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Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N)

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ABSTRACT: The combined effects of predicted ocean acidification and global warming on the larvae of the cold-eurythermal spider crab *Hyas araneus* L. were investigated in 2 populations: a southernmost around Helgoland (North Sea, 54° N) and a northernmost at Svalbard (North Atlantic, 79° N). Larvae were exposed at temperatures of 3, 9 and 15°C to present day normocapnia (380 ppm CO_2) and to CO_2 conditions predicted for the near or medium-term future (710 ppm by the year 2100, 3000 ppm by 2300 and beyond). Larval development time, growth and C/N ratio were studied in the larval stages Zoea I, II, and Megalopa. Permanent differences in instar duration between both populations were detected in all stages, likely as a result of evolutionary temperature adaptation. With the exception of Zoea II at 3°C and under all CO_2 conditions, development in all instars from Svalbard was delayed compared to those from Helgoland. Most prominently, development was much longer and fewer specimens morphosed to the first crab instar in the Megalopa from Svalbard than from Helgoland. Enhanced CO_2 levels (particularly 3000 ppm) extended the duration of larval development and reduced larval growth (measured as dry mass) and fitness (decreasing C/N ratio, a proxy of the lipid content). Such effects were strongest in the zoeal stages of Svalbard larvae, and during the Megalopa instar of Helgoland larvae. The high sensitivity of megalopae from the Svalbard population to warming and of those from Helgoland to enhanced CO_2 levels suggests that this larval instar is a physiologically sensitive bottleneck within the life cycle of *H. araneus*.

KEY WORDS: Ocean acidification · CO_2 · Larval development · CHN · Growth · Helgoland · Svalbard

INTRODUCTION

The ongoing increase of anthropogenic CO_2 in the atmosphere causes an accumulation of CO_2 in the oceans and an acidification trend, which develops in parallel with global warming (IPCC 2001, 2007). Caldeira & Wickett (2005) calculated CO_2 concentrations in the atmosphere and oceans according to various emission scenarios. They predicted a CO_2 concentration of 710 ppm in the atmosphere and oceans by the year 2100; values of about 3000 ppm CO_2 might be reached by the year 2300 (Caldeira & Wickett 2005). CO_2 is absorbed into the ocean surface water by air-to-sea equilibration and is distributed by ocean circulation (Orr et al. 2001). The associated ocean acidification leads to questions about its effect on marine ecosystems in times of ocean warming (cf. Pörtner et al. 2005, Pörtner 2008).

Studies on adult crustaceans have detected negative effects of CO_2 on various physiological processes and performances (*Chionoectes tanneri*: Pane & Barry 2007, *Cancer pagurus*: Metzger et al. 2007, *Necora puber*: Spicer et al. 2007, *Palaemon pacificus*: Kurihara et al. 2008, *Semibalanus balanoides*: Findlay et al. 2009, *Hyas araneus*: Walther et al. 2009). Development of early stages in invertebrates, i.e. larvae, is the basis for a succesful life cycle (Fabry et al. 2008). However, little information exists on the effect of enhanced CO_2 levels in combination with global warming on the

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physiology of early developmental stages of invertebrates, especially of crustaceans. Existing studies have focused on echinoderms (Dupont et al. 2008, Kurihara 2008), copepods (Kurihara et al. 2004, Mayor et al. 2007), lobsters (Arnold et al. 2009) and barnacles (Findlay et al. 2009) and have demonstrated negative effects of CO_2 on development, morphology, growth, hatching success or survival of early developmental stages. Furthermore, sensitivity to CO_2 may be highest where a species experiences extreme temperatures (Pörtner & Farrell 2008) and lives close to the border of its temperature dependent distribution range, e.g. along a latitudinal gradient.

The spider crab *Hyas araneus* (L.) is a cold-eurythermal species and seems to be a good model organism for such studies because it shows a particularly wide geographic range. In the eastern North Atlantic, it is distributed from the temperate southern North Sea (Helgoland 54° N) to the sub-Arctic waters of Svalbard (79° N) (Christiansen 1969). The temperature in the southern North Sea varies seasonally between 3 and 18°C (Wiltshire & Manly 2004). Near Svalbard, by contrast, the temperatures range from 0 to 6°C (Svendsen et al. 2002). Adult individuals live on stony, sandy and soft bottoms from <1 m down to 360 m, most commonly at depths <50 m (Christiansen 1969). The ovigerous females release their larvae upon hatching of the first zoeal stage after a 2-yr embryonic development period (Petersen 1995). The rate at which Zoea I moults to Zoea II is temperature-dependent. The zoeal stages are larger than in other decapods (Anger 2001) and use thoracopods (maxillipeds I and II) to swim in the water column (Christiansen 1971). After the moult to the Megalopa stage, they assume a semi-benthic life style, selecting a suitable habitat for the benthic juvenile and adult life-history stages, before they metamorphose to the first crab instar (Anger 2001).

Growth and biochemical composition during larval development appear as suitable indicators to study the combined effects of temperature and ocean acidification (Anger 1998, 2001). It is well known that these traits are influenced by temperature (Anger 1987, 2001), but little is known about specific or additional effects of CO_2. A study on lobster larvae showed a negative effect of CO_2 on their dry mass (Arnold et al. 2009). Previous studies on the larval biology of *Hyas araneus* have used individuals from the temperate region of Helgoland (Anger & Nair 1979, Anger 1983, 1987) or from Oslo (Christiansen 1971), while no data have been available from the sub-Arctic region of Svalbard. In the present study, we investigated whether populations of *H. araneus* from the southern temperate and northern cold limits of distribution, respectively, differ in their characteristics of temperature adaptation and in their responses to elevated CO_2 levels.

MATERIALS AND METHODS

Obtaining and maintaining crabs. Ovigerous females (15 ind.) of *Hyas araneus* were dredged in January 2008 at 30 to 50 m depth near Helgoland (German Bight, North Sea, 54° 11′ N, 7° 53′ E) (Fig. 1). Each female was kept in a flow-through aquarium at ambient water temperature (4 to 6°C) and salinity (32‰) at the Biologische Anstalt Helgoland (Alfred-Wegener-Institute [AWI], Germany). Experiments were performed with larvae from 3 females that hatched from the end of January to the end of February 2008.

In July 2008, ovigerous females (60 ind.) of *Hyas araneus* were caught by divers in the Kongsfjorden (Svalbard, Norway, 78° 55′ N, 11° 57′ E) (Fig. 1). Females were transported to the AWI, Bremerhaven and kept for 8 mo at 5°C and 32‰ salinity in flow-through seawater aquaria. Before larvae hatched, the females were transported to the Helgoland Marine Station (Biologische Anstalt Helgoland, AWI). Here the larvae hatched during the period from the end of February to the beginning of April 2009. For the experiments, larvae from 4 females were used.

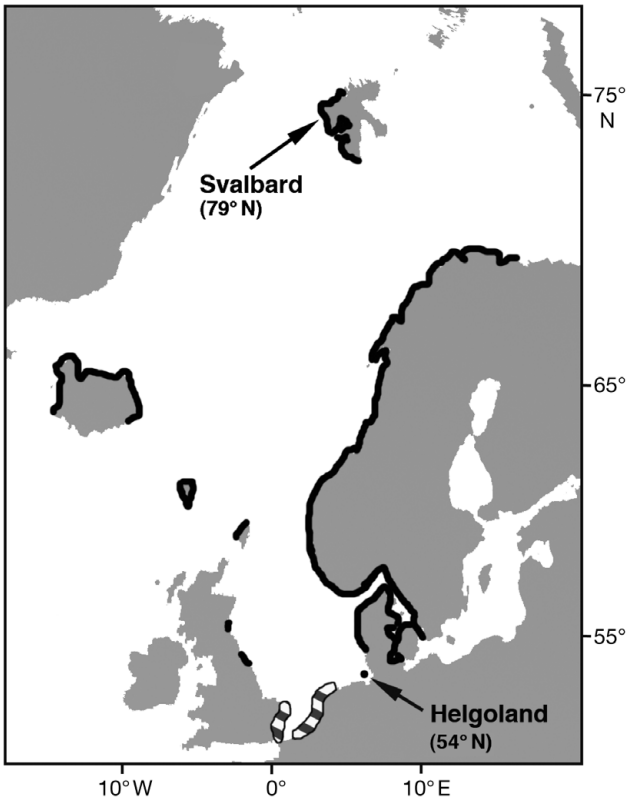


Fig. 1. *Hyas araneus.* Distribution in the North Atlantic modified after Christiansen (1969). Original distribution range from the English Channel (dashed line area) to the Arctic (Svalbard, 79°N) (black lines) and the present, postulated distribution from the German Bight (Helgoland, 54°N) to Svalbard (black lines only)

Due to extended periods of reproduction, the number of females contributing to sufficient numbers of offspring was small: the first 3 females from Helgoland and the first 4 females from Svalbard. From each female, a total of 1350 freshly hatched larvae were used in the experiments. Per combination of treatments (3 temperatures, 3 CO_2 conditions), each 50 larvae from a single female (3 from Helgoland; 4 from Svalbard in total) were distributed over seven 0.5 l Kautex-flasks (7 × 50 larvae per female × 9 treatments). Every day, we changed seawater, removed dead larvae and fed larvae with freshly hatched *Artemia* sp. Nauplii (50 to 100 food ind. ml^{-1}) (San Francisco Bay Brand).

Zoea I larvae from the 7 flasks of each female and treatment that moulted to the next instar Zoea II on the same day were pooled together into a new flask. When <12 larvae moulted into Zoea II, they were transferred into 0.2 l Kautex flasks. When >12 to 30 individuals moulted, we used 0.5 l Kautex flasks. After moulting from Zoea II to the Megalopa instar, we pooled the Megalopa of each female and treatment in 0.2 l Kautex flasks (1 to 5 ind.) or 0.5 l Kautex flasks (6 to 15 ind.). Exposure of megalopae at 9 and 15°C continued until metamorphosis to the Crab I stage occurred. In this way, not only the duration of larval development was recorded, but also the survival rate of the megalopae prior to metamorphosis into the Crab 1 stage. At 3°C, megalopae were reared only till the Day 14 because of extended development.

Treatments. Larvae were reared at 3 different temperatures (3, 9 and 15°C) in combination with 3 CO_2 conditions (380, 710 and 3000 ppm CO_2). For normocapnic conditions (380 ppm CO_2), seawater filtered at 0.2 µm was used. For a CO_2 concentration of 710 ppm, 60 l Kautex bottles were filled with filtered seawater and equilibrated with a gas mixture (0.071% CO_2, 21% oxygen in nitrogen). For exposure <3000 ppm, a gas mixture (0.3% CO_2, 21% oxygen in nitrogen, provided by AIR LIQUIDE) was used. The flasks were closed with a lid to avoid contamination with air and to ensure stable water conditions over 24 h. Each day the seawater was changed, Kautex bottles were refilled, and pH was measured (WTW 340i, WTW SenTix HWS). Alkalinity samples were taken, fixed with a $HgCl_2$ solution (0.02%), and stored at 3°C in 250-ml borosilicate flasks. Total alkalinity was later measured by potentiometric titration (Brewer et al. 1986) and calculated from linear Gran plots. The carbonate system was calculated from temperature, pH, alkalinity and salinity using the CO2Sys program (Lewis & Wallace 1998) using equilibrium constants provided by Mehrbach et al. (1973) and refitted by Dickson & Millero (1987). The parameters of the carbonate system applied during the various treatments are given in Table 1.

CHN analysis. All larval stages were subjected to biochemical analyses. For each treatment, 5 replicate samples were collected after hatching of the Zoea I (with 4 larvae $replicate^{-1}$), after moulting to Zoea II (2 larvae $replicate^{-1}$), and in the Megalopa (1 larva $replicate^{-1}$) within a few hours after moulting (Day 0, denoted as M0), on Day 7 (M7) and Day 14 (M14). Larvae were briefly rinsed in Millipore water, blotted on filter paper, and stored frozen at –20°C in preweighted tin cartridges. The samples were freeze-dried over night (CHRIST freeze-dryer ALPHA 1-4 LSC). After determination of the dry wt to the nearest 0.1 µg on a Sartorius SC2 microbalance, carbon (C) and nitrogen (N) were measured in a CHN analyzer (Elementar Vario MICRO CUBE).

Statistical analysis. Statistical analyses were performed with GraphPad Prism (version 4, GraphPad Software) and STATISTICA (version 7.1, StatSoft). Prior to the analyses, the data were tested for female-specific effects, as larvae from individual females were grouped throughout the experimental period. For this reason, we used a 1-way ANOVA with females as factor levels. Where assumptions for normality and homogeneity of variances were not met, a Kruskal-Wallis test was conducted. The results showed that there were differences between individual females from Helgoland and between individuals from Svalbard. Accordingly, it cannot be excluded that the results are biased by individual geno- and phaenotypes. Although the comparison of data from larvae originating from individual females showed significant differences between females, actual values were very similar between females from one region. Accordingly, we assume that differences between individuals do not obscure the differences between regions and climate regimes.

Table 1. Seawater carbonate system parameters calculated from temperature, pH, total alkalinity (TA) and salinity (32‰) using the CO2Sys program (Lewis & Wallace 1998). Means ± SD, n = 10. 380 ppm CO_2 = normocapnia

Temp. (°C)	CO_2 treatment (ppm)	pH	TA (µmol kg^{-1})	pCO_2 (µatm)
3	380	8.11 ± 0.05	2405 ± 7	354 ± 65
	710	7.81 ± 0.03	2405 ± 4	754 ± 37
	3000	7.33 ± 0.03	2405 ± 6	237 8 ± 164
9	380	8.12 ± 0.07	2404 ± 8	346 ± 80
	710	7.81 ± 0.03	2403 ± 6	786 ± 54
	3000	7.35 ± 0.04	2406 ± 8	2443 ± 238
15	380	8.05 ± 0.04	2409 ± 2	401 ± 30
	710	7.79 ± 0.04	2409 ± 4	846 ± 42
	3000	7.34 ± 0.04	2409 ± 2	2637±160

The duration of larval development in Zoea I and II was analysed with respect to the factors temperature, CO_2 condition and population using 3-way ANOVAs. Dry wt and C/N ratios were analysed using 4-way ANOVAs testing the effects of the factors temperature, CO_2 condition, population and developmental stage. In all tested data sets, assumptions of normal distribution of data were met. Inspections of quantile-plots indicated no obvious deviations from normality, although Bartlett's test of homogeneity of variances indicated clear deviations from this assumption in all data sets ($p < 0.05$) also when transformed. We nonetheless chose to use the parametric ANOVAs. According to Underwood (1997, cited in Coleman et al. 2006), large designs incorporating 3 or more factors are robust against the consequences of deviation from this assumption.

In case of significant results, subsequent pairwise comparisons were made using Tukey's post hoc test in order to identify the differences. The analysis of the survival of the Megalopa and the metamorphosis to the first crab instar were tested by Pearson's chi-square test. Graphs were designed with GraphPad Prism (version 4). Average values of the development data are given as arithmetic means ± 1 SD. Values of dry wt and C/N ratios are given as means ± 1 SD. All analyses were tested at the 0.95% confidence level.

RESULTS

Development and survival

The larval development of *Hyas araneus* through the instars Zoea I and II depends on temperature and CO_2 concentration (Fig. 2). The analysis revealed that the duration of the first instar (Zoea I) lasted longer in individuals from Svalbard than from Helgoland, that temperature had a significant effect on the duration of development and that increased CO_2 levels caused increased development times (for all 3 main factors, $p < 0.001$; Table 2). Overall, the lengths of developmental periods of larvae originating from different populations and exposed to different CO_2 levels responded differently to temperature (interactions population × temperature and temperature × CO_2 with $p < 0.01$ each; Table 2).

In actual fact, under all CO_2 conditions, Zoea I larvae from Svalbard (north) developed significantly slower at 3°C (61.4 to 66.8 d) and 9°C (20 to 20.9 d) than the larvae from Helgoland (south) (3°C: 46.9 to 50 d, 9°C: 16.8 to 17.4 d) ($p < 0.05$) (Fig. 2a, Table 3). At 15°C, the difference between the Helgoland (10.5 d) and Svalbard (13.8 d) population was significant only at 3000 ppm CO_2, with a longer development in the Svalbard larvae ($p < 0.05$). The development of Zoea I at 3°C was in both populations significantly slower at 3000 ppm compared to normocapnia or 710 ppm CO_2 ($p < 0.05$).

The analyses of the duration of the second instar (Zoea II) revealed significances for all factors and factor combinations apart from population × CO_2 (Table 2). The results indicate that the developmental duration of Zoea II from individuals originating from Svalbard and Helgoland was differently affected by temperature and CO_2 levels. Only at 3°C, the duration of Zoea II instar differed significantly between populations and CO_2 conditions (Fig. 2b, $p < 0.001$, Table 3). In contrast to the pattern seen in the other larval stages, development was significantly slower in Zoea II from Helgoland (72.5 to 75.8 d) than in the Svalbard larvae (59.3 to 69 d) ($p < 0.001$). Svalbard Zoea II reared under 3000 ppm CO_2 developed significantly more slowly (69 d) than under 380 (59.3 d) or 710 ppm CO_2 (62.9 d) ($p < 0.001$).

The survival and duration of the development of Megalopa and their metamorphosis to the first crab instar showed significant differences between Helgo-

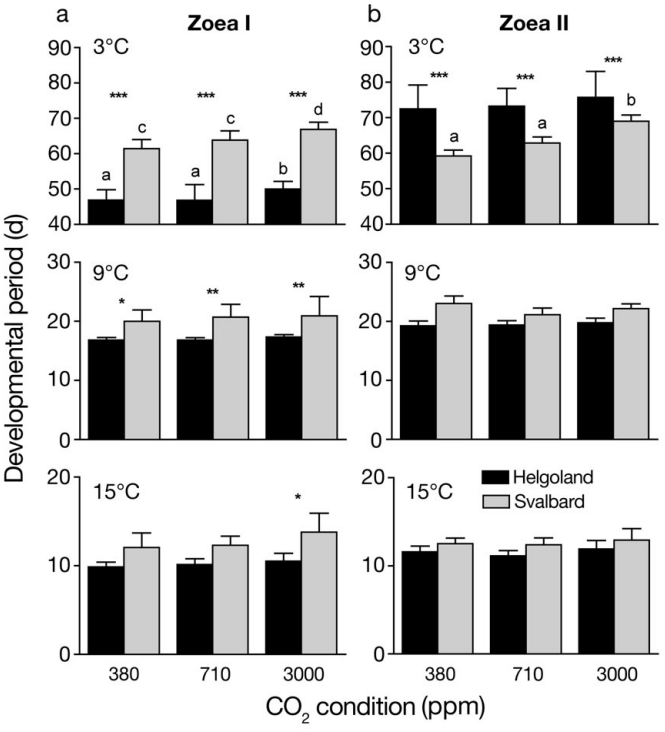


Fig. 2. *Hyas araneus*. Larval development through zoea stages I and II reared from hatching at 3, 9 and 15°C and under different CO_2 conditions (380 normocapnia, 710 and 3000 ppm) for Helgoland (black) and Svalbard (grey) populations. Significant differences (*) between populations in (a) Zoea I (n = 479–980) at each temperature, (b) Zoea II (n = 104–662) at 3°C only. Significant differences between different CO_2 conditions within a population (lower case letters) at 3°C only. Four-way ANOVA, p-values in Table 3

land larvae reared at 3000 ppm CO_2 and those reared under the other CO_2 conditions, at 9°C (Fig. 3, $p < 0.05$, Table 3). Under 3000 ppm, the development of the Megalopa stage (49.1 d) was extended, and significantly fewer Megalopa moulted to the Crab I stage (8 out of 357 ind.) compared to those under 380 (41 d, 67 out of 312 ind.) or 710 ppm CO_2 (42 d, 73 out of 279 ind.) ($p < 0.001$). Differences between Helgoland and Svalbard larvae in the survival and development time of the Megalopa stage were found under all CO_2 conditions. In Svalbard larvae under all conditions, the duration of Megalopa development was significantly extended compared to larvae from Helgoland, at 9°C ($p < 0.05$). Metamorphosis to the first crab instar occurred after ~30 d (normocapnia) in the Helgoland population, in contrast to 40 d (normocapnia) in the larvae from Svalbard ($p < 0.05$). In the Svalbard Megalopa, no differences in development time could be identified between the various CO_2 conditions. The number of Crab I juveniles obtained from Svalbard megalopae was similar and, when compared to those from Helgoland, generally low at 380 (3 out of 252 ind.), 710 (3 out of 172), and 3000 ppm CO_2 (0 out of 120). By comparison, 8 to 73 ind. (less at higher CO_2 levels, see above) developed successfully to the Crab I stage in the population from Helgoland.

At 15°C (Fig. 3, Table 3) Megalopa survival, development time and metamorphosis to the Crab I stage showed a pattern similar to the one at 9°C. The survival rate of the Megalopa from Helgoland was significantly less under 710 than under 380 ppm CO_2; development took significantly longer under 3000 (30.1 d) than under both 380 (26.8 d) and 710 ppm CO_2 (26.7 d) ($p < 0.05$). Significant differences between populations could be identified in the survival of the Megalopa under all CO_2 conditions and in the success of metamorphosis under normocapnia and 710 ppm. Similar to observations at 9°C, only few larvae (1 out of 145 ind. at normocapnia, 2 out of 224 at 710 ppm, and 2 out of 140 at 3000 ppm) moulted to the first crab instar in the Svalbard population. In contrast, 79 out of 335 ind. (normocapnia), 65 out of 224 (710 ppm) and 38 out of 281 (3000 ppm) developed successfully in the Helgoland population.

Table 2. *Hyas araneus*. Results of 3-way ANOVAs concerning development duration of Zoea I and II and 4-way ANOVAs concerning dry wt and C/N ratio. Significant values in **bold**

Source of variation	df	SS	MS	*F*	p
Development duration of Zoea I					
Population	1	2951.1	2951.1	656.03	**<0.001**
Temperature	2	81978.5	40989.3	9111.81	**<0.001**
CO_2	2	163.5	81.8	18.17	**<0.001**
Population × Temperature	2	2061.3	1030.7	229.11	**<0.001**
Population × CO_2	2	16.8	8.4	1.87	0.16
Temperature × CO_2	4	97.9	24.5	5.44	**<0.001**
Population × Temperature × CO_2	4	12.6	3.2	0.70	0.59
Residuals	198	890.7	4.5		
Development duration of Zoea II					
Population	1	247.0	247.0	31.05	**<0.001**
Temperature	2	134227.4	67113.7	8438.24	**<0.001**
CO_2	2	208.7	104.3	13.12	**<0.001**
Population × Temperature	2	1737.9	868.9	109.25	**<0.001**
Population × CO_2	2	30.1	15.0	1.89	0.15
Temperature × CO_2	4	335.1	83.8	10.53	**<0.001**
Population × Temperature × CO_2	4	111.4	27.8	3.50	**<0.01**
Residuals	198	1574.8	8.0		
Dry weight (µg ind.$^{-1}$)					
Stage	3	7646605	2548868	635.60	**<0.001**
Population	1	461772	461772	115.15	**<0.001**
Temperature	2	576620	288310	71.89	**<0.001**
CO_2	2	402686	201343	50.21	**<0.001**
Stage × Population	3	405112	135037	33.67	**<0.001**
Stage × Temperature	6	500121	83353	20.79	**<0.001**
Population × Temperature	2	652833	326417	81.40	**<0.001**
Stage × CO_2	6	327346	54558	13.60	**<0.001**
Population × CO_2	2	75106	37553	9.36	**<0.001**
Temperature × CO_2	4	60944	15236	3.80	**<0.01**
Stage × Population × Temperature	6	148187	24698	6.16	**<0.001**
Stage × Population × CO_2	6	106149	17692	4.41	**<0.001**
Stage × Temperature × CO_2	12	169639	14137	3.53	**<0.001**
Population × Temperature × CO_2	4	43176	10794	2.69	**<0.05**
1 × 2 × 3 × 4	12	75578	6298	1.57	0.09
Residuals	908	3641237	4010		
C/N ratio					
Stage	3	14.89	4.96	40.9	**<0.001**
Population	1	16.39	16.39	135.1	**<0.001**
Temperature	2	39.18	19.59	161.5	**<0.001**
CO_2	2	2.56	1.28	10.6	**<0.001**
Stage × Population	3	2.79	0.93	7.7	**<0.001**
Stage × Temperature	6	27.57	4.60	37.9	**<0.001**
Population × Temperature	2	0.21	0.11	0.9	0.42
Stage × CO_2	6	2.67	0.44	3.7	**<0.01**
Population × CO_2	2	0.76	0.38	3.1	**<0.05**
Temperature × CO_2	4	0.93	0.23	1.9	0.10
Stage × Population × Temperature	6	2.57	0.43	3.5	**<0.01**
Stage × Population × CO_2	6	1.44	0.24	2.0	0.07
Stage × Temperature × CO_2	12	1.79	0.15	1.2	0.26
Population × Temperature × CO_2	4	2.63	0.66	5.4	**<0.001**
1 × 2 × 3 × 4	12	3.78	0.32	2.6	**<0.01**
Residuals	906	109.87	0.12		

Table 3. *Hyas araneus*. Significant results from pairwise comparisons (Tukey's test) focusing on differences that had all been identified as significant in preceding ANOVAs and chi-square tests. Statistical significances in development, dry wt and C/N ratios between 3 different temperatures (*T*; 3, 9, 15°C) and instars (Zoea I, II [ZI, ZII]; Megalopa Day 0, 7 and 14 [M0, M7, M14]); comparison between Helgoland and Svalbard populations and between different CO_2 conditions (380 [= normocapnia], 710 and 3000 ppm CO_2)

T (°C)	Location	CO_2 treatment (ppm)	p
Development duration of Zoea I (3-way ANOVA)			
3	Helgoland–Svalbard	380	0.000036
		710	0.000036
		3000	0.000036
3	Helgoland	380–3000	0.033092
		710–3000	0.27101
3	Svalbard	380–3000	0.000036
		710–3000	0.047041
9	Helgoland–Svalbard	380	0.031423
		710	0.001099
		3000	0.005142
15	Helgoland–Svalbard	3000	0.018803
Development duration of Zoea II (3-way ANOVA)			
3	Helgoland–Svalbard	380	0.000036
		710	0.000036
		3000	0.000036
3	Svalbard	380–3000	0.000047
		710–3000	0.000036
Survival of Megalopa (chi–square–test)			
9	Helgoland–Svalbard	380	0.0004
		710	0.0138
		3000	< 0.0001
9	Helgoland	380–3000	< 0.0001
		710–3000	< 0.0001
15	Helgoland–Svalbard	380	< 0.0001
		710	< 0.0001
		3000	0.0485
15	Helgoland	380–710	0.0139
	Svalbard	380–3000	0.0006
		710–3000	0.0218
Metamorphosis to Crab 1 (chi–square–test)			
9	Helgoland–Svalbard	380	0.0093
		710	0.0002
9	Helgoland	380–3000	< 0.0001
		710–3000	< 0.0001
15	Helgoland–Svalbard	380	0.0207
		710	0.0094
15	Helgoland	380–3000	< 0.0001
		710–3000	< 0.0001
Dry wt (µg ind.$^{-1}$) at Normocapnia (4-way ANOVA)			
9	Helgoland–Svalbard	M7	0.000031
		M14	0.000031
15	Helgoland–Svalbard	M0	0.000717
		M7	0.000098
		M14	0.000041
Dry wt (µg ind.$^{-1}$) of Helgoland larvae (4-way ANOVA)			
3	M7	380–3000	0.009827
	M14	380–3000	0.00205
		710–3000	0.000035
9	M7	380–3000	0.000031
		710–3000	0.002273
	M14	380–3000	0.000031
		710–3000	0.004209
15	M7	380–3000	0.003527
		710–3000	0.000031
C/N ratio at Normocapnia (4-way ANOVA)			
9	Helgoland–Svalbard	M7	0.005616
		M14	0.000186
C/N ratio of Helgoland larvae (4-way ANOVA)			
9	M14	380–3000	0.000051

The cumulative duration of larval development is shown in Fig. 4. Under all CO_2 conditions, total larval development of Svalbard larvae was prolonged at 9 and 15°C, with a further extension under 3000 ppm as compared to the other CO_2 treatments. The longest zoea phase was found in larvae from Svalbard incubated at 3000 ppm.

Growth and elemental composition

The analyses of biomass (dry wt in µg ind.$^{-1}$) revealed significances for all factors and factor combinations apart from the interactions of all 4 factors (Table 2). The results indicate that dry wt of individuals originating from Svalbard and Helgoland were differently affected by temperature and CO_2 levels and that this was different between the 2 developmental stages under study (see interactions of 3 factors, Table 2).

Dry wt increased from Zoea I to Zoea II and throughout the Megalopa stage (M0–M7–M14; Fig. 5a). At 3°C, no differences were detectable between populations. At 9°C, the dry wt of Helgoland larvae increased significantly during the Megalopa instar (from M0 to M14) whereas it did not in the Svalbard larvae, which grew much more slowly ($p < 0.001$; Table 3). In both populations, the differences between dry wt of Megalopa at 7 d and 14 d were highly significant ($p < 0.001$). Larvae reared at 15°C showed a similar picture. The increment in dry wt of larvae from Helgoland was significantly larger from Megalopa at 0 d to 7 d and 14 d compared to the Svalbard population ($p < 0.001$).

All factors affected the C/N ratios significantly (for all main factors $p < 0.001$; Table 2). Although not all interactions between 2 or 3 factors were significant, the results indicate that the C/N ratios of individuals originating from Svalbard and Helgoland were affected differently by temperature and CO_2 levels and that this was different between the 2 developmental stages under study (for interaction of all 4 factors: $p < 0.01$; Table 2). The C/N ratio at all temperatures showed an increase from Zoea I (3.86) to Zoea II (~5.0) (Fig. 5b). During the Megalopa instar, the C/N ratio was nearly constant, except at 9°C when the values in the Megalopa from Helgoland measured at Days 7 (5.38) and 14 (5.45) increased slightly and those in the Svalbard megalopae (M7: 4.72; M14: 4.61) decreased significantly ($p < 0.05$).

At 3 and 9°C, dry wt of the Helgoland Megalopa at Days 7 and 14 was significantly lower under 3000 ppm CO_2 as compared to normo-

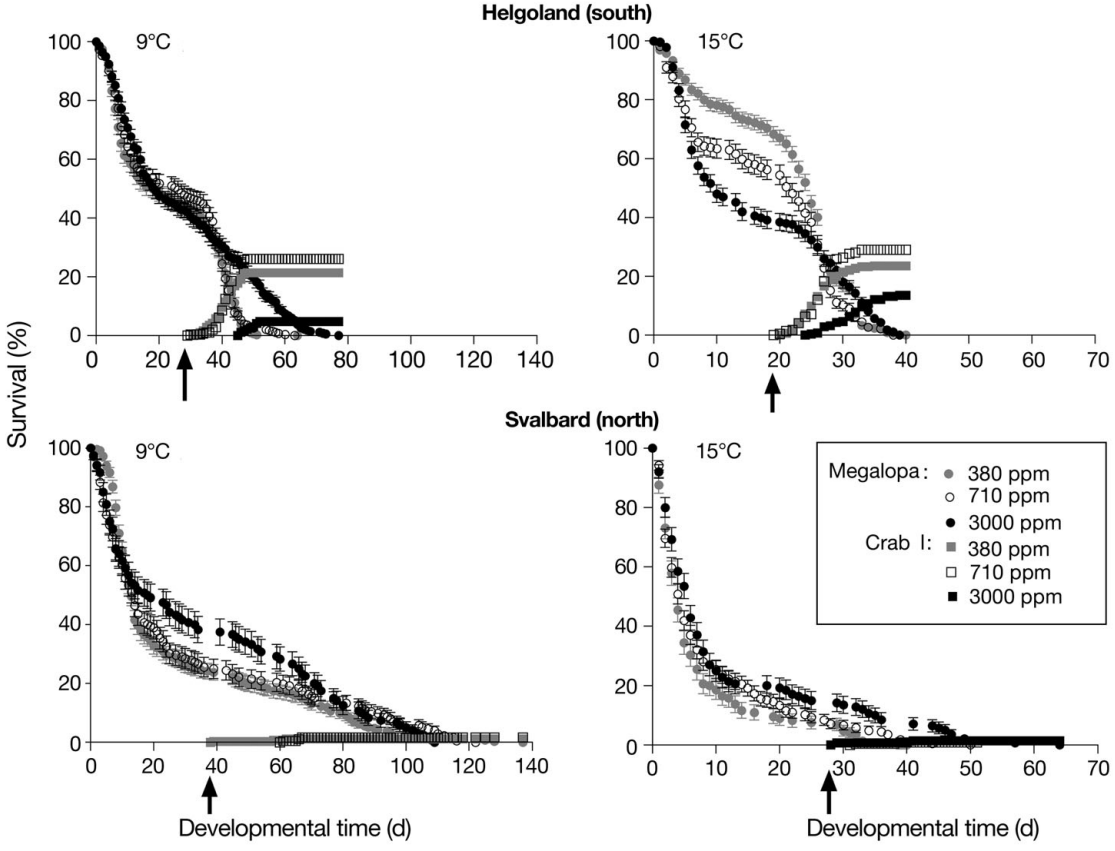
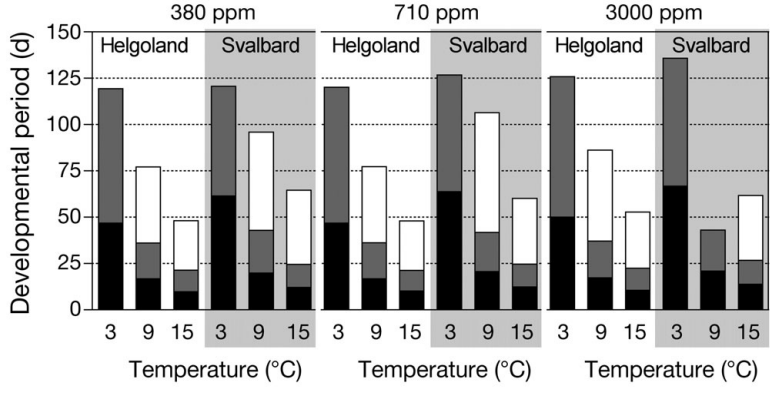


Fig. 3. *Hyas araneus*. Percent survival through Megalopa stage and moulting to Crab I, reared from hatching under 380 (normocapnia ●, ■), 710 (○, □) and 3000 (●, ■) ppm CO_2, at 9 and 15°C for Helgoland (top) and Svalbard (bottom) populations. Values ± SE (initial n = 120–367). Arrows: initiation of metamorphosis of megalopae to Crab I. Chi-squared test, p-values in Table 3



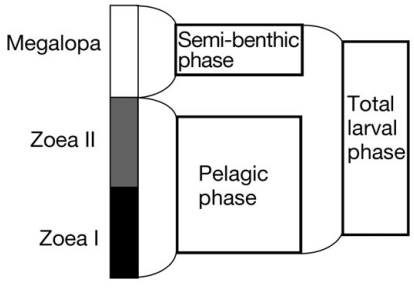


Fig. 4. *Hyas araneus*. Cumulative duration of larval development under 380 (= normocapnia), 710 and 3000 ppm CO_2 and at 3, 9 and 15°C for Helgoland (white background) and Svalbard (grey background) populations. Zoea I (black) and II (dark-grey) represent the fully pelagic larval phases; Megalopa (white) (not recorded for 3°C) is semi-benthic

capnia and 710 ppm (Fig. 6a, $p < 0.01$, Table 3). At 15°C, dry wt of Megalopa at Day 7 was only significantly lower at 3000 CO_2 compared to normocapnia and 710 ppm ($p < 0.01$). A significant difference between C/N ratios measured at the 3 CO_2 conditions was seen in M14 at 9°C, being lower at 3000 (4.55) than under 380 (5.45) or 710 ppm CO_2 (4.96) ($p < 0.001$). For the Svalbard population, no significant differences in the C/N ratio could be identified between the various CO_2 conditions.

DISCUSSION

Population comparison

The ovigerous females of the Helgoland population released their larvae in mid-winter, from late January to late February. This differs from the hatching period observed about 30 yr ago, which occurred

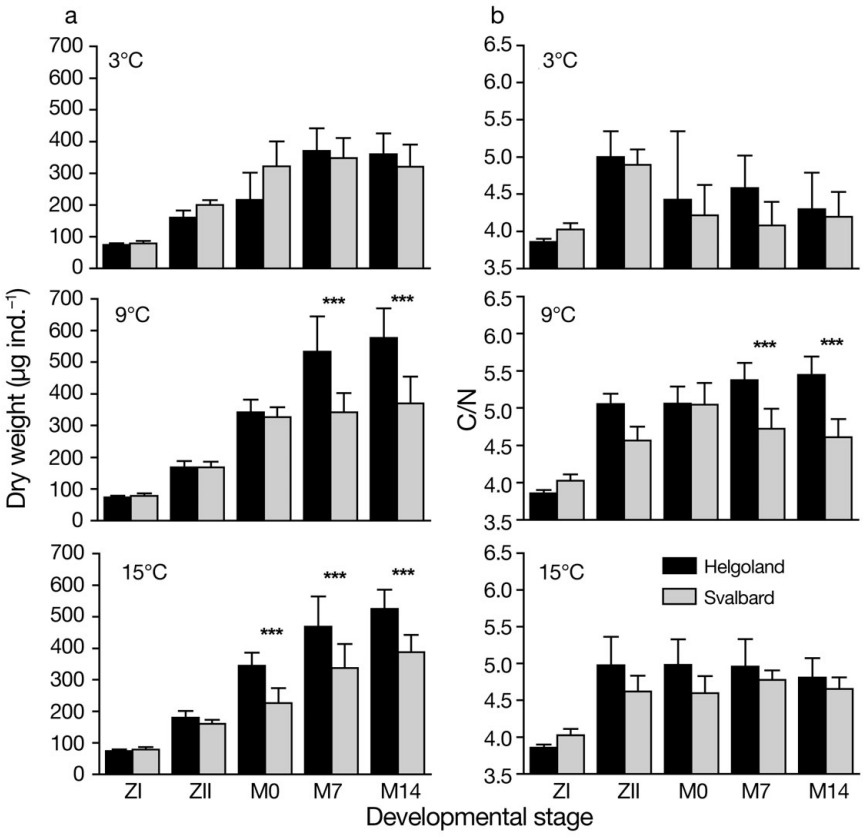


Fig. 5. *Hyas araneus*. Comparison of (a) dry wt and (b) C/N ratio of zoeal stages I and II (ZI, ZII), Megalopa Day 0, 7 and 14 (M0, M7, M14) reared at 3, 9 and 15°C under normocapnia, for Helgoland (black) and Svalbard (grey) populations. Significant differences in dry wt at 9 and 15°C and in the C/N ratio at 9°C. Four-way ANOVA, n = 10, p-values in Table 2

later, mainly from mid-February to mid-March (Anger & Nair 1979, Anger 1986). This shift in phenology might be caused by an increase in the average winter temperature in the southern North Sea from 2 to 4°C (Wiltshire & Manly 2004). In *Hyas araneus*, the seasonal warming in late winter and spring seems to trigger hatching. It relates to the onset of the plankton bloom, and hence, an increasing food supply to the larvae (Starr et al. 1994, Anger 2001). This trigger may now occur earlier during winter, ending the diapause in its 2-yr embryonic development (Petersen 1995). Similar strategies of a biennial reproductive cycle, which is connected to seasonal plankton blooms, have also been observed in other decapods from high latitudes, e.g. *Chionoecetes opilio* (Taylor et al. 1985) and *Paralithodes platypus* (Jensen & Armstrong 1989).

In the Svalbard population, the ovigerous females release their larvae between late February and early April, which coincides with the beginning of the spring plankton bloom in the Arctic region (Hop et al. 2002). Again, this appears to be adaptive, as all larval stages of *Hyas araneus* depend on food supply. As an alternative developmental strategy, other crustaceans at high latitudes pass through a lecithotrophic (i.e. food-independent) larval phase (Anger et al. 2003). This has been observed, for instance, in Lithodidae from sub-Arctic and Antarctic waters (*Lithodes maja*, *L. santolla*, *Paralomis granulosa*), which may release their larvae throughout the year, independent of seasonal plankton blooms (Anger 1996, Thatje et al. 2003).

The larval development of *Hyas araneus* in all stages is generally temperature-dependent, with a shortening at increasing temperature, similar to other crabs (e.g. Nakanishi 1981, Okamoto 1993, Vinuesa et al. 1985, Anger et al. 2003). Larvae from Helgoland and Svalbard differ significantly in the duration of development at the same temperature and within the same stage (Figs. 2 & 3). With only one exception (Zoea II at 3°C), duration was longer in the larvae from Svalbard. This extended development in the Svalbard population may be indicative of a trend towards permanent cold adaptation in the Arctic population. Enhanced energy efficiency seen in polar stenotherms is realized at the expense of extended rates of reproduction and development (cf. Pörtner 2006). This elevated energy efficiency is associated with the narrow temperature range that the species experiences during embryonic development in the North (0 to 6°C; Svendsen et al. 2002), compared to the wider temperature range experienced in the South (3 to 18°C; Wiltshire & Manly 2004). Studies on *Jasus edwardsii* (Smith et al. 2002) and *Rhithropanopeus harrisii* (Laughlin & French 1989) illustrated the correlative effect of temperature on embryonic and larval development periods. Thus, 2 yr of cold temperature presumably cause a reduction of embryonic development, with the consequence of an extended larval developmental period, as necessary for the formation of body compartments.

The larval development of *Hyas araneus* begins after hatching during the coldest time of the year in January to March at Helgoland (Anger & Nair 1979, Anger 1983). The Zoea II is found in April to May, when temperatures are increasing to ~9°C, and the Megalopa develops from May to July at temperatures up to ~16°C. The zoeal stages of *H. araneus* may thus be pre-adapted to low temperatures, while the Megalopa should prefer warmer conditions.

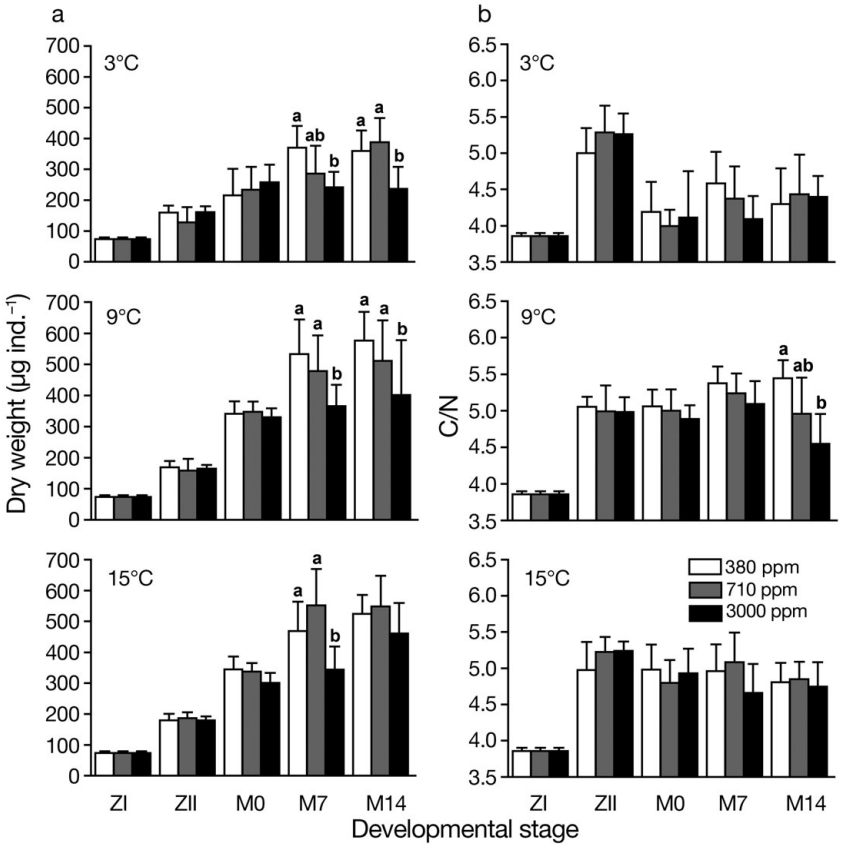


Fig. 6. *Hyas araneus*. (a) Dry wt and (b) C/N ratio at ZI, ZII, M0, M7 and M14 in Helgoland larvae reared at 3, 9 and 15°C under 380 (white), 710 (grey) and 3000 (black) ppm CO_2. Significant differences between different CO_2 condition within each developmental time (lower case letters): 4-way ANOVA, n = 10, p-values in Table 2. See Fig. 5 for stage definitions

The Megalopa instar changes from a pelagic to a semi-benthic phase. During this time, the megalopae select a suitable habitat where they settle and later metamorphose to the first crab instar (Anger & Dawirs 1982, Anger 1983, Sulkin 1984). The delayed development of the Svalbard megalopae may enhance their sensitivity to variability in the physical conditions and exposes them for longer periods to potential predators in their pelagic phase (Morgan 1995). Higher mortality observed in the Svalbard Megalopa at 9 and 15°C and in the Helgoland population at temperatures >15°C may indicate heat stress, which disturbs the enzymatic or hormonal systems that regulate the moult cycle (Anger 1987).

Differences in development periods between Helgoland and Svalbard larvae concur with different growth rates (Anger 1987, 2001). During the development of each instar, dry wt and the C/N ratio showed a decrease during premoult and an increase during the postmoult phase (Anger 1987, Anger et al. 1989), which coincides with patterns of lipid storage (Anger & Hirche 1990), mainly in the hepatopancreas (Storch & Anger 1983). The C/N ratio is a proxy for the lipid to protein ratio, reflecting the fitness of the larvae (Anger 2001). Dry wt and C/N ratio are higher in Helgoland larvae compared to those from Svalbard, in particular during the Megalopa instar (Fig. 5). This is reflected in higher carbon values (authors' unpubl. data), higher C/N ratios, and higher lipid contents, thus indicating a higher fitness level. Anger et al. (1983) found similar levels of dry wt in *Hyas araneus* larvae from Helgoland reared at 12, 9 or 15°C. However, the C/N values reported by Anger et al. (1983) were much lower than in our material from Helgoland, but similar to those from Svalbard. Hence, it seems that larvae from Helgoland reared at 9 to 15°C now have a higher lipid content than about 20 yr ago. This suggests an improved adaptation of the Helgoland population at temperatures up to 16°C, which might enhance their tolerance to changes associated with global warming (Anger 1983, Wiltshire & Manly 2004).

Low levels of dry wt and C/N ratio in megalopae reared at 3°C (for both populations) reflect a lower tolerance of the Megalopa to very low temperatures (Fig. 5). Our data indicate that this temperature is also low for Svalbard megalopae, where the temperature reaches up to 6°C in summer (Svendsen et al. 2002). Svalbard megalopae are thus experiencing temperatures between 3 and 6°C. This is a narrower range than around Helgoland, where the larvae experience 9 to 15°C (Fig. 5). The level of temperature adaptation in each larval stage may be genetically pre-determined, comparable with the genetic pre-adaptation to different salinities observed in crab larvae (Charmantier 1998, Anger & Charmantier 2000, Charmantier et al. 2002).

The metamorphosis of the Megalopa from Svalbard to the first crab instar is disturbed by temperatures >9°C. Global warming may therefore cause reduced survival or migrations of sub-Arctic populations towards colder regions, for instance to the northeast of Greenland (Christiansen 1982). This region is influenced by the extremely cold East Greenland Current, which apparently excludes an occurrence of brachyuran crabs (Christiansen 1982). Warming in the North Atlantic may thus create new habitats for the cold-adapted population of *Hyas araneus* in Arctic regions. Its long planktonic phase ('long-distance larvae'; Thorson 1961) supports dispersal over a wide geographic

range. Our study indicates that the populations from Helgoland and Svalbard show different levels of temperature adaptation, raising the question whether these populations are also genetically distinct. Molecular studies are currently underway in our group to address this question.

CO_2 effects

The Zoea I and Megalopa of the Helgoland population were most severely affected under 3000 ppm CO_2 (Figs. 2, 3, 6). In Svalbard larvae, enhanced CO_2 levels extended the duration of the zoeal stages (Fig. 2). However, in Svalbard Megalopa, delayed developmental time was primarily induced by increased temperature (Fig. 3), which was confirmed by the observation that CO_2 caused no differences in dry wt and C/N ratio (data not shown). Upon closer inspection of the total period of larval development, the prolonging effect of 3000 ppm CO_2 on the cumulative period of larval development is conspicuous (Fig. 4). This developmental delay implies extended exposure to predators in the pelagic environment.

A CO_2 level of 710 ppm showed no significant effects on the developmental parameters studied (Figs. 2, 3 & 6). The question arises whether a threshold concentration exists above which larval development and growth in this species are disturbed. Arnold et al. (2009) demonstrated a decrease in the dry wt of larval lobsters *Homarus gammarus* exposed to 1200 ppm CO_2, but no differences were detected in the period of planktonic development. Presumably, CO_2 first affects larval growth before the duration of development is extended. This is in line with our data, where a trend for dry wt and C/N ratio to decrease was detectable at 710 ppm CO_2 (Fig. 6).

CO_2 diffuses into the larval body, where it acidifies the haemolymph and other compartments (Pörtner et al. 2004). It may thereby interfere with enzymatic or hormonal systems, which are essential for the moulting cycle (Anger 1987). This may be due to the pH-dependence of enzymatic processes and the dependence of hormonal mechanisms on enzyme activities. Prior to moulting, larval metabolism appears to switch from lipid storage to an increasing production of protein (enzymes and structural proteins relevant during moulting), which is reflected in decreasing C/N ratios (Anger 1987, 2001, Anger et al. 1989). CO_2 might affect these metabolic processes, e.g. through metabolic depression (Pörtner et al. 2004). It may thereby reduce the C/N ratios and dry wt (Fig. 6). Lower dry wt might also indicate a thinner and less calcified exoskeleton (Arnold et al. 2009), causing greater susceptibility to predators and disease.

CONCLUSIONS

The discussion above suggests that the Megalopa stage of *Hyas araneus* is more warm adapted and has a narrower thermal window than the 2 zoeal stages, so that this larval instar may be a bottleneck in the life cycle. This putative pattern is schematically illustrated in Fig. 7. In the Helgoland population, ocean acidification should affect larval growth (dry wt) and physiological condition (C/N ratio; Fig. 6). The Svalbard population, by contrast, seems to respond more sensibly to thermal stress than to enhanced CO_2 levels, evidenced by the thermal disturbance of development during the Megalopa stage (Fig. 3) and no differences in fitness related data (dry wt and C/N ratio) under elevated CO_2 levels (data not shown). Further during ontogeny, we may expect that the thermal tolerance window is narrower in the spawner (i.e. the ovigerous females) than in males and non-ovigerous females (cf. Pörtner & Farrell 2008). This hypothesis is supported by observations of ovigerous females that drop their eggs within a few days when they are kept at 18°C (Kunisch & Anger 1984). This projection results from the principles of oxygen- and capacity-limited thermal tolerance: The brooding of the egg masses by crustacean females (Wheatly 1981, Fernández et al. 2000) enhances oxygen demand at constant oxygen supply capacity and, thereby, exacerbates any oxygen limitation. This conclusion is supported by the observation that spawners increase their oxygen consumption rate during the embryonic development (Baeza & Fernández 2002) and especially during warming (Wheatly 1981).

Furthermore, elevated CO_2 causes a narrowing of the thermal tolerance window in adult *Hyas araneus*, illustrated by a shift of the critical temperature from above 25°C to 21°C under 3000 ppm CO_2 (Walther et al. 2009).

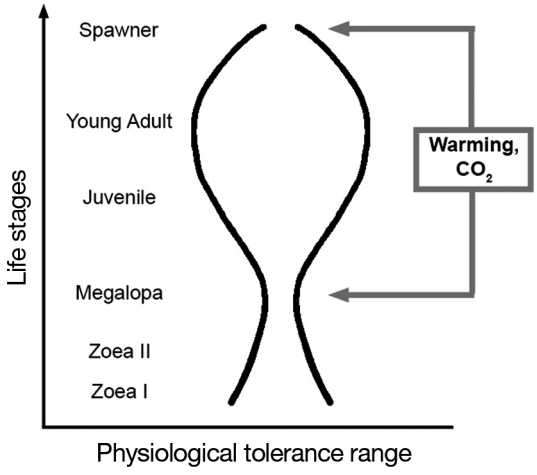


Fig. 7. *Hyas araneus*. Schematic model of ontogenetic changes in the physiological tolerance range. Warming and CO_2 primarily affect the Megalopa stage and adult spawners

At ecosystem level, this narrowing of the thermal tolerance window of *H. araneus* by increasing CO_2 concentrations will thus primarily affect ovigerous females and the Megalopa (Figs. 3 & 6). In conclusion, ocean warming and acidification endanger the recruitment of the benthic life stages of the species. The warming of the North Sea by 1.1°C during the last 40 yr (Wiltshire & Manly 2004) has already led to a drastic decrease in the abundance of *H. araneus* around Helgoland. Additionally, the southernmost distribution limit, which Christiansen (1969) saw in the English Channel, might have shifted north accordingly (Fig. 1). Ongoing ocean acidification trends may exacerbate this trend and shift this limit further and even north of Helgoland (54°N).

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