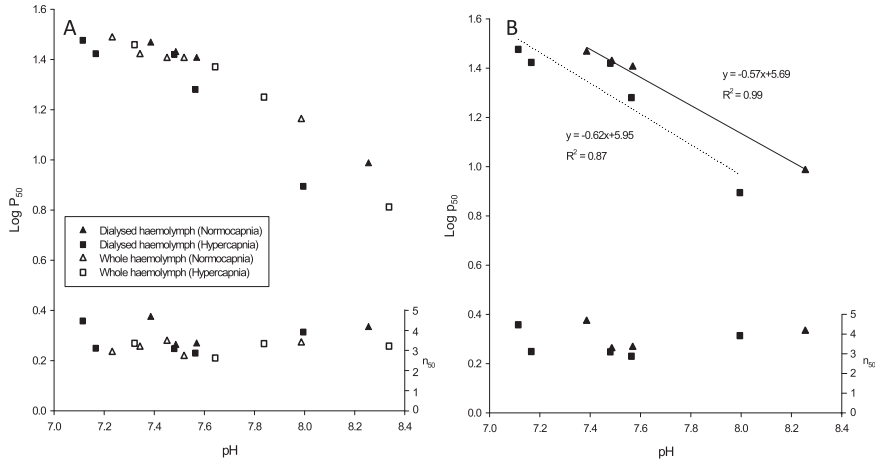

Fig. 2. pH-dependence of oxygen affinity of haemocyanin from whole and dialysed haemolymph from juvenile lobsters incubated for 28 weeks in normocapnic and hypercapnic seawater, respectively. (A) Relationship of haemocyanin-oxygen affinity as log P_{50} and pH (upper part and left y-axis) and cooperativity of haemocyanin-oxygen binding and pH (lower part and right y-axis) for whole blood. (B) Bohr effect (upper part) and cooperativity (lower part) of haemocyanin from dialysed haemolymph only from juvenile lobsters after normocapnic and hypercapnic treatment, respectively.

4. Discussion

Our study reveals, for the first time, adjustments in the haemolymph acid-base balance of a palinurid crustacean during chronic hypercapnic exposure. During 28 weeks of exposure to a water pH level of about 7.3, the following changes in the haemolymph of juvenile *J. lalandii* occurred:

1. Adjustment of the acid-base balance by increased bicarbonate
2. A change in quantity and oxygen binding properties of haemocyanin

4.1. Regulation of acid-base balance

Extracellular pCO_2 is maintained above environmental pCO_2 to ensure an outward gradient for CO_2 removal [20,21] whereas oxygen affinity has to be safeguarded in the presence of a pronounced Bohr shift in *J. lalandii* haemocyanin (Fig. 2B). Under hypercapnia, this requires extra buffering achieved by bicarbonate and non-bicarbonate buffers [22]. In adult *J. lalandii*, acute environmental hypercapnia causes acidosis driven by CO_2 increase but is adjusted by increased [HCO_3^-] within 5 h (*unpublished data*). Here, we show that a secondary [HCO_3^-] increase (i.e. above the non-bicarbonate buffer line) is present as chronic adjustment of extracellular pH in juveniles. Compared with lobsters kept in normocapnic seawater, those under hypercapnic conditions doubled their [HCO_3^-]. Non-bicarbonate buffer capacity, however, is almost exclusively achieved by haemocyanin in crustaceans [23], whose concentration decreased under hypercapnia here. This may be compensated by an increase of other non-bicarbonate buffer substances, such as proteins or inorganic phosphate. Variation in [HCO_3^-] between individuals is large in the hypercapnic group (Fig. 1B), whereas it is small in normocapnic individuals (Fig. 1A). The values for hypercapnic lobsters spread along the 4 Torr pCO_2 isopleth, suggesting that individual differences in pH were primarily due to differences in [HCO_3^-]. Individual [HCO_3^-] values are not correlated to [Ca^{2+}] (not shown), indicating, as does the spread of moult stages in both groups, that this is not a moult effect. Data suggest that, after non-respiratory compensation, hypercapnic juveniles had reached a steady state at a more acidic level (i.e. only partial compensation) after exposure, similar to other crustacean species [21], and maintained it thereafter. In other crustaceans, an elevated [HCO_3^-] could not be maintained over an extended period, see Ref. [1]. The pCO_2 data for water and haemolymph from Tables 1 and 2 show that the CO_2 gradient across the gills increases from 1.4 Torr to 2.3 Torr, changing from normocapnia to hypercapnia. This could indicate that metabolism also increased during hypercapnic exposure as would energetically be expected to fuel the ionic pumping of bicarbonate from sea water.

4.2. Properties of haemocyanin

Fig. 3 summarizes the calculated changes in haemolymph oxygen affinity together with *in vivo* pH changes. In whole blood P_{50} remains relatively constant even though haemolymph pH drops by 0.1 units. Without compensation P_{50} would decrease by

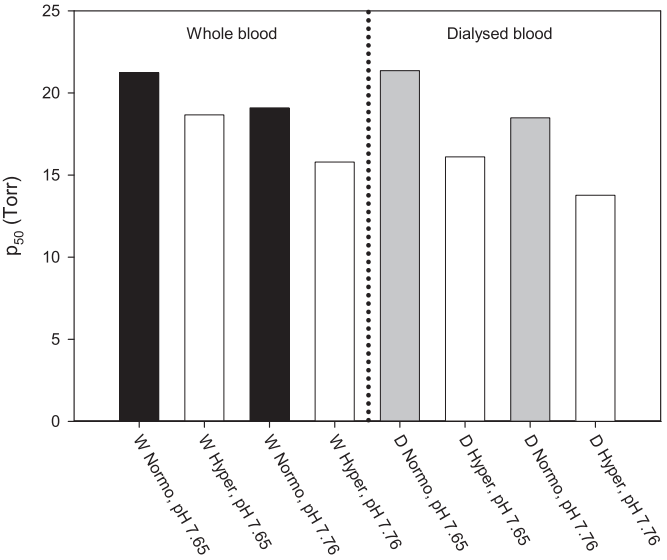

Fig. 3. Calculated P_{50} values for respective treatments at *in vivo* pH conditions. Values were calculated for the different pH values using the regression equations shown in Fig. 2. *In vivo* pH values (indicated in column label) were taken from Table 2. Labels: W = whole blood, D = dialysed blood, Normo = normocapnia, Hyper = hypercapnia.

Table 3
Comparison of oxygen affinity (P_{50}) of haemocyanin from different crustacean species measured at a specific pH and temperature.

Species	P_{50} (Torr)	pH	T (°C)	Source	Whole (W)/ dialysed (D)
juvenile West Coast rock lobster (Jasus lalandii)	14.8	8.0	15	current study	W
adult West Coast rock lobster (*Jasus lalandii*)	14.1	7.8	15	unpublished data	W
European spiny lobster (*Palinurus elephas*)	6.5	8.0	20	[25]	D
(Australian) Spiny rock lobster (*Jasus edwarsii*)	25.8	7.8	20	[28]	W
European Lobster (*Homarus vulgaris*)	9.3	7.9	15	[32]	W
Intertidal prawn (*Palaemon elegans*)	9.0	7.8	10	[19]	W
European shore crab (*Carcinus maenas*)	10.9	7.8	15	[33]	W

approximately 2.5 Torr. Since calcium levels are 28% lower in the hypercapnic group and decreases in calcium will lead to a lowering of oxygen affinity [24,25], one would expect an even greater change to the detriment of oxygen transport. The role of other co-factors such as lactate [2] can be ruled out since these levels are similar in both normocapnia and hypercapnia. For dialysed blood at constant levels of Mg^{2+} and Ca^{2+}, it is clear from Fig. 3, that an increase in intrinsic affinity by almost 5 Torr occurred. Haemocyanin concentration was low in normocapnic juveniles compared with adult lobsters (70 mg ml^{-1}, *unpublished data*). Such differences between life- and moult stages, however, are not uncommon [26,27]. In terms of oxygen affinity, haemocyanin of juvenile and adult *J. lalandii* is similar (Table 3). Compared with other palinurids, affinity of *J. lalandii* haemocyanin (P_{50} ~14 Torr) is much lower than the very high affinity of the European spiny lobster, *Palinurus elephas* ($P_{50} = 6.5$ Torr in dialysed blood, using Tris-Buffers, [25] and, taking into account the difference in incubation temperature, should be in the low affinity range as its Australian relative *Jasus edwardsii* ($P_{50} = 26$ Torr, [28]). Oxygen affinity is generally higher in hypoxic environments [29] and the difference between the three palinurid species could therefore be an expression of the oxygen availability in their respective environments. Compared with crustacean species that live in shallow waters or in the intertidal, haemocyanin of juvenile *J. lalandii* has a low oxygen affinity, an indication that these juveniles are normally exposed to well-oxygenated seawater.

Haemocyanin was termed the “interface” of crustacean physiology due to its molecular and biochemical flexibility [2]. This flexibility is present in *J. lalandii* haemocyanin: Dialysed haemocyanin from juvenile lobsters kept in hypercapnic seawater for 28 weeks had an increased oxygen affinity compared with that from normocapnic juveniles. A possible explanation is a modification of the intrinsic structure of the haemocyanin molecule [2,30] and this could be a mechanism to conserve energy under environmental stress. Also, such structure change could explain the observed increase in oxygen affinity [31].

In hypercapnic conditions, a decline of pH by 0.1 units (27% acidification) was observed. It is probably not energetically possible to maintain constant ‘normal’ pH in the long term. The lower pH could also just be a result of the increased extracellular pCO_2. The latter ensures a gradient for removal of respiratory CO_2 under hypercapnia which becomes necessary when environmental pCO_2 almost reaches normocapnic extracellular pCO_2 levels (1.6 *vs* 1.7 Torr). There is also a potential influence of molecular modulators on haemocyanin properties: The reduced [Ca^{2+}] may be due to energetic costs, too, or as a result of calcification processes in the exoskeleton. The slightly elevated [Mg^{2+}], meanwhile, may ensure an increased Bohr coefficient and oxygen affinity of haemocyanin [24]. In the crab *C. maenas*, Ca^{2+} increases oxygen affinity whereas Mg^{2+} increases oxygen affinity AND Bohr coefficient [24]. Protecting the Bohr shift guarantees loading of haemocyanin with oxygen at the gills and unloading in the tissues.

Although it was not the aim of this study to investigate the effect of the various molecular modulators of oxygen affinity in detail, the differences between full and dialysed haemolymph show that oxygen affinity is not decreased in hypercapnic juvenile lobsters when these modulators are removed. Cooperativity values were similar in all treatments.

In conclusion, we have shown that, to ensure functioning of respiration during prolonged hypercapnia, juvenile lobsters are capable of bicarbonate buffering of their haemolymph to provide optimum pH conditions for oxygen binding in the presence of a strong Bohr Effect. In addition, modification of the intrinsic structure of the haemocyanin molecule, and possibly the presence of molecular modulators, seems to improve oxygen affinity under conditions of elevated pCO_2.

Conflict of interest

None.

Acknowledgments

The project was financially supported by the NRF of South Africa (grant 71239 to L.A.), the IB of the BMBF of Germany (grant SUA 09/009 to C.R.B.) and student support by Stellenbosch University (J.L.K.). We are grateful to Mr T. Tonen and staff from Saldanha Bay Oyster Company for opportunity and help to collect lobsters from oyster rafts and to Mr A. Busby and team from the Seapoint research aquarium for support.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.025.

References

[1] N.M. Whiteley, Physiological and ecological responses of crustaceans to ocean acidification, Mar. Ecol. Prog. Ser. 430 (2011) 257–271.
[2] C.R. Bridges, Modulation of haemocyanin oxygen affinity: properties and physiological implications in a changing world, J. Exp. Biol. 204 (2001) 1021–1032.
[3] R. Melville-Smith, L. van Sittert, Historical commercial West Coast rock lobster *Jasus lalandii* landings in South African waters, Afr. J. Mar. Sci. 27 (2005) 33–44.
[4] L.B. Holthuis, An annoted and illustrated catalogue of species of interest to fisheries known to date, Marine Lobsters of the World, in: FAO Species Catalogue, vol. 13, 1991, pp. 1–292.
[5] G.C. Pitcher, T.A. Probyn, Red tides and anoxia: an example from the southern Benguela current system, in: P. Pagou, G. Hallegraeff (Eds.), 14th Int. Conf. Harmful Algae, International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, 2010, pp. 175–177.
[6] A. Bakun, Global climate change and intensification of coastal ocean upwelling, Science 247 (1990) 198–201.

[7] M. Rhein, S.R. Rintoul, S. Aoki, et al., Observations: ocean, in: T.F. Stocker, D. Qin, G.-K. Plattner, et al. (Eds.), Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge and New York, 2013, pp. 255–315.
[8] K. Caldeira, M.E. Wickett, Oceanography: anthropogenic carbon and ocean pH, Nature 425 (2003) 365.
[9] G. Sarazin, G. Michard, F. Prevot, A rapid and accurate spectroscopic method for alkalinity measurements in sea water samples, Water Res. 33 (1999) 290–294.
[10] D. Pierrot, E. Lewis, D.W.R. Wallace, MS Excel Program Developed for CO_2 System Calculations, ORNL/CDIAC-105a, US Dept. Energy, Oak Ridge, Tennessee, 2006.
[11] A.G. Dickson, F.J. Millero, A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, Deep Sea Res. 34A (1987) 1733–1743.
[12] C. Mehrbach, C.H. Culberson, J.E. Hawley, R.M. Pytkowicx, Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure, Limnol. Oceanogr. 18 (1973) 897–907.
[13] A.G. Dickson, Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K, Deep Sea Res. 37A (1990) 755–766.
[14] H.G. Marco, The moult cycle of *Jasus lalandii* (H. Milne Edwards, 1837) (Decapoda, Palinuridae), a cold water spiny lobster species: changes in ecdysteroid titre and setogenesis, Crustaceana 85 (2012) 1221–1239.
[15] J.P. Truchot, Carbon dioxide combining properties of the blood of the shore crab Carcinus maenas (L): carbon dioxide solubility coefficient and carbonic acid dissociation constants, J. Exp. Biol. 64 (1976) 45–57.
[16] K.W. Nickerson, K.E. van Holde, A comparison of molluscan and arthropod hemocyanin - I. Circular dichroism and absorption spectra, Comp. Biochem. Physiol. 39B (1971) 855–872.
[17] H. Sick, K. Gersonde, Method for continuous registration of O_2- binding curves of hemoproteins by means of a diffusion chamber, Anal. Biochem. 32 (1969) 362–376.
[18] C.R. Bridges, J.E.P.W. Bicudo, G. Lykkeboe, Oxygen content measurement in blood containing haemocyanin, Comp. Biochem. Physiol. 62A (1979) 457–462.
[19] C.R. Bridges, S. Morris, M.K. Grieshaber, Modulation of haemocyanin oxygen affinity in the intertidal prawn *Palaemon elegans* (Rathke), Respir. Physiol. 57 (1984) 189–200.
[20] R.P. Henry, J.N. Cameron, The role of carbonic anhydrase in respiration, ion regulation and acid-base balance in the aquatic crab *Callinectes sapidus* and the terrestrial crab *Gecarcinus lateralis*, J. Exp. Biol. 103 (1983) 205–223.
[21] J.N. Cameron, Acid-base equilibrium in invertebrates, in: N. Heisler (Ed.), Acid-base Regulation in Animals, Elsevier, Michigan, 1986, pp. 357–394.
[22] F. Melzner, M.A. Gutowska, M. Langenbuch, et al., Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosci. Discuss. 6 (2009) 4693–4738.
[23] J.P. Truchot, Carbon dioxide combining properties of the blood of the shore crab, *Carcinus maenas* (L.): CO_2-dissociation curves and Haldane effect, J. Comp. Physiol. 112B (1976) 283–293.
[24] J.P. Truchot, Factors controlling the *in vitro* and *in vivo* oxygen affinity of the hemocyanin in the crab, Respir. Physiol. 24 (1975) 173–189.
[25] A. Wänke, Ca^{2+}, Mg^{2+} und Urat: Modulatoren des Hämocyanins der Europäischen Languste (*Palinurus elephans*), Heinrich-Heine-Universität, Düsseldorf, 2008, pp. 1–119. PhD thesis.
[26] J.S. Djangmah, The effects of feeding and starvation on copper in the blood and hepatopancreas, and on blood proteins of *Crangon vulgaris* (Fabricius), Comp. Biochem. Physiol. 32 (1970) 709–731.
[27] J.-C. Chen, S. Cheng, Studies on haemocyanin and haemolymph protein levels of *Penaeus japonicus* based on sex, size and moulting cycle, Comp. Biochem. Physiol. 106B (1993) 293–296.
[28] S. Morris, S. Oliver, Respiratory gas transport, haemocyanin function and acid-base balance in *Jasus edwardsii* during emersion and chilling: simulation studies of commercial shipping methods, Comp. Biochem. Physiol. 122A (1999) 309–321.
[29] W. Burggren, B. McMahon, D. Powers, Environmental and metabolic animal physiology, in: C.L. Prosser (Ed.), Environmental and Metabolic Animal Physiology, fourth ed., Wiley-Liss, Illlinois, 1991, pp. 437–508.
[30] J.P. Truchot, Respiratory function of arthropod hemocyanins, in: C.P. Mangum (Ed.), Blood and Tissue Oxygen Carriers, thirteenth ed., Springer, Berlin Heidelberg, 1992, pp. 377–404.
[31] L.E. Burnett, Integrated function of the respiratory pigment hemocyanin in crabs, Am. Zool. 32 (1992) 438–446.
[32] J.Y. Bouchet, J.P. Truchot, Effects of hypoxia and L-lactate on the haemocyanin-oxygen affinity of the lobster, *Homarus vulgaris*, Comp. Biochem. Physiol. 80A (1985) 69–73.
[33] F. Lallier, J.P. Truchot, Hemolymph oxygen transport during environmental hypoxia in the shore crab, *Carcinus maenas*, Respir. Physiol. 77 (1989) 323–336.