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ORIGINAL PAPER

Tolerance of *Hyas araneus* zoea I larvae to elevated seawater PCO_2 despite elevated metabolic costs

Melanie Schiffer · Lars Harms · Hans O. Pörtner · Magnus Lucassen · Felix C. Mark · Daniela Storch

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Abstract Early life stages of marine crustaceans respond sensitively to elevated seawater PCO_2. However, the underlying physiological mechanisms have not been studied well. We therefore investigated the effects of elevated seawater PCO_2 on oxygen consumption, dry weight, elemental composition, median developmental time (MDT) and mortality in zoea I larvae of the spider crab *Hyas araneus* (Svalbard 79°N/11°E; collection, May 2009; hatch, December 2009). At the time of moulting, oxygen consumption rate had reached a steady state level under control conditions. In contrast, elevated seawater PCO_2 caused the metabolic rate to rise continuously leading to a maximum 1.5-fold increase beyond control level a few days before moulting into the second stage (zoea II), followed by a pronounced decrease. Dry weight of larvae reared under high CO_2 conditions was lower than in control larvae at the beginning of the moult cycle, yet this difference had disappeared at the time of moulting. MDT of zoea I varied between 45 ± 1 days under control conditions and 42 ± 2 days under the highest seawater CO_2 concentration. The present study indicates that larval development under elevated seawater PCO_2 levels results in higher metabolic costs during premoulting events in zoea I. However, *H. araneus* zoea I larvae seem to be able to compensate for higher metabolic costs as larval MDT and survival was not affected by elevated PCO_2 levels.

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M. Schiffer (✉) · L. Harms · H. O. Pörtner · M. Lucassen · F. C. Mark · D. Storch
Department Integrative Ecophysiology, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
e-mail: Melanie.Schiffer@awi.de

Introduction

Since colder seawater displays higher solubility for CO_2, it has been predicted that the largest pH changes due to absorption of atmospheric CO_2 over the twenty-first century will occur in the Arctic Ocean. Furthermore, the Arctic Ocean is predicted to become increasingly undersaturated with respect to aragonite and calcite in the coming decades (Steinacher et al. 2009), which might especially affect calcifying Arctic species (Lischka et al. 2010).

Various effects of elevated PCO_2 on the physiology of marine organisms have already been described. Affected processes include calcification, but also acid–base regulation and metabolism, growth and reproduction (Melzner et al. 2009). It is known that elevated seawater PCO_2 leads to decreasing extracellular pH in crustaceans (Cameron 1978; Wheatly and Henry 1992; Pane and Barry 2007). There is also evidence that an uncompensated drop in extracellular pH has a depressing effect on aerobic energy metabolism of some tissues like muscle (Reipschläger and Pörtner 1996) and isolated liver cells (Langenbuch and Pörtner 2003), through effects on the mode and rate of proton equivalent ion exchange (Pörtner et al. 2000) and a decrease in protein synthesis (Langenbuch et al. 2006). This may result in decreasing whole animal oxygen consumption (Pörtner et al. 1998; Michaelidis et al. 2005) or, if compensated for by the rise in energy demanding processes (e.g. calcification), an increase in whole organism oxygen demand (Thomsen and Melzner 2010, Lannig et al. 2010; Stumpp et al. 2011) and an associated shift in energy budgets. So far, it is not known to what extent these patterns also hold for early life stages of crustaceans, which have to allocate considerable amounts of energy to growth and morphological changes and may be the more sensitive life stages under ocean acidification (Walther et al. 2010).

Therefore, assessing energy turnover during larval development under elevated seawater PCO_2 may help to predict possible consequences of ocean acidification for this marine group.

Adult crustaceans seem to be relatively tolerant of ocean acidification, presumably due to an expansive ability to compensate for acid–base disturbances. In two efficiently ion-regulating prawn species, extracellular acidosis was fully compensated after 30 days of exposure to a PCO_2 of 3,000 µatm (Dissanayake et al. 2010). Also poor ion-regulators like the decapod species *Necora puber* and *Cancer magister* were able to recover their haemolymph pH over 24 h at a seawater PCO_2 of 8,000 µatm (Pane and Barry 2007, Spicer et al. 2007). However, crustacean species adapted to a stable environment like the deep sea seem to be unable to compensate haemolymph acid–base disturbances during hypercapnia and might therefore be especially vulnerable to ocean acidification (Pane and Barry 2007).

While early life stages of crustaceans may be more sensitive to high seawater PCO_2 levels than adults, the physiological background of their sensitivity has not been investigated. An increase in larval mortality and a decrease in hatching success under elevated seawater PCO_2 have been observed in two species of marine copepods (Kurihara et al. 2004). Other crustacean larvae showed altered growth and calcification rates at high seawater CO_2 concentrations at 1,200–3,000 µatm (Arnold et al. 2009, Walther et al. 2010, 2011). Growth rates were also reduced in early life stages of the barnacle *Semibalanus balanoides* reared at 1,000 µatm (Findlay et al. 2010). In larvae from two populations of the spider crab *Hyas araneus*, extended development times in zoea I and zoea II were recorded at low temperatures of 3 °C and the highest CO_2 treatment of 3,000 µatm CO_2, while elevated PCO_2 values reduced larval growth in megalopa but not in zoea I and zoea II (Walther et al. 2010). By sampling once per larval stage, the authors concluded that the megalopa stage might be a physiologically sensitive bottleneck. However, total larval development was delayed and the megalopa stage reached with some time delay under elevated CO_2. Larval weight, elemental composition and energy demand vary with age (Anger et al. 1989) within and between each life stage (Arnold et al. 2009; Walther et al. 2010, 2011). Developmental timing events may also vary between CO_2 treatments, and therefore several time points should be used for such comparisons to avoid misleading conclusions (Pörtner et al. 2010).

Development in crustaceans is characterised by regular moulting events including metamorphosis. From moult to moult, the larvae undergo growth and/or morphological changes (Anger 1983), which are accompanied by changing rates of oxygen consumption depending on larval stage, day of development and, accordingly, day within the moulting cycle (Anger et al. 1989; Anger 2001). Metabolic requirements of crustacean larval development with respect to abiotic factors like temperature and salinity have been studied in detail (Jacobi and Anger 1985; Anger et al. 1998). However, ongoing ocean acidification may entail new challenges for successful development. During their complex life cycle, crustaceans face variable environmental conditions. The first larval stages (zoea larvae) are pelagic and most of them are actively swimming in the upper water column. The megalopa larvae mark the transition from the pelagic to the benthic mode of life. Since Arctic surface waters are predicted to undergo larger pH changes than other oceanic regions (Steinacher et al. 2009), the early larval stages of Arctic crustaceans could be among the first to show significant effects of ocean acidification.

We therefore investigated the effect of elevated CO_2 levels on energy turnover during development of zoea I of the spider crab *H. araneus* from an Arctic population of Svalbard (Norway). *H. araneus* is a continental shelf species, which inhabits rocky, sandy and muddy bottoms and has a wide distribution range from the temperate southern North Sea to subarctic waters (Christiansen 1969). Broad knowledge exists on its larval development depending on several abiotic conditions (Anger 2001; Walther et al. 2010, 2011). This makes it an adequate model organism to study the effects of elevated seawater PCO_2 on the development of crustacean larvae. Ovigerous females carry the egg masses for approximately 2 years (Petersen 1995). After the embryonic phase, zoea I hatch from the eggs and are released into the water column. Larvae undergo two zoeal stages, and one megalopa stage before settling as juveniles in the adult habitat. We investigated potential changes in sensitivity and the specific patterns of physiological response during the time course of the first larval stage, the zoea I. We followed the course of oxygen consumption, weight and elemental composition in developing zoea I larvae of Arctic *H. araneus* and determined mortality and median developmental time (MDT) under different CO_2 levels. With our data, we have been able to distinguish age-related effects from the effects of elevated CO_2 levels.

Materials and methods

Larval collection and maintenance

Ovigerous females of Arctic *H. araneus* were collected in Kongsfjorden (Ny Alesund, Svalbard 78°55 N, 11°56 E) by scientific divers during May 2009 and transferred to the Alfred Wegener Institute in Bremerhaven. They were

maintained in flow-through aquaria at 4 °C, which corresponds to the seawater temperature in the Kongsfjorden during summer, 32 psu and a constant dark/light cycle (12 h:12 h). Four females were placed individually in 2 l flow-through aquaria shortly before larval hatching. Larvae hatched during December 2009. Equal numbers of newly hatched larvae of the four females were pooled and subsequently transferred into 0.5 l culture vessels at a density of 30 individuals per vessel. Experiments were conducted with zoea I larvae that had hatched within 24 h. They were reared in 27 culture vessels, respectively, filled with seawater of different CO_2 concentrations (27 culture vessels for each CO_2 treatment: 490 µatm, 1,100 µatm, 2,400 µatm CO_2) at a constant temperature of 6.2 ± 0.1 °C. Out of these 27 vessels, three vessels were used for mortality observation and developmental time, and individuals from the remaining 24 vessels were used for oxygen consumption, dry weight and C:N ratio measurements. Seawater was provided from reservoir tanks (60 l) kept at the same temperature as culture vessels and bubbled with air/CO_2 mixture using a mass flow controller (HTK 6 channel, HTK Hamburg GmbH, Germany). Water in culture vessels was changed daily, and dead larvae and moults were removed. Larvae were fed daily ad libitum with freshly hatched *Artemia* sp. (Sanders Brine Shrimp Company, Ogden, Utah, USA). Days of development and age of larvae are given as days post hatching (dph). Water physicochemistry was monitored weekly by determinations of pH and the collections of water samples for the determination of dissolved inorganic carbon (DIC) and total alkalinity. Water PCO_2 was calculated from DIC and pH_{NBS} using the program CO_2 SYS (Lewis and Wallace 1998, Table 1).

Mortality and median developmental time (MDT)

Three culture vessels per CO_2 concentration (of about 30 larvae each) were used for investigating the effect of elevated CO_2 on the mortality and MDT of the first larval stage. Mortality and moulting (number of zoea II) were recorded on a daily basis until all larvae were either dead or moulted into the zoea II. Dead larvae and zoea II were removed. Larval daily mortality and total mortality were calculated for each vessel and expressed as percentage. For larval daily mortality, the relative (percentual) proportion refers to number of zoea I remaining after each day. Culture vessels were used as replicates, and total mortality was determined as means (±SE) of the three replicates.

Median developmental time was calculated according to Landry (1983). Age was measured from the day of development the larvae started hatching, and MDT is defined as the time when 50 % of the zoea I larvae have moulted to zoea II. It was estimated from least-square regressions of the cumulative proportion of all larvae that had not yet passed the zoea I stage plotted against time. MDT was obtained from the exponential part of the moulting curve, and data below the 40 % moulting level were left out. MDT for larvae of corresponding seawater CO_2 concentration was determined as means of the three replicates. Moulting range is given as first day when zoea I started moulting until the day when the last zoea I moulted to the second stage.

Oxygen consumption

Oxygen consumption rates of individual zoea I were measured according to Storch et al. (2009). Hamilton high-precision microlitre syringes (500 µl, Hamilton-Bonaduz, Switzerland) were used as closed respiratory chambers. Oxygen saturation was recorded by oxygen micro-optodes (needle-type NTH-PSt1-L5-TF-NS55x0,80-PC3,1-YOP, fibre-optic microsensor, flat broken tip, diameter: 140 µm, PreSens GmbH, Regensburg, Germany), connected to a 4-Channel Microsensor Oxygen Meter (PreSens GmbH, Regensburg, Germany). The larvae were transferred from the culture vessel into a plastic vessel, which was placed in a temperature-controlled seawater bath, containing seawater of the corresponding CO_2 condition. The plastic vessel was used for a careful introduction of the larvae into the barrel of the Hamilton syringe. This handling took place under water to avoid air bubbles. Afterwards the plunger of the high-precision syringe was inserted, and the volume of the chamber was reduced to 50 µl. The needle of the microsensor was inserted into the syringe through the cannula, and the sensitive tip of the optode was placed in the middle of the chamber. The syringe was placed into the temperature-controlled seawater bath (6 °C). Respiration

Table 1 Seawater parameters measured during incubation

Incubation	Temperature (C°)	pH_{NBS}	DIC (µmol/kg)	Alkalinity (µmol/kg)	P_{CO2} (µatm)
Control	6.13 ± 0.17	8.10 ± 0.03	2320 ± 63	2417 ± 6	489 ± 21
CO_2 1100	5.96 ± 0.24	7.80 ± 0.04	2435 ± 33	2482 ± 6	1026 ± 107
CO_2 2400	6.3 ± 0.13	7.44 ± 0.02	2524 ± 77	2483 ± 5	2343 ± 130

Values are given in mean ± SD. $N = 9$

NBS National Bureau of Standards, *DIC* dissolved inorganic carbon, *PCO_2* partial pressure of CO_2

measurements were stopped when the oxygen saturation of the chamber water reached about 80 %. Before and after two measurements, blanks were run to consider bacterial oxygen consumption. Oxygen consumption was expressed as $\mu gO_2/Ind^{-1}\ h^{-1}$ as there was no correlation with larval dry weight. At all sampling days, eight larvae from each CO_2 treatment were used to measure oxygen consumption. The same eight individuals were used for dry weight and C:N ratio measurements. For each day of measurement, eight larvae were taken from a particular vessel. Vessels were sampled between one and four times throughout the experiment (i.e. on one to four different days).

Dry weight and C:N ratio

After respiration measurements, all larvae were removed from the chamber and briefly rinsed with deionised water. Excessive water was removed using a paper towel. Subsequently, larvae were stored at −20 °C in preweighed tin cartridges for analyses. Individual larvae within the tin cartridges were freeze-dried over night, weighed on a high-precision balance (Mettler Toledo AG, Greifensee, CH-8606, CH), and C:N ratios were measured in a CN analyzer (Euro EA-CN analyzer), using acetanilide as standard. C and N are given as µg per individual and as percent of dry weight (%DW). Dry weight is given as µg per individual. Unfortunately, no data on dry weight and C and N content could be obtained for the first 8 days of development in the present study due to loss of samples.

Statistical analyses

Results were analysed using GraphPad Prism 4 (GraphPad Software Inc.). All data were checked for outliers by use of Nalimov's test (Noack 1980). A two-way ANOVA was used to investigate the effects of CO_2 concentration and larval age on larval oxygen consumption, dry weight, C and N content and C:N ratio. Bonferroni tests were used for a posterior analyses. When the interaction between factors was significant, an a posterior Bonferroni test was run separately for each CO_2 concentration to detect differences among days of development. A one-way ANOVA was conducted to analyse the effect of CO_2 on MDT and daily mortality of larvae, followed by an a posterior Bonferroni test.

Results

Mortality and median developmental time (MDT)

There was no effect of CO_2 on daily mortality (ANOVA: $F_{2,35} = 1{,}131$; $p = 0.3349$) and on total mortality (ANOVA: $F_{2,8} = 0{,}2090$; $p = 0.8170$). Mean daily mortality was 0.3 ± 0.7 % in larvae exposed to 490 µatm CO_2, 0.2 ± 0.6 % in larvae exposed to 1,100 µatm and 0.2 ± 0.6 % in larvae exposed to 2,400 µatm CO_2, while total mortality was 18.7 ± 2.3 % in larvae exposed to 490 µatm CO_2, 17.5 ± 9.5 % in larvae exposed to 1,100 µatm CO_2 and 13.4 ± 4.0 % in larvae from the 2,400 µatm CO_2 treatment.

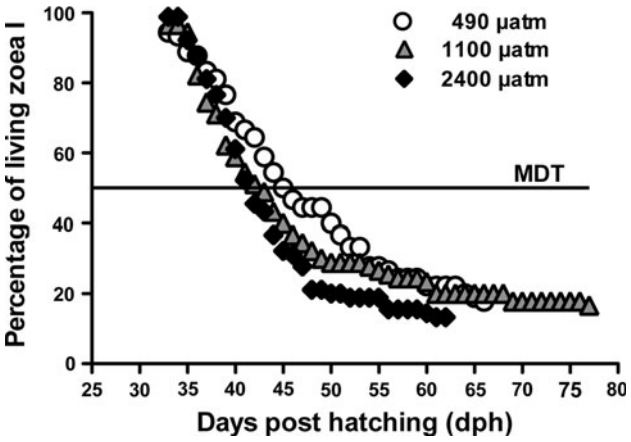

Fig. 1 Percentage of living zoea I larvae of *H. araneus* reared under three different seawater PCO_2 (490 µatm *white circles*, 1,100 µatm *grey triangle*, 2,400 µatm *black rectangle*) during time of development. *Line* indicates time when 50 % of the zoea I larvae have moulted to zoea II (median developmental time, MDT)

Seawater CO_2 concentration had no effect on the MDT of the first larval stage (ANOVA: $F_{2,8} = 1{,}217$; $p = 0.3600$). MDT of zoea I at 490 µatm CO_2 was 45 ± 1 days and 43 ± 4 at 1,100 µatm CO_2, whereas larvae exposed to 2,400 µatm CO_2 moulted on day 42 ± 2 (Fig. 1). First moulting into zoea II occurred at day 33 in all three CO_2 treatments but moulting ranged between day 33 and day 66 in larvae reared at 490 µatm CO_2, between day 33 and day 77 in larvae reared at 1,100 µatm CO_2 and between day 33 and day 62 in larvae reared at 2,400 µatm CO_2 (Fig. 1).

Oxygen consumption

Oxygen consumption varied greatly with developmental time but in different ways for the three CO_2 treatments (Fig. 2a–c). Oxygen consumption rates in larvae reared at 490 µatm CO_2 (controls) decreased during the first few days after hatching, increased during further development and reached a steady state level before moulting. In contrast, oxygen consumption of larvae reared under higher CO_2 conditions followed a similar pattern during the first days but showed a significant increase in oxygen consumption followed by a pronounced decrease a few days before moulting. Consequently, the 2-way ANOVA showed a significant interaction between day of development and

CO_2 concentration (ANOVA II: $F_{28,289} = 2{,}107; p = 0.0013$) (Table 2).

The Bonferroni test showed a significant increase in oxygen consumption at 490 µatm CO_2 between 2- and 22-day-old larvae ($p < 0.05$) followed by a constant rate in 22–42-day-old larvae ($p > 0.05$, Fig. 2a). Oxygen consumption rates from larvae incubated at 1,100 µatm CO_2 also increased significantly between day 2 and day 22 ($p < 0.05$, Fig. 2b), remained constant between day 22 and day 27 ($p > 0.05$) and increased again significantly between day 27 and day 36 ($p < 0.001$), where oxygen consumption peaked at 0.234 ± 0.01 $\mu gO_2/Ind^{-1}\ h^{-1}$ followed by a significant decrease on day 42 ($p < 0.001$, Fig. 2b). A similar trend could be found in larvae incubated at 2,400 µatm CO_2 (Fig. 2c). Oxygen consumption rates increased significantly between day 4 and day 24 ($p < 0.05$), levelled off between day 24 and 34 ($p > 0.05$) and increased again on day 36 ($p < 0.001$). A significant decline in oxygen consumption rates on day 42 ($p < 0.001$) was observed. At 2,400 µatm CO_2, the highest oxygen consumption rate was found on day 36 (0.231 ± 0.03 $\mu gO_2/Ind^{-1}\ h^{-1}$, Fig. 2c). An unpaired t test showed a significant difference between oxygen consumption rates of the three treatments on day 36, with higher oxygen consumption in larvae from each CO_2 treatment compared with those of control larvae (unpaired t test: 1,100 µatm CO_2: $p < 0.05$; 2,400 µatm CO_2: $p < 0.05$, Fig. 2a–c).

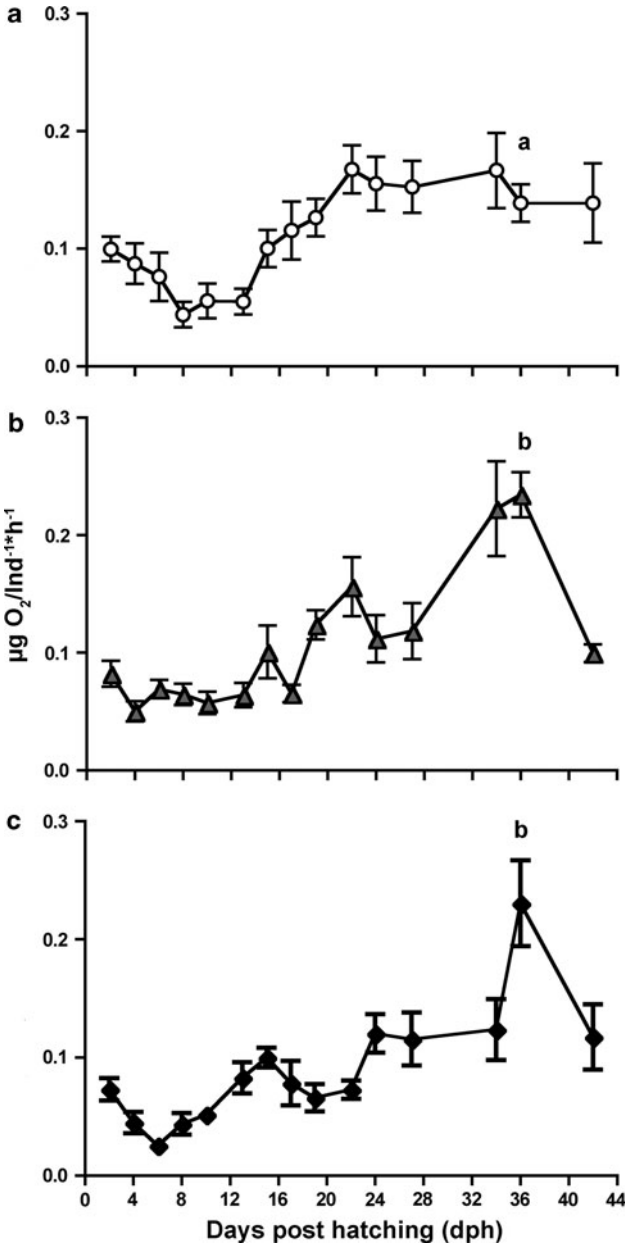

Fig. 2 Individual oxygen consumption during development of zoea I larvae of *H. araneus* reared under three different levels of seawater PCO_2 (**a** 490 µatm, **b** 1,100 µatm, **c** 2,400 µatm). Different l*owercase letters* indicate significant differences between treatments on the same developmental day. Mean ± SE

Dry weight, C:N ratio, C + N (%) of dry weight

Changes in larval dry weight over time followed bell-shaped curves in all three treatments (Fig. 3). There was a significant interaction detected by 2-way ANOVA between larval age and CO_2 concentration (ANOVA II: $F_{14,155} = 2{,}064$; $p = 0.0166$) (Table 2). The Bonferroni test showed two peaks in dry weight in larvae exposed to 490 µatm CO_2. Dry weight increased between day 8 and 22 ($p < 0.001$), decreased on day 24 ($p < 0.001$) and remained constant between day 24 and 34 ($p > 0.05$). Subsequently, dry weight rose on day 36 ($p < 0.05$) followed by a significant drop in weight by day 42 ($p < 0.001$) (Fig. 3). Dry weight of larvae from incubations under 1,100 and 2,400 µatm CO_2 increased significantly between day 8 and 22 ($p < 0.001$) and remained constant thereafter. Larvae under 2,400 µatm CO_2 displayed constant dry weight between day 22 and 36 ($p > 0.05$), followed by a decrease on day 42 ($p < 0.01$). In larvae under 1,100 µatm CO_2, the decline could already be seen on day 27 ($p < 0.001$). An unpaired t test showed that on day 8, dry weight of larvae under 2,400 µatm CO_2 remained significantly below that of larvae under 490 µatm CO_2 and 1,100 µatm CO_2 (unpaired t test: 490 µatm CO_2: $p < 0.05$; 1,100 µatm CO_2: $p < 0.001$, Fig. 3).

While CO_2 had no direct effect, larval age significantly affected C and N contents of zoea I larvae (ANOVA II: C: $F_{7,165} = 19{,}43$; $p < 0.0001$; N: $F_{7,166} = 22{,}56$; $p < 0.0001$; Table 2, Fig. 4a, b). In larvae exposed to 490 µatm CO_2, C content increased between day 19 and 22 ($p < 0.01$), remained constant until day 34 and increased again on day 36 ($p < 0.01$) followed by a significant drop on day 42 ($p < 0.001$). In larvae from both high CO_2 treatments, C content increased significantly between day 8

Table 2 Results of 2-way ANOVAs conducted to investigate effects of CO_2 and larval age on oxygen consumption, dry weight, carbon content (C), nitrogen content (N), C:N ratio and weight-specific sum of C and N of *H. araneus* zoea I larvae

Response variable	CO_2 effect			Day of development			Interaction		
	F	*df*	*p*	*F*	*df*	*p*	*F*	*df*	*p*
Individual oxygen consumption	5,519	2	**0.0044**	15.73	14	**<0.0001**	2,107	28	**0.0013**
Dry weight	7,691	2	**0.0007**	40.21	7	**<0.0001**	2,064	14	**0.0166**
C	1,227	2	0.2959	19.43	7	**<0.0001**	1,742	14	0.0518
N	0,413	2	0.6618	22.56	7	**<0.0001**	1,516	14	0.1100
C:N	2,225	2	0.1113	13.30	7	**<0.0001**	6,816	14	**<0.0001**
Sum of weight-specific C and N	6,065	2	**0.0029**	11.10	7	**<0.0001**	3,671	14	**<0.0001**

Significant values with $p < 0.05$ are given in bold

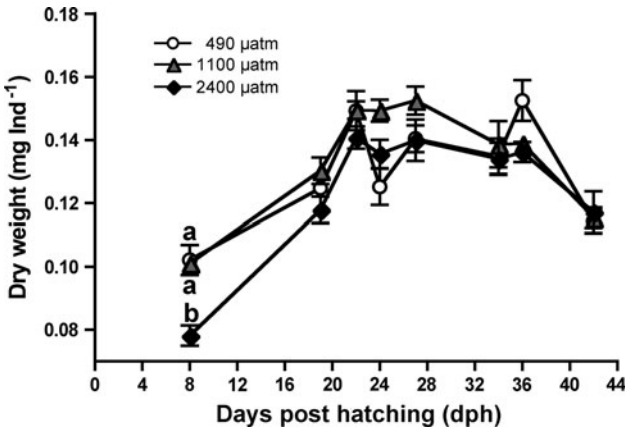

Fig. 3 Dry weight of zoea I larvae of *H. araneus* reared under three different seawater PCO_2 (490 µatm *white circles*, 1,100 µatm *grey triangle*, 2,400 µatm *black rectangle*) during time of development. *Different letters* indicate significant differences between treatments on the same developmental day. Mean ± SE

and 19 ($p < 0.01$), levelled off between day 19 and 36, and dropped on day 42 (1,100 µatm CO_2: $p < 0.01$; 2,400 µatm CO_2: $p < 0.05$, Fig. 4a). In all treatments, N content increased significantly between day 8 and 22 ($p < 0.001$, Fig. 4b). Afterwards it remained constant and finally decreased between day 36 and 42 in larvae exposed to 490 µatm CO_2 ($p < 0.01$) and between day 34 and 42 in larvae exposed to 1,100 µatm CO_2 ($p < 0.001$), respectively. In larvae exposed to 2,400 µatm CO_2, a stable N content could be found between day 22 and 42.

There was a significant interaction in the 2-way ANOVA between larval age and CO_2 concentration on larval C:N ratio (ANOVA II: $F_{14,166} = 6{,}816$; $p < 0.0001$, Ta. 2, Fig. 4c). Under 490 µatm CO_2, the C:N ratio decreased between day 8 and 19 ($p < 0.001$) and again between day 22 and 24 ($p < 0.001$). It remained constant between day 24 and 34 and finally increased between day 34 and 36 ($p < 0.001$). In larvae from 1,100 µatm CO_2, the C:N ratio stayed constant between day 8 and 34, followed by an increase between day 34 and 36 ($p < 0.001$) and a drop on day 42 ($p < 0.01$). Under 2,400 µatm CO_2, an increase in the C:N ratio could be found between day 8 and 19 ($p < 0.001$), followed by a decrease on day 22 ($p < 0.001$) and a constant C:N ratio between day 22 and 42.

On the sum of weight-specific C and N content (Fig. 5a–c), there was a significant interaction in the 2-way ANOVA between larval age and CO_2 concentration (ANOVA II: $F_{14,154} = 3{,}671$; $p < 0.0001$, Table 2, Fig. 5a–c).

An unpaired *t* test showed a significant difference between the sum of C and N content (%DW) of the three treatments on day 8, with higher sum of C and N content (%DW) in larvae from 2,400 µatm CO_2 compared with those of 490 µatm CO_2 larvae ($p < 0.01$) and 1,100 µatm CO_2 larvae ($p < 0.001$) (Fig. 5a–c). On day 22, an unpaired *t* test revealed a significant reduced sum of C and N content (%DW) in larvae reared at 2,400 µatm CO_2 compared with larvae at 490 µatm CO_2 ($p < 0.05$) as well as a significantly higher sum of C and N content (%DW) on day 34 in larvae from each high CO_2 treatment compared with those of at the larvae at 490 µatm CO_2 (1,100 µatm CO_2: $p < 0.05$; 2,400 µatm CO_2: $p < 0.01$) (Fig. 5a–c).

Discussion

Our data demonstrate the importance to disentangle the effects of larval age from those of abiotic factors on physiological parameters during the analysis of time series of a single larval stage. This has been revealed by former studies on sea urchin larvae with continuous development (Stumpp et al. 2011; Martin et al. 2011), but to our knowledge, the present study is the first one on a species with discrete development. Larval age had a strong effect on all measured parameters in comparison with CO_2. Therefore, the transient increase in oxygen consumption caused by CO_2 might not have been detected by sampling only once during each developmental stage. Since the development of crustacean larvae involves drastic

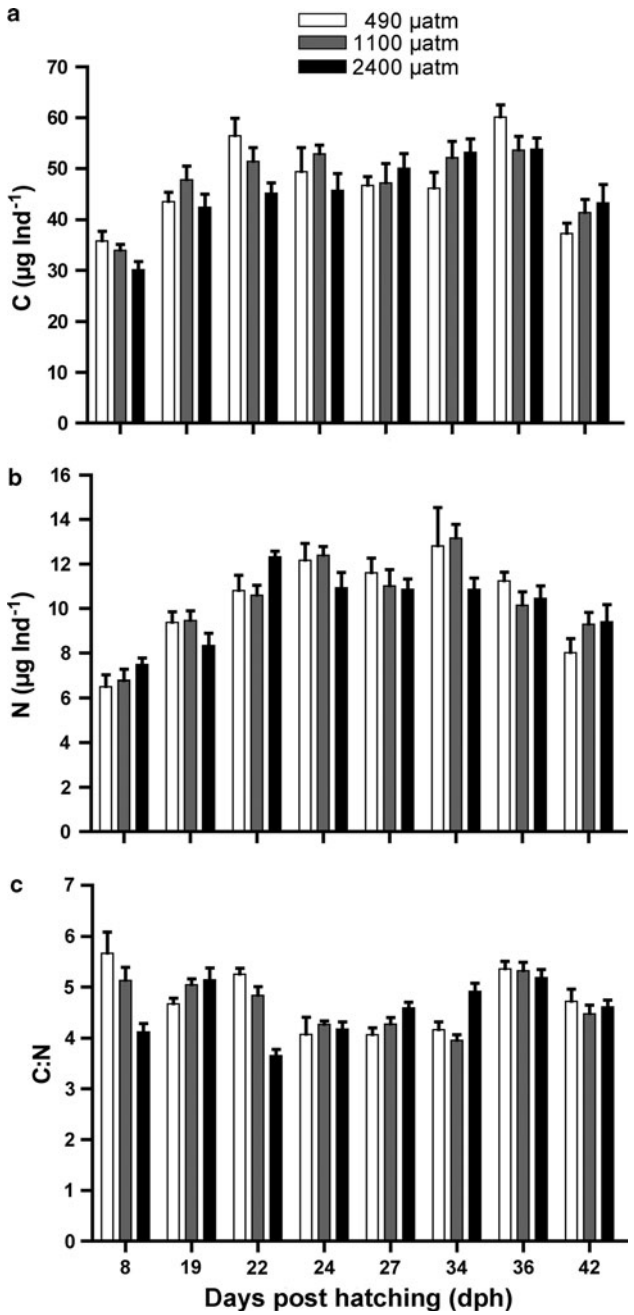

Fig. 4 Carbon content (**a**), nitrogen content (**b**) and C:N ratio (**c**) of *H. araneus* zoea I larvae reared under 490 µatm (*white*), 1,100 µatm (*grey*) and 2,400 µatm (*black*) during time of development. Mean ± SE

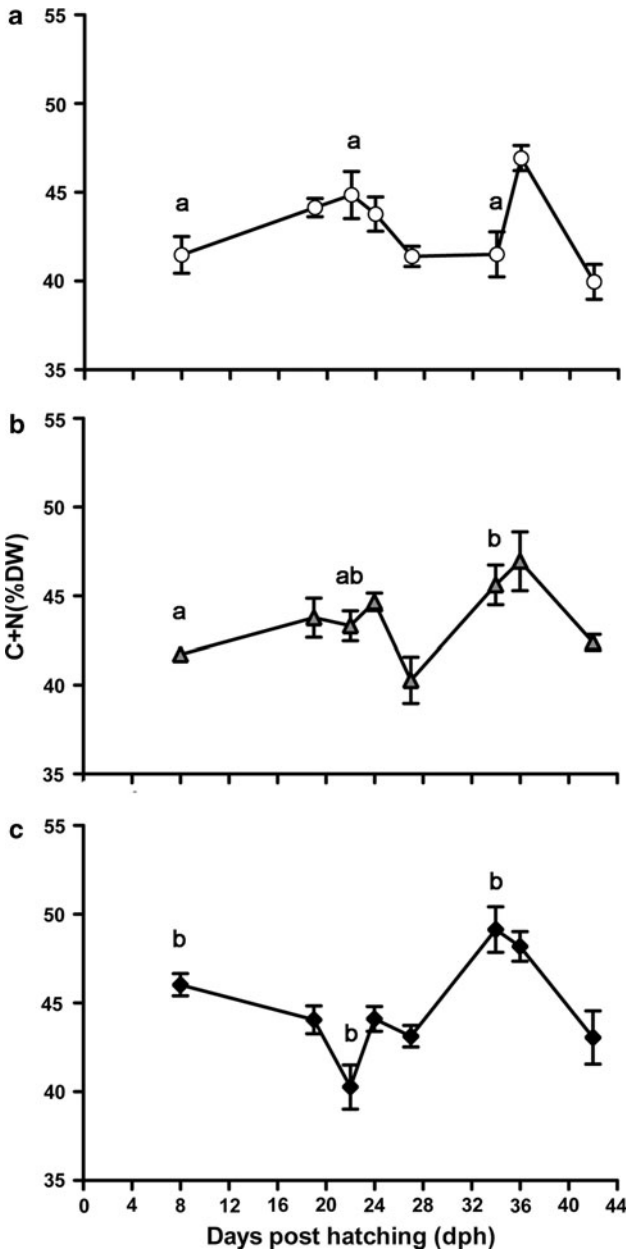

Fig. 5 Sum of carbon and nitrogen (% of dry weight) in *H. araneus* zoea I larvae reared under three different levels of seawater PCO_2 (**a** 490 µatm, **b** 1,100 µatm, **c** 2,400 µatm). *Different letters* indicate significant differences between treatments on the same developmental day. Mean ± SE

morphological changes, which can be classified in decapods according to Drach (1939), the high respiration rates at the end of the moult cycle suggest that the energy demand for morphological changes is enhanced by hypercapnic conditions. In *H. araneus* zoea I from Helgoland, the late premoult phase, which is characterised by the appearance of new cuticle, accounted for 26 % of total developmental time (Anger 1983). Assuming similar relationships in Svalbard zoea I, the premoult phase would set in approximately on day 31, followed by a peak in oxygen consumption under elevated CO_2. Thus, it is very likely that increased oxygen consumption in larvae reared under high seawater CO_2 concentrations indicates high metabolic costs associated with the appearance of the new cuticle.

Shortly before moulting, the larvae possess two cuticles, and the epidermis reaches its greatest retraction from the

old cuticle (Anger 1983). As respiratory gas exchange is mainly accomplished via the body surface in this larval stage, diffusion distances for respiratory gas (CO_2 and O_2) might be enlarged. A limited gas exchange would resemble hypoxic conditions and would cause the haemolymph O_2 level to decrease and CO_2 level to increase as it has been found in hypoxia studies (Varley and Greenaway 1992; Luquet and Ansaldo 1997). It is known that elevated seawater PCO_2 also leads to an increase in haemolymph PCO_2 in crustaceans (Pane and Barry 2007). Thus, it is likely that the zoea I larvae reared at 1,100 µatm and 2,400 µatm CO_2 experienced haemolymph acid–base disturbances during the premoult phase caused by a high extracellular PCO_2. Spicer and Eriksson (2003) found the thin cuticle of the telson to be the main site for respiratory gas exchange in early life stages of the Norway lobster *Nephrops norvegicus*. They found a correlation between pleopod activity, and seawater oxygen saturation with increasing pleopod activity at decreasing oxygen saturation. Storch et al. 2009 found higher oxygen consumption rates at increased maxilliped activity in zoea I of the Chilean kelp crab *Taliepus dentatus* under unfavourable low temperature. Enhanced maxilliped activity probably favours the gas exchange during unfavourable conditions like a limitation in oxygen supply. Thus, it is conceivable that increased oxygen consumption during the premoult phase in *H. araneus* zoea I larvae reared under high seawater CO_2 concentrations is due to enhanced larval activity to improve the haemolymph CO_2 release.

Growth and elemental composition are classic parameters to investigate effects of abiotic factors on the development of crustacean larvae (Anger et al. 1998). So far, a PCO_2-induced decrease in dry weight has only been found in late developmental stages (Arnold et al. 2009; Walther et al. 2010). Although overall larval growth and elemental composition were not affected by high seawater PCO_2 in the present study, a significant change in larval dry weight could be found in the zoea I on day 8 (Fig. 3) under the highest CO_2 concentration (2,400 µatm) when larval dry weight was considerably reduced. Interestingly, the difference was visible in dry weight only, whereas the elemental composition (C and N content) remained the same. Anger et al. (1989) found a strong increase in dry weight at the beginning of each moult cycle in *H. araneus,* while organic components (i.e. C and N) did not increase as rapidly. This leads to a decrease in the percentage of organic components in larval dry weight. The decrease in C and N at increasing dry weights was explained by an initial phase of rapid uptake of inorganic substances at the beginning of the moult cycles of all larval stages (zoea I, zoea II and megalopa; cf. Anger et al. 1989). This assumption is supported by an increased mineral (ash) content in percent of dry weight at the beginning of each moult cycle (Anger 2003). In the present study, we found a higher percentage of organic components in larval dry weight on day 8 only at the highest seawater CO_2 concentration of 2,400 µatm followed by an initial decrease in organic components in larval dry weight. Accordingly, it is likely that the low dry weight of zoea I on day 8 of development was due to a less pronounced uptake of inorganic substances in larvae reared under the highest CO_2 concentration as dry weight equalled with progressing development in all treatments, and no differences could be found on day 22. There might be an extended period of mineral uptake in larvae from the highest CO_2 concentration. This suggests a shift in the energetic balance. It is thus possible that zoea I have slowed down energy demanding processes like mineral uptake in favour of other processes like acid–base regulation.

In the Svalbard population of *H. araneus* from Kongsfjorden, zoea I larvae hatch between late February and early April (Walther et al. 2010). This corresponds to the Arctic spring bloom in the Kongsfjorden (Hop et al. 2002), which presents a sufficient food supply for small zooplankton. In crustaceans, different larval feeding strategies can be found. While larvae development of some crustacean species at high latitudes comprises a lecithotrophic phase (Anger et al. 2003), Arctic *H. araneus* larvae depend on food out of the water column for a successful development. Former studies indicated the crucial importance of a sufficient food supply for development and survival of crustacean larvae (Anger and Dawirs 1981; Dawirs 1983). Larvae in the present study were fed ad libitum to guarantee a suitable food supply, which should be consistent with natural food ability as the estimated biomass (dry mass) in Kongsfjorden is relatively high with 8.8 ± 5.1 g m^{-2} (Hop et al. 2002). However, larvae might be differently affected by elevated CO_2 when food supply is limited.

Measurements of larval dry weight should be a suitable indicator for growth and feeding in crustacean larvae to ensure adequate food supply. However, to date the only studies focusing on growth in *H. araneus* have been carried out with the Helgoland population at higher temperatures compared with the present study (Anger and Dawirs 1982; Anger and Jacobi 1985). The overall growth of Svalbard larvae during the zoea I stage followed a bell-shaped curve independent of seawater CO_2 concentration, while *H. araneus* zoea I from waters around Helgoland displayed almost exponential growth curves, and bell-shaped growth curves were only common in megalopa (Anger and Dawirs 1982; Anger and Jacobi 1985). A simple explanation could be the considerably longer developmental time of Svalbard zoea I compared with Helgoland zoea I. While it took 44 days under control conditions to moult into zoea II in the Svalbard population (6 °C), Helgoland zoea I need only

10 days at a rearing temperature of 12 °C. It is known that *H. araneus* zoea I feed continuously until the middle of the moult cycle (Anger et al. 1989), which can explain the significant increase in dry weight between day 8 and day 22. Afterwards larval feeding decreases. Assuming similar patterns in larvae from Svalbard, a longer period between the reduction in feeding and the moulting event might explain their bell-shaped growth curve. The almost exponential growth curve in Helgoland larvae is supported by their rapid development.

The temporary increase in oxygen consumption under elevated CO_2 was not accompanied by elevated mortalities or increased developmental time of zoea I larvae under both CO_2 conditions of 1,100 and 2,400 µatm. Although not significant, mortality and MDT decreased with increasing CO_2 concentration. However, the low number of statistical replicates might explain the lack of significance. This finding is in line with those of other ocean acidification studies on larval crustaceans. Mortality during zoea development of the shrimp *Pandalus borealis* also remained unaffected by elevated seawater PCO_2 of 1,300 µatm (Bechmann et al. 2011). Mortality of shrimp zoea during an experimental period of 35 days was in fact less under high seawater PCO_2 on the last day of experiment. In the copepod *Acartia tsuensis,* high seawater CO_2 concentration of about 2,300 µatm had no effect on survival or developmental speed through all life stages (Kurihara and Ishimatsu 2008). Also, survival of larval Decapoda seems to be largely unaffected by elevated seawater PCO_2. Survival and developmental time of all zoeal stages of the European lobster *Homarus gammarus* were not affected by seawater CO_2 concentration of 1,200 µatm (Arnold et al. 2009). However, the previous study on the larvae of the spider crab *H. araneus* demonstrated negative impacts on larval development (Walther et al. 2010). At a higher CO_2 concentration of 3,000 µatm and lower temperature of 3 °C than in our study they found an increased developmental time in both zoeal stages, but also no effect on mortality. The influence of elevated seawater PCO_2 on larval developmental time vanished at higher temperatures (Walther et al. 2010), which is in accordance with our findings.

In previous studies, effects of elevated PCO_2 on dry weight and mineral content in larvae of *H. gammarus* and *H. araneus* were only found in the latest stages (Arnold et al. 2009; Walther et al. 2010). Therefore, the megalopa stage of *H. araneus* was postulated to represent the larval stage most sensitive to high seawater PCO_2 (Walther et al. 2010). Our monitoring of the time course of zoea I development indicates that CO_2 effects may set in earlier, although at first sight, CO_2 acidified seawater had no negative effect on the successful development of *H. araneus* zoea I, as moulting into zoea II was similarly successful under all conditions. However, the course of oxygen consumption in developing zoea I indicates that development under elevated seawater PCO_2 level seemed to be more costly during premoulting. The effects of elevated seawater PCO_2 on dry weight and metabolism seen in the present study did not influence zoea I survival. It seems that the zoea I stage of *H. araneus* is able to compensate for the elevated costs associated with the development in a high PCO_2 environment. However, as it has been found by Dupont et al. (2012) this compensation might be an energy consuming process and might therefore affect performance of later stages by carry-over effects due to depletion of energy reserves.

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